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# Hydrothermal Focusing of Chemical and Chemiosmotic Energy, Supported by Delivery of Catalytic Fe, Ni, Mo/W, Co, S and Se, Forced Life to Emerge

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**Abstract** Energised by the protonmotive force and with the intervention of inorganic catalysts, at base Life reacts hydrogen from a variety of sources with atmospheric carbon dioxide. It seems inescapable that life emerged to fulfil the same role (i.e., to hydrogenate $CO_2$) on the early Earth, thus outcompeting the slow geochemical reduction to methane. Life would have done so where hydrothermal hydrogen interfaced a carbonic ocean through inorganic precipitate membranes. Thus we argue that the first carbon-fixing reaction was the molybdenum-dependent, proton-translocating formate hydrogenlyase system described by Andrews et al. (Microbiology 143:3633–3647, 1997), but driven in reverse. Alkaline on the inside and acidic and carbonic on the outside - a submarine chambered hydrothermal mound built above an alkaline hydrothermal spring of long duration - offered just the conditions for such a reverse reaction imposed by the ambient protonmotive force. Assisted by the same inorganic catalysts and potential energy stores that were to evolve into the active centres of enzymes supplied variously from ocean or hydrothermal system, the formate reaction enabled the rest of the acetyl coenzyme-A pathway to be followed exergonically, first to acetate, then separately to methane. Thus the two prokaryotic domains both emerged within the hydrothermal mound—the acetogens were the forerunners of the Bacteria and the methanogens were the forerunners of the Archaea.

**Keywords** Origin of life · Hydrothermal vent · Chemiosmosis · Chemoautotrophy · LUCA

> We are forced to work from energy upward into form, because, at the beginning, form is nothing, energy is everything. H. F. Osborn 1917, p. xv

## Introduction

While Darwin wrote in 1872 that "It is no valid objection {to the theory of natural selection} that science as yet throws no light on the far higher problem of the essence or origin of life" (Darwin 1872, p. 421; Yokey 1995), Haeckel was already considering an autogenic thesis (Haeckel 1870, p. 302). Translated by Lankester in Haeckel 1876, p. 339), this reads: "By autogeny we understand the origin of a most simple organic individual in an inorganic formative fluid, that is, in a fluid which contains the fundamental substances for the composition of the organism dissolved in simple and loose combinations (for example, carbonic acid, ammonia, binary salts, etc.)". Leduc added a further liquid of contrasting chemistry (and pH) to Haeckel's recipe to assert: "the study of life may be best begun by the study of those physico-chemical phenomena which result from the contact of two different fluids" (Leduc 1911, p. xiv). Recalling the synthesis by Traube in 1867 of the first inorganic "artificial" cellular structures, he introduced the idea of a complex of cellular cavities separated by inorganic osmotic membranes—comparable in operation to life's membranes—encapsulating an ever-elaborating and evolving chemistry

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(Leduc 1911, p. 150). To him "The reduction of carbonic acid" was the most important problem facing "synthetic biology" (Leduc 1911, p. 157).

Mining the same autogenic vein from the microbiological perspective, Mereschkowsky (1910, p. 360) considered the first cells to have been anaerobic autotrophs. Harvey (1924) extended this line of thought, suggesting "the origin of living things" might have been in sulphidic hot springs where hydrolytic and other enzymes may not have been required for chemosynthesis and where the source of organic carbon was carbon dioxide. To quote "The chemosynthetic forms of the iron and sulphur bacteria… considered… to closely approach the type of physiological processes demanded for a primitive organism… are able to fix atmospheric nitrogen and synthesize their carbon compounds… using the oxidation of inorganic material such as $H_2S$ as the source of energy for synthesis" (1924, p. 482). And from the geochemical vector, Goldschmidt (1952) was also to favour carbon dioxide (and its nearest derivatives) as the primary substrate for emergent life.

Although these autogenic theorists of life's emergence were paying their considered attention to the material and energetic conditions that obtained on our wet, rocky planet, there was no common explanation for how normally recalcitrant carbon dioxide might be reduced to the organic molecules required for the first sustainable metabolic pathways. In the first model detailing a possible initial autogenic biochemistry Wächtershäuser (1988) suggested reduction was by nascent hydrogen (as $2H^+$ and $2e^-$), produced as sulphide in FeS was oxidized to $S^-$ during sulphidation to pyrite ($FeS_2$). A two-dimensional surface metabolism was held to have ensued (Wächtershäuser 1988). He pointed out that $H_2$ itself could not have been the source of electrons as its reducing potential is not sufficient for hydrogenating $CO_2$, CO or $R{\cdot}COO^-$.

In contrast, Russell et al. (1989) did favour hydrogen as an electron carrier, derived from water on the oxidation of ferrous iron in silicate minerals during serpentinization of the early ocean's crust, and delivered in a solution to a chambered and partly closed hydrothermal mound produced above the site of submarine exhalation at around 100°C. In this case too charge was assumed to have been split by FeS, though the mackinawite nanocrysts (FeS)—acting as a prebiotic hydrogenase—were not oxidized to pyrite, but were the semiconductors that transferred electrons to electron acceptors on the outside of the inorganic compartments. While there is still uncertainty regarding other likely electron carriers, from a geochemical perspective it seems likely that acetate and methane were the first effluents from a rudimentary metabolic system likely to have involved the acetyl coenzyme-A pathway (Russell and Martin 2004; Martin and Russell 2007). Nevertheless, energy derived from this pathway appears inadequate to drive reductions, condensations and biosyntheses required of an emergent metabolic cooperative. Moreover, acetate has defied chemical synthesis in aqueous solution directly from $CO_2$ and $H_2$ in the laboratory; nor has abiotic acetate been recorded in natural hydrothermal conditions (Lang et al. 2007). For an autonomous metabolism to emerge and quicken within the hydrothermal mound without the hydrothermal contribution of primers such as formate and methyl sulphide would have required the exploitation of the ambient protonic energy (Russell and Hall 1997, 2006). But other energies would also have impacted on emerging life. Indeed, during the period that life was first differentiating and emerging from the rocky surface of the planet, it had no autonomy and no way of discriminating against the energetic and material supplies delivered to what we take to be its hydrothermal hatchery. Indeed, earliest life may on occasion have required the exploitation of electrochemical (i.e., chemiosmotic) energies other than the ambient protonmotive force, against which anyway it could have mounted no particular defence (Russell et al. 1993, 1994).

Thus the purpose of this paper is to suggest that the emergence of energy harvesting and chemiosmotic processing concomitant with the origin of life was implicit to the initial conditions of our wet and rocky planet. These initial conditions decreed that protons from the acidulous and carbonic earliest ocean (Walker 1985), streamed downgradient through inorganic precipitate membranes to the alkaline, hydrogen-bearing chambered interior of a submarine hydrothermal mound, wherein the generation of formate from $CO_2$ at a molybdenum site was driven with electrons supplied from hydrothermal $H_2$ via a Ni–Fe mineral cluster acting as a proto-hydrogenase. This proton gradient would also drive pyrophosphate (PPi) formation, and thereby perhaps, the synthesis of a formyl group as well as other prebiotic molecules (Martin and Russell 2007).

## The Top–Down Approach; Its Power and Limitations

True to the Darwinian postulate that extant life is shaped by its evolutionary past, we consider that memories from the origins of chemiosmosis can still be drawn from present-day mechanisms. We will therefore in the following, review general principles of life's ways to harvest energy through chemiosmosis, analyse phylogenetic histories of the respective processes and energies and then attempt the retracing of steps back to their inorganic roots.

The last two to three decades have witnessed an explosion of our knowledge and understanding concerning the extent of the prokaryotic world (Boone et al. 2001). In particular, the impressive diversity of mechanisms dedicated to the harvesting of energy in prokaryotes began to be

truly recognized (Madigan et al. 2008). While this functional diversity and versatility of bioenergetic processes unfolded in front of our eyes, it soon became clear that the manifold of differing mechanisms was based on a quite restricted number of enzymes and even of elementary building components (i.e. protein subunits) thereof (Beinert et al. 1997; Baltscheffsky et al. 1999; Baymann et al. 2003; Volbeda and Fontecilla-Camps 2006; McGlynn et al. 2009).

