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On the origins of cells: a hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells

William Martin[1*] and Michael J. Russell[2]

[1]*Institut für Botanik III, Heinrich-Heine Universitaet Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany*
[2]*Scottish Universities Environmental Research Centre, Scottish Enterprise Technology Park, Rankine Avenue, East Kilbride, Glasgow G75 0QF, UK* (*m.russell@suerc.gla.ac.uk*)

All life is organized as cells. Physical compartmentation from the environment and self-organization of self-contained redox reactions are the most conserved attributes of living things, hence inorganic matter with such attributes would be life's most likely forebear. We propose that life evolved in structured iron monosulphide precipitates in a seepage site hydrothermal mound at a redox, pH and temperature gradient between sulphide-rich hydrothermal fluid and iron(II)-containing waters of the Hadean ocean floor. The naturally arising, three-dimensional compartmentation observed within fossilized seepage-site metal sulphide precipitates indicates that these inorganic compartments were the precursors of cell walls and membranes found in free-living prokaryotes. The known capability of FeS and NiS to catalyse the synthesis of the acetyl-methylsulphide from carbon monoxide and methylsulphide, constituents of hydrothermal fluid, indicates that pre-biotic syntheses occurred at the inner surfaces of these metal-sulphide-walled compartments, which furthermore restrained reacted products from diffusion into the ocean, providing sufficient concentrations of reactants to forge the transition from geochemistry to biochemistry. The chemistry of what is known as the RNA-world could have taken place within these naturally forming, catalytic-walled compartments to give rise to replicating systems. Sufficient concentrations of precursors to support replication would have been synthesized *in situ* geochemically and biogeochemically, with FeS (and NiS) centres playing the central catalytic role. The universal ancestor we infer was not a free-living cell, but rather was confined to the naturally chemiosmotic, FeS compartments within which the synthesis of its constituents occurred. The first free-living cells are suggested to have been eubacterial and archaebacterial chemoautotrophs that emerged more than 3.8 Gyr ago from their inorganic confines. We propose that the emergence of these prokaryotic lineages from inorganic confines occurred independently, facilitated by the independent origins of membrane-lipid biosynthesis: isoprenoid ether membranes in the archaebacterial and fatty acid ester membranes in the eubacterial lineage. The eukaryotes, all of which are ancestrally heterotrophs and possess eubacterial lipids, are suggested to have arisen *ca*. 2 Gyr ago through symbiosis involving an autotrophic archaebacterial host and a heterotrophic eubacterial symbiont, the common ancestor of mitochondria and hydrogenosomes. The attributes shared by all prokaryotes are viewed as inheritances from their confined universal ancestor. The attributes that distinguish eubacteria and archaebacteria, yet are uniform within the groups, are viewed as relics of their phase of differentiation after divergence from the non-free-living universal ancestor and before the origin of the free-living chemoautotrophic lifestyle. The attributes shared by eukaryotes with eubacteria and archaebacteria, respectively, are viewed as inheritances via symbiosis. The attributes unique to eukaryotes are viewed as inventions specific to their lineage. The origin of the eukaryotic endomembrane system and nuclear membrane are suggested to be the fortuitous result of the expression of genes for eubacterial membrane lipid synthesis by an archaebacterial genetic apparatus in a compartment that was not fully prepared to accommodate such compounds, resulting in vesicles of eubacterial lipids that accumulated in the cytosol around their site of synthesis. Under these premises, the most ancient divide in the living world is that between eubacteria and archaebacteria, yet the steepest evolutionary grade is that between prokaryotes and eukaryotes.

Keywords: hydrothermal vents; acetyl-CoA pathway; origin of life; RNA-world; chemoautotrophic origins; holochirality

1. INTRODUCTION

The Earth is 4.5 billion years (Gyr) old and the first ocean had condensed by *ca*. 4.4 Gyr (Wilde *et al.* 2001). There are good reasons to believe that life arose here by *ca*.

*Author for correspondence (w.martin@uni-duesseldorf.de).

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3.8 Gyr, because carbon isotope data provide evidence for biological CO_2 fixation in sedimentary rocks of that age (Mojzsis *et al.* 1996; Rosing 1999; Nisbet & Sleep 2001; Ueno *et al.* 2002). By 3.5 Gyr, stromatolites were present, preserved microbial mats indicative of deposition by photosynthetic prokaryotes (Walter 1983; Nisbet & Sleep 2001). By *ca*. 1.5 Gyr, so-called acritarchs became reasonably abundant, microfossils of unicellular organisms that are almost certainly eukaryotes (Javaux *et al.* 2001) and probably algae because of an easily preserved cell wall. By 1.2 Gyr, spectacularly preserved multicellular organisms appear that were very probably red algae (Butterfield 2000). There have been reports of more ancient remains claimed to be eukaryotes, but they are equivocal. For example *Grypania* is a 2.1 Gyr fossil (Han & Runnegar 1992), but could just as easily be a filamentous prokaryote as a filamentous eukaryote, because the cellular structure of the fossil is not preserved. More recently, steranes were found in 2.7 Gyr sediments and were claimed to provide evidence for the existence of eukaryotes at that time (Brocks *et al.* 1999), but several groups of prokaryotes including methanotrophic proteobacteria (see Schouten *et al.* 2000), myxobacteria (Kohl *et al.* 1983) and cyanobacteria (Hai *et al.* 1996) make the same kinds of compounds (for example cholesterol) claimed to be eukaryote-specific, such that the sterane evidence for the age of eukaryotes is questionable at best. Ultra-light carbon material that bears the isotopic signature of biological consumption of biologically produced methane goes back to 2.7 Gyr (Hayes 1994), providing evidence for the distinctness of eubacteria (methanotrophs) and archaebacteria (methanogens) by that time at the very least, and furthermore indicating that arguments for an origin of archaebacteria only 850 million years (Myr) ago (Cavalier-Smith 2002) must be in error.

Those geological benchmarks provide rough but robust orientation for the process of early evolution: the origin of prokaryotes by at least 3.5 Gyr and the origin of eukaryotes by at least 1.5 Gyr. But biologists and geologists alike would like to know a few more details. What happened during those first 2.5 Gyr? How did life get off the ground to begin with? Why did it take eukaryotes so long to appear? Why are the two groups of contemporary prokaryotes (eubacteria and archaebacteria) so genetically coherent yet so biochemically different? Why are there no intermediate forms between prokaryotic and eukaryotic organization? Where did the nucleus come from? Here, we discuss evolutionary processes that may underlie the differences between eubacteria and archaebacteria and that may underlie the differences between prokaryotes and eukaryotes as well as the mosaic patterns of attributes (Zillig 1991) shared between the three.

2. COMMONALITIES AND DIFFERENCES LEAD QUICKLY TO DEEPER PROBLEMS

Understanding the evolutionary differences between archaebacteria and eubacteria requires a concrete picture of their last common ancestor. This is because one can only discuss the differentiation of their attributes on the basis of the attributes possessed by their progenitor. But because there is no direct evidence for the nature of their last common ancestor, its attributes can only be addressed through logical inference (Penny & Poole 1999).

What did the ancestor of prokaryotes possess for sure? It certainly possessed the universal genetic code, tRNA (Eigen & Schuster 1978; Woese *et al.* 1990; Eigen 1992) and ribosomes with a battery of ribosomal proteins (Woese 2002), most of which are neatly encoded in a conserved superoperon that is present and conserved in gene order across many genomes (Wächtershäuser 1998). Furthermore, the universal ancestor surely possessed DNA (Reichard 1997; Poole *et al.* 2000) and DNA polymerases, RNA polymerases, and a battery of accessory translation factors such as EF-Tu and EF-G that are present in all prokaryotes (Poole *et al.* 1998, 1999), probably along with other more or less ubiquitous prokarytotic proteins such as F_1–F_0-type ATPases (Gogarten *et al.* 1989) as well as prokaryotic forms of the signal recognition particle, which is an accessory to translation (Brinkmann & Philippe 1999). Furthermore, it had to have had all of the pathways necessary for efficient nucleotide and amino-acid biosyntheses, a core carbon metabolism to provide the biosynthetic precursors, and the cofactors required for those biosynthetic pathways: but this is the point where things get very complicated very quickly, because although archaebacteria and eubacteria do many things similarly when it comes to the processing of genetic information (Woese 2002), they do many things fundamentally differently when it comes to central metabolism.

One simple example for such core metabolic differences between the two groups of prokaryotes is the breakdown of glucose to pyruvate, the glycolytic (Embden–Meyerhof) pathway, which is universal among eukaryotes, but not among prokaryotes. If we look among prokaryotic genomes for the familiar glycolytic enzymes that occur in eukaryotes, we see that they have good homologues among a wide spectrum of eubacterial genomes, but many of the corresponding homologues are missing in archaebacteria (figure 1). This observation might seem puzzling to some (Canback *et al.* 2002) but is easily attributable to two simple factors. First, although archaebacteria possess pathways from glucose to pyruvate, they have several alternative pathways that involve different enzymatic steps, hence different enzymes not present in glycolysis (Daniel & Danson 1995; Schönheit & Schäfer 1995; Kengen *et al.* 1996; Selig *et al.* 1997). These include: (i) the modified Embden–Meyerhof pathway, which employs ADP- instead of ATP-dependent steps and a glyceraldehyde-3-phosphate oxidoreductase in place of the steps catalysed by GAPDH and 3-phosphoglycerate kinase; (ii) the Entner–Doudoroff pathway, which does not require the enzymatic steps catalysed by GPI, PFK, FBA or TPI; and (iii) the non-phosphorylated Entner–Doudoroff pathway, which shares only two enzymatic steps in common with glycolysis (Selig *et al.* 1997), those catalysed by enolase (Hannaert *et al.* 2000) and pyruvate kinase (Schramm *et al.* 2000). Second, several archaebacteria have complete glycolytic pathways, but for many steps they use totally different enzymes that are unrelated to the familiar homologues found in eubacteria (and eukaryotes) (Schönheit & Schäfer 1995; Selig *et al.* 1997). These include: (i) an ADP-dependent (rather than ATP-dependent) hexokinase with no sequence similarity to the eubacterial enzyme (Ito *et al.* 2001); (ii) an ADP-dependent PFK that lacks

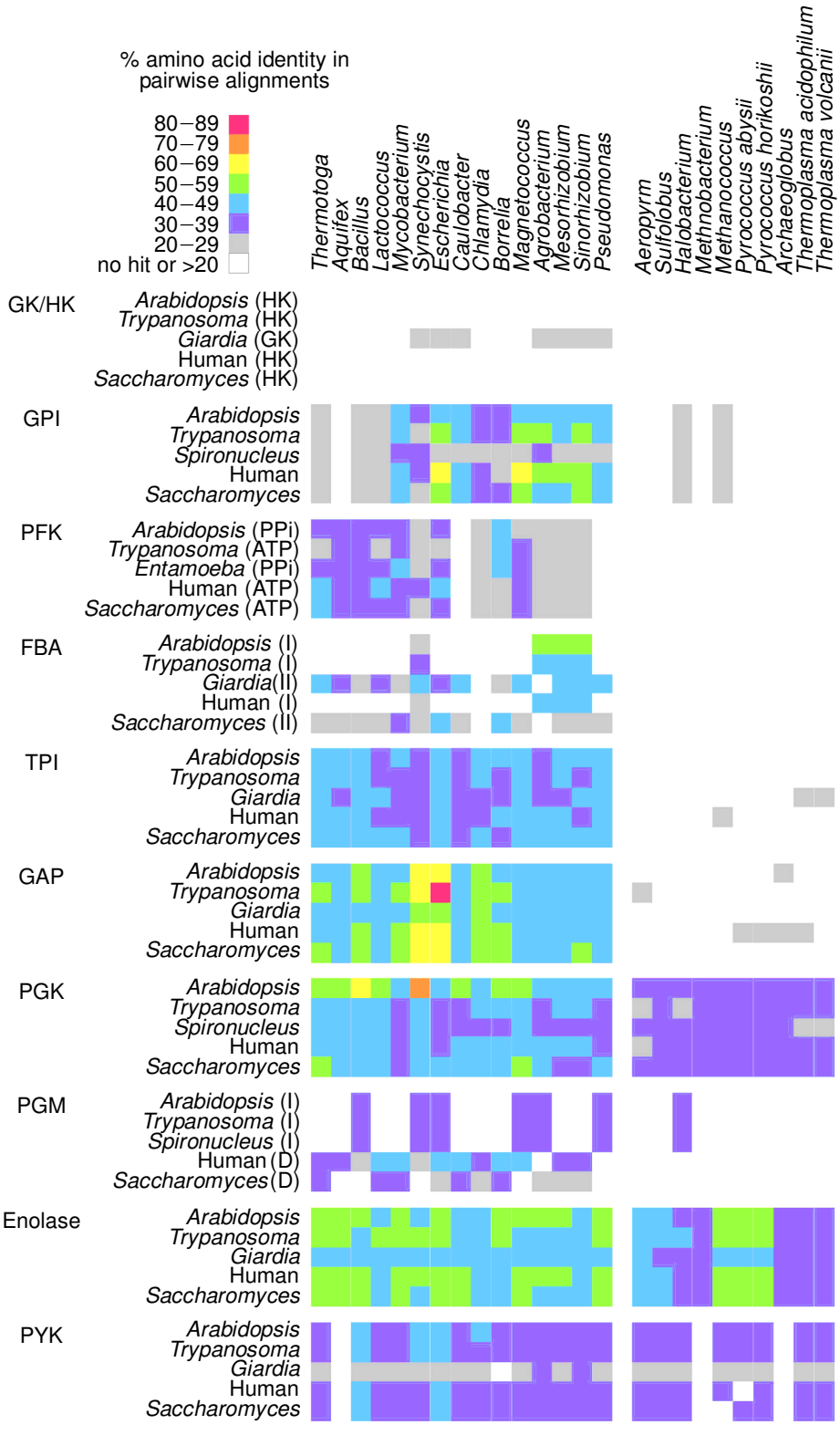

Figure 1. Global patterns of similarity between the enzymes of the glycolytic pathways in eukaryotes and the corresponding enzymes from several completely sequenced eubacterial and archaebacterial genomes. The eukaryotic amino-acid sequences were compared with all peptides from the corresponding genome with gapped BLAST (Altschul *et al.* 1997), the percentage amino acid identity from the pairwise alignment to the best match was recorded and colour coded. Only such matches were counted as aligned over 100 amino acids or more. Note that the eukaryotic sequences are much more similar to their eubacterial homologues. (GK (EC 2.7.1.2); HK, hexokinase (EC 2.7.1.1); GPI (EC 5.3.1.9); PFK, 6-phosphofructo-1-kinase (ATP–PFK, EC 2.7.1.11; PPi–PFK, EC 2.7.1.90); FBA (EC 4.12.1.13, with class I (Schiff-base intermediate) and class II (endiol intermediate) types); TPI (E.C. 5.3.1.1); GAPDH (phosphorylating, EC 1.2.1.12); PGK, 3-phospho-D-glycerate kinase (EC 2.7.2.3); PGM, 3-phospho-D-glycerate mutase (EC 5.4.2.1; 2,3-bisphospho-D-glycerate dependent (D) and independent (I) types); enolase, 2-phospho-D-glycerate hydrolase (EC 4.2.1.11); PYK, pyruvate kinase (EC 2.7.1.40).)

sequence similarity to eukaryotic or eubacterial PFK but is related to archaebacterial ADP-dependent GK instead (Tuininga *et al.* 1999); (iii) an aldolase that shares no sequence similarity with either of the typical eubacterial enzymes (Siebers *et al.* 2001); and (iv) a TPI that shares only residual (*ca.* 20%) amino-acid sequence similarity to the eubacterial enzyme (Kohlhoff *et al.* 1996). So what did the universal ancestor possess in its pathway from glucose to pyruvate, and where did the glucose come from in the first place? We will return to these questions shortly.

