Biol. Chem., Vol. 382, pp. 1521 – 1539, November 2001

Review

An Overview of Endosymbiotic Models for the Origins of Eukaryotes, Their ATP-Producing Organelles (Mitochondria and Hydrogenosomes), and Their Heterotrophic Lifestyle

William Martin*, Meike Hoffmeister, Carmen Rotte and Katrin Henze

Institut für Botanik III, Universität Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany

* Corresponding author

The evolutionary processes underlying the differentness of prokaryotic and eukaryotic cells and the origin of the latter's organelles are still poorly understood. For about 100 years, the principle of endosymbiosis has figured into thoughts as to how these processes might have occurred. A number of models that have been discussed in the literature and that are designed to explain this difference are summarized. The evolutionary histories of the enzymes of anaerobic energy metabolism (oxygen-independent ATP synthesis) in the three basic types of heterotrophic eukaryotes – those that lack organelles of ATP synthesis, those that possess mitochondria and those that possess hydrogenosomes – play an important role in this issue. Traditional endosymbiotic models generally do not address the origin of the heterotrophic lifestyle and anaerobic energy metabolism in eukaryotes. Rather they take it as a given, a direct inheritance from the host that acquired mitochondria. Traditional models are contrasted to an alternative endosymbiotic model (the hydrogen hypothesis), which addresses the origin of heterotrophy and the origin of compartmentalized energy metabolism in eukaryotes.

Key words: Compartmentalization / Endosymbiosis / Eukaryotes / Evolution / Hydrogenosomes / Organelles.

Introduction

In the macroscopic world, almost all biological diversity can be explained by Darwin's principles of natural variation and natural selection. But because Darwin was not primarily concerned with microbes, there are two important principles of microbial evolution that he neither discovered nor anticipated – endosymbiosis and lateral (or horizontal) gene transfer. Recent overviews on the role of lateral gene transfer in microbial evolution are available (Doolittle, 1999a,b; Eisen, 2000; Ochman *et al*., 2000). Good overviews of the geochemical setting of early life and early cell evolution are also available (Russel and Hall, 1997; Nisbet and Sleep, 2001). This article addresses a narrow (but to some extent representative) segment of the literature that uses endosymbiosis as an explanatory principle in cell evolution. The issue of how endosymbiosis has figured into attempts to bridge the great evolutionary gap between prokaryotic and eukaryotic cell organization is considered, as is the evolutionary significance of anaerobic eukaryotes that lack mitochondria.

A Diversity of Models to Derive Eukaryotes and Their Organelles

Historians of science have dealt elsewhere in detail with the turbulent past of endosymbiotic theories (Sapp, 1994; Höxtermann, 1998). Schimper (1883) was among the first to cautiously lean toward the notion that some organelles (plastids) of what we now call eukaryotes might have once been free-living cells. But the young Russian biologist Constantin Mereschkowsky was probably the first to publish a thoroughly argued case that some cells arose through the intracellular union of two different kinds of cells (endosymbiosis). His 1905 publication (Mereschkowsky, 1905) is exceptional and exciting to read. At the most basic level, Mereschkowsky (1905) said three things: (i) plastids are without question (not might be) reduced cyanobacteria that early in evolution entered into a symbiosis with a heterotrophic host; (ii) the host that acquired plastids was itself the product of an earlier symbiosis between a larger, heterotrophic, amoeboid host cell and a smaller 'micrococcal' endosymbiont that gave rise to the nucleus, and (iii) the autotrophy of plants is an inheritance *in toto* from cyanobacteria. Under Mereschkowsky's scheme, which was more fully elaborated but basically unchanged in his 1910 series (Mereschkowsky, 1910), there were two kinds of fungi – those that evolved a nucleus without endosymbiosis (today we call them the true fungi) and those that once possessed plastids but became secondarily nonphotosynthetic (today we call them the oomycetes, fungus-like organisms related to red algae; van't Klooster *et al.,* 2000). His figure summarizing the 1910 series is re-

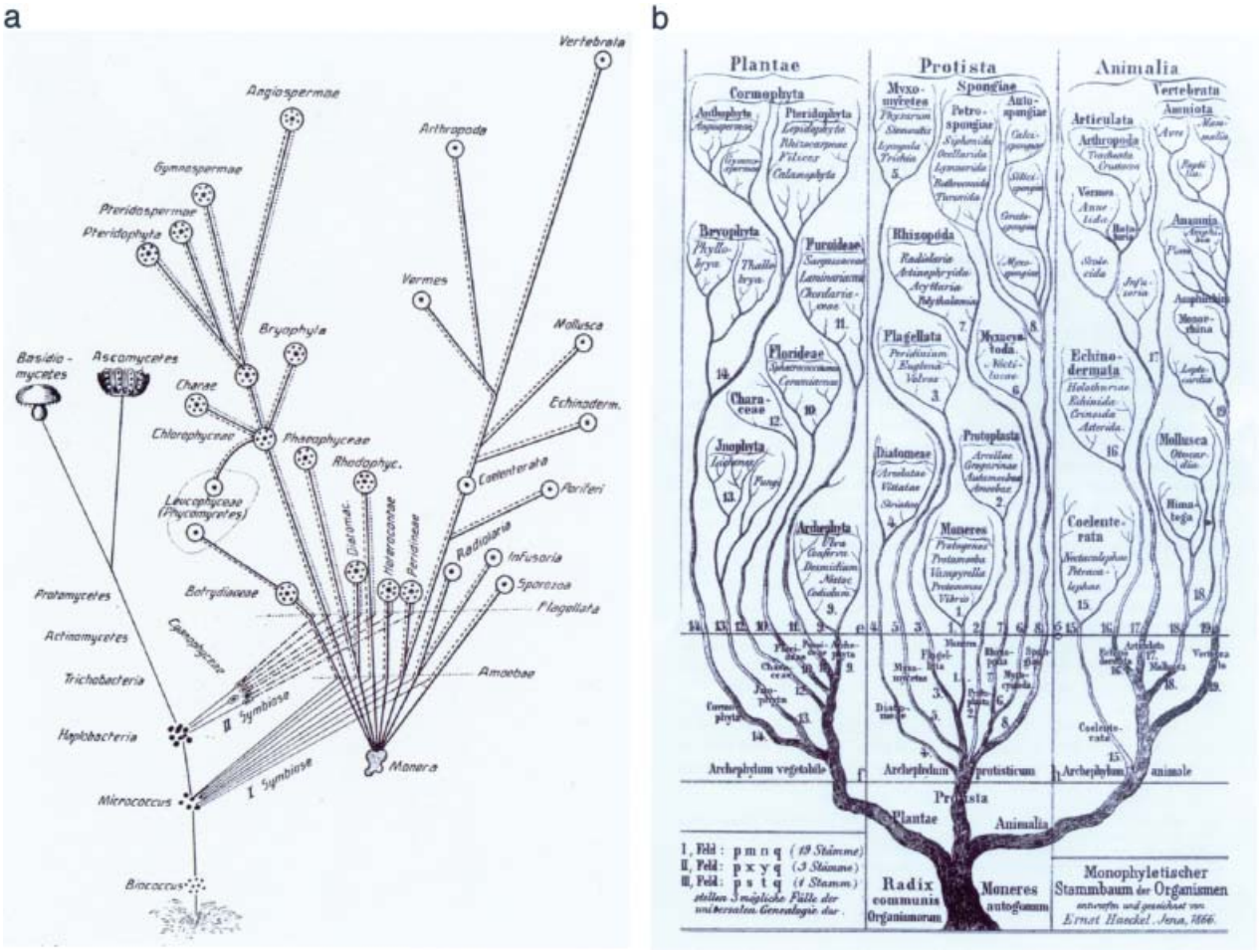

Fig. 1 A Comparison of Bifurcating-Only and Bifurcating-Plus-Anastomozing Evolutionary Trees Depicting the Relatedness of Organisms.

(a) Constantin Mereschkowsky's not-so-famous 1910 tree depicting an additional, combinatorial mechanism of evolution among unicellular organisms – endosymbiosis (for details see text). (b) Ernst Haeckel's famous 1866 tree depicting the evolutionary history of organisms under the assumption that evolution works in a solely bifurcating manner through descent with modification only.

produced in Figure 1a. The major point where it differs from Darwin's single bifurcating mechanism of evolution, as manifested in Haeckel's elaboration of Darwin's principle (Figure 1b), is that the branches in Mereschkowsky's tree occasionally anastomose (unite) *via* endosymbiosis to produce fundamentally and radically new kinds of organisms (plants, for example), whereas the branches in Haeckel's tree do not.

Clearly, (endo)symbiotic theory begins with Mereschkowsky. But this theory missed the mark on a few points. First, it missed the dichotomy of life into prokaryotes and eukaryotes (terms that were introduced by several decades later), because Mereschkowsky argued the true fungi to descend directly from prokaryotes without endosymbiosis. Second, he inferred that contemporary life forms arose twice independently (rather than once as we know today based on the universality of the genetic code), which can be seen as a statement of the differentness of prokaryotes and eukaryotes and thus as related to the first problem. Third, he did not recognize mitochondria as descendants of endosymbionts. He also believed that the different colored plastids of different algal groups (green, red, golden, brown) arose from different free-living cyanobacteria. A distilled cartoon of his model is presented in Figure 2a.

Ivan Wallin (1927) recognized mitochondria as descendants of endosymbiotic bacteria, but he interpreted chloroplasts as evolutionary modifications of mitochondria, rather than as independent endosymbionts. It is hard to tell from his book what Wallin thought about the host. E. B. Wilson, in his highly regarded standard college textbook of the day, bitterly criticized endosymbiotic hypotheses for the origins of organelles as too fanciful (Wilson, 1928). Possibly as a direct consequence, endosymbiosis for the origins of organelles fell out of grace and remained that way for several decades (see Lederberg, 1952) – grotesquely, even in the literature devoted specifically to the study of intracellular symbiosis (see Buchner, 1953). Endosymbiotic theory was repopularized in 1967 by Lynn Sagan (later to be named Margulis) (Sagan, 1967). However, another little-noted article appeared in the same year that argued mitochondria and chloroplas-

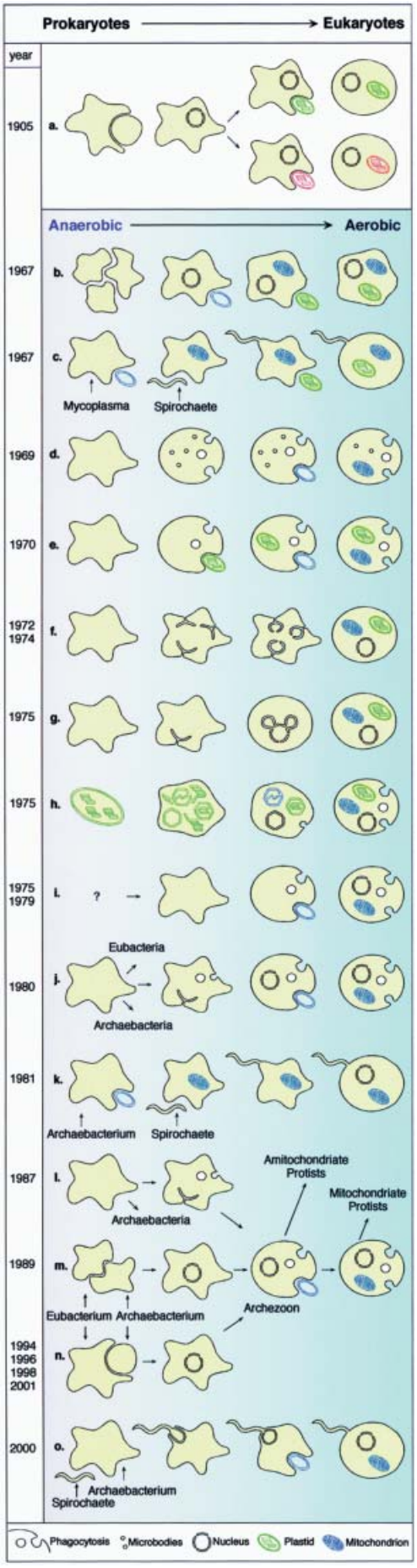

Fig. 2 A Schematic Summary of Some Models That Are Designed to Explain Various Aspects of the Differentness of Prokaryotes and Eukaryotes and the Origins of the Latter's Organelles.
The reader is warned that this schematic highly condensed (and graphically uniform) representation of the respective models does not do justice to any of them, it just documents that the model was elaborated. Furthermore, there are many, many additional models in the literature that are not covered either in this article or in the Figure. References to models summarized are: (a), Mereschkowsky (1905, 1910); (b), Goksøyr (1967); (c), Sagan (1967); (d), de Duve (1969); (e), Stanier (1970); (f), Raff and Mahler (1972); Uzzel and Spolsky (1974); (g), Bogorad (1975); (h), Cavalier-Smith (1975); (i) John and Whatley (1975); Whatley *et al*. (1979); (j), Doolittle (1980); Van Valen and Maiorana (1980); (k), Margulis (1981); (l), Cavalier-Smith (1987b); Van Valen and Maiorana (1980); (m), Zillig *et al*. (1989); (n), Lake and Rivera (1994); Gupta and Golding (1996); Moreira and Lopez-Garcia (1998); Horiike *et al*. (2001); (o), Margulis *et al*. (2000).

ts to be endosymbiotic prokaryotes that donated most of their genes to the chromosomes of their host, which in turn was viewed as the product of agglomerated prokaryotes (Goksøyr, 1967; Figure 2b). Like Goksøyr, Sagan (1967) also argued that mitochondria and chloroplasts descend from different prokaryotes. Her host for the origin of mitochondria was a heterotrophic anaerobe (a prokaryote perhaps similar to modern *Mycoplasma*), which later acquired a spirochaete that gave rise to eukaryotic flagella (Figure 2c). Christian de Duve (1969) argued that an amitochondriate (but peroxisome-bearing) eukaryote could have arisen from a heterotrophic prokaryote through loss of the cell wall and the evolution of membrane invagination processes (endocytosis) that ultimately gave rise to a primitive form of compartmentalized aerobic metabolism (peroxisomal respiration) and the ability to engulf (phagocytose) the mitochondrial symbiont (Figure 2d). Stanier (1970) proposed an interesting variant that had the origins of chloroplasts in an anaerobic, heterotrophic host before the origin of mitochondria, his reasoning being that since mitochondria use oxygen, and since eukaryotes probably arose in anaerobic times during Earth's history, there must have been a continuous local source of oxygen available in order for mitochondria to become useful (Figure 2e).