Some 20 years ago, our understanding of the phylogeny of the prokaryotic world was revolutionized by the introduction of the small subunit rRNA (ssu rRNA) molecule as the standard marker to analyse family relationships among microorganisms (Woese 1987; Olsen and Woese 1993). Overlaying bioenergetic mechanisms on the obtained phylogenetic tree of species allowed tracing the evolutionary pathways of these processes, somewhat reminiscent of analyzing the propagation of haemophilia in royal families. The recognition of the possibility and even high probability of exchange of genetic material between unrelated species, so-called horizontal gene transfer (HGT) (Doolittle 1999), admittedly complicated these analyses but in many cases did not fully blur the picture of vertical inheritance of specific bioenergetic traits.

Most pertinent to the concerns of this contribution is the resulting possibility to conclude on the bioenergetic inventory existing at the root of the phylogenetic tree, i.e. in the entity which is now commonly referred to as LUCA, the Last Universal Common Ancestor of Bacteria and Archaea. The high sophistication of LUCA with respect to all cellular processes emerging from these kinds of analyses clearly precludes imagining LUCA as a single, individual cell. LUCA is presently rather envisaged as a huge conglomerate of individual cells subject to intense HGT and correspondingly sharing a common "gene pool" (Koonin and Martin 2005).

Several strongly different scenarios have been put forward describing this gene pooled community of cells and its influence on, and interaction with, the amino acid pool (Forterre 2002; Di Giulio 2003, 2008). The scenario underlying the analysis presented in this contribution assumes that LUCA corresponded to the biological entity which had originated in the FeS-bearing structures of a warm hydrothermal mound, structures that evolved though a peptidic stage toward compartments that were essentially proteinaceous (Russell et al. 1994; Milner-White and Russell 2008).

The gene pool property of LUCA implies that the evolutionary analysis of metabolic processes in general and of bioenergetic mechanisms in particular almost fully breaks down for the time interval from the origin of life to the diversification of LUCA into Bacteria and Archaea, the two fundamental prokaryotic domains. Just as the "dark ages" of recombined hydrogen in the early universe constitute an insurmountable barrier for analyzing the first hundreds of million years following the assumed Big Bang through visible light; the total gene mixing in LUCA prevents us from pursuing the phylogenetic approach all the way back to the origin of life. It is this time window for which our contribution seeks to develop possible and plausible scenarios by inferring likely correspondences between the bioinorganic roots of somewhat helpless life in the mound and bioenergetic principles in LUCA as well as evolutionary trajectories between these time boundaries of the "empirical black-out zone".

## Chemiosmosis Drove ATP Synthesis Already in the Common Ancestor

### Rotor/Stator Type ATP Synthases

Extant life on this planet drives endergonic cellular processes by exploiting the Gibbs free energy contained in a strong thermodynamic disequilibrium between ATP and ADP. This disequilibrium thus being constantly exhausted for maintaining the entropy decrease characteristic for life, an "outside" energy source must be tapped into to keep ATP/ADP ratios high and thus permit living cells to continuously uphold vital cellular processes. In all extant life, this disequilibrium is maintained by chemiosmotic, i.e. trans-membrane ionmotive potential driven, ATP synthesis (Mitchell 1967). The comparatively energy-inefficient fermentative mechanisms, which furthermore depend on a nutrient-rich, "heterotrophic" environment is the proverbial exception that probes the rule. In the vast majority of cases, the ion gradient consists in proton disequilibria, i.e. pH differences between the two opposite sides of the membrane, although $Na^+$ gradients have also been found to play an equivalent role in a few selected cases.

The enzyme coupling ATP synthesis to the ion gradients is the rotor/stator ATP synthase, a complicated multi-subunit nano-turbo-motor composed of a membrane integral ion channel, a cytoplasm-exposed ATPase module and a stalk coupling ion transport to ATP synthesis via a rotary movement of the first two modules with respect to each other (Elston et al. 1998).

In keeping with its crucial role in maintaining life far from thermodynamic equilibrium, ATP synthase is a ubiquitous enzyme. The genes encoding its constituent protein subunits are readily recognized in and retrieved from sequenced genomes allowing large-scale phylogenetic analyses to be performed (Lolkema et al. 2003; Müller and Grüber 2003). Although some of these analyses emphasize the existence of HGT in ATP synthase phylogenetic trees, the majority and in particular the most recent

(and hence based on the largest datasets) studies conclude on the presence of ATP synthase in LUCA and therefore suggest chemiosmotic coupling to be an ancient process.

Since a pH gradient across the FeS-bearing boundaries is an inherent properties of the foam-like mineral structures in the hydrothermal mound, it is tempting to assume that this ancestry of chemiosmotic coupling extends all the way back to the inorganic roots of life (Russell et al. 1994). A nagging problem for this point of view, however, consists in the overwhelming complexity of the ATP synthase enzyme and the lack of a plausible inorganic counterpart for its catalytic centres in the hydrothermal structures.

## $H^+$-Translocating Pyrophosphatases

The missing link between chemiosmosis at life's inorganic origins and fully fledged, high-efficiency, chemiosmotic ATP synthesis in LUCA is possibly provided by a family of enzymes called "$H^+$-translocating pyrophosphatases". Although the archetypal representative of this family was discovered back in 1966 (Baltscheffsky et al. 1966), its wide, albeit sporadic, phylogenetic distribution was only recognized during the last few years (Baltscheffsky et al. 1999; Hedlund et al. 2006). Thus, as pointed out by Baltscheffsky (1996), $H^+$-translocating pyrophosphatases are a promising candidate for a living molecular fossil permitting insight into the evolutionary time interval stretching from life's presumed origins in the hydrothermal mound to the last common ancestor of prokaryotes. Both their molecular structure and their substrate/product are more "primitive" than ATP synthase and ATP. Schultz and Baltscheffsky (2003) and Baltscheffsky et al. (2004) demonstrate that a bacterial membrane-bound $H^+$-pyrophosphatase, with a conserved loop containing DVGADLVGKVE, representing the phosphate-binding site was the likely precursor synthetase. Examinations of the catalytic PPi synthase site found in *Pyrobaculum aerophilum* and *Thermotoga maritima* (Baltscheffsky et al. 1999) and *Streptomyces coelicolor* (Hirono et al. 2007) show that it occurs attached to the membrane on its cytosol side. Milner-White and Russell (2008) suggest that a racemic or glycine-bearing peptide would automatically form a P-loop when exposed to phosphate ions. Thus, protons driven through a natural channel in an early partly peptidic membrane could have generated PPi in a natural P-loop on the alkaline interior, trussed to the inner side of an otherwise inorganic precipitate membrane (Fig. 1).

As already mentioned, $H^+$-translocating pyrophosphatases are single-subunit, membrane-integral proteins with extended cytoplasm-exposed domains coupling $H^+$-translocation to the phosphorylation of inorganic orthophosphate (Pi) to PPi. PPi itself has been shown to be able to

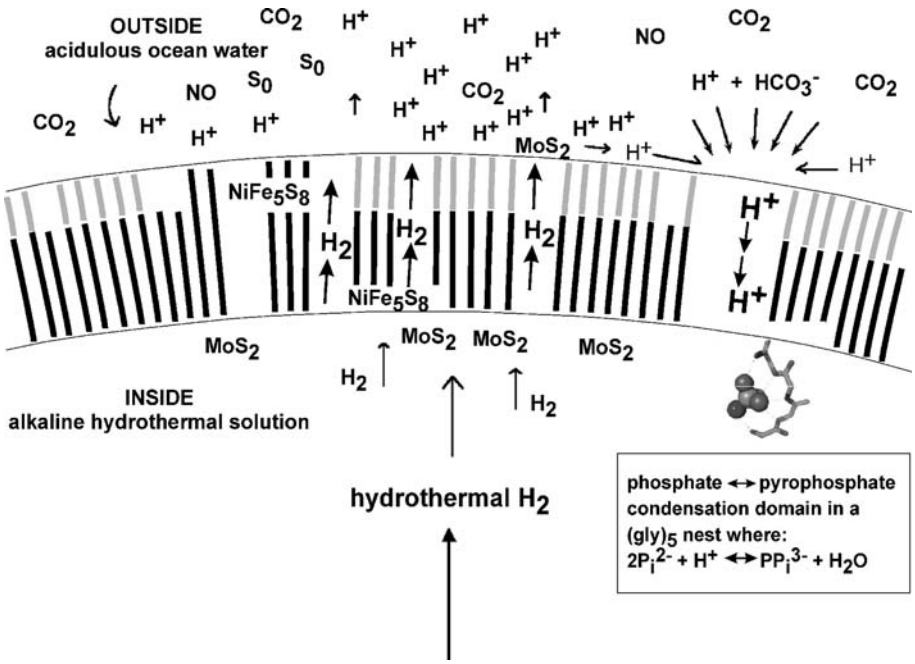


**Fig. 1** Sketch of the earliest sulphidic precipitate membrane considered to have oxidized hydrogen diffusing through the membrane, to protons at nickel/iron clusters. The protons generated on the outside augment those present in the acidulous carbonic Hadean Ocean. The proton gradient is spent through channels where they condense phosphate to pyrophosphate, first in the mineral membrane and later in peptidic phosphate/pyrophosphate nests shown here (Milner-White and Russell 2008). The iron minerals in the membrane would inhibit hydration (de Zwart et al. 2004). The membrane (Mielke et al., in prep.) comprising ferric oxyhydroxides set in carbonates and amorphous silica, harboured mackinawite (Fe(Ni)S), a mineral readily sulphidized to greigite ($\sim NiFe_5S_8$) (Rickard et al. 2001)

