Review Article

The hydrogenosome

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Introduction

It was noted two decades ago that several trichomonad species, parasitic unicellular eukaryotes, contain no mitochondria but have another type of membrane-bounded organelle, which was termed the hydrogenosome (Lindmark & Müller, 1973, 1974*b*; Lindmark *et al.*, 1975). This organelle is defined by its unusual function: under anaerobic conditions it produces molecular hydrogen by oxidizing pyruvate or malate. More recently organelles with similar biochemical properties have been found in a number of additional protist groups, all from oxygen-poor or anoxic environments: certain free-living ciliates (van Bruggen *et al.*, 1983; Finlay & Fenchel, 1989; Fenchel & Finlay, 1991*a*) and rumen ciliates (Ciliata) (Yarlett *et al.*, 1981, 1983, 1984; Snyers *et al.*, 1982; Paul *et al.*, 1990) and chytrid fungi (Chytridiomycota) (Yarlett *et al.*, 1986*a*; Marvin-Sikkema *et al.*, 1992). Their presence in additional protist groups is likely. An interesting case is the amoeboid flagellate *Psalteriomonas lanterna* (Broers *et al.*, 1990), which possibly contains both hydrogenosomes and mitochondria. No hydrogenosomes have yet been found in multicellular animals or plants. The broad systematic distribution of hydrogenosomes raises a number of interesting questions: what is their functional contribution to the protist cell, are all hydrogenosomes homologous organelles, and what is their evolutionary origin. In this review, I summarize our knowledge on hydrogenosomes and explore its evolutionary implications. Various aspects of hydrogenosomes have been discussed in earlier reviews (Müller, 1980; Čerkasov *et al.*, 1980; Williams, 1986; Müller, 1988; Lindmark *et al.*, 1989; Fenchel & Finlay, 1991*a*; Finlay & Fenchel, 1993; Johnson *et al.*, 1993; Yarlett, 1994).

Ultrastructure

The morphology of hydrogenosomes varies from group to group (Fig. 1*a–d*). No common tell-tale features enable us to identify hydrogenosomes by their morphology alone. Trichomonad and other parabasalid hydrogenosomes (Fig. 1*a*) were first described as microbody-like in view of their relatively uniform matrix, apparently single membrane and lack of extensive intra-organellar membrane systems (Müller, 1973). Subsequent studies of sectioned and freeze-fractured material revealed two closely apposed unit membranes around the organelle (Benchimol & de Souza, 1983; Honigberg *et al.*, 1984). Often there is a flattened vesicle at the periphery. The morphology of the organelle in rumen ciliates has received less attention (Fig. 1*b*) but they seem to be similar to the trichomonad organelles (Anderson & Dumont, 1966; Yarlett *et al.*, 1981, 1983, 1984; Paul *et al.*, 1990). In rumen fungi, hydrogenosomes vary in their appearance depending on the life-cycle stages of the organism (Yarlett *et al.*, 1986*a*; Marvin-Sikkema *et al.*, 1992). In the hyphae they are microbody-like (Fig. 1*c*) while in the zoospores they are elongated and have a peduncular extension (Fig. 1*h* in Marvin-Sikkema *et al.*, 1992). In both the ciliates and fungi the hydrogenosomes are often described as microbody-like with the implication that they are surrounded by a single membrane (Yarlett *et al.*, 1981; Marvin-Sikkema *et al.*, 1992), but in the ciliate *Polyplastron multivesiculatum* a double membrane has been noted (Paul *et al.*, 1990). This point needs further studies. There is little similarity to mitochondria in the above cases. In contrast, hydrogenosomes of free-living ciliates show a more elaborate internal organization with a surrounding double membrane and they often closely resemble mitochondria (Fig. 1*d*) (Finlay & Fenchel, 1989). These morphological differences could reflect fundamental differences in the biological nature of hydrogenosomes of various organisms.