These ideas were hotly debated in the literature of the 1970s. Mostly they found a positive echo, but they also prompted the search for alternative and less radical models that avoided endosymbiosis altogether and preserved the Darwinian view of pure descent with modification. Both Raff and Mahler (1972) and Uzzel and Spolsky (1974) argued good cases for the non-symbiotic origins of organelles through compartmentalization arising in the wake of endocytosis in a heterotrophic prokaryote (Figure 2f). Bogorad (1975) presented a good model to account for the curious finding – one that still puzzles many biologists today – that some organelle proteins (for example Rubisco) are encoded in part in the nuclear DNA and in part in the organelle DNA. The model involved a

budding-like process of one genome into three (Figure 2g). Cavalier-Smith (1975) articulated a very detailed model that derived the nucleus, the mitochondrion and the chloroplast through restructuring of thylakoids in a cyanobacterium (Figure 2h).

In the same year, John and Whatley (1975) published an explicit and detailed model that envisaged the host of mitochondrial symbiosis as a mitochondrion-lacking 'protoeukaryote', perhaps similar to the anaerobic, fermenting amitochondriate giant amoeba *Pelomyxa palustris*. Although they did not address the origin of that host – that is, the way in which the host and its suspectedly *Paracoccus*-like (or possibly *Rhodobacter*-like) symbiont are related (Figure 2i) – the nature of the metabolic association between host and symbiont (fermentation plus respiration) that John and Whatley envisaged (and illustrated in their paper) has been at the heart of most thinking on this topic since. An exception is Woese (1977) who suggested that mitochondria might descend from a photosynthetic prokaryote-like that evolved the ability to respire O_2 after becoming an endosymbiont, rather than before (whereby the host was envisaged as a nondescript engulfing cell).

To some, Cavalier-Smith's 1975 model might seem very odd from today's standpoint, but prior to 1970, the predominating view in evolutionary cell biology was that the simplest eukaryotes arose from the most complex prokaryotes (cyanobacteria). The foregoing statement can be found in a noteworthy paper by W. Ford Doolittle (1980) that summarized some of the early molecular data which had been collected to test the endosymbiotic origins of organelles. Doolittle's publication furthermore tied the emerging significance of archaebacteria (Woese *et al*., 1978) neatly into endosymbiotic theories by positing how archaebacteria might be related to the host (Figure 2j). In another very noteworthy article that same year, Van Valen and Maiorana (1980) also incorporated archaebacteria into endosymbiotic theories, suggesting that they are the sister group of the host that acquired mitochondria, a notion that became very popular in the 1990s. Van Valen and Maiorana (1980) were very explicit about the evolution of ATP-synthesizing pathways, suggesting that glycolysis was an ancestral state of ATP synthesis and the prime energy source in a hypothetical heterotrophic common ancestor of all cells – but that assumption, which is implicit in many models, might be wrong. For various reasons, today it seems more likely that the common ancestor of all cells was autotrophic (Kandler, 1994; Russel and Hall, 1997; Wächtershäuser, 1998), heterotrophy arising later in evolution, after autotrophs had produced something for cells to eat.

Margulis (1981) also incorporated achaebacteria into endosymbiotic theories in that the host for the mitochondrial (and later flagellar, spirochaete) symbiont in her model was posited to be an archaebacterium (Figure 2k), possibly similar to contemporary *Thermoplasma* (no evidence in support of this view was found in the *Thermoplasma* genome; Cowan, 2000). Because only few evolutionary cell biologists have vested any belief in the notion that the eukaryotic flagellum was ever a free-living prokaryote, Doolittle's 1980 model, which does not entail a symbiotic origin of flagella, probably comes closest to what most people now think when they hear or use the term 'endosymbiont hypothesis'.

Doolittle's 1980 model (like John and Whatley's 1975 model and Van Valen and Maiorana's 1980 model) contained a very important hypothetical organism – a cytoskeleton-bearing, nucleated (eukaryotic) cell that did not possess mitochondria. This kind of hypothetical cell became a central element of views on the origins of eukaryotes and their organelles. When Woese and coworkers studied the ribsomal RNA of a contemporary eukaryote that lacks mitochondria (a member of the protist group called microsporidia) and found it to branch most deeply on the eukaryotic branch of the tree of ribosomal RNA sequences (Vossbrink *et al*., 1987) biologists became confident that the nature of the host which acquired mitochondria had been revealed. (Today it appears that microsporidia are not primitive at all, but are in fact highly derived fungi; Germot *et al*., 1997; Hirt *et al*., 1999). But even before that, contemporary eukaryotes that were supposedly primitively amitochondriate and supposedly direct descendants of the (n.b.) assumedly eukaryotic host which acquired mitochondria had been given a rank and a name, they were called the archezoa (see Cavalier-Smith, 1987a).

The archezoa model was fully articulated (Cavalier-Smith, 1987b). It entailed the origin of a cytoskeleton, endocytosis and nucleus in an early member of the archaebacterial lineage, giving rise to a primitively amitochondriate eukaryote (Figure 2l), but it also contained very important elements of Van Valen and Maiorana's 1980 model. Initially, it received some direct support from molecular phylogenetics of several proteins, most notably proteins that are related by ancient duplications (Gogarten *et al*., 1989; Iwabe *et al*., 1989) as well as proteins involved in transcription (Langer *et al*., 1995) and translation (Baldauf *et al*., 1996). But the part of the model that was most difficult for some people to accept had to do with lipids. Archeabacteria have different lipids from eukaryotes, including amitochondriate ones. Archaebacteria possess glycerol isoprene ethers while eukaryotes and eubacteria have glycerol fatty acid esters (and the stereochemistry of the glycerol backbone in the lipid is also different; Koga *et al*., 1998). In order to explain that, the archezoa model, like Van Valen and Maiorana's 1980 model, posited that archezoa diverged from the archaebacterial lineage at a time when the latter still possessed eubacterial-type lipids. It was argued that archezoa inherited the eubacterial-type lipids directly, whereby the ancestral archaebacterial lineage reinvented its lipid biochemistry prior to the diversification of contemporary forms.

The lipid problem and other kinds of data, for example that some eukaryotic enzymes involved in energy metabolism (ATP production) were more similar to eubacterial than to archaebacterial homologs (Hensel *et al*., 1989) (also among members of the archezoa) spawned a new class of models for the origin of eukaryotes. These were designed to derive an archezoon that possessed a chimeric pattern

of characters shared with archaebacteria and eubacteria. The first of these was probably Zillig's (1989) fusion model (Figure 2m), which simply argued that an archaebacterium and a eubacterium fused during the origin of archezoa, and that one member of that archezooid group acquired the mitochondrion whereas others remained primitively amitochondriate. This fusion model was followed by models for the origin of archezoa that entailed the origin of the nucleus through endosymbiosis of an archaebacterium in a eubacterial host (Lake and Rivera, 1994; Figure 2n), many variants of which have subsequently been articulated (Gupta and Golding, 1996; Moreira and Lopez-Garcia, 1998; Horiike *et al*., 2001; see also critique in Martin, 1999a). Margulis *et al*. (2000) modified the archaebacterium-spirochaete symbiosis so as to derive the flagellum and the nucleus prior to the origin of mitochondria (Figure 2o). This small sample of symbiotic models by no means covers the full spectrum of conceptual diversity that can be found about the origin of eukaryotes and mitochondria (for further models and their discussion, see chapters in Wagner *et al*., 1999), but it does show that the problem can be viewed from many different angles.

To sum up thus far, endosymbiosis is a good explanatory principle when it comes to accounting for the overall similarity between chloroplasts and mitochondria to free-living cyanobacteria and α-proteobacteria, respectively. The overall physiology of both organelle types (John and Whatley, 1975; Gray and Doolittle, 1982), in addition to the sequence and structure of their genomes, attest beyond all reasonable doubt that these organelles were indeed once free-living prokaryotic cells (Martin *et al*., 1998; Gray *et al*., 1999). Furthermore, secondary endosymbiosis – the engulfment of a eukaryote by a eukaryote (Gibbs, 1978) – fully explains why the complex plastids of some eukaryotes are more similar to eukaryotic cells than they are to prokaryotic cells (Gilson *et al*., 1997). But endosymbiosis has also been overworked as an explanatory principle to account for the origins of various parts of a eukaryotic cell. In addition to the nucleus and the flagellum, almost every piece of a eukaryotic cell has been suggested at some time to have been an inheritance from an endosymbiotic prokaryote. Examples can be found for peroxisomes and glycosomes (Cavalier-Smith, 1987c, 1997), the endoplasmic reticulum (Gupta *et al*., 1994; Gupta, 1998), and elements of the cytoskeleton (Doolittle, 1998). But the only organelles or structures of eukaryotic cells that we really know to be of truly endosymbiotic origin are plastids, including the complex ones, and mitochondria, including their anaerobic cousins, hydrogenosomes – organelles of anaerobic ATP synthesis in amitochondriate protists (see below).

Other Models

There are a number of other models that address various aspects of the relatedness of eukaryotes and prokaryotes that are not summarized in Figure 2 but that definitely should be mentioned. The model of Vellai *et al*. (1998) is well-articulated. It operates with a purely archaebacterial (as opposed to a eukaryotic or a chimeric) host and a eubacterial symbiont, it takes hydrogenosomes and secondary loss of mitochondria into account, and it specifically addresses genome size differences between prokaryotes and eukaryotes, focussing on the hypothetical benefit of additional ATP provided by mitochondria as a resource to increase genome size in eukaryotes.

The thermoreduction model (Forterre, 1995; Forterre and Philippe, 1999; Poole *et al*. 1999) was also not discussed above. It focusses more on aspects of gene and genome organization rather than on cellular organization, interpreting many aspects of RNA processing in eukaryotes as direct inheritances from an RNA world. As its arguably most radical element, thermoreduction posits that eukaryotes are the most ancient cellular lineage of all and that prokaryotes descend from eukaryotes. The thermoreduction model does not specifically address the origin of organelles, rather it focusses on the derivation of prokaryotic traits through reductive evolution in response to thermophily from a hypothetical mesophilic ancestor with eukaryotic-like cell organization. Since prokaryotes derive from eukaryotes under this model, the origin of eukaryotes would necessarily have had to antedate the origin of mitochondria by quite a bit, meaning that the most ancient eukaryotes would have been primitively amitochondriate (archezoa) and would have had ample time to diversify prior to the origin of mitochondria, meaning that it would predict primitively amitochondriate eukaryotic lineages (archezoa) to have persisted to the present.

Another model not specifically discussed above is the ox-tox model of Kurland and Andersson (1999). The ox-tox model posits that the ancestor of mitochondria was an aerobic α-proteobacterium, perhaps similar to the obligate intracellular parasite *Rickettsia prowazeckii*, and that the host was a strictly anaerobic, primitively amitochondriate eukaryote (an archezoon) that is presumed to have existed but whose origin is a given, not an explanandum, of the model (Andersson and Kurland, 1999; Kurland and Andersson, 2000). In this sense, ox-tox addresses the origin of mitochondria but not the origin of eukaryotes and is thus very similar to John and Whatley's 1975 model (Figure 2i). In essence, the ox-tox model argues that the prime benefit provided by the α-proteobacterial symbiont to its eukaryotic host was the consumption of molecular oxygen, which is toxic to anaerobes, thus enabling a strictly anaerobic eukaryote to survive as oxygen levels were globally rising about 2 billion years ago. Ox-tox places considerable importance upon the origin of the mitochondrial ADP-ATP translocator, which, as Whatley *et al.* (1979) argued, was an important step *en route* to converting an intracellular symbiont to an organelle. However, the ADP-ATP translocator of *Rickettsia* is not related to the mitochondrial translocator, rather it is related to the chloroplast ADP-ATP translocator (Winkler and Neuhaus, 1999). The ox-tox model also stresses the finding that many of the proteins localized in the yeast mitochondrion

do not have recognizeable homologs in prokaryotes by the criterion of BLAST searching (Karlberg *et al*., 2000). Its proponents interpret this as evidence that such proteins are inventions specific to the eukaryotic lineage, from which the argument is generated that the ancestor of mitochondria made a very slight contribution of genes to the eukaryotic genome (Kurland and Andersson, 2000). However, very few of the proteins that eukaryotes inherited from their organelles belong to the conservatively evolving class of proteins (Rujan and Martin, 2001). Thus, many eukaryotic proteins that share no or only barely detectable sequence similarity to prokaryotic homologs still might ultimately come from organelles, their origins having been blurred by sequence divergence. Examples of this kind of extreme divergence are found in components of the protein import machinery of chloroplasts (Reumann and Keegstra, 1999) and in genome-wide analyses (Rujan and Martin, 2001). The ox-tox model has been argued by its proponents to possess particular virtues (Andersson and Kurland, 1999) but it also has been criticized (Rotte *et al*., 2000). One problem specific to the ox-tox model is that contemporary anaerobes do not typically acquire oxygen-consuming symbionts in order to cope with oxygen, rather they usually possess one or several oxygen-detoxifying enzymes (Martin, 2000a).

Five Problems with Traditional Endosymbiotic Models

Traditional symbiotic models such as those outlined in Figure 2 and above have five main problems, which can be labeled as archezoa, heterotrophy, oxygen, hydrogenosomes, and anaerobic mitochondria. Each of these five issues, individually and in their entirety, pose difficulties for traditional models.