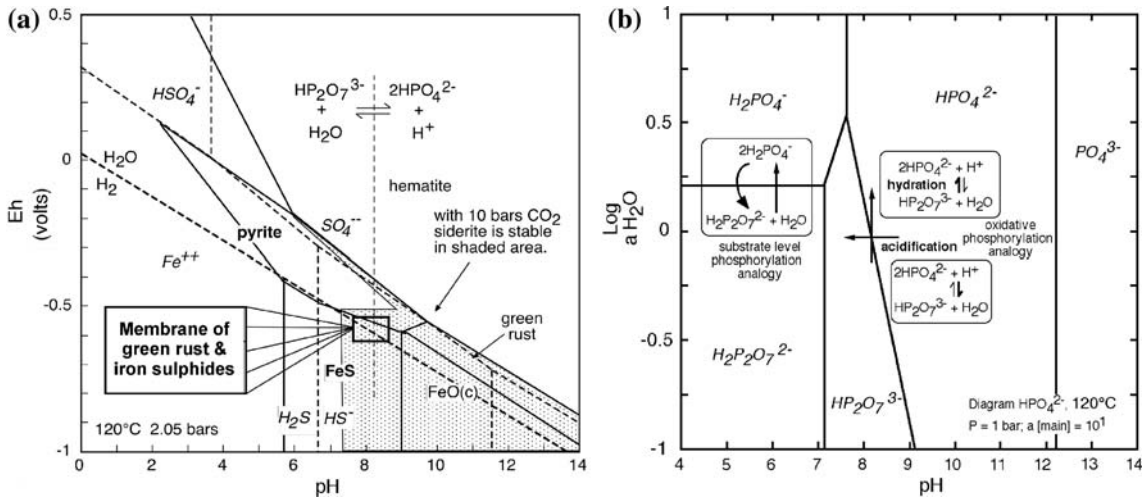


**Fig. 2** Pourbaix (Eh/pH) diagrams (Bethke 1996) to illustrate the stability fields of phosphate and pyrophosphate relative to **a** pH vs. redox and **b** pH vs. water activity in an inorganic membrane containing various embedded iron minerals. From Russell and Hall (1997, 2006)

drive a number of metabolic reactions (Baltscheffsky 1967; Wood 1977), i.e. to fulfil a role rather similar to that of ATP. This does not really come as a surprise given the similarity of the functional inorganic group of ATP to inorganic polyphosphates. The pKa of the diphosphate is ~9 at low water activity (Russell and Hall 1997) (Fig. 2). Initially the channel may have comprised cavities through which the protons were forced to follow by rotational/translational diffusion of water/hydronium molecules adhering to the crystallite surfaces comprising the precipitate membrane (da Silva and Williams 1991, p. 103) (Fig. 1).

Several phylogenetic analyses of $H^+$-translocating pyrophosphatases have been reported (Drozdowicz and Rea 2001; Hedlund et al. 2006). Whereas all authors conclude on a likely pre-divergence origin of $H^+$-translocating pyrophosphatases, that is, its presence in LUCA, the reported phylogenetic trees are far from convincing with respect to this claim. Figure 3 shows a phylogeny of the enzyme based on a different rooting. This tree suggests a differing scenario for the evolutionary relationship between the two subclasses, i.e., the $K^+$-dependent and the $K^+$-independent groups, but eliminates contradictions in previously reported trees and strongly indicates a pre-LUCA origin.

In summary, the $H^+$-translocating pyrophosphatase enzyme family underscores the ancestry of $H^+$ driven chemiosmotic synthesis of polyphosphates and it probably preceded rotor/stator type ATP synthases on the evolutionary time scale (Baltscheffsky 1996). Rotor/stator ATP synthases are structures combining an ATPase module to an ion channel and these modules may therefore well have arisen separately outside of a chemiosmotic context. The single-subunit $H^+$-translocating pyrophosphatase, by contrast, suggests an intrinsic coupling of protonmotive force to PPi synthesis (Hirono et al. 2007). Going one step

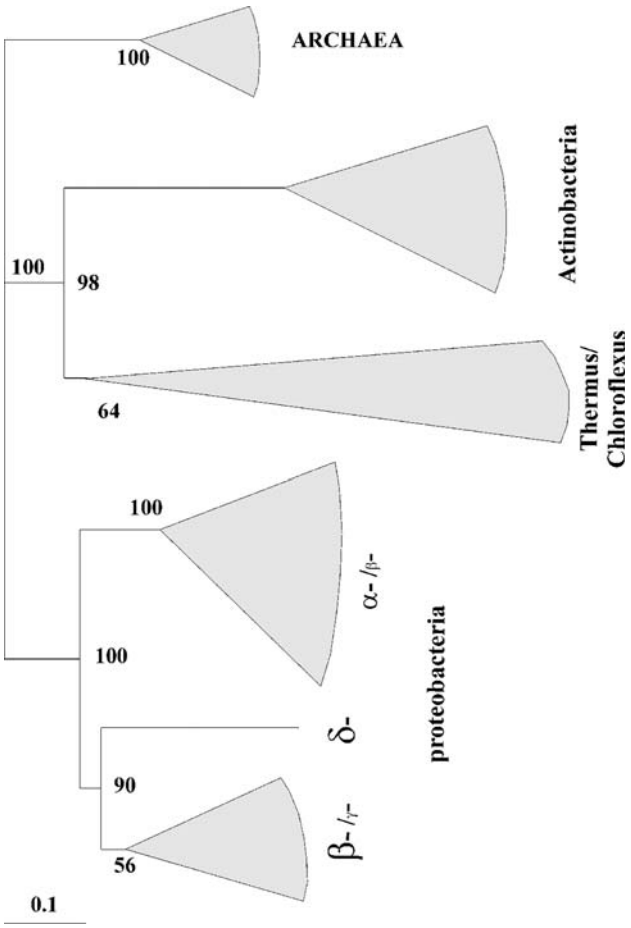


**Fig. 3** Neighbour-joining phylogenetic tree of $H^+$-translocating pyrophosphatases considering only the $K^+$-independent subclass

further from this assessment, we infer that this intrinsic coupling already operated in the inorganic context (Russell and Hall 2006) (Fig. 1a), a scenario augmenting that

envisaging bulk-phase-produced acetyl phosphate as the primordial "chemical" energy (Martin and Russell 2007).

## The Evolutionary Approach; the Geochemical Conditions

This expectation viewed from the geochemical perspective appears to converge with the model favouring $H^+$-translocating pyrophosphatases as a way of harnessing protonic potential, for the alkaline hydrothermal mound taken to be the hatchery of life was bathed in an ocean of protons as well as potential electron acceptors beyond just $CO_2$ such as nitrate, polysulphides and ferric iron (Osborn 1917; Braterman and Cairns-Smith 1983; Zachara et al. 2002; Martin et al. 2007; Duval et al. 2008; Ducluzeau et al. 2009) (Fig. 4a).

Phosphate, derived from PPi in volcanic gases and dissolved from volcanic glasses, would have been present in millimolar quantities in the acidulous Hadean Ocean and been precipitated at the margins of the hydrothermal mound along with a variety of ferrous and ferric iron minerals (Staudigel et al. 1981; Spivack and Staudigel 1994; Yamagata et al. 1991; Hagan et al. 2007). It has been argued that phosphate precipitated in these conditions would be reconverted to PPi in the iron-bearing membrane by protons streaming through from the Hadean Ocean (Figs. 1, 2a, b), thus augmenting that produced during substrate level phosphorylation (Russell and Hall 1997, 2006) (Fig. 2b). Ferrous ions and ferrous iron minerals in the precipitate membrane would protect any PPi so produced from simple hydrolysis (de Zwart et al. 2004).