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Fig. 1. Hydrogenosomes (H) in various protists. (a) *Trichomonas vaginalis* (parabasalid flagellate from human genito-urinary tract); (b) *Isotricha ruminantium* (ciliate from sheep rumen); (c) *Neocallimastix* sp. L2 hypha (fungus from llama rumen); (d) *Trimyema* sp. (freshwater ciliate) (M, methanogenic symbiont). Unpublished micrographs by Helen Shio (a), Bernard Vigués (b) and Bland Finlay (see Finlay *et al.*, 1993) (d). (c) With permission from Marvin-Sikkema *et al.* (1992). Bars, 1 μ m.

Biochemistry

Trichomonad flagellates

Isolated intact hydrogenosomes of *Trichomonas vaginalis* and *Tritrichomonas foetus* produce under anaerobic conditions approximately equimolar amounts of CO₂, H₂ and acetate from pyruvate (Fig. 2). This process is accompanied by disappearance of ADP from the medium and appearance of ATP in it (Steinbüchel & Müller, 1986b). Usually somewhat less H₂ than CO₂ is produced. Anaerobic metabolism of isolated hydrogenosomes is dependent on the presence of ADP and phosphate, as well as of catalytic amounts of succinate (Steinbüchel & Müller, 1986b). In the presence of oxygen the organelles also produce CO₂ and acetate and phosphorylate ADP to ATP. No H₂ is formed, however, and oxygen is consumed, i.e. the organelle assumes a respiratory function (Čerkasov *et al.*, 1978; Yarlett *et al.*, 1986b). This aerobic metabolism is rapidly inactivated indicating that oxygen is toxic for the isolated organelles.

Fig. 3 shows a minimal metabolic pathway that accounts for the observed functions of isolated hydrogenosomes. Pyruvate, formed by glycolysis, enters the organelle where it is oxidatively decarboxylated (reaction 1). The acetyl group is linked to coenzyme A thus forming acetyl-CoA from which free acetate is liberated (reaction 5). The CoA moiety of acetyl-CoA is subsequently transferred to succinate (reaction 5) forming succinyl-CoA. The latter serves as substrate for a substrate-level phosphorylation reaction producing ATP (reaction 6). The isolated organelle can also produce malate through reductive carboxylation of pyruvate (reaction 2). Reducing equivalents derived from the oxidation of pyruvate (reaction 1) are transferred to protons with the formation of H₂ (reaction 4) under anaerobic conditions. If O₂ is present, it serves as an electron acceptor and is reduced, probably not to H₂O but to H₂O₂ or O₂⁻. In the presence of NAD⁺, isolated hydrogenosomes can utilize malate as substrate. Malate is oxidatively decarboxylated to pyruvate (reaction 2) which serves as substrate for reaction (1). The re-oxidation of NADH is probably via reduction of ferredoxin (reaction 3). Whether pyruvate or malate is the substrate *in situ* has not been settled. Although pyruvate kinase is present in *T. vaginalis* (Mertens *et al.*, 1992) it could not be detected in *T. foetus* (Hrdý & Mertens, 1993); thus in this species at least, malate might be the substrate for hydrogenosomes *in situ*.

Studies on the biochemical composition of trichomonad hydrogenosomes have underscored the unusual nature of these organelles. All enzyme activities which can account for the proposed anaerobic metabolic processes have been detected and characterized in some detail.

Fig. 2. Anaerobic metabolism of isolated *Trichomonas vaginalis* hydrogenosomes. An organelle-enriched fraction was incubated under osmotic protection with pyruvate as a substrate in the presence of ADP. (a) Production of H₂ (□), CO₂ (●) and acetate (■) in almost equimolar quantities; ○, malate. (b) Utilization of ADP (△) and production of ATP (▲); ▼, AMP. For further details see Steinbüchel & Müller (1986b). Reproduced with the permission of Elsevier Science publishers.

