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The Chemical Basis of Membrane Bioenergetics

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Abstract. All organisms rely on chemiosmotic membrane systems for energy transduction; the great variety of participating proteins and pathways can be reduced to a few universal principles of operation. This chemical basis of bioenergetics is reviewed with respect to the origin and early evolution of life. For several of the cofactors which play important roles in bioenergetic reactions, plausible prebiotic sources have been proposed, and it seems likely that these cofactors were present before elaborate protein structures. In particular, the hydrophobic quinones require only a membrane-enclosed compartment to yield a minimum chemiosmotic system, since they can couple electron transport and proton translocation in a simple way. It is argued that the central features of modern bioenergetics, such as the coupling of redox reactions and ion translocation at the cytoplasmic membrane, probably are ancient features which arose early during the process of biogenesis. The notion of a thermophile root of the universal phylogenetic tree has been discussed controversially, nevertheless, thermophiles are interesting model organisms for reconstructing the origin of chemiosmotic systems, since they are often acidophiles and anaerobic respirers exploiting iron–sulfur chemistry. This perspective can help to explain the prominent role of iron–sulfur proteins in extant biochemistry as well as the origin of both respiration and proton extrusion within the context of a possible origin of life in the vicinity of hot vents.

Key words: Chemiosmotic theory — Electron transport — Origin of life — Photosynthesis — Prebiotic chemistry — Proton translocation — Respiration — Sodium bioenergetics — Thermophiles

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Introduction

Several fundamental features of membrane bioenergetics are ubiquitous among bacteria, archaea, and eukaryotes:

(1) electron transport chains, composed of membrane proteins and soluble electron carriers and driven by redox energy or light;
(2) coupling of transmembrane ion translocation to this electron transport;
(3) use of the resulting ion gradients for driving ATP synthesis; and
(4) use of ATP and NAD(P)H as universal currencies for short-term storage and transport of energy.

The title of Monod's book, *Chance and Necessity,* captures two reasons that could account for such biological universalities: they may represent either "frozen accidents" that were passed on to all extant organisms from the last common ancestor (LCA) or optimum solutions for the respective problems, which may have arisen independently in several lineages. In recent years, the spread of genes by lateral gene transfer (LGT) has emerged as an important third explanation for widely distributed biochemical features. These three possibilities are not mutually exclusive: certain biological features may be universal because they are inherited from the LCA, but they may have been present in the LCA exactly because they were favored by natural selection.

Likewise, certain genes should have had higher chances of becoming a permanent acquisition after LGT because of the functional advantages they offered.

In the present review, I discuss the early evolution of bioenergetic membranes, with a focus on the possible origin of the abovementioned universal features. There is a vast literature on extant proteins, pathways, and organisms; in particular, an immense number of sequence data has become available recently. This literature, especially papers that appeared recently, is used to explicate some fundamental aspects of bioenergetic evolution. However, such considerations cannot bridge the gap between the LCA, which was probably already a complex cell similar to modern ones, and the very first protocellular entities. Therefore, the review of data on extant systems has to be combined with a physicochemical perspective, which addresses issues of function and of possible prebiotic/early biotic reactions. It seems that in fact many biochemical inventions of prebiotic and early biotic times are optimum solutions that were, from a geochemical and physiochemical point of view, superior to conceivable alternatives (Berry 1997; de Duve 1995; Pace 2001). This kind of reasoning is applied here to the design of bioenergetic systems to elucidate the origins of present-day bioenergetic reactions.

Thermophiles and the Origin of Life

Based on RNA sequences of the ribosomal small subunit, a universal tree of all living organisms can be constructed (Woese 1998, 2000), which places many thermophile and hyperthermophile bacteria and archaea near the root, supporting the notion of a "hot origin" of life (Hartman 1998; Nisbet and Fowler 1996; Stetter 1998). The deep phylogenetic position of (hyper)thermophile prokaryotes is supported by sequence analysis of protein encoding genes (Bhuiya et al. 2000; Bocchetta et al. 2000), and an analysis of the origin of the genetic code also supports the hot origin of life (Di Giulio 2000). However, in addition to some more technical problems, the hot origin scenario faces two general objections: First, reconstruction of the phylogenetic tree using sequence data can be problematic due to LGT events, and the universal tree has to be interpreted with caution (Doolittle 1999; Woese 1998, 2000). Second, phylogenetic reconstruction using comparative biochemistry cannot go beyond the LCA (Lazcano and Miller 1999), which is not identical to the first living cell (the progenote), and even if a thermophile root of the universal tree implies a thermophile LCA, no direct conclusions with respect to the progenote are possible.

Nevertheless, there are interesting aspects of the hot origin independent of issues of phylogenetic reconstruction. The solar system and the Earth formed about 4.5 billion years (Ga) ago (Nisbet 1985; Sleep et al. 2001); recent evidence indicates the presence of oceans as early as 4.3–4.4 Ga ago (Mojzsis et al. 2001; Wilde et al. 2001). On the other hand, certain 3.5-Ga-old microfossils seem to represent cyanobacteria indistinguishable from extant species (Nisbet and Sleep 2001; Schopf 1998). Obviously, a complex sequence of events took place within the first 700 million years: formation of the primordial atmosphere and ocean, prebiotic evolution and the origin of life, diversification of the LCA into bacteria and archaea, the invention of chlorophyll-based photosynthesis, radiation of the phototrophic bacteria, and, finally, the invention of oxygenic photosynthesis and emergence of the cyanobacteria. The young Earth was warm due to the formation of the planet by collision of planetesimals (Delsemme 1998; Nisbet 1985; Nisbet and Sleep 2001), and additionally, even if there was a rapid initial cooling (Sleep et al. 2001), the heavy meteorite bombardment during the first 500 million years must have heated the primordial ocean repeatedly, implying that terrestrial organisms had to pass through hot-ocean bottlenecks (Nisbet and Sleep 2001). Not only does a "warm" young Earth mean higher temperatures than today, but also there was more geological activity and a frequent occurrence of geothermal environments which are discussed as possible birthplaces of life (Arndt 1998; Nisbet 1985; Nisbet and Fowler 1996). Putting all this evidence together, one wonders how life could have originated in an environment that was *not* quite warm.

In any case, the deep-branching thermophiles show several features (Schäfer et al. 1996, 1999; Stetter 1998, 1999), which make them interesting model organisms for the reconstruction of early bioenergetics: First, all of them rely on respiration and there are no phototrophs among them. Second, the majority of them is capable of anaerobic respiration, either facultatively or obligatorily; obligatory aerobic respiration is rare. Both observations fit the "respiration early" scenario, according to which anaerobic/microaerobic respiration preceded oxygenic photosynthesis (Castresana and Saraste 1995). Third, many thermophiles are acidophilic; this may provide a clue concerning the origin of chemiosmotic proton pumping. Finally, redox reactions of iron and sulfur play a prominent role in the energy metabolism of these thermophiles (Adams et al. 2001; Beinert 2000; Huber et al. 2000; Stetter 1998); evidence for thermophilic chemotrophic prokaryotes has also been found in a 3.235-Ga-old sulfide deposit (Rasmussen 2000). This is interesting because iron–sulfur chemistry in combination with geothermal settings has also been implicated in the prebiotic production of organic molecules (Blöchl et al. 1992; Clark et al. 1998; Wächtershäuser 1998), and the central role of FeS proteins in bioenergetics indicates that they are ancient. Therefore, iron–sulfur chemistry links prebiotic chemistry, hot vents, and early energy metabolism. Some reactions utilized by (hyper)thermophilic bacteria and archaea are as follows (Huber et al. 2000; Reysen-

bach et al. 2000; Schäfer et al. 1996, 1999; Stetter 1998, 1999)