As a first step, this PPi could have polymerized amino acids produced in the alkaline mound through the amination of alpha-keto acids (Huber and Wächtershäuser 2003; Huber et al. 2003). These short peptides could then have sequestered mono- and pyrophosphate, thus improving their condensing efficiency (Milner-White and Russell 2008) (Fig. 1). At the same time such peptides could have improved the efficiency of the precipitate membrane itself. To understand how requires that we consider the makeup of these precipitate membrane barriers. In the hydrothermal model for the emergence of life favoured here, semiconducting iron sulphide nanocrysts such as mackinawite (comprising $[Fe \gg Ni]_2S_2$ rhombs) and greigite ($NiFe_5S_8$ partly comprised of cubanes), and oxyhydroxides such as green-rust ($Fe^{II}_4Fe^{III}_2(OH)_{12}{\cdot}CO_3{\cdot}2H_2O$) contributed to the inorganic osmotic and chemiosmotic baffles between the hydrothermal fluids and the acidulous Hadean Ocean (Fig. 4). These nanocrysts—assumed to have acted as hydrogenases and dehydrogenases (Russell et al. 1994; Russell and Arndt 2005)—were the first components of a redox protein construction kit (Baymann et al. 2003). In these conditions, electrons lost from hydrothermal thiolates to acceptors on the outside of the cells would lead to the assembly of thiolated metal sulphide clusters within the membranes (Bonomi et al. 1985; Russell et al. 1994). Such thiolated metal sulphides would then be the more easily sequestered by random peptides produced within the same system heralding the organic takeover (Mulholland et al.

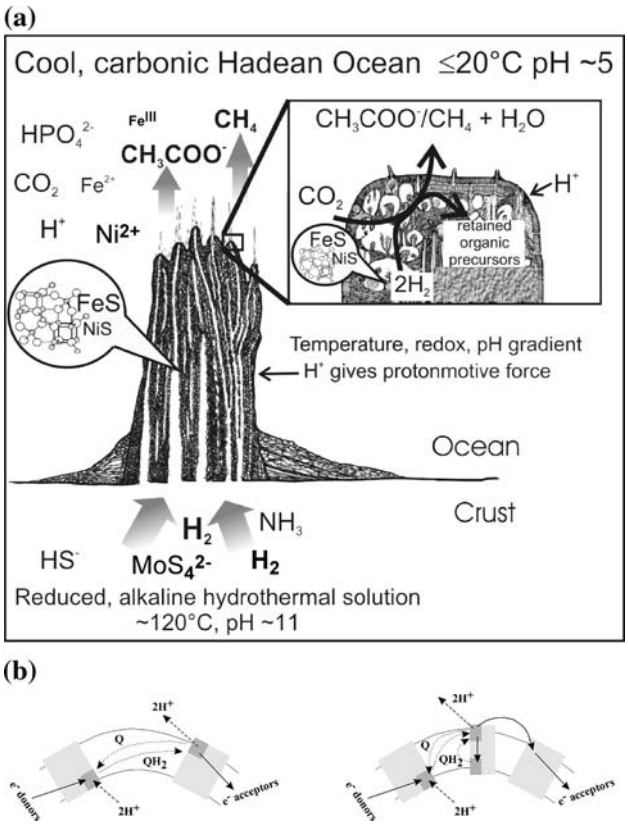


**Fig. 4** **a** Model environment for the emergence of life at a submarine seepage on the ocean floor. The walls of the complex three-dimensional semiconducting and semipermeable inorganic compartments comprising parts of the mound and occupied by the flux of alkaline, hydrogen-bearing hydrothermal solution comprise nanocrysts of iron sulphides and hydroxides. The mound acts as a natural self-restoring flow reactor and fractionation column. Note that the ambient gradients imposed on the outer margins of the mound include a natural potential protonmotive force and high redox gradients resulting from the presence of dissolved $CO_2$, as well as nitrate, $S^0$ and $Fe^{III}$ (Braterman and Cairns-Smith 1983; Duval et al. 2008; Ducluzeau et al. 2009). While phylogenetic evidence points to the involvement of NO and its derivatives as well as of elemental sulphur as electron acceptors in LUCA, there is no such evidence for $Fe^{III}$ although it is a common electron acceptor for prokaryotes near the base of the evolutionary tree (Vargas et al. 1998). After Russell and Martin (2004), Russell and Hall (2006, 2009), Mielke et al. (in prep). **b** Schematic representation of the basic principle of proton gradient build-up in bioenergetic systems, relying on the vertically asymmetric arrangement of quinone reducing and quinol oxidising sites. In the primordial setting (cf. **a**), this asymmetry was imposed by the environment of the mound, i.e. alkaline hydrogen-bearing, hydrothermal solution from the inside separated from the acidulous carbonic ocean on the outside by the precipitate membrane

1999; Huber et al. 2003; Milner-White and Russell 2008). Eventually these peptides could, as amyloidal 'protein', have taken over the role of baffles between the alkaline hydrothermal solution and the acidulous ocean.

## The (Present and Past) World of Chemiosmotic Potential Build-Up

Whereas the hydrothermal mound scenario provides chemiosmotic potential "for free" (Fig. 4), extant organisms need to generate their own pH gradient. Invariably they do this through various kinds of energy-conserving mechanisms. These are based on collapsing electrochemical disequilibria between redox substrates either delivered by the environment or created internally relying on the energy of photons. As so often with life's principles, this "general mechanism" is only imperfectly general, since the archaeal light-driven proton pumps (bacteriorhodopsins) do not fit into this scheme. Although we voluntarily neglect this rather isolated bioenergetic process, we do clearly (and literally) see the utmost importance of this protein for the way more complex organisms perceive the outside world.

At first sight one might argue that the need to actively generate chemiosmotic potential arose only when cells first struggled free from the hydrothermal mound. However, as we shall see below, a number of the ensemble of mechanisms that build up protonmotive force appear to have been already operating in the common ancestor. According to several lines of argument presented previously (Martin and Russell 2007), LUCA was almost certainly confined to the mound. This means that prior to the transition to free-living cells, life in the mound had implemented reactions collapsing electrochemical potential. Whereas the benefit of these reactions may not have been to increase the protonmotive force but rather to reduce $CO_2$, as will be discussed below, the majority of them is likely to have worked as chemiosmotic generators. It seems sensible to assume that these mechanisms have helped life withstand an occasional dearth of protons, i.e. a loss of the "free" pH gradient, induced for example, through major leakages of alkaline hydrothermal solution at the base of the mound.

As was to be anticipated, modern organisms have learnt to tap into virtually every available electrochemical energy source to drive electron transfer coupled to $H^+$ (or, more rarely, $Na^+$) translocation with the aim of producing a transmembrane gradient, positive on the outside and thus perfectly resembling the situation in the hypothetic "hatchery-of-life" hydrothermal system. A (still growing) plethora of bioenergetic, that is, chemiosmotic potential generating, electron transfer chains have correspondingly been discovered during the last decades, which sometimes even use "exotic" substrates such as arsenics, selenates or chlorate/perchlorate (Wallace et al. 1996; Oremland et al. 1994, 2000; Coleman et al. 2003; Lebrun et al. 2003; Duval et al. 2008).

The observation that many enzymes involved in these ancient redox reactions use inorganic catalysts such as nickel, cobalt, molybdenum, tungsten or iron sulphur clusters (e.g. Schönheit et al. 1979) has already been taken to suggest that the corresponding electrochemical reactions are deeply rooted within the inorganic origins of life (Russell et al. 1994; Russell and Hall 1997, 2006; Volbeda and Fontecilla-Camps 2006; McGlynn et al. 2009). Before coming back to the possible details of an early inorganic coupling between redox reactions and the chemiosmotic gradient, we will attempt a summary of common features and idiosyncracies of pre-LUCA electron transfer chains and to identify inorganic counterparts of the respective bioenergetic processes.