Archezoa

Most (but not all) traditional endosymbiotic models assume that the host was a primitively amitochondriate eukaryote – an archezoon. But there is currently no evidence of any type to indicate that the cell that acquired the mitochondrion was in fact a eukaryote. On the contrary, there is very clear evidence to indicate that all eukaryotes studied to date either (i) possess a mitochondrion or (ii) possessed a mitochondrial symbiont (the same mitochondrial symbiont) in their evolutionary past but subsequently lost the organelle. In other words, mitochondrion-lacking nucleated cells, such as the microsporidia and the diplomonads, possess genes in their nuclear chromosomes that clearly are of mitochondrial origin. Such findings started to arise in 1995 (Clark and Roger, 1995; Henze *et al*., 1995) and were substantiated in subsequent studies on many different lineages of amitochondriate eukaryotes (summarized in Embley and Hirt, 1998; Lang *et al*., 1999; Müller and Martin, 1999; Roger, 1999; Philippe *et al*., 2000; Rotte *et al*., 2000). In fact, one eukaryote that was thought to lack mitochondria, *Entamoeba histolytica*, was later found to possess a reduced, nonfunctional mitochondrion termed a mitosome (Tovar *et al*., 1999; Müller, 2000) (or crypton; Mai *et al*., 1999). Since all eukaryotes examined to date possess(ed) a mitochondrion and since they all possess a nucleus, there is currently no way to tell which came first in evolution: the mitochondrion or the nucleus (Martin, 1999a; Roger, 1999).

Heterotrophy

Despite their many differences, all of the endosymbiotic models outlined in Figure 2 (except Mereschkowsky's model, because he did not believe in an endosymbiotic origin of mitochondria) have one thing in common: they assume that the host that acquired the mitochondrion was a heterotrophic cell (Stanier's host was also a heterotroph, but acquired the plastid first). Heterotrophs are organisms that satisfy their carbon and energy needs through the oxidative breakdown of reduced carbon compounds. Some of the models in Figure 2 assume the host to have been a heterotrophic eukaryote, some assume it to have been a heterotrophic prokaryote. That the host of mitochondrial symbiosis – be it a prokaryote or be it a eukaryote – was a heterotroph is a premise, an assumption made for the purpose of argument, it is not a known fact that is founded in experiment, observation, or anything else. This is an important point. There is no clear cut evidence, molecular, biochemical or otherwise, to indicate that the host cell that acquired the mitochondrion was a heterotroph (Martin, 2000a). One alternative model has been proposed entailing an autotrophic host (Martin and Müller, 1998); it will be discussed in a later section.

Oxygen

Traditional endosymbiotic models focus on the role of aerobic respiration in the origin of mitochondria, and the increased ATP yield (38 *versus* 2 mol of ATP per mol of glucose) over fermentative ATP synthesis through glycolysis – the pathway that the host is assumed to have used in models that posit the host to have been heterotrophic. But models that posit the host to have been a fermenting cell also assume it to have been anaerobic. It is very difficult to imagine how an anaerobic host could have realized the advantage of increased ATP yield from glucose if its mitochondrial symbiont needed oxygen to generate that ATP. It is furthermore difficult to imagine how an aerobic cell could have associated with an anaerobic one in the first place, since the requirement for one (O_2) is a toxin for the other (see Blackstone, 1995, for a good discussion of this problem). In fact, it was evident early on that ATP was very unlikely to have been the initial advantage that the symbiont conferred to the host in the first place, because that view entails the notion that the symbiont was able to export ATP to its environment so that the host could realize that benefit (John and Whatley, 1975; Whatley *et al*., 1979). Indeed, no cell is known that exports ATP to the environment. Furthermore, in such models the

symbiont must have produced ATP in excess so that it could donate some to the host, and the host must have been unable to synthesize sufficient amounts of ATP by itself. No cell is known that synthesizes more ATP than it needs, nor is one known that cannot satisfy its ATP needs given sufficient substrate. Overall, whether implicitly or explicitly, the models outlined in Figure 2 (except Uzzel and Spolsky's 1974 model) view the origin of mitochondria as coinciding with the transition from anaerobic to aerobic energy metabolism in eukaryotes. But there are many eukaryotes that synthesize ATP without the help of oxygen, which brings us to the fourth point.

Hydrogenosomes and Energy Metabolism in Eukaryotes That Lack Mitochondria

There are several groups of unicellular eukaryotes that are known that lack mitochondria (amitochondriate protists) and that do not require oxygen for ATP synthesis (Fenchel and Finlay, 1995). They thrive in anaerobic environments such as marine or freshwater sediments, salt marshes, rumen, and intestinal tracts of metazoa; some are parasitic. Amitochondriate protists satisfy their ATP needs through anaerobic fermentations (Müller, 1988, 1993, 1998). Like mitochondriate eukaryotes, they also obtain 2 mol of ATP from glycolysis in the cytosol, but they differ from mitochondriate eukaryotes with respect to the fate of pyruvate (Figure 3). Whereas mitochondrion-bearing eukaryotes metabolize pyruvate in mitochondria *via* pyruvate dehydrogenase (PDH), eukaryotes that lack mitochondria oxidize pyruvate with the O_2-sensitive enzyme pyruvate:ferredoxin oxidoreductase (PFO). Like PDH, PFO yields CO_2 and acetyl-CoA, but the electrons that are removed from pyruvate are not transferred to NAD^+ as in the PDH reaction, but rather to ferredoxin.

Among amitochondriate protists, two (unnatural) groups of organisms are known that differ in the compartmentalization of core energy metabolism. They were designated by Müller (1998) as Type I and Type II amitochondriate protists (Figure 3). The human intestinal para-

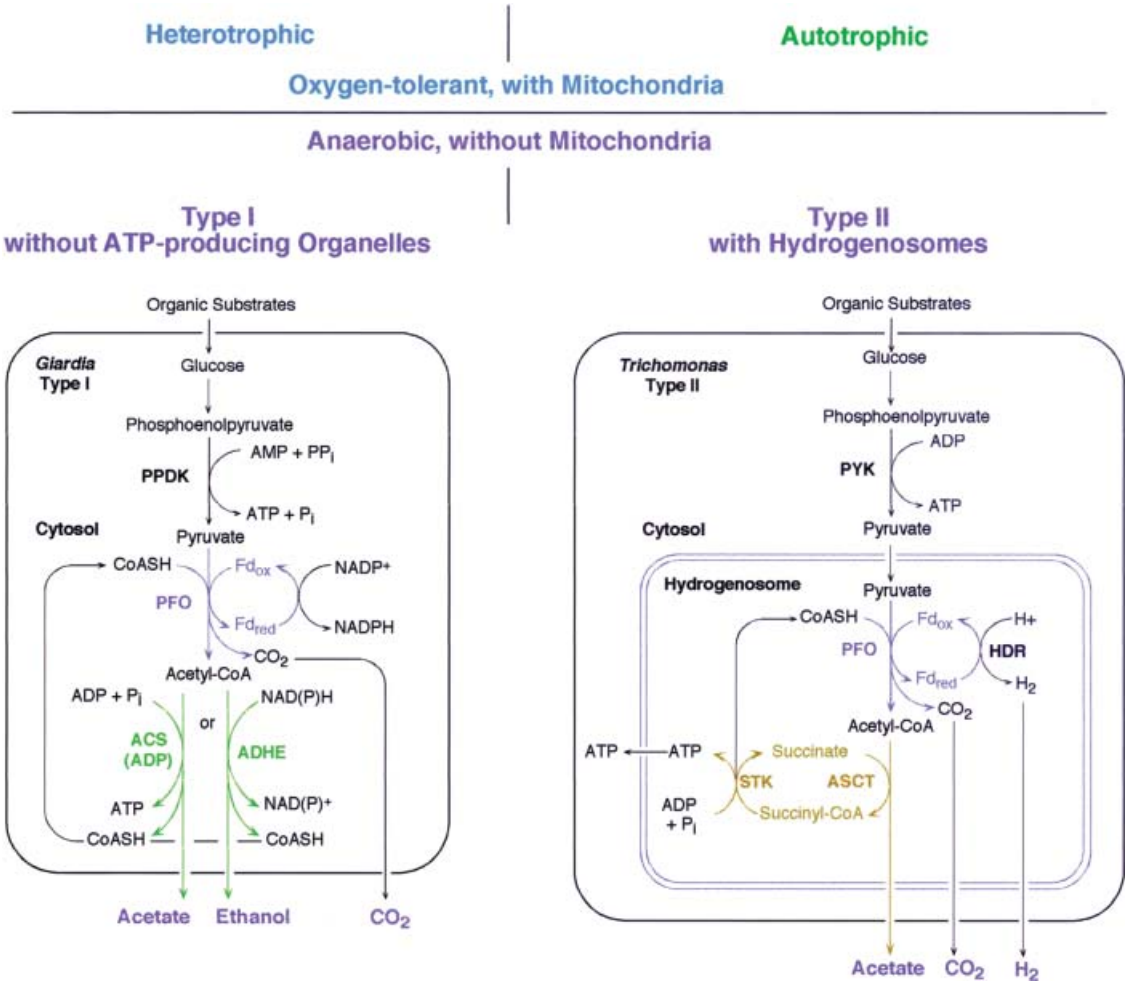

Fig. 3 The Basic Kinds of Compartmentalized Energy Metabolism in Eukaryotes (Modified and Slightly Simplified from Müller, 1998) (See Text).

Abbreviations: PPDK, pyruvate:pyrophosphate dikinase; PFO, pyruvate:ferredoxin oxidoreductase; ACS, actetyl CoA synthase (ADP-forming); ADHE, bifunctional aldehyde reductase/alcohol dehydrogenase; PYK, pyruvate kinase; STK, succinate thiokinase (also called succinyl-CoA synthase); ASCT, acetate:succinate CoA-transferase; HDR, hydrogenase.

site *Giardia lamblia* is a well-studied representative of the Type I organisms (Figure 3, left panel). They do not possess organelles involved in core energy metabolism, and in these organisms PFO decarboxylates pyruvate in the cytosol. The resulting acetyl-CoA is further converted into a mixture of ethanol and acetate. Ethanol is produced with the help of a bifunctional acetyl-CoA reductase/alcohol dehydrogenase (ADHE; Müller, 1998; Sánchez, 1998). Acetate is produced by an acetyl-CoA synthase (ADP-forming, ADP-ACS; Müller, 1998; Sánchez *et al*., 1999, 2000) that yields the synthesis of one additional ATP through substrate level phosphorylation. Both reactions regenerate CoASH for PFO. The relative amounts of ethanol and acetate produced depend upon environmental conditions, specifically the trace amounts of oxygen available for maintaining redox balance (Lloyd, 1996; Martin, 2000a), so that the additional ATP yield is between 0 and 2 additional mol of ATP per mol of glucose (Müller, 1996, 1998).

Type II amitochondriate eukaryotes are also anaerobes, but harbor double membrane-bounded, ATP- and H_2-producing organelles called hydrogenosomes (Müller, 1993). A well-studied representative of this group is the parabasalid *Trichomonas vaginalis*, a common pathogen of the human urogenital tract (Figure 3, right panel). In Type II organisms, PFO is localized in the hydrogenosome where it converts pyruvate to CO_2, acetyl-CoA, and reduced ferredoxin. Ferredoxin is reoxidized by hydrogenase, producing the H_2 characteristic of the organelle. The CoA moiety of acetyl-CoA is transferred to succinate by an acetate-succinate CoA transferase (ASCT), yielding acetate and succinyl-CoA. CoASH is regenerated by succinate thiokinase (STK; also called succinyl:CoA synthase, SCS), that synthesizes ATP (in some species GTP), utilizing the energy of the thioester bond in succinyl-CoA. Per mol of glucose, pyruvate metabolism in hydrogenosomes thus yields two additional mol of ATP and two mol each of H_2, CO_2, and acetate as waste products (Figure 3).

Obviously, hydrogenosomes as double membrane-bounded, pyruvate-metabolizing organelles of anaerobic ATP synthesis are relevant to endosymbiotic theory (Whatley *et al*., 1979; Müller, 1988), even though most hydrogenosomes studied to date do not contain their own DNA (Clemens and Johnson, 2000). But although hydrogenosomes were discovered almost 30 years ago (Lindmark and Müller, 1973) they were neglected by mainstream endosymbiotic theory, which focussed on the origin of aerobic mitochondria and which was not designed to account for anaerobic organelles. Hydrogenosomes are an explanandum that traditional symbiotic models, on the whole, have not addressed.

Anaerobic Mitochondria

Anaeobic mitochondria were also neglected by mainstream endosymbiotic theory. Anaerobic mitochondria have no need for oxygen in ATP synthesis and use alternative terminal acceptors for mitochondrial electron transport. Several ciliates (Finlay *et al*., 1983) and denitrifying fungi as *Fusarium oxysporum* and *Cylindrocarpon tonkinense* perform nitrate respiration in the absence of oxygen (Kobayashi *et al*., 1996; Takaya *et al*., 1999). The facultatively anaerobic mitochondria of the nematode *Ascaris suum*, in some stages of its life cycle, use fumarate as electron acceptor (Amino *et al*., 2000). Fumarate respiration is also known from the mitochondria of fresh-water snails, some lower marine organisms, and parasitic flatworms (plathelminths), which switch from aerobic to anaerobic metabolism during their life cycle (for a good review see Tielens and Van Hellemond, 1998). During aerobic phases of the life cycle, plathelminth energy metabolism is typical of that in most eukaryotes – glycolysis proceeds to pyruvate in the cytosol, pyruvate is imported into mitochondria where it is converted to acetyl-CoA by pyruvate dehydrogenase (PDH), which enters the Krebs cycle, and oxygen is the end acceptor of the respiratory chain. But during anaerobic phases, energy metabolism shifts rather dramatically to a different mode – anaerobic respiration (Tielens and Van Hellemond, 1998). Glycolysis in the cytosol proceeds to phosphoenolpyruvate which is converted by phosphoenolpyruvate carboxykinase (PEPCK) to oxaloacetate and further to malate that is then imported into the mitochondrion. Part of the malate is converted by malic enzyme (ME) and PDH to acetyl-CoA. Acetyl-CoA then enters the same two enzyme ASCT/STK system as is found in hydrogenosomes (Steinbüchel and Müller, 1986; Lahti *et al*., 1992; 1994).

