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Mitochondria and hydrogenosomes are two forms of the same fundamental organelle

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Published data suggest that hydrogenosomes, organelles found in diverse anaerobic eukaryotes that make energy and hydrogen, were once mitochondria. As hydrogenosomes generally lack a genome, the conversion is probably one way. The sources of the key hydrogenosomal enzymes, pyruvate : ferredoxin oxidoreductase (PFO) and hydrogenase, are not resolved by current phylogenetic analyses, but it is likely that both were present at an early stage of eukaryotic evolution. Once thought to be restricted to a few unusual anaerobic eukaryotes, the proteins are intimately integrated into the fabric of diverse eukaryotic cells, where they are targeted to different cell compartments, and not just hydrogenosomes. There is no evidence supporting the view that PFO and hydrogenase originated from the mitochondrial endosymbiont, as posited by the hydrogen hypothesis for eukaryogenesis. Other organelles derived from mitochondria have now been described in anaerobic and parasitic microbial eukaryotes, including species that were once thought to have diverged before the mitochondrial symbiosis. It thus seems possible that all eukaryotes may eventually be shown to contain an organelle of mitochondrial ancestry, to which different types of biochemistry can be targeted. It remains to be seen if, despite their obvious differences, this family of organelles shares a common function of importance for the eukaryotic cell, other than energy production, that might provide the underlying selection pressure for organelle retention.

Keywords: hydrogenosomes; mitochondria; eukaryotic evolution; hydrogenase

1. INTRODUCTION

Hydrogenosomes are anaerobic organelles that make energy and excrete molecular hydrogen, aiding redox balancing, which are found in phylogenetically diverse eukaryotes (Müller 1993; Biagini *et al.* 1997*a*). The best studied hydrogenosome-containing groups are trichomonads, ciliates and chytrid fungi but other eukaryotes living in anaerobic habitats probably also contain them (see Broers *et al.* 1993; Fenchel & Finlay 1995; Roger & Silberman 2002). Hydrogenosomes have thus been frequently invented independently during eukaryotic evolution, and the question of how we currently think eukaryotes have achieved this is the focus of this short review. Two key issues must be addressed:

(i) the source(s) of the organelle compartment housing the hydrogen-producing biochemistry; and
(ii) the source(s) of the biochemistry itself, in particular the key enzymes (Müller 1993), PFO and hydrogenase.

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2. ORIGIN OF THE ORGANELLE COMPARTMENT

Shortly after hydrogenosomes were discovered in *Tritrichomonas foetus* by Lindmark & Müller (1973) it was suggested that these hydrogenosomes were descended from endosymbiotic clostridia, a eubacterial group that also makes hydrogen using hydrogenase and PFO, providing a common origin for organelles and biochemistry (Whatley *et al.* 1979). The subsequent demonstration of a double membrane around the hydrogenosomes of *T. foetus* (Benchimol & De Souza 1983), was seen as being consistent with an endosymbiotic origin. At that time, *Trichomonas* and related trichomonads such as *Tritrichomonas* that compose the Parabasalia, were thought never to have had any mitochondria. Parabasalids were one of four protozoan groups placed by Cavalier-Smith (1983) into the eukaryotic subkingdom Archezoa, the others being Archamoebae (e.g. *Entamoeba*), Metamonada (e.g. *Giardia*, *Spironucleus*) and Microspora (e.g. *Vairimorpha*, *Trachipleistophora*). Archezoa were defined as being primitively without mitochondria because they split from other eukaryotes before the mitochondrial symbiosis (Cavalier-Smith 1983).

The first molecular data to suggest that *Trichomonas vaginalis* (and by inference its relatives) once contained the mitochondrion endosymbiont, were discoveries of genes encoding mitochondrial-type heat shock proteins (Hsp70, Hsp60 and Hsp10), on its nuclear genome (Bui *et al.* 1996; Germot *et al.* 1996; Horner *et al.* 1996; Roger *et al.* 1996). In aerobic organisms, these proteins have key roles

in mitochondria, and phylogenetic analysis betrays their common origin from the endosymbiont that gave rise to the organelle (Boorstein *et al.* 1994; Viale & Arakaki 1994). The *Trichomonas* Hsp60 formed a monophyletic group with mitochondrial Hsp60, which was most closely related to the homologous protein GroEL from α-proteobacteria. This is the bacterial group from which the mitochondrial endosymbiont is thought to have originated (Viale & Arakaki 1994; Andersson *et al.* 1998; Gray *et al.* 1999). The simplest interpretation of these data is that the gene for *Trichomonas* Hsp60 also came from the mitochondrial endosymbiont. The GroEL from clostridia clustered in a separate part of the tree, well away from the *Trichomonas* and mitochondrial proteins (Horner *et al.* 1996). The localization of the Hsp60 in *Trichomonas* hydrogenosomes (Bui *et al.* 1996; Bozner 1997), provided the first strong data that the organelle itself shared common ancestry with mitochondria. *Trichomonas* hydrogenosomes, like mitochondria, divide by segmentation and partition (Benchimol *et al.* 1996).

The hypothesis that *Trichomonas* Hsp60 and Hsp70 originated from the same symbiont that gave rise to mitochondria is the simplest interpretation of these data. By contrast, alternative theories have posited horizontal gene transfer from another α-proteobacterium (Sogin 1997), from food bacteria (Doolittle 1998) or from 'more casual donors' (Kurland & Andersson 2000), to explain the presence of Hsp genes on the genomes of *Trichomonas* and other Archezoa. However, none of these alternatives are founded in phylogenetic analyses that actually indicate an alternative donor to the mitochondrial endosymbiont. Recent Hsp60 trees containing *Trichomonas* and *Giardia* are consistent with current ideas of their relationships (Embley & Hirt 1998; Roger 1999), and thus with vertical inheritance of genes from ancestors that contained the mitochondrial endosymbiont (see Horner & Embley 2001). There are now additional data that strongly support a link between *Trichomonas* hydrogenosomes and mitochondria.

Trichomonas hydrogenosomes lack an associated genome (Clemens & Johnson 2000), so their proteins must be synthesized in the cytosol and then correctly targeted and imported. In mitochondria there are two main protein import pathways, each of which is based upon a sophisticated multicomponent pathway that shares some common proteins (Pfanner & Geissler 2001). The mitochondrial import pathways are distinct from the machineries that sort proteins to other organelles such as plastids and peroxisomes. Mitochondrial proteins that are destined for the inner mitochondrial membrane carry cryptic internal targeting signals within the mature proteins that are necessary for import. Other proteins, destined for the mitochondrial matrix, are synthesized as pre-proteins carrying a positively charged targeting sequence at their amino terminus that is cleaved during import. The mitochondrial protein import machinery is so complicated, and correct targeting so important for the host cell, that the same import machinery is unlikely to have evolved twice in different organelles.