The enzyme responsible for pyruvate oxidation, a key enzyme of the organelle (reaction 1), has been identified as pyruvate:ferredoxin oxidoreductase in several species (Lindmark & Müller, 1973, 1974b; Lindmark *et al.*, 1975) and has been purified to homogeneity from *T. vaginalis* (Williams *et al.*, 1987). It is a homodimer of two 120 kDa subunits and contains thiamin pyrophosphate and iron-sulphur centres but no lipoate or lipoamide. This oxygen-sensitive enzyme catalyses the oxidative decarboxylation of pyruvate with the formation of acetyl-CoA or, in the reverse direction, the formation of pyruvate from acetyl-CoA and CO₂ (Lindmark & Müller, 1973; Williams *et al.*, 1987). *In vivo* probably only the forward reaction occurs. Its natural electron acceptor is ferredoxin. The *T. foetus* pyruvate:ferredoxin oxidoreductase reaction is unusual, involving two free radical intermediates (Docampo *et al.*, 1987). Related enzymes are found only in a few other anaerobic protists but are widespread in bacteria, primarily in obligate anaerobes, and in archaeobacteria. There seems to be no kinship between trichomonad pyruvate:ferredoxin oxidoreductase and the pyruvate dehydrogenase complex found in mitochondria and in various aerobic and facultative anaerobic bacteria.

Of the enzymes involved in the further metabolism of acetyl-CoA, acetate:succinate CoA transferase (reaction 5) has been detected but not studied in any detail (Lindmark, 1976). An ADP-specific succinate thiokinase (succinyl-CoA synthase) is responsible for substrate-level phosphorylation in trichomonad hydrogenosomes (reaction 6). The *T. vaginalis* enzyme is a 150 kDa heterotetramer of α - and β -subunits (Jenkins *et al.*, 1991). The amino acid sequence of the β -subunit shows 43 %

Fig. 3. Schematic metabolic map of hydrogenosomes. 1, Pyruvate:ferredoxin oxidoreductase; 2, malate dehydrogenase (decarboxylating) (NAD); 3, NAD:ferredoxin oxidoreductase; 4, H_2 :ferredoxin oxidoreductase; 5, acetate:succinate CoA-transferase; 6, succinate thiokinase. Arrows indicate assumed physiological directions *in vivo*. Dashed arrows indicate the *in vitro* detected formation of malate via a reversed reaction (2), and a postulated adenyl nucleotide transfer. The box reflects only the organellar localization of the enzyme activities but does not reflect their relationship to the organelle envelope. Modified from Steinbüchel & Müller (1986b). Reproduced with the permission of Elsevier Science publishers.

identity to the same subunit from *Escherichia coli* (Lahti *et al.*, 1992). The same enzyme in *T. foetus* is a 75 kDa dimer (Jenkins *et al.*, 1991).

Pyruvate oxidation by pyruvate:ferredoxin oxidoreductase leads to the reduction of an iron-sulfur centre in the enzyme. This is re-oxidized by transferring the electrons to a [2Fe-2S]ferredoxin. Trichomonad ferredoxins are related to [2Fe-2S]ferredoxins that participate in mixed-function oxidase systems present in the bacterium *Pseudomonas putida* and in vertebrate mitochondria. This is shown by their size, characteristic EPR spectrum and marked similarities in their amino acid sequences (Marczak *et al.*, 1983; Gorrell *et al.*, 1984; Johnson *et al.*, 1990). Interestingly, ferredoxins of this type are not constituents of H_2 -producing bacterial pathways.

Hydrogenases of trichomonads are oxygen-sensitive enzymes which utilize reduced ferredoxin as an electron donor (Lindmark & Müller, 1973, 1974b; Lindmark *et al.*, 1975). Partially purified *T. vaginalis* hydrogenase was characterized as an Fe-hydrogenase (Payne *et al.*, 1993). The nature of the oxidase involved in hydrogenosomal respiration remains unknown. It is not susceptible to

inhibitors of mitochondrial respiration (Čkerkasov *et al.*, 1978) and has a high affinity for oxygen (Yarlett *et al.*, 1986b).

Complex EPR spectra of the isolated organelles indicate that not all hydrogenosomal redox components have yet been recognized (Ohnishi *et al.*, 1980; Chapman *et al.*, 1986).

