Mitochondria as we don't know them

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Biochemistry textbooks depict mitochondria as oxygen-dependent organelles, but many mitochondria can produce ATP without using any oxygen. In fact, several other types of mitochondria exist and they occur in highly diverse groups of eukaryotes – protists as well as metazoans – and possess an often overlooked diversity of pathways to deal with the electrons resulting from carbohydrate oxidation. These anaerobically functioning mitochondria produce ATP with the help of proton-pumping electron transport, but they do not need oxygen to do so. Recent advances in understanding of mitochondrial biochemistry provide many surprises and furthermore, give insights into the evolutionary history of ATP-producing organelles.

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In textbooks, mitochondria are usually viewed as oxygen-consuming, ATP-producing organelles. Indeed, typical mitochondria, such as those found in mammalian cells, require oxygen to function. They use pyruvate dehydrogenase (PDH) for oxidative decarboxylation of pyruvate to acetyl-CoA, which is then completely oxidized to CO_2 through the Krebs cycle. Most of the energy is produced by oxidative phosphorylation: the electrons from NADH are transported to oxygen by the proton-pumping electron transport chain, and the backflow of the pumped protons results in ATP formation by the mitochondrial ATP synthase (Fig. 1). Oxygen deprivation in classical mitochondria rapidly results in halted mitochondrial ATP synthesis and often in cell death.

Yet numerous groups of eukaryotes exist that are less sensitive to the absence of oxygen. Some, such as yeast and goldfish, can survive by using simple fermentations in which the electrons from glycolysis are transferred to pyruvate, with lactate or ethanol excreted as end products. But we will not consider cytosolic fermentations here. Rather, we focus on the diversity and biochemistry of mitochondrial ATP synthesis in eukaryotes that thrive in oxygen-poor (hypoxic) or oxygen-free (anoxic) conditions. In nature, these organisms inhabit, during all or part of their life cycle, environments that do not contain enough O_2 to support aerobic ATP synthesis. Such habitats include, for example, marine and fresh-water sediments, or the digestive tract of animals. In addition, some of these eukaryotes, such as parasitic worms, lack a circulatory system and respiratory organs, and they are simply too large to maintain an aerobic energy metabolism using oxygen diffusion. The mitochondria of anaerobic eukaryotes produce ATP with the help of proton-pumping electron transport, but they do not need O_2 to do so. Instead, they use terminal electron acceptors other than O_2, so that their excreted end product of electron transport is not H_2O, but nitrite (NO_2^-), nitric oxide (NO), succinate and the like.

In addition to anaerobic mitochondria, another type of anaerobic ATP-producing organelle exists – the hydrogenosome [1–7]. Hydrogenosomes are H_2-producing, membrane-enclosed organelles, evolutionarily related to mitochondria. They occur in a wide spectrum of anaerobic protists, including ciliates, amoeboflagellates, chytridiomycete fungi and parabasalids, the group in which they were discovered. Hydrogenosomes are highly relevant to the general topic of anaerobic energy metabolism in eukaryotes, but because they do not possess a membrane-associated electron-transport chain, they will not be considered further here.

Mitochondria – both aerobic and anaerobic – are regarded as a major characteristic of eukaryotic cells. Here we summarize the basic biochemistry of anaerobic mitochondrial ATP synthesis on the basis of a few well-studied examples, draw attention to recent developments in the field and discuss the possible evolutionary origins of these peculiar organelles.

Anaerobic mitochondria in protists and metazoa

The list of eukaryotes with anaerobic mitochondria spans unicellular (protists) and multicellular organisms, including flatworms (platyhelminths), parasitic nematodes and invertebrates such as mussels and snails (see Table 1). Anaerobic mitochondria are known to exist in some algae, such as *Euglena* (see below), but are not found in land plants. It is not known whether this possible absence has an evolutionary relevance or whether it is a mere fluke of sampling.

