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Review

Hydrogen, metals, bifurcating electrons, and proton gradients: The early evolution of biological energy conservation

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ABSTRACT

Life is a persistent, self-specified set of far from equilibrium chemical reactions. In modern microbes, core carbon and energy metabolism are what keep cells alive. In very early chemical evolution, the forerunners of carbon and energy metabolism were the processes of generating reduced carbon compounds from CO_2 and the mechanisms of harnessing energy as compounds capable of doing some chemical work. The process of serpentinization at alkaline hydrothermal vents holds promise as a model for the origin of early reducing power, because Fe^{2+} in the Earth's crust reduces water to H_2 and inorganic carbon to methane. The overall geochemical process of serpentinization is similar to the biochemical process of methanogenesis, and methanogenesis is similar to acetogenesis in that both physiologies allow energy conservation from the reduction of CO_2 with electrons from H_2. Electron bifurcation is a newly recognized cytosolic process that anaerobes use generate low potential electrons, it plays an important role in some forms of methanogenesis and, via speculation, possibly in acetogenesis. Electron bifurcation likely figures into the early evolution of biological energy conservation.

1. Introduction

Life is a process of harnessing energy. All models for the chemical-evolutionary transition from the elements on the early Earth to free living cells recognize the requirement for a sustained source of energy. Among the main possible sources of energy that have been discussed, ultraviolet radiation [1], electric discharge [2], pyrite (FeS_2) synthesis [3], and molecular hydrogen (H_2) [4] currently stand in the foreground [5]. Although energy is essential for the origin of life, energy alone is not enough. To be useful for biological systems, energy needs to be conserved, or harnessed, in some form of biochemical currency that can be translated into chemical syntheses and metabolic work. Accordingly, concepts regarding the source of energy that powered early evolution tend to be paired with a concept about carbon metabolism: the ultraviolet radiation and electric discharge theories assume that the first organisms were fermenting heterotrophs while the pyrite and H_2 theories assume that the first organisms were autotrophs that obtained their carbon from CO_2. Pyrite synthesis as an energy source is conceptually linked with the reverse citric acid cycle [6,7], while the idea that H_2 was the first main biological energy source is paired with the Wood–Ljungdahl (acetyl-CoA) pathway [4,8]. The purpose of this paper is to contrast and explore ideas about the nature of early biological energy conservation in the context of some newer findings about how modern anaerobic autotrophs conserve chemical energy.

2. No soup for starters, but maybe vents

Theories involving organic soup or prebiotic broth [9] entail a two stage process: energy input from ultraviolet light [1], electric discharge [2] or interstellar processes [10] to make organic soup, then a second stage in which biologically useful energy is somehow gleaned from that stable, preformed collection of reduced organic compounds, either via oxidation entailing compounds like Fe^{3+} as oxidants [10], or via disproportionations. Both variants correspond to heterotrophy in modern metabolism. Because the simplest forms of heterotrophy involve fermentations, heterotrophy first models have assumed that the first organisms were fermenters that conserved their biochemical energy through substrate level phosphorylations (SLPs) alone. Heterotrophic origin theories thus have two distinct phases of energetic processes at the onset of life: environmental energy spent to reduce CO_2, a portion of which remains stored in organic molecules, and is later harnessed when organic soup serves as a substrate for fermentation.

The list of problems with heterotrophy first models is long [11,12]. From the bioenergetic standpoint, two problems appear sufficiently severe that prebiotic broth can be excluded from further consideration. First, prebiotic soup models assume that

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after the synthesis of organic soup, the process of life somehow starts from a mixture of carbon, hydrogen, oxygen and nitrogen that already has reacted; organic soup is at or very close to equilibrium. Life, by contrast, is a far from equilibrium process [13]. If we homogenize any organism so as to destroy the cellular structure, but keeping all the molecular constituents needed for life intact, and wait for any amount of time in anticipation of those constituents reorganizing into living cells once again, we will be disappointed. Living systems are open systems undergoing carbon and energy flux through very small volumes in space (corresponding to the size of a cell); the site and chemistry of life's origin should have those properties. Second, anaerobic fermenters like *Clostridia* have traditionally been viewed as representing both ancient lineages and ancient fermentative metabolic types [14], and it was long assumed that their fermentations entail solely SLP. But it turns out that clostridial type fermentations entail chemiosmosis, the generation of ion gradients across the cytoplasmic membrane and the harvesting of those gradients via rotor–stator type ATPases [15]. Fermenters that conserve their ATP via substrate level phosphorylations alone are very rare and even such reduced fermentative specialists as *Mycoplasma genitalium* make use of chemiosmosis and rotor–stator type ATPases to generate membrane potential for import and maintenance of ion homeostasis [16,17]. In modern biology, all free living fermenters are either themselves chemiosmotic [15] or are strictly dependent upon organisms that produce fermentable substrates with the help of chemiosmotic energy harnessing. That circumstance is very problematic for theories holding that the first cells were pure fermenters that emerged from a puddle, a pond, or an ocean of organic soup.

The discovery of submarine hydrothermal vents revealed chemically reactive environments, far from equilibrium conditions, as well as temperature, redox and pH gradients [18] and had lasting impact on theories concerning the origin of life. Such far from equilibrium conditions have more in common with life processes than organic soup, and vents might also provide a window into conditions on the early Earth [18]. While submarine hydrothermal vents brought modern biology and thoughts on life's chemical origins closer together, there remained considerable debate about temperature [19], because the black smoker types of vents harbor water that is 350 °C hot. At the same time, thoughts about the nature of the most ancestral kinds of metabolism gradually shifted away from fermentations and towards autotrophy [11,18], especially with the characterization of prokaryotes living in such chemically reactive environments [20]. The characterization of hydrogen rich alkaline hydrothermal vents with mild temperatures of around 70 °C [21,22] underscored once more the possible relevance of submarine vents in early evolution [23,24]. Geochemistry thus offered fresh chemical, energetic, and thermodynamic perspectives on biochemical origins [25]. During those developments, two concepts emerged with regard to the source of energy at the origin of life: pyrite and hydrogen.

3. Pyrite and the reverse citric acid cycle

Wächtershäuser's theory posits that the exergonic synthesis of pyrite (FeS_2) from iron monosulfide (FeS) and H_2S fuelled the first metabolic cycles [3,6,7,26] according to the reaction

$$FeS + H_2S \rightarrow FeS_2 + H_2 \quad (1)$$

with an estimated $\Delta G'_o = -38.4\ kJ\ mol^{-1}$ at pH 7 [7]. That idea is closely linked with the idea that CO_2 entered metabolism via the reverse TCA cycle in an overall reaction that was pulled by pyrite synthesis :

$$4HCO_3^- + 2H^+ + 7H_2S + 7FeS \rightarrow \text{succinate}^{2-} + 7FeS_2 + 8H_2O \quad (2)$$

with an estimated $\Delta G'_o = -429\ kJ\ mol^{-1}$ [6]. The thermodynamic favorability of that reaction has, however, come under scrutiny. Schoonen et al. [27] pointed out a number of difficulties with the temperature dependence and kinetic properties of pyrite-dependent CO_2 reduction. Kalapos [28] pointed out some stoichiometric issues with FeS_2 synthesis and obtained less favorable thermodynamic values for the production of oxalacetate

$$4CO_2 + 5H_2S + 5FeS \rightarrow \text{oxalacetate}^{2-} + 2H^+ + 5FeS_2 + 3H_2O \quad (3)$$

with an estimated $\Delta G'_o = -219\ kJ\ mol^{-1}$, and malate

$$4HCO_3^- + 2H^+ + 6H_2S + 4FeS \rightarrow \text{malate}^{2-} + 2S^0 + 4FeS_2 + 7H_2O \quad (4)$$

with an estimated $\Delta G'_o = -138\ kJ\ mol^{-1}$ [28] via the reductive tricarboxylic acid (rTCA) cycle. Michalkova et al. [29] examined the details of acetate synthesis from CH_3SH and CO with respect to pyrite-pulled reactions and found unfavorable thermodynamic values with regard to pyrite. But it should be recalled that the synthesis of acetate (and the thioester methylthioacetate) did go forward [30], whereby it is often overlooked that some of the best yields reported [30] were obtained with $NiSO_4$ alone as the catalyst, conditions under which pyrite (for lack of either iron or sulfide in the experiment) could not have been produced.

Wächtershäuser's theory of a pyrite pulled rTCA cycle predicts the presence of the rTCA cycle in the common ancestor of all prokaryotes [26]. But in contrast to earlier assumptions [31] the rTCA cycle is not found among archaebacteria known so far [32,33]. Another problem is that in modern microbes, the rTCA cycle is, in known cases, always supported by an independent and chemiosmotic energy metabolism. When chemiosmosis was incorporated into Wächtershäuser's theory, it came as a casual afterthought and with the proton gradient backwards at first (acid inside), requiring the assumption that cells somehow inverted their cytosol from outside to inside, and without specifying any redox partners, squeezing the origin of bioenergetics as it exists in the real world into a footnote [26, p. 1797]. Concerning the source of electrons for CO_2 reduction in primordial biochemistry, Wächtershäuser states that the half reaction

$$H_2 \rightarrow 2H^+ + 2e^- \quad (5)$$

can be "excluded as the first source of electrons since its reducing potential is not sufficient for reducing CO_2" [34, p. 466; 26, p. 1790–1791]. But hydrogenotrophic methanogens and homoacetogens reduce CO_2 with H_2 for a living, they even conserve energy while reducing CO_2 with H_2 [35–37] it is their core bioenergetic process, raising interesting questions as to how they do that.

4. H_2 and Wood–Ljungdahl pathway

Several authors have mentioned in passing that H_2 could have been a source of energy for primordial biochemical systems [38–41]. If H_2 was the electron donor, what was the electron acceptor? From the geochemical standpoint, Sleep et al. [42] underscored the energetic potential of geologically produced H_2 stemming from the hydrothermal process of serpentinization to fuel early evolutionary processes, for example methane production. From the chemical thermodynamic standpoint, Shock and colleagues [43,44] underscored the organic-synthetic potential of hydrothermal systems to generate reduced carbon compounds because of the H_2 accrued in the reducing environment provided by massive amounts of Fe^{2+} in the Earth's crust, showing for example that amino acid biosynthesis is an exergonic reaction under hydrothermal vent conditions [45]. From the biological perspective, Fuchs [46,47] underscored the simplicity and biochemical antiquity of the Wood–Ljungdahl pathway [48], which is the pathway of CO_2 fixation employed by

homoacetogenic clostridia and by methanogens. The antiquity of the Wood–Ljungdahl pathway in the context of an origin of life in hydrothermal vents [4,8] fits well with the kind of chemistry observed in alkaline vents today [21–25,49].

The Wood–Ljungdahl pathway has several properties that make it attractive as an – or probably the most – ancient pathway of CO_2 fixation currently known [4,46,47], eight are listed in the following.

1. The Wood–Ljungdahl (or acetyl-CoA) pathway is a linear pathway of CO_2 fixation [48], not a cyclic one, its most complicated intermediate is a methyl group, and from CO_2 through to acetyl-CoA and on to pyruvate and phosphoenolpyruvate (the next steps in methanogen and homoacetogen CO_2 fixation), there are no chiral carbon atoms in any of the pathway intermediate carbon atoms – albeit many in the cofactors, yet not in their active moieties [4].
2. The enzymes of the pathway are replete with FeS and FeNiS centers [48,50], consistent with the notion that transition metal sulfides and clusters such as in ferredoxin are ancient relics from the earliest phases of biochemical evolution [51]. Furthermore, some of its most important reactions, including the reduction of CO_2 [52] or the synthesis of acetyl thioesters [30], have been performed in the laboratory using only transition metals (and sulfides thereof) as catalysts.
3. In the comparison of the pathway as it is manifested in acetogens (eubacteria) and methanogens (archaebacteria), the pterin cofactors tetrahydrofolate and tetrahydromethanopterin are similar [53], the reactions involved are chemically quite similar and possibly homologous, but the enzymes involved are – for the most part – independently evolved and altogether unrelated at the level of sequence and structure. Exceptions are the starting points (CO_2 reduction) and the endpoints (thioester synthesis). The MoCo binding protein of W-dependent formylmethanofuran dehydrogenase from *Methanothermobacter thermoautotrophicus* is related to clostridial formate dehydrogenase [54] and carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) enzymes from methanogens and acetogens are also clearly related [55]. The lack of obvious sequence similarity for the enzymes catalyzing the steps from formylation of the pterin cofactor up to the methyl transfer to CODH/ACS in acetogen–methanogen comparisons suggests that the basic chemistry of the pathway preceded the origin of genes and proteins [4], consistent with the view that when genes and proteins arose, some of them just added catalysis (hence speed and specificity) to reactions that were already occurring anyway, so that reactions that tend to occur spontaneously could occur more rapidly [31]. The selective/replicative advantage for genetically specified catalysts that increased the flux of carbon into reactive intermediates from which energy could be harnessed is evident for a pathway that generates reactive C1 intermediates, reactive methyl groups, and energy rich thioesters.
4. With the elucidation of several new pathways of CO_2 fixation in archaebacteria and eubacteria [32,33], the Wood–Ljungdahl pathway is currently the only pathway of core CO_2 fixation known to be present in both eubacteria and archaebacteria. This argues strongly in favor of its antiquity. This also argues strongly in favor of the view that methanogens and acetogens represent the most ancestral autotrophs within the archaea and bacteria, respectively. If we embrace the concept of autotrophic origins, this would argue for the ancestral position of acetogens and methanogens within their respective domains, consistent with newer phylogenetic reports for the basal position of methanogens among archaebacteria [56] and the basal position of firmicutes, which contain the clostridial hydrogenotrophic acetogens, among eubacteria [57].
5. In both acetogens and methanogens, which use the Wood–Ljungdahl pathway, there are forms known that lack cytochromes. The acetogens that lack cytochromes lack quinones [35,58]. The methanogens that lack cytochromes lack the quinone functional quinone analog methanophenazine [59]. Acetogens and methanogens are the only autotrophs known (to this author) that harness energy with the help of chemiosmosis but without the help of quinones or quinone analogs. By virtue of simplicity – no quinones, no cytochromes, no soluble electron carriers in the membrane to pump ions, just single ion pumping protein complexes –, that appears to be an ancient energy metabolic configuration, more ancient than with cytochromes and quinones.
6. Abiogenic methane and formate are produced from inorganic carbon at Lost City [49,60,61]. At Lost City geochemical CO_2 reduction is driven by serpentinization, a geochemical process as ancient as water on Earth [42] in which Fe^{2+} in the Earth's crust reduces water in hydrothermal systems to H_2 [42,62], and CO_2 to carbon compounds that leave the vent through the effluent. Lost City effluent contains about 1 mM methane of abiogenic origin [49,60,61]. This is important because the theory that life could have arisen at Lost City types of vents carries with it the prediction that such vents should still be able to carry out some of the same chemical reactions today. The circumstance that abiogenic CO_2 reduction is observed at Lost City strengthens the case for hydrothermal origins, because an observable, exergonic geological process at hydrothermal vents – methane synthesis through serpentinization – is similar in overall chemistry to a biological process (methanogenesis). Methane production at Lost City narrows, but does not close, the gap between biological and geological methane production and is consistent with geochemical data indicating evidence for methanogenesis in rocks 3.5 billion years of age [63].
7. The thermodynamics for the overall synthesis of cell mass under Lost City type conditions (alkaline, reducing, moderate temperature effluent interface with ocean water) is favorable. Amend and McCollom [25] and colleagues [64] have found that the overall reaction for the synthesis of the chemical constituents of a microbial cell is exergonic (energy-releasing) at temperatures of around 50–100 °C for exactly the kind of alkaline hydrothermal vent (chemically similar to Lost City) at the source of current consideration. In other words, the synthesis of life's substance (protein, nucleic acids, cell wall, lipids, etc.) from the concentrations of CO_2, H_2S, HPO_4^{2-}, NH_4^+, H^+ and electrons (H_2) as would be found at the vent ocean interface of such an alkaline hydrothermal system *releases* energy, even without considering main energy metabolic reactions, just considering anabolic cell mass accumulation. That is very important because many people assume that at life's origin one had to add a special kind of energy somehow (for example in the form of lightning), rather than harnessable chemical energy being available naturally, all the time, via H_2 stemming from serpentinization [65].
8. Importantly, the Wood–Ljungdahl pathway is the only pathway of core CO_2 fixation that is also a source of energy conservation [48,66]. All other known pathways of CO_2 fixation require supporting energetic input in the form of an independent energy metabolism, for example photosynthesis, sulfate reduction or other respiration [33,67]. Acetogens and methanogens use the Wood–Ljungdahl pathway not only as their core pathway of CO_2 fixation, it is also their source of energy conservation: they synthesize ATP at the expense of CO_2 fixation, energetically they get a free lunch that they are paid to eat [44]. In the reaction of H_2 with CO_2, the equilibrium lies on the side of acetate and methane, acetogens and methanogens use this energy to generate ion gradients that are used for

chemiosmotic ATP synthesis. Acetogens and methanogens live from reactions that involve very small free energy changes [68]. The overall methanogenic reaction is summarized as

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (6)$$

with $\Delta G'_o = -131$ kJ mol^{-1} [67]. The overall acetogenic reaction

$$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 2H_2O \quad (7)$$

is somewhat less exergonic with $\Delta G'_o = -104.6$ kJ mol^{-1} [69]. The reactions of the Wood–Ljungdahl pathway to the level of the energy-rich thioester

$$2CO_2 + 4H_2 + CoASH \rightarrow CH_3COSCoA + 3H_2O \quad (8)$$

are exergonic with an estimated thermodynamic value of $\Delta G'_o = -59.2$ kJ mol^{-1} [66]; this is enough energy to pump a couple of ions across a membrane, but it is not enough energy to make thioesters for net carbon incorporation and simultaneously to conserve energy through substrate level phosphorylation [69]. That is why acetogens and methanogens are dependent upon chemiosmosis for their energy metabolism.

5. The early formyl pterin problem revisited

The notion that the Wood–Ljungdahl pathway is a viable model for prebiotic chemical evolution is not without energetic problems [4]. The problem concerns the synthesis of formylated pterins in early biochemical evolution, because the synthesis of N^{10}-formyl-H_4F in acetogens and of N^5-formyl-H_4MPT in methanogens from H_2 and CO_2 each entails thermodynamically very unfavorable steps. The problem remains interesting, but a new and exciting solution (exciting from the standpoints of physiology and early chemical evolution) is now in sight.

Specifically, for acetogens, the synthesis of N^{10} -formyltetrahydrofolate from formate and tetrahydrofolate (H_4F) is endergonic with $\Delta G'_o = +22$ kJ mol^{-1} [53]. This is an extremely steep bioenergetic barrier. The reaction catalyzed by N^{10}- formyl-H_4F synthetase goes forward only because it is coupled to ATP hydrolysis. That ATP hydrolysis – by analogy to *Clostridium cylindrosporum* N^{10}-formyl-H_4F synthetase [70,71] – at the $N^1$0- formyl-H_4F synthetase step appears to involve the synthesis of formyl phosphate as the active intermediate. In the absence of enzymes, formyl phosphate will spontaneously formylate H_4F specifically at the N^5-position [72]. But without chemiosmotic coupling, no net ATP synthesis for any (anabolic) process other than acetate synthesis is possible [58]. And although the exact nature of coupling in acetogens growing on H_2 and CO_2 is still not known [73], it almost certainly involves Rnf, a protein complex that pumps ions while reducing NAD^+ with electrons from ferredoxin [74,75], and possibly involves an important and newly characterized mechanism of energy conservation, first described in *Clostridium kluyveri* [76], but turning up in many anaerobes [36,77,78] called electron bifurcation [15,73].

In methanogens, the synthesis of formylmethanofuran from methanofuran (MFR), CO_2 and 2[H], catalyzed by formylmethanofuran dehydrogenase, is also highly endergonic ($\Delta G'_o = +20$ kJ mol^{-1}) if 2[H] is H_2 [36]. The subsequent transfer of the formyl group to tetrahydromethanopterin (H_4MPT) by formylmethanofuran:H_4MPT formyltransferase is slightly exergonic, with $\Delta G'_o = -3.5$ kJ mol^{-1}. Even though free formate does not occur in the methanogen pathway, the synthesis of N^5-formyl-H_4MPT from formate is endergonic with $\Delta G'_o = +9$ kJ mol^{-1} [53]. The reaction catalyzed by formyl-MFR dehydrogenase is coupled to later steps of methane synthesis, which involve chemiosmosis, but the nature of that coupling is, significantly, now known [36] to be different to what was recently thought; an earlier paper [4] assumed that the formyl-MFR dehydrogenase reaction mechanism in hydrogenotrophic methanogens involves chemiosmotic flux of ions into the cytosol through the formyl-MFR dehydrogenase protein, but that was incorrect, as newer findings show [36].

The problem – the early formyl pterin problem [4] – is this: If the Wood–Ljungdahl pathway is genuinely ancient, and if we turn back the clock to the time before early metabolically competent and replicating systems could harness chemiosmosis (which requires genes and proteins), how did they overcome the steeply uphill energetic barriers to synthesizing formyl pterins as critical intermediates in their core CO_2 reduction and energy conservation pathways? The answer appears to lie in a newly recognized aspect of bioenergetics in methanogens and in clostridias (and probably in clostridial acetogens): electron bifurcation.

6. Electron bifurcation in methanogens and clostridial fermenters

The principle of electron bifurcation is familiar. It is a means to drive an endergonic reaction forward by coupling it to an exergonic reaction, but the "energetic currency" of coupling is neither a high energy bond such as a thioester or an anhydride, nor is it a chemisomotic ion gradient, it is a soluble reduced low potential ferredoxin [15,36,73,76–78]. A simple explanation of electron bifurcation, which was only recently proposed an as energy conserving mechanism in clostridial-type fermenters [15,76], is given by Kaster et al. [36], who addressed the problem of how modern hydrogenotrophic methanogens synthesize formylmethanofuran at the highly endergonic formylmethanofuran dehydrogenase step

$$CO_2 + H_2 + MFR \rightarrow formyl\text{-}MFR + H_2O \quad (9)$$

with $\Delta G'_o = +20$ kJ/mol^{-1} [36]. They note that reaction (9) consists of two partial reactions involving Fd (ferredoxin) as an electron carrier

$$H_2 + Fd_{ox} \rightarrow Fd^{2-}_{red} + 2H^+ \quad (10)$$

with $\Delta G'_o = +16$ kJ mol^{-1} [59] and

$$Fd^{2-}_{red} + 2H^+ + CO_2 + MFR \rightarrow Fd_{ox} + formyl\text{-}MFR + H_2O \quad (11)$$

with $\Delta G'_o = 0$ kJ mol^{-1} [59], and point out that the ferredoxin involved in reaction (10) is so far not characterized, whereby *Clostridium pasteurianum* ferredoxin with two reduced FeS clusters (Fd^{2-}_{red}) was used in their enzyme assays [36]. In (over)simplified terms, the synthesis of formyl-methanofuran with electrons from a reduced low potential ferredoxin (instead of from H_2 directly) is not an energetic problem, but the generation of reduced low potential ferredoxin with electrons from H_2 is, which is why many organisms use chemiosmotic coupling (ion influx) to reduce ferredoxin when the electrons stem from H_2 [59]. So where on Earth does the Fd_{red} for the formyl-MFR dehydrogenase reaction come from?

Kaster et al. [36] show that the energetic problem posed by reaction (9) is solved by coupling the endergonic reaction (10) to a highly exergonic reaction (12) catalyzed by heterodisulfide reductase

$$H_2 + CoM\text{-}S\text{-}S\text{-}CoB \rightarrow CoM\text{-}SH + CoB\text{-}SH \quad (12)$$

with $\Delta G'_o = -55$ kJ mol^{-1} [36], whereby CoM and CoB are the thiol cofactors coenzyme M and coenzyme B, respectively. The mechanism of coupling entails neither ATP nor chemiosmosis, it entails electron bifurcation. The soluble, cytoplasmic enzyme complex that catalyzes reaction (12) in *Methanothermobacter marburgensis* (the MvhADG/HdrABC complex) contains FAD as a cofactor, and one of the electrons in the reduced FAD ($FADH_2$) goes in the direction of the highly exergonic reaction (12) while the other electron from $FADH_2$ goes to a low potential ferredoxin in the direction of the endergonic reaction (10). Repeating those uphill and downhill electron routes gives the gray arrows in the scheme shown in Fig. 1a which is redrawn in simplified form from Fig. 4 of Ref. [36].

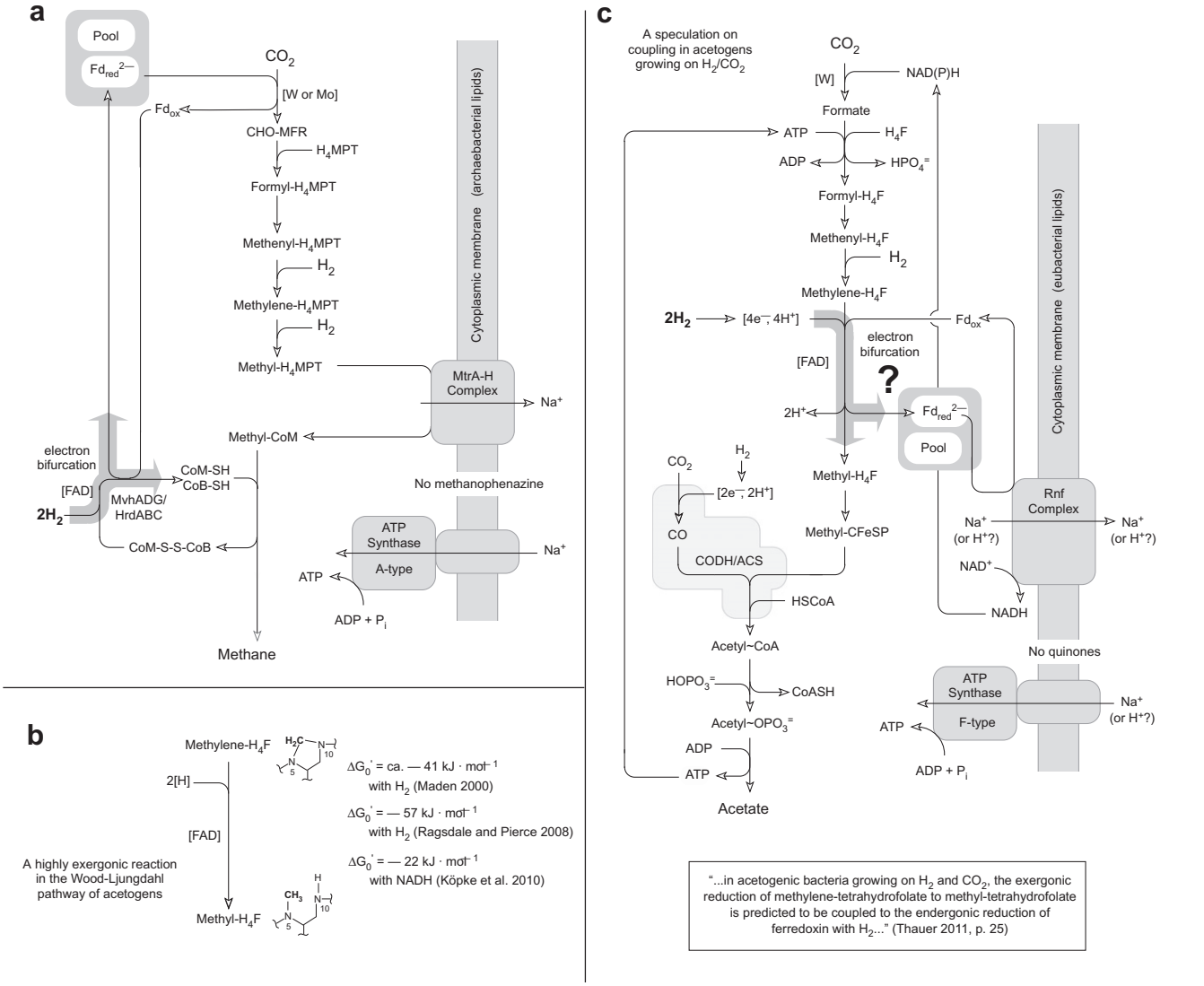

Fig. 1. A sketchy comparison of energy metabolism in hydrogenotrophic methanogens and acetogens. (a) Simplified scheme of energy metabolism in *Methanothermobacter marburgensis* redrawn from Kaster et al. [36]. Electron bifurcation is indicated by a broad gray arow. (b) A highly exeronic reaction in the Wood–Ljungdahl pathway with some estimates from the literature for the corresponding $\Delta G_o'$. (c) Simplified scheme of energy metabolism in acetogens growing on H_2 and CO_2 compiled from references [36,37,50,58,74,75] with an attempt to incorporate the suggestion of Thauer [73] that electron bifurcation might accompany the reduction of methylene tetrahydrofolate (see quote at bottom); the scheme was not endorsed by anyone.

Thus, the overall reaction of formyl-MFR synthesis from H_2 and CO_2 is thermodynamically favorable because reaction (12) is still exergonic even when driving reaction (10) on the side. But this CO_2-fixing reaction, though ultimately driven by H_2, requires Fd_{red} to go forward efficiently, and the reduction of low potential ferredoxin with H_2 requires energy input. That energy is gleaned, and conserved as Fd_{red}, from more favorable steps later in methanogenesis.

Herrmann et al. [15] list several examples in which ferredoxin reduction is coupled to various exergonic reactions via electron bifurcation, and they suggest that electron bifurcation can be viewed as a third form of energy conservation, in addition to substrate level phosphorylation and chemiosmotic coupling. The principle of bifurcating electrons from a reduced cofactor to two different acceptors, one energetically uphill and the other energetically downhill, is not fundamentally new, because the same thing occurs with quinones in the Q-cycle [79] at the cytochrome bc_1 complex of respiration and at the cytochrome b_6f complex of photosynthesis [80]. The idea that reduced ferredoxin is a currency of energy was formulated over four decades ago by Margaret Dayhoff, who wrote about ferredoxin from *Clostridium pasteurianum* [51, p. 363–364]:

> *This protein seems to have arisen at an earlier times than many others which have been studied. We draw this inference from the following considerations. (1) Ferredoxin occurs in primitive anaerobic organisms, both photosynthetic and non-photosynthetic. It must have been present in simpler organisms, the extinct common ancestors of these. (2) Ferredoxin contains iron and sulfur, bonded to the protein at its active site. Ferrous sulfide, FeS, is a widely dispersed mineral, a catalyst which would have been readily available to the most primitive organism. (3) The functions of ferredoxin are basic to cell chemistry. The reduction of ferredoxin is the key photochemical event in photosynthesis by chloroplasts. All the energy is channeled through this compound to other cellular energy storage mechanisms. Ferredoxin is the most highly reducing stable compound so far found in the cell, having a reducing potential near that of molecular hydrogen. This suggests that its function may have evolved at a very early time when the earth's atmosphere*

was still strongly reducing. It reduces nicotinamide adenine dinucleotide (NAD), a ubiquitous reducing agent in the cell. Therefore, it may be even more primitive than NAD. It catalyzes adenosine triphosphate (ATP) formation by radiation. This indicates possible relation to primitive energy transfer processes. It catalyzes the synthesis of pyruvate from carbon dioxide and acetylcoenzyme-A. This indicates its involvement with one of the simplest, most primitive synthetic processes in intermediary metabolism, the fixation of CO_2. It participates in nitrogen fixation and hydrogenase-linked reactions.

Dayhoff (then) expressed the (now) conventional wisdom that iron–sulfur centers represent catalytically active, inorganic relics from the mineral origins of life. Her comment that in photosynthesizers, all the energy is channeled through ferredoxin to other cellular energy storage mechanisms, now curiously applies to hydrogenotrophic methanogens, too. The downhill flow of electrons from Fd_{red} in cyanobacteria and hydrogenotrophic methanogenesis harbors something very familiar, but the chemical means of generating Fd_{red} could hardly be more different – photochemistry at photosystem I vs. electron bifurcation driven by (hetero-)disulfide reduction. Proteins involved in electron bifurcation are turning up in an increasingly diverse spectrum of prokaryotic anaerobes [81], suggesting that this mechanism of generating low potential reduced ferredoxins is even more widespread than initially suspected.

7. Early bioenergetic evolution and electron bifurcation

The prevalence of electron bifurcation in the energy metabolism of clostridia and relatives [15], its prominent essentiality in hydrogenotrophic methanogens [36] as sketched in Fig. 1a, and its predicted significance for acetogens growing on H_2 and CO_2 [73] as sketched in Fig. 1b and c, prompts a brief evolutionary reflection. Could the first free-living cells really have lived from H_2 and CO_2 alone and was the Wood–Ljungdahl pathway really at the root of carbon and energy metabolism at an alkaline hydrothermal vent?

In hydrogenotrophic methanogens, carbon metabolism and energy metabolism are not independent of one another because the anabolic hydrogenases used for net carbon fixation depend upon the ion gradient generated from methane production [36]. In acetogens the situation is very similar, net carbon and energy gain from H_2 and CO_2 are only possible through chemiosmosis [58,82]. In both physiological groups, the Wood–Ljungdahl pathway stands central. Assuming for the sake of argument that biochemistry and life did indeed arise at an alkaline hydrothermal vent [4], we can explore the sequence of events.

The universality of chemiosmotic coupling via ATP synthases among eubacteria and archaebacteria leaves little (or no) doubt that the first *free-living* cells were able to harness ion gradients. The naturally chemiosmotic nature of alkaline hydrothermal vents – roughly pH 9–10 on the vent effluent side [21,22] and roughly pH 6 in the Hadean ocean [83] leaves little doubt such proton gradients did exist at the vent-ocean interface of Archean (>3.8 Ga old) alkaline serpentinizing systems. Geochemists furthermore tell us that serpentinization has been going on since there was water on Earth [42]. So we can assume (safely) that chemiosmotic potential was available in alkaline hydrothermal settings before there were genes and proteins. But the view that naturally preexisting chemiosmotic potential could be harnessed in a biochemically relevant manner without the help of genetically specified proteins [84] is problematic for this author, who contends that we have to get to genes and proteins without bioenergetic help from chemiosmosis. Once genetically specified proteins exist, harnessing geochemically generated chemiosmotic potential is not a problem [4]. But without proteins, the mechanism of coupling remains problematic.

The hydrothermal delivery of chemically reactive methyl groups, such as methyl sulfide, to the vent-ocean interface could, in principle, solve the energetic problem presented by the synthesis of formyl pterins in a chemiosmosis-independent manner [4] – we just leave the hard chemistry of reducing CO_2 to higher temperature and pressure processes (serpentinization, Fe^{2+}-dependent reductions) deeper in the Earth's crust. The circumstance that Lost City effluent contains about 1 mM methane of a biogenic origin, derived from serpentinization [49,60,61], provides evidence supporting that view. But if all the hard chemistry of CO_2 reduction is taking place 3–5 km deep in the Earth's crust, what use is the H_2/CO_2(H_2 from serpentinization and very high marine CO_2 levels from volcanoes) redox couple at the vent ocean interface? Electron bifurcation appears to provide the solution.

Electron bifurcation is a means to generate low potential Fd_{red} in hydrogenotrophic methanogens (and probably acetogens [73]) and Fd_{red} appears to be the key compound linking carbon and energy metabolism in both groups, which lack cytochromes and quinones. At the kind of alkaline vent upon which the present considerations are founded, the business end of reduced ferredoxin – iron monosulfide – comprises the substance of the hydrothermal mound in which, for the purposes of this paper, the origin of gene and proteins is assumed to have taken place to begin with [4,83]. So naturally forming inorganic compartments consisting of freshly precipitated FeS in a highly reducing environment replete with H_2 are not a problem, as they are hard wired into the model. But a chemistry that depends upon physical contact to a hydrothermal mound for catalysis and electrons can only go so far en route to free living cells. At some point electrons have to get from H_2 to CO_2 via proteins and cofactors. That requires electron bifurcation in modern methane and acetate producing biochemistry. Maybe the same was true at biochemical origins.

Can electron bifurcation possibly operate in the absence of aromatic cofactors such as FAD or quinones? It has been suggested [84] that other compounds in addition to quinones and FAD, including W^{IV}, Mo^{IV}, and possibly even H_2 might be capable of electron bifurcation, by virtue of their two-electron donating redox reactions [85]. Indeed, the entry of carbon into metabolism in acetogens and methanogens starts with W- (or Mo-) dependent reactions. Formylmethanofuran dehydrogenase catalyzes the first CO_2-reducing step in methanogens, accepts electrons from Fd_{red}, and requires either Mo or W [86]. Formate dehydrogenase (FDH) in acetogens is a soluble enzyme that catalyzes the energetically unfavorable synthesis of formate from CO_2 and NADPH [87] and requires W [88]; the enzyme from other sources requires Mo [50]; the enzymes in methanogens (formyl-MRF dehydrogenase) and acetogens (FDH) share related components [54] even though they catalyze different reactions. Yet even if W^{IV} and Mo^{IV} could provide electron-bifurcating catalysis, what would serve as the exergonic reaction to permit the generation of Fd_{red} from H_2? Nitrogen oxides [83] is one suggestion, but neither methanogens nor acetogens require nitrogen oxides for growth. Geochemically delivered disulfides of simple organic thiols (such as methane thiol or similar), in analogy to the case of methanogens (Fig. 1a) would be another.

If so, that would explain why Wächtershäuser [26] was partially right concerning the claim that the half-reaction (5) (H_2 as a reductant) cannot reduce CO_2. By itself, it probably cannot, but in the presence of simple organic disulfides, it certainly can in methanogens; the reoxidation of the thiols is achieved through an exergonic carbon reduction step (reduction of a methylthiol to methane). The implication is that in laboratory regimens the same should be true, because enzymes and cofactors do not make impossible reactions possible, they just allow reactions that tend to occur anyway to occur more rapidly. There can be no question that acetogens and methanogens do make cell mass out of H_2, CO_2, and (in diazotrophic forms) N_2 and that the overall reaction is exergonic. But some

individual steps can be steeply uphill, and the mechanistic details of how modern anaerobes that live from H_2, N_2, and CO_2 overcome those hurdles ought to hold insights into the very ancient past.

Taking that thought one step further, and looking at Kaster et al.'s [36] scheme for energy metabolism in methanogens (Fig. 1a) in comparison to what the situation in acetogens might approximate (Fig. 1c), the simplicity of the methanogen pathway is striking. For generation of the ion gradient, no ATP is involved, no NAD(P)H is involved, not even thioesters or acyl phosphates. Were the methyl group transfer from H_4MPT to CoM to be short circuited so as to omit ion pumping we would have an overall reaction identical to methane production at Lost City, which has its own natural pH gradient of ca. 10 (effluent) vs. ca. 8 (today's ocean). At face value, this simplicity speaks for a greater antiquity of methanogenesis (Fig. 1a) compared to the scheme for acetogenesis (Fig. 1c).

It is noteworthy that the acetogen *Moorella thermoacetica* encodes a heterodisulfide reductase of unknown function (HdrABC homologs of the *M. marburgensis* enzyme) directly next to the genes for acetyl-CoA synthase [37] and genes with sequence similarity to the subunits HdrABC and MvrADG of the soluble *M. marburgensis* heterodisulfide reductase are readily found in database searches among firmicutes and other eubacteria that use the acetyl-CoA pathway. Given that heterodisulfide reductase supports electron bifurcation in methanogens [36], its presence in acetogens raises the question of whether electron bifurcation plays more than one role in their carbon and energy metabolism. This issue is all the more pressing with regard to the path of electrons from H_2 to CODH, which is sometimes said to entail Fd_{red} [50], yet leaving the issue open whether chemiosmotic coupling is tapped to move electrons uphill from H_2 to reduce ferredoxin, or whether electron bifurcation coupling might be used [36]. This potential commonality between acetogens and methanogens points to a very ancient role for disulfide reduction in metabolism, as it might play a key role in the energetics of organisms other than methanogens [81]. In the present context, the connection of disulfide reduction to CO_2 reduction as outlined in Fig. 2 seems most important.

Only the energy metabolic routes are sketched in Fig. 1. Both groups have to assimilate net carbon via carbon monoxide dehydrogenase/acetyl CoA synthase (CODH/ACS), which accepts methyl groups from methyl-H_4MPT [85] or from methyl-CFeSP (corrinoid iron sulfur protein [50]), generates CO from CO_2 with electrons (from H_2, but via Fd_{red}?), catalyzes condensation of the carbonyl and methyl groups to an enzyme bound acetyl group that is removed via thiolysis with CoASH yielding the thioester acetyl-CoA [50]. Although N_2 fixation has not been shown for Lost City or similar vents, the transition metal nature of nitrogenase catalysis and laboratory results [90] suggest that it should, in principle, be possible.

In methanogens and acetogens, net energy conservation with concomitant net carbon assimilation from H_2 and CO_2 strictly depends upon chemiosmotic coupling [89,91]. Because chemiosmotic coupling requires proteins, the ability to harness natural chemiosmotic potential at a Hadean alkaline hydrothermal vent should be an genetic invention in the world of genes and proteins. Harnessing chemiosmotic potential also requires semipermeable membranes consisting of a hydrophobic barrier; such hydrophobics are today synthesized in metabolism, but in Lost City effluent alkanes up to pentane are detected [49], suggesting that abiotic synthesis of hydrophobic compounds in the Hadean capable of generating an organic membrane should not pose an insurmountable problem. Net carbon and energy gain through ancestral (inorganically catalyzed) versions of the Wood–Ljungdahl pathway entailing substrate level phosphorylation via thioesters and acyl phosphates [4] would appear possible at a Hadean hydrothermal vent, provided that chemically accessible methyl groups are delivered via serpentinization, for example in the form of methyl sulfide or similar. In that case, the origin of genes and proteins could have been energetically feasible based on soluble reactions (and with time, enzymatically catalyzed versions thereof) that do not require membrane-associated chemiosmotic coupling. Electron bifurcation

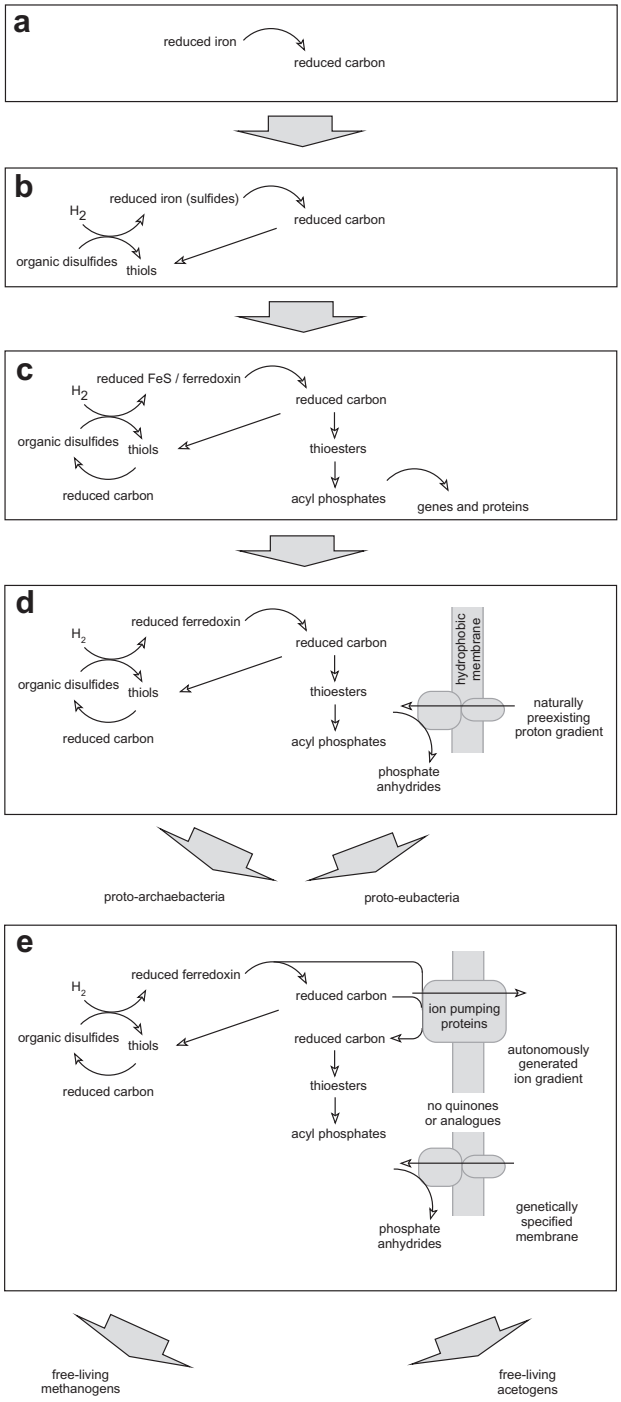

Fig. 2. A hypothetical sereies of events in carbon and energy metabolism, based on earlier schemes [4] but taking newer data from mathanogens and aetogens into account (see legend to figure 1). Reduced iron refers to Fe^{2+} minerals (iron magnesium silicates) that reduce H_2O and CO_2 during the process of serpentinization [49,60–62]. The starting reaction "reduced iron yields reduced carbon" refers both to CO_2 reduction via serpentinization, and to the circumstance that CO_2 reduction process in acetogens and methanogens involves ferredoxin, even though the electrons ultimately stem from H_2. The arrows indicate associated processes and flux.

coupled to highly exergonic reactions without recourse to membrane potential is an alternative to chemiosmotic coupling for generating reduced ferredoxin, and probably represents a relic from the time of carbon and energy metabolism before there were free living cells. The involvement of electron bifurcation to reduce ferredoxin in the carbon and energy metabolism of clostridia [15], methanogens [26] and probably acetogens [73] speaks in favor of its antiquity. A rough general sketch for a possible sequence of stages in early carbon and energy metabolism is given in Fig. 2, the arrows indicate associations as much as flux.

8. Conclusion

Some modern cells overcome the energetically uphill reaction of generating low potential FeS clusters (ferredoxin) with electrons from H_2 via electron bifurcation, it seem possible that early biochemical systems did as well. While modern electron bifurcation entails FAD (or quinones), there is room for (testable) speculation that simpler inorganic catalysts (transition metals) might have been able to do the job. In very early chemical evolution, the problem of whence reduced carbon has priority over whence RNA-like bases, whose origin is conspicuously disregarded here. From thermodynamics, the synthesis of amino acids is the main energy releasing reaction when it comes to the synthesis of cell mass under reducing hydrothermal conditions [25,45], their synthesis compensates for the endergonic synthesis of bases [25]. In metabolism, the bases come from amino acids and carbamates [4], in early evolution a similar sequence can be envisaged. However, in the beginning there is just no way that base synthesis could have been highly specific. The early real RNA world (assuming it existed) must have consisted of messy bases synthesized spontaneously without the help of genes. The chemical environment at the heart of the present considerations is full of reactive C1 and methyl groups (on their way to becoming methane, acetyl groups and acetate), and it is noteworthy that ribosome–tRNA interactions at the heart of the genetic code will not work properly without the myriad base modifications in tRNA and rRNA, the vast majority of which are methylations [92]. That a chemical imprint of the environment in which genes and proteins arose is preserved in the chemical structure of modern tRNA and rRNA bases is something to consider. Biological energy conservation, anaerobes, ferredoxin, and Fe–S proteins are topics that stood central in the interests of Antonio V. Xavier, to whose memory this volume is dedicated. It is hoped that he would have enjoyed this paper.

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References

[1] Haldane, J.B.S. (1929) The Origin of Life. Rationalist Annual 3, 3–10.
[2] Miller, S.L. (1953) A production of amino acids under possible primitive Earth conditions. Science 117, 528–529.
[3] Wächtershäuser, G. (1988) Pyrite formation, the first energy source for life: a hypothesis. Syst. Appl. Microbiol. 10, 207–210.
[4] Martin, W. and Russell, M.J. (2007) On the origin of biochemistry at an alkaline hydrothermal vent. Phil. Trans. Roy. Soc. Lond. B 367, 1887–1925.
[5] Madigan, M.T., Martinko, J.M., Dunlap, P.V. and Clark, D.P. (2009) Brock Biology of Microorganisms, 12th ed, Pearson International Edition.
[6] Wächtershäuser, G. (1990) Evolution of the first metabolic cycles. Proc. Natl. Acad. Sci. USA 87, 200–204.
[7] Wächtershäuser, G. (1992) Groundworks for an evolutionary biochemistry – The iron- sulfur world. Prog. Biophys. Mol. Biol. 58, 85–201.
[8] Russell, M.J. and Martin, W. (2004) The rocky roots of the acetyl-CoA pathway. Trends Biochem. Sci. 29, 358–363.
[9] Orgel, L.E. (2008) The implausibility of metabolic cycles on the prebiotic earth. PLoS Biol. 6, e18.
[10] de Duve, C. (1994) Vital Dust: Life as A Cosmic Imperative, Basic Books, Harper Collins.
[11] Maden, B.E.H. (1995) No soup for starters? Autotrophy and the origins of metabolism. Trends Biochem. Sci. 20, 337–341.
[12] Shapiro, R. (2006) Small molecule interactions were central to the origin of life. Q. Rev. Biol. 81, 105–125.
[13] Lane, N., Allen, J.F. and Martin, W. (2010) How did LUCA make a living? Chemiosmosis and the origin of life. BioEssays 32, 271–280.
[14] Hall, D.O., Cammack, R. and Rao, K.K. (1971) Role of ferredoxins in the origin of life and biological evlution. Nature 233, 136–138.
[15] Herrmann, G., Elamparithi, J., Galina, M. and Buckel, W. (2008) Energy conservation via electron-transferring flavoprotein in anaerobic bacteria. J. Bacteriol. 190, 784–791.
[16] Harold, F.M. (1986) The Vital Force: A Study of Bioenergetics, WH Freeman, New York.
[17] Paulsen, I.T., Sliwinski, M.K. and Saier, M.H. (1998) Microbial genome analyses: global comparisons of transport capabilities based on phylogenies, bioenergetics and substrate specificities. J. Mol. Biol. 277, 573–592.
[18] Baross, J.A. and Hoffman, S.E. (1985) Submarine hydrothermal vents and associated gradient environments as sites for the origin and evolution of life. Origins Life Evol. Biosphere 15, 327–345.
[19] Bada, J.L. and Lazcano, A. (2002) Some like it hot, but not the first biomolecules. Science 296, 1982–1983.
[20] Stetter, K.O. (2006) Hyperthermophiles in the history of life. Philos. Trans. R. Soc. London Ser. B 361, 1837–1842.
[21] Kelley, D.S. (2001) An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. Nature 412, 145–149.
[22] Kelley, D.S. (2005) A serpentinite-hosted ecosystem: The Lost City hydrothermal field. Science 307, 1428–1434.
[23] 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.
[24] Martin, W., Baross, J., Kelley, D. and Russell, M.J. (2008) Hydrothermal vents and the origin of life. Nat. Rev. Microbiol. 6, 805–814.
[25] Amend, J.P. and McCollom, T.M. (2009) Energetics of biomolecule synthesis on early EarthChemical Evolution II: From the Origins of Life to Modern Society, pp. 63–94, American Chemical Society, berlin.
[26] Wächtershäuser, G. (2006) From volcanic origins of chemoautotrophic life to Bacteria, Archaea and Eukarya. Phil. Trans. R. Soc. Lond. B 361, 1787–1806.
[27] Schoonen, M.A.A., Xu, Y. and Bebie, J. (1999) Energetics and kinetics of the prebiotic synthesis of simple organic acids and amino acids with the FeS-H2S/FeS_2 redox couple as reductant. Orig. Life Evol. Biosph. 29, 5–32.
[28] Kalapos, M.P. (2007) The energetics of the reductive citric acid cycle in the pyrite-pulled surface metabolism in the early stage of evolution. J. Theor. Biol. 248, 251–258.
[29] Michalkova, A., Kholod, Y., Kosenkov, D., Gorb, L. and Leszczynski, J. (2011) Viability of pyrite pulled metabolism in the 'iron–sulfur world' theory: quantum chemical assessment. Geochim. Cosmochim. Acta 75, 1933–1941.
[30] Huber, C. and Wächtershäuser, G. (1997) Activated acetic acid by carbon fixation on (Fe, Ni) S under primordial conditions. Science 276, 245–247.
[31] Morowitz, H.J., Kostelnik, J.D., Yang, J. and Cody, G.D. (2000) The origin of intermediary metabolism. Proc. Natl. Acad. Sci. USA 97, 7704–7708.
[32] Hiigler, M. and Sievert, S.M. (2011) Beyond the Calvin cycle: autotrophic carbon fixation in the ocean. Annu. Rev. Mar. Sci. 3, 261–289.
[33] Berg, I.A., Kockelkorn, D., Ramos-Vera, W.H., Say, S.F., Zarzycki, J., Hugler, M., Alber, B.E. and Fuchs, G. (2010) Autotrophic carbon fixation in archaea. Nat. Rev. Microbiol. 8, 447–460.
[34] Wächtershäuser, G. (1988) Before enzymes and templates: theory of surface metabolism. Microbiol. Rev. 52, 452–484.
[35] Koepke, M., Held, C. and Hujer, S. (2010) *Clostridium ljungdahlii* represents a microbial production platform based on syngas. Proc. Natl. Acad. Sci. USA 107, 13087–13092.
[36] 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. 108, 2981–2986.
[37] Pierce, E., Xie, G. and Barabote, R.D. (2008) The complete genome sequence of *Moorella thermoacetica* (f. *Clostridium thermoaceticum*). Environ. Microbiol. 10, 2550–2573.
[38] Koch, A.-L. (1985) Primeval cells – possible energy-generating and cell-division mechanisms. J. Mol. Evol. 21, 270–277.
[39] Russell, M.J., Hall, A.J. and Turner, D. (1989) In vitro growth of iron sulphide chimneys: possible culture chambers for origin-of-life experiments. Terra Nova 1, 238–241.
[40] Deamer, D.W. (1997) The first living systems: a bioenergetic perspective. Microbiol. Mol. Biol. Rev. 61, 239–261.
[41] Kaufmann, M. (2009) On the free energy that drove primordial anabolism. Int. J. Mol. Sci. 10, 1853–1871.
[42] 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.

[43] Shock, E.L. and Schulte, M.D. (1998) Organic synthesis during fluid mixing in hydrothermal systems. J. Geophys. Res. 103, 28513–28527.

[44] 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 (Wiegel, J. and Adams, M.W.W., Eds.), pp. 59–76, Taylor and Francis, Washington.

[45] Amend, J.P. and Shock, E.L. (1998) Energetics of amino acid synthesis in hydrothermal ecosystems. Science 281, 1659–1662.

[46] Fuchs, G. and Stupperich, E. (1985) Evolution of autotrophic CO_2 fixation in: Evolution of prokaryotes (Schleifer, K.H. and Stackebrandt, E., Eds.), FEMS Symposium No. 29, pp. 235–251, Academic Press, London, UK.

[47] Fuchs, G. (1986) CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol. Rev. 39, 181–213.

[48] Ljungdahl, L.G. (2009) A life with acetogens, thermophiles, and cellulolytic anaerobes. Annu. Rev. Microbiol. 63, 1–25.

[49] Proskurowski, G., Lilley, M.D., Seewald, J.S., Frih-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.

[50] Ragsdale, S.W. and Pierce, E. (2008) Acetogenesis and the Wood–Ljungdahl pathway of CO_2 fixation. Biochim. Biophys. Acta 1784, 1873–1898.

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

[52] 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.

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

[54] Hochheimer, A., Schmitz, R.A., Thauer, R.K. and Hedderich, R. (1995) The tungsten formylmethanofuran dehydrogenase from *Methanobacterium thermoautotrophicum*contains sequence motifs characteristic for enzymes containing molybdopterin dinucleotide. Eur. J. Biochem. 234, 910–920.

[55] Lindahl, P.A. (2002) The Ni-containing carbon monoxide dehydrogenase family: light at the end of the tunnel? Biochemistry 41, 2097–2105.

[56] Kelly, S., Wickstead, B. and Gull, K. (2011) Archaeal phylogenomics provides evidence in support of a methanogenic origin of the Archaea and a thaumarchaeal origin for the eukaryotes. Proc. Roy. Soc. Lond. B 278, 1009–1018.

[57] Ciccarelli, F.D., Doerks, T., Mering, von C., Creevey, C.J., Snel, B. and Bork, P. (2006) Toward automatic reconstruction of a highly resolved tree of life. Science 311, 1283–1287.

[58] Miller, V. (2003) Energy conservation in acetogenic bacteria:. Appl. Environ. Microbiol. 69, 6345–6353.

[59] 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.

[60] Bradley, A.S. and Summons, R.E. (2010) Multiple origins of methane at the Lost City hydrothermal field. Earth Planetary Sci. Lett. 297, 34–41.

[61] 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.

[62] Bach, W., Paulick, H., Garrido, C.J., Ildefonse, B., Meurer, W.P. and Humphris, S.E. (2006) Unraveling the sequence of serpentinization reactions: petrography, mineral chemistry, and petrophysics of serpentinites from MAR 15°N (ODP Leg 209, Site 1274). Geophys. Res. Lett. 33, L13306.

[63] Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S. and Isozaki, Y. (2006) Evidence from fluid inclusions for microbial methanogenesis in the early archaean era. Nature 440, 516–519.

[64] Amend, J.P., McCollom, T.M., Hentscher, M. and Bach, W. (2011) Catabolic and anabolic energy for chemolithoautotrophs in deep-sea hydrothermal systems hosted in different rock types. Geochim. Cosmochim. Acta. 75, 5736–5748.

[65] Russell, M.J., Hall, A.J. and Martin, W. (2010) Serpentinization as a source of energy at the origin of life. Geobiology 8, 355–371.

[66] Fuchs, G. (1994) Variations of the acetyl-CoA pathway in diversely related microorganisms that are not acetogens in: Acetogenesis (Drake, G., Ed.), pp. 506–538, Chapman and Hall, New York.

[67] Amend, J.P. and Shock, E.L. (2001) Energetics of overall metabolic reactions of thermophilic and hyperthermophilic archaea and bacteria. FEMS Microbiol. Rev. 25, 175–243.

[68] Deppenmeier, U. and Miller, V. (2007) Life close to the thermodynamic limit: how methanogenic archaea conserve energy. Result. Probl. Cell Differ. 45, 121–152.

[69] Thauer, R.K., Jungermann, K. and Decker, K. (1977) Energy-conservation in chemotropic anaerobic bacteria. Bacteriol. Rev. 41, 100–180.

[70] Mejillano, M.R., Jahansouz, H., Matsunaga, T.O., Kenyon, G.L. and Himes, R.H. (1989) Formation and utilization of formyl phosphate by N^{10}-formyltetrahydrofolate synthetase: evidence for formyl phosphate as an intermediate in the reaction. Biochemistry 28, 5136–5145.

[71] Smithers, G.W., Jahansouz, H., Kofron, J.L., Himes, R.H. and Reed, G.H. (1987) Substrate activity of synthetic formyl phosphate in the reaction catalyzed by formyltetrahydrofolate synthetase. Biochemistry 26, 3943–3948.

[72] Jahansouz, H., Scheriibel, D.M. and Himkes, R.M. (1990) Formylation of tetrahydrafolate by formyl phosphate. FEBS 262, 366–368.

[73] Thauer, R.K. (2011) A novel mechanism of energetic coupling in anaerobes. Environ. Microbiol. Rep. 3, 24–25.

[74] Biegel, E., Schmidt, S., Gonzalez, J.M. and Miiller, V. (2011) Biochemistry, evolution and physiological function of the Rnf complex, a novel ion-motive electron transport complex in prokaryotes. Cell. Mol. Life Sci. 68, 613–634.

[75] Biegel, E. and Miiller, V. (2010) Bacterial Na(+)-translocating ferredoxin: NAD(+) oxidoreductase. Proc. Natl. Acad. Sci. USA 107, 18138–18142.

[76] 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.

[77] Schut, G.J. and Adams, M.W.W. (2009) The iron-hydrogenase of Thermotoga maritima utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production. J. Bacteriol. 191, 4451–4457.

[78] Wang, S., Huang, H., Moll, J. and Thauer, R.K. (2010) NADP+ reduction with reduced ferredoxin and NADP+ reduction with NADH are coupled via an electron bifurcating enzyme complex in Clostridium kluyveri. J. Bacteriol. 192, 5115–5123.

[79] Mitchell, P. (1975) Protonmotive redox mechanism of the cytochrome b–c_1 complex in the respiratory chain: protonmotive ubiquinone cycle. FEBS Lett. 59, 137–139.

[80] Allen, J.F. (2004) Cytochrome b6f: structure for signalling and vectorial metabolism. Trends Plant Sci. 9, 130–137.

[81] Pereira, I.A.C., Ramos, A.R., Grein, F., Marques, M.C., da Silva, S.M. and Venceslau, S.S. (2011) A comparative genomic analysis of energy metabolism in sulfate reducing bacteria and archaea. Frontiers Microbiol. 2, 69.

[82] Schmidt, S., Biegel, E. and Miiller, V. (2009) The ins and outs of Na (+) bioenergetics in *Acetobacterium woodii*. BBA-Bioenergetics 1787, 691–696.

[83] Russel, 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. Lond. 154, 377–402.

[84] 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.

[85] Lord, R.L., Schultz, F.A. and Baik, M.H. (2010) Two-electron redox energetics in ligand-bridged dinuclear molybdenum and tungsten complexes. Inorg. Chem. 49, 4611–4619.

[86] Hochheimer, A., Hedderich, R. and Thauer, R.K. (1998) The formylmethanofuran dehydrogenase isoenzymes in *Methanobacterium wolfei* and *Methanobacterium thermoautotrophicum*: induction of the molybdenum isoenzyme by molybdate and constitutive synthesis of the tungsten isoenzyme. Arch. Microbiol. 170, 389–393.

[87] Thauer, R.K. (1972) CCVreduction to formate by NADPH. The initial step in the total synthesis of acetate from CO_2 in *Clostridium thermoaceticum*. FEBS Lett. 27, 1.

[88] Yamamoto, I., Saiki, T., Liu, S.M. and Lindahl, L.G. (1983) Purification and properties of NADP-dependent formate dehydrogenase from *Clostridium thermoaceticum*, a tungsten–selenium–iron protein. J. Biol. Chem. 258, 1826–1832.

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

[90] Dorr, M., Kassbohrer, J., Grunert, R., Kreisel, G., Brand, W.A., Werner, R.A., Geilmann, H., Apfel, C., Robl, C. and Weigand, W. (2003) A possible prebiotic formation of ammonia from dinitrogen on iron sulfide surfaces. Angew. Chem. Int. Ed. 42, 1540–1543.

[91] Ferry, J.G. and House, C.H. (2006) The step-wise evolution of early life driven by energy conservation. Mol. Biol. Evol. 23, 1286–1292.

[92] Grosjean, H., Gupta, R. and Maxwell, E.S. (2008) Modified nucleotides in archaeal RNAsArchaea New models for prokaryotic biology, pp. 171–196, Caister Academic Press, Norfolk, UK.