(1) Anaerobic reactions:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (1)$$

$$H_2 + S^0 \rightarrow H_2S \quad (2)$$

$$H_2 + 6FeO(OH) \rightarrow 2Fe_3O_4 + 4H_2O \quad (3)$$

(2) Aerobic reactions:

$$2H_2 + O_2 \rightarrow 2H_2O \quad (4)$$

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \quad (5)$$

$$FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4 \quad (6)$$

(3) Quasiaerobic reactions:

$$4H_2 + H_2SO_4 \rightarrow H_2S + 4H_2O \quad (7)$$

$$3H_2 + H_2SO_3 \rightarrow H_2S + 3H_2O \quad (8)$$

$$4H_2 + H_2S_2O_3 \rightarrow 2H_2S + 3H_2O \quad (9)$$

$$H_2 + HNO_3 \rightarrow HNO_2 + H_2O \quad (10)$$

$$5H_2 + 2HNO_3 \rightarrow N_2 + 6H_2O \quad (11)$$

$$3H_2 + 2HNO_2 \rightarrow N_2 + 4H_2O \quad (12)$$

$$4H_2 + HNO_3 \rightarrow NH_3 + 3H_2O \quad (13)$$

$$FeCO_3 + HNO_3 + 3H_2O \rightarrow 2Fe(OH)_3 + HNO_2 + 2CO_2 \quad (14)$$

"Quasiaerobic" respiration is not dependent on molecular dioxygen but requires a highly oxidized electron acceptor such as sulfate or nitrate. The picture of a strongly reducing primordial atmosphere is no longer the consensus view, and carbon dioxide was probably a major atmospheric ingredient (Delsemme 1998; Holland 1984; Kasting and Ackerman 1986; Walker 1985), but the exact redox status of the primitive atmosphere is controversial (Catling et al. 2001; Clark et al. 1998; Kakegawa et al. 1999; Nisbet and Sleep 2001; Österberg 1997; Shen et al. 2001; Watanabe et al. 1997) and it is unclear what amount of electron acceptors such as sulfate and nitrate was available. The "strictly anaerobic," sulfate-reducing bacterium *Desulfovibrio gigas* was recently shown to have an aerobic respiratory chain, including a terminal oxidase of the cytochrome (cyt) *bd* type (Lemos et al. 2001), and this may indicate that the gap between aerobic and quasiaerobic modes of metabolism is not as large as previously thought. On the other hand, anaerobic regeneration of sulfate is possible via

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2H_2SO_4 + 12H^+ \quad (15)$$

(Bottrell et al. 2000), explaining the existence of sulfate reducing microbes already in the early Archaean (Kakegawa et al. 1999; Shen et al. 2001; Watanabe et al. 1997).

Proteins and Cofactors

Despite the enormous variety of bioenergetic membrane systems, most of their chemistry can be understood in terms of a handful of reactions, since a limited number of cofactors accounts for most bioenergetic processes. The rather conserved set of cofactors in all organisms probably indicates that these substances are an ancient feature. For understanding a reaction completely, detailed knowledge of the three-dimensional protein structure is necessary, but for a first approximation one may say that proteins provide a passive scaffold, while energy transducing reactions take place in the form of cofactor chemistry, with the cofactors being bound to this matrix. This minimalist view ignores important aspects of protein function, but several lines of evidence indicate that there is a justification for this approach as long as one is interested in the chemical basis of bioenergetics.

(1) For many proteins the dependence of the rate constant of electron transfer (k_{ET}) on the distance *(R)* between donor and acceptor obeys a universal law,

$$k_{ET} \propto \exp(-\beta R) \quad (16)$$

where $\beta \approx 1.4$ Å^{-1} (Moser et al. 1995; Page et al. 1999; Winkler 2000), implying that the distance between redox centers is the major factor controlling the turnover rate; details of the protein structure are of secondary importance. For instance, in the cyt bc_1 and cyt b_6f complexes a similarity of function concomitant with deep differences in protein structure is seen: the subunits containing the *c*-type hemes, cyt c_1 and cyt *f,* respectively, have no homology, and even the heme binding in cyt *f* is completely different from that in other *c*-type cytochromes, but the function of both subunits within their complexes is identical and the spectroscopic and electrochemical characteristics are similar (Breyton 2000; Soriano et al. 1999).

(2) Artificial α-helical peptides have been constructed which mimic the biophysical properties of natural bioenergetic proteins but which have only some critical residues for cofactor binding in common with their natural counterparts. Such "maquettes" can reproduce the heme–heme interaction of *b*-type cytochromes (Kalsbeck et al. 1996; Robertson et al. 1994), they can bind FeS clusters (Gibney et al. 1995), they show a coupling of proton exchange to redox reactions as in cytochromes (Shifman et al. 1998), and they can transfer electrons from a donor to oxygen (Gibney et al. 2000).

(3) Some basic bioenergetic reactions, such as conversion of light energy into a proton gradient, can be simulated even by protein-free model systems, which contain only the cofactors (Rotello 1999; Seta et al. 1985; Steinberg-Yfrach et al. 1997; Sun and Mauzerall 1996).

The feasibility of a minimalist approach, ignoring details of protein structure, has important implications for reconstructing the origin of bioenergetics, because the prebiotic formation of several cofactors seems likely, while it is unlikely that complex proteins have already been present at the same time. In this view, the cofactors came first, perhaps attached to unspecialized proteins or random polymers, and the elaborate present-day protein structures were later built around these cofactors to optimize performance.

Nucleotides and Phosphorylation Equivalents

Several important cofactors are nucleotides, such as ATP, NAD, NADP, FAD, FMN, CoA, SAM, and PAPS. The central role of nucleotides is probably a remnant from the RNA world, the widely accepted scenario for the origin of the genetic system (Benner et al. 1989: Ferris 1998; James and Ellington 1998; Schwartz 1998; Yarus 1999). Although prebiotic syntheses for the building blocks of nucleotides at moderate temperatures have been proposed, they are unstable at high temperatures (Miller 1998) and this is an obstacle for finding a plausible overall scenario linking the hot origin and the RNA world. Adenine and derived cofactors are of outstanding importance in biochemistry, because they link bioenergetics and genetics. This may reflect that adenine is the most stable nucleobase (Pullman 1972) and also the one which is formed with the highest yield in some prebiotic syntheses (Miller 1987; Orgel and Lohrmann 1974). However, the other nucleobases also have a dual function, as, in addition to their role in nucleic acids, they are involved in energy transfer in the biosynthesis of proteins (GTP), saccharides (UTP), and phospholipids (CTP).

ATP is the universal energy currency today (Skulachev 1996), but pyrophosphate may have preceded ATP as the carrier of phosphorylation equivalents (Baltscheffsky 1996; de Duve 1991; Morowitz 1992). Pyrophosphate can be formed abiotically from hot magma (Yamagata et al. 1991). Another pathway of PP_i formation could have been (Fox 1988)

$$PO_4^{3-} + SO_3^{2-} + 2Fe^{3+} \rightarrow {}^{-}O_3S\text{–}O\text{–}PO_3^{2-} + 2Fe^{2+} \quad (17)$$

$${}^{-}O_3S\text{–}O\text{–}PO_3^{2-} + PO_4^{3-} \rightarrow P_2O_7^{4-} + SO_4^{2-} \quad (18)$$

This is a hypothetical forerunner of the formation of ATP by sulfite oxidation (via APS as the activated intermediate), as in *Thiobacillus.* A third possibility is the formation of pyrophosphate from acyl phosphates (de Duve 1991, 1998),

$$R\text{–}CO\text{–}O\text{–}PO_3^{2-} + PO_4^{3-} \rightarrow RCOO^{-} + P_2O_7^{4-} \quad (19)$$

which can be formed from thioesters (see below). [A critical voice with respect to the prebiotic availability of phosphorylation equivalents is given by Keefe and Miller (1995).]

Membrane-bound pyrophosphatase (Baltscheffsky et al. 1999; Drozdowicz and Rea 2001) has a simple architecture, and it has been speculated that there was an evolution from a PP_i-synthesizing enzyme to a primitive ATP-synthase, which later underwent gene splitting and gene duplication (Nelson 1994; Taiz and Nelson 1996) to give rise to the multisubunit rotatory ATPases/ATP-synthases. However, the evolutionary relation of a hypothetical ancestral ATP-synthase to the membrane-bound pyrophosphatase is elusive (Baltscheffsky et al. 1999). The rotatory ATP-synthases/ATPases of the A type (archaea), F type (bacteria, mitochondria, chloroplasts), and V type (eukaryotic organelles and some bacteria) form a large family that was already present in the LCA (Castresana and Moreira 1999; Nelson and Harvey 1999; Taiz and Nelson 1996). Another family of ATPases is the P-type enzymes, which serve as pumps for various cations and phospholipids. Most P-ATPases have a single subunit, and due to this simple structure, P-ATPases might be considered an early invention. However, it has been suggested that they evolved rather late as auxiliary systems for maintaining cellular homeostasis (Nelson 1994). This seems reasonable since P-ATPases are unable to synthesize ATP and are dependent on other ATP-providing enzymes.

In addition to ATP synthesis driven by transmembrane H^+ flux, generation of ATP is possible by glycolysis. It is generally assumed that substrate-level phosphorylation is more ancient than chemiosmotic ATP synthesis (de Duve 1991; Fox 1988; Skulachev 1996): it does not require the complicated structure of membrane-bound ATP-synthase, and its low energetic efficiency and the fact that it functions under anaerobic conditions also appear as primitive features.