There is now good evidence, mainly from Johnson and co-workers, that *Trichomonas* hydrogenosomes import proteins by both mitochondrial pathways. Thus, a number of hydrogenosomal proteins carry N-terminal leader sequences and some of these have also been shown to be cleaved during transit (Plumper *et al.* 1998). For example, *in vitro* import experiments on hydrogenosomal ferredoxin show that it carries a cleavable eight amino-acid N-terminal extension, that is necessary for import into isolated *Trichomonas* hydrogenosomes (Bradley *et al.* 1997). The same targeting sequence can also sort a marker protein into yeast mitochondria (Häusler *et al.* 1997). A member of the mitochondrial carrier family of proteins has recently been isolated from *Trichomonas* hydrogenosomes (Dyall *et al.* 2000). These proteins are found in the inner membrane of mitochondria and are imported using cryptic internal signals (Pfanner & Geissler 2001). The *Trichomonas* protein, which is of unknown function, can also be imported into yeast mitochondria, strongly suggesting that these signals are conserved. In the reciprocal experiment, *Trichomonas* hydrogenosomes can import the yeast AAC protein, confirming that the import machinery required to recognize the yeast-import signals is also present in the hydrogenosome (Dyall *et al.* 2000).

At least four phylogenetically distinct groups of anaerobic ciliates contain hydrogenosomes (see Embley *et al.* 1995), suggesting that the transition from aerobe with mitochondria to anaerobe with hydrogenosomes, is fairly easy to make within this group of predominantly aerobic eukaryotes. Hydrogenosomal ciliates are ecologically significant, providing a niche for endosymbiotic methanogens that use the hydrogen and carbon dioxide liberated by the hydrogenosomes, to make methane and energy (Fenchel 1993; Embley & Finlay 1994). It has been argued that the facility by which ciliates make hydrogenosomes must result from modification of pre-existing mitochondria (Embley *et al.* 1995), and in some species, for example *Metopus contortus* and *Cyclidium porcatum*, the hydrogenosomes strongly resemble mitochondria in their morphology (Finlay & Fenchel 1989; Fenchel & Finlay 1995). The hydrogenosomes of *M. contortus* also resemble mitochondria physiologically, being calcium stores and possessing a membrane potential and an alkali lumen (Biagini *et al.* 1997*b*). At least one ciliate, *Nyctotherus ovalis*, apparently contains a mitochondrial small subunit ribosomal RNA gene (Akhmanova *et al.* 1998). Although the gene product has not been localized to the ciliate hydrogenosome, such genes are normally encoded by the mitochondrial genome (Gray *et al.* 1998). Thus, in at least one case, there is the possibility that a ciliate hydrogenosome may have retained a genome. The question of what it might encode is an intriguing one, as mitochondrial genomes typically encode components of the respiratory chain, thought to be absent in hydrogenosome-containing organisms. However, the mitochondrial genomes of the aerobic ciliates *Paramecium* and *Tetrahymena* are already known to share several open reading frames of as yet unknown function (Burger *et al.* 2000).

The evolutionary origin of chytrid fungal hydrogenosomes has been the subject of considerable debate. Reports of a single 'microbody-like' boundary membrane around the hydrogenosomes of *Neocallimastix* (Yarlett *et al.* 1986; Marvin-Sikkema *et al.* 1993*b*), fuelled suggestions that fungal hydrogenosomes were modified peroxisomes (Cavalier-Smith 1987; Hackstein *et al.* 1998). Support for this hypothesis was claimed from reports of an SKL peroxisomal targeting motif, detected using a het-

erologous antibody, on a putative nickel–iron–selenium [Ni–Fe–Se]-hydrogenase from *Neocallimastix frontalis* L2 (Marvin-Sikkema *et al.* 1993*b*). However, neither protein nor gene was isolated and sequenced to confirm their identity or the presence of the SKL motif. A carboxyl terminal peroxisomal targeting motif has also been claimed for genes encoding chytrid hydrogenosomal adenylate kinase, although the genes themselves are apparently of mitochondrial ancestry (Hackstein *et al.* 1998). Unfortunately, neither these data nor an associated and intriguing claim for the presence of elements of the peroxisomal-import machinery in chytrids (Hackstein *et al.* 1998) has yet been fully published.

By contrast, two separate laboratories have published electron micrographs showing two boundary membranes around *Neocallimastix* hydrogenosomes (Benchimol *et al.* 1997; Van der Giezen *et al.* 1997*b*). These membranes look like the two membranes that surround *Trichomonas* hydrogenosomes (Benchimol *et al.* 1996). Hydrogen production by *Neocallimastix* hydrogenosomes is reduced by bongkrekic acid and carboxyatractylate, which are inhibitors of the mitochondrial AAC in aerobic eukaryotes (Marvin-Sikkema *et al.* 1994). Recent attempts to confirm the presence of a [Ni–Fe–Se]-hydrogenase in *Neocallimastix* have failed and it has been suggested that the data supporting the presence of this enzyme and its SKL motif are ambiguous (Davidson *et al.* 2002; Voncken *et al.* 2002*b*). *Neocallimastix* has now been shown to contain an iron-only [Fe]-hydrogenase, a type that is distinct from [Ni–Fe–Se]-hydrogenases (Davidson *et al.* 2002; Voncken *et al.* 2002*b*). This protein lacks any SKL motif at its carboxyl terminus—in fact *none* of the published hydrogenosomal proteins contain this motif. Other properties that fungal hydrogenosomes share with mitochondria, but not peroxisomes, include the existence of a transmembrane pH gradient and an alkaline lumen. Free Ca^{2+} pools and calcium phosphate precipitates have also been detected in fungal hydrogenosomes suggesting that, like mitochondria, they accumulate this intracellular messenger (Biagini *et al.* 1997*a*).

As for *Trichomonas* hydrogenosomes, the strongest evidence that fungal hydrogenosomes were once mitochondria comes from the demonstration that they import proteins in the same ways. *Neocallimastix* hydrogenosomes also lack a genome (Van der Giezen *et al.* 1997*b*), so any proteins that they contain must be synthesized in the cytosol and imported. Hydrogenosomal ME is encoded in the nucleus and targeted to the hydrogenosome using a cleaved amino-terminal targeting signal, which resembles those of yeast mitochondrial proteins (Van der Giezen *et al.* 1997*a*). Hydrogenosomal ME is also selectively imported into mitochondria, rather than peroxisomes, in the heterologous host *Hansenula polymorpha,* in an N-terminal presequence dependent manner (Van der Giezen *et al.* 1998). Other hydrogenosomal proteins, including hydrogenase and β-succinyl CoA synthetase, also have N-terminal extensions resembling mitochondrial leader sequences (Brondijk *et al.* 1996; Davidson *et al.* 2002; Voncken *et al.* 2002*b*). These data suggest that *Neocallimastix* hydrogenosomes use the classic N-terminal cleaved transit peptide import pathway that is found in mitochondria.

Strong evidence that hydrogenosomes also use the second 'cryptic internal signal' mitochondrial pathway for membrane proteins comes from recent studies of the fungal hydrogenosomal AAC (Van der Giezen *et al.* 2002). This protein transports ADP and ATP across the hydrogenosome membrane; a key function for any energy generating organelle. It is now clear that fungal hydrogenosomes and yeast mitochondria use the same protein to carry out this function. Thus, the hydrogenosomal AAC has similar properties in primary structure, mode of transport and sensitivity towards inhibitors as its mitochondrial counterpart (Van der Giezen *et al.* 2002; Voncken *et al.* 2002*a*). Phylogenetic analyses demonstrate its common ancestry with mitochondrial AAC from aerobic fungi, in a tree that is consistent with its vertical inheritance from a common mitochondrial ancestor (see Van der Giezen *et al.* 2002). Crucially, when the hydrogenosomal protein is expressed in an AAC-deficient yeast, it is correctly imported into the yeast mitochondrial inner membrane and restores its ability to exchange ATP for ADP—making a functional mitochondrion (Van der Giezen *et al.* 2002).