Furthermore, ATP-producing organelles intermediate between typical, aerobic mitochondria and hydrogenosomes are known. Such facultatively anaerobic organelles can be found *inter alia* in the photosynthetic flagellate *Euglena gracilis* and trypanosomes, the sister group of the euglenids. In the facultatively anaerobic mitochondrion of *Euglena*, pyruvate is oxidized by an oxygen-sensitive enzyme, pyruvate:$NADP^+$ oxidoreductase (PNO; Inui *et al*., 1984; Nakazawa *et al*., 2000; Rotte *et al*., 2001), an enzyme closely related to PFO from amitochondriate protists. Notably, *Euglena* PNO functions both under aerobic and anaerobic conditions and substitutes for pyruvate dehydrogenase (PDH) in the oxidative decarboxylation reaction of these mitochondria (Inui *et al*., 1990). Trypanosome mitochondria, during part of their life cycle, use the two enzyme system ASCT/STK instead of the Krebs cycle to regenerate CoASH for the PDH reaction (van Hellemond *et al*., 1998). This enzyme system was previously thought to be specific for hydrogenosomes. The mitochondrion of the ciliate *Nyctotherus ovalis* even possesses a hydrogenase that mediates the transfer of electrons to protons, thereby producing hydrogen like hydrogenosomes do (Akhmanova *et al*., 1998). However, unlike all other hydrogenosomes studied to date, the hydrogenosomes of *Nyctotherus* contain DNA (Akhmanova *et al*., 1998; Embley and Martin, 1998).

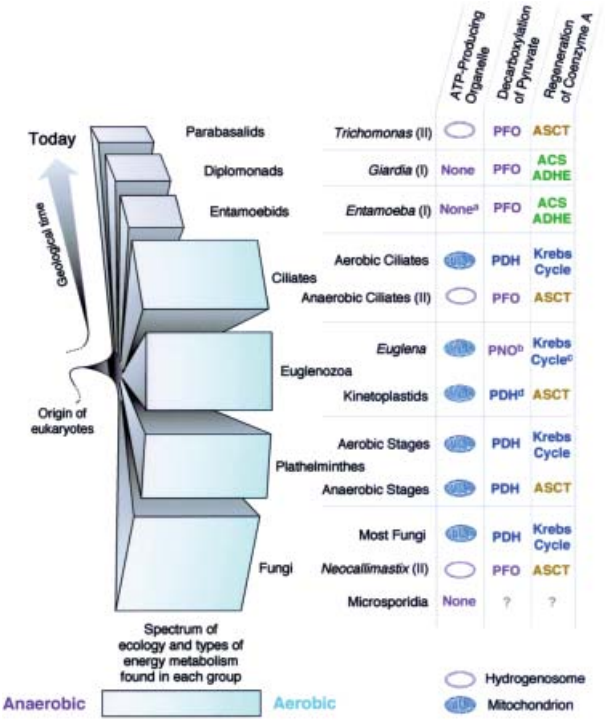

Fig. 4 Schematic Representation of the Distribution of Organelles and Enzymes Involved in Energy Metabolism in Selected Eukaryotic Lineages (Slightly Modified from Embley and Martin, 1998).

Abbreviations: PFO, pyruvate:ferredoxin oxidoreductase; ACS, actetyl CoA synthase (ADP-forming); ADHE, bifunctional aldehyde reductase/alcohol dehydrogenase; ASCT, acetate:succinate CoA-transferase; PDH, pyruvate dehydrogenase complex. Type I and Type II eukaryotic compartmentalization is indicated as (I) and (II) next to the respective organism names. The schematic tree at the right indicates that all eukaryotes share a single common ancestor, but does not indicate evolutionary relationships. The spectrum of aerobic and anaerobic energy metabolism in each group is roughly indicated with shading.

Footnotes: [a]*Entamoeba* possesses a highly reduced mitochondrion, but it does not appear to be involved in energy metabolism (Tovar *et al.,* 1999; Müller, 2000). [b]*Euglena* PNO is a translational fusion of PFO and an $NADP^+$-reducing domain (Rotte *et al*., 2001). [c]The Krebs cycle in *Euglena* is slightly unusual in that 2-oxoglutarate dehydrogenase is replaced by 2-oxoglutarate decarboxylase and succinate semialdehyde dehydrogenase (Kitaoka *et al.,* 1989). [d]In Rotte *et al*. (2001) it was reported that a homolog of PFO was detected in the genome sequencing data of *Trypanosoma brucei*, but attempts to detect that PFO gene in *T. brucei* DNA and additional computer analyses (C. Rotte, W. Martin, unpublished data) suggest that the sequence in the *T. brucei* genome sequencing data is possibly a bacterial contamination, rather than a genuine *T. brucei* gene. Color coding of enzymes corresponds to that in Figure 3.

A schematic representation of the distribution of Type I and Type II compartmentalized energy metabolism and the components of pyruvate metabolism in aerobic and anaerobic eukaryotes are shown in Figure 4. Note that the figure makes no statement about the phylogeny of these groups. This is because the phylogeny of eukaryotes, particularly the suspectedly early-branching forms, is not known with any certainty whatsoever, although it is reasonably certain that the phylogeny of eukaryotes as depicted in traditional ribosomal RNA trees is fundamentally flawed (Embley and Hirt, 1998; Baldauf *et al*., 2000; Philippe *et al*., 2000). The color coding of enzyme designations in Figure 4 is consistent with that in Figure 3 in order to underscore the finding that there is no absolutely clear distinction between pyruvate metabolism in Type I, Type II or mitochondrion-bearing eukaryotes.

What Are Hydrogenosomes and How to Account for Them under Endosymbiotic Theory?

Because hydrogenosomes synthesize ATP using the same kind of H_2-producing fermentations as some free-living prokaryotes, it was once thought that they might represent remnants of a third kind of symbiotic event, a symbiont different from those that gave rise to chloroplasts and mitochondria (Whatley *et al*., 1979; Müller, 1988). However, work in many laboratories over the past years has shown that hydrogenosomes are in fact anaerobic forms of mitochondria (summarized *inter alia* in Embley *et al*., 1995, 1997; Doolittle, 1996; Müller, 1996, 1997, 2000; Biagini *et al*., 1997; Embley and Hirt, 1998; Embley and Martin, 1998; Akhmanova *et al*., 1998; Hackstein, *et al*., 1999; Müller and Martin, 1999; Roger *et al*., 1998; Roger, 1999; Rotte *et al*., 2000).

Hydrogenosomes and mitochondria share not only the general function of pyruvate metabolism and ATP production (albeit by different means) but also a number of other characters. Despite their divergent metabolism, several proteins including ferredoxin (Johnson *et al*., 1990), succinate thiokinase (Lahti *et al*., 1992, 1994), acetate:succinate CoA transferase (Steinbüchel and Müller, 1986), malic enzyme (Hrdy and Müller, 1995; van der Giezen *et al*., 1997) and adenylate kinase (Länge *et al*., 1994), have been identified in hydrogenosomes as well as in mitochondria. Among these, ferredoxin and succinate thiokinase from hydrogenosomes show distinct affinity to mitochondrial homologs in phylogenetic analyses. Protein import into hydrogenosomes is mediated by transit peptides which are shorter than mitochondrial presequences but have been demonstrated to target proteins into kinetoplastid and yeast mitochondria (Hausler *et al*., 1997; van der Giezen *et al*., 1998). This transport, like in mitochondria, is dependent on ATP and an electrochemical membrane potential (Bradley *et al*., 1997). Furthermore, the heat shock proteins Hsp10, Hsp60, and Hsp70 from hydrogenosomes are most closely related to their mitochondrial homologs in phylogenetic reconstructions (Bui *et al*., 1996; Germot *et al*., 1996; Horner *et al*., 1996; Roger *et al*., 1996) indicating a common origin of both types of organelle. And the hydrogenosomes of the ciliate *Nyctotherus ovalis* even contain a relic genome that reveals these organelles to share a common ancestor with mitochondria (Akhmanova *et al*., 1998; van Hoek *et al*., 2000). Furthermore, enzymes of cytosolic energy metabolism in Type I eukaryotes, such

as PFO (Horner *et al*., 1999; Rotte *et al*., 2001) and hydrogenase (Horner *et al*., 2000), and enzymes of the glycolytic pathway (Martin and Müller, 1998) indicate a common eubacterial ancestry.

At face value, these data suggest that the enzymes essential to all three known types of eukaryotic energy metabolism were acquired from eubacteria and that the free-living common ancestor of hydrogenosomes and mitochondria was capable of producing sufficient ATP in both anaerobic and aerobic environments. The simplest interpretation of these findings is that the three forms of energy metabolism found in eukaryotes today were inherited from the common ancestor of hydrogenosomes and mitochondria, that possessed the enzymes involved therein. From that would follow that in the case of Type II amitochondriate protists, the respiratory pathway and hydrogenosomal genome have been lost, whereas in the case of Type I amitochondriate protists, the entire organelle has been lost in addition. The phylogenetic distribution of Type I and Type II amitochondriate protists indicates that these losses have occurred many times independently in separate eukaryotic lineages (Fenchel and Finlay, 1995; Embley *et al*., 1995, 1997; Cavalier-Smith *et al*., 1996; Müller, 1996, 1998), as did the transitions between aerobic, facultatively anaerobic and anaerobic lifestyles among eukaryotes (Embley *et al*., 1997; Martin and Embley, 1998; Rotte *et al*., 2001) whereby, for the purposes of this review, the order of these losses and the order of these transitions is irrelevant (as oulined in Figure 4).

There are ways to invoke special pleas for horizontal gene transfer in such a manner as to account for the distribution of organelles and anaerobic energy metabolism in eukaryotes and thus to save the concept of a primitively amitochondriate, eukaryotic (archezoon) host, and discussion of explanations that lean in this direction can be found (Doolittle, 1998b; Andersson and Kurland, 1999; Roger *et al.*, 1999). But given the overall number and severity of problems with archezoon-like models, it is also possible (in our view probable) that there is simply something fundamentally wrong with traditional views on the origin of eukaryotes, the origin of their genes, and the origin of their compartmentalized ATP-producing pathways (Martin, 1996; Martin and Schnarrenberger, 1997; Doolittle, 1997, 1998a; Martin and Müller, 1998).

Indeed, traditional endosymbiotic hypotheses have had reasonably good success when it comes to explaining the origins of (aerobic) mitochondria, but they have had little or no success when it comes to explaining the origins of anaerobic organelles or the origin of the host. Given the newer findings summarized above, it is not unreasonable to explore alternative models that depart completely from traditional views and to pursue fundamentally different avenues of thought.

The following section describes a model for the origins of eukaryotes and their ATP-producing organelle (in its aerobic and anaerobic manifestations) that explores the possibilities which ensue if we entertain the notions, just briefly, (i) that the host was not a eukaryote, (ii) that the host was not heterotrophic, and (iii) that neither ATP synthesis nor oxygen had anything whatsoever to do with the origin of mitochondria (and hydrogenosomes). At the most basic level, the model (Martin and Müller, 1998) is based upon three simple observations:

(i) many contemporary methanogens (autotrophic archaebacteria with a strictly H_2-dependent energy and carbon metabolism) are strictly dependent upon the H_2 that is produced by hydrogenosomes and by free-living, fermenting eubacteria (Embley *et al*., 1995; Fenchel and Finlay, 1995).

(ii) The enzymes of the glycolytic pathway in the eukaryotic cytosol are more similar to eubacterial than to archaebacterial homologs (Martin and Cerff, 1986; Markos *et al*., 1993; Henze *et al*., 1995, 2001; Martin and Schnarrenberger, 1997; Keeling and Doolittle, 1997; Nowitzki *et al.,* 1998).

(iii) Energy metabolism in eukaryotes in their entirety is astonishingly less diverse than energy metabolism in prokaryotes (see Schönheit and Schäfer, 1995; Schäfer *et al*., 1999), encompassing only one very, very small sample (less than one bacterium's worth) of prokaryotic genetic and biochemical diversity – heterotrophy with the help of the Embden-Meyerhof pathway. This third point cannot be sufficiently stressed. The reader is invited to pick up a microbiology textbook and look at the almost countless (and still incompletely explored) ways that prokaryotes can synthesize ATP. By contrast, in eukaryotes, all known diversity in ATP biosynthetic pathways boils down to glycolysis and three alternative fates of pyruvate (Müller, 1996, 1998; Martin and Müller, 1998) (Figure 3). The dramatic discrepancy between prokaryotes and eukaryotes with regard to diversity in energy metabolic pathways is a surprising (and long-overlooked) explanandum in its own right.

The Hydrogen Hypothesis: A Facultatively Anaerobic Symbiont and an Autotrophic Host

The hydrogen hypothesis (Martin and Müller, 1998) is an alternative to traditional endosymbiotic models (Doolittle, 1998a). It specifically addresses the compartmentalization and the ancestral state of eukaryotic ATP synthesis and it specifically addresses the origins of enzymes for ATP synthesis in both hydrogenosomes and mitochondria (including anaerobic ones) and in the cytosol of both mitochondrion-bearing and amitochondriate eukaryotes. Not unreasonably, the common eubacterial ancestor of hydrogenosomes and mitochondria is viewed as a free-living, facultatively anaerobic proteobacterium that was able to satisfy its energy needs under aerobic or anaerobic conditions, like many (if not most) proteobacteria can do today. It furthermore posits that the host was a *bona fide* archaebacterium, not a eukaryote. It differs uniquely and most radically from previous views in that it posits the archaebacterial host to have been an autotroph – an

obligate autotroph that was strictly dependent upon H_2 as an energy and electron source, like many contemporary archaebacteria (such as methanogens) are.

In a nutshell, the hydrogen hypothesis entails a hypothetical symbiosis between a free-living, H_2- and CO_2-producing eubacterium (the symbiont) and a methanogenic archaebacterium (the host). They would have to meet in anaerobic environments where CO_2 and geological H_2 are abundant, so that the host is viable from the start. But once the pair is physically removed from the H_2 source (by whatever means), the host becomes immediately and strictly dependent upon the heterotrophic eubacterial symbiont, in particular upon H_2 generated from the symbiont's fermentative energy metabolism (Figure 5a). This provides a strong selective force (hydrogen dependence) that irreversibly associates symbiont and host. If the symbiont escapes, the host starves immediately. Such hosts are thus most successful that (i) stick tightly to symbionts, and (ii) can reap the greatest benefit from them. This could conceivably select host cell shapes of large surface area that tend to surround symbionts (not endocytose them, because archaebacteria have no cytoskeleton), increasing contact, so that more H_2 and CO_2 could be filtered through the host's cytosol (Figure 5b).