Interconversion of malate and pyruvate is catalysed in hydrogenosomes by a malate dehydrogenase (decarboxylating) which in *T. foetus* can utilize either NAD^+ or $NADP^+$ (Hrdý & Mertens, 1993). Malate dehydrogenase has also been detected in *T. foetus* hydrogenosomes (Hrdý *et al.*, 1993). Trichomonad hydrogenosomes also contain an adenylate kinase. The *T. vaginalis* enzyme is a 26 kDa protein, similar in size to mitochondrial and most bacterial enzymes and larger than the enzyme found in the cytosol of eukaryotes (Declerck & Müller, 1987). Part of the iron-superoxide dismutase content of trichomonad cells is localized in the hydrogenosomes (Lindmark & Müller, 1974a).

Enzymes of the tricarboxylic acid cycle (Müller, 1973) as well as cytochromes, cytochrome oxidase (Lloyd *et al.*, 1979b) and F_0F_1 ATPase (Lloyd *et al.*, 1979a) have not

been detected in trichomonad hydrogenosomes. The only exceptions are the above-mentioned succinate thiokinase and malate dehydrogenase. Enzymes characteristic of peroxisomes have not been detected (Müller, 1973). Although the evidence is not definitive, the search for DNA in these organelles has given negative results (Turner & Müller, 1983; Wang & Wang, 1985).

The composition of the membranes of trichomonad hydrogenosomes remains to be studied. No cardiolipin was detected (Paltauf & Meingassner, 1982). The membrane probably contains a number of transporters. The presence of an adenine nucleotide transporter, exchanging ADP for ATP is shown by the utilization of extra-organellar ADP and release of ATP (Fig. 2*b*) by intact isolated hydrogenosomes. This transporter is moderately susceptible to inhibition by atractyloside (Čkerkasov *et al.*, 1978). Entry of pyruvate or malate and the release of acetate have not been studied. The flattened vesicular structures below the hydrogenosomal membrane contain high levels of Mg, Ca and P (Chapman *et al.*, 1985) and possibly function in intracellular calcium regulation.

Rumen protists

Hydrogenosomes of rumen ciliates and fungi have also been studied with biochemical methods. The available information is more restricted than for trichomonads (Williams, 1986; Lloyd *et al.*, 1989; Yarlett, 1993). Hydrogenosomes of the isotrich ciliate *Dasytricha ruminantium* utilize malate as substrate and contain the complete pathway from malate through pyruvate to H₂ (Yarlett *et al.*, 1981; Lloyd *et al.*, 1989); they also respire if O₂ is present (Yarlett *et al.*, 1982; Lloyd *et al.*, 1989). They differ from trichomonad hydrogenosomes in two major aspects. First, the enzymes detected in them show that conservation of the energy of the thioester bond of acetyl-CoA as ATP is by a pathway known from anaerobic bacteria but previously not detected in eukaryotes. Acetyl-CoA is converted to acetyl phosphate by phosphotransacetylase. The latter serves as a phosphoryl donor in a reversed ADP-dependent acetate kinase reaction producing acetate and ATP (Yarlett *et al.*, 1982). The other specific aspect is that in addition to acetate, *D. ruminantium* hydrogenosomes also produce short-chain fatty acids (butyrate etc.) by condensation and further conversion of two acetyl-CoA molecules (Yarlett *et al.*, 1985). Other isotrich (Yarlett *et al.*, 1983) and entodiniomorphid rumen ciliates (Snijders *et al.*, 1982; Yarlett *et al.*, 1983; Paul *et al.*, 1990) also have *bona fide* hydrogenosomes, but these have not been studied in detail. None of the hydrogenosomal enzymes

of rumen ciliates have been purified, characterized or sequenced.

Hydrogenosomes of rumen fungi of the genus *Neocallimastix* [*N. patriciarum* (Yarlett *et al.*, 1986*a*) and *Neocallimastix* sp. L2 (Marvin-Sikkema *et al.*, 1993*a*)] also utilize malate as a substrate and have the complete malate through pyruvate to H₂ pathway. The reported lack of pyruvate:ferredoxin oxidoreductase in another species (*N. frontalis*) is an unexpected exception (O'Fallon *et al.*, 1991). Isolated organelles of *Neocallimastix* sp. L2 can establish a transmembrane proton gradient due to the presence of a proton-translocating ATPase (Marvin-Sikkema *et al.*, 1993*b*). It is not clear whether this process is specific to fungal hydrogenosomes since it has not been studied in other hydrogenosome-containing organisms.