A more striking and much more significant example involves lipid biosynthesis. Lipid biosynthesis is important because lipids are the constituents of membranes and all cells—with no exception—are surrounded by membranes. But archaebacteria and eubacteria possess completely different lipids (Kandler 1998). Archaebacterial lipids consist of isoprenoids and various derivatives connected to *sn*-glycerol-1-phosphate by an ether bond. Eubacterial lipids consist of fatty acids connected to *sn*-glycerol-3-phosphate by an ester bond (Kates 1979). The biochemical pathways leading to these two kinds of lipid could not differ more. Their lowest common denominators are acetyl-CoA for the non-polar component and dihydroxyacetone phosphate, which is non-chiral, for the backbone. Eubacteria synthesize their fatty acids from acetyl-CoA by carboxylating it to malonyl-CoA which is used to elongate the growing chain by two carbon units at a time with reduction of the 3-ketoacyl intermediates. Archaebacteria synthesize their isoprenoids via the condensation of IPP and its isomer, dimethylallyl diposphate, C5 units that are synthesized from acetyl-CoA via the MVA pathway (Langworthy *et al.* 1982). Notably, eubacteria also synthesize isoprenoids, but they do so through a completely unrelated pathway, the DXP pathway, intermediates of which are precursors for thiamin diphosphate and pyridoxal diphosphate biosynthesis (Lange *et al.* 2000). Conversely, archaebacteria generally do not synthesize fatty acids at all. Regarding the glycerol backbone, *sn*-glycerol-1-phosphate and *sn*-glycerol-3-phosphate are stereoisomers. Eubacteria synthesize *sn*-glycerol-3-phosphate from dihydroxyacetone phosphate with *sn*-glycerol-3-phosphate dehydrogenase, archaebacteria synthesize the corresponding stereoisomer with *sn*-glycerol-1-phosphate dehydrogenase, which shares no sequence similarity with *sn*-glycerol-3-phosphate dehydrogenase (Koga *et al.* 1998). Finally, the ether and ester linkages are highly distinct chemical bonds. Of course many archaebacteria possess the derived tetraether lipids, and various modifications of the polar head have long been known in both groups (Langworthy *et al.* 1982; Zillig 1991). Add to this the finding that murein, the typical peptidoglycan of the eubacterial cell wall, is missing in archaebacteria—one of the criteria through which the group was discovered (Kandler & König 1978), they possess pseudomurein and other constituents instead (Kandler 1982)—and we arrive from the top-down perspective at an exceedingly severe explanandum: given that there is no similarity whatsoever in the components with which archaebacteria and eubacteria uniformly compartmentalize their cytosol from the environment, what on Earth could have been the precursor of their membrane and cell wall in their last common ancestor?

This question leads directly to a second, even more severe, problem from the bottom-up perspective: given that life evolved from the geochemicals, and given that something like an RNA-world (Gilbert 1986; Poole *et al.* 1998; Bartel & Unrau 1999; Gesteland *et al.* 1999; but see also Orgel & Crick 1993) ever existed in which catalytic self-replicating systems could get off the ground, how on Earth was it possible to attain sufficient and sustained concentrations of the building blocks of life that are needed for any self-assembling prebiotic system to get to the stage that it could perform a single round of replication?

3. UNANSWERED, BUT NOT UNANSWERABLE, QUESTIONS

The second problem is quite well known in the literature on the origin of life and is known as the concentration problem, as addressed by De Duve (1991, 1994), Maynard-Smith & Szathmary (1995) and others (Cairns-Smith 1982; Shapiro 1986). Various solutions to this problem have been proposed, among which the currently most popular are probably surface catalysis, that is, the concentration of reactants on inorganic surfaces allowing them to react (Bernal 1951; Weber 1995; Ferris *et al.* 1996; Huber & Wächtershäuser 1998), and evaporites, that is, the evaporation of terrestrial ponds containing organic precursors synthesized by Miller–Urey-like reactions (Nelson *et al.* 2001). A fundamental drawback to the surface catalysis model is that once two molecules have reacted on a surface, they diffuse away into the Hadean ocean, never to react again. A fundamental drawback to the evaporite model is the sustainability of catalysis: any pond so envisaged has to dry up and refill without a washout very many times before something like a living system can arise, which is hard to imagine given the absence of continents, high and highly frequent tides and 10 km deep oceans at *ca.* 4 Gyr (Gaffey 1997; Bounama *et al.* 2001; Glasby & Kasahara 2001; Kamber *et al.* 2001). The notion of Hadean oceans chock-full of Oparin's prebiotic soup still enjoys some popularity (Bada & Lazcano 2002), but the question remains of how a solution at equilibrium can start doing chemistry. Put another way, once autoclaved, a bowl of chicken soup left at any temperature will never bring forth life. Comets and meteorites contain organic compounds (Deamer 1985), as Oró (1961, 1994) suggested as a possible source of some of life's building blocks, but it is not obvious how to get such compounds to the necessary concentrations to generate the chemistry of life, nor how to select those few active, simple, multipurpose building blocks that make up the basis of life from an interplanetary chemical supply house.

The severity of the problem concerning the differentness of archaebacterial versus eubacterial membrane lipids and cell walls is, with few exceptions (see Koga *et al.* 1998), not as widely recognized as it perhaps should be. What is generally recognized as difficult is the step to cellular organization (Bernal 1960; De Duve 1991; Oró 1994; Segret & Lancet 2000), that is, the origin of *bona fide* membrane-bounded cells. Coacervates or spontaneous self organization of abiotically formed lipids, through whatever prebiotic chemistry, is imaginable (Deamer 1986; Oberholzer *et al.* 1995), but what is not imaginable is putting inside that first little lipid droplet

exactly the right mixture of self-replicating molecules possessing all that is needed (a source of energy, for example, once isolated from the surrounding aqueous phase) to make more of themselves, including the lipids in which they would have been encased.

Getting the universal ancestor into the membranous or other cloak that it has to have at some time under all models for the origin of life and the origin of cells poses seemingly insurmountable problems. In models for the origin of cells, the membranes that surround living systems—under whatever chemical premise (Calvin 1969) and if they are addressed at all (Deamer 1985, 1986)—just seem to come from thin air, and the ultimate contents of cells so derived have to arise as a free-living cytosol that needs to get inside, even in well-argued cases (Segret & Lancet 2000).

Indeed, if we look at all truly living things, which are composed of cells (that is, if we exclude viruses and the like, which are derived from cells upon which they depend for replication), the principle of semi-permeable compartmentation from the aqueous environment is even more strictly conserved than the universal genetic code, because there are rare deviations from the universal code (Hao *et al.* 2002; Srinivasan *et al.* 2002), but there are no exceptions whatsoever to the principle of semi-permeable compartmentation from the environment. Furthermore, an attribute of life that all cells possess (but viruses do not) is the capacity to perform redox chemistry—the transfer of electrons from an external donor to an available acceptor. In fact, a very simple definition of a living system might be: compartments separated from their surroundings that spontaneously multiply with energy gleaned through self-contained, thermodynamically favourable redox reactions. If nothing about life is more conserved than compartmentalized redox chemistry, what was the precursor compartment at the origin of life? We have a suggestion.

4. IRON MONOSULPHIDE: 3D CELLS, NOT JUST 2D SURFACES

In 1981, 360 Myr old hydrothermally formed iron sulphide chimneys were reported from ore deposits near the town of Silvermines, Ireland (Larter *et al.* 1981; Boyce *et al.* 1983). These structures (figure 2*a*) now consist of mostly of pyrite (FeS_2), although they probably precipitated as the monosulphide (FeS) in the Carboniferous sea. Some of the chimneys contain sphalerite (ZnS). They were formed through the hydrothermal exhalation of metal-bearing fluid, originally derived from seawater that had been convectively recycled through the crust (Banks *et al.* 2002). Iron, zinc and lead sulphides were precipitated as the hydrothermal fluid exhaled into a 60 °C brine pool containing bacteriogenic hydrogen sulphide (Boyce *et al.* 1983; Russell 1983; Samson & Russell 1987) to produce the ore deposits found today. These discoveries had been inspired by the much hotter (*ca.* 380 °C) mineral-laden black smoker fluids emanating from chimneys in the eastern Pacific photographed by Ballard & Grassle (1979) (see also Spiess *et al.* 1980). Although fundamentally similar, the chimneys developing at the hydrothermal vents at oceanic ridge crests were much larger than the centimetre-sized structures in Ireland. Nevertheless, the possible evolutionary importance of both types of spring site as

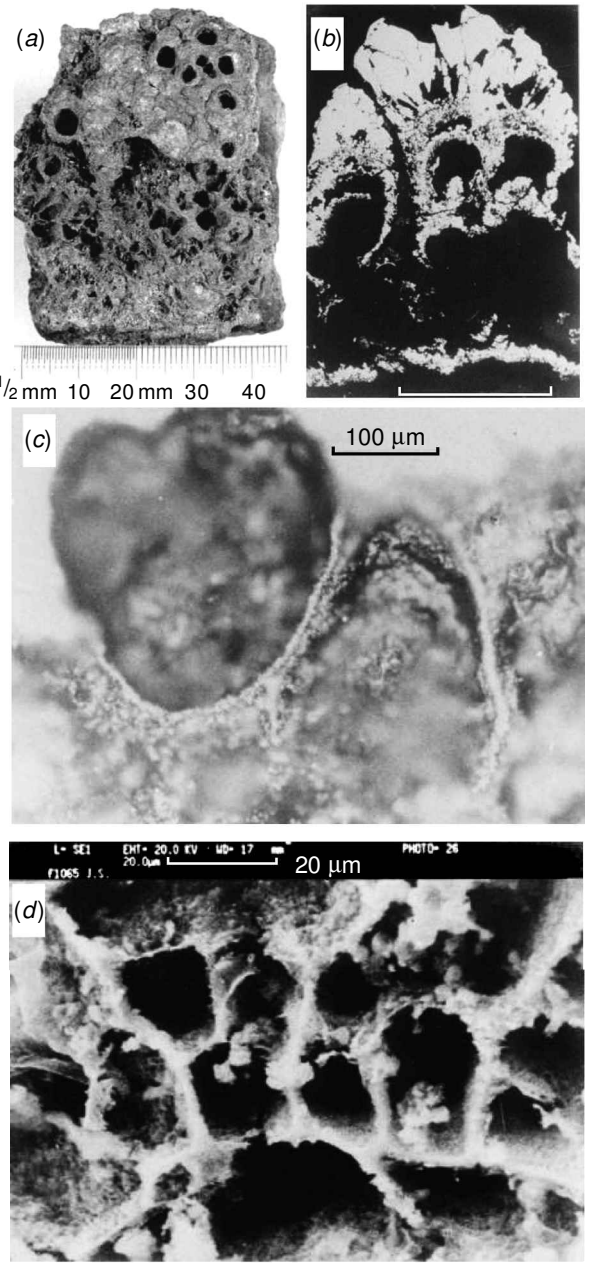

Figure 2. Iron monosulphide precipitates. (*a*) A 360 Myr old hydrothermally formed iron sulphide chimney from Silvermines, Ireland, (Boyce *et al.* 1983). (*b*) Electron micrograph of a thin section of a 360 Myr old pyrite precipitate found at the Tynagh ore deposit, Ireland (Banks 1985). (*c*) Enlargement of (*b*). (*d*) Electron micrograph of a structure formed in the laboratory by injecting Na_2S solution (500 mM, representing hydrothermal fluid) into $FeCl_2$ solution (500 mM, representing the iron-bearing Hadean ocean) (Russell & Hall 1997). High concentrations were used to produce an examinable structure. Similar structures are produced at submarine hydrothermal vents by porous clay (Geptner *et al.* 2002).

chemically reactive 'hatcheries' for the origin of life rather quickly became a popular notion (Corliss *et al.* 1981; Russell *et al.* 1988), although not without staunch critics (Miller & Bada 1988).

Picking up on a long biochemical tradition of thoughts concerning the suspectedly primitive nature of redox reactions catalysed by iron sulphur centres and their possible

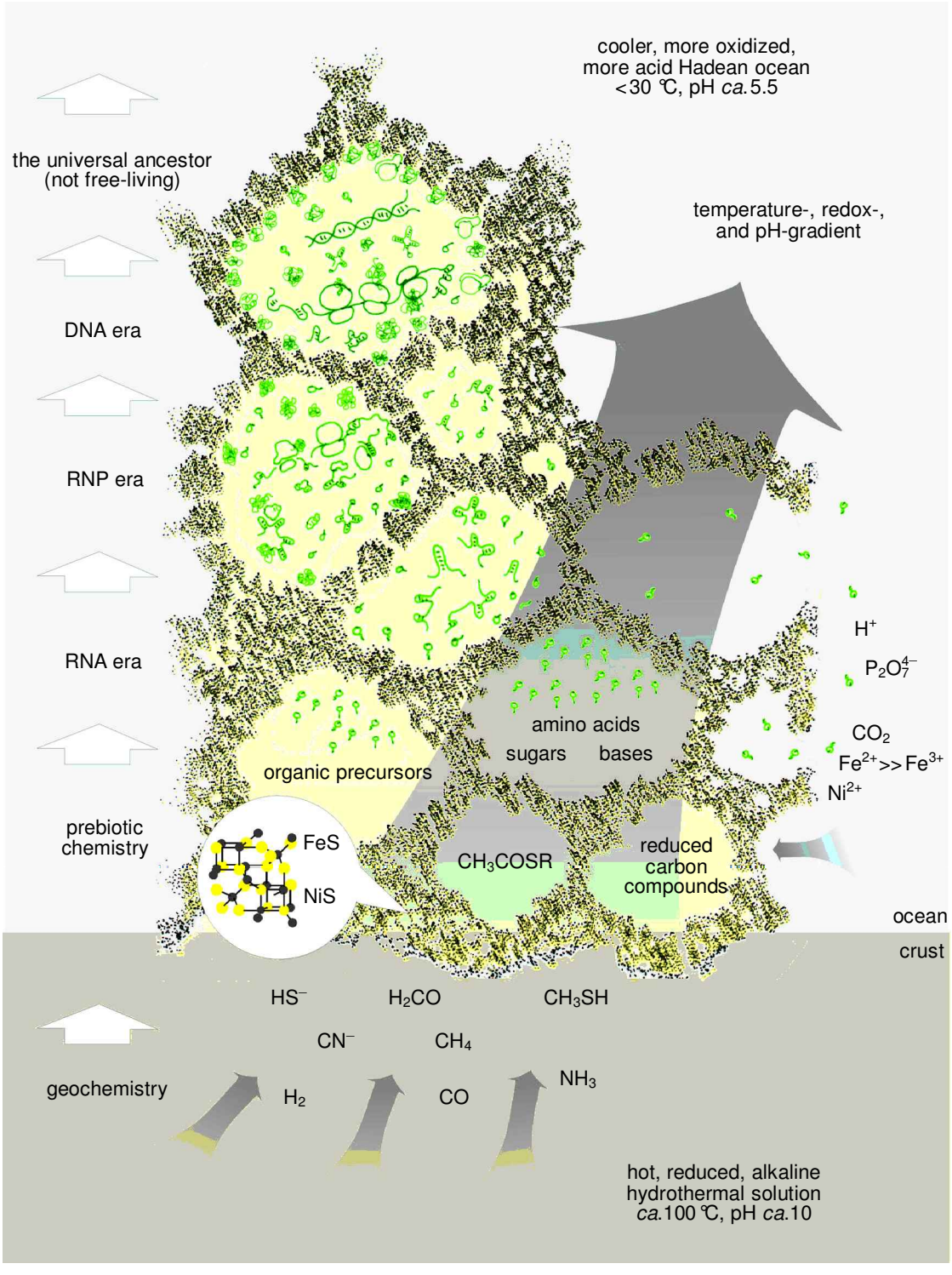

Figure 3. A model for the origin of life at a redox, pH and temperature gradient at a submarine hydrothermal vent. See Russell & Hall (1997) and Russell *et al.* (2003) for details. The terms RNA, RNP and DNA era (instead of 'world') are used to emphasize that no nucleic acid evolution is possible without a supporting geochemistry, later biogeochemistry and finally biochemistry to provide a steady flow of adequate concentrations of polymerizeable precursors (for example nucleotides) and thus to underpin any sort of replication.