Tetrapyrrol Pigments

The hemes of cytochromes and the chlorophylls (including bacteriochlorophylls and pheophytins) of photosynthetic reaction centers are tetrapyrrols, rendering this substance class one of the most important ones in bioenergetics. Hemes are also found in other proteins functioning in energy metabolism and detoxification, such as catalase, peroxidase, and the cytochrome P450 family, and in hemoglobin and myoglobin. Linear tetrapyrrols

serve as antenna pigments in the phycobilisomes of cyanobacteria and red algae, and they are the chromophores of the ubiquitous light-sensing phytochrome system. Linear tetrapyrrols are synthesized starting from cyclic ones by ring opening; the phycobilisome pigments are synthesized starting from heme (rather than chlorophyll). The corrin ring of vitamin B_{12} is similarly synthesized by heme ring opening, removal of one C atom, and ring closure. The water-splitting apparatus of photosystem (PS) 2 contains a manganese cluster, and it has been speculated that even this Mn_4 cluster was derived from Mn-porphyrins (Olson 1970), which are photooxidizable *in vitro* (Maliyackel et al. 1986). However, other candidates, such as a superoxide dismutase containing nonheme manganese (Blankenship and Hartman 1998; Rutherford and Nitschke 1996) and the possible electron donor $Mn(II)_2(HCO_3)_4$ (Dismukes et al. 2001), are more likely precursors of the photosynthetic Mn cluster.

Since tetrapyrrol pigments are ubiquitous compounds, they may be an old feature of life. This hypothesis is supported by the universal tree of tetrapyrrol biosynthesis, which presumably reflects the evolution of utilization of these pigments (Avissar and Moberg 1995; Battersby and McDonald 1979; Mauzerall 1992; Xiong et al. 2000). However, the implication of porphyrins in prebiotic times is controversial, and it has been concluded that no adequate prebiotic synthesis for them yet exists (Miller 1987, 1998). There is a correlation between the optimum growth temperature (OGT) of (hyper)thermophile organisms and the presence of porphyrin biosynthesis genes: at a high OGT, the number of genes related to heme metabolism is reduced, and in *Pyrococcus* (OGT ca. 100°C) no genes for heme biosynthesis have been identified (Kawashima et al. 2000). Similarly, the respiratory complex II (SDH) of the thermoacidophilic archaeon *Sulfolobus* sp. Strain 7 contains flavin and FeS centers but lacks the *b*-type cytochrome usually found in SDH (Iwasaki et al. 1995). These findings may imply that porphyrins played no prominent role during a hot origin of life.

On the other hand, some authors have discussed the abiotic emergence of porphyrins (Calvin 1969; Hodgson and Ponnamperuma 1968) and their participation in bioenergetic reactions. The resonance stabilization of porphyrins (Pullman 1972) has been considered as a factor that would have facilitated the accumulation of abiotically formed porphyrins. The presumed prebiotic synthesis of porphyrin can be improved by the addition of metals, and since porphyrins with chelated metals are redox active, this might have provided an autocatalytic route of porphyrin formation (Calvin 1969). Porphyrins have also been implicated in primordial photosynthesis (Masinovsky et al. 1989; Mauzerall 1992; Mercer-Smith and Mauzerall 1984; Sun and Mauzerall 1996; Telegina et al. 2000).

FeS Proteins and the Chemistry of Iron and Sulfur

Iron–sulfur clusters are often found in bioenergetic proteins (Beinert 2000; Hall et al. 1974; Sticht and Rösch 1998). Three of four complexes (NADH dehydrogenase = NDH, SDH, and cyt bc_1) in the mitochondrial electron transport chain (ETC) and two of three in oxygenic photosynthesis (cyt b_6f and PS1) have FeS clusters. Thirteen or fourteen FeS clusters are involved in mitochondrial respiration (Beinert 2000), and FeS clusters occur frequently in menaquinone-reductases and menaquinol-oxidases of anaerobes (Cammack 1996). In addition to membrane-bound FeS clusters, soluble proteins such as ferredoxin, rubredoxin, and high-potential iron–sulfur protein (HiPIP) play a crucial role, and FeS proteins are also involved in regulatory processes (Beinert and Kiley 1999). Since iron–sulfur clusters participate in many pathways, an ancient origin of FeS proteins is generally assumed (Cammack 1996), in particular, for ferredoxins (Hall et al. 1974).

Several proposals link the prominent modern role of FeS proteins to prebiotic reactions of iron and sulfur (Cammack 1996). The particular model of a complex, self-organized protometabolic network on pyrite surfaces of Wächtershäuser and colleagues (Blöchl et al. 1992; Wächtershäuser 1994, 1997, 1998) has met much skepticism (de Duve and Miller 1991; Lazcano and Miller 1999; Miller 1998; Orgel 2000; Österberg 1997), but this does not rule out the general idea that iron and sulfur played important prebiotic roles, in particular, in connection with hot vents, which show a complex FeS chemistry. Pyrite formation via

$$FeS + H_2S \rightarrow FeS_2 + 2e^- + 2H^+ \qquad (20)$$

has been proposed as a primordial energy source (Wächtershäuser 1998). When this reaction occurs in the presence of nitrate, the formation of ammonia is possible (Blöchl et al. 1992; Stetter 1998). Sulfur also plays a central role in the "thioester world" proposed by de Duve (1991, 1998). Thioesters can be formed via

$$R'\text{–}SH + R\text{–}COOH \rightarrow R'\text{–}S\text{–}CO\text{–}R + H_2O \qquad (21)$$

and are suitable for many syntheses, such as the formation of amino acids via α-keto acids:

$$R'\text{–}S\text{–}CO\text{–}R + CO_2 + 2e^- + 2H^+ \rightarrow R'\text{–}SH + R\text{–}CO\text{–}COOH \qquad (22)$$

$$R\text{–}CO\text{–}COOH + NH_3 + 2e^- + 2H^+ \rightarrow R\text{–}CH(NH_2)\text{–}COOH + H_2O \qquad (23)$$

An interesting feature of the thioester world is that, in addition to implying a crucial role of sulfur; the synthesis of thioesters according to Eq. (21) is facilitated by elevated temperatures and low pH levels (de Duve 1991,

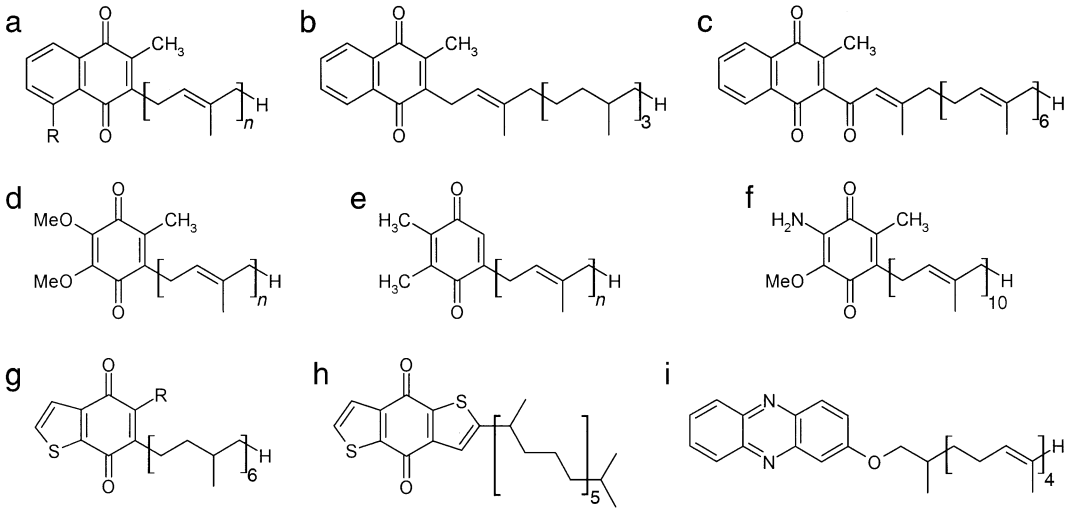

Fig. 1. Bioenergetic quinones. **a** Menaquinone (R = H). n = 6–10 in bacteria and archaea; the latter also use variants with saturated terpene chains, and in the genus *Thermoplasma* thermoplasmaquione (n = 7; R = CH_3) is also found. **b** Phylloquinone, the quinone cofactor A_1 in RC1 and PS1. **c** Chlorobiumquinone, used by the phototrophic chlorobiaceae in addition to phylloquinone. **d** Ubiquinone. n = 6–10 in bacteria and 7 or 8 in archaea; most eukaryotes utilize UQ-10. **e** Plastoquinone. n = 9 dominates in both cyanobacteria and higher plants. **f** Rhodoquinone, first isolated from the purple bacterium *Rhodosprillium rubrum,* but also synthesized by anaerobic eukaryotes. **g, h** Benzothiophene quinones of the archaeal genera *Sulfolobus* and *Acidianus*: sulfolobusquinone (g; R = CH_3), caldariellaquinone (g; R = SCH_3), and tricycloquinone (h). **i** Methanophenazine, used by methanogens instead of quinones. See text for references.