3. ORIGIN OF PFO

Several microaerophilic microbial eukaryotes including *Entamoeba*, *Giardia* and *Trichomonas* use the oxygen-sensitive enzyme PFO to decarboxylate pyruvate to acetyl-CoA (Müller 1998). In *Trichomonas*, PFO is localized within hydrogenosomes whereas in *Entamoeba* and *Giardia* it is thought to be cytosolic. In aerobic eukaryotes the decarboxylation of pyruvate is catalysed in the mitochondrion by the non-homologous enzyme PDH (Kerscher & Oesterhelt 1982). The distribution of PFO and PDH in eukaryotes is so far mutually exclusive, although some eubacteria, for example *Escherichia coli,* use both enzymes under different circumstances (Kerscher & Oesterhelt 1982). When it was thought that *Entamoeba*, *Giardia* and *Trichomonas* were Archezoa and thus primitively without mitochondria (Cavalier-Smith 1983), the differential distribution of PFO and PDH was explained by the hypothesis that PFO was the ancestral eukaryotic enzyme, being replaced in aerobic eukaryotes by PDH from the protomitochondrion (Kerscher & Oesterhelt 1982).

The story became more complicated when it was shown that all of the species that contain PFO also contain genes of mitochondrial ancestry (Clark & Roger 1995; Bui *et al.* 1996; Germot *et al.* 1996; Horner *et al.* 1996; Roger *et al.* 1996, 1998; Horner & Embley 2001). It is also now apparent that the evidence for these species branching before other eukaryotes with *bona fide* mitochondria is weak. *Entamoeba* is related to the aerobic mitochondria-containing slime mould *Dictyostelium* on the basis of multiple gene trees (Horner & Embley 2001; Bapteste *et al.* 2002) and on morphological data (Cavalier-Smith 1998). The hypothesis that *Giardia* and *Trichomonas* branch before other eukaryotes is based mainly upon gene trees that were constructed using unrealistic assumptions about how genes evolve. Furthermore, the identification of an early branching eukaryote assumes that we know where the root of the eukaryotic tree lies. Currently, there is no consensus as to where this root might be, but there are no compelling published data that it lies on either the *Giardia* or *Trichomonas* branches (Kumar & Rzhetsky 1996; Hirt

et al. 1999). The presence of a derived gene fusion that affects dihydrofolate reductase and thymidylate synthase (Philippe *et al.* 2000), has recently been used to argue that the root splits animals, fungi and choanoflagellates from other eukaryotes, including *Giardia* and *Trichomonas* (Stechmann & Cavalier-Smith 2002).

There are also several gene trees that suggest that *Giardia* (representing metamonads) and *Trichomonas* (parabasalids) are related to each other (Embley & Hirt 1998; Roger 1999; Henze *et al.* 2001; Horner & Embley 2001). If *Giardia* and *Trichomonas* are related, and the *Trichomonas* hydrogenosome is a modified mitochondrion, then *Giardia* must have either lost mitochondria, or they persist but have not been recognized as such. Another former archezoan, the microsporidian *Trachipleistophora hominis,* is now known to contain a tiny, and previously overlooked, organelle of mitochondrial ancestry and unknown function (Williams *et al.* 2002). There are also suggestions from the earlier literature that candidate structures exist in *Giardia*. Cheissen (1965) described structures that he suggested were 'changed mitochondria' produced in response to low-oxygen conditions. So it is probably worth looking more closely at *Giardia* using modern techniques to see if it really lacks an organelle (Lloyd & Harris 2002).

Several hypotheses have been proposed for the origin of PFO in anaerobic eukaryotes. Eukaryotic enzymes more closely resemble eubacterial homodimeric PFO than they do the multimeric PFOs that have been described for archaebacteria (see Kletzin & Adams 1996), and most hypotheses have posited eubacterial donors. An origin of PFO (and hydrogenase) from clostridia was predicted by Whatley *et al.* (1979), based upon their hypothesis of a separate endosymbiotic origin for the *Trichomonas* hydrogenosome. Rosenthal *et al.* (1997) analysed eight PFO sequences, including those from *Entamoeba*, *Giardia* and *Trichomonas* and concluded that there was little evidence for the common ancestry of these eukaryotic PFO, preferring separate origins from different prokaryotes, and in the case of *Entamoeba* possibly from an enterobacterium. The hydrogen hypothesis (Martin & Müller 1998) for the contemporaneous origin of eukaryotes and the acquisition of the mitochondrial endosymbiont posits that PDH and PFO (and hydrogenase) were donated by this endosymbiont, a possibility also discussed by Embley *et al.* (1997). The most recent phylogenetic analyses of PFO sequences (Horner *et al.* 1999; Rotte *et al.* 2001) recovered eukaryotic sequences together in a single cluster. We find the same in figure 1, where we have analysed all of the currently available versions of this protein and its homologues from the databases and genome projects.

Monophyly of the eukaryotic sequences make it probable that PFO was already present in early eukaryotes. However, the sparse sampling of eukaryotic species and genomes make it difficult to draw strong inferences about the mode of inheritance of this gene *within* the eukaryotic cluster. The weak clustering of the metamonad and parabasalid enzymes is consistent with the posited relationship between these two groups, and thus with vertical inheritance of their PFO genes from a common ancestor. The precise branching pattern between the *Entamoeba* PFO and the N-terminal PFO domains of the PNO fusion proteins of *Cryptosporidium* and *Euglena* is not resolved; for example, our tree for these three sequences is different from that of Rotte *et al.* (2001). As in previous analyses, the eukaryotic sequences are not the nearest neighbours of sequences from the α-proteobacteria (Horner *et al.* 1999; Rotte *et al.* 2001). Thus, the currently available data provide no support for the hypothesis (Embley *et al.* 1997; Martin & Müller 1998) that eukaryotic PFO originated from the mitochondrial endosymbiont.

