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, 1974b; 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, 1991a) 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., 1986a; Marvin-Sikkema et al., 1992). Their presence in additional protist groups is likely. An interesting case is the amoeboflagellate Psalteriornonas Ianterna (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, 1991a; Finlay & Fenchel, 1993; Johnson et al., 1993; Yarlett, 1994).

Ultrastructure

The morphology of hydrogenosomes varies from group to group (Fig. 1a–d). No common tell-tale features enable us to identify hydrogenosomes by their morphology alone. Trichomonad and other parabasalid hydrogenosomes (Fig. 1a) were first described as microbody-like in view of their relatively uniform matrix, apparently single membrane and lack of extensive intragranellar 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. 1b) 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., 1986a; Marvin-Sikkema et al., 1992). In the hyphae they are microbody-like (Fig. 1c) while in the zoospores they are elongated and have a peduncular extension (Fig. 1h 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 Polyplostron 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. 1d) (Finlay & Fenchel, 1989). These morphological differences could reflect fundamental differences in the biological nature of hydrogenosomes of various organisms.
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 Vigues (b) and Bland Finlay (see Finlay et al., 1993) (d). (c) With permission from Marvin-Sikkema et al. (1992). Bars, 1 μm.
Biochemistry

Trichomonad flagellates

Isolated intact hydrogenosomes of *Trichomonas vaginalis* and *Tririchomonas 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.

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 oxido-reductase 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 oxido-reductase 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%
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 H₂-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 FoF₁ ATPase (Lloyd et al., 1979a) have not

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**Fig. 3.** Schematic metabolic map of hydrogenosomes. 1, Pyruvate:ferredoxin oxidoreductase; 2, malate dehydrogenase (decarboxylating) (NAD); 3, NAD:ferredoxin oxidoreductase; 4, H₂: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.
been detected in trichomonad hydrogenosomes. The
only exceptions are the above-mentioned succinate
thiokinase and malate dehydrogenase. Enzymes charac-
teristic 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

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. 2b) 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 mem-
brane 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 rumin-
antium* 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
eukaryotes. Acetyl-CoA is converted to acetyl phosphate
by phosphotransacetylase. The latter serves as a phos-
phoryl 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 (Snyers 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 *Neo-
callimastix* [*N. patriciarum* (Yarlett et al., 1986a) and
*Neocallimastix* sp. L2 (Marvin-Sikkema et al., 1993a)
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 *Neo-
callimastix* sp. L2 can establish a transmembrane proton
gradient due to the presence of a proton-translocating
ATPase (Marvin-Sikkema et al., 1993b). 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., 1990b; 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. 1d), 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 F₄₃₀ (van
Bruggen et al., 1983; Goosen et al., 1990a; 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.,

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 H₂ producing bacteria and H₂-utilizing archaeobacteria. With the use of specific inhibitors the methanogen 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 pH₂ 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 H₂ 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 H₂ under anaerobic conditions. Even under aerobic conditions the reduction of O₂ 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 H₂ 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 hydrogensomal 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 hydrogensomes make to the energy balance of the cell.

A pharmacological role of the hydrogensome 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 hydrogensomal 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 hydrogensomes (Johnson *et al.*, 1993) is probably similar in nature to mitochondrial biogenesis. The two hydrogensomal 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 synthesised 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 hydrogensomal 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 hydrogensomal 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 hydrogensome-containing organisms is the demonstration of an immune reactivity of hydrogensomal hydrogenase with the Ser-Lys-Leu antibody in *Neo-callimastix* sp. L2 (Marvin-Sikkema *et al.*, 1993c). This challenging observation suggests a relationship of fungal hydrogensomes to microbodies.

**Distribution and origins**

The distribution of hydrogensomes among protists follows ecological and physiological principles. All organisms that harbour hydrogensomes 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 hydrogensomes 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 hydrogensome-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 hydrogensomes was a late event in these groups. Among the ciliates, hydrogensomes 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 hydrogensomes and (b) what was the evolutionary origin of these organelles.

The presence of hydrogensomes 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 hydrogensomes 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 hydrogensome
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 H₂ 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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