As long as the symbiont finds sufficient organic substrates, this symbiosis of prokaryotes is indefinitely sustainable, but a limitation becomes evident. Host benefit from increased surface area to the symbiont concomitantly decreases the latter's ability to effervesce gaseous life into its host, because surface contact to the environment for fueling its own metabolism (and producing hydrogen to fuel the host) is impaired. If competition for organic substrates ensues, so will selection for hosts that find a means of utilizing their own environmental surface to import fermentable organic substrates (something which contemporary methanogens cannot do; Thauer *et al*., 1993; Schönheit and Schäfer, 1995) for the symbiont. This could be done by evolutionary invention of something that did not exist prior to the initial meeting (invention of importers of reduced carbon in methanogens), or without invention, by merely genetically rearranging preexisting components: if eubacterial genes for the symbiont's carbon importers are transferred by whatever mechanism to the archaebacterial chromosomes of the host's cytosol, are expressed there, and if the products are functional in the archaebacterial membrane, then the host would in principle be able to feed its symbiont with organics and thus feed itself with H_2 and CO_2 (and acetate, depending upon the capability of the host). This is neither outrageously improbable, nor does it involve an evolutionary invention. It merely requires the genetic systems of eubacterium and archaebacterium to be sufficiently compatible as to allow expression of the transferred gene(s). Such genetic compatibility may be lesser today than it was two or three billion years ago, at which time symbiont and host may have shared a common ancestor only one or two billion years prior. Furthermore, the type of endosymbiotic gene transfer invoked here, *i. e.* without return of the gene product to the cell compartment that donated the gene, is very well-documented among contemporary eukaryotes (Brinkmann and Martin, 1996; Martin and Schnarrenberger, 1997).

But importers alone do not allow the host to feed its symbiont. This is because, in contrast to the heterotrophic metabolism of the symbiont that generates ATP from

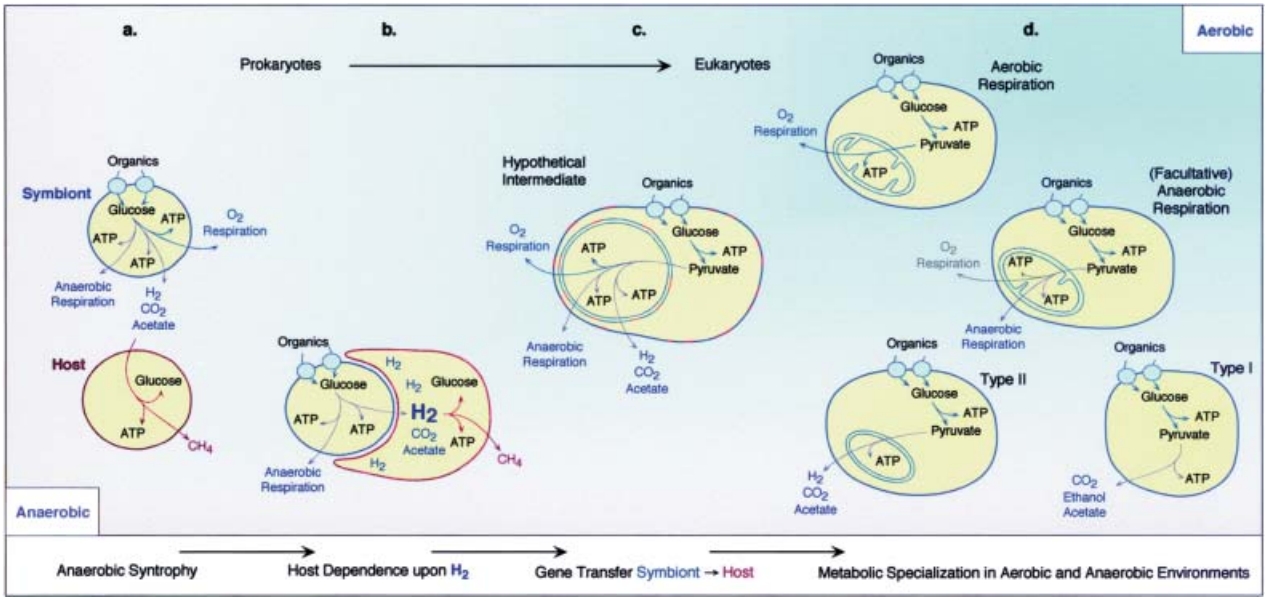

Fig. 5 An Evolutionary Model for the Origins of Mitochondria, Hydrogenosomes, Eukaryotes and Their Heterotrophic Lifestyle (Modified from Martin and Müller, 1998).
Note that in contrast to Figure 2, the transition from anaerobic to aerobic environments does not run parallel to the transition from prokaryotic to eukaryotic cell organization. For details see text.

carbohydrates, the autotrophic metabolism of the host is specialized toward synthesizing carbohydrates from CO_2 at the expense of ATP gained by other means (Thauer *et al*., 1993; Schönheit and Schäfer, 1995). As a consequence, imported carbon flows in the wrong direction – host and symbiont alike will starve unless carbon flux to the symbiont is established, providing strong selection for the latter to occur. To achieve this, either (i) the host's carbohydrate metabolism must acquire, step-by-step, the regulatory properties necessary to make it run backwards (a series of evolutionary inventions), or (ii) the symbiont's carbohydrate metabolism must simply be transferred to the cytosol, again *via* straight endosymbiotic gene transfer (single-step relocation of pre-existing components). This still does not completely solve the problem, because two pathways of carbon metabolism are now running in opposite directions (catabolic and anabolic) in the same cytosol. The result is futile cycling (glucose + ADP $\rightarrow$ C3 compounds + ATP $\rightarrow$ glucose + ADP), selection dictates that one of these pathways must be eliminated. But only if the host's pathway is eliminated, can the symbiosis survive.

This leads to a curious situation. The selective pressure that associated the partners from the start and that drove the integration of eubacterial genes into archaebacterial chromosomes was the host's strict dependence upon hydrogen produced by the symbiont. But by transferring the symbiont's importers and glycolysis to the cytosol in order to satisfy that dependence, the host suddenly can meet both its carbon and energy needs from organic substrates. The functions of both methanogenesis and autotrophy have been replaced, and there is no obvious selective pressure to retain either. The host has irreversibly become heterotrophic, and hydrogen is once again a waste product, but now of a compartmentalized energy metabolism (Figure 5c).

The result of this effortless metabolic endeavour is a hydrogenosome with a genome in an archaebacterial host with cytosolic chromosomes – a cell that is organized in a manner strikingly similar to the amitochondriate eukaryote *Trichomonas vaginalis* (Müller, 1993). That this hypothetical primitive eukaryote does not possess a nuclear membrane is not disturbing; the hydrogen hypothesis simply derives a different stage of the eukaryotic cell cycle (open mitosis) than previous hypotheses do. Not a single evolutionary invention was necessary to deduce this organelle-bearing cell.

Through specialization to aerobic and anaerobic habitats, the forms of energy metabolism found in eukaryotes today can easily be derived. From the generalized ancestral state (a facultatively anaerobic, organelle-bearing heterotroph), differential loss could lead to aerobic or anaerobic energy metabolism in mitochondria or to hydrogenosomes (Type II protists), whereby loss of the organelle altogether would yield the compartmentation of energy metabolism found in Type I protists (Figure 5d). Various manifestations of the organelle, including forms that are biochemically intermediate to mitochondria and hydrogenosomes, as outlined in Figures 3 and 4, are directly accounted for under this model (Martin and Müller, 1998).

What About the Cytoskeleton, the Nucleus, and Other Specifically Eukaryotic Traits?

Of course, the organelle-bearing cell inferred in the hydrogen hypothesis is not strictly a eukaryote because it does not possess a nucleus, but as outlined previously (Martin, 1999a), it is not too difficult to derive a nucleus in that cell. The model in Figure 5 entails straightforward selective pressures (hydrogen dependence) that drive the transfer and fixation of eubacterial genes for the heterotrophic lifestyle to the archaebacterial chromosomes of the host. If copies of the genes for enzymes of eubacterial lipid synthesis were also transferred (*via* chance, hitchhiking or otherwise) from the symbiont's genome to the cytosolic chromosomes of the host, and were expressed there, the immediate result would have been the synthesis of eubacterial lipids in a compartment (the largely archaebacterial cytosol) that very likely was unprepared to accommodate them. In principle, this could have led to the incorporation of eubacterial lipids into the plasma membrane, or the accumulation (by simple phase separation) of the eubacterial lipids as droplets, sheaths, or vesicles surrounding their site of synthesis in the cytosol. In that event, the further accumulation of such lipids would have led, through vesicle fusions, to the seeds of a primitive endomembrane system that, upon continued accumulation, ultimately could be expected to have surrounded the chromosomes harboring the genes that encoded the proteins of the pathway (Martin, 1999a).

That vesicular model to derive a primitive endomembrane system in eukaryotes would follow as a consequence of selection for the transfer of eubacterial genes from the symbiont to the archaebacterial chromosomes of the host. This model would not demand (but would not preclude) the existence of a cytoskeleton prior to the existence of primitive endomembrane vesicles. However, the cell inferred under these premisses would obviously need to evolve some form of primitive cytoskeleton before anything similar to a truly structured endomembrane system and a truly nuclear-like compartment could arise. The backbone of such a cytoskeleton could easily be derived from pre-existing prokaryotic gene products (see below). Considering that the cell in which these processes are assumed here to have occurred is a facultatively anaerobic heterotroph (Martin and Müller, 1998), the first factor limiting its survival would therefore likely have been the ability to obtain sufficient amounts of oxidizable organic compounds to produce ATP for fueling all other cellular processes. This would suggest that primitive endocytosis-like (feeding) processes could have led to the fixation of a cytoskeleton, as de Duve (1969) and Cavalier-Smith (1987b) have argued. But under the views stated here, this would have occurred in a mitochondrion-

bearing cell. Once cytoskeleton, endomembrane vesicles, and their routing in the cell were in place, then perhaps something similar to a nuclear compartment could have arisen. This model is just as speculative as all other models for nuclear origins are (Martin, 1999a), but it differs from them and is not obviously worse. It has the curious property that the mechanism of the inferred origin of the nuclear compartment during evolution and the physical origin of the nuclear envelope during the cell cycle of modern eukaryotes would be very similar – proximal fusion of preformed distal vesicles consisting of eubacterial lipids. Under these premisses, the eukaryotic endomembrane system would have arisen as a fortuitous result of the strongly selected transfer of genes from the genome of a heterotrophic mitochondrial symbiont to the genome of a chemolithoautotrophic archaebacterial host, and hence necessarily occurred subsequent to the origin of mitochondria, not prior.

The cytoskeleton is another eukaryotic-specific attribute, but its most basic components, tubulin and actin, do have homologs among prokaryotes – the cell division proteins FtsZ and FtsA. Protofilaments formed by prokaryotic FtsZ are strikingly similar to those formed by eukaryotic tubulins (Lu *et al*., 2000). Although the sequence similarity between FtsZ and tubulin is slight (Doolittle, 1998; Faguy and Doolittle, 1998), the three-dimensional structures of the proteins are dramatically similar (Burns, 1998). Moreover, a further component of the prokaryotic cell division machinery, FtsA, shares similarity with actin (Doolittle, 1998; Faguy and Doolittle, 1998). Since a true cytoskeleton is one of the attributes that distinguishes eukaryotes from prokaryotes, the transition of these two prokaryotic proteins into their eukaryotic homologs is envisaged by biologists as one of the crucial steps in eukaryotic evolution. Yet with newer data pushing the origin of mitochondria as far back as the origin of eukaryotes themselves (Martin and Müller, 1998; Roger, 1999), there is currently less unanimity as to what came first, the cytoskeleton or the mitochondrion. Though FtsZ seems to occur in all prokaryotes, the gene for FtsA is more prevalent among eubacteria that possess peptidoglycan (Erickson, 1997; Lutkenhaus, 1998), pointing to an intriguing eubacterial (mitochondrial?) connection surrounding the origin of eukaryotic actin. Notably, some mitochondria have been found that still use proteobacterial FtsZ for division (Beech *et al*., 2000).

The eukaryotic 9+2 flagellum is another eukaryote-specific attribute. Well-reasoned models have been proposed that derive the basic components of this structure from simpler, pre-existing prokaryotic components (Rizzotti, 1995), rather than through endosymbiosis with a spirochaete. As far as eukaryotic mitosis goes, there is nothing even vaguely similar in prokaryotes, hence nothing from which it can obviously be derived.

But of course not everything in a eukaryotic cell must be an inheritance from prokaryotes – Darwin's principle of descent with modification includes the possibility of invention. After all, there must have been a time when the ancestor of contemporary eukaryotes did not possess a nucleus or any other typically eukaryotic trait. The question is whether that cell possessed a mitochondrial (hydrogenosomal) symbiont or not.

A Few Predictions and Expectations

The hydrogen hypothesis generates several explicit predictions. One of them is that comparative genome analysis should ultimately reveal evolutionary links between eukaryotes and methanogens; histones, which methanogens possess (Sandman and Reeve, 1998; Sandman *et al*., 1998), can be considered as such a link. Furthermore, Moreira and López-García (1998) argued that in addition to histones, topoisomerases and aspects of lipid biochemistry provide links between eukaryotes and methanogens. Another prediction is that the enzymes integral to energy metabolism in hydrogenosomes in various eukaryotic lineages should reflect a single common origin, rather than multiple origins as models invoking lateral gene transfer to account for anaerobic metabolism in eukaryotes (Doolittle, 1998a; Andersson and Kurland, 2000) predict; this is in line with the present observations for hydrogenase (Horner *et al*., 2000) and for PFO (Horner *et al*., 1999; Rotte *et al*., 2001).