There are no published PFO sequences from chytrid fungi or from hydrogenosomal ciliates, but there are biochemical data that are consistent with the presence of PFO in hydrogenosomes of some species. Thus, PFO and hydrogenase are enriched in the fraction containing hydrogenosomes, in extracts from the ciliates *Dasytricha ruminantium* (Yarlett *et al.* 1981; Lloyd *et al.* 1989), *Eudiplodinium* and *Epidinium* (Yarlett *et al.* 1984) and *Isotricha* (Yarlett *et al.* 1983). PFO is also enriched with hydrogenase in hydrogenosomal fractions from the chytrid fungus *Neocallimastix patriciarum* (Yarlett *et al.* 1986) and *N. frontalis* L2 (Marvin-Sikkema *et al.* 1993*a*). In contrast, O'Fallon *et al.* (1991) failed to detect PFO in *N. frontalis* EB188. Whereas Akhmanova *et al.* (1999) have recently suggested that *Piromyces* sp. E2 and *N. frontalis* L2, the same strain studied by Marvin-Sikkema *et al.* (1993*a*), use pyruvate formate lyase, rather than PFO, as the key pyruvate-metabolizing enzyme in their hydrogenosomes. The apparent conflict regarding the presence or absence of PFO in the hydrogenosomes of *N. frontalis* L2 may be due in part to differing growth conditions of the two experiments. For example, it has already been shown for *N. patriciarum* that PFO activity is suppressed by growth under CO_2 (Yarlett *et al.* 1986). Moreover, Akhmanova *et al.* (1999) did not actually assay for PFO activity but only failed to detect PFO genes by PCR. The available data on whether or not *N. frontalis* L2 hydrogenosomes contain PFO are thus incomplete. In the absence of PFO it has been suggested that reducing equivalents are generated by the oxidation of malate to pyruvate by ME (O'Fallon *et al.* 1991; Williams & Lloyd 1993; Akhmanova *et al.* 1999). The NADPH/NADH could then be coupled to hydrogen production either indirectly (O'Fallon *et al.* 1991) or directly (Akhmanova *et al.* 1999).

4. ORIGIN OF EUKARYOTIC HYDROGENASE

Genes encoding hydrogenases have now been cloned and characterized for *T. vaginalis* (Bui & Johnson 1996; Horner *et al.* 2000), the hydrogenosomal ciliate *N. ovalis* (Akhmanova *et al.* 1998) and the chytrid fungi *N. frontalis* L2 and *Piromyces* sp. E2 (Davidson *et al.* 2002; Voncken *et al.* 2002*b*). All of these genes encode iron-only [Fe]-hydrogenases of a type found in eubacteria but not in archaebacteria, and each contains the distinctive [Fe–S]-cluster that constitutes the active site or 'hydrogen' cluster (Nicolet *et al.* 2000). In the hydrogenosomes of *Trichomonas,* pyruvate is oxidized by PFO and electrons are transferred via ferredoxin and hydrogenase to protons resulting in the generation of molecular hydrogen (Müller 1993; Kulda 1999). *T. vaginalis* contains genes for at least three [Fe]-hydrogenases displaying length variation at the N-terminus. A 'long-form' [Fe]-hydrogenase contains four putative accessory [FeS]-clusters at the N-terminus, like some eubacterial enzymes (see Horner *et al.* 2002).

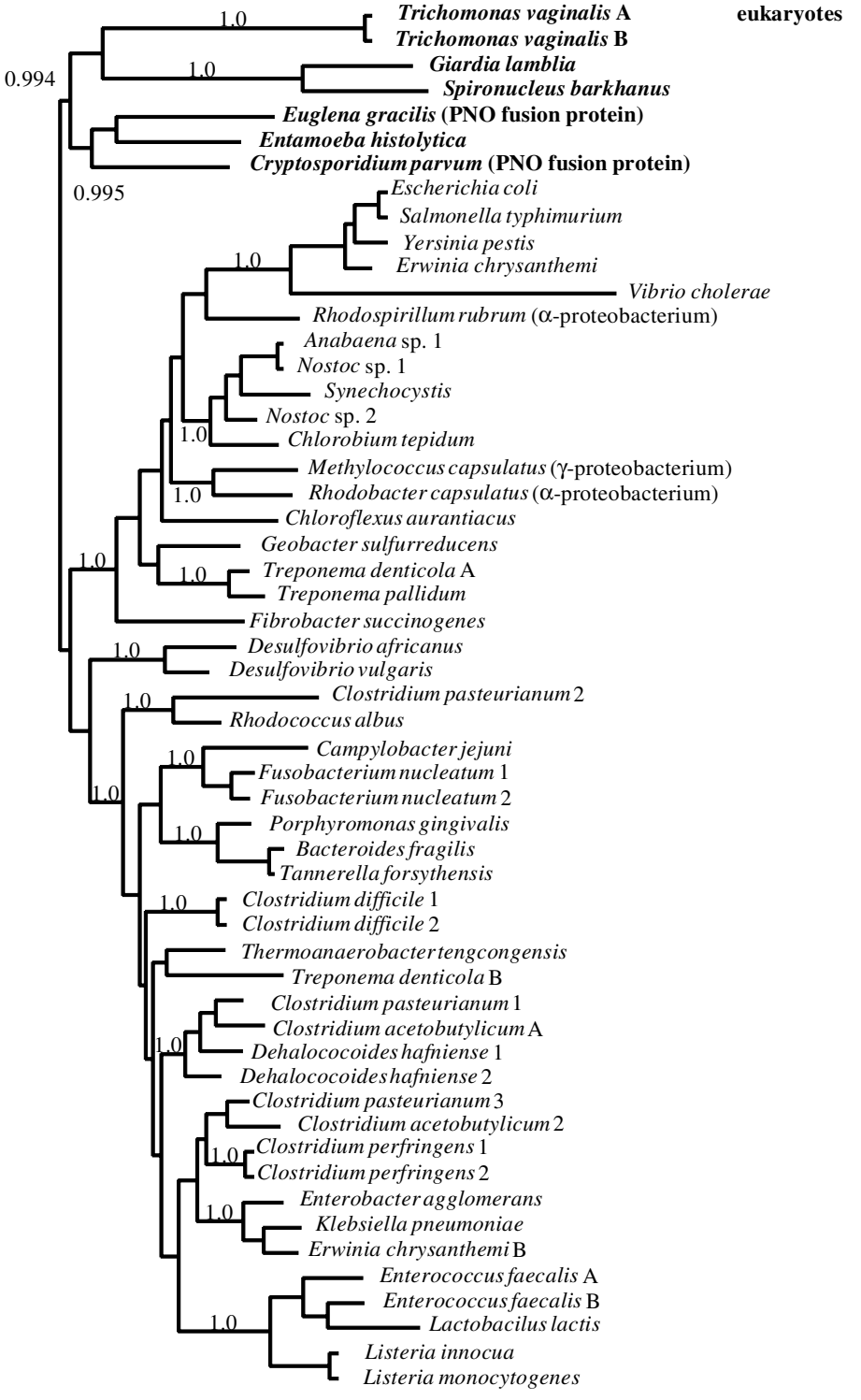

Figure 1. A phylogenetic tree showing the relationships between PFOs from eukaryotes and eubacteria. For Bayesian phylogenetic analysis of the PFO and hydrogenase sequences aligned positions were recoded from the amino acid data into the six Dayhoff groups: C, STPAG, NDEQ, HRK, MILV and FYW. This is based on the notion that changes within the groups will be common and noisy, whereas changes between groups will be rarer and so have less saturation. The approach thus has similarities to the more commonly used transversion-only analysis of DNA sequences. The model used a 6×6 reversible rate matrix with 14 free parameters and 5 free composition parameters. Among-site rate variation was modelled with a free proportion of invariable sites, and a four-category discrete γ-distribution for rates at variable sites, with a free-shape parameter. The analysis used the Metropolis-coupled Markov chain Monte Carlo variant (Huelsenbeck & Ronquist 2001), in software available on request from P. G. Foster (pfoster@nhm.ac.uk). All model parameters were optimized during the running of the chain, which was allowed to run for 2 million generations, sampled every 500 generations, with the first 3000 samples being discarded as burn-in. The results show the consensus of the remaining sampled trees. The posterior probabilities for some groups are shown on both trees, with a value of 1.0 representing maximum support. The strongly supported groups have also been recovered in previously published analyses using different methods, including maximum likelihood and different support measures (Horner *et al.* 1999, 2000; Rotte *et al.* 2001; Voncken *et al.* 2002*b*; Davidson *et al.* 2002).