Anaerobically functioning mitochondria need an alternative terminal oxidase to be able to use substrates other than oxygen as final electron acceptor. Organisms with anaerobic mitochondria can be broadly divided into two different classes: those that use an electron acceptor present in the environment, such as NO_3^-, and those that use an endogenously produced organic electron acceptor, such as fumarate (Table 1). Examples of the first class can be found among protists and nitrate respiration has been reported from several ciliates [8]. The best studied examples are, however, the denitrifying mitochondria of the fungi *Fusarium oxysporum* and *Cylindrocarpon tonkinense*, which use NO_3^- or NO_2^- as the terminal electron acceptor of a membrane-associated electron-transport chain, producing nitric oxides as reduced end products [9,10] (Fig. 2). These mitochondria contain a nitrite reductase (NiR), which reduces NO_2^- to NO by deriving electrons from the cytochrome *c* pool, some also contain a nitrate reductase (NaR), which reduces NO_3^- to NO_2^- by

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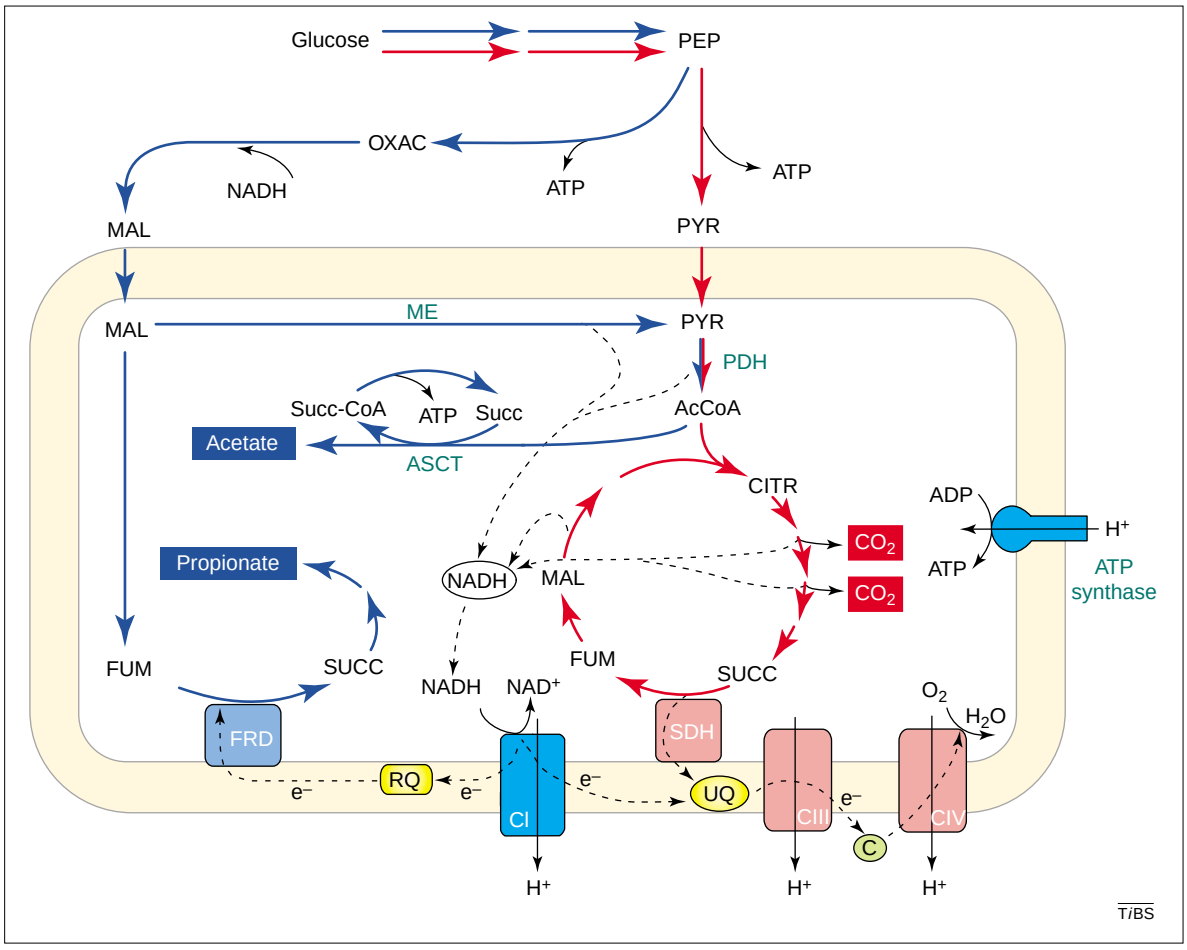

Fig. 1. Comparison of mitochondrial carbohydrate metabolism in aerobically and anaerobically functioning mitochondria. As an example, the main pathways of aerobic (red arrows) and anaerobic (blue arrows) metabolism in *Fasciola hepatica* are shown [11]. Transport of electrons is shown in dashed arrows and end products are shown in boxes. Abbreviations: AcCoA, acetyl-CoA; ASCT, acetate:succinate CoA-transferase; C, cytochrome *c*, C I, complex I; C III, complex III; CIV, complex IV; CITR, citrate; FRD, fumarate reductase; FUM, fumarate; MAL, malate; ME, malic enzyme; OXAC, oxaloacetate; PDH, pyruvate dehydrogenase; PEP, phosphoenolpyruvate; PYR, pyruvate; RQ, rhodoquinone; SDH, succinate dehydrogenase; SUCC, succinate; Succ-CoA, succinyl-CoA; UQ, ubiquinone.