role in early metabolism (Hall *et al.* 1971; Marczak *et al.* 1983), but radically departing from notions of organic soup, electric discharge or photocatalysis for the origin of the first biomolecules, Wächtershäuser (1988*a*) suggested that redox reactions between iron sulphide minerals, resulting in FeS_2 (pyrite) formation, could have provided the free energy and electrons needed for primordial carbon fixation. More fully developed as a theory of surface catalysis (Wächtershäuser 1988*b*), this work laid down the case for a chemoautotrophic origin of life, but it is not without its problems. The reduction of CO_2 with an FeS-H_2S/FeS_2 redox couple was shown to be a far less energetically favourable process than originally suggested by Wächtershäuser (Schoonen *et al.* 1999). More importantly, the surface catalysis model still failed to solve the problem of how to achieve sufficient concentrations of reacted pro-

ducts to get life off the ground. Criticizing the notion of 2D life on surfaces and referring to the fate of reacted products on surfaces, De Duve & Miller (1991, p. 10 015) noted 'These, once detached, are considered irretrievably lost'.

At that point, geochemists studying iron sulphide precipitates as they arise and occur in nature took careful note of these developments and suggested, on the basis of good evidence, a different '…role for iron sulphide, that of nucleating the membrane of the earliest cell walls' (Russell *et al.* 1990, p. 387). An important part of that good evidence is shown in figure 2. Iron monosulphide as it naturally precipitates from submarine hydrothermal exhalates forms striking structures (Russell 1983). Figure 2*b* shows an electron micrograph of a thin section of a 360 Myr old pyrite precipitate found at the Tynagh ore deposit, Ireland (Banks 1985) and reveals a highly compartmentalized inner fabric. An enlargement of figure 2*b* is shown in figure 2*c*, which shows a spectrum of internal compartments that are incompletely separated from one another by membrane-like sheaths of iron sulphide precipitate.

Iron sulphide structures of this type are familiar to scientists in the field of economic geology, but it is very worthwhile for biologists and biochemists to have a good look at such images, because most biologists and chemists would imagine an FeS precipitate as a mere clump of mineral with no internal structure: at the extreme a cube with six faces for surface chemistry.

Naturally formed, geologically occurring 3D FeS structures of this type are extremely relevant to the origins of life. As discussed in great detail (Russell & Hall 1997), they provide a very plausible solution to the problem of achieving concentrations of reactants synthesized through FeS chemistry that are sufficient to generate a molecule population of the complexity that prebiotic chemistry requires, if it is ever going to evolve life. For example, iron sulphides are known to have high affinities for organophosphates, cyanide (Woods 1976; Leja 1982), amines (Wark & Wark 1935) and formaldehyde (Rickard *et al.* 2001).

However, the Tynagh and Silvermines chimneys (figure 2*a–c*) were formed from *ca.* 200 °C exhaling fluid containing dissolved iron, zinc and lead, which precipitated as it met cooler (*ca.* 60 °C) sulphide-bearing brine during the late Devonian. At the origin of life some 4 Gyr ago, the conditions were somewhat different, as Fe^{2+} concentrations in the oceans were high and the exhaling fluid would have contained hydrosulphide (HS^-) (Russell & Hall 1997). Simulating Hadean conditions in the laboratory by injecting Na_2S solution (approximating hydrothermal fluid) into $FeCl_2$ solution (approximating the iron-bearing ocean) resulted in highly structured iron monosulphide precipitates (Russell & Hall 1997), an electron micrograph of which is shown in figure 2*d*. Of course, the precise conditions at seepage sites which existed 4 Gyr ago, and whether they would produce exactly such structures, is not known. But deep ocean hydrothermal convective currents and their seepage sites should have existed in the Hadean (Nisbet & Sleep 2001), and strikingly structured clay precipitates have been reported at active submarine seepage sites in Iceland (Geptner *et al.* 2002). It is hardly unreasonable to assume that metal sulphides would have formed at seepage site hydrothermal mounds in the Hadean.

5. ON THE ORIGIN OF LIFE AND CELLS

Founded in geology, geochemistry and thermodynamics, a model for the (chemoautotrophic) origin of life was proposed by Russell & Hall (1997), the salient elements of which are as follows: (i) life emerged as hot, reduced, alkaline, sulphide-bearing submarine seepage waters interfaced with the colder, more oxidized, more acid, $Fe^{2+} \gg Fe^{3+}$-bearing water at deep (*ca.* 4 km) floors of the Hadean ocean *ca.* 4 Gyr ago; (ii) the difference in acidity, temperature and redox potential of these waters provided a gradient of pH (*ca.* four units), temperature (*ca.* 60 °C) and redox potential (*ca.* 500 mV) at the interface of these waters that was sustainable over geological time-scales, providing the continuity of conditions conducive to organic chemical reactions needed for the origin of life; (iii) the exhalate was part of a typical hydrothermal system that brought water from the ocean floor moderately deep into the crust (*ca.* 5 km), where it was subjected to very high pressures and temperatures, and thus contained outgassing CO, H_2, N_2, along with reduced nitrogen (NH_3, CN^-) (Schulte & Shock 1995), reduced carbon (CH_3COO^-, H_2CO, short alkyl sulphides), CH_4 (Kelley 1996; Appel *et al.* 2001), and HS^-, which are hardly unreasonable premises and which were individually cited and justified on geochemical grounds; (iv) colloidal FeS precipitate formed at the ocean floor interface was inflated by the emergent exhalate to produce structured iron monosulphide precipitates possibly similar in morphology to those shown in figure 2; and (v) the sustained redox gradient of about half a volt across the walls of the FeS precipitate, which of course would have contained NiS and sulphides of other metals present in the Hadean ocean as well, provided a persistent source of electrons for prebiotic chemistry in addition to catalytic surfaces of great area and, most importantly, provided a continuously forming ('growing') network of 3D compartments within which reactants could remain concentrated. A slightly adapted version of Russell & Hall's (1997) model is presented in figure 3. Notably, a proton-motive force founded in a natural pH gradient exists across the outer FeS membrane.

Only few minor modifications with respect to the original model have been introduced here. One of these concerns the possible temperature of the exhalate (*ca.* 70–100 °C instead of *ca.* 150 °C) and the temperature of the ocean floor water (*ca.* 0–30 °C versus *ca.* 100 °C) (Russell *et al.* 2003). Modern hydrothermal exhalates can be hot, exceeding 350 °C, or much cooler, for example 70 °C (Kelley *et al.* 2001; Geptner *et al.* 2002), whereby the latter have a pH of 9.8 or more. Owing to the very poor stability of critical building blocks of life such as RNA, temperatures closer to 50 °C seem more conducive to the assembly and sustained stability of early organic molecules and self-reproducing systems (Moulton *et al.* 2000; Bada & Lazcano 2002). Thus, it seems more reasonable to assume more moderate temperatures for the hydrothermal exhalate. Regarding the temperature of the ocean, there is considerable uncertainty. Because the sun was much dimmer 4 Gyr ago than it is now (Newman & Rood 1977;

Sagan & Chyba 1997), it is possible that the Hadean oceans were intermittently frozen in global glaciation, in which case the ocean floor temperature would have been very cold, or that they were fluid in a much warmer climate due to high levels of greenhouse gases such as CO_2, CO and CH_4 in the atmosphere (Nisbet & Sleep 2001). The model would merely require the ocean temperature to be below that of the exhalate. A local gradient of temperatures exists at all submarine springs and seepages.

Another change involves the removal of the reverse citric acid (TCA or Krebs) cycle as an early CO_2 fixation pathway. Wächtershäuser (1988*b*) made a strong case for the reverse TCA cycle being perhaps the first biochemical pathway, based on its construeable similarity to FeS chemistry and thermodynamic attributes. But like any cyclic pathway of CO_2 fixation such as the Calvin cycle, 3-hydroxypropionate pathway or pentose monophosphate cycle, the reverse TCA cycle requires pre-existing, stereochemically correct chemical intermediates of the cycle to get started. A case can be made for the view that formol condensation-like reactions could have fuelled early CO_2-fixation (Russell *et al.* 2003). However, the acetyl-CoA pathway of CO_2 fixation, better known to many as the Wood–Ljungdahl pathway, is also very attractive as a starting point of metabolism because it is linear, using electrons from H_2 to reduce 2 mol of CO_2 to produce an acetyl moiety that is transferred to the thiol group of coenzyme A (Ljungdahl 1994). In the CO_2-fixing direction, the pathway has the net reaction:

$$2CO_2 + 8[H] + CoASH \rightarrow CH_3COSCoA + 3H_2O,$$

with estimated thermodynamic values of $\Delta G'_o = -59.2$ kJ mol^{-1} if $2[H] = H_2$ or $\Delta G'_o = +13.2$ kJ mol^{-1} if $2[H] = NADH$ (Fuchs 1994). The pathway is flexible and occurs among various eubacteria and various archaebacteria. Among other things, it can be: (i) the basis of chemoautotrophy in sulphate-reducing eubacteria and archaebacteria, in methanogens, and in homoacetogens such as *Clostridium thermoautotrophicum* when growing chemoautotrophically (Ljungdahl 1994); (ii) the source of chemiosmotic potential in homoacetogens when growing fermentatively (Ljungdahl 1994); (iii) a pathway of acetyl-CoA oxidation if CO_2 and H_2 levels are kept extremely low through consumption by other microbes; (iv) a pathway of acetate disproportionation to H_2 and CO_2 in acetoclastic methanogenesis; (v) a pathway for the assimilation of various C_1 compounds; and (vi) a source of electrons for carbon fixation through oxidation of CO to CO_2 (reviewed in Fuchs 1994; Ragsdale 1994; Ferry 1995).

The key enzyme of the acetyl-CoA pathway is an extremely intriguing protein called ACS/CODH (Ljungdahl 1994; Ragsdale 1994; Ferry 1995; Lindahl 2002). In the CODH domain, ACS/CODH employs five FeS clusters including an unusual 4FeNi5S cluster that binds CO_2 and reduces it to CO. As part of the proposed reaction mechanism, the ACS domain of ACS/CODH condenses Ni-bound methyl and Ni-bound CO, yielding an Ni-bound acetyl moiety that is transferred to CoASH yielding acetyl-CoA (Lindahl 2002). Emulating the ACS/CODH enzymatic reaction, Huber & Wächtershäuser (1997) showed *inter alia* that micromolar amounts of methyl thioacetate (CH_3COSCH_3) could be formed overnight without pressure at 100 °C from 4.5 mmol CO and 100 μmol CH_3SH (Heinen & Lauwers 1996) in the presence of FeS and NiS, making this type of reaction in FeS precipitates extremely plausible as a prebiotic source of basic carbon building blocks. However, the experiment produced no pyrite. CH_3COSCH_3 contains a thioester bond and can be considered as homologous to the business end of acetyl-CoA. In most organisms that use the acetyl-CoA pathway today, the next step of CO_2 fixation is catalysed by pyruvate synthase, also called PFOR, an FeS protein that combines the acetyl moiety of acetyl-CoA with CO_2 and two electrons from ferredoxin, another FeS protein, to yield pyruvate (Schoenheit & Schäfer 1995; Yoon *et al.* 1999). At very high temperature and pressure, traces of pyruvate were synthesized from CO and alkyl thiols by FeS catalysts (Cody *et al.* 2000). In contrast to other views (Wächtershäuser 1988*b*; Huber & Wächtershäuser 1997), we see no need for the presence of cyclic CO_2 fixation pathways at this stage, because a linear route of carbon fixation would suffice, given continuous geochemical input.

There is a vast literature regarding plausible models for prebiotic syntheses of sugars (formol condensation (Quayle & Ferenci 1978; Müller *et al.* 1990)), bases (cyanide condensation (Oró & Kimball 1962; Sanchez *et al.* 1967)), amino acids (Hennet *et al.* 1992; Marshall 1994) and other molecules (Sutherland & Whitfield 1997), and a vast literature on the broad catalytic capabilities of RNA (Bartel & Unrau 1999; Gesteland *et al.* 1999), none of which we can deal with adequately here. We surmise that it is conceivable and not implausible that using such conceptual tools one could get to something like an RNA-world (Gilbert 1986), provided that its monomeric building blocks are steadily replenished and remain in sufficient concentrations, conditions that structured FeS precipitates could fulfil because of their sustained energy input though the natural redox gradient and by their compartmentalizing, reactant-retaining nature (Russell & Hall 1997).