Free-living ciliates

A positive histochemical reaction for hydrogenase is observed in the hydrogenosomes of free-living ciliates, just as in trichomonad hydrogenosomes, showing that they are indeed hydrogenosomes (Zwart *et al.*, 1988). Endosymbiont-free anaerobic ciliates produce H₂ (Goosen *et al.*, 1990*b*; Fenchel & Finlay, 1992) indicating the functioning of a typical hydrogenosomal pathway. The biochemistry of these organelles has not been explored, however.

Hydrogenosome-containing free-living protists almost invariably contain intracellular symbionts. The nature and role of these endosymbionts have been studied in ciliates in greatest detail (Fenchel & Finlay, 1991*a*; Finlay & Fenchel, 1993). Electron microscopic images show that hydrogenosomes and the endosymbionts are usually in close proximity (Fig. 1*d*), sometimes even forming stacks indicating their intimate physiological interaction. The endosymbionts were shown to be methanogenic archaeobacteria by their characteristic fluorescence due to their content of cofactor F₄₂₀ (van Bruggen *et al.*, 1983; Goosen *et al.*, 1990*a*; Fenchel & Finlay, 1991*b*; Finlay *et al.*, 1993), by rRNA sequencing (Embley *et al.*, 1992; Finlay *et al.*, 1993) and by cultivation (van Bruggen *et al.*, 1984). Washed cells of several protist species harbouring the above association have been shown to produce CH₄, demonstrating the functional activity of the symbionts (Wagener *et al.*, 1990; Fenchel & Finlay, 1992).

The biochemistry of the above association remains to be studied. It is likely that the hydrogenosome produces H₂ and CO₂, as it does in trichomonads and the rumen protists, while the endosymbiont utilizes these with the production of CH₄ (Wagener *et al.*, 1990; Fenchel & Finlay, 1992). This association represents a case of intracellular interspecific hydrogen transport, analogous

to the well-known interspecific hydrogen transport between H_2 producing bacteria and H_2 -utilizing archaeobacteria. With the use of specific inhibitors the methanogens can be eliminated from the association (Fenchel & Finlay, 1992). Organisms 'cured' of the endosymbionts do not show any major functional or morphological disturbances. Their rate of multiplication is slower than that of organisms harbouring the complete association, indicating that the host cell derives some benefit from the presence of the endosymbiont (Finlay & Fenchel, 1993). The nature of this benefit remains elusive. Maintenance of a low intracellular pH_2 has been mentioned as a possible factor enhancing hydrogenosomal metabolism (Finlay & Fenchel, 1993).

Comparison with mitochondria

The biochemistry of hydrogenosomes from various protists reveals a number of significant differences but also some similarities with mitochondria. The following comparison is based primarily on trichomonad hydrogenosomes. The more-limited information on the rumen protists is in essential agreement with the conclusions made here. The biochemistry of free-living protists remains to be studied but the presence of hydrogenase and production of H_2 indicate that they share significant biochemical traits with other hydrogenosomes.

Pyruvate derived from glycolysis or from malate is a major substrate of both hydrogenosomes and mitochondria and its oxidative decarboxylation yields acetyl-CoA as a primary product in both. The subsequent fate of acetyl-CoA is different, however. In hydrogenosomes it is converted to acetate, while in mitochondria it enters the tricarboxylic acid cycle. One ATP is formed in a substrate-level phosphorylation step catalysed by succinate thiokinase in both organelles, though succinyl-CoA, the proximal substrate of this reaction, is formed by different mechanisms in hydrogenosomes and mitochondria. The fate of electrons derived from pyruvate oxidation differs fundamentally, both in the nature of the electron transport components and of the terminal electron acceptor. The *raison d'être* of mitochondria, i.e. electron-transport-linked oxidative phosphorylation is absent in hydrogenosomes in which the electrons derived from pyruvate oxidation reduce protons through the action of hydrogenase and are eliminated as H_2 under anaerobic conditions. Even under aerobic conditions the reduction of O_2 is not linked to ATP formation.