deriving electrons from the ubiquinol pool [11] (Fig. 2). Both the nitrite and nitrate reductase resemble their bacterial counterparts in structure and function [9]. The reduction of NO, a product of nitrite reduction, is catalyzed in denitrifying mitochondria by an NO reductase, which is similar in sequence to cytochrome P450. By contrast, bacteria use a distinct NO reductase, which is similar in sequence to cytochrome *bc* type complexes [10]. Numerous eubacteria, including the α-proteobacteria from which mitochondria arose, use nitrite respiration as part of their metabolic repertoire. Hence, nitrite respiration in mitochondria could be an inheritance from the ancestral mitochondrial endosymbiont [10]. However, during eukaryotic evolution, additional components (such as P450-type NO reductases) could also have been recruited into these anaerobic electron transport chains [10].

Fumarate-respiring mitochondria – a closer look at metabolism

The best studied examples of the second class of anaerobic mitochondria, those that use an endogenously produced final electron acceptor, are the anaerobic mitochondria of parasitic helminths such as *Fasciola hepatica* and *Ascaris suum* [12–15] (Table 1). Whereas free-living and some larval stages possess mitochondria that use oxygen, the adult stages of most parasitic helminths are able to survive the anaerobic conditions in their habitat by using a pathway called malate dismutation. This entails the use of a specialized electron-transport chain and the reduction of endogenously produced fumarate as the electron sink. Here, glycolysis in the cytosol proceeds to phosphoenolpyruvate, which is converted by phosphoenolpyruvate carboxykinase (PEPCK) to oxaloacetate and then reduced further to malate. The malate is imported into mitochondria for further degradation (Fig. 1).

To maintain redox balance, some of the malate is oxidized and some is reduced (dismutation). The oxidation of malate is catalyzed by malic enzyme (ME) and PDH and results in acetyl-CoA formation. Acetyl-CoA then enters a two enzyme cycle: the CoA moiety of acetyl-CoA is transferred to succinate by an acetate:succinate CoA-transferase (ASCT), yielding acetate and succinyl-CoA [1,14,16]. Regeneration of CoASH is performed by a succinate:thiokinase

Table 1: Characteristics of various types of mitochondria

	Examples	Group	Respiratory Chain		Main end products[a]	Characteristic components[b]	Refs
			e^- donor	e^- acceptor			
Aerobic (classical)							
Unicellular	*Saccharomyces*	Yeast	NADH, $FADH_2$	O_2	CO_2, H_2O	–	[56]
Metazoa	*Homo*, *Arabidopsis*	Mammals, plants	NADH, $FADH_2$	O_2	CO_2, H_2O	–	
Aerobic (partial substrate oxidation)							
Unicellular	Insect stages of: *Leishmania*, *Trypanosoma*	Trypanosomatids	NADH, $FADH_2$	O_2	Acetate, succinate, H_2O	ASCT	[16,39]
Metazoa	Juvenile *Fasciola*	Juvenile parasitic worms	NADH, $FADH_2$	O_2	Acetate, H_2O	ASCT	[12]
Chemolithotrophic (facultative) anaerobic							
Unicellular	unknown						
Metazoa	*Arenicola*, *Geukensia*, *Bythograea*	Bivalves, polychaete worms, crustaceans	S^{2-}, NADH	fumarate, (O_2)	$S_2O_3^{2-}$, succinate, (CO_2, H_2O)	SO, RQ, FRD	[42–44]
(Facultative) anaerobic with external e- acceptor							
Unicellular	*Fusarium*, *Cylindrocarpon*	Fungi	NADH	NO_3^-, NO_2^-, (O_2)	N_2O, (CO_2, H_2O)	NiR, NaR	[9–11]
Metazoa	unknown						
(Facultative) anaerobic with internal e- acceptor							
Unicellular	*Euglena*	Euglenids	NADH	fatty acid synthesis, fumarate, (O_2)	wax esters, succinate, (CO_2, H_2O)	PNO, RQ, FRD	[32,33]
Metazoa	Adult *Ascaris*, Adult *Fasciola*, *Mytilus*	Adult parasitic worms, bivalves, snails	NADH	fumarate, (O_2)	acetate, succinate, propionate, (CO_2, H_2O)	FRD, RQ, ASCT	[12,13, 15,57]

[a]CO_2 indicates carbon dioxide produced by Krebs cycle activity; brackets indicate end products in facultative anaerobes produced only when oxygen is present.
[b]ASCT, acetate:succinate CoA-transferase; FRD, fumarate reductase; NaR, nitrate reductase; NiR, nitrite reductase; PNO, pyruvate:$NADP^+$ oxidoreductase; RQ, rhodoquinone; SO, sulphide oxidase; Succ, succinate.