6. HOLOCHIRALITY

Another addition to the proposal of Russell & Hall (1997) is that an unresolved problem—the holochirality problem—needs to be addressed (see also Russell *et al.* (2003) for another perspective on this issue). Life today consists of L-amino acids and D-sugars, but the molecules at the very beginning of life almost certainly did not. It has been argued that asymmetric surfaces could give rise to stereospecific prebiotic synthesis (Hazen *et al.* 2001), but given the surfaces of iron sulphide, we find it unconvincing that one or the other stereoisomer of either class should tend to be synthesized at early stages. Thus, our model up to this point could in principle lead to a sustainable and compartmentalized RNA-world, but a badly scrambled, primitive one of racemic sugars coexisting with a racemate of amino acids and everything else. A possible way out of the racemic world could have involved the origin of the peptidyl transferase reaction of the large ribosomal subunit. Among the myriad of molecules that existed in the RNA-world, a population of them was involved in the origin of translation, whereby it has been sensibly suggested that the original function of the ribosome was RNA

replication via triplets, not translation (Poole *et al.* 1999). We can take it as a given that at some point a precursor of the peptidyl transferase reaction (Muth *et al.* 2000) of an RNA molecule did evolve, and the chance stereochemistry of that molecule would determine the decision of whether it could catalyse the polymerization of D- or L-amino acids into peptides. That filter would be sufficient to tip the holochirality scale, because despite the presence of a racemate, only amino acids of the same α-carbon configuration would preferentially end up in peptides, yielding a population of distinctly handed peptides, some of which would eventually need to feed back in a hypercycle-like manner (Eigen & Schuster 1978) into favoured synthesis of their stereochemically correct polymerizing template.

Concerning D-sugars, if we assume that the FeS-catalysed synthesis of acetyl-CoA and pyruvate was possible as above, then the origin of chirality should be sought at the three-carbon stage. Pyruvate is not chiral, nor is its phosphorylated derivative, phospho*enol*pyruvate. In organisms that use the acetyl-CoA pathway, the next step of autotrophic carbon metabolism involves the stereospecific addition of a water molecule to the double bond in phospho*enol*pyruvate, yielding 2-phospho-D-glycerate. Today, that reaction is catalysed by the enzyme enolase, which is by far the most highly conserved of all glycolytic enzymes (figure 1) and as far as we are aware, is the only enzyme of core carbon metabolism that is truly ubiquitous among all free-living organisms. For the whole remainder of central carbon metabolism in all organisms, the stereochemistry at C2 of 2-phospho-D-glycerate never changes. This approach to the holochirality problem implicates the peptidyl transferase reaction of the ribosome as the pacemaker for amino-acid stereochemistry and a simpler precursor of enolase as the pacemaker of sugar stereochemistry. It is probably just curious coincidence that enolase is the only enzyme of carbon metabolism, other than adenylate kinase, that balances AMP, ADP and ATP levels, that is encoded in the highly conserved ribosomal protein superoperon in archaebacteria (Hannaert *et al.* 2000), which might also carry a faint trace of chiral origins. At any rate, our suggestion for the origin of holochirality starts with the peptidyl transferase reaction and is thus largely congruent with Woese's (2002, p. 8745) assessment 'The evolution of modern cells, then, had to begin with the onset of translation'. It was certainly a decisive step.

One particularly interesting thought emerges from the foregoing, namely that the primitive peptides synthesized by the kind of a universal ancestor shown in figure 3 (particularly the sulphhydryl-containing ones) would have provided a chiral, proteinaceous surface associated with an FeS (or other metal sulphide) centre. This would have provided dramatic synergy for biochemical diversity, and should have initiated an era of biochemical discovery, with Darwinian backcoupling (via translation) to the encoding nucleic acids. The very clear prediction of this notion is that experiments of the type performed by Heinen & Lauwers (1996) or Huber & Wächtershäuser (1997), when supplemented with small synthetic holochiral peptides (random mixtures of varying complexity), should catalyse the synthesis of a much more diverse spectra of biologically relevant molecules than control experiments lacking the synthetic peptides. Experiments of this type might provide insights as to how biochemistry evolved, and might even reveal various primitive analogues of contemporary active centres.

7. THE UNIVERSAL ANCESTOR: THE LAST COMMON ANCESTOR OF PROKARYOTES ONLY

By the time that a ribosome had evolved, a genuine ribonucleoprotein world, the step to a DNA world and the last common ancestor would not be far off. But here we have to depart completely from some long-held and widely regarded views on the early evolution of cells (Woese 2002), because the weight of evidence precludes the existence of eukaryotes anywhere near the origin of cells. The reasons for this are at least threefold.

First, without question, eukaryotes are ancestrally heterotrophs, the only autotrophy in the entire group having been acquired through endosymbiosis during the origin of plastids. All eukaryotes that lack plastids and some that possess plastids are heterotrophs, and without exception the backbone of their ATP synthesis is the oxidative breakdown of reduced carbon compounds through the glycolytic pathway (Martin & Müller 1998). Embracing the view of a chemoautotropic origin of life, the first organisms necessarily had to be chemoautotrophs (Kandler 1998) and heterotrophy as a microbial lifestyle could only have arisen after autotrophs had evolved a metabolism sufficient to support the free-living lifestyle and had subsequently produced sufficient quantities of reduced carbon compounds in the environment to provide heterotrophs—prokaryotic and eukaryotic—with something to eat.

Second, all of the eukaryotes whose origin needs to be explained (contemporary ones) seem to have possessed a mitochondrion in their evolutionary past (see Embley & Hirt 1998; Roger 1999; Williams *et al.* 2002). The origin of eukaryotes is thus hardly separable from the origin of mitochondria (Gray *et al.* 1999; Lang *et al.* 1999). This would mean that the origin of eukaryotes would necessarily post-date the diversification of the α-proteobacteria from which the mitochondrion is thought to descend, meaning that eukaryotes must substantially post-date prokaryotes in origin.

Third, if we look beyond the diversity of the genetic system and consider the diversity of metabolic pathways that prokaryotes use to synthesize the ATP that is the backbone of their survival, then we are faced with the more than 150 different redox reactions involving inorganic donors and acceptors used by the thermophilic prokaryotes alone for their core energy metabolism (Amend & Shock 2001) versus the complete diversity of eukaryotic energy metabolism, which does not comprise more than one single pathway of glucose oxidation to pyruvate and three basic metabolic pathways of subsequent pyruvate metabolism (Martin & Müller 1998). The full diversity of eukaryotic energy metabolism, including the diversity of enzymes catalysing the individual steps (figure 1), does not exceed that of a single, generalist α-proteobacterium. With such a narrow biochemical diversity at the root of their ATP synthesis, it seems completely at odds with common sense to assume that eukaryotes are anywhere nearly as old as prokaryotes. Prokaryotes are easily seen as holdovers from the phase of evolution where novel biochemical

routes to glean energy from the environment were still being invented, and many such relics can be found among prokaryotes today. The argument by Kandler (1998), that the complete lack of chemoautotrophy among eukaryotes prescribes their origin subsequent to the origin of prokaryotes is compelling. But the question of how eukaryotes and their defining features might have arisen is a different matter to be discussed in a later section.

There are no eukaryotes that can live from the elements alone without the help of prokaryotes (or prokaryotic endosymbionts). By contrast, there are many eubacteria and archaebacteria that can live from the elements alone (chemoautotrophs), and it is hence eminently reasonable to assume that organisms able to live from the elements alone arose before those organisms that cannot. The converse argument that eukaryotes arose before prokaryotes (as for example argued in Forterre & Philippe (1999)) is completely unreasonable from the standpoint of microbial physiology and is only tenable if one looks at genetic systems and genetic systems only, leaving microbial physiology aside. But physiology is just as important for early evolution as genetic systems are, because no genetic system operates without a physiological system to support it with energy in the form of ATP and precursors for doing the things that genetic systems do. Arguing that eukaryotes predated prokaryotes, which underwent some mysterious reductive process, is like arguing that land animals predated land plants, which nourished themselves from animal excrements. The most fundamental difference between prokaryotic and eukaryotic physiology from the standpoint of energy metabolism is illustrated in figure 4, where only a very small sample of the various redox couples used by prokaryotes as summarized by Amend & Shock (2001) are contrasted to the entire diversity of energy metabolism found in eukaryotes (see also Schäfer *et al.* (1999) for an overview of energy metabolism in archaebacteria). Clearly, under our premise, embracing a chemoautotrophic origin of life, eukaryotes simply cannot have arisen at even approximately the same time as prokaryotes. At any rate, common sense and a bit of biochemical reasoning dictates that eukaryotes must have arisen long after prokaryotes did, as most biologists have always reasonably assumed and as the microfossil record indicates.

Our model for the origin of life to this point has specifically addressed the arguably most important problems that early self-organizing and self-replicating systems faced, namely: (i) where did the energy come from that is needed to continuously synthesize the building blocks of evolving genetic systems (natural redox and pH gradient); and (ii) how did the chemical constituents of life manage to stay sufficiently concentrated to permit any kind of replication to occur (structured FeS precipitates).

The universal ancestor as depicted in figure 3 was, in our view, an organism—though not a free-living one—that should have possessed all of the attributes that are common to all eubacterial and archaebacterial chemoautotrophs: the genetic code, the ribosome, DNA, a supporting core and intermediate metabolism (nitrogen fixation, modern amino-acid, nucleic-acid and cofactor biosyntheses) needed to supply the constituents of its replication (doubling of mass), compartmentation from the environment, redox chemistry and ATPases, which could have used the naturally existing proton gradient (alkaline inside, acid outside) at the seepage site.

This non-free-living universal ancestor would have found itself at the dawn of the biochemical revolution where genes and proteins were diversifying into a myriad of functions, where RNA and metal sulphide catalysts were being replaced by proteins, where new pathways and cofactors were being invented to augment and substitute their mineral and RNA precursors, where FeS from the mother lode was being incorporated into proteins as FeS clusters and where biochemistry started to diversify into the forms that were both possible and useful. From the standpoint of protein structure, this age of invention would have witnessed the origin of basic building blocks of biochemical function that: (i) are conserved at the level of a 3D structure among archaebacteria and eubacteria; and (ii) are recognizable as modules of function in various electron-transporting proteins as discussed by Baymann *et al.* (2003). From the standpoint of amino-acid sequence conservation, this age of invention would have been a phase of molecular evolution where proteins were diversifying (e.g. Habenicht *et al.* 1994) and getting better at what they do, rather than not getting worse at what they do, as is thought to be the case for the evolution of today's proteins. Put another way, most modern proteins are thought to accept amino-acid substitutions because the new amino acid does not significantly impair the pre-existing function (neutral or nearly neutral molecular evolution). At the very beginning of protein evolution, positively selected mutations that improve the function of the protein might have been the rule rather than the exception, because without advantageous mutations, adaptive evolution cannot occur (Nei 1987). Accordingly, the mechanistic basis of sequence divergence among the most ancient proteins (intense positive selection) would differ fundamentally from that in recently diverged proteins (near neutrality).

8. FROM A NON-FREE-LIVING UNIVERSAL ANCESTOR TO FREE-LIVING CELLS

At some point, this biochemical diversification would have included the invention of pathways that catalyse the synthesis of lipids and cell wall constituents (figure 5), both of which would have begun to insulate the cytosol from its FeS encasement, requiring a new category of proteins: membrane-associated proteins involved in redox chemistry. These would have been rich in FeS (and other metal sulphide) centres so as to replace the functions originally associated with the original FeS redox chemistry germane to the original FeS 'cell wall'. As a consequence, the catalysis performed by (Fe ≫ Ni)S (Russell *et al.* 2003) would have been gradually separated from the mineral wall and step-by-step incorporated into proteins, an imprint of which would be reflected in the FeS centres of ancient proteins under this view, as schematically indicated in figure 6. Protein modules such as ferredoxins and hydrogenase are very common in today's membrane-associated electron transport chains (Baymann *et al.* 2003). Only when a sufficient battery of redox carriers had become assembled into the membrane to allow the compartments to sustain ATP synthesis via chemiosmosis outside the confines of their 3D (Russell & Hall 1997; see also

eubacteria

$S^0 + O_2 + H_2O \leftrightarrow HSO_3^- + H^+$
$CO + 0.5O_2 \leftrightarrow CO_2$
$H_2S + 0.5O_2 \leftrightarrow S^0 + H_2O$
$2NO_2^- + O_2 \leftrightarrow 2NO_3^-$
$NH_3 + 1.5O_2 \leftrightarrow H^+ + NO_2^- + H_2O$
$S_2O_3^{2-} + 2O_2 + H_2O \leftrightarrow 2SO_4^{2-} + 2H^+$
$6S_2O_3^{2-} + 5O_2 \leftrightarrow 4SO_4^{2-} + 2S_4O_6^{2-}$
$5S_2O_3^{2-} + H_2O + 4O_2 \leftrightarrow 6SO_4^{2-} + 2H^+ + 4S^0$
$S_3O_6^{2-} + 2O_2 + 2H_2O \leftrightarrow 3SO_4^{2-} + 4H^+$
$2S_4O_6^{2-} + 6H_2O + 7O_2 \leftrightarrow 8SO_4^{2-} + 12H^+$
$S^0 + 1.5O_2 + H_2O \leftrightarrow SO_4^{2-} + 2H^+$
$H_2S + 2O_2 \leftrightarrow SO_4^{2-} + 2H^+$
$2H_2S + 2O_2 \leftrightarrow S_2O_3^{2-} + H_2O + 2H^+$
$2Fe^{2+} + 0.5O_2 + 2H^+ \leftrightarrow 2Fe^{3+} + H_2O$
$SCN^- + 2O_2 + 2H_2O \leftrightarrow SO_4^{2-} + CO_2 + NH_4^+$
$COS + 2O_2 + H_2O \leftrightarrow SO_4^{2-} + CO_2 + 2H^+$
$CH_4 + 2O_2 \leftrightarrow CO_2 + 2H_2O$
2 pyrite + $7.5O_2 + H_2O \leftrightarrow 2Fe^{3+} + 4SO_4^{2-} + 2H^+$
pyrite + $3.5O_2 + H_2O \leftrightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$
2 chalcopyrite + $8.5O_2 + 2H^+ \leftrightarrow 2Cu^{2+} + 2Fe^{3+} + 4SO_4^{2-} + H_2O$
covellite + $2O_2 \leftrightarrow Cu^{2+} + SO_4^{2-}$
sphalerite + $2O_2 \leftrightarrow Zn^{2+} + SO_4^{2-}$
galena + $2O_2 \leftrightarrow Pb^{2+} + SO_4^{2-}$
$H_2 + 0.5O_2 \leftrightarrow H_20$
$SCN^- + H_2O \leftrightarrow H_2S + OCN^-$
$COS + H_2O \leftrightarrow SO_4^{2-} + CO_2 + H_2S$
$NO_3^- + H_2S \leftrightarrow NO_2^- + H_2O + S^0$