Trichomonas also contains two 'short-form' [Fe]-hydrogenases that lack the terminal [2Fe2S]-cluster and the first [4Fe4S]-cluster (Bui & Johnson 1996). The functional significance of this structural variation for hydrogen production by *Trichomonas* is unknown, but the long-form hydrogenase may correspond to the 64 kDa enzyme previously purified from *T. vaginalis* (Payne *et al.* 1993).

The *N. ovalis* [Fe]-hydrogenase is unique among eukaryotic enzymes in that it carries additional domains at its carboxyl terminus (Akhmanova *et al.* 1998). It was initially claimed that these were the 51 kDa and 24 kDa subunits of the mitochondrial respiratory chain (Hackstein *et al.* 1999), encouraging the suggestion that hydrogenases may have been 'stitched together' more than once from bits and pieces of mitochondrial genes (Andersson & Kurland 1999). Phylogenetic analysis provides no support for this hypothesis (Horner *et al.* 2000). The 51 kDa subunit of the *Nyctotherus* enzyme is not part of the cluster containing mitochondrial 51 kDa subunits and NuoF proteins from α-proteobacteria. Instead, the *Nyctotherus* protein clusters with the NuoF components of the C-terminal domains of other eubacterial hydrogenases. Thus, although the *Nyctotherus* NuoF-like domain is certainly related to mitochondrial proteins, it is not of mitochondrial ancestry. It has been suggested that the *Nyctotherus* enzyme uses its NuoF- and 24 kDa/NuoE-like components to interact with NADP(H) directly, as do some eubacterial hydrogenases (Pilkington *et al.* 1991; Albracht *et al.* 1997). Thus, unlike the *Trichomonas* enzyme, the *Nyctotherus* fusion protein may not depend on ferredoxin as an electron donor (Akhmanova *et al.* 1998, 1999).

The [Fe]-hydrogenase in *N. patriciarum* has not been cloned but has been suggested to function like the *Trichomonas* enzyme, with both ferredoxin and PFO involved (Yarlett *et al.* 1986; Williams & Lloyd 1993). By contrast, it was suggested that chytrid hydrogenases might be similar to the *Nyctotherus* fusion protein in containing accessory domains that are able to interact directly with NADP(H), as depicted in fig. 10 in Akhmanova *et al.* (1999). The [Fe]-hydrogenase from *N. frontalis* L2 has now been cloned and shown to lack accessory domains at its carboxyl terminus (Davidson *et al.* 2002; Voncken *et al.* 2002*b*). It thus resembles the long-form *Trichomonas* enzyme in its primary structure, rather than the *Nyctotherus* fusion protein.

It has been known for a long time that green algae can produce hydrogen (Gaffron & Rubin 1942). The enzyme is located in the chloroplast stroma and is linked via ferredoxin to the photosynthetic electron transport chain (Happe *et al.* 1994). Green algal plastids thus make hydrogen under anaerobic conditions in the light. The genes from *Chlorella fusca*, *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* code for very short [Fe]-hydrogenases, which lack all accessory Fe–S clusters at the N-terminus (Florin *et al.* 2001; Happe *et al.* 2002; Winkler *et al.* 2002). They appear to function satisfactorily in the absence of these clusters, with the catalysis of hydrogen production and other biochemical results being similar to *Clostridium pasteurianum* (Florin *et al.* 2001). Electrons are transferred directly from ferredoxin to the catalytic domain of the protein. The presence of [Fe]-hydrogenases in plastids is surprising, because cyanobacteria use [Ni–Fe]-hydrogenases in their hydrogen-generating pathways and lack [Fe]-hydrogenases. The [Ni–Fe]-hydrogenase of the cyanobacterial endosymbiont was apparently replaced by a host-encoded [Fe]-hydrogenase, early in the evolution of green algal plastids.

One of the most surprising developments of recent years concerning hydrogenases was the finding of [Fe]-hydrogenases in non-photosynthetic eukaryotes that do not contain hydrogenosomes. Genes encoding [Fe]-hydrogenases have now been discovered in *Giardia* (J. E. Nixon, J. Field, J. Samuelson and M. L. Sogin, unpublished data), in its relative *Spironucleus* and in the intestinal parasite *Entamoeba histolytica* (Horner *et al.* 2000). The genes from *Entamoeba* and *Spironucleus* are expressed at the mRNA level and they contain all of the residues known to be important to the function of [Fe]-hydrogenases (Horner *et al.* 2000). It has now been shown that *Giardia* can make hydrogen at a rate that is about tenfold less (2 nmol min^{-1} per 10^7 *Giardia*) than *T. vaginalis* (Lloyd *et al.* 2002*a*). The key to its detection was the development of a highly sensitive and selective way of measuring hydrogen (Lloyd *et al.* 2002*a*). Given that the organization of the *Entamoeba* and *Spironucleus* genes closely resemble that of *Giardia*, it seems probable that these species can also make hydrogen. Possible reasons for hydrogen production include a role in redox balancing by getting rid of any excess reducing equivalents (Lloyd *et al.* 2002*b*).

Equally remarkable are recently discovered genes bearing the hallmark signatures of [Fe]-hydrogenases on our own genome and in the genomes of other aerobic eukaryotes (Barton & Worman 1999; Horner *et al.* 2000). These genes that are here termed NARF-like after the first one to be discovered—nuclear prelamin A recognition factor (Barton & Worman 1999), contain the unique hydrogen cluster that is found in all [Fe]-hydrogenases (Horner *et al.* 2000). Database searches have revealed that NARF-like genes are present in the genomes of a wide variety of different eukaryotes (Horner *et al.* 2002), including the smallest eukaryotic genome sequenced so far, that of the intracellular microsporidian parasite *Encephalitozoon cuniculi* (Katinka *et al.* 2001). The function of these proteins in different eukaryotes, and if they can make hydrogen, is unknown. Human NARF is localized to the nucleus and interacts with prenylated prelamin A, the precursor form of lamin involved in the maintenance of the structural integrity of the metazoan nucleus (Barton & Worman 1999). Yeast apparently lacks lamin but does contain a NARF-like gene, the deletion of which is lethal in a haploid background (Winzeler *et al.* 2000).