1998), and all three aspects point to the chemistry of hot vents.

Iron in the form of Fe^{2+} is often considered as a possible prebiotic reagent (de Duve 1991; Hartman 1998), in particular, as a light-driven reductant. On the other hand, several hyperthermophiles closely related to the LCA are able to use Fe^{3+} as an electron acceptor, and even *Thermotoga maritima,* which was for a long time considered to rely exclusively on fermentation, can respire using Fe^{3+} (Vargas et al. 1998). Therefore, Fe^{3+} may have been an important early respiratory substrate.

Thermophiles frequently utilize nonheme iron proteins, such as ferredoxin, for functions which are fulfilled by NAD(P) in mesophiles. This might be called a "special adaptation" of thermophiles, because nucleotides are unstable at high temperatures (Daniel and Cowan 2000; Stetter 1999). However, in the light of the hot origin theory, the importance of FeS proteins in these organisms simply reflects the prominent role of such proteins in early times (Beinert 2000), and later mesophiles switched to NAD(P) in certain pathways. The use of FeS centers instead of nucleotide cofactors thus corresponds to a trend similar to that seen when comparing FeS centers versus porphyrins.

Terpenoids

Terpenoids are an ancient and diverse family of biomolecules, but their direct participation in energy transducing mechanisms is rare. It occurs only in bacteriorhodopsin and related archaeal proteins, which are light-driven pumps of protons (bacteriorhodopsin, sensory rhodopsin) or chloride ions (halorhodopsin), using the *cis–trans* isomerization of the sesquiterpene retinal as the principle of operation (Kolbe et al. 2000; Lanyi 2000). Until recently, it was assumed that these proteins occur only in halophilic archaea, but rhodopsin was also identified in marine bacteria (Béjà et al. 2000). It remains to be seen whether this finding can be explained by sporadic LGT or whether retinal-based photosynthesis is common among bacteria, as proposed by Béjà et al. (2000, 2001).

However, terpenes have many auxiliary functions in bioenergetics. One important subclass is the carotenoids, which are tetraterpenes. In all phototrophic bacteria and eukaryotes they have the dual role of funneling energy into the reaction centers and mitigating the effects of high light intensity and UV radiation. A protective role is also fulfilled by the antioxidant function of carotenoids. As polyprenyl side chains, terpenes are found in many bioenergetic compounds, such as chlorophylls, hemes A and O, and quinones (Fig. 1). Moreover, terpenoids occur in cell membranes, where they serve as mechanical reinforcements, but also, in archaea, as basic building blocks (Dannenmuller et al. 2000; Ourisson and Nakatani 1994).

Quinones

Quinones (Fig. 1) operate in almost all electron transport chains as mobile electron carriers. Biotic quinones have lipophilic terpenoid side chains to anchor them in the membrane, but the quinone moiety itself is lipophilic, and artificial quinones without a membrane anchor can also act as electron carriers within membranes. The pH-

dependent oxidation of quinol at cyt bc_1 and cyt b_6f complexes is a rate-limiting step of the whole ETC, giving rise to the regulatory mechanisms known as "respiratory control" and "photosynthetic control." The quinone pool often occupies a central position of branched electron chains with multiple quinone reductases and/or multiple quinol oxidases (Otten et al. 1999; Poole and Cook 2000). The ETCs of archaea are less well characterized than their bacterial and eukaryotic counterparts, but here also quinone pools have a central position (Lübben 1995; Schäfer et al. 1999). Bioenergetic quinones can generally be classified as either benzo- or naphthoquinones. The essential difference is in the standard redox potential, which is about 150 mV lower in naphthoquinones. Due to this low potential, reduced naphthoquinones are less stable in the presence of oxygen, and they are used mainly in anaerobic metabolism. Organisms which can switch between aerobic and anaerobic modes usually can synthesize both types (Poole and Cook 2000; Shestopalov et al. 1997). Benzoquinones seem to have been invented independently in different bacterial lines after the atmosphere became increasingly oxygenated (Schütz et al. 2000). Many archaea contain naphthoquinones (menaquinone and thermoplasmaquinone), but in the genera *Sulfolobus* and *Acidianus* unusual S-heterocyclic quinones, such as caldariellaquione (CQ), have been found (Lübben 1995; Schäfer et al. 1999). CQ has a high standard potential like benzoquinones (about +100 mV), suited for the aerobic metabolism of the archaea where it occurs. These sulfur-containing quinones may therefore represent a third, independent transition from low-potential naphthoquinones to high-potential quinones, in addition to ubiquinone and plastoquinone (Schütz et al. 2000).

Low-potential quinones are indispensable in anaerobic respiration using low-potential electron acceptors. Prokaryotes use the sequence menaquinol $\rightarrow$ SDH $\rightarrow$ fumarate in fumarate reduction (Lemma et al. 1990; Wissenbach et al. 1990), which is the reversal of the mitochondrial pathway succinate $\rightarrow$ SDH $\rightarrow$ ubiquinone. While most eukaryotes are obligatory aerobes, some anaerobic eukaryotes also utilize fumarate reduction, but they cannot synthesize menaquinone and have invented rhodoquinone as a low-potential quinone, which is an amino-substituted ubiquinone (Takamiya et al. 1999; Tielens and Van Hellemond 1998). In methanogenic archaea no quinones have been detected, and they utilize the *ortho*-quinoid methanophenazine (MP) as the membrane-integral carrier of protons and electrons (Abken et al. 1998; Murakami et al. 2001; Schäfer et al. 1999). The redox potential of MP (ca. −250 mV) is even lower than that of naphthoquinones, and the use of MP is probably an adaptation to the energetic constraints of methanogenesis. The archaeal complex which transfers electrons from reduced coenzyme F_{420} to MP in *Methanosarcina mazei* shows a high degree of homology to NADH:ubiquinone oxidoreductase (NDH-1) from bacteria and eukaryotes (Bäumer et al. 2000), supporting the derived nature of MP usage.

Given the biochemical significance of the quinones, it is surprising that they have received only little attention within an origin-of-life context. Accepting the "naphthoquinones first" view (Schütz et al. 2000), one should look for abiotic sources, in particular, of naphthoquinones. Naphthalene derivatives occur in cometary and meteoritic matter (Clemett et al. 1998; Cottin et al. 1999), and the production of naphthoquinones in simulated interstellar ice has been shown (Bernstein et al. 1999), indicating that there is a possible prebiotic source of naphthoquinones, which thus may have been present before the rise of enzyme-mediated metabolism. The use of excreted naphthoquinone as a soluble redox mediator, transferring electrons from the cell to extracellular electron acceptors such as ferric oxides, has been reported for *Shewanella putrefaciens* (Newman and Kolter 2000). It is unknown whether this pathway is common among bacteria [other species can similarly use the quinoid structures of humic substances as extracellular redox mediators (Lovley et al. 1996)], but these findings indicate that quinones may have participated in a similar way in prebiotic redox reactions even before the emergence of membranes. On the other hand, several authors favor an early emergence of membranes (see below), where quinones may have been utilized. The reaction

$$QH_2 \rightleftharpoons Q + 2e^- + 2H^+ \tag{24}$$

makes quinones an ideal site for coupling electron transport and proton transport at biological membranes. Quinone redox chemistry represents the principles of chemiosmosis *in nuce* (Fox 1988; Mitchell 1976; Trumpower 1982) and without the necessity for proton pumping protein structures. Therefore, chemiosmotic circuits may be a basic feature of terrestrial life like other characteristics such as the use of nucleic acids for information storage.