$2NO_3^- + 5H_2S + 2H^+ \leftrightarrow N_2 + 5S^0 + 6H_2O$
$8NO_3^- + 5H_2S \leftrightarrow 4N_2 + 5SO_4^{2-} + 4H_2O + 2H^+$
$4SO_3^{2-} + 2H^+ \leftrightarrow 3SO_4^{2-} + H_2S$
$SO_2 + H_2O + S^0 \leftrightarrow H_2S_2O_3$
$S_2O_3^{2-} + H_2O \leftrightarrow SO_4^{2-} + H_2S$
$S_2O_3^{2-} \leftrightarrow SO_3^{2-} + S^0$
$4S_2O_4^{2-} + 4H_2O \leftrightarrow 3H_2S + 5SO_4^{2-} + 2H^+$
$S_3O_6^{2-} + H_2O + \leftrightarrow SO_4^{2-} + S_2O_3^{2-}2H^+$
$4S^0 + 4H_2O \leftrightarrow SO_4^{2-} + 3H_2S + 2H^+$
$5S_2O_3^{2-} + 8NO_3^- + H_2O \leftrightarrow 10SO_4^{2-} + 4N_2 + 2H^+$
$5S^0 + 6NO_3^- + 2H_2O \leftrightarrow 5SO_4^{2-} + 3N_2 + 4H^+$
$4H_2 + 2CO_2 \leftrightarrow CH_3COOH + 2H_2O$
$H_2 + 2Fe^{3+} \leftrightarrow 2H^+ + 2Fe^{2+}$
$H_2 + UO_2^{2+} \leftrightarrow$ uraninite $+ 2H^+$
$H_2O_2 + H_2 \leftrightarrow 2H_2O$
$NO_3^- + H_2 \leftrightarrow NO_2^- + H_2O$
$NO_3^- + 2.5H_2 + H^+ \leftrightarrow 0.5N_2 + 3H_2O$
$NO_3^- + 4H_2 + H^+ \leftrightarrow NH_3 + 3H_2O$
$NO_2^- + 0.5H_2 + H^+ \leftrightarrow NO + H_2O$
$NO_2^- + 1.5H_2 + H^+ \leftrightarrow 0.5N_2 + 2H_2O$
$NO + 0.5H_2 \leftrightarrow 0.5N_2O + 0.5H_2O$
$N_2O + H_2 \leftrightarrow N_2 + H_2O$
$N_2 + 3H_2 \leftrightarrow 2NH_3$
$SO_4^{2-} + 4H_2 + 2H^+ \leftrightarrow H_2S + 4H_2O$
$SO_3^{2-} + 3H_2 + 2H^+ \leftrightarrow H_2S + 3H_2O$
$S_4O_6^{2-} + H_2 \leftrightarrow 2S_2O_3^{2-} + 2H^+$
$S^0 + H_2 \leftrightarrow H_2S$
$S_2O_3^{2-} + 2H^+ + 4H_2 \leftrightarrow 2H_2S + 3H_2O$
$NH_3 + NO_2^- + H^+ \leftrightarrow N_2 + 2H_2O$

archaebacteria

$2S_4O_6^{2-} + 6H_2O + 7O_2 \leftrightarrow 8SO_4^{2-} + 12H^+$
$2Fe^{2+} + 0.5O_2 + 2H^+ \leftrightarrow 2Fe^{3+} + H_2O$
2 chalcopyrite + $8.5O_2 + 2H^+ \leftrightarrow 2Cu^{2+} + 2Fe^{3+} + 4SO_4^{2-} + H_2O$
2 pyrite + $7.5O_2 + H_2O \leftrightarrow 2Fe^{3+} + 4SO_4^{2-} + 2H^+$
pyrite + $3.5O_2 + H_2O \leftrightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$
pyrrhotite + $2O_2 \leftrightarrow Fe^{2+} + SO_4^{2-}$
sphalerite + $2O_2 \leftrightarrow Zn^{2+} + SO_4^{2-}$
$H_2 + 0.5O_2 \leftrightarrow H_2O$
$N_2 + 3H_2 \leftrightarrow 2NH_3$

$SO_4^{2-} + 4H_2 + 2H^+ \leftrightarrow H_2S + 4H_2O$
$SO_3^{2-} + 3H_2 + 2H^+ \leftrightarrow H_2S + 3H_2O$
$S^0 + 1.5O_2 + H_2O \leftrightarrow SO_4^{2-} + 2H^+$
$S^0 + H_2 \leftrightarrow H_2S$
$S_2O_3^{2-} + 2H^+ + 4H_2 \leftrightarrow 2H_2S + 3H_2O$
$CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O$
$4CO + 2H_2O \leftrightarrow CH_4 + 3CO_2$
$CO + 3H_2 \leftrightarrow CH_4 + H_2O$
$S_4O_6^{2-} + 10H_2O + 14Fe^{3+} \leftrightarrow 4SO_4^{2-} + 20H^+ + 14Fe^{2+}$
$S^0 + 6Fe^{3+} + 4H_2O \leftrightarrow HSO_4^- + 7H^+ + 6Fe^{2+}$

eukaryotes

No chemoautotrophs.
No representatives capable of sustained chemotrophy.
Heterotrophy through glucose oxidation via the Embden–Meyerhof pathway to pyruvate (2 ATP per glucose), followed by either simple fermentation (with no additional ATP yield) or further oxidation of pyruvate (with additional ATP yield) in mitochondria, in hydrogenosomes, or in the cytosol.

Figure 4. A comparison of a narrow segment of the redox reactions involving inorganic donors and acceptors that a narrow segment of prokaryotes use for sustained ATP synthesis (excerpted from Amend & Shock (2001)) versus the entire breadth of reactions that non-photosynthetic eukaryotes *in toto* use for sustained ATP synthesis. Note that there are rare cases where mitochondria can operate chemotrophically, but only transiently, not for sustained ATP production (Doeller *et al.* 2001).

Koch & Schmidt 1991), naturally chemiosmotic housing would they have been able to make first attempts at life in the wild. With the invention of pathways for biosynthesis of redox cofactors, quinones, hemes and other electron-transporting centres, these early membranes might have begun to look reasonably similar to modern prokaryotic membranes, at least in terms of basic architecture.

Notably, our model for the independent origins of membrane-bounded compartmentation in archaebacteria and eubacteria predicts that the electron- and proton-

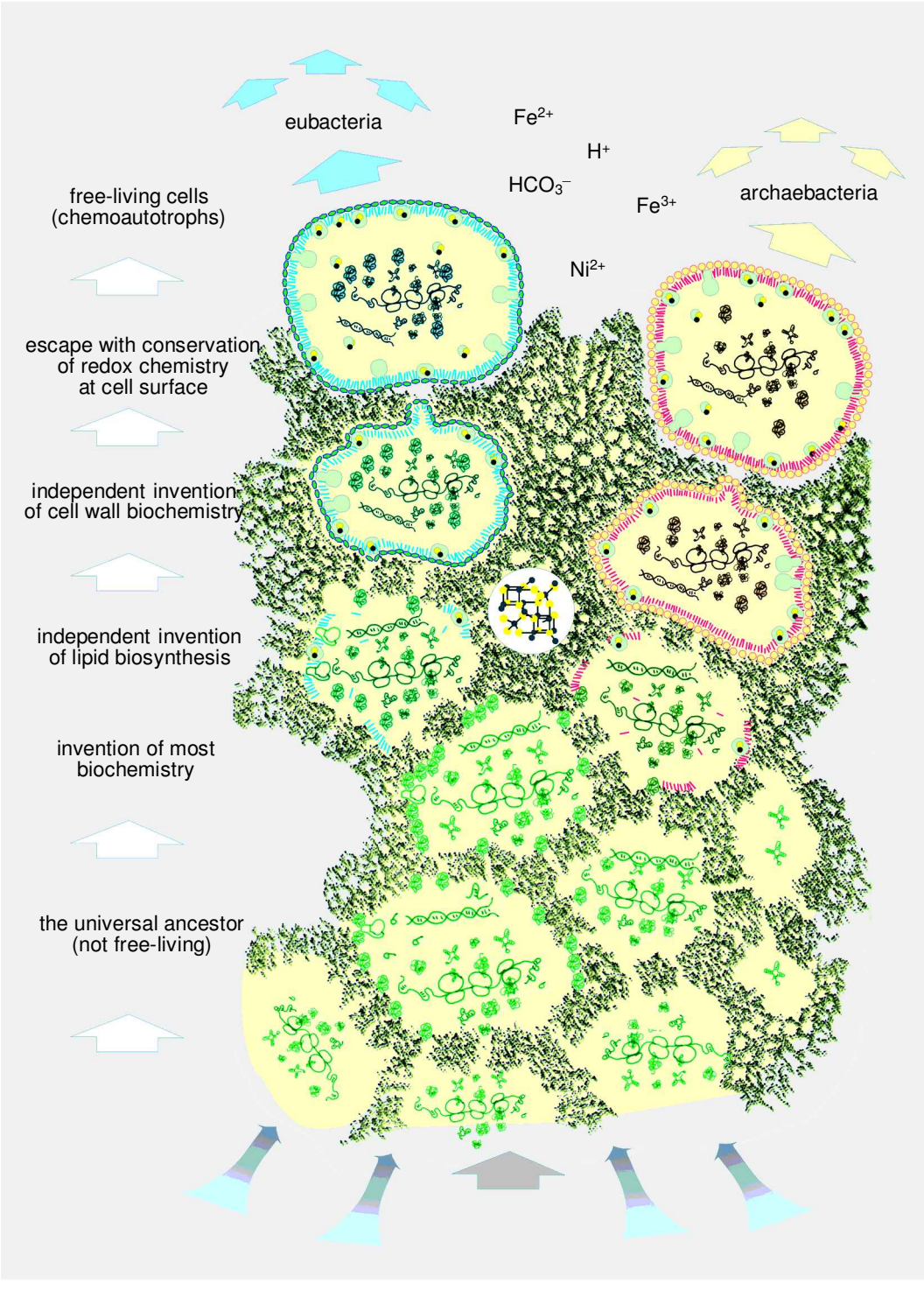

Figure 5. A model for the origin of membrane-bounded prokaryotic cells from iron monosulphide compartments within which the chemoautotrophic origin of life could have occurred. The drawing implies (but does not show) very large populations of replicating systems and unspecified physical distances (within, however, a single submarine seepage site) between diversifying replicating systems *en route* to free-living eubacterial and archaebacterial cells. Various kinds of genetic exchange that have been repeatedly postulated (Woese 2002) before the origin of a truly membrane-bounded cellular organization of prokaryotes could be easily accommodated by the model. Note that iron monosulphide precipitates are initially colloidal (Russell & Hall 1997) and inflated to the general types of structure shown in figure 2 by exhaling hydrothermal fluid.

transporting quinones of their independently evolved membranes should also differ substantially. That prediction fares quite well. The quinones of both groups of prokaryotes possess isoprene side chains (Schütz *et al.* 2000; Berry 2002). However these isoprenes—like the lipids themselves—are synthesized by different, completely unrelated biochemical pathways in the two groups. Eubacteria synthesize IPP via the DXP pathway, which starts from D-glyceraldehyde-3-phosphate and pyruvate via a decarboxylating transketolase reaction (DXP synthase), whereas archaebacteria synthesize IPP via the MVA pathway, which starts with the condensation of three molecules

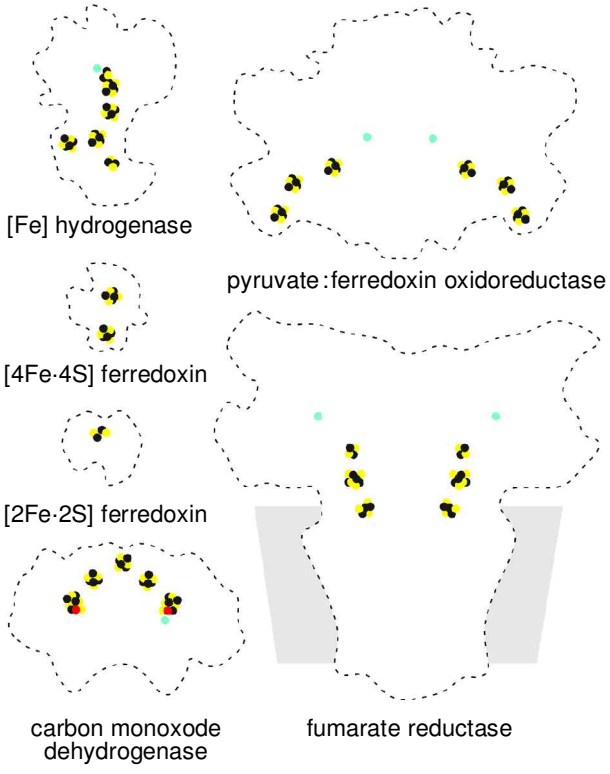

Figure 6. The position of FeS centres in some proteins that catalyse redox reactions. Figures were sketched from: [Fe]-hydrogenase (Peters 1999); PFOR (Chabriere *et al.* 1999); [4Fe·4S] ferredoxin (Macedo-Rieiro *et al.* 2001); [2Fe·2S] ferredoxin (Kostic *et al.* 2002); CODH (Lindahl 2002); and fumarate reductase, a membrane protein (Lancaster *et al.* 1999). Blue dots indicate active sites.

of acetyl-CoA (Lange *et al.* 2000). Not only do the isoprene side chain pathways differ in the two groups, the proton- and electron-transferring aromatic heads of the quinones also differ in archaebacteria and eubacteria: although some archaebacteria possess menaquinone and ubiquinone like eubacteria, others use sulphur-containing heterocyclics such as the benzothiophenes of *Sulfolobus* and *Acidianus* (Schütz *et al.* 2000), whereby the methanogens use methanophenazine, which is not a quinone at all (Berry (2002), q.v. for quinone structural comparisons). Interestingly, a key enzyme of the MVA pathway, 3-hydroxy-3-methylglutaryl-CoA synthase is distantly related to β-ketoacyl-acyl carrier protein synthase III of eubacteria (Lange *et al.* 2000), which catalyses a similar condensation reaction to produce the fatty acid precursor acetoacetyl-ACP from acetyl-ACP and malonyl-CoA as substrates, indicating the presence of an ancestral acetyl-CoA condensing protein in the non-free-living universal ancestor. Furthermore, DXP synthase of the DXP isoprenoid route is distantly related to PFOR, a ferredoxin-dependent enzyme catalysing the $C_2 \rightarrow C_3$ step subtending the acetyl-CoA pathway in many chemoautotrophs, indicating the presence of this primitive C_2-transferring step in the non-free-living universal ancestor. Because both DXP synthase and PFOR require TPP in their C_2-transferring catalysis, the prediction clearly follows that functional and/or structural analogues of the TPP coenzyme should be obtainable from mineral catalysts under Huber & Wächtershäuser (1997) type conditions in the presence of suitable nitrogenous compounds.