## A Minimal, Archetypal Electron Transfer Chain for Building-Up a Chemiosmotic Potential

The common theme for setting up a bioenergetic electron transfer chain goes like this: look around to see what is out there in the environment in terms of electrochemical disequilibrium, i.e. search for a redox couple which is present essentially in the reduced state (and call it "the donor") and a second one for which the reverse is true (call it "acceptor"). Ideally but not indispensably, the donor should have a lower standard redox midpoint potential than the acceptor. Whether for given concentrations and reduced/oxidised ratios of donor and acceptor, the electron transfer will be exergonic can be calculated by the ordinary textbook equations. Then take two different trans-membrane proteins and insert catalytic sites (typically some kind of metal or clusters of metals) suited to oxidize the donor on one of them and reduce the acceptor on the other. The catalytic sites shall naturally be positioned on the side of the membrane where the respective substrate is accessible. The next step is to create a quinone-binding site on each of these trans-membrane structures, situated inside for the donor-oxidising and outside for the acceptor-reducing protein. If the catalytic sites and the quinone-binding sites turn out to be on opposite sides of the membrane, a trans-membrane wire needs to be added (usually in the form of hemes). This system will create a proton gradient while it collapses the electrochemical disequilibrium merely by the fact that the quinone will have to suck a proton off the cellular inside while it gets reduced and deliver it to the outside when reoxidized (Fig. 1b left scheme). Admittedly, evolutionary optimization of efficiency has added more bits and pieces such as for example direct proton pumping (in heme copper $O_2$ reductases or Complex I). The basic setup of a bioenergetic chain, however, is as outlined above and

many chains indeed function only relying on the described principle of vectorial $H^+$ transport by quinones (Fig. 1b left scheme).

Aficionados of bioenergetics may protest that this scheme is oversimplified since it omits the quasi-ubiquitous and almost certainly pre-LUCA Rieske/cytochrome *b* complexes. These entities, however, do not directly deal with the electrochemical substrates but serve as a chemiosmotic turbo-charger, increasing the efficiency of energy conversion to the thermodynamic limit (see below). The way it does this well illustrates the principle outlined above of membrane–anisotropic quinone-binding sites and we therefore schematically present this mechanism in Fig. 1b (right scheme).

Chemiosmotic electron transfer chains being strictly linked to the presence of quinones, we tend to think of the quinone as the quintessential factor of energy-conserving electron transport. Concerning the question of the chemical nature of the quinone in LUCA, it seems almost inevitable to us to stipulate that this was menaquinone (Nitschke et al. 1995; Schütz et al. 2000). Almost all prokaryotic phyla use menaquinone for bioenergetic needs and in only a few localized regions of the tree do different chemical types of quinones appear (Fig. 5).

Three of these "aberrant" quinones (i.e. ubi-, plasto- and caldariella-quinone) represent merely independent evolutionary solutions to the challenge of producing quinones with a substantially higher redox midpoint potential (Schoepp-Cothenet et al. 2009). The evolutionary driving force for this redox adaptation almost certainly comes from the much later oxygenation of the Earth's surface and the ensuing increase in ambient redox potential (Williams and Frausto da Silva 2003). In the fourth region of Fig. 5, the "new" membrane-soluble hydrogen carrier, methanophenazine, is chemically speaking not even a quinone. Although we do not discuss the evolutionary bearing of this molecule further, this molecule does fulfil exactly the same role and binds to sites on enzymes which in other species are reserved for the quinones.

## Consanguinity of Particular Redox Properties Between $H_2$, Mo/W-Pterin, Quinones and Flavins

In the early days of chemiosmotic research, the quinone was considered as just an electron and proton shuttle for the lipid phase of membrane bioenergetics and was condescendingly called "coenzyme Q". Through the study of specific bioenergetic enzymes such as the purple bacterial reaction centres and the cytochrome $bc_1$ complex (Crofts et al. 1983, Robertson et al. 1984), it soon became clear that one specific property of quinones was pivotal to the function of bioenergetic chains in general; this property consists in the extreme versatility of the two-electron redox reactions and their coupling to the protonation state of the quinone. Depending on their environment (e.g. to the detailed make-up of the binding sites on specific redox enzymes), quinones either go through consecutive single redox steps (therefore involving a stable intermediate "semiquinone" state) or, if the electrostatic environment destabilizes this semiquinone form, the potentials of the individual steps are modified so as to end up in a concerted, two-electron redox reaction which goes together with the appearance of redox phenomena that almost appeared magical when first observed in chemiosmotic systems. A classic example is the counterintuitive observation of the reduction of low potential heme centres in mitochondria after addition of the strong oxidant ferricyanide (Hurt and Hauska 1982; von Jagow et al. 1984). This phenomenon, termed "oxidant-induced reduction" was dealt with in numerous articles of the early

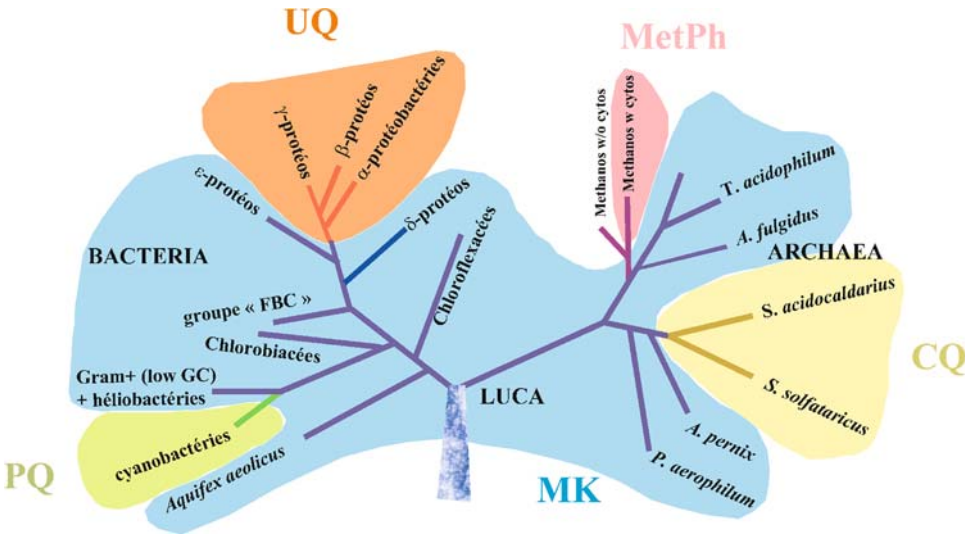


**Fig. 5** Distribution of different chemical types of quinone and the functionally related methanophenazine on the tree of species. *Methanopyrus kandleri*, a hyperthermophilic methanoarchaeon growing on $H_2$ and $CO_2$ has been proposed as being closely related to LUCA (Slesarev et al. 2002; Wong et al. 2007). This species is located on the branch denoted "Methanos w/o cytos"

chemiosmotic literature and was eventually rationalized via the redox properties of the quinone bound to the so-called $Q_o$-site in Complex III, a site which strongly destabilizes the semiquinone form. Oxidation of quinol in this site by the relatively oxidising iron–sulphur centre of the enzyme (or alternatively by the redox chemical ferricyanide in the early experiments) thus induced the generation of an extremely destabilized and hence strongly reducing semiquinone species able to reduce the low potential heme centres. This redox chemistry indeed turned out to be crucial to the capacity of the Rieske/cyt*b* (including Complex III) enzymes to squeeze the very last drop of Gibbs free energy out of a given couple of redox substrates and to convert it into chemiosmotic driving force.

In the $Q_A$ and $Q_B$ binding sites of photosynthetic reaction centres, by contrast, the redox properties of the quinones are completely different but again optimized to the specific functional requirements of the respective reactions. Other redox enzymes such as nitrate reductase or photosystem I harbour quinones with again very differing redox properties further illustrating the extent of the variability of the quinones' electrochemical capacities.

We therefore think it is fair to say that the intrinsic redox properties of the coupled two-electron transitions of quinones are fundamental to bioenergetic chains building up chemiosmotic potential. However, the quinone is not unique in possessing these useful redox characteristics. Flavins display related redox properties and may be regarded as the "quinone of the aqueous phase". Molybdenum with its two-redox transitions from MoIV to MoVI also can do these fancy electrochemical tricks, just as tungsten can (Schulzke 2005), and this may in part explain why molybdoenzymes are functionally so versatile. Most intriguingly but structurally not astonishingly, molecular hydrogen also belongs to this group of redox wizards and may possibly even be regarded as the penultimate ancestor of quinone-type electrochemistry. The oxidation of $H_2$ in [NiFe] hydrogenases is generally considered to proceed via a heterolytic cleavage resulting in the formation of the strongly reducing (−2.25 V), extremely unstable intermediate hydride, $H^-$, very much reminiscent of the quinone reaction at the $Q_o$-site.