Equation (24) describes a reaction with a H^+/e^- stoichiometry of 1, but a higher coupling ratio, of 2, can be achieved by the reaction

$$2QH_{2(o)} + Q_{(i)} + 2H^+_{(in)} \rightarrow 2Q_{(o)} + QH_{2(i)} + 2e^- + 4H^+_{(out)} \tag{25}$$

In this mechanism, which requires two binding sites, "o" and "i," and intraprotein transfer of electrons from o to i, the net transfer of two electrons is coupled to deposition of four protons. This "Q-cycle" was proposed for cyt bc_1 complexes (Mitchell 1976; Trumpower 1990); it is also performed by cyt b_6f complexes (discussed by Berry and Rumberg 2001) and probably by quinol-oxidizing terminal oxidases (Lübben 1995; Musser and Chan 1998; Saraste et al. 1996; Schultz and Chan 1998).

Membranes and Ion Translocation

The Role of Membranes in the Origin of Life

Lipid membranes are a basic feature of terrestial life, and they are crucially involved in the energy metabolism of cells, rather than being only passive enclosures of the cytoplasm. An early emergence of a cell membrane and of bioenergetic membrane processes was proposed by several authors (Deamer 1997; Fox 1988; Goldacre 1958; Koch 1985; Morowitz 1992; Norris and Raine 1998; Segré et al. 2001). Prebiotic membrane compartments have also been discussed, independent of bioenergetic considerations, in the context of genetic self-organization. Several authors have concluded that the formation of macromolecules would have been assisted by or even dependent on compartmentation (Blocher et al. 2000; Eigen et al. 1980; Kauffman 1993; Norris and Raine 1998; Segré et al. 2001; Szathmáry and Maynard Smith 1995; Szostak et al. 2001; Wicken 1985; Yarus 1999).

Spontaneous formation of the phospholipids, which constitute biomembranes today, may be problematic under plausible prebiotic conditions. If, on the other hand, such lipids were present, then one could ask how the necessary transmembrane transport of molecules was possible, with no specialized transporter proteins being available. This dilemma, however, is only an apparent one. Abiotic amphiphils, being able to form membrane vesicles in water, have been isolated from the Murchison meteorite (Deamer 1997), and similar membrane forming molecules were obtained from experimental simulations of cometary ice (Dworkin et al. 2000, 2001). This indicates that the modern phospholipids are no necessary ingredient and that other compounds may be suitable for building prebiotic membranes. Another route of prebiotic membrane formation is described in the "terpenoid theory" (Ourisson and Nakatani 1994). Archaeal membranes are composed of polyprenyl phospholipids (Daniel and Cowan 2000; Dannenmuller et al. 2000), and hopanoid triterpenes have also been identified as molecular fossils (Ourisson and Nakatani 1994; Ourisson et al. 1987; Rohmer et al. 1979). According to the terpenoid theory, the origin of the first cells was related to the formation of the cell membrane from terpenoids, which may have been available from the spontaneous oligomerization of isopentenol.

The abiotic membranes formed, for instance, from Murchison meteorite extracts are much more permeable than genuine phospholipid membranes, and so the problem of solute exchange with the surroundings is not crucial. There is evidence indicating that nutrient uptake by passive diffusion was feasible (Monnard and Deamer 2001; Pohorille and Wilson 1995). Moreover, the permeability of membranes can be modulated by short RNA molecules (Khvorova et al. 1999); this effect could have facilitated membrane transport and provides a link between the RNA world and transport processes at the primitive plasma membrane.

Coupling Ions: A Gang of Four

The ions H^+, Na^+, K^+, and Cl^- are the major players in membrane bioenergetics, and in this section some possible reasons why this particular set has been chosen are discussed.

Protons. Quinone redox chemistry is related to proton exchange, but this is also true for the nucleotide cofactors NAD(P)H, FAD, and FMN,

$$\mathrm{NAD(P)H} \rightleftharpoons \mathrm{NAD(P)^+} + 2e^- + H^+ \qquad (26)$$

$$\mathrm{FlavinH_2} \rightleftharpoons \mathrm{flavin} + 2e^- + 2H^+ \qquad (27)$$

and for methanophenazine (Abken et al. 1998; Murakami et al. 2001; Schäfer et al. 1999),

$$\mathrm{MPH_2} \rightleftharpoons \mathrm{MP} + 2e^- + 2H^+ \qquad (28)$$

Protons are also involved in several inorganic reactions [Eqs. (2) and (5)–(14)] which could have been energy sources for primitive life. Therefore, simple stoichiometric reasons may account for the coupling of fluxes of electrons and protons at membranes. However, there are additional advantages of the proton as a coupling ion: although the concentration of "free" protons in water is low, there is a much higher number of "latent" protons to be readily mobilized from water molecules. In water as well as on protein or membrane surfaces, extensive hydrogen-bond networks form that enable fast H^+ conductance (Brzezinski 2000; Chaplin 1999; Gutman and Nachliel 1995; Williams 1988). The classical explanation for this rapid H^+ conductance is the Grotthus or bucket-brigade mechanism, but quantum mechanical effects (delocalization of the H^+ ions) also play a role (Chatzidimitriou-Dreismann and Brändas 1990).

But these considerations do not yet explain the *direction* of proton translocation: the polarity is "positive outside," i.e., protons are extruded from the cytoplasm (and into the lumen of organelles such as the thylakoid, which are topologically equivalent to invaginations of a plasma membrane). It is possible that proton extrusion originated simply because of the sequence of redox reactions and the availability of reaction partners. For instance, it has been proposed that quinones in microspheres were oxidized by external Fe(III) (Fox 1988), giving rise to external acidification via

$$\mathrm{QH_2} + 2\mathrm{Fe^{3+}_{(out)}} \rightarrow \mathrm{Q} + 2\mathrm{Fe^{2+}_{(out)}} + 2\mathrm{H^+_{(out)}} \qquad (29)$$

Likewise, oxidation of H_2 at the outside of a primitive cell would induce external acidification (Koch 1985).

However, an interesting clue is the fact that geothermal environments are usually acidic, and (hyper)thermophiles are typically acidophilic (Stetter 1998, 1999). Therefore, the hot origin scenario offers an explanation for the invention of proton extrusion, which could have stabilized the cytoplasm of the first cells at about-neutral pH. Another hint, independent of the chemistry at hot vents, comes from the notion that the early ocean was acidic because of a high atmospheric CO_2 content (Delsemme 1998; Holland 1984; Kasting and Ackerman 1986; Walker 1985).

A large amount of energy is released when the P–O–P bond of oligophosphates is hydrolyzed, and this sufficiently explains why pyrophosphate and ATP emerged as cellular energy currencies. However, the evolutionary pathway that coupled the regeneration of "high-energy" phosphates to proton flow is less clear. Skulachev (1996) has proposed that the membrane-spanning F_0 part of ATP-synthases evolved first as a channel for efflux of protons from the cell, which were generated during glycolysis. Since passive H^+ diffusion was not efficient enough, in the second stage the efflux of protons was driven by ATP hydrolysis, after the membrane-extrinsic catalytic F_1 part had been added to F_0. In this scenario, the F_0F_1 ATP-synthase was initially a H^+-pumping ATPase and later acquired the function of a H^+-driven ATP-synthase. Such a reversal of function is conceivable, but the first step in the scenario is problematic: assuming that the first cells lived in an acidic environment, passive H^+ efflux would never have been feasible and therefore the initial function of F_0 as a mere H^+ channel is not supported. It seems likely that an already existing proton gradient was utilized for the synthesis of ATP, replacing less efficient reactions of substrate-level phosphorylation (de Duve 1991). According to the proposal of Koch (1985), generation of phosphorylating equivalents could have been coupled to phosphate uptake and proton flow at a primitive cell membrane by the reaction sequence

Outside:

$$HPO_4^{2-} + 3H^+ + Z \rightarrow HO\text{–}ZH^+\text{–}PO_3H_2 \quad (30)$$

Inside:

$$HO\text{–}ZH^+\text{–}PO_3H_2 + A\text{–}OH \rightarrow Z + A\text{–}PO_4H^- + 2H^+ + H_2O \quad (31)$$

which requires a membrane-embedded carrier molecule, "Z," and a cytosolic phosphate acceptor, "A." An early link among pH gradients, intracellular accumulation of phosphate, and formation of P–O–P bonds is also discussed by Morowitz (1992).

Sodium. Na^+ is the second major coupling ion at membranes; sodium bioenergetics is typically found in alkaliphiles, halophiles, anaerobes, thermophiles, and pathogens (Dimroth 1997; Häse and Barquera 2001; Hicks and Krulwich 1995; Skulachev 1994, 1996). The polarity of Na^+ transport is positive outside, as in the case of protons. Considering the origin of sodium bioenergetics, two questions arise: (1) Why is Na^+ extruded from cells? and (2) Which was the primordial coupling ion, H^+ or Na^+?