Under this view, the evolutionary basis of the attributes shared by prokaryotes would be founded in their inheritance from the last common ancestor. The evolutionary basis for the fundamental differences that distinguish eubacteria and archaebacteria, but which more or less uniformly unite the respective groups, could be sought in the age of biochemical discovery during the persistence of proto-eubacterial and protoarchaebacterial lineages living within the confines of their energy-laden mineral incubator. Such differences would include their use of many fundamentally different cofactors such as pterin derivatives versus tetrahydrofolate, coenzyme B, methanofuran, coenzyme F420, coenzyme M and cobamids in many archaebacteria (see DiMarco *et al.* 1990; Thauer 1998; White 2001), their use of different pathways for the same metabolic intermediates such as IPP (Lange *et al.* 2000) or their differences in purine biosynthesis (White 1997), their use (archaebacteria) or disuse (eubacteria) of small nucleolar RNAs for the modification of ribosomes (Omer *et al.* 2000), the differences between their DNA maintenance and repair machineries (Tye 2000), the differences between their transcriptional regulatory apparatus (Thomm 1996; Bell *et al.* 2001) and RNA polymerases (Langer *et al.* 1995; Bell & Jackson 1998), their quinones (Schütz *et al.* 2000; Berry 2002), or, and what is probably the most important point for this paper, the differences between their membrane lipid biosynthetic pathways and cell-wall constituents (Kandler 1982). Many other such differences could be listed. The assembly of FeS-clusters into proteins, an ancient biochemical function (Tachezy *et al.* 2001), would have been among those attributes essential to both lineages, a function possibly provided by the recently discovered SUF pathway of FeS cluster assembly (Takahashi & Tokumoto 2002). Efficient means of N_2-reduction, perhaps through primitive molybdenum–iron, vanadium–iron or iron-only nitrogenases (Chien *et al.* 2000) would have also been prerequisite to the transition to a free-living habit.

Once the crucial step of membrane synthesis and a turgor-resistant cell wall had been taken, independently, the two kinds of prokaryotic stem lineage could have begun making their attempts at the transition to the free-living state (independently) and begun trying their luck at life as—chemoautotrophic—free-living cells (figure 5). If one cell can escape, so can the next, and the next, continuously until the well at the seepage site ran dry, at which point the origin of life would have been over. Those free-living cells that possessed or invented the biochemical tools needed to survive outside their initial confines would have found themselves in a very standard kind of Darwinian struggle, but one with a particular reward for ingenuity at tapping new kinds of inorganic redox resources in an uninhabited, competitorless, ocean-floor world. This would have been a time where much of the oxygen-independent metabolic diversity among the prokaryotes might have arisen (figure 5). The age of biochemical discovery would have found a veritable gold mine at the oceans's surface, and the origin of chlorophyll-based photosynthesis in the eubacteria emerged apparently quite early (Dismukes *et al.* 2001; Russell & Hall 2001; Xiong & Bauer 2002). With that, the prokaryotic world would have

been off and running, changing the chemistry of the oceans, and eventually supplying enough reduced carbon sediment to allow the origin of heterotrophic prokaryotic lifestyles. At some early time, oxygenic photosynthesis would have been providing low local concentrations of a new and powerful electron acceptor (O_2), with which early microbes had to cope, probably using exactly the same strategies as modern organisms: either through the use of oxygen-detoxifying enzymes (Chapman *et al.* 1999; Jenny *et al.* 1999), through the avoidance of oxygen-containing niches, or through the gradual adaptation to oxygen, harnessing its utilitous oxidative power as many oxygen-dependent microbes (figure 4) have done.

9. EUKARYOTES: CELLS WITH SOMETHING INSIDE

The foregoing model for the origin of cells (prokaryotic ones) embraces the view of a chemoautotrophic origin of life and metabolism in general and views organic chemistry catalysed by FeS (and NiS) as ancient and ancestral in biochemistry. It accounts for the attributes shared by eubacteria and archaebacteria as inheritances from their non-free-living common ancestor (figure 5), and accounts for the differences of eubacteria and archaebacteria through divergence from that common ancestor. In this section, we address the evolutionary basis for the attributes: (i) that are shared by eubacteria and eukaryotes; (ii) that are shared by archaebacteria and eukaryotes; and (iii) that distinguish eukaryotes from prokaryotes.

Thus, this section boils down to the question of the origin of eukaryotes, one of biology's messiest problems. To introduce the issue as briefly as possible, one can try to distill the essence of what Otto Kandler and Carl Woese, who discovered the distinctness of archaebacteria from other then-known cells, and who proposed the three-domain classification of life (Woese *et al.* 1990), have recently written on the topic. Considering the characters shared by various lineages, such as those considered by Zillig (1991), Kandler (1998, p. 20) wrote:

> …each of the three statistically possible pairs among the three domains appears to be a sister group depending on the basic phenetic characters considered. The quasi-random phenetic mosaicism among the three domains that this indicated was confirmed at the genomic level when the orthologous genes of the archaeon *Methanococcus jannaschii*, of the eukaryote *Saccharomyces cerevisiae*, and of several bacterial species were aligned (Clayton *et al.* 1997). While the majority of the genes of *Methanococcus* are shared with either bacteria and/or eukaryotes, only a minority of genes seem to be confined to the domain Archaea (Bult *et al.* 1996). Such a quasi-random distribution of genes among the three domains cannot be explained satisfactorily by any order of dichotomous branching of a common ancestral cell or by chimaerism originating from fusion or engulfment among prokaryotes at early evolutionary stages as was suggested by various authors (Zillig *et al.* 1992; Sogin 1994; Golding & Gupta 1995; Doolittle 1996; Margulis 1996). The distribution pattern rather calls for a revival of the previous proposal of a tripartition at an early pre-cellular stage of life (Fox *et al.* 1980), resulting in three dichotomously branched separate lineages forming a phylogenetic 'bush' rather than a tree.

Firmly embracing the notion of a chemoautotrophic origin of life, the nagging matter of lacking chemoautotrophy in eukaryotes prompted Kandler to note (p. 25):

> The domain Eukarya contains neither any genuine chemolithoautotrophic nor thermophilic members growing optimally above 50 °C. […] Hence, founder groups of the domain Eukarya most likely consisted of highly derived mesophilic heterotrophic pre-cells, adapted to an almost 'modern', at least late Archaean or early Proterozoic, environment of a predominantly mesophilic temperature regime favourable for the evolution of intracellular membrane systems typical of eukaryotes.

Woese (2002, p. 8742), while considering the immense problems of cellular evolution, surmized:

> Initial attempts to frame the issue have typically been in the classical Darwinian mode, and the focus to date has almost been exclusively on modeling the evolution of the eukaryotic cell. The reason, of course, is clear—the appeal of the endosymbiosis concept. Because endosymbiosis has given rise to the chloroplast and mitochondrion, what else could it have done in the more remote past? Biologists have long toyed with an endosymbiotic (or cellular fusion) origin for the eukaryotic nucleus, and even for the entire eukaryotic cell. These classical explanations have three characteristics: they (i) invoke cells that are basically fully evolved; (ii) evolve the essential components of the eukaryotic cell well after its archaeal and bacterial counterparts (as has always been connoted by the term 'prokaryote'); and (iii) focus attention on eukaryotic cellular evolution, which implies that the evolutions of the 'prokaryotic' cell types, are of a different character—simpler, and, it would seem, less interesting. [emphasis from the original]

Both authors dismiss endosymbiosis as possible explanatory principles to account either for the characters shared by eubacteria and eukaryotes, or to account for those characters shared by archaebacteria and eukaryotes. They also dismiss fusion models, as do we, because fusion models are exactly that: they entail the fusion of two cells, each surrounded by its own membrane into a single cytoplasm surrounded by one membrane, like putting two smaller soap bubbles together to get one bigger one. Eukaryotes do occasionally fuse cytoplasms (for example at fertilization), but prokaryotes do not, and there is no reason to suspect that they ever did in the past either. But both authors do something that is very common in the literature: they lump endosymbiotic models and fusion models for the origin of eukaryotes into one basket. An example of an explicit fusion model can be found in the right panel of fig. 3 in Lopez-Garcia & Moreira (1999), where a free-living consortium of prokaryotes just starts fusing away, uniting up their cytoplasms.

Nobody doubts any more that the principle of endosymbiosis as elaborated by Mereschkowsky (1905), condemned by Wilson (1928) and revived by Margulis (Sagan 1967) is real and that chloroplasts and mitochondria are descended from free-living prokaryotes. But the endosymbionts did not fuse with their host, rather they remained intact as membrane-bounded cell compartments within their host, a general principle in cell evolution termed membrane heredity (Cavalier-Smith 2000). Yet rejections of the notion that endosymbiosis might have given rise not

only to the mitochondrion but also to the bipartite cell which became a eukaryote, are sometimes reminiscent of Wilson's (1928) rejection of the notion that mitochondria were endosymbionts to begin with:

> More recently Wallin ('22) has maintained that chondriosomes [mitochondria] may be regarded as symbiotic bacteria whose association with the other cytoplasmic components may have arisen in the earliest stages of evolution (p. 712). To many, no doubt, such speculations may appear too fantastic for present mention in polite biological society; nevertheless it is within the range of possibility that they may someday call for more serious consideration. (p. 739).

The question at the root of the problem on the origin of eukaryotes—and the reason why biologists have to this day been unable to connect up archaebacteria, eubacteria and eukaryotes into a unified scheme of cell evolution that is generally accepted by all—concerns the host: the cell that acquired the mitochondrion. Less fundamentally problematical, but none the less a decade-long problem for endosymbiotic theory are hydrogenosomes: pyruvate-oxidizing, hydrogen- and ATP-producing organelles of anaerobic eukaryotes that were discovered by Müller (Lindmark & Müller 1973). Long suspected to be a third kind of endosymbiotic organelle distinct from mitochondria and chloroplast and perhaps descended from clostridial ancestors (Whatley *et al.* 1979; Müller 1988), recent advances have shown that they are simply an anaerobic manifestation of the same endosymbiont that gave rise to mitochondria (Embley *et al.* 1997; Van der Giezen *et al.* 2002).

On the bottom line, there are only two possibilities for the nature of the host that acquired the organelle ancestral to mitochondria and hydrogenosomes: it was either a prokaryote or a eukaryote. The long-held view that the host was a eukaryote drew support primarily from the existence of eukaryotes without mitochondria (Cavalier-Smith 1987) and from the finding that they branched deeply in the eukaryotic branch of the 'universal tree' of ribosomal RNA (Vossbrink *et al.* 1987; Sogin *et al.* 1989). But all of the mitochondrion-lacking and suspectedly primitive eukaryotes that built the basis for traditional views on the origins of mitochondria, for example microspridians, diplomonads, entamoebids and the like, have turned out either to have possessed mitochondria in their past or to still possess the organelle in reduced form (Embley & Hirt 1998; Roger *et al.* 1998; Roger 1999; Müller 2000; Philippe *et al.* 2000). They are thus not direct descendants of an amitochondriate host. Furthermore, the seemingly deep branching of such organisms in the 'universal tree' is in many if not all cases apparently just an artefact of tree reconstruction (Embley & Hirt 1998; Philippe & Laurent 1998). A prime example of this problem are microsporidians, organisms that started many people believing that mitochondrion-lacking eukaryotes are both ancient and primitive on the basis of their rRNA phylogeny (Vossbrick *et al.* 1987). But the microsporidia are in fact highly derived fungi (Keeling 2001), as revealed by genome sequencing (Katinka *et al.* 2001), and the seemingly deep position of their rRNA sequence is a phylogenetic artefact.

However, the 'great similarity of the archaeal and eukaryal information processing systems' (Woese 2002, p. 8744) is definitely not an artefact, it is an incontrovertible observation founded in the comparative analysis of transcription and translation machineries. Yet, in which light this observation should be interpreted is a different matter: were the host a eukaryote or were it an archaebacterium, we would arrive at the same observation. Models for the origin of eukaryotes and the nature of the host have recently been reviewed elsewhere (Martin *et al.* 2001).

The starting point for the remainder of this section was the study of the phylogenies of various enzymes of carbohydrate metabolism in higher plants, starting with GAPDH (Martin & Cerff 1986; Martin *et al.* 1993) in the context of their homologues in eubacteria and archaebacteria and extended to the complete glycolytic pathway, the Calvin cycle, gluconeogenesis, the oxidative pentose phosphate pathway, the glyoxylate cycle and the citric acid cycle as reviewed elsewhere (Martin & Schnarrenberger 1997; Martin & Herrmann 1998; Henze *et al.* 2001; Schnarrenberger & Martin 2002). At the same time, similar studies were being conducted on the glycolytic enzymes from amitochondriate protists, as reviewed by Müller (1998). For every glycolytic enzyme (except enolase) (Hannaert *et al.* 2000) the same observation was scored: the eukaryotic enzymes are more similar to their eubacterial homologues than they are to their archaebacterial homologues, as shown once more in figure 1 for the glycolytic pathway, and in many cases the archaebacteria do not even possess the homologous enzyme.

To account for the findings: (i) that eukaryotes possess an archaebacterial genetic apparatus; (ii) that eukaryotes possess eubacterial ATP-producing pathways in mitochondria, in hydrogenosomes and in the cytosol; (iii) that mitochondria and hydrogenosomes descend from a common ancestral organelle; and (iv) that amitochondriate eukaryotes possessed a mitochondrion in their evolutionary past, a hypothesis was formulated, which explored the possibility that the host might have been an archaebacterium, an anaerobic and autotrophic one with a carbon and energy metabolism that was dependent upon molecular hydrogen as an electron donor (Martin & Müller 1998). The microbial physiology of the model was based on phenomena abundantly observable among modern prokaryotes: hydrogen transfer and anaerobic syntrophy (Fenchel & Finlay 1995). The selective pressure associating the host to its eubacterial symbiont was posited to have been the host's strict dependence upon H_2 produced as a waste product of the fermentative metabolism of a facultatively anaerobic symbiont. This model for the origin of mitochondria and for the origin of the heterotrophic lifestyle in eukaryotes was fortuitously also a model for the origin of eukaryotes. It is shown in figure 7, which is largely self-explanatory.

Only few minor modifications with respect to the original figure, but not to the original model, have been introduced here. First, the original figures (Martin & Müller 1998) did not specifically indicate DNA or ribosomes in the cells (corrected here) with the eubacterial components shaded blue and the archaebacterial components shaded red. There is a limit to the amount of detail that can be included in such a figure, hence the Gram-negative wall and lipopolysaccharide layer of the symbiont are symbolized.