The phylogeny of [Fe]-hydrogenases is difficult to resolve because the gene sequences are mutationally saturated, and subject to compositional biases that are not fully mitigated at the amino-acid level (Horner *et al.* 2000). These problems translate into trees where there is little support for most relationships. However, the tree we show here (figure 2) and the previously published ones (Horner *et al.* 2000; Davidson *et al.* 2002; Horner *et al.* 2002; Voncken *et al.* 2002*b*), do suggest that eukaryotic [Fe]-hydrogenases are not monophyletic to the exclusion of bacterial sequences. Simulation and likelihood ratio test of monophyly have also been used to show that the hydrogenase data can reject the null hypothesis of eukaryotic monophyly (Horner *et al.* 2000). Thus, although the current data cannot exclude a common origin for eukaryotic

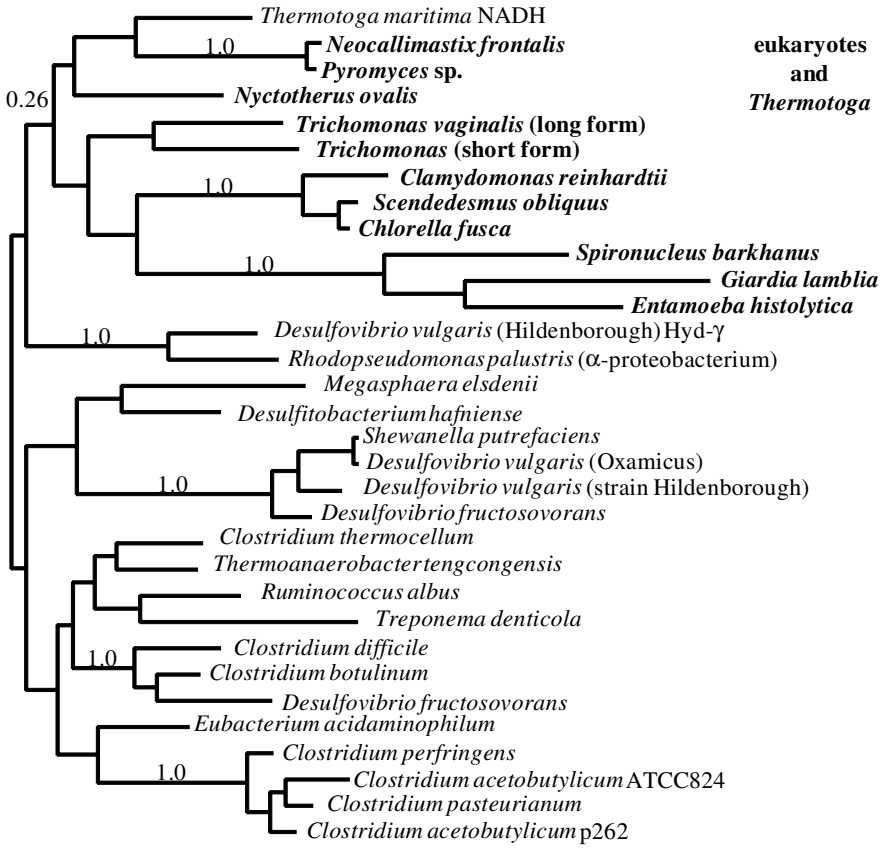

Figure 2. The relationships between [Fe]-hydrogenases from eukaryotes and eubacteria. See legend to figure 1 for details of analysis.

enzymes, the trees also suggest that *Thermotoga maritima* might have acquired its hydrogenase gene by horizontal gene transfer from a eukaryote. The hydrogen hypothesis (Martin & Müller 1998) posits that hydrogen transfer from the mitochondrial endosymbiont to an archaebacterial 'host' was the first step in eukaryogenesis, and thus it predicts an α-proteobacterial origin for eukaryotic hydrogenase. Our analyses provide no support for a close relationship between the eukaryotic sequences and the only α-proteobacterial [Fe]-hydrogenase sequence from *Rhodopseudomonas palustris*. The latter forms a strongly supported relationship (Davidson *et al.* 2002) with one of two different [Fe]-hydrogenases (hyd-γ) from *Desulfovibrio vulgaris* (strain Hildenborough), a member of the δ-proteobacteria. It is not clear whether the short NARF-like proteins, which are not included in figure 2, share a common origin with other eukaryote Fe-hydrogenases, although they do appear to share a single common origin among themselves and an early origin in eukaryotes (Horner *et al.* 2002; Voncken *et al.* 2002*b*). The other genes encoding [Fe]-hydrogenases also show considerable heterogeneity in length. The presence of two types of hydrogenase in *T. vaginalis*, the loss of all accessory FeS clusters in the algal enzymes, and the formation of the hydrogenase fusion protein in *N. ovalis* all suggest that structural modifications are common aspects of [Fe]-hydrogenase evolution in eukaryotes.

Within the eukaryotic cluster there is strong support for the monophyly of fungal sequences suggesting a single common origin for the *Neocallimastix* and *Piromyces* enzymes (Voncken *et al.* 2002*b*). A single origin for the three green algal enzymes is also strongly supported (Horner *et al.* 2002). Support for monophyly of the long- and short-form *Trichomonas* enzymes is not so strong, but the best tree found indicates a single origin followed by structural divergence. The position of the enzyme from the ciliate *Nyctotherus* is unstable. The strongly supported relationship between the two metamonad sequences, *Giardia* and *Spironucleus*, and the one from *Entamoeba* is surprising, as there are no published data that suggest that these species share a common ancestor exclusive of other eukaryotes. Moreover, it appears from the tree that the sequence from *Giardia* is more closely related to the *Entamoeba* sequence than to the other metamonad *Spironucleus* sequence. One possible explanation for this topology is to posit a horizontal gene transfer to an ancestor of *Entamoeba* from an ancestor of *Giardia*. However, the relationship between *Giardia* and *Entamoeba* was lost when we used a method that allowed different composition and rate matrices across the tree (not shown). In these analyses *Giardia* and *Spironucleus* clustered together, with *Entamoeba* the outgroup to this pair. Further work is needed to understand the basis for this behaviour and to investigate the evolution of eukaryotic [Fe]-hydrogenases.

5. CONCLUSIONS

Current data suggest that hydrogenosomes and mitochondria share common ancestry. As there are no strong data indicating that hydrogenosomal eukaryotes branch

before mitochondrial eukaryotes, it appears that all hydrogenosomes are secondary adaptations of mitochondria. For ciliates this is almost certainly true, because hydrogenosomal lineages are clearly descended from mitochondria-containing ancestors, and the nearest relatives of ciliates, the apicomplexa and dinoflagellates, also contain mitochondria. As hydrogenosomes lack a genome, with one possible exception in *Nyctotherus*, the conversion is probably one way only. There are also less drastic modifications of mitochondrial biochemistry (Embley & Martin 1998; Tielens & Van Hellemond 1998). These include the mitochondria of flatworms, which can use fumarate as terminal electron acceptor (Tielens & Van Hellemond 1998), and the mitochondria of ciliates and fungi, which can use nitrates (Finlay *et al.* 1983; Kobayashi *et al.* 1996). Organelles of mitochondrial ancestry but unknown function have now been described in the anaerobic amoeba *Entamoeba histolytica* (Mai *et al.* 1999; Tovar *et al.* 1999), and a tiny organelle has been found in the microsporidian parasite *Trachipleistophora hominis* (Williams *et al.* 2002). The jury remains out as to whether *Giardia* contains an organelle—but it does contain genes (Roger *et al.* 1998; Horner & Embley 2001; Morrison *et al.* 2001), whose products (Hsp60, Hsp70) localize to an organelle in other eukaryotes. It is possible that all eukaryotes will eventually be found to contain an organelle of mitochondrial ancestry, providing a flexible compartment to which different types of biochemistry can be conveniently located.