(STK, also named succinyl-CoA synthase, SCS), which generates ATP (or GTP) through substrate-level phosphorylation, using the energy of the thioester bond in succinyl-CoA.

The rest of the malate is reduced to succinate, which is often metabolized further to propionate [12]. The reduction of malate to succinate occurs in two reactions that reverse part of the Krebs cycle, whereby the reduction of fumarate is the essential NADH-consuming reaction for maintaining redox balance. Fumarate reduction is linked to the electron transport chain, proton-pumping by NADH dehydrogenase (complex I) and ATP-formation (Fig. 1). Furthermore, malate dismutation is also accompanied by substrate-level phosphorylation. Overall, the anaerobic degradation of glucose to acetate and propionate results in ~5 mol ATP per mol glucose degraded.

Compared with typical aerobic mitochondria, the three main distinctions of these anaerobic mitochondria are: (1) the enzyme catalyzing the conversion of fumarate to succinate, (2) the quinone that connects this electron transfer to the enzyme complex in the electron transport chain, and (3) the presence of ASCT – an enzyme otherwise typical of hydrogenosomes – which converts acetyl-CoA into acetate [1].

Under aerobic conditions, the reducing equivalents of both mitochondrial complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase complex) are transferred to ubiquinone. However, the redox potential of ubiquinone is too high for an efficient transfer of electrons to fumarate [14,17,18] (Fig. 2). In prokaryotes, this problem is solved by using different quinones with a lower redox potential, namely menaquinone and demethylmenaquinone [19], which are lacking in eukaryotes. Rhodoquinone (RQ), like menaquinone, has a lower redox potential than ubiquinone and therefore can function as an electron donor to fumarate [14,20] (Fig. 2). It was shown that RQ is essential for electron-transport chain associated fumarate-reduction in eukaryotes in general [21]. RQ is present not only in all investigated parasitic helminths, but also in all other eukaryotes examined that reduce fumarate under anaerobic conditions *in vivo*, such as the sea mussel *Mytilus edulis*, the oyster *Crassostrea angulata*, the lugworm *Arenicola marina*, and the fresh-water snail *Lymnea stagnalis* (Table 1) [21]. RQ is also present in unicellular eukaryotes that reduce fumarate during anoxia, such as *Euglena gracilis*, whereas those unicellular eukaryotes that do not reduce fumarate during anoxia do not possess RQ [20,21]. Apparently, eukaryotic fumarate reductases (FRD) interact with RQ (a benzoquinone), whereas most prokaryotic FRDs interact with the naphthoquinones menaquinone and