The ability to bifurcate two-electron transfer reactions and thus recover electrochemical driving force for strongly endergonic processes was certainly essential to bioenergetic transport operating in LUCA but to our minds is likely to already have played an equally pivotal role in the redox reactions within the mound frequently operating at the thermodynamic edge. The fact that the $H_2$ and Mo/W delivered in alkaline solution to the mound both dispose of these extraordinary redox capacities further supports this point of view. However, for this redox bifurcation to allow seemingly endergonic reactions to proceed, thermodynamics require the presence of a sufficiently oxidising electron acceptor, i.e. a substrate pulling out the first electron and thereby creating the out-of-equilibrium semi-reduced state. Nitrogen oxides may have been these oxidising substrates (Ducluzeau et al. 2009). The structural and functional similarities between the Mo/W-(bi)pterin, flavins and menaquinone might be taken to indicate an evolutionary development, possibly driven by a dearth of molybdenum/tungsten supply as the mound itself grows further from the succour of the hydrothermal solution.

## Enzymes Coupling Substrate Electrochemistry to the Redox Conversion of Quinones

Whereas the quinone thus represents the constant core of electrochemical build-up of chemiosmotic potential, the coupling sites of substrate to quinone redox reactions are extremely variable. The entities depicted as light grey boxes in Fig. 1b indeed correspond to a plethora of different membrane-integral redox enzymes performing the oxidation or reduction of redox substrates. Some of these redox enzymes may indeed be old, that is, may have been present in LUCA whereas others certainly have more recent origins as a result of shifts in the geochemical environment and the concomitant appearance of previously unavailable redox couples (Williams and Frausto da Silva 2003; Duval et al. 2008). Figure 6 represents a sub-sample of such enzyme systems which appear to have pre-LUCA origins and which perform redox reactions that possibly occurred in the mound.

Once again, enzyme phylogenies potentially provide clues for distinguishing the former from that latter group of redox enzymes. Unfortunately, the sample of bioenergetic redox enzymes for which phylogenies have been reconstructed is still rather limited. In the following, we will attempt an inventory of bioenergetic enzymes for which either phylogenetic trees suggest pre-LUCA origins or which, judging from the chemical nature of their electrochemical substrates, are likely to have played a role in ancient energy converting redox conversions.

### Bona Fide pre-LUCA Bioenergetic Enzymes

The list of enzymes which bear convincing signs of pre-LUCA origins comprises most notably the group I [NiFe] hydrogenases (Brugna-Guiral et al. 2003; Vignais and Billoud 2007) as well as complexes reducing moderately oxidized sulphur compounds, such as polysulphide reductases or tetrathionate reductases (Hedderich et al. 1999; Duval et al. 2008). Venerably old roots for these enzymes make palaeogeochemical sense since their substrates are likely to have been abundant in the Hadean and early

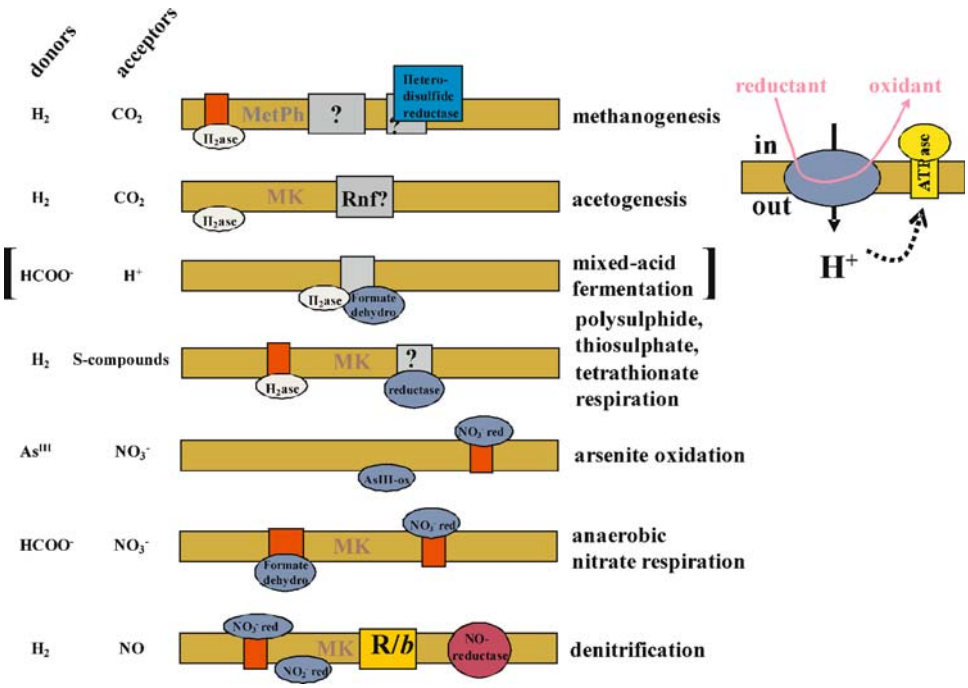


**Fig. 6** Membrane-integral bioenergetic chains performing the oxidation or reduction of inorganic redox substrates as seen in extant organisms chosen to demonstrate the likely systems in LUCA. *Ovals* and *boxes* indicate common structural motifs and cofactors. Note that in this article we argue that the mixed-acid fermentation pathways operated in reverse in the earliest cells recalling the inorganic beginnings of life (Stetter and Gaag 1983; Andrews et al. 1997; Müller et al. 2003; Ducluzeau et al. 2009)

Archaean and since furthermore their active centres ([NiFe]-clusters and molybdenum/tungsten ions) are revealingly congruent with the bioinorganic catalysts which would have been supplied to the hydrothermal mounds—the ferrous iron, with some Ni supplied from the ocean (ultimately derived from 360–400°C hydrothermal vents) and the remainder from the alkaline hydrothermal solution as $Mo^{IV}/W^{IV}S/Se_{3-x}O_x^{2-}$ or $Mo^{VI}/W^{VI}S/Se_{4-x}O_x^{2-}$ (Nekrasov and Konyushok 1982; Seward and Barnes 1997; Russell and Hall 1997, 2006; Helz et al. 1996; Beverskog and Puigdomenech 1997; Erickson and Helz 2000).

## Potential (Judging from Their Redox Centres) pre-LUCA Bioenergetic Enzymes

The enzymes arsenite oxidase and polysulphide reductase phylogenetically are predicted to have existed prior to the Archaea/Bacteria divergence. The catalytic subunit of these enzymes harbours a molybdopterin cofactor and is part of the large ensemble of proteins called the "DMSO-reductase superfamily" (Boyington et al. 1997). The fact that the basic building block of this superfamily is a pre-LUCA protein raises the possibility that further members of the superfamily also functioned in the common ancestor. From the phylogenetic analysis of the heme copper oxygen reductase superfamily (Ducluzeau et al. 2008, 2009), we recently concluded that at least parts of the denitrification pathway operated in the common ancestor, providing a geochemically more plausible alternative to oxygen as a strongly oxidizing electrochemical substrate for bioenergetic chains. In this framework, it is tempting to envisage that nitrate reductase, a member of the DMSO superfamily and the first enzyme of the denitrification chain, also functioned in LUCA (Weiner et al. 2006). The next step in this chain, the reduction of nitrite to nitric oxide is performed alternatively by two very different enzymes, that is either by the heme-containing $cd_1$-type or by the multicopper-type nitrite reductases. The phylogeny of the $cd_1$ enzyme is indeed in line with its presence in LUCA, although the sample of recognized prokaryotic cases is still rather limited (Jones et al. 2008; Ducluzeau 2009). The multi-copper enzyme, however, shows a very complicated pattern of species distribution and the fact that its redox centres are exclusively composed of copper ions disqualifies this complex as a pre-divergence enzyme. In the absence of $O_2$ in the Hadean and Archaean eras, copper would have been essentially insoluble and thus unavailable for biological processes (Anbar 2008). The same reasoning applies to the enzyme performing the denitrification step following the reduction of nitric oxide to nitrous oxide. Nitrous oxide reductase as well is a multi-copper enzyme and we assume that the denitrification chain in the Hadean and Archaean was shorter than that observed in extant organisms and ended with the release of nitrous oxide, a gas that would eventually escape from the biosphere back to the atmosphere.