One of the premisses of the hydrogen hypothesis, namely that eukaryotes possess an archaebacterial genetic apparatus (with archaebacterial ribosomes in the cytosol) and eubacterial energy metabolism, has been borne out in previous studies involving yeast (Rivera *et al*., 1998; Ribiero and Golding, 1998; Horiike *et al*., 2001), whereby those data can be interpreted in different ways (Rotte and Martin, 2001). Of course, further data and analyses, particularly of complete genomes, are needed to test the model.

One of the things that the hydrogen hypothesis does not predict – although it has been asserted that it does (Kurland and Andersson, 2000) – is that all eukaryotic proteins which are more similar to eubacterial than to archaebacterial homologs should branch specifically with α-proteobacterial homologs in phylogenetic analyses. There reasons for this are twofold.

First, the phylogenetic signal contained within individual proteins is limited, such that recovering a particular ~2 billion year-old branch for a particular individual protein is not going to be easy, even if the protein were evolving in a completely clock-like manner in all lineages. This can be illustrated with the example of chloroplast genomes. In an analysis of 45 proteins encoded in sequenced chloroplast genomes and commonly inherited from cyanobacteria, only 11 were found to produce the tree that is produced by the entire data set, although all 45 proteins are almost certainly related by identical evolutionary histories (Martin *et al*., 1998). Similar results have been noted in multigene analyses of eukaryotic phylogeny (Baldauf *et al.*, 2000).

Second, there has been quite a bit of horizontal gene

transfer among free-living eubacteria since the origin of mitochondria (Brown and Doolittle, 1997; Martin, 1999b; Doolittle, 1999a,b; Nelson *et al*., 1999; Ochman *et al*., 2000; Eisen, 2000). Thus, the expectation that all eukaryotic genes that, during history, were in fact acquired from the single ancestor of mitochondria (and hydrogenosomes) should branch specifically with homologs found in α-proteobacterial genomes today is based upon the (extremely unlikely) premise that there has been no horizontal gene transfer whatsoever between the free-living relatives of mitochondria and other prokaryotes in the ~2 billion years since mitochondria arose. In other words, it is unlikely that any contemporary eubacterium has exactly the same complement of genes that the ancestor of mitochondria and hydrogenosomes did (Martin, 1999b). This is an important issue, because there have been claims that the presence of eubacterial genes in eukaryotic genomes that do not branch specifically with α-proteobacterial homologs might provide evidence in favor of the view that a bacterium distinct from the ancestor of mitochondria was involved in eukaryote (or organelle) origins or evolution (Gupta, 1998; Andersson and Kurland, 1999; Morrison *et al*., 2001). The problem with this kind of reasoning (for which some of us are partially responsible: Henze *et al*., 1995), is that with increasing numbers of genes and genomes studied all prokaryotes whose genomes have been sequenced will appear to have contributed genes to eukaryotes. For various reasons, this is unlikely to be true (Rotte *et al*., 2001; Rujan and Martin, 2001). Rather, we currently think that lacking phylogenetic resolution of individual proteins, as argued by Forterre and Philippe (1999), and lateral gene transfer between prokaryotes are probably at the root of most of such observations (but we might be wrong).

A specific premise of the hydrogen hypothesis that has been viewed with skepticism (*inter alia* in Roger, 1999) is the argument that it should be possible for an α-proteobacterial symbiont to enter into an intracellular symbiosis with a prokaryotic host through a mechanism that does not require the host to be capable of eukaryotic-like endocytosis, something for which there was no precedent. Surprisingly, von Dohlen *et al*. (2001) recently found γ-proteobacterial symbionts that live within the cytosol of β-proteobacterial hosts. Although the biochemical basis of that intriguing symbiosis is not known, it demonstrates that a prokaryotic symbiont can become established within the cytosol of a prokaryotic host (von Dohlen *et al*., 2001). Analogous to our arguments regarding the nucleus, this again raises the question *ab initio* of what came first in eukaryotic evolution, the cytoskeleton or the mitochondrion (Martin, 2000b).

A clear and explicit prediction of the hydrogen hypothesis is that eubacterial genes for glycolytic enzymes and for importers of reduced carbon compounds (i) should be able to be expressed in archaebacterial (specifically methanogen) chromosomes, (ii) that such gene products should be active in the methanogen cytosol and in the ether lipid membrane, respectively, (iii) that such transfers should have occurred naturally, and (iv) that they should have impaired new biochemical and physiological attributes to the methanogen recipients. The genome sequences of *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum* did not contain obvious cases of such transfers, but the prediction still stands that methanogen genomes will eventually reveal evidence for the occurrence of such lateral gene transfers.

Of course, the most robust falsification of the hydrogen hypothesis would be the finding of a eukaryote for which conclusive evidence can be marshalled that it never possessed a mitochondrial symbiont.

Conclusion

Available data indicate that all eukaryotes studied to date possess either a mitochondrion or a hydrogenosome (an anaerobic form of mitochondria) or that they possessed such an organelle in their evolutionary past. Thus, models for the origin of eukaryotes and mitochondria no longer need to entail the notion that primitively amitochondriate eukaryotes ever existed. Eukaryotes obtain their ATP with the help of glycolysis and through oxidative pyruvate metabolism using either (i) PFO and fermentations in the cytosol, or (ii) PFO and fermentations in hydrogenosomes, or (iii) PDH and a respiratory chain (sometimes without O_2 as the terminal electron acceptor) in mitochondria. Surprisingly, the total diversity of energy metabolism across all eukaryotes studied to date is lesser than that which can be found in a single contemporary free-living proteobacterium such as *Rhodobacter* or even *E. coli*. Both archaebacteria and eubacteria can synthesize ATP in almost countless ways; over 150 different kinds of redox reactions that thermophilic and hyperthermophilic prokaryotes alone employ to make ATP were summarized recently by Amend and Shock (2001). In light of that prokaryotic diversity, eukaryotic energy metabolism is a sample – an extremely narrow sample – of prokaryotic energy metabolism. Much like the photosynthesis in eukaryotes, which is a narrow sample of prokaryotic diversity in photosynthetic physiology, the narrow sample of prokaryotic energy metabolism in eukaryotes suggests that this was a direct inheriance from prokaryotes, perhaps most plausibly through endosymbiosis. Previous evolutionary studies of eukaryotic glycolytic enzymes indicate that, with very rare exceptions (Wu *et al*., 2001), they are a single common inheritance from prokaryotes *in toto* and furthermore, with very rare exceptions (Hannaert *et al*., 2000), that they are an inheritance from eubacteria rather than from archaebacteria. It is possible that eukaryotes obtained not only their mitochondrion (hydrogenosome) and the genes for the enzymes contained therein from an endosymbiotic eubacterium, but that they also obtained from that symbiont the heterotrophic lifestyle that is the backbone of their survival.

Acknowledgements

Work in the authors' laboratory is funded by the Deutsche Forschungsgemeinschaft and the Sonderforschungsbereich Transregio 1. C.R. is the recipient of a stipend from the Studienstiftung des deutschen Volkes.

References

Akhmanova, A., Voncken, F., van Alen, T., van Hoeck, A., Boxma, B., Vogels, G.D., Vennhuis, M., and Hackstein, J.P.G. (1998). A hydrogenosome with a genome. Nature *396*, 527–528.

Amend, J.P., and Shock, E.L. (2001). Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and Bacteria. FEMS Microbiol. Rev. *25*, 175–243.

Amino, H., Wang, H., Hirawake, H., Saruta, F., Mizuchi, D., Mineki, R., Shindo, N., Murayama, K., Takamiya, S., Aoki, T., Kojima, S., and Kita, K. (2000). Stage-specific isoforms of *Ascaris suum* complex. II: the fumarate reductase of the parasitic adult and the succinate dehydrogenase of free-living larvae share a common iron-sulfur subunit. Mol. Biochem. Parasitol. *106*, 63–76.

Andersson, S.G.E., and Kurland, C.G. (1999). Origins of mitochondria and hydrogenosomes. Curr. Opin. Microbiol. *2*, 535–541.

Baldauf, S.L., Palmer, J.D., and Doolittle, W.F. (1996). The root of the universal tree and the origin of eukaryotes based on elongation factor phylogeny. Proc. Natl. Acad. Sci. USA *93*, 7749–7754.

Baldauf, S.L., Roger, A.J., Wenk-Siefert, I., and Doolittle, W.F. (2000). A kingdom-level phylogeny of eukaryotes based on combined protein data. Science *290*, 972–977.

Beech, P.L., Nheu, T., Schultz, T., Herbert, S., Lithgow, T., Gilson, P.R., and McFadden, G.I. (2000). Mitochondrial FtsZ in a chromophyte alga. Science *287*, 1276–1279.

Biagini, G.A., Finlay, B.J., and Lloyd, D. (1997). Evolution of the hydrogenosome. FEMS Microbiol. Lett. *155*, 133–140.

Blackstone, N. (1995). A units-of-evolution perspective on the endosymbiont theory of the origin of the mitochondrion. Evolution *49*, 785–796.

Bogorad, L. (1975). Evolution of organelles and eukaryotic genomes. Science *188*, 891–898.

Bradley, P.J., Lahti, C.J., Plumper, E., and Johnson, P.J. (1997). Targeting and translocation of proteins into the hydrogenosome of the protist *Trichomonas*: similarities with mitochondrial protein import. EMBO J. *16*, 3484–3493.

Brinkmann, H., and Martin, W. (1996). Higher plant chloroplast and cytosolic 3-phosphoglycerate kinases: a case of endosymbiotic gene replacement. Plant. Mol. Biol. *30*, 65–75.

Brown, J.R., and Doolittle, W.F. (1997). Archaea and the prokaryote-to-eukaryote transition. Microbiol. Mol. Biol. Rev. *61,* 456–502.

Buchner, P. (1953). Endosymbiose der Tiere mit pflanzlichen Mikroorganismen (Basel, Switzerland: Birkhäuser).

Bui, E.T., Bradley, P.J., and Johnson, P.J. (1996). A common evolutionary origin for mitochondria and hydrogenosomes. Proc. Natl. Acad. Sci. USA *93*, 9651–9656.

Burns, R. (1998). Synchronized division proteins. Nature *391*, 121–122.

Cavalier-Smith, T. (1975). The origin of nuclei and of eukaryotic cells. Nature *256*, 463–468.

Cavalier-Smith, T. (1987a). Eukaryotes with no mitochondria. Nature *326*, 332–333.

Cavalier-Smith, T. (1987b). The origin of eukaryote and archaebacterial cells. Ann. NY Acad. Sci. *503*, 17–54.

Cavalier-Smith, T. (1987c). The simultaneous symbiotic origin of mitochondria, chloroplasts and microbodies. Ann. NY Acad. Sci. *503*, 55–71.

Cavalier-Smith, T. (1997). Cell and genome coevolution: facultative anaerobiosis, glycosomes and kinetoplastan RNA editing. Trends Genet. *13*, 6–9.

Cavalier-Smith, T., and Chao, E.E. (1996). Molecular phylogeny of the free-living archaezoan *Trepomonas agilis* and the nature of the first eukaryote. J. Mol. Evol. *43*, 551–562.

Clark, C.G., and Roger, A.J. (1995). Direct evidence for secondary loss of mitochondria in *Entamoeba histolytica*. Proc. Natl. Acad. Sci. USA *92*, 6518–6521.

Clemens, D.L., and Johnson, P.J. (2000). Failure to detect DNA in hydrogenosomes of *Trichomonas vaginalis* by nick translation and immunomicroscopy. Mol. Biochem. Parasitol. *106*, 307–313.

Cowan, D. (2000). Use your neighbor's genes. Nature *407*, 466–467.

de Duve, C. (1969). Evolution of the peroxisome. Ann. NY Acad. Sci. *168*, 369–381.

Doolittle, R.F. (1998). Microbial genomes opened up. Nature *392*, 339–342.

Doolittle, W.F. (1980). Revolutionary concepts in evolutionary biology. Trends Biochem. Sci. *5*, 146–149.

Doolittle, W.F. (1996). Some aspects of the biology of cells and their possible evolutionary significance. In: Evolution of Microbial Life, 54th Symp. Soc. Gen. Microbiol., D. Roberts, P. Sharp, G. Alserson and M. Collins, eds. (Cambridge, UK: Cambridge University Press), pp. 1–21.

Doolittle, W.F. (1997). Fun with genealogy. Proc. Natl. Acad. Sci. USA *94*, 12751–12753.

Doolittle, W.F. (1998a). A paradigm gets shifty. Nature *392*, 15–16.

Doolittle, W.F. (1998b). You are what you eat: a gene transfer ratchet could account for eubacterial genes in eukaryotic genomes. Trends Genet. *14*, 307–311.

Doolittle, W.F. (1999a). Phylogenetic classification and the universal tree. Science *284*, 2124–2128.

Doolittle, W.F. (1999b). Lateral genomics. Trends Biochem. Sci. *24*, M5–M8.

Eisen, J. (2000). Horizontal gene transfer among microbial genomes: new insights from complete genome analysis. Curr. Opin. Genet. Dev. *10*, 606–611.

Embley, T.M., and Hirt, R.P. (1998). Early branching eukaryotes? Curr. Opin. Genet. Dev. *8*, 655–661.

Embley, T.M., and Martin, W. (1998). A hydrogen-producing mitochondrion. Nature *396*, 517–519.

Embley, T.M., Finlay, B.J., Dyal, P.L., Hirt, R.P., Wilkinson, M., and Williams, A.G. (1995). Multiple origins of anaerobic ciliates with hydrogenosomes within the radiation of aerobic ciliates. Proc. R. Soc. Lond. B *262*, 87–93.

Embley, T.M., Horner, D.A., and Hirt, R.P. (1997). Anaerobic eukaryote evolution: hydrogenosomes as biochemically modified mitochondria? Trends Ecol. Evol. *12*, 437–441.

Erickson, H.P. (1997). FtsZ, a tubulin homologue in prokaryote cell division. Trends Cell Biol. *7*, 362–369.

Faguy, D.M., and Doolittle, W.F. (1998). Cytoskeletal proteins: the evolution of cell division Curr. Biol. *8*, R338–R341.

Fenchel, T. and Finlay, B.J. (1995). Ecology and Evolution in Anoxic Worlds (Oxford, UK: Oxford University Press).