Hydrogenosomes lack a number of processes and constituents characteristic of mitochondria. These could be absent due to secondary losses arising as adaptations to anaerobic metabolism. But these organelles also

contain at least two enzymes, key components of their metabolism, which are not found in mitochondria. These are pyruvate:ferredoxin oxidoreductase and hydrogenase. Certain enzymes and components present in both organelles are similar, however, e.g. ferredoxin, succinate thiokinase and adenylate kinase. The first two have already been shown to be homologous in the two organelles or at least to belong to the same protein family.

Role of hydrogenosomes *in vivo*

The benefits to the cell of having hydrogenosomes remains an elusive question, in contrast to mitochondria and plastids. This situation is reminiscent of that in the 1960s, when animal peroxisomes were still actors in search of a role. It might turn out that in addition to oxidizing pyruvate, thus contributing to cellular ATP formation, hydrogenosomes have additional functions, possibly even, as do peroxisomes, different ones in different organisms.

Hydrogenosomes participate in the overall metabolism of the cell to a significant extent. This can be evaluated by comparing the rate of carbohydrate utilization with the elimination of H_2 and acetate, typical end products of hydrogenosomal processes. In *T. foetus* up to one-half of the glycolytic end-products can pass through the organelle (Ryley, 1955), but in the other species hydrogenosomal flow represents a lower proportion (Müller & Gorrell, 1983; Marvin-Sikkema *et al.*, 1993a).

Hydrogenosomal processing of glycolytically formed pyruvate and malate are not indispensable for the organisms discussed, at least for *T. vaginalis* and *T. foetus*. If cultured for extended periods in the presence of the anti-anaerobic drug metronidazole, these organisms develop hydrogenosomal deficiencies. A number of enzymes of the organelle lose activity and the core metabolism of the organism becomes entirely cytosolic (Čerkasovova *et al.*, 1984; Kulda *et al.*, 1993). Hydrogenosome-deficient lines of *T. foetus* are vigorous, though their growth *in vitro* is slower, while similar *T. vaginalis* lines are difficult to maintain *in vitro*. These results point toward decreased efficiency of metabolism but they are not sufficient to establish the real causes behind the observed effects.

The known functions of the hydrogenosome are consistent with the notion that it contributes to ATP generation: it is thus an organelle of energy metabolism. Since the organelle has no electron-transport-linked phosphorylation, this contribution is limited to 1 mole of ATP per mole of pyruvate oxidized. Under the maximal level of hydrogenosomal carbon flow, metabolizing half of the glycolytic products, this represents 1 mole of ATP per hexose utilized, a 30 % increase of ATP yield over the

3 moles of ATP produced in *T. foetus* without hydrogenosomal metabolism (Mertens, 1993). Actual metabolic balances indicate that this is rarely attained. If intracellular redox balances are also considered, the contribution becomes even lower. The formation of glycerol, one of the products of cytosolic metabolism, plays a role in redox maintenance (Steinbüchel & Müller, 1986a). In this process one ATP is ultimately wasted. Decreased glycerol production by *T. vaginalis* in the presence of low levels of O₂ possibly explains its beneficial effects (Paget & Lloyd, 1990). Further detailed studies are necessary to establish the exact contributions hydrogenosomes make to the energy balance of the cell.

A pharmacological role of the hydrogenosome needs to be mentioned briefly. Metronidazole and other 5-nitroimidazoles are used extensively as selective antimicrobial agents against anaerobic bacteria and protists, including *T. vaginalis*. These compounds are by themselves not toxic but are activated to short-lived toxic derivatives by nitroreduction (Müller, 1986). Electrons for this reduction originate from pyruvate oxidation by pyruvate:ferredoxin oxidoreductase and are transferred via ferredoxin to the nitro group of the drugs. The hydrogenosomal location of this pathway in trichomonads makes this organelle a major factor in the mode of action of these drugs.