Sodium ions inhibit many proteins, but this toxicity is a *consequence* of the fact that proteins have evolved for billions of years in an intracellular low-sodium milieu rather than a causal *explanation* for the invention of sodium extrusion. The salt content of the oceans is thought to stem from weathering of rocks, and a likely explanation for the origin of sodium extrusion is a gradual increase in the external sodium concentration in the environment of the first cells, evoking the evolution of homeostatic sodium extrusion systems. The salinity of the primordial ocean is controversial, but it is assumed that the salt concentration increased quickly to present levels (Holland et al. 1986; Stanley 1989). Minor changes have occurred over geological time, for instance, in the Br^-/Cl^- and I^-/Cl^- ratios (Channer et al. 1997), but the general property of seawater being essentially a ca. 0.5 *M* solution of NaCl was probably more or less constant. However, the exact timing of the crucial events—origin of the first cells, increase in ocean salinity, evolution of proton and sodium extrusion—is not known. In any case, using sodium gradients generated by maintaining low cytosolic Na^+ levels at a high external Na^+ concentration offered itself as a second system in addition to H^+ bioenergetics, even if no particular reasons made sodium extrusion a biochemical necessity.

There are several reasons to assume that H^+ bioenergetics came first (Skulachev 1996). First, many organisms rely exclusively on H^+ gradients for ATP synthesis, and even when Na^+ bioenergetics is utilized, the sodium gradient is often produced by Na^+/H^+ antiporters (Padan et al. 2001) and thus dependent on H^+ extrusion. This is the case in alkaliphiles (Krulwich et al. 1996, 2001), which have normal H^+-translocating respiratory chains and ATP-synthases. An exclusive use of sodium gradients occurs only in some organisms living in special environments. Second, Na^+ pumping is frequently performed by means of "exotic" proteins, which couple Na^+ translocation to reactions such as decarboxylation of oxaloacetate or malonate (Buckel 2001; Dimroth 1997, 2001); some reaction steps in methanogenesis are also Na^+ linked (Dimroth 1997; Gottschalk and Thauer 2001). In most cases these proteins have no evolutionary relation to the vast number of respiratory and photosynthetic protein complexes of H^+ bioenergetics. Some bacteria, such as *E. coli* and *Klebsiella pneumoniae,* seem to utilize the normal complex I (NDH-1) for sodium extrusion (Steuber 2001), but other species, such as *Vibrio harveyi* and *Haemophilus influenzae,* use a special Na^+-translocating NADH:ubiquinone oxidoreductase with no homology to NDH-1 (Bogachev et al. 2001; Hayashi et

al. 2001). Na^+ translocation is coupled to a normal H^+-linked redox chemistry in the reaction catalyzed by these enzymes (Dimroth 1997; Zhou et al. 1999):

$$NADH + UQ + H^+ + nNa^+_{(in)} \rightarrow NAD^+ + UQH_2 + nNa^+_{(out)} \quad (32)$$

This demonstrates another crucial point: while the coupling of H^+ translocation and electron transport may have developed for simple stoichiometric reasons, there exists no analogous reaction of the type

$$Donor^{red}Na_2 \rightleftharpoons Donor^{ox} + 2e^- + 2Na^+ \quad (33)$$

which could provide a primary link between sodium exchange and electron transport. Therefore, sodium movement requires proteins with specialized structures for pumping. Finally, the F- and V-type Na^+–ATP-synthases/ATPases of some bacteria are single branches within the universal family of rotatory ATP-synthases/ATPases, which are generally H^+ dependent (Müller et al. 2001; Murata et al. 2001; Ubbing-Kok et al. 2000).

All in all, the "protons first" view is well founded, but, accepting the hot origin of life, one line of evidence supports an early invention of sodium bioenergetics: the H^+ permeability of cytoplasmic membranes increases with temperature and therefore some thermophiles use Na^+ gradients (Tolner et al. 1997). However, proton leakage at high temperatures can be overcome by other mechanisms (adjustment of membrane composition, increased rate of H^+ pumping), demonstrating that sodium bioenergetics is not a necessary consequence of (hyper) thermophily.

Potassium. Cells actively concentrate potassium in the cytosol; this process is as universal as the extrusion of sodium (Schachtman 2000; Welsh 2000). The abundances of both metals in the Earth's crust are similar, but, due to their different geochemical behavior, the concentration of potassium in seawater is much lower than that of sodium. This explains why a bioenergetics based on potassium extrusion was neither feasible nor necessary, and potassium has assumed a role complementary to that of H^+ and Na^+ (Hediger 1994; Nelson 1994). Transport of K^+ into the cytoplasm may have evolved for osmotic stabilization, but the electrical compensation of the charge dislocation arising from extrusion of H^+ and Na^+ is also important. A charge compensation is necessary because, due to the high numerical value of the Faraday constant, even small amounts of unbalanced charges across a membrane give rise to dangerously high electrical potential differences.

Chloride. The direct participation of chloride in active transport processes is very rare, it occurs in the chloride-pumping halorhodopsin of halophilic archaea (Kolbe et al. 2000) and in the Cl^--transporting F-ATPase of the alga *Acetabularia acetabulum* (Moritani et al. 1997). The existence of a Cl^--translocating P-type ATPase has also been proposed (Gerencser and Zhang 2001), but no unequivocal molecular identification exists yet. The role of chloride in bioenergetic ion fluxes is mainly passive; like potassium it serves for compensating the electrical charges arising from the movement of H^+ and Na^+ by passive cotransport. Two major reasons may explain why chloride offered itself for this role: chlorine is abundant, both in the Earth's crust in general and in seawater in particular, and it can easily diffuse across lipid membranes (Paula et al. 1998).

The Diversification of Bioenergetic Pathways

Respiratory Complexes

Mitochondrial respiration requires a certain level of free oxygen, and therefore, it is generally accepted that it could develop only after the invention of oxygenic photosynthesis, which gradually oxygenated the biosphere. This, however, does not exclude that anaerobic (using electron acceptors such as NO) or microaerobic (based on traces of photochemically produced oxygen) respiration preceded photosynthesis. There is evidence for this "respiration early" hypothesis (Castresana et al. 1995; Castresana and Moreira 1999): chlorophyll-based photosynthesis occurs only among some bacteria (including the eukaryotic chloroplasts, which are derived from endosymbiotic cyanobacteria), while homologues of respiratory cytochromes are found universally among bacteria and archaea. A reconstruction of the protein inventory of the LCA indicates that it had several respiratory chains, enabling a flexible use of various substrates, but there is no hint that the LCA performed photosynthesis (Castresana et al. 1995; Castresana and Moreira 1999; Castresana and Saraste 1995). Anaerobic and microaerobic metabolism in a number of thermophiles (Reysenbach et al. 2000; Stetter 1998) links the respiration early scenario and the hot origin scenario.

The respiratory cytochrome complexes can be grouped into families; for instance, the cyt *c* oxidases of the cyt aa_3 type form a family together with other heme–copper oxidases. It is a tempting speculation that these families ultimately might be linked by one global phylogenetic tree to reconstruct THE ancestral cytochrome complex. Musser and Chan (1998) have presented a model of respiratory protein evolution, starting with a quinol oxidase similar to *E. coli* cyt bo_3; the multitude of present-day cytochrome complexes is explained by repeated splitting of multisubunit proteins into independent complexes. An attractive feature of this model is the identification of the ancestral terminal oxidase as a quinol oxidase: as discussed above, the redox reactions of a quinone/quinol couple in a membrane represent in a

nutshell the logic of chemiosmotic energy transduction, and it would be nice to have evidence that the ancestral terminal oxidase was a quinol oxidase. However, phylogenetic analyses of other groups do not indicate an ancestral quinol-oxidizing heme–copper oxidase: quinol-oxidases, such as SoxM and SoxABCD from the archaeon *Sulfolobus acidocaldarius,* are interspersed among cyt *c*-oxidizing complexes (Castresana et al. 1995; Lübben 1995; Saraste et al. 1996; Schäfer et al. 1996). Similarly, quinol-oxidizing bacterial NO reductases (NOR) are a branch of a tree with a cyt *c*-oxidizing root (Hendricks et al. 2000). The situation is further complicated by the fact that two complexes (cyt caa_3 and cyt cbb_3) in the bacterium *Rhodotermus marinus* use a FeS protein (HiPIP) as electron donor (Pereira et al. 2000a, b; Santana et al. 2001); it has been proposed that this substrate may represent the earliest electron donor of heme–copper oxidases (Santana et al. 2001). (This would be in line with a greater importance of iron–sulfur proteins at early stages of bioenergetic evolution, as discussed above.)