Finlay, B.J., Span, A.S.W., and Harman, J.M.P. (1983). Nitrate respiration in primitive eukaryotes. Nature *303*, 333–336.

Forterre, P. (1995). Thermoreduction, a hypothesis for the origin of prokaryotes. CR Acad. Sci. III *318*, 415–422.

Forterre, P., and Philippe, H. (1999). Where is the root or the universal tree of life? Bioessays *21*, 871–879.

Germot, A., Philippe, H., and Le Guyader, H. (1996). Presence of

a mitochondrial-type 70-kDa heat shock protein in *Trichomonas vaginalis* suggests a very early mitochondrial endosymbiosis in eukaryotes. Proc. Natl. Acad. Sci. USA *93*, 14614–14617.

Germot, A., Philippe, H., and Le Guyader, H. (1997). Evidence for loss of mitochondria in microsporidia from a mitochondrial-type HSP70 in *Nosema locustae*. Mol. Biochem. Parasitol. *87*, 159–168.

Gibbs, S.P. (1978). The chloroplast of *Euglena* may have evolved from symbiotic green algae. Can. J. Bot. *56*, 2883–2889.

Gilson, P.R., Maier, U.-G., and McFadden, G.I. (1997). Size isn't everything: lessons in genetic miniaturisation from nucleomorphs. Curr. Opin. Genet. Dev. *7*, 800–806.

Gogarten, J.P., Kibak, H., Dittrich, P., Taiz, L., Bowman, E.J., Bowman, B.J., Manolson, M.F., Poole, R.J., Date, T., Oshima, T., Konishi, J., Denda, K., and Yoshida, M. (1989). Evolution of the vacuolar H^+-ATPase: implications for the origin of eukaryotes. Proc. Natl. Acad. Sci. USA *86*, 6661–6665.

Goksøyr, J. (1967). Evolution of eucaryotic cells. Nature *214*, 1161.

Gray, M.W., and Doolittle, W.F. (1982). Has the endosymbiont hypothesis been proven? Microbol. Rev. *46*, 1–42.

Gray, M.W., Burger, G., and Lang, B.F. (1999). Mitochondrial evolution. Science *283*, 1476–1481.

Gupta, R.S. (1998). Protein phylogenies and signature sequences: a reappraisal of evolutionary relationships among archaebacteria, eubacteria, and eukaryotes. Microbiol. Mol. Biol. Rev. *62*, 1435–1491.

Gupta, R.S., and Golding, G.B. (1996). The origin of the eukaryotic cell. Trends Biochem. Sci. *21*, 166–171.

Gupta, R.S., Aitken, K., Falah, M., and Singh, B. (1994). Cloning of *Giaria lamblia* heat shock protein HSP70 homologs: implications regarding origins of eukaryotic cells and of endoplasmic reticulum. Proc. Natl. Acad. Sci. USA *91,* 2895–2899.

Hackstein, J.H.P., Akhmanova, A., Boxma, B., Harhangi, H.R., and Voncken, F.G.J. (1999). Hydrogenosomes: eukaryotic adaptations to anaerobic environments. Trends Microbiol. *7*, 441–447.

Hannaert, V., Brinkmann, H., Nowitzki, U., Lee, J.A., Albert, M.-A., Sensen, C., Gaasterland, T., Müller, M., Michels, P., and Martin, W. (2000). Enolase from *Trypanosoma brucei*, from the amitochondriate protist *Mastigamoeba balamuthi*, and from the chloroplast and cytosol of *Euglena gracilis*: pieces in the evolutionary puzzle of the eukaryotic glycolytic pathway. Mol. Biol. Evol. *17*, 989–1000.

Hausler, D., Stierhoff, Y., Blattner, J., and Clayton, C. (1997). Conservation of mitochondrial targeting sequence function in mitochondrial and hydrogenosomal proteins from the early-branching eukaryotes *Crithidia*, *Trypanosoma* and *Trichomonas*. Eur. J. Cell Biol. *73*, 240–251.

Hensel, R., Zwickl, P., Fabry, S., Lang, J., and Palm, P., (1989). Sequence comparison of glyceraldehyde-3-phosphate dehydrogenase from the three urkingdoms: evolutionary implication. Can. J. Microbiol. *35*, 81–85.

Henze, K., Badr, A., Wettern, M., Cerff, R. and Martin, W. (1995). A nuclear gene of eubacterial origin in *Euglena gracilis* reflects cryptic endosymbioses during protist evolution. Proc. Natl. Acad. Sci. USA *92*, 9122–9126.

Henze, K., Schnarrenberger, C., and Martin, W. (2001). Endosymbiotic gene transfer: a special case of horizontal gene transfer germane to endosymbiosis, the origins of organelles and the origins of eukaryotes. In: Horizontal Gene Transfer, M. Syvanen and C. Kado, eds. (London, UK: Academic Press), pp. 343–352.

Hirt, R.P., Logsdon Jr, J.M., Healy, B., Dorey, M.W., Doolittle, W.F., and Embley, T.M. (1999). Microsporidia are related to fungi: evidence from the largest subunit of RNA polymerase II and other proteins. Proc. Natl. Acad. Sci. USA *96*, 580–585.

Horiike, T., Hamada, K., Kanaya, S., and Shinozawa, T. (2001). Origin of eukaryotic cell nuclei by symbiosis of Archaea in Bacteria is revealed by homology hit analysis. Nature Cell Biol. *3*, 210–214.

Horner, D.S., Hirt, R.P., Kilvington, S., Lloyd, D., and Embley, T.M. (1996). Molecular data suggest an early acquisition of the mitochondrion endosymbiont. Proc. R. Soc. Lond. B *263*, 1053–1059.

Horner, D.S., Hirt, R.P., Embley, T.M. (1999). A single eubacterial origin of eukaryotic pyruvate:ferredoxin oxidoreductase genes: implications for the evolution of anaerobic eukaryotes. Mol. Biol. Evol. *16*, 1280–1291.

Horner, D.S., Foster, P.D., and Embley, T.M. (2000). Iron hydrogenase and the evolution of anaerobic eukaryotes. Mol. Biol. Evol. *17*, 1695–1709.

Höxtermann, E. (1998). Konstantin S. Merezkovskij und die Symbiogenesetheorie der Zellevolution. In: Bakterienlicht und Wurzelpilz, A. Geus, ed. (Marburg, Germany: Basiliken-Presse), pp. 11–29.

Hrdy, I., and Müller, M. (1995). Primary structure of the hydrogenosomal malic enzyme of *Trichomonas vaginalis* and its relationship to homologous enzymes. J. Eukaryot. Microbiol. *42*, 593–603.

Inui, H., Miyatake, K., Nakano, Y., and Kitaoka, S. (1984). Occurence of oxygen-sensitive, $NADP^+$-dependent pyruvate dehydrogenase in mitochondria of *Euglena gracilis*. J. Biochem. *96*, 931–934.

Inui, H., Miyatake, K., Nakano, Y., and Kitaoka, S. (1990). Pyruvate:$NADP^+$ oxidoreductase from *Euglena gracilis*: mechanism of O_2-inactivation of the enzyme and its stability in the aerobe. Arch. Biochem. Biophys. *280*, 292–298.

Iwabe, N., Kuma, K.-I., Hasegawa, M., Osawa, S. and Miyata, T. (1989). Evolutionary relationship of archaebacteria, eubacteria and eukaryotes inferred from phylogenetic trees of duplicated genes. Proc. Natl. Acad. Sci. USA *86*, 9355–9359.

Jain, R., Rivera, M.C., and Lake, J.A. (1999). Horizontal transfer among genomes: the complexity hypothesis. Proc. Natl. Acad. Sci. USA *96*, 3801–3806.

John, P., and Whatley, F.R. (1975). *Paracoccus denitrificans* and the evolutionary origin of the mitochondrion. Nature *254*, 495–498.

Johnson, P., d'Oliveira, C., Gorrell, T., and Müller, M. (1990). Molecular analysis of the hydrogenosomal ferredoxin of the anaerobic protist *Trichomonas vaginalis*. Proc. Natl. Acad. Sci. USA *87*, 6097–6101.

Kandler, O. (1994). The early diversification of life. In: Early Life on Earth, S. Bengtson, ed. (New York, USA: Columbia University Press), pp. 152–160.

Karlberg, O., Canback, B., Kurland, C.G., and Andersson, S.G.E. (2000). The dual origin of the yeast mitochondrial proteome. Yeast *17*, 170–187.

Keeling, P.W., and Doolittle, W.F. (1997). Evidence that eukaryotic triosephosphate isomerase is of alpha-proteobacterial origin. Proc. Natl. Acad. Sci. USA *94*, 1270–1275.

Kitaoka, S., Nakano, Y., Miyatake, K., and Yokota, A. (1989). Enzymes and their functional location. In: The Biology of Euglena, Vol. 6, Subcellular Biochemistry and Molecular Biology, D.E. Buetow, ed. (San Diego, USA: Academic Press), pp. 2–135.

Kobayashi, M., Matsuo, Y., Takimoto, A., Suzuki, S., Maruo, F., and Shoun, H. (1996). Denitrification, a novel type of resipiratory metabolism in fungal mitochondrion. J. Biol. Chem. *271*, 16263–16267.

Koga, Y., Kyuragi, T., Nishihara, M. and Sone, N. (1998). Did ar-

chaeal and bacterial cells arise independently from noncellular precursors? A hypothesis stating that the advent of membrane phospholipid with enantiomeric glycerophosphate backbones caused the separation of the two lines of descent. J. Mol. Evol. *46*, 54 – 63.

Kurland, C.G., and Andersson, S.G.E. (2000). Origin and evolution of the mitochondrial proteome. Microbiol. Mol. Biol. Rev. *64*, 786 – 820.

Lahti, C.J., d'Oliveira, C.E., and Johnson, P.J. (1992). Beta-succinyl-coenzyme-A synthetase from *Trichomonas vaginalis* is a soluble hydrogenosomal protein with an amino-terminal sequence that resembles mitochondrial presequences. J. Bacteriol. *174*, 6822 – 6830.

Lahti, C.J., Bradley, P.J., and Johnson, P.J. (1994). Molecular characterization of the alpha-subunit of *Trichomonas vaginalis* hydrogenosomal succinyl CoA synthetase. Mol. Biochem. Parasitol. *66*, 309 – 318.

Lake, J.A., and Rivera, M.C. (1994). Was the nucleus the first endosymbiont? Proc. Natl. Acad. Sci. USA *91*, 2880 – 2881.

Lang, B.F., Gray, M.W., and Burger, G. (1999). Mitochondrial genome evolution and the origin of eukaryotes. Annu. Rev. Genet. *33,* 351 – 397.

Länge, S., Rozario, C., and Müller, M. (1994). Primary structure of the hydrogenosomal adenylate kinase of *Trichomonas vaginalis* and its phylogenetic relationships. Mol. Biochem. Parasitol. *66*, 297 – 308.

Langer, D., Hain, J., Thuriaux, P., and Zillig, W. (1995). Transcription in Archaea: similarity to that in Eukarya. Proc. Natl. Acad. Sci. USA *92*, 5768 – 5772.

Lederberg, J. (1952). Cell genetics and hereditary symbiosis. Physiol. Rev. *32,* 403 – 430.

Lindmark, D.G., and Müller, M. (1973). Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate, *Tritrichomonas foetus* and its role in pyruvate metabolism. J. Biol. Chem. *248*, 7724 – 7728.

Lloyd, D. (1996). Obligate anaerobe or not? Nature *381*, 121 – 122.

Lu, C., Reedy, M., and Erickson, H.P. (2000). Straight and curved conformations of FtsZ are regulated by GTP hydrolysis. J. Bacteriol. *182*, 164 – 170.

Lutkenhaus, J. (1998). Organelle division: from coli to chloroplasts. Curr. Biol. *8*, R619 –R621.

Mai, Z., Ghosh, S., Frisardi, M., Rosenthal, B., Rogers, R., and Samuelson, J. (1999). Hsp60 is targeted to a cryptic mitochondrion-derived organelle ('crypton') in the microaerophilic protozoan parasite *Entamoeba histolytica*. Mol. Cell. Biol. *19*, 2198 – 2205.

Margulis, L. (1981). Symbiosis in Cell Evolution (San Francisco, USA: W.H. Freeman).

Margulis, L., Dolan, M.F., and Guerrero, R. (2000). The chimeric eukaryote: origin of the nucleus from the karyomastigont in amitochondriate protists. Proc. Natl. Acad. Sci. USA. *97*, 6954 – 6959.

Markos, A., Miretsky, A., and Müller, M. (1993). A glyceraldehyde-3-phosphate dehydrogenase with eubacterial features in the amitochondriate eukaryote *Trichomonas vaginalis*. J. Mol. Evol. *37*, 631 – 643.

Martin, W. (1996). Is something wrong with the tree of life? Bioessays *18*, 523 – 527.

Martin, W. (1999a). A briefly argued case that mitochondria and plastids are descendants of endosymbionts, but that the nuclear compartment is not. Proc. R. Soc. Lond. B *266*, 1387 – 1395.

Martin, W. (1999b). Mosaic bacterial chromosomes – a challenge en route to a tree of genomes. Bioessays *21*, 99 – 104.

Martin, W. (2000a). Primitive anaerobic protozoa: the wrong host for mitochondria and hydrogenosomes? Microbiology *146*, 1021 – 1022.

Martin, W. (2000b). A powerhouse divided. Science *287*, 1219.

Martin, W., and Cerff, R. (1986). Prokaryotic features of a nucleus encoded enzyme: cDNA sequences for chloroplast and cytosolyic glyceraldehyde-3-phosphate dehydrogenases from mustard (*Sinapis alba*). Eur. J. Biochem. *159*, 323 – 331.

Martin, W., and Müller, M. (1998). The hydrogen hypothesis for the first eukaryote. Nature *392*, 37 – 41.

Martin, W., and Schnarrenberger, C. (1997). The evolution of the Calvin cycle from prokaryotic to eukaryotic chromosomes: a case study of functional redundancy in ancient pathways through endosymbiosis. Curr. Genet. *32,* 1 – 18.

Martin, W., Stoebe, B., Goremykin, V., Hansmann, S., Hasegawa, M., and Kowallik, K.V. (1998). Gene transfer to the nucleus and the evolution of chloroplasts. Nature *393*, 162 – 165.