Another change is that a model for the origin of the endomembrane system in eukaryotes, including the

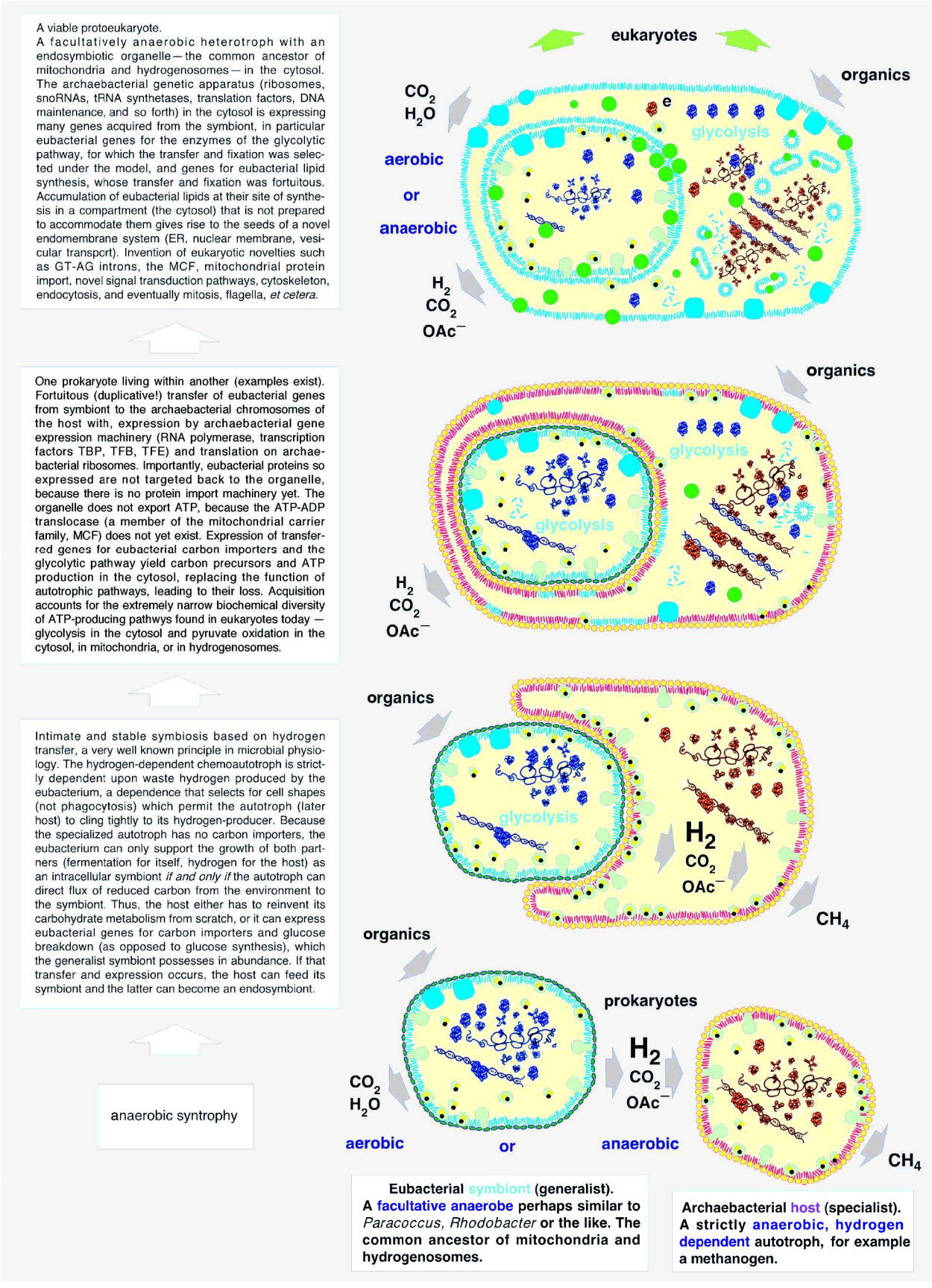

Figure 7. A summary of a model suggested for the origin of eukaryotes (Martin & Müller 1998), including a possible route for the principle underlying the origin of the nucleus under that model as discussed (Martin 1999*a*), where it was suggested that the origin of the endoplasmic reticulum endomembrane system preceded the origin of the nuclear compartment. See text.

nuclear membrane, which is a single folded membrane that is contiguous with the endoplasmic reticulum, is sketched here in the top panel of the figure. That model for the origin of the nuclear membrane (the vesicular model) was elaborated and contrasted with other models elsewhere (Martin 1999*a*); its basic tenets are recapitulated in figure 7.

Another change is that the eubacterial and archaebacterial lipids were indicated somewhat more clearly although their relevance was stated in the original. The lipids are important, because according to textbook depictions of cell evolution (the 'universal tree'), eukaryotes should have archaebacterial lipids, just like they have an archaebacterial genetic apparatus: but they do not. Eukaryotes have eubacterial lipids. Few evolutionary biologists address this issue. One suggestion to account for this is that the common ancestor of all cells was a eubacterium, and that the single common ancestor of archaebacteria reinvented its entire lipid and cell wall chemistry subsequent to the divergence from the eukaryotic lineage on an rRNA-like tree (Van Valen & Maiorana 1980; Cavalier-Smith 2002). Our explanation for the origin of eubacterial lipids in eukaryotes is that the genes for their biosynthesis were transferred to the host's chromosomes and that pathway became functional there (acetyl-CoA, NAD(P)H and dihydroxyacetone phosphate as the precursors is in ample supply in all cells) and is somewhat more explicit but not principally different from either Zillig's (1991) or Koga *et al.*'s (1998) explanation. Another change is that the presence of eubacterial and archaebacterial genes in the cytosolic chromosomes is more clearly indicated than in the original, where it was simply stated in words. Note that the term 'gene transfer' is not exactly correct, because gene translocation from an organelle genome to the hosts's chromosomes is always a duplicative process; in the strict sense the transferred genes are copied, not transferred, because an active copy has to remain in the organelle until its function can be substituted by the copy residing in the host's chromosomes (Allen 1993). During the phase of evolution before the origin of a protein routing apparatus to direct cytosolic proteins into the mitochondrion (Henze & Martin 2001), the process of gene copying from the organelle to the host, which occasional organelle death or lysis renders inevitable (Doolittle 1999), would have meant a continuous downpour of eubacterial genes into the host's chromosomes, constantly providing new raw genetic starting material for the evolution of eukaryotic-specific traits, while providing the opportunity to retain originally encoded functions as well. (The converse transfer process from host to organelle is extremely unfavourable: events of occasional host death or lysis do not lead to transfers, they lead to no progeny.) This view implicates gene transfers from the organelle and the integration of those genomes within a single cellular confines (Herrmann 1997) as major genetic mechanisms underlying the generation of eukaryotic novelties.

Another change is that histones, which are present in methanogens and relatives (Reeve *et al.* 1997; Fahrner *et al.* 2001) as well as in eukaryotes, are sketched onto the host's chromosomes. Another change is that inventions specific to the eukaryotic lineage are symbolized as green dots. These include for example spliceosomal introns that are probably descended from eubacterial group II introns, that were argued to have entered the eukaryotic lineage via the mitochondrial symbiont (Cavalier-Smith 1991; Roger & Doolittle 1993). Other inventions include the cytoskeletal components tubulin and actin, which are probably descended from their prokaryotic homologues FtsZ and MreB (Erickson 2001), the protein import apparatus of the two mitochondrial membranes (Truscott *et al.* 2001; Pfanner & Truscott 2002), the mitochondrial carrier family of proteins (Van der Giezen *et al.* 2002; Voncken *et al.* 2002), and what-all. The cell inferred under this model and sketched at the top of figure 7 has an archaebacterial genetic apparatus, eubacterial energy metabolism, eubacterial lipids, the seeds of an endomembrane system from which the nucleus is inferrable and ample genetic starting material (two highly divergent and partially merged prokaryotic genomes) to evolve cytological and genetic traits that are specific to the eukaryotic lineage.

Thus, to deliver the goods promised at the outset of this section, under the premise stated here: (i) the evolutionary basis for the attributes that are shared by eubacteria and eukaryotes lies in gene acquisition from the eubacterial common ancestor of mitochondria and hydrogenosomes; (ii) the evolutionary basis for the attributes that are shared by archaebacteria and eukaryotes lies in the archaebacterial nature of the host that acquired the mitochondrion; and (iii) the evolutionary basis for the attributes that distinguish eukaryotes from prokaryotes are founded in novelties that arose specifically in the eukaryotic lineage subsequent to its origin, which given the fossil evidence, probably occurred between 1.5 and 2 Gyr ago.

10. GETTING ACCUSTOMED TO THE POSSIBILITY OF A PROKARYOTIC AND AUTOTROPHIC HOST

The model presented here for mitochondrial (and eukaryote) origins differs in several fundamental aspects from traditional models (Doolittle 1998), which usually entail a phagocytosing eukaryote as the host, rather than an H_2-dependent, autotrophic archaebacterium, and as such it has received welcome criticism (Cavalier-Smith 2002). Regarding a descent of the host lineage from H_2-dependent, autotrophic archaebacteria, it is indeed curious that some proteins, such as group II chaperonins (Leroux *et al.* 1999) and histones (Sandmann & Reeve 1998) do link up eukaryotes with methanogens and their relatives, but obviously genome-wide analyses are needed to derive further support for this view. Another question to some was whether anaerobic syntrophy would provide sufficient selective pressure to select for the unsual kinds of prokaryotic morphologies required by the model to associate an archaebacterium and a eubacterium, but truly striking anaerobic syntrophic associations involving methanogens and hydrogen dependence (albeit by sulphate reducers) are becoming known in the kinds of environments predicted (Boetius *et al.* 2000; Michaelis *et al.* 2002). Another criticism was that at the time the model was presented, there was no precedence for any prokaryote living inside another prokaryote, raising questions as to whether it is possible that an endosymbiosis between two prokaryotes would be possible (Doolittle 1998). In the meantime, a clear example has become known where one prokaryote lives stably as an endosymbiont within

another (von Dohlen *et al.* 2001). Although the mechanism of entry is still unknown, that example demonstrates that it is possible to establish an endosymbiosis with a non-phagocytotic, prokaryotic host, as predicted. Another prediction was that mitochondria should be found that possess and use both pyruvate dehydrogenase and PFOR, evidence that is forthcoming (M. Hoffmeister and C. Rotte, unpublished data). Another prediction was that evidence for a trace of glycolysis should be found in mitochondria, which has been found (Liaud *et al.* 2000). Another prediction was that autotrophic archaebacteria should be able to acquire and express eubacterial genes, and that such aquisitions may expand the biochemical repertoire of the recipient, as the genome sequence of *Methanosarcina mazei*, a methanogen with *ca.* 30% eubacterial genes in its genome, has been confirmed (Deppenmeier *et al.* 2002). Furthermore, the brunt of genes that *M. mazei* has acquired from eubacteria are largely associated with its nutritional lifestyle, in line with the predictions of the model (but also include proteins such as GroEL and DnaK). The clearest, most easily falsifiable (and most often overlooked) prediction of the hydrogen hypothesis is that it predicts all eukaryotes to possess a mitochondrion (or hydrogenosome) or to have possessed one in their past. Other current models for the origin of eukaryotes do not generate that prediction.

From the start, it was clear that eukaryotes had archaebacterial ribosomes, linking these two lineages. But it was also well-known early on (Zillig 1991) that equally numerous, possibly even more (Rivera *et al.* 1998), traits link eukaryotes and eubacteria. In some anaerobic protists, traces of the archaebacterial host lineage have been retained in energy metabolism as well (see Hannaert *et al.* 2000; Sanchez *et al.* 2000; Suguri *et al.* 2001), clearly indicating mosaicism in eukaryotic biochemistry. Zillig (1991) argued that eukaryotes acquired their eubacterial traits, including lipid biosynthesis and some of their glycolytic genes, from a eubacterial symbiont; but because he thought that *Giardia* was primitively amitochondriate, he invoked a eubacterial donor that was distinct from the mitochondrion. Today it is quite clear that *Giardia* in fact did possess a mitochondrion (Hashimoto *et al.* 1998; Roger *et al.* 1998; Tachezy *et al.* 2001). Had Zillig been aware of that, it is doubtful that he would have invoked the additional endosymbiont. The hydrogen hypothesis (figure 7) merely simplifies Zillig's scheme of cell evolution by one endosymbiont, accounts for the origin of hydrogenosomes, and specifies the possible ecological context of symbiosis by making predictions about the nature of the cells involved. But it uses the same explanatory principle of gene acquisition from an endosymbiont, the common ancestor of mitochondria and hydrogenosomes, to account for the numerous biochemically eubacterial traits in eukaryotes that are too often disregarded.

11. HORIZONTAL GENE TRANSFER: COMPLICATING BUT NOT OBSCURING EARLY EVOLUTION

The common ancestor of mitochondria and hydrogenosomes was a member of the α-proteobacteria (Yang *et al.* 1985), and therefore it may have been photosynthetic or non-photosynthetic, autotrophic or heterotrophic, anaerobic or aerobic, or most probably all of the above, as is the case for many contemporary representatives of the group, such as *Rhodobacter*, but it at least should have had the physiological potential of a bacterium like *Paracoccus* (John & Whatley 1975). The α-proteobacteria are a rather heterogeneous group (Woese 1987): some have acquired hefty chunks of DNA from methanogens (see Chistoserdova *et al.* 1998) and some have evolved very elaborate mechanisms of lateral transfer, for example the gene transfer agents of *Rhodobacter*, which slice the genome up into nicely transmissible 4 kilobase pieces under suitable conditions (Lang & Beatty 2001). These days, it has become popular to invoke an independent horizontal gene transfer from prokaryotes to eukaryotes from a new and different donor every time one sees a eukaryotic gene that is not archaebacterial-like, but does not branch with homologues from α-proteobacteria (Baughn & Malamy 2002), also by staunch opponents of lateral gene transfer (Kurland 2000), and particularly when it suits the argument (Canback *et al.* 2002). The main problem with such *ad hoc* use of that explanatory principle is that the eukaryotic genes involved usually share a single common eubacterial origin (Rotte *et al.* 2001; Horner *et al.* 2002), meaning that eukaryotes acquired most of their eubacterial genes only once, and in their common ancestor, in line with the model set out here. Horiike *et al.* (2001) analysed the yeast genome and fulfilled the predictions set out by Martin & Müller (1998) for the origins of functional classes of eukaryotic genes, yet Horiike *et al.* (2001) did not notice that they had confirmed those predictions (Rotte & Martin 2001).

There is another interesting variant of lateral gene transfer as an explanatory principle as it applies to eukaryotic genes: namely to take all of the eubacterial genes in eukaryotes that do not branch with α-proteobacterial homologues, lump them together and attribute their origin to one single hypothetical donor, usually an imaginary organism (Hartman & Federov 2002; Hedges *et al.* 2001; Horiike *et al.* 2001). Given lateral transfer and the problems of deep phylogenies, it makes more sense to attribute such genes to the mitochondrial endosymbiont. Mitochondria arose only once (Gray *et al.* 1999; Lang *et al.* 1999) and lateral transfer is real among free-living prokaryotes (see Doolittle 1999; Jain *et al.* 1999; Eisen 2000; Ochman *et al.* 2000). Because lateral transfer occurs today, we should assume it to have also existed in the past. If we incorporate that reasonable but mildly complicating assumption to our views on early evolution, it suddenly seems silly to assume: (i) that any contemporary α-proteobacterium should have exactly the same genes as it had 2 Gyr ago; and (ii) that all genes possessed by the single α-proteobacterium that gave rise to mitochondria should be found today only among α-proteobacterial genomes (Martin 1999*b*). The same relatively insevere problem applies to cyanobacteria (Rujan & Martin 2001) when it comes to genes that plants acquired from plastids. Horizontal gene transfer was 'a pain in the neck' for molecular evolutionists 20 years ago (Dickerson 1980) and will still be in 20 more; it has not dashed all hopes that genome sequences will help unravel evolutionary history, it has just left a bit of tea in the cup, and swirling leaves are more difficult to read. After all, the distinction between ancient gene transfers and outright phylogenetic artefacts in gene

trees that span 2 Gyr or more of evolution is anything but simple (Forterre & Philippe 1999 Poole *et al.* 1999), indicating that both general (pathways) and specific (enzymes) biochemical characters in addition to broad-scale phylogenetic comparisons, which have revealed widespread mosaicism in eukaryotes (Brown & Doolittle 1997; Doolittle 1997; Feng *et al.* 1997) should weigh in evidence of early cell evolution.