Biogenesis

The biogenesis of trichomonad hydrogenosomes (Johnson *et al.*, 1993) is probably similar in nature to mitochondrial biogenesis. The two hydrogenosomal proteins studied so far, ferredoxin and the β -subunit of succinate thiokinase of *T. vaginalis*, are synthesised on free ribosomes (Lahti & Johnson, 1991). The sequence of the corresponding message reveals an amino-terminal extension of eight or nine amino acids beyond the amino-terminal residues of the proteins purified from the organism (Johnson *et al.*, 1990; Lahti *et al.*, 1992). This extension is probably a signal peptide which plays a role in the import of newly synthesized proteins into the organelle. *In vitro* protein synthesis in heterologous systems gives products which contain the amino-terminal extension. The amino-terminal extensions of the two hydrogenosomal enzymes are similar: they start with Met-Leu-Ser, a tripeptide often found in mitochondrial presequences and they have an arginine residue at the -2 position. Trichomonad hydrogenosomal proteins do not react with antibodies against the carboxy-terminal Ser-Lys-Leu tripeptide which is a peroxisomal (microbody) targeting signal in various organisms (Keller *et al.*, 1991).

The only related information available for other hydrogenosome-containing organisms is the demon-

stration of an immune reactivity of hydrogenosomal hydrogenase with the Ser-Lys-Leu antibody in *Neocallimastix* sp. L2 (Marvin-Sikkema *et al.*, 1993c). This challenging observation suggests a relationship of fungal hydrogenosomes to microbodies.

Distribution and origins

The distribution of hydrogenosomes among protists follows ecological and physiological principles. All organisms that harbour hydrogenosomes live in environments of low oxygen content, i.e. anaerobic or microaerobic habitats. At the same time their distribution does not correspond to any phylogenetic principles. Groups of species with hydrogenosomes are located on independent branches of the eukaryotic tree and can be very distant from each other. This is clearly revealed by classical taxonomy and supported by small and large subunit rRNA sequence data.

According to rRNA-based phylogenetic trees (Baroin *et al.*, 1988; Sogin, 1991; Leipe *et al.*, 1993; Viscogliosi *et al.*, 1993), trichomonads and other parabasalids represent an early branch. This branch could well have separated from the main trunk before mitochondria became established. Hydrogenosome-containing fungi and ciliates are bona fide members of these crown groups and separated late in eukaryotic evolution. The ancestors of these organisms must have had mitochondria for a long time. Phylogenetic analysis reveals that hydrogenosome-containing fungi (Doré & Stahl, 1991) and ciliates (Baroin-Tourancheau *et al.*, 1992) are nested among species with mitochondria. This forces the conclusion that the appearance of hydrogenosomes was a late event in these groups. Among the ciliates, hydrogenosomes are present in representatives of almost all major groups (Fenchel & Finlay, 1991a; Finlay & Fenchel, 1993). The above survey leaves us with several unanswered questions. The two most intriguing ones are: (a) what is the advantage for a protist to have hydrogenosomes and (b) what was the evolutionary origin of these organelles.

The presence of hydrogenosomes in unrelated eukaryotic groups necessitates the conclusion that they have arisen independently on several occasions. Differences in their morphology and biology suggest that not all hydrogenosomes are homologous. Finding a common mechanism for their appearance in separate lineages would satisfy a quest for parsimony but reality might just be more complicated.

The presence in different eukaryotes of hydrogen-producing organelles that probably have arisen in independent events implies that such organelles give a significant selective advantage to organisms living in oxygen-poor niches. The presence of the hydrogenosome

in unrelated lineages indicates that its emergence is not only possible but probable in appropriate niches. The advantage for these organisms of having hydrogenosomes instead of mitochondria remains enigmatic. Generating some extra ATP by hydrogenosomal processes supplementing that produced by glycolysis is probably beneficial but the extent of this gain might not be very large. It is well-known that extended anaerobic glycolysis with the generation of additional ATP is not hydrogenosome dependent but can also be achieved by eukaryotic organisms which contain no organelles of energy metabolism (Müller, 1988) or have mitochondria (Behm, 1991). An anaerobic mode of eukaryotic life is not dependent on hydrogenosomes, yet hydrogenosomes have arisen in several lineages leading an anaerobic or microaerophilic life.