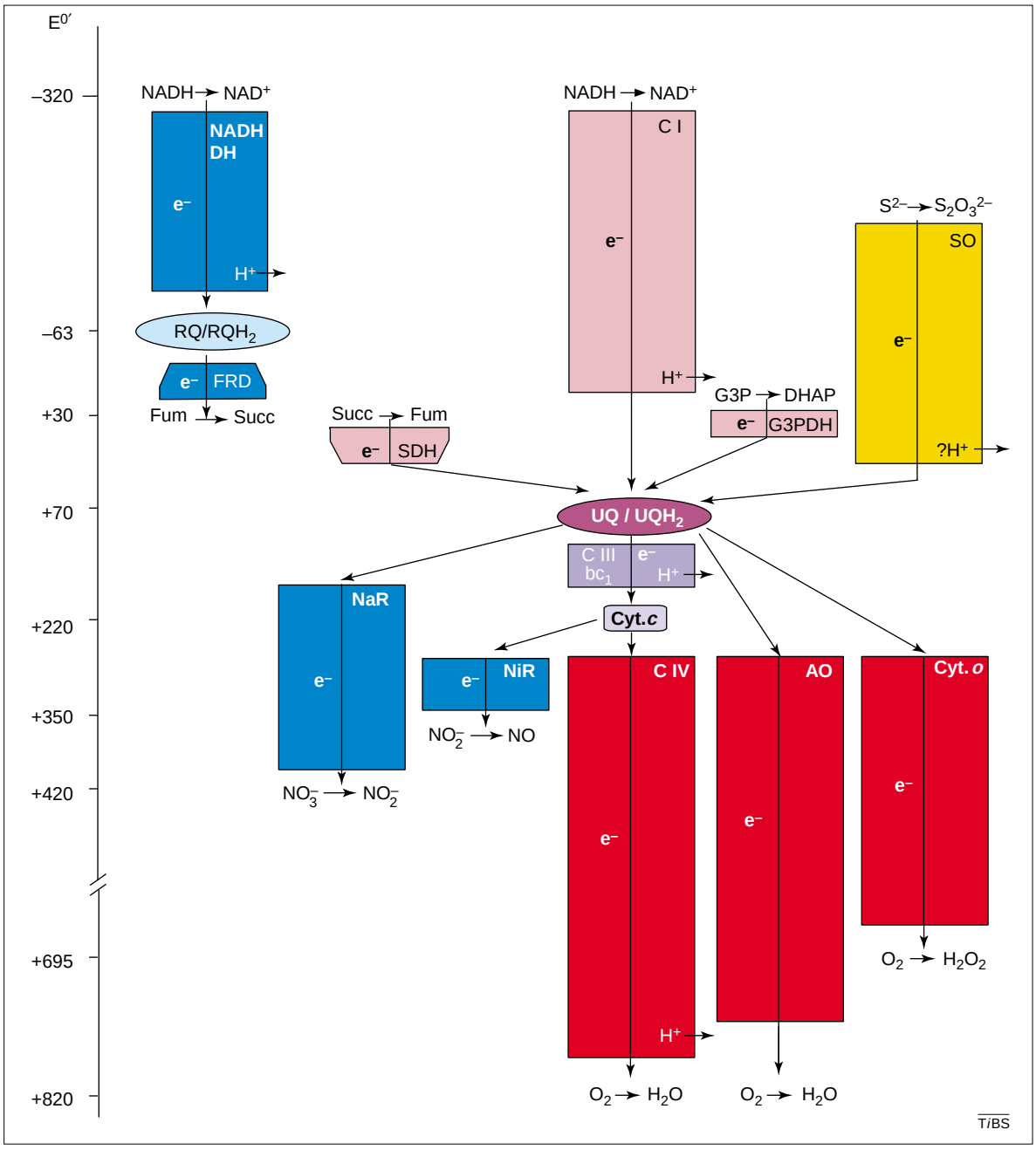

Fig. 2. Mitochondrial respiratory chains. Boxes indicate electron-transport chain complexes, ovals represent the electron transporters ubiquinone (UQ) and rhodoquinone (RQ). The three pink boxes represent complexes involved in entry of e^- transport from organic substrates and the yellow box represents entry from inorganic substrates. Red boxes represent complexes with oxygen as final e^- acceptor, dark blue boxes represent complexes involved in anaerobic electron transport. The vertical bar shows a scale for the standard redox potentials in mV. Translocation of protons by the complexes is indicated by $H^+\rightarrow$. Abbreviations: AO, plant-like alternative oxidase; C I, complex I of the respiratory chain; bc_1, complex III of the respiratory chain; cyt *c*, cytochrome *c*; cyt *o*, cytochrome *o*; DHAP, dihydroxyacetonephosphate; Fum, fumarate; G3P, glycerol-3-phosphate; G3PDH, glycerol-3-phospate dehydrogenase; NADH DH, NADH dehydrogenase; NaR, nitrate reductase; NiR, nitrite reductase; RQ, rhodoquinone, SO, sulphide oxidase; Succ, succinate; UQ, ubiquinone.

demethylmenaquinone [14]. Low levels of ubiquinone have also been detected in various anaerobic protists, including hydrogenosome-bearing parabasalids, and in some that lack ATP-producing organelles altogether, including *Giardia* [22]. However, in these organisms ubiquinone probably has no role in electron transport as these protists seem to lack an electron-transport chain [1]. It is uncertain whether ubiquinone functions as defence mechanism against oxidative stress in these organisms, as it does in many other organisms.

Succinate dehydrogenase versus fumarate reductase

In aerobic energy metabolism, electrons coming from succinate generated in the Krebs cycle are transferred to ubiquinone through complex II (succinate dehydrogenase) of the respiratory chain (Fig. 1). However, in anaerobic energy metabolism fumarate serves as the terminal electron acceptor and electrons are transferred in the reverse direction, from a quinone