## Did a Step in the Inorganic Carbon Fixation Pathway Turn Bioenergetic?

Reasoning along the lines of a likely pre-LUCA presence of the molybdopterin module as outlined above, one group

of enzymes from the DMSO reductase superfamily, the formate dehydrogenases (Fdhs) appear to have very deep evolutionary roots, almost all the way back to the bioinorganic origins of life. Fdhs catalyze the oxidation of formate ($HCOO^-$) to $CO_2$, that is, the reverse reaction to what was most probably the first step in the ancestral bioinorganic carbon-fixation pathway. In the hydrothermal mound scenario, $H_2$ naturally lends itself as the electron donor for reducing $CO_2$ to $HCOO^-$. Most intriguingly, an enzyme has been described in *E. coli* which seems to impersonate the ancestral carbon-fixing system, only working in reverse. This enzyme is referred to as formate hydrogenlyase and performs what is called mixed-acid fermentation (Andrews et al. 1997; Bagramyan and Trchounian 2003). Formate hydrogenlyase uses electrons from the oxidation of $HCOO^-$ (yielding $CO_2$) to reduce protons (yielding $H_2$). Concomitant to this redox reaction, protons appear to be translocated across the membrane, building up chemiosmotic potential (Fig. 7a). This reaction is almost at thermodynamic equilibrium under standard conditions (concentration and pH) ($E_{m,pH7}$ $[HCOO^-{\cdot}CO_2] = -430$ mV and $E_{m,pH7}$ $[H^+/H_2] = -410$ mV). Under physiological conditions, the carbon redox couple will be strongly on the reducing side whereas the equilibrium of the hydrogen redox pair will strongly lean towards the oxidized species (both $CO_2$ and $H_2$ rapidly diffusing out and partitioning into the atmosphere). The available Gibbs free energy difference thus will strongly exceed the negligible 20 mV of the midpoint potential difference, rendering proton translocation energetically likely.

The two half-reactions are carried out separately within a molybdopterin-carrying molecule (for the formate to $CO_2$ conversion) and a [NiFe]-type hydrogenase (for the reduction of $2H^+$ to $H_2$), i.e. reactions solely relying on basically inorganic redox cofactors. In the hydrothermal mound system, both $CO_2$ and $H_2$ will dominate over formate and protons, yielding a working potential for the $HCOO^-/CO_2$ couple more positive than $-430$ mV and that for the $H_2/H^+$ couple substantially more negative than $-410$ mV. In such a system, $CO_2$ reduction to formate by $H_2$ may be energetically favourable in the presence of molybdenum and [NiFe] cofactors, at least until formate starts to accumulate. However, the example of formate hydrogenlyase suggests that the amount of produced formate can reach high values if the reaction is further driven by the "natural" chemiosmotic potential of the FeS bubbles in the mound. The parallelism of the formate hydrogenlyase reaction and the ancestral $CO_2$ reduction to formate is presented in Fig. 8.

Reduction of $CO_2$ to formate represents the energetically most unfavourable step in the total chain from $CO_2$ to methane (Fig. 8) (Maden 2000; Martin and Russell 2007). A potential reaction scheme producing formate from $CO_2$, $H_2$ and a chemiosmotic potential and catalyzed by molybdenum or tungsten and [NiFe] clusters therefore removes a major obstacle for conceiving an ancestral $CO_2$ fixing pathway. Navigating the acetogenic pathway would have faced the even higher energetic hurdle to the formyl group, perhaps negotiated through formyl phosphate with the help of PPi, itself coupled through the ambient protonmotive force (Fig. 7b) (Maden 2000; Martin and Russell 2007) (Fig. 8).

To our knowledge, the molybdopterin subunit of formate hydrogenlyase has not been studied with respect to phylogeny so far. The [NiFe] hydrogenase subunit of this enzyme belongs to group 4 adopting the nomenclature introduced by Vignais et al. (2001). This group also contains subunits of Complex I which hampers a straightforward interpretation of the respective phylogenetic trees with respect to pre- or post-LUCA origins. We therefore consider that this specific enzyme deserves a more in-depth phylogenetic analysis to eventually assay whether this complex truly is as ancient as its catalytic reaction appears to suggest.

## Overcoming Energy Deficits/Gradient Decay Toward the Mound's Interior

### Enzymes Dealing with Strong Oxidants as Redox Substrates: From LUCA Back Toward Bioinorganic Roots

The formate hydrogenlyase system provides a nice example for an inorganic redox reaction having subsequently evolved into a bioenergetic mechanism. Most of the remaining, presumably pre-LUCA, energy-conserving (i.e. chemiosmotic potential-building) electron transfer enzymes (Fig. 6) are difficult to rationalize in a comparable scheme. This difficulty raises the question, 'misguided' by evolutionary thinking, why on earth a proto-cell in the mound would bother to generate an $H^+$-gradient if it is already provided anyway? But this would be to look at the transition from inorganic reactions to biochemistry from the wrong direction. From the geochemical (bottom up) point of view, several of the extant oxidants were certainly or very probably present in substantial concentrations in and around the mound. These comprise (as indicated in Fig. 6) sulphur, polysulphides, FeIII and nitrogen oxides (Braterman and Cairns-Smith 1983; Pavlov and Kasting 2002; Martin et al. 2007) (Fig. 9). The mere presence of the oxidants together with the reductant $H_2$ and the appropriate inorganic catalysts would render reactions having the effect of collapsing these redox disequilibria irresistible. Once harnessed and then tuned to the needs of the protocells, they may indeed have been beneficial in an evolutionary sense. This is because the escape from the hydrothermal hatchery would have almost

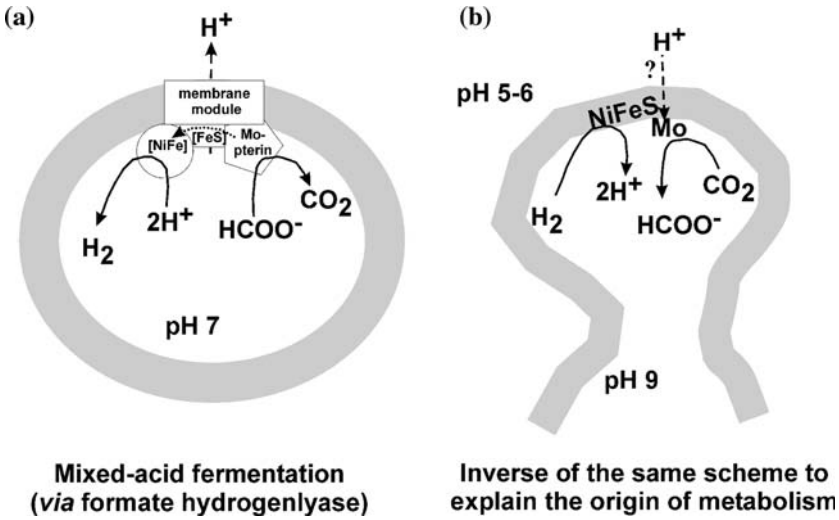


**Fig. 7** Informed by the arrangement of metalloenzymes (formate hydrogenlyase system/module) on the cytoplasmic side of an *E. coli* membrane (**a**) (Andrews et al. 1997), we argue (**b**) that an ambient proton gradient (acidulous ocean juxtaposed to the alkaline, hydrogen-bearing interior) would drive the generation of formate from $CO_2$ with electrons supplied from $H_2$ via a Ni–Fe mineral cluster acting as a protohydrogenase (e.g., Fe(Ni)S = mackinawite), cf. Fig. 1. Note, the two-electron transfer reactions are dependent on the presence of a molybdenum redox entity (cycling between Mo(IV, V and VI)) supplied, in the latter case, by the alkaline solution