12. CONCLUSION

In this paper, we have undertaken the task of sketching one possible route to get from the chemical elements to eukaryotes in *ca*. 2 Gyr, focusing on aspects of the origin of life and the origin of cells that we consider to be particularly important: sustained sources of redox potential (energy) and physical compartments for every step along the way. The catalytic capabilities of RNA (Cech 1986) and the importance of the ribosome, possibly as a replicator first (Poole *et al.* 1999), and certainly as a translating coupler between genotype and phenotype (Woese 2002), brought biology out of the chicken-and-egg problem with respect to DNA and protein. But where there is anything like replication going on, something is doubling in mass, so it should be kept in mind that a steady flow of precursors must be coming from somewhere, that their synthesis requires sustained energy input, sustained reducing power in the form of electrons, sustained concentrations of precursors sufficient to allow any pair of reactants to meet, and physical compartments to retain the products formed.

We have argued strongly in favour of a chemoautotrophic but mesophilic origin of life, and upon that premise we see no way in which eukaryotes, none of which are chemoautotrophic as Kandler (1998) has pointed out, could have possibly arisen at the same time as prokaryotes, let alone before prokaryotes, as some biologists currently contend. We have argued in favour of a single origin of life, which gave rise to a non-free-living universal ancestor that was confined to structured FeS precipitates at a submarine seepage site within which it arose. We have argued that the archaebacterial and eubacterial lineages of prokaryotes independently surmounted the transition to the free-living state based on the chemical dissimilarity of their lipids, as Koga *et al.* (1998) have suggested. However, the universal common ancestor we propose was not of a non-cellular organization, because we consider it impossible that any form of life or self-replicating system, regardless of how primitive, could glean energy from its environment and retain chemical constituents for its proliferation without a pre-existing, inorganic cellular scaffold. We have argued against 'fusion' or 'chimaeric' models for the origin of eukaryotes. Instead, we have argued for an endosymbiotic origin of mitochondria in an archaebacterial host. Under these premises, the living world's most ancient evolutionary divide is that between eubacteria and archaebacteria and its steepest evolutionary grade is that between prokaryotes and eukaryotes (Mayr 1998).

All life is organized as cells—compartments separated from their surroundings that spontaneously multiply with energy gleaned through self-contained, thermodynamically favourable redox reactions. From our viewpoint, physical compartmentation from the environment and self-organization of self-contained redox reactions are the most conserved attributes of living things, hence inorganic matter with such attributes would be life's most likely forebear.

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Discussion

D. Horner (*Dipartimento di Fisiologia e Biochimica Generali, University of Milan, Milan, Italy*). I want to understand why the core of glycolysis has to come from this symbiont and also why, apart from a parsimony argument, it is not possible for the endosymbiosis giving rise to the mitochondrion to have happened more than once.

W. Martin. Let me take the second comment first. It is justified and can be reworded as follows: 'Dr Martin, if you're saying this is so likely that this could give rise to eukaryotes, how come it doesn't happen all the time?'. That the eukaryotes arose only once; that all eukaryotes that have been studied to date have a mitochondrion; and that all mitochondria arose only once are observations. It is an observation, it is an explanandum i.e. something that needs to be explained. We have to generate a hypothesis that can explain that observation. We have heard from Tom [Cavalier-Smith] that secondary symbiosis is, compared with primary symbiosis, a very widespread phenomenon. Primary endosymbiosis happened twice among countless cells in 4 billion years. It is excruciatingly rare. I suspect that the rareness of a free-living bacterium becoming an organelle lies in the difficulties of sorting out the genetic integration of these systems into something that will survive, because we know of many eukaryotes that have bacterial symbionts, but we do not know of many eukaryotes that have acquired independent organelles. It comes down to chloroplasts and mitochondria. So, I think, the second comment simply boils down to an interesting observation.

The first question is, why did the core enzymes of glycolysis have to come from the mitochondrial symbiont? That is the application of Occam's razor to suggestions, which are common in the literature, that an inability to get a citric acid cycle enzyme to branch with the alphaproteobacterial homologue means that another endosymbiotic event has to be invoked. Every time they see a new, unusual, gene, whose phylogeny they cannot explain, they invoke a new endosymbiont. I have looked at a lot of genes, and if I have to invoke a new symbiont every time I see a strange branch, then we would get a conglomeration that just does not make sense. My suspicion is that what is wrong is that our models for protein evolution, in particular for glycolytic enzymes, are so wrong that they are producing artefacts regularly, and we just do not understand how these molecules are evolving. So what we are doing is, rather than entertaining a notion of a new endosymbiosis every time we see a new gene phylogeny—I have been there, done that—we say what is wrong are the gene phylogenies; and let us take a minimalist view where we have one archaebacterial host and one eubacterial symbiont, and see how much we can explain with that.

R. Blankenship (*Department of Chemistry and Biochemistry, Arizona State University, AZ, USA*). Do you conclude from the fact that the archaeal glycolytic enzymes are apparently unrelated to the eubacterial ones, that glycolysis was independently invented two separate times?

W. Martin. Yes. I embrace the view of a chemoautotrophic origin of life, that is, we start with carbon monoxide. What do I think was the first pathway? I think the first pathway was the acetyl-CoA pathway of CO_2 fixation. This is a pathway you find among clostridia and it is widespread among archaebacteria. Its key enzyme is carbon monoxide dehydrogenase. And what carbon monoxide dehydrogenase does, is it reduces CO_2 with molecular hydrogen to carbon monoxide, takes a methyl group from a methyl donor, adds that to an iron–sulphur centre, transfers the methyl group to the carbon monoxide, and makes an acetyl group, all in one reaction. Add a sulphide in the form of CoA, and you get two molecules of CO_2 in, and one molecule of acetyl CoA out. It is a linear reaction, so that is a good start, and that is exactly the reaction that the Huber–Wächtershäuser experiment modulates, so start with C_2. The next reaction, in metabolism in organisms that have the acetyl-CoA pathway, is pyruvate synthase. Pyruvate synthase is also an iron–sulphur enzyme that just adds CO_2 with electrons from ferredoxin, to make pyruvate. So we go up the glycolytic pathway. OK, so yes, I think that if we embrace the notion of a chemoautotrophic origin of life, we have to embrace the notion that this common ancestor we infer, living in its iron sulphide housing that it embedded a myriad of ways to make carbon backbones, not just to break them down from Oparin's organic soup. I would like to point out one very interesting finding. The most conserved enzyme of the glycolytic pathway is without doubt enolase. Enolase catalyses the first stereochemically specific reaction in carbon metabolism. It takes a water molecule and adds it to phospho*enol*-pyruvate, which is not chiral, and the product is 2-D-phosphoglycerate, and the stereochemistry at C_2 of that carbon compound never changes for the rest of metabolism. And, strangely, enolase, the most conserved glycolytic enzyme, and the only one that is universally distributed, is not encoded in glycolytic operons in archaebacteria, it sits in the same operon as the ribosomal proteins. It sits in the same operon as the ribosomal proteins. And if you ask me, that is the key to the holochirality problem from sugars. And the selection, at the origin of translation, for incorporation of only L-amino acids into proteins from a racemate, might be a possible solution,

which we suggest in the paper, for the holochirality problem as it arises in the amino acids. Short question, long answer. Yes, independent origins, these enzymes and the whole biochemistry are not related.

T. Cavalier-Smith (*Department of Zoology, University of Oxford, Oxford, UK*). I have a comment and a question. The comment relates to the fact that you rightly criticize Carl Woese for assuming that eukaryotes are as old as eubacteria, and I have similarly criticized him for assuming that archaebacteria are that old, also. You have made that same assumption and I think my criticisms of him apply to you as well….

W. Martin. I will address that. OK, go ahead.

T. Cavalier-Smith. And you are aware of the idea that I put forward in 1987, and since have written about more recently, which is that archaebacteria and eukaryotes are sisters, and not that eukaryotes evolved from an archaebacterial ancestor?

W. Martin. May I reply?

T. Cavalier-Smith. Yes.

W. Martin. What Tom is saying is that he has always placed very great importance on the same explanandum that we are addressing here, namely that eubacteria have one kind of lipid, archaebacteria have a different kind of lipid, and eukaryotes have eubacterial lipids. To explain that, he has had something called the neomuran theory, which, in this element, is not different from Lee van Valen's and Moriana's 1980 paper, seven years before, in *Nature*, where they said exactly the same thing. I am not criticizing the novelty, but I am just saying that this explanandum has been around for a long time. Zillig has also addressed it. Tom explains this by saying that eukaryotes and archaebacteria share a common ancestor and that that common ancestor was a eubacterium. Thus, he postulated a eubacterial common ancestor of eukaryotes and archaebacteria that has many archaebacterial attributes but eubacterial lipids. This ancestor is then argued to have brought forth the common ancestor of all eukaryotes and the common ancestor of all archaebacteria, whereby the latter are argued to have completely reinvented their lipid biochemical pathways prior to archaebacterial diversification. I do not believe that, but we cannot necessarily prove that it is wrong. What we can prove is wrong is an element of your most recent formulation of this model, in which you propose that this happened 800 million years ago. Now, some geochemists are here, and, tell me if I am wrong, the carbon isotope record for ultra-light carbon, i.e. $\delta^{13}C$ values of –50‰ or –60‰ goes back well beyond 2.7 billion years; it goes right down into the same carbon levels where Schidlowski found $\delta^{13}C$ values that are typical of present-day CO_2 fixation pathways using inorganic C with present-day δ^{13} C values. This ultra-light carbon is generally interpreted, as there is no other process that makes it, as the trace of methanogenesis. And that means that archaebacteria as a group must be at least three billion years old, as common sense would dictate. Another problem, Tom, with the view that archaebacteria are only 800 million years old, is the things they can do with a wide range of inorganic donors and acceptors. You can say 'yes', this is due to adaptation to extreme environments, but just looking at the biochemical characters, which most people who are involved in this early cell evolution business do not do, it is like taking the group with the least diversity of characters and making it the most ancient and saying that there has been rapid evolution of all of the characters that are clearly ancestral on biogeochemical grounds. The kinds of chemistry we are talking about here are consuming pyrite or sphalerite, or even sustained ATP production using carbon monoxide. These are ancient pathways. So that is how I would address those two criticisms.

T. Cavalier-Smith. Well, OK. My question was that, in a recent paper (Cavalier-Smith 2002), I mentioned a couple of gene splittings, which I think argue against the idea that eukaryotes came from within archaebacteria, and I wonder how you would refute that argument. As for the carbon isotope data, I would really like to hear from any geochemists about the validity of these values.

W. Martin. Dr Nisbet is raising his hand.

T. Cavalier-Smith. Good, but may I just make one point about it? The basic argument is not direct evidence for archaebacteria; it is based on an assumption about ecological interactions between archaebacteria and methylotrophs. It is a rather complex argument based on several assumptions, and I do not think that the geochemists have looked critically at alternative hypotheses. They have assumed that archaebacteria are old, therefore it would be a reasonable assumption. Strauss *et al.* (1992), when reviewing this very point, do say that other explanations are in principle possible.

W. Martin. OK, I would like to have Dr Nisbet reply to the evidence, and at the same time recall that it is not only methylotrophs that can produce this trace. The association between methanogens and sulphate-reducers actually causes methanogenesis to run backwards. So anaerobic methanotrophy works, but it works through reverse methanogenesis and it produces this trace without methanotrophy in the strict sense.

E. Nisbet (*Department of Geology, Royal Holloway, University of London, Egham, Surrey, UK*). I am a geologist and I do not know very much biology, but certainly at 2.7 billion years both the carbon and the sulphur isotopes look very comparable in many ways to what we get in the modern Earth, with a wide diversity of carbon pathways, including things that fractionate carbon very substantially, and also very substantial self-refractionation. If you go back past 3.5 billion years, the story is more complex and some of the outcrops are beautiful. At 3.7 billion years, at Issua in Greenland, there is clear evidence for light carbon, but the rocks are metamorphosed so you cannot say much more than that there was some sort of biological process. It looks like acetyl CoA metabolism, but that is a pure guess.

T. Cavalier-Smith. That is not specifically archaebacterial.

E. Nisbet. No, if you want to say it is specifically archaebacterial, you can say at least at 2.7 billion years. There is also, I should mention, Brock's finding of chemicals that look as if they are eukaryotic at 2.7 billion years. We have oil at 2.7 billion years.

T. Cavalier-Smith. But sterols can be made by three groups of bacteria.

E. Nisbet. Yes, I know they can be made by Archaea as well.

W. Martin. We agree on that.

T. Cavalier-Smith.What about the gene splitting as evidence for eukaryotes being sisters, not derived from archaebacteria?

W. Martin. Well, I do not know exactly which data you are referring to, but we have seen operons put together independently in many groups of organisms.

T. Cavalier-Smith. This is splitting up genes into separately coded proteins.

W. Martin. Well, Tom—independent? In enolase we have seen independent insertions at exactly the same site. I also consider independent splittings to be possible. I was hoping you would ask why do the eukaryotes in the ribosomal RNA tree branch below the archaebacteria, when our model actually says they should branch within the archaebacteria, and the answer is exactly the same as for the microsporidia. The microsporidia belong at the top of the eukaryotic tree, but because of odd evolutionary processes in their rRNA they branch artefactually at the bottom. In the model for the origin of eukaryotes that I presented, archaebacterial ribosomes are evolving in a chimaeric cytosol. Thus, the same thing is expected to happen as in the case of the microsporidian RNA molecule. The ancestral eukaryotic rRNA molecule goes through a phase of dramatic change, it acquires a very long branch relative to its archaebacterial homologues, and branches artefactually deep on the archaebacterial side of the rRNA tree.

Additional references

Cavalier-Smith, T. 2002 The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *Int. J. Syst. Evol. Microbiol.* **52**, 297–354.

Strauss, H., Des Marais, D. J., Hayes, J. M. & Summons, R. E. 1992 The carbon-isotopic record. In *The Proterozoic biosphere* (ed. J. W. Schopf & C. Klein), pp. 117–127. Cambridge University Press.

GLOSSARY

ACS: acetyl-CoA synthase
CODH: carbon monoxide dehydrogenase
DXP: 1-deoxyxylulose-5-phosphate
FBA: fructose-1,6-bisphosphate aldolase
GAPDH: glyceraldehyde-3-phosphate dehydrogenase
GK: glucokinase
GPI: glucose-6-phosphate isomerase
IPP: isopentenyl diphosphate
MVA: mevanolate
PFK: phosphofructokinase
PFOR: pyruvate : ferredoxin oxidoreductase
TCA: tricarboxylic acid
TPI: triosephosphate isomerase
TPP: thiamine pyrophosphate