The second major group of cytochrome complexes is the "*bc*"-type quinol oxidases. Their functional core is given by the modules cyt *b* and FeS protein (Schäfer 1996; Schütz et al. 2000), often—but not always—with an additional *c*-type cytochrome. [A similar chimaeric composition from various proteins is also found for the NDH-1-type NAD(P)H dehydrogenases (Friedrich and Scheide 2000; Friedrich and Weiss 1997).] The Rieske FeS proteins of the cyt bc_1/b_6f complexes show some homologies, and high degrees of homology are found for the cyt *b* subunit of the complexes, but there is no homology between cyt c_1 and cyt *f,* indicating that they were recruited independently (Castresana et al. 1995; Furbacher et al. 1996; Gatti et al. 1998; Schütz et al. 2000). The green sulfur bacterium *Chlorobium limicola* seems to have no *c*-type cytochrome in its complex of cyt b_6 and a Rieske protein (Schütz et al. 1994, 2000) [even for the normal cyt b_6f complex of the green alga *Chlamydomonas reinhardtii,* a novel electron transfer pathway from the Rieske FeS cluster to plastocyanin has been proposed recently, which would bypass cyt *f* (Fernández-Velasco et al. 2001)]. A functional unit of a Rieske-type FeS protein and a cyt *b*-like protein is also found in the quinol-oxidizing complexes SoxABCD and SoxM, which, additionally, contain subunits of typical heme–copper oxidases (Castresana et al. 1995; Lübben et al. 1992; Saraste et al. 1996). As for the heme–copper oxidases, quinol oxidation in the *bc*-type complexes appears as a derived feature, since, in particular, the Rieske modules are older than the first quinol-oxidizing *bc* complexes (Schmidt and Shaw 2001; Schütz et al. 2000). On the other hand, under functional aspects it is still likely that quinone utilization is an ancient pathway (Musser and Chan 1998). Interestingly, the Gram-positive *Bacillus azotoformans* contains a novel NO reductase of the heme–copper family, which can oxidize both menaquinol and cyt *c,* and it was proposed that this "omnipotent" complex represents the precursor of the heme–copper oxidase family (Suharti et al. 2001). And if, as concluded by Pereira et al. (2001), the heme–copper oxidases are a specific bacterial invention that was not present in the LCA, then the phylogenesis of this protein family is not at all suited for elucidating early bioenergetic evolution. Moreover, as discussed above, the participation of hemes (or other porphyrins) in the earliest stages of metabolism is not certain, and it is not necessary that the most primitive quinol oxidase was a cytochrome. A candidate could have been a protein similar to the eukaryotic alternative quinol oxidase, which is a non-heme iron protein (Berthold et al. 2000; Siedow and Umbach 2000).

Photosynthetic Reaction Centers

Assuming that respiration came first, the question arises how photosynthesis would have best fit into the existing respiratory ETCs. In the case of archaeal retinal-based photosynthesis, this may have been straightforward, since bacteriorhodopsin provided an additional proton-pumping device without interfering with electron transport. For bacterial chlorophyll-based photosynthesis, the situation is more complicated. The following events in photosystem evolution seem to be likely.

(1) A monomeric ancestral reaction center (RC) with a single branch of redox centers became homodimeric, thus having two identical branches for electron transfer.
(2) The homodimeric RC underwent divergent evolution in different strains, giving rise to the ferredoxin-reducing RC1 of green sulfur bacteria and heliobacteria and the quinone-reducing RC2 of purple bacteria and green filamentous bacteria.
(3) By gene duplication and divergent evolution of the duplicated genes, homodimeric RCs became heterodimers where in general only one of the two electron transfer branches is active. This seems to have happened independently in the lines of RC1 and RC2.
(4) A bacterial RC2 acquired a water-splitting Mn_4 cluster to become an oxygenic PS2.
(5) PS2 and RC1/PS1 were connected in series within the ancestor of cyanobacteria, probably by LGT from another bacterium, to form the oxygenic photosynthetic ETC, the so-called Z scheme.

This sequence may be a reasonable overall picture, but the available data do not allow for the exact placement of each step. It is controversial whether the divergence RC1–RC2 (step 2) or the monomer–homodimer transition (step 1) came first and whether the homodimer–

heterodimer transition (step 3) occurred before or after the RC1–RC2 bifurcation. However, the homodimeric RC1 in two separate lines (green sulfur bacteria and heliobacteria) supports the notion that the heterodimers were invented independently after the separation of RC1 and RC2. Finally, it is also unknown whether the water-splitting device was acquired by a RC2-type reaction center before or after the connection of two RCs in the Z scheme (step 4 vs step 5) and whether this RC2 was already heterodimeric or not. For further discussion see Blankenship (1992), Dismukes et al. (2001), Mathis (1990), Nitschke et al. (1996), Rutherford and Nitschke (1996), Schubert et al. (1998), Vermaas (1994), and Xiong et al. (2000).

Focusing on the chemical basis of the bioenergetic pathways, an important question is, What was the function of the ancestral RC, quinone reduction as in RC2 or ferredoxin reduction as in RC1? Apparently, complete respiratory chains already existed when chlorophyll-based photosynthesis originated, and therefore the incorporation of the primordial RC at the donor end of the chain may have been advantageous: a primitive RC as a light-driven quinone reductase (Blankenship 1992) would have enabled the utilization of energetically "cheap" electron donors with a high redox potential. One candidate is Fe(II) (Blankenship and Hartman 1998; Hartman 1998). The redox potential of the Fe(II)/Fe(III) couple is too positive for a direct reduction of quinones, but this difficulty would be overcome in a light-driven version of the reaction. In fact, this reaction is utilized by some extant purple bacteria (Ehrenreich and Widdel 1994; Straub et al. 1999). Likewise, manganese could have been used as an electron donor, which is similarly abundant as iron. The redox potential of the Mn(II)/Mn(IV) couple is even higher than that of Fe(II)/Fe(III), but the complex $Mn(II)_2(HCO_3)_4$ may have been the electron donor of the ancestor of PS2 (Dismukes et al. 2001).

Alternatively, the first RC could have served for reducing NAD or other low potential acceptors as in some extant phototrophs, in particular, those which have only RC1. However, assuming this reaction as ancestral, the presence of quinones in all RCs and photosystems remains enigmatic. These firmly bound quinones, one molecule Q_A in RC2/PS2 and two molecules A_1 in RC1/PS1, can be interpreted as evolutionary vestiges of a primitive, quinone-reducing reaction center. And while quinones in most RC1/PS1 are firmly bound, in the green sulfur bacterium *Chlorobium tepidum* there is evidence of mobile menaquinone in RC1, which exchanges with the quinone pool in a manner similar to that of the Q_B of RC2/PS2 (Hager-Braun et al. 1998). Assuming the sequence (1) anaerobic/microaerobic respiration using naphthoquinone, (2) anoxygenic photosynthesis using naphthoquinone, (3) oxygenic photosynthesis, (4) aerobic respiration and invention of benzoquinones, the phylogenetic distribution of quinone cofactors in RCs also makes sense: the divergence of RC1 and RC2 took place before the oxygenation of the atmosphere, and thus all RC1/PS1 contain bound naphthoquinones as evolutionary relics, even when the pool naphthoquinone of the respective organism was later substituted by a benzoquinone. Likewise, in some purple bacteria, such as *Rhodopseudomonas viridis,* the Q_A of RC2 is a naphthoquinone, although the pool consists of ubiquinone. Other species of purple bacteria and the cyanobacteria have made a complete transition and use benzoquinones for the pool/Q_B and for Q_A.

Primordial Photosynthesis?

It seems well established that respiration preceded chlorophyll-based (and, independently, retinal-based) photosynthesis, but this does not preclude the possibility that other types of light utilization prevailed at even earlier stages of bioenergetic evolution, as proposed by several authors. Light-induced production of reducing equivalents by Fe(II) is one possibility (de Duve 1991; Hartman 1998), for instance, in the reaction

$$2Fe^{2+} + 2H^+ + 2h\nu \rightarrow 2Fe^{3+} + H_2 \qquad (34)$$

This type of reaction has also been proposed as a pathway for nitrogen fixation (Mauzerall 2000). As noted above, photooxidation of Fe(II) is found in some extant purple bacteria (Ehrenreich and Widdel 1994; Straub et al. 1999). A related concept is the protophotosynthetic unit based on magnetite (Fe_3O_4), which was one of the earliest models for the origin of photosynthesis (Granick 1965; Mauzerall 1992). Other proposals for primordial light-driven reactions include the following.