Some of these organelles can make energy, but the details of what else they can do, their detailed biochemistry, including their similarities and differences, are still poorly understood. For example, it is not known if they all carry out other essential functions for the host cell, providing, despite their many differences, a common selection pressure to retain the organelle under diverse living conditions. The maturation of Fe–S clusters is reported to be an essential function of yeast mitochondria (Lill & Kispal 2000). However, it is currently not known if the various hydrogenosomes, and the other recently described organelles in anaerobes and parasites, also fulfil this function. *Trichomonas* and *Giardia* contain elements of this pathway and the *Trichomonas* IscS protein contains a plausible hydrogenosomal targeting sequence (Tachezy *et al.* 2001). The genome of the highly reduced microsporidian parasite *Encephalitazoon* also contains genes for making Fe–S clusters, and it was on this basis that a putative organelle with a role in Fe–S cluster assembly was first predicted (Katinka *et al.* 2001). An organelle of mitochondrial ancestry has now been found in the microsporidian *Trachipleistophora hominis* (Williams *et al.* 2002), so this *in silico* prediction can now be tested. Data from the ongoing *Entamoeba* genome project should also provide the starting material to begin investigating the functions of its organelle of mitochondrial ancestry, the mitosome (Tovar *et al.* 1999).

Genes bearing the hallmark signatures of [Fe]-hydrogenases and PFO are found on the genomes of aerobic as well as anaerobic eukaryotes. [Fe]-hydrogenases and PFO are closely integrated into the fabric of diverse eukaryotes, and not just those with hydrogenosomes. This is consistent with a long history for both proteins within the eukaryotic cell. The so far mutually exclusive distribution of NARF-like genes and longer hydrogenases on available genomes indicates that there might be some overlap in function, perhaps related to redox sensing or balancing, but it is not known if all of the encoded proteins can actually make hydrogen. The mitochondria of *Euglena* contain a pyruvate : NADP oxidoreductase, a fusion protein containing PFO domains linked to a C-terminal NADPH-cytochrome P450 reductase domain (Rotte *et al.* 2001). The same fusion is found in the unrelated apicomplexan *Cryptosporidium parvum* but its cell localization is unknown (Rotte *et al.* 2001). In *Saccharomyces* and other fungi, PFO domains are combined with fragments of redox proteins to form fusion proteins that participate in methionine biosynthesis (Kobayashi & Yoshimoto 1982; Hansen *et al.* 1994; Horner *et al.* 1999).

The origins of PFO and hydrogenase are not clearly resolved by current data and methods of phylogenetic analysis. A common origin for eukaryotic enzymes cannot be rejected, but the source of the genes is unclear given poor sampling and our inability to resolve the roots of the gene trees. The genes could be ancestral to eukaryotes, or the result of one or more early horizontal gene transfers from eubacteria. Archaebacteria contain different kinds of PFO and hydrogenase and so are less likely donors. There is no evidence from current trees and gene sampling that the genes for PFO and [Fe]-hydrogenase came together from the mitochondrial endosymbiont, as suggested previously (Embley *et al.* 1997) and by the hydrogen hypothesis for eukaryogenesis (Martin & Müller 1998). However, the sample of eubacteria is still small and the trees for PFO and hydrogenase already reveal a very complex picture for the evolution of these enzymes. For example, there is evidence of multiple copies of genes, which cluster in different parts of the tree, on the same eubacterial genome (figures 1 and 2). Of potentially greater concern are claims of rampant horizontal gene transfer among prokaryotes (Doolittle 1999), which could have been even more prevalent in the deep past when barriers to transfer might have been lower (Martin 1999). Under this scenario the mitochondrial endosymbiont might have already contained genes originating from other prokaryotes (Martin 1999), making the testing of hypotheses, like the hydrogen hypothesis, much more difficult using phylogenetic analysis. However, it might still be too early to be so pessimistic. Claims of large-scale HGT have often been based upon BLAST similarity scores, an approach that is potentially flawed when applied crudely, or other surrogates, instead of detailed phylogenetic analysis (Eisen 2000; Koski & Golding 2001; Ragan 2001*a*,*b*; Salzberg *et al.* 2001). Thus, although no one can doubt that HGT between prokaryotes does occur, its extent and effect on prokaryotic genomes and phylogeny are still being determined.

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Discussion

A. G. M. Tielens (*Faculty of Biology, Utrecht University, Utrecht, The Netherlands*). Martin, thanks for a lovely talk, but you mentioned that certain ciliates contain hydrogenosomes, while others contain mitochondria. What could be the reason that there are no ciliates, nor in my opinion any other organisms that contain both organelles, because even nowadays there will be many environments that will be at one time aerobic and at another time anaerobic, and there are organisms that are adjusted to that, so why not by means of having these two types of organelle?

T. M. Embley. Well, I guess that if one is converted from the other. Are you suggesting that some in a population may be converted and others not? It is difficult to see how that could happen.

A. G. M. Tielens. Or you could still have the intermediate form, if you do not want to have the two extremes.

T. M. Embley. I cannot think of any good reason why you cannot … but no, there are no cases. What there are cases of, are ciliates that are aerobic that can colonize anaerobic environments. They change the morphology of their mitochondria, are more electron dense and they lose cristae, but there is no evidence that they can make hydrogen. Bill, do you have a suggestion for why we do not get intermediate forms?

W. Martin (*Institute of Botany III, Heinrich-Heine Universität, Düsseldorf, Düsseldorf, Germany*). Well, as we have been saying in publications for a couple of years now, I think we do have intermediate forms in *Euglena*. Euglenas have PFO; that is published data. They also have pyruvate dehydrogenase, so they do not make hydrogen, which is the functional definition of a hydrogenosome. However, they do have one of the key enzymes that until now was specific to hydrogenosomes, namely the PFO fusion enzyme. So I would agree with you that we would be unlikely to see both organelles in the same cell, but it would not be surprising to see transitions between the different forms.

A. G. M. Tielens. That is what I meant, having a second option.

T. M. Embley. I did not show the data. Ciliates are special because you get nesting of hydrogenosomes with this predominantly aerobic radiation, and one thing that confused us is that the *Nyctotherus* hydrogenase appears to be somewhat different from other published hydrogenases. We have recently found a hydrogenase from *Trimyema* a ciliate that is completely unrelated to *Nyctotherus*, and this hydrogenase is exactly the same type. And if you make a tree, it forms a monophyletic group with the *Nyctotherus* hydrogenase. Now my interpretation of this is that ciliates got their hydrogenases at the start of the radiation, and have retained this enzyme. We are looking for additional evidence of this hydrogenase in aerobic ciliates; certainly the *Trimyema* and *Nyctotherus* hydrogenases are very, very similar, and in trees they form a monophyletic group.