So what can be said of the evolutionary origin of this organelle? The ever-present alternatives of endogenous versus endosymbiotic origin clearly apply to this problem also. Whatever the path was that led to the existence of hydrogenosomes, their establishment has at least two complementary requirements. The first is the availability of a membrane-bounded entity with single or double membrane, probably of endosymbiotic origin. The second is the establishment in the nuclear genome of a set of genes coding for hydrogenosomal components and providing them with signals which target the expressed proteins to the organelle. Although an endogenous origin cannot be rigorously excluded at present, an endosymbiotic origin or origins seem to be more likely as is currently accepted for mitochondria, chloroplasts and peroxisomes. Two major possibilities proposed so far are the acquisition of an endosymbiont with the biochemical characteristics of the organelle and its subsequent integration into the host cell (Müller, 1980; Whatley *et al.*, 1979) and conversion of an already established and integrated organelle by transformation of its biochemical composition (Cavalier-Smith, 1987; Finlay & Fenchel, 1989).

In the first case it is assumed that the membrane-bounded entity descended from a bacterium that contained much of the enzymic equipment of the later hydrogenosome. After a successful endosymbiotic period its genetic information became incorporated into the nucleus and the proteins acquired the proper targeting sequences. In brief, a scenario is proposed that corresponds to the accepted notion of mitochondrial and chloroplast origins, but with a complete transfer of genetic information to the nucleus.

A conversion or replacement of pre-existing organelles, mitochondria or peroxisomes, is also a distinct possibility. Transformation of a pre-existing organelle implies the elimination of expression of most mitochondrial or peroxisomal constituents and the expression

of a new set of proteins with proper targeting signals. The organism of course had to acquire the corresponding nuclear genes either by descent or transfer. Replacement would correspond to the first scenario with the added step of eliminating the expression and assembly of an organelle present previously.

The proposed origins are all complex and the establishment of hydrogenosomes must have been a long process consisting of multiple steps. The available limited information hardly permits a choice among the above scenarios. Morphological data are of little help, since a morphology similar to mitochondria (Finlay & Fenchel, 1989), for instance, could be interpreted as showing a conversion from mitochondria or independent origin of an organelle from another bacterial precursor. Biochemical data are restricted to the functional enzymes, but we know little of the properties and composition of the hydrogenosomal membrane. Preliminary information on targeting signals of hydrogenosomal constituents is available for two groups, and is conflicting. The presence or absence of an organellar genome has been explored only in trichomonads and even there the results are not conclusive. Hydrogenosomes of other protists could well contain DNA. Hydrogenosomal enzymes of different organisms are similar in their functional properties but their genetic distances and even homologies remain to be established.

The late appearance of hydrogenosomes in certain fungi and ciliates, that is, in organisms which must have derived from mitochondrion-containing ancestors, is perhaps more easily explained by conversion of pre-existing organelles (Cavalier-Smith, 1987; Finlay & Fenchel, 1989). Parsimony would favour a similar origin for the hydrogenosomes of the early branching trichomonads. In view of the great phylogenetic distances between trichomonads and the other hydrogenosome-bearing protists, differences in the origins of their hydrogenosomes are not impossible, however. We might also have to consider the direct endosymbiotic hypothesis seriously.

Conclusions

Hydrogenosomes, discovered two decades ago, are membrane-bounded organelles which produce H_2 under anaerobic conditions. They are present in a variety of unrelated free-living and parasitic protist groups, all living in anoxic or oxygen-poor environments. Their enzymic composition, not established for all hydrogenosomes, differs markedly from that of mitochondria and peroxisomes. This justifies the use of a special term to designate these organelles. It is, however, likely that hydrogenosomes of different organisms are not homologous and have a polyphyletic origin. The contri-

butions of hydrogenosomes to the cells, their biological nature and evolutionary history remain largely unexplored. All in all, hydrogenosomes are an exciting area for future studies, which probably will give unexpected results, but promise new insights into the evolution of eukaryotic cells and the functional significance of membrane-bounded organelles.

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