- Light-induced redox reactions and ion pumping involving various porphyrins, in particular, those which occur early in the biosynthetic pathway (Masinovsky et al. 1989; Mauzerall 1992; Mercer-Smith and Mauzerall 1984; Sun and Mauzerall 1996; Telegina et al. 2000)
- Aromatic hydrocarbons as UV and blue light-absorbing pigments (Deamer 1992, 1997; Volkov et al. 1995)
- Function of pteridine and flavine pigments, formed abiotically from amino acid thermolysis, in light-driven redox reactions (Heinz et al. 1979; Kritsky et al. 2000)
- UV light-induced formation of ATP from ADP + P_i via an intermediate arising from a reaction of the amino group of excited adenine with phosphate (Skulachev 1996)

A discussion of such systems is given by Deamer (1997) and Morowitz (1992). Of particular interest is the possible link between primordial light utilization and the

origin of chemiosmotic proton translocation. Various chemical model systems are able to couple proton translocation to photochemistry (Seta et al. 1985; Steinberg-Yfrach et al. 1997; Sun and Mauzerall 1996). Proton gradients across a membrane can arise from a scalar photochemistry (Deamer 1997): proton uptake may be undirected and occur with equal probability on both sides of the membrane, but a proton gradient would nevertheless be formed due to the difference in the volumes of the interior and exterior phase. The same amount of removed protons might give rise to a substantial alkalization of a small vesicle, while the proton concentration in the external phase (the ocean) would be unaffected.

A general problem is that various types of hypothetical early photosynthesis have left no trace in modern biochemistry, and even the feasibility under prebiotic conditions is not always obvious. In particular, the use of UV-radiation by pigment systems must be considered problematic (Broda 1975). Several prebiotic light-driven reactions are conceivable, but no strong argument demonstrates that the chemical disequilibrium at hot vents alone, which today can sustain complex ecosystems in a light-independent way (Luther et al. 2001), was no sufficient energy source for the first metabolism.

Discussion

Sequence data have proved extremely useful for reconstructing phylogenesis, and molecular genetics also had a strong impact on the reconstruction of the evolution of bioenergetics. In particular, the characterization of those organisms, which live in extreme habitats and which therefore are both difficult to collect and difficult to cultivate, is making rapid progress nowadays. In the present paper the literature has been reviewed using a combined approach, which joins genetic and biochemical data on extant organisms with physicochemical, functional considerations. Such a complementation is useful for understanding the origin of bioenergetic features, in particular, because phylogenetic reconstruction based on sequence comparison between extant organisms cannot go back farther than to the LCA. For reconstructing the first stages of evolution, one has to resort to speculations on prebiotic conditions. As discussed above, and as also stressed by Baltscheffsky (1996), de Duve (1991), Deamer (1997), Fox (1988), Koch (1985), Morowitz (1992), Norris and Raine (1998), and Ourisson and Nakatani (1994), the molecular design of life is deeply related to bioenergetics and membrane processes.

The main issues addressed in this paper can be summarized as a catalog of alternatives:

(1) Compartmentation by membranes early or late in prebiotic evolution?
(2) First cells thermophilic or mesophilic?
(3) Participation of quinones as carriers of electrons and protons early or late?
(4) Naphthoquinones or benzoquinones first?
(5) Protons or sodium ions as primeval coupling ion?
(6) Respiration or photosynthesis first?
(7) First respiratory complexes quinol oxidizing or cyt *c* oxidizing?

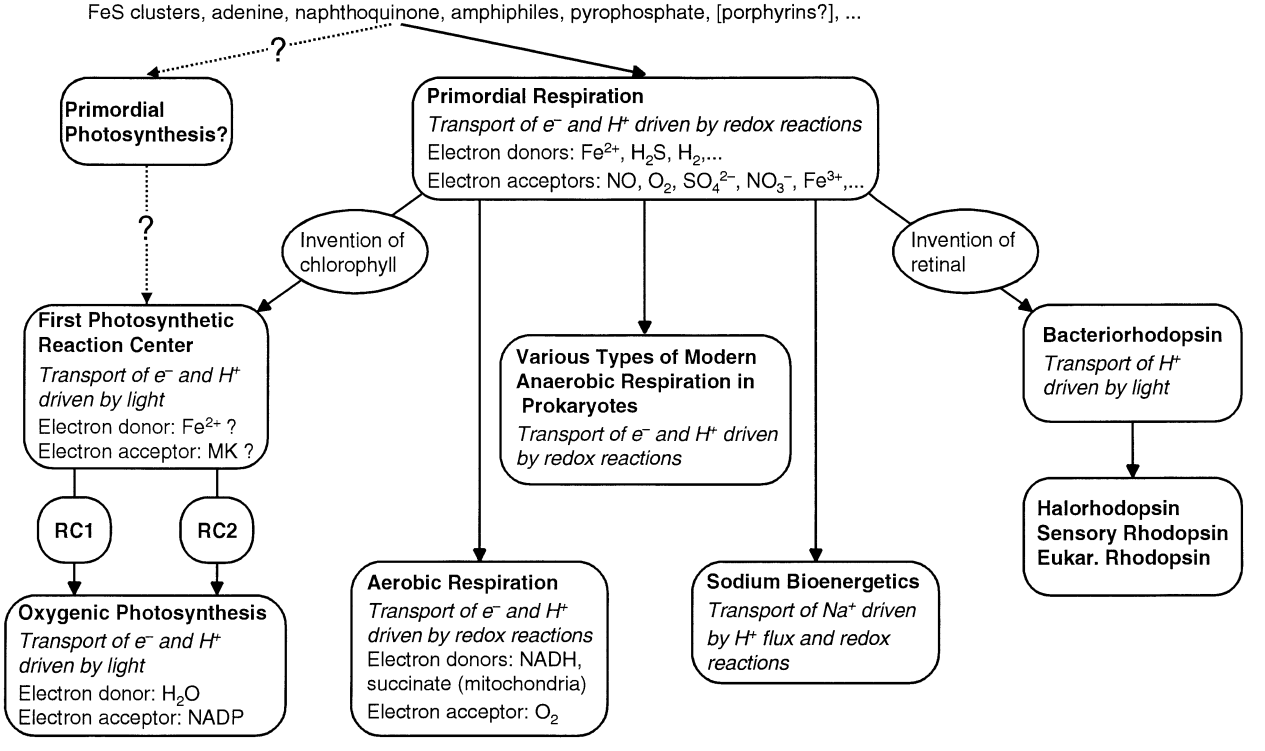

Fig. 2. Hypothetical relations among various bioenergetic modes, based on the principle of parsimony.

(8) First photosynthetic reaction centers quinone reducing or ferredoxin reducing?
(9) Anoxygenic or oxygenic photosynthesis first?

As discussed in the previous sections, in each of these nine cases the first of the given alternatives is more likely, but these options can also be combined into a consistent overall picture, supporting each other's plausibility. For instance, the following hypotheses and observations form a cluster, where the individual elements fit together: the hypothesis of a hot origin of life, the role of iron and sulfur in the chemistry of hot vents, the possible formation of prebiotic compounds driven by FeS chemistry, the role of FeS clusters in many bioenergetic proteins, the utilization of iron and sulfur chemistry in the predominantly anaerobic bioenergetics of deep-branching thermophiles, and the hypothesis that respiration precedes photosynthesis. A similar cluster is as follows: acidophily in many thermophiles, a hypothetical acidic environment of the first cells as the cause for inventing proton extrusion, the role of membranes for compartmentation of the genetic apparatus, the participation of protons in many bioenergetic reactions, quinones as possible early transmembrane proton carriers, and the central role of quinones in modern ETCs.

An outline of bioenergetic evolution, as it emerges from these considerations, is shown in Fig. 2. In this model some major innovations (chlorophyll-based and retinal-based photosynthesis, sodium bioenergetics, aerobic respiration) are derived directly from anaerobic/microaerobic H^+-translocating respiration as the ancestral bioenergetic membrane system. Although alternative hypotheses are conceivable (such as photosynthesis before respiration and sodium before protons), in general they require a greater number of evolutionary transitions to explain the multitude of extant pathways. The model in Fig. 2 thus can serve as an integrative framework which helps to put the plethora of currently available detail information in order.

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