Mereschkowsky, C. (1905). Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. Biol. Centralbl. *25,* 593 – 604. [English translation in Martin, W., and Kowallik, K.V. (1999). Annotated English translation of Mereschkowsky's 1905 paper 'Über Natur und Ursprung der Chromatophoren im Pflanzenreiche.' Eur. J. Phycol. *34*, 287 – 295].

Mereschkowsky, C. (1910). Theorie der zwei Plasmaarten als Grundlage der Symbiogenesis, einer neuen Lehre von der Entstehung der Organismen. Biol. Centralbl. *30*, 278 – 288, 289 – 303, 321 – 347, 353 – 367.

Moreira, D., and Lopez-Garcia, P. (1998). Symbiosis between methanogenic archaea and δ-proteobacteria as the origin of eukaryotes: the syntrophic hypothesis. J. Mol. Evol. *47*, 517 – 530.

Morrison, H.G., Roger, A.J., Nystul, T.G., Gillin, F.D., and Sogin, M.L. (2001). *Giardia lamblia* expresses a proteobacterial-like DnaK homolog. Mol. Biol. Evol. *18*, 530 – 541.

Müller, M. (1988). Energy metabolism of protozoa without mitochondria. Annu. Rev. Microbiol. *42*, 465 – 488.

Müller, M. (1993). The hydrogenosome. J. Gen. Microbiol. *139*, 2879 – 2889.

Müller, M. (1996). Energy metabolism of amitochondriate protists, an evolutionary puzzle. In: Christian Gottfried Ehrenberg-Festschrift anläßlich der 14. Wissenschaftlichen Jahrestagung der Deutschen Gesellschaft fur Protozoologie, 9.-11. March 1995 in Delitzsch/Saxony, M. Schlegel and K. Hausmann, eds. (Leipzig, Germany: Leipziger Universitätsverlag), pp. 63 – 76.

Müller, M. (1997). The evolutionary origin of trichomonad hydrogenosomes. Parasitol. Today *13*, 166 – 167.

Müller, M. (1998). Enzymes and compartmentation of core energy metabolism of anaerobic protists – a special case in eukaryotic evolution? In: Evolutionary relationships among protozoa, G.H. Coombs, K. Vickerman, M.A. Sleigh and A. Warren, eds. (Dordrecht, The Netherlands: Kluwer), pp. 109 – 131.

Müller, M. (2000). A mitochondrion in *Entamoeba histolytica*? Parasitol. Today *16*, 368 – 369.

Müller, M., and Martin, W. (1999). The genome of *Rickettsia prowazekii* and some thoughts on the origin of mitochondria and hydrogenosomes. Bioessays *21*, 377 – 381.

Nakazawa, M., Inui, H., Yamaji, R., Yamamoto, T., Takenaka, S., Ueda, M., Nakano, Y., and Miyatake, K. (2000). The origin of pyruvate:$NADP^+$ oxidoreductase in mitochondria of *Euglena gracilis*. FEBS Lett. *479*, 155 – 157.

Nelson, K.E., Eisen, J.A., Fraser, C.M. *et al*. (1999). Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima.* Nature *399*, 323 – 329.

Nisbet, E.G., and Sleep, N.H. (2001). The habitat and nature of early life. Nature *409*, 1083 – 1091.

Nowitzki, U., Flechner, A., Kellermann, J., Hasegawa, M.,

Schnarrenberger, C., and Martin, W. (1998). Eubacterial origin of eukaryotic nuclear genes for chloroplast and cytosolic glucose-6-phosphate isomerase: Sampling eubacterial gene diversity in eukaryotic chromosomes through symbiosis. Gene *214*, 205–213.

Ochman, H., Lawrence, J.G., and Groisman, E.A. (2000). Lateral gene transfer and the nature of bacterial innovation. Nature *405*, 299–304.

Philippe, H., Germot, A., and Moreira, D. (2000). The new phylogeny of eukaryotes. Curr. Opin. Genet. Dev. *10*, 596–601.

Poole, A., Jeffares, D., and Penny, D. (1999). Prokaryotes, the new kids on the block. Bioessays *21*, 880–889.

Raff, R.A., and Mahler, H.R. (1972). The non symbiotic origin of mitochondria. Science *177*, 575–582.

Reumann, S., and Keegstra, K. (1999). The endosymbiotic origin of the protein import machinery of chloroplastic envelope membranes. Trends Plant Sci. *4*, 302–307

Ribiero, S., and Golding, G.B. (1998). The mosaic nature of the eukaryotic nucleus. Mol. Biol. Evol. *15*, 779–788.

Rivera, M.C., Jain, R., Moore, J.E., and Lake, J.A. (1998). Genomic evidence for two functionally distinct gene classes. Proc. Natl. Acad. Sci. USA *95*, 6239–6244.

Rizzotti, M. (1995). Cilium: origin and 9-fold symmetry. Acta Biotheor. *43*, 227–240.

Roger, A.J. (1999). Reconstructing early events in eukaryotic evolution. Am. Nat. *154*, S146–S163.

Roger, A.J., Clark, C.G., and Doolittle, W.F. (1996). A possible mitochondrial gene in the early-branching protist *Trichomonas vaginalis*. Proc. Natl. Acad. Sci. USA *93*, 14618–14628.

Roger, A.J., Svärd, S.G., Tovar, J., Clark, C.G., Smith, M.W., Gillin, F.D., and Sogin, M.L. (1998). A mitochondrial-like chaperonin 60 gene in *Giardia lamblia*: evidence that diplomonads once harbored an endosymbiont related to the progenitor of mitochondria. Proc. Natl. Acad. Sci. USA *95*, 229–234.

Rotte, C., Henze, K., Müller, M., and Martin, W. (2000). Origins of hydrogenosomes and mitochondria. Curr. Opin. Microbiol. *3*, 481–486.

Rotte, C., and Martin, W. (2001). Does endosymbiosis explain the origin of the nucleus? Nature Cell Biol. *8*, E173–174.

Rotte, C., Stejskal, F., Zhu, G., Keithly, J.S., and Martin, W. (2001). Pyruvate:NADP$^+$ oxidoreductase from the mitochondrion of *Euglena gracilis* and from the apicomplexan *Cryptosporidium parvum*: a fusion of pyruvate:ferredoxin oxidoreductase and NADPH-cytochrome P450 reductase. Mol. Biol. Evol. *18*, 710–720.

Rujan, T., and Martin, W. (2001). How many genes in *Arabidopsis* come from cyanobacteria? An estimate from 386 protein phylogenies. Trends Genet. *17*, 113–120.

Russel, M.J., and Hall, A.J. (1997). The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. J. Geol. Soc. Lond. *154*, 377–402.

Sagan, L. (1967). On the origin of mitosing cells. J. Theoret. Biol. *14*, 225–274.

Sánchez, L.B. (1998). Aldehyde dehydrogenase (CoA-acetylating) and the mechanism of ethanol formation in the amitochondriate protist, *Giardia lamblia*. Arch. Biochem. Biophys. *354*, 57–64.

Sánchez, L.B., Galperin, M.Y., and Müller, M. (2000). Acetyl-CoA synthetase from the amitochondriate eukaryote *Giardia lamblia* belongs to the newly recognized superfamily of acyl-CoA synthetases (nucleoside diphosphate-forming) J. Biol. Chem. *275*, 5794–5803.

Sánchez, L.B., Morrison, H.G., Sogin, M.L., and Müller, M. (1999). Cloning and sequencing of an acetyl-CoA synthetase (ADP-forming) gene from the amitochondriate protist, *Giardia lamblia*. Gene *233*, 225–231.

Sandman, K. and Reeve, J.N. (1998). Origin of the eukaryotic nucleus. Science *280*, 501–503.

Sandman, K., Periera, S.L. and Reeve, J.N. (1998). Diversity of prokaryotic chromosomal proteins and the origin of the nucleosome. Cell. Mol. Life Sci. *54*, 1350–1364.

Sapp, J. (1994). Evolution by Association: A History of Symbiosis (New York, USA: Oxford University Press).

Schäfer, G., Engelhard, M., and Müller, V. (1999). Bioenergetics of the Archaea. Microbiol. Mol. Biol. Rev. *63*, 570–620.

Schimper, A.F.W. (1883). Ueber die Entwickelung der Chlorophyllkörner und Farbkörper. Bot. Ztg. *41*, 105–114.

Schönheit, P., and Schäfer, T. (1995). Metabolism of hyperthermophiles. World. J. Microbiol. Biotechnol. *11*, 26–57.

Stanier, Y. (1970). Some aspects of the biology of cells and their possible evolutionary significance. Symp. Soc. Gen. Microbiol. *20*, 1–38.

Steinbüchel, A., and Müller, M. (1986). Anaerobic pyruvate metabolism of *Tritrichomonas foetus* and *Trichomonas vaginalis* hydrogenosomes. Mol. Biol. Parasitol. *20*, 57–65.

Takaya, N., Suzuki, S., Kuwazaki, S., Shoun, H., Maruo, F., Yamaguchi, M., and Takeo, K. (1999). Cytochrome P450nor, a novel class of mitochondrial cytochrome P450 involved in nitrate respiration in the fungus *Fusarium oxysporum*. Arch. Biochem. Biophys. *372*, 340–346.

Thauer, R.K, Hedderich, R. and Fischer, R. (1993). Reactions and enzymes involved in methanogenesis from CO_2 and H_2. In: Methanogenesis: Ecology, Physiology, Biochemistry and Genetics, J.G. Ferry, ed. (New York, USA: Chapman & Hall), pp. 209–252.

Tielens, A.G.M., and Van Hellemond, J.J. (1998). The electron transport chain in anaerobically functioning eukaryotes. Biochim. Biophys. Acta *165*, 71–78.

Tovar, J., Fischer, A., and Clark, C.G. (1999). The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. Mol. Microbiol. *32*, 1013–1021.

Uzzel, T., and Spolsky, C. (1974). Mitochondria and plastids as emdosymbionts: a revival of special creation? Am. Sci. *62*, 335–343.

Van der Giezen, M., Rechinger, K., Svendsen, B., Durand, R., Hirt, R., Fèvre, M., Embley, T.M., and Prins, R. (1997). A mitochondrial-like targeting signal on the hydrogenosomal malic enzyme from the anaerobic fungus *Neocallimastix frontalis*: support for the hypothesis that hydrogenosomes are modified mitochondria. Mol. Microbiol. *23*, 11–21.

Van der Giezen, M., Kiel, J., Sjollema, K., and Prins, R. (1998). The hydrogenosomal malic enzyme from the anaerobic fungus *Neocallimastix frontalis* is targeted to mitochondria of the methylotrophic yeast *Hansenula polymorpha*. Curr. Genet. *33*, 131–135.

Van Hellemond, J.J., Opperdoes, F.R., and Tielens, A.G.M. (1998). Trypanosomatidae produce acetate *via* a mitochondrial acetate:succinate CoA transferase. Proc. Natl. Acad. Sci. USA *95*, 3036–3041.

van Hoek, A.H.A.M., Akhmanova, A.S., Huynen, M.A., and Hackstein, J.H.P. (2000). A mitochondrial ancestry of the hydrogenosomes of *Nyctotherus ovalis*. Mol. Biol. Evol. *17*, 202–206.

Van Valen L.M., and Maiorana V.C. (1980). The Archaebacteria and eukaryotic origins. Nature *287*, 248–250.

van't Klooster, J.W., van den Berg-Velthuis, G., van West, P., and Govers, F. (2000). *tef1*, a *Phytophthora infestans* gene encoding translation elongation factor 1 α. Gene *249*, 145–151.

Vellai, T., Takács, K., and Vida, G. (1998). A new aspect on the origin and evolution of eukaryotes. J. Mol. Evol. *46*, 499–507.

von Dohlen C.D, Kohler, S., Alsop, S.T., and McManus, W.R.

(2001). Mealybug β-proteobacterial symbionts contain γ-proteobacterial symbionts. Nature *412*, 433 – 436.

Vossbrinck, C.R., Maddox, J.V., Friedman, S., Debrunner-Vossbrinck, B.A., and Woese, C.R. (1987). Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. Nature *326,* 411 – 414.

Wagner, E., Norman, J., Greppin, H., Hackstein, J.H.P., Herrmann, R.G., Kowallik, K.V., Schenk, H.E.A., and Seckbach, J. (1999). In: Endocytobiology VII: From Symbiosis to Eukaryotism (Geneva, Switzerland: Geneva University Press).

Wallin, I.E. (1927). Symbionticism and the Origin of Species (London, UK: Bailliére, Tindall and Cox).

Wächtershäuser, G. (1998). The case for a hyperthermophilic, chemolithoautotrophic origin of life in an iron-sulfur world. In: Thermophiles, J. Wiegel and M.W.W. Adams, eds. (London, UK: Taylor and Francis), pp. 47 – 57.

Whatley, J.M., John, P., and Whatley, F.R. (1979). From extracellular to intracellular: the establishment of chloroplasts and mitochondria. Proc. R. Soc. Lond. B *204*, 165 – 187.

Wilson, E.B. (1928). The Cell in Development and Heredity. 3rd revised edition. Macmillan, New York. 1232 pp. Reprinted in 1987 by Garland Publishing, New York.

Winkler, H.H., and Neuhaus, H.E. (1999). Non-mitochondrial ATP transport. Trends Biochem. Sci. *24*, 64 – 68.

Woese, C.R. (1977). Endosymbionts and mitochondrial origins. J. Mol. Evol. *10*, 93 – 96.

Woese, C.R., Magrum, L.J., and Fox, G.E. (1978). Archaebacteria. J. Mol. Evol. *11,* 245 – 251.

Wu, G., Henze, K., and Müller, M. (2001). Evolutionary relationships of the glucokinase from the amitochondriate protist, *Trichomonas vaginalis.* Gene *264*, 265 – 271.

Zillig, W., Klenk, H.P., Palm, P., Leffers, H., Pühler, G., Gropp, F., and Garrett, R.A. (1989). Did eukaryotes originate by a fusion event? Endocyt. Cell Res. *6*, 1 – 25.