W. Martin. Notwithstanding the problems of the *Thermotoga* enzyme, we go back to the hydrogenase tree, and we look at *Rhodopseudomonas*, that was your red. I think that you only have to invoke one lateral transfer from the *Rhodopseudomonas* lineage to *Desulfovibrio* which I think was its sister, which has apparently acquired a lot of hydrogenase genes; so if you were to take the *Desulfovibrio* out, then it would look more like what we were expecting, but I believe in these trees as little as you do.

T. M. Embley. Yes, you can invoke a small number of events and make these trees say whatever you want. All I would say is there is no strong positive support from them that can distinguish between any of these hypotheses that are out there. But what is interesting to me is the widespread distribution of iron hydrogenase, which we thought was restricted to a small number of eukaryotes, and the relationships between the eukaryotic enzymes…. *Giardia* has it, as do *Spironucleus* and *Entamoeba*: what is it doing in these organisms?

W. Martin. Is it known what the α-proteobacterial iron hydrogenase does? Is it just a genome sequence?

T. M. Embley. No; yes.

J. Tovar (*School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, UK*). How does the evolution—what we know of the evolution of PFO—fit with the evolution of the bag itself and of the hydrogenase?

T. M. Embley. It looks like eukaryotic PFO is monophyletic; you cannot exclude that possibility in any analysis that we have done and that we have published. Some of the relationships within the eukaryotic clade are rather unusual, but then there are different types of PFO. There are some types of PFO that are fusions; yeast have taken bits of PFO and have made a sulphite reductase by fusing it to domains of other proteins, so there is again a complex history going on. The data, I think, are consistent with an early acquisition of PFO, with retention in various lineages. Again, we cannot get a sister-group relationship with α-proteobacteria, and here there is better sampling of α-proteobacterial genes. I think there are three, and none of them falls into a monophyletic group with the PFO from eukaryotes. One thing I would like to say, and it echoes back to something Ford Doolittle said, and which I think Bill Martin alluded to, and that is that the sampling is pitiful here. It would be extremely surprising if we had actually sampled a contemporary organism that was related to the ancestor that gave rise to this eukaryotic enzyme. And even if it was an α-proteobacterium, we have got such a small sample that I am very cautious when interpreting these trees. They do not provide strong sup-

port for any of the hypotheses, but cannot robustly reject any of them either.

A. E. Douglas (*Department of Biology, University of York, York, UK*). You quite clearly indicated that peroxisomes are very unlikely to be allied to hydrogenosomes. Do you have any views about the origins of peroxisomes?

T. M. Embley. I do not think there is any strong evidence for an endosymbiotic origin of peroxisomes. Tom [Cavalier-Smith] has written about this—is there any strong evidence now?

T. Cavalier-Smith (*Department of Zoology, University of Oxford, Oxford, UK*). I do not think so, no. I have tended to have gone away from that to the idea that they come from the endomembrane system, but I think that there are a number of problems.

A. E. Douglas. And that would add circumstantial support to your more direct evidence that hydrogenosomes, even in the chytrids, are allied to mitochondria.

T. M. Embley. Yes, I do not think there is any strong evidence out there that suggests chytrid hydrogenosomes have elements of peroxisomes involved.

A. Barbrook (*Department of Biochemistry, University of Cambridge, Cambridge, UK*). How sensitive is your parametric bootstrap to the initial tree that you model the data on?

T. M. Embley. Parametric bootstrapping is not reported to perform correctly if the model does not fit the data, and we did test that. The problem is that the tests that are out there to test the fit of the data and the model are fairly insensitive themselves, so we used the Goldman test, which is the classic one, and we found that the general time-reversible model seemed to fit, and, along with the tree that we used, plausibly could have given rise to the data we observe in front of us. And we have also done some Bayesian tests recently and again it seems to fit; the data seem to fit that particular model, and really that is all we can think of doing.

A. Barbrook. I just wondered if you have tried changing the tree slightly.

T. M. Embley. Yes, the parameters would not change very much.

A. E. Douglas. Just before this session, you indicated that perhaps we would be able to answer some of your questions. Do you have any questions that you wish to ask the audience?

T. M. Embley. Yes, I wanted someone to tell me what they believe was the core function of the mitochondrial organelle. If it is not oxidative phosphorylation, and it is not production of hydrogen, what could it be? Mitochondria do all sorts of things, haem biosynthesis, producing iron–sulphur clusters. Does anyone out there have an idea? Let us suppose all eukaryotes have got a mitochondrial organelle. Whatever it does, it is an organelle bag derived from the mitochondrial endosymbiont. What would people predict it would be doing? What would be a general selection pressure for the retention of this organelle? Roland Lill has suggested that iron–sulphur cluster assembly is a key function of yeast mitochondria, so does anyone know if the machinery for iron–sulphur cluster assembly is mitochondrial in origin? That raises the question of what did the host cell do before it got the mitochondrial endosymbiont. If it was an archaean, did it make iron–sulphur clusters in the way archaebacteria do? So what happened to those genes and proteins in eukaryotes?

J. F. Allen (*Plant Biochemistry, Lund University, Lund, Sweden*). Why do you rule out ATP synthesis?

T. M. Embley. Well, I do not rule out ATP synthesis, but hydrogenosomes do not carry out oxidative phosphorylation; they make ATP by substrate-level phosphorylation. It is fine that they can do that, but with the *Entamoeba* mitosome, there is only one per cell, so it is unlikely to be involved in energy metabolism. But it is of mitochondrial ancestry.

W. Martin. Was it not David Lloyd who recently showed that *Giardia* makes hydrogen?

T. M. Embley. Yes, I referred to that.

W. Martin. OK. I must have missed that. And is there any evidence for localization of that hydrogen production, compartmentalized within the cell? Does anybody know?

T. M. Embley. David [Lloyd] could not find any, and people working on *Giardia* have looked at the localization of cpn60. Soltys and Gupta have looked at it, and they found a punctate distribution for cpn60, which I guess is consistent with potential localization within a compartment. Andrew Roger reported diffuse localization, if my memory serves me correctly, and Dave Lloyd, in the papers recording hydrogen production by *Giardia*, said he could not localize it to a particular compartment. They tried a number of antibodies, but they did not try an antibody to the hydrogenase.

D. S. Horner (*Dipartimento di Fisiologia e Biochimica Generali, University of Milan, Milan, Italy*). As an adjunct to that, I have a feeling that David published in a separate paper this year some Mitotracker experiments, which showed something—not localization, but something that was potentially a bag.

N. Lane (*Department of Surgery, Royal Free and University College Medical School, London, UK*). Whatever mitochondria started out as, what their purpose was, all kinds of degenerate purposes may now be the main one. There was a paper published fairly recently (by Kristina Peachman and colleagues) but showing, I think, that eosinophils have completely lost their oxidative phosphorylation, and exist basically for the purpose of apoptosis now.

A. E. Douglas. It seems that the chief function of organelles is to be a membrane-bound compartment.

T. M. Embley. Yes, you can organize all kinds of neat biochemistry in there.

GLOSSARY

AAC: ADP–ATP carrier
HGT: horizontal gene transfer
ME: malic enzyme
NARF: nuclear prelamin A recognition factor
PDH: pyruvate dehydrogenase
PFO: pyruvate : ferredoxin oxidoreductase
PNO: pyruvate : NADP+ oxidoreductase
SKL: serine, lysine, leucine