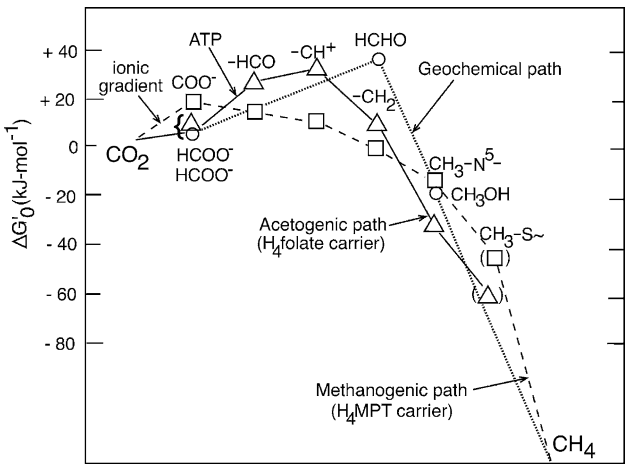


**Fig. 8** Left to right free-energy profiles of acetogenic and methanogenic reduction (hydrogenation) pathways (the eastern tributary to the acetyl coenzyme-A pathway, Ragsdale 1997), compared to the geochemical pathway (cf. Seewald et al. 2006). Recast from Maden (2000; Fig. 2)

certainly been downward and sideways into the ocean crust to inaugurate the 'deep biosphere' rather than into the desert of the early ocean (Russell and Arndt 2005). Once within the basaltic and komatiitic lavas and sediments comprising the ocean crust, the prokaryotes, now weaned from direct hydrothermal succour, would still be bathed in fluids rich in hydrogen from beneath (Krumholz et al. 1999; Morita 2000) and carbon dioxide and other electron acceptors percolating from the ocean above (Russell and Hall 2006). The only thing missing would have been the protons now spent dissolving groups 1 and 2 elements from minerals comprising the crust (Staudigel et al. 1981). Now chemiosmosis can really come into its own!

## An Alternative Scenario

A heterotrophic alternative to the autogenic hypothesis for the emergence of life detailed herein has been advanced by Wong et al. (2007). Such a scenario stems from Wong's case against an autogenic origin based on a triple convergence of evidence favouring the separate origins of what he terms Phase 1 and Phase 2 amino acids (Wong 2009a, 2009b). The phase 1 amino acids—Gly, Ala, Ser, Asp, Glu, Val, Leu, Ile, Pro and Thr—happen to be those that occur naturally in meteorites and spark discharge experiments. From this evidence Wong (2009a) suggests that these amino acids were first utilized in a putative primordial soup (Bada and Lazcano 2003) by emerging heterotrophs living some distance from a hydrothermal mound. They are presumed to have derived their energy from the oxidation of carboxylic and amino acids in this milieu (Wong 2009a). Once the soup was depleted, these primitive heterotrophs were forced to migrate to a hydrothermal mound where they developed methanogenesis, thus giving rise to an autotrophic methanogenic LUCA. Under this view a combined heterotrophic origin of life is speculated upon culminating in an autotrophic methanogenic LUCA: a triple-convergence or "hot-cross" origin of life (Mat et al. 2008). However, even in this heterotrophic scenario, oxidants such as nitrogen oxides would have been required (Ducluzeau et al. 2009). Only later, when the cellular

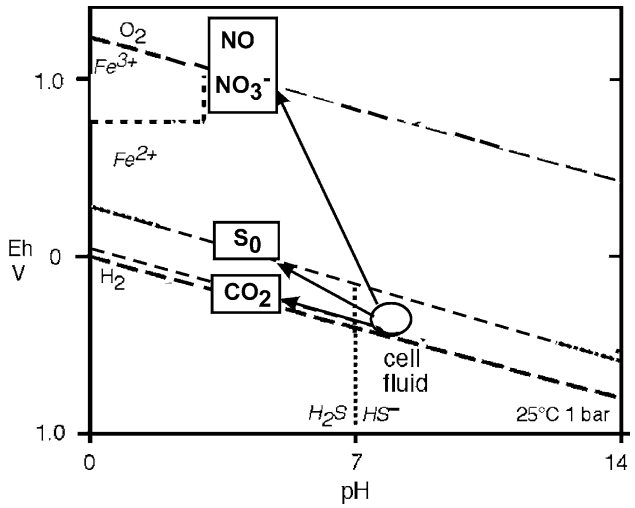


**Fig. 9** Redox potential/pH vectors between the cellular fluid of LUCA (comparable to the hydrogen-bearing alkaline hydrothermal fluid feeding the mound) to demonstrate the requirement for the two-electron redox propensity of the molybdenum cluster, in order to bifurcate electrons of contrasting energies delivered from the reductant. The first electron is extracted by one of the available high potential acceptors inducing the generation of a strongly reducing $Mo^{V}$ to $Mo^{VI}$ transition thereby permitting reduction of carbon dioxide. Whether $Fe^{III}$ was another possible electron acceptor remains unknown. Modified from Russell and Hall (2006)

amino acid biosynthetic pathways produced the Phase 2 amino acids, would these also be involved in order to increase the chemical versatility of proteins (Wong 2009b). Apart from the requirement for an external potent oxidant, both the autogenic hypothesis favoured here as well as Wong's heterotrophic alternative converge on the idea that acetogenesis preceded methanogenesis and that both processes were involved in the LUCA. We note in one defense of our hypothesis that all of these "Phase 1" amino acids have also been produced in hydrothermal experiments (Hennet et al. 1992; Marshall 1994; Huber and Wächtershäuser 2003; cf. Davis 2005).

Our more profound discomfort with this as well as with all other kinds of heterotrophic scenarios arises from thermodynamics. The oxidation of organic molecules may well provide an (albeit weak) energy source for a fully-fledged organismal entity. However, in our minds it falls desperately short of allowing life to originate. In the spatial unit cell where life comes into being, the appearance of a walled, structured and metabolizing entity represents an enormous decrease in entropy. The second law of thermodynamics allows such spontaneous structure-formation only in the presence of a very strong flux of enthalpy (think for example Jupiter's Great Red Spot or the alkaline hydrothermal convection cell itself). Such a spatial and temporal constancy in free enthalpy supply in the form of electrochemical disequilibrium between $H_2$ and $CO_2$ is the basic premise of the hydrothermal autogenic scenario and we presently do not know of any other hypotheses for the origin of life on Earth (or elsewhere) which proposes a comparatively credible energetic driving force for structure-formation—or as Dobzhansky might have said: Nothing in the origin of life makes sense except in the light of (bio)energetics.

## Conclusions

In our view the setting for the emergence of life was a long-lived steady-state ~100°C hydrothermal feed comprising, in descending order of concentration, hydrogen, sulphide, formate, a methyl group and metal ligands of nickel, cobalt, tungsten and molybdenum. These solutions exhaled into a carbonic ocean containing protons, phosphate, elemental sulphur and nitric oxide and its derivatives as well as ferrous iron sulphides dosed with nickel and cobalt along with minor molybdenum sulphide and/or selenide (Braterman and Cairns-Smith 1983; Russell and Hall 1997; Martin et al. 2007; Hagan et al. 2007; Konhauser et al. 2009). Mixing of the two solutions was strongly inhibited by precipitates of amorphous silica, clays, ephemeral carbonates and iron sulphides and hydroxides, which together constituted a growing chambered mound above the warm spring. Nevertheless, controlled transactions were made across the iron (nickel, molybdenum) sulphide and hydroxide barriers precipitated at this site of exhalation. We have argued here that a reversal of the mixed-acid fermentation through the molybdenum enzyme, formate hydrogenlyase, described by Andrews et al. (1997) was the initial mechanism of carbon dioxide reduction to formate by an emerging metabolizing system hosted by the chambered hydrothermal mound. The energetics of this reaction, involving an unstable intermediate $Mo^{V}$ state, required the involvement of a high potential oxidant (nitrogen oxides and/or elemental sulphur) in order to allow a low potential electron to be delivered to carbon dioxide for its reduction to formate and beyond.

Such a reduction took place either *directly* through the synthesis of similar intermediates on the path to methane or *indirectly* through PPi generation to generate the kinetic intermediates on the way to acetate. These were the first steps toward the initiation of the acetyl coenzyme-A pathway, considered by Fuchs to be the one with the longest pedigree (Fuchs 1989). In the wings, awaiting a more complex and cooperative system, were other electron acceptors such as nitric oxide and its derivatives, elemental sulphur and possibly ferric iron. Within the growing hydrothermal mound, before genetic honing, the assimilatory and dissimilatory use by the relatively inept early

cellular cooperatives of these elements was somewhat indiscriminate.

The eventual bifurcation of the archaea and bacteria probably took place in the mound before the diaspora, and was brought about by the differentiation of function. Although both domains used the acetyl coenzyme-A pathway the main effluent of the proto-bacteria was probably acetate whereas the proto-Archaea developed the pathway further to reduce carbon dioxide all the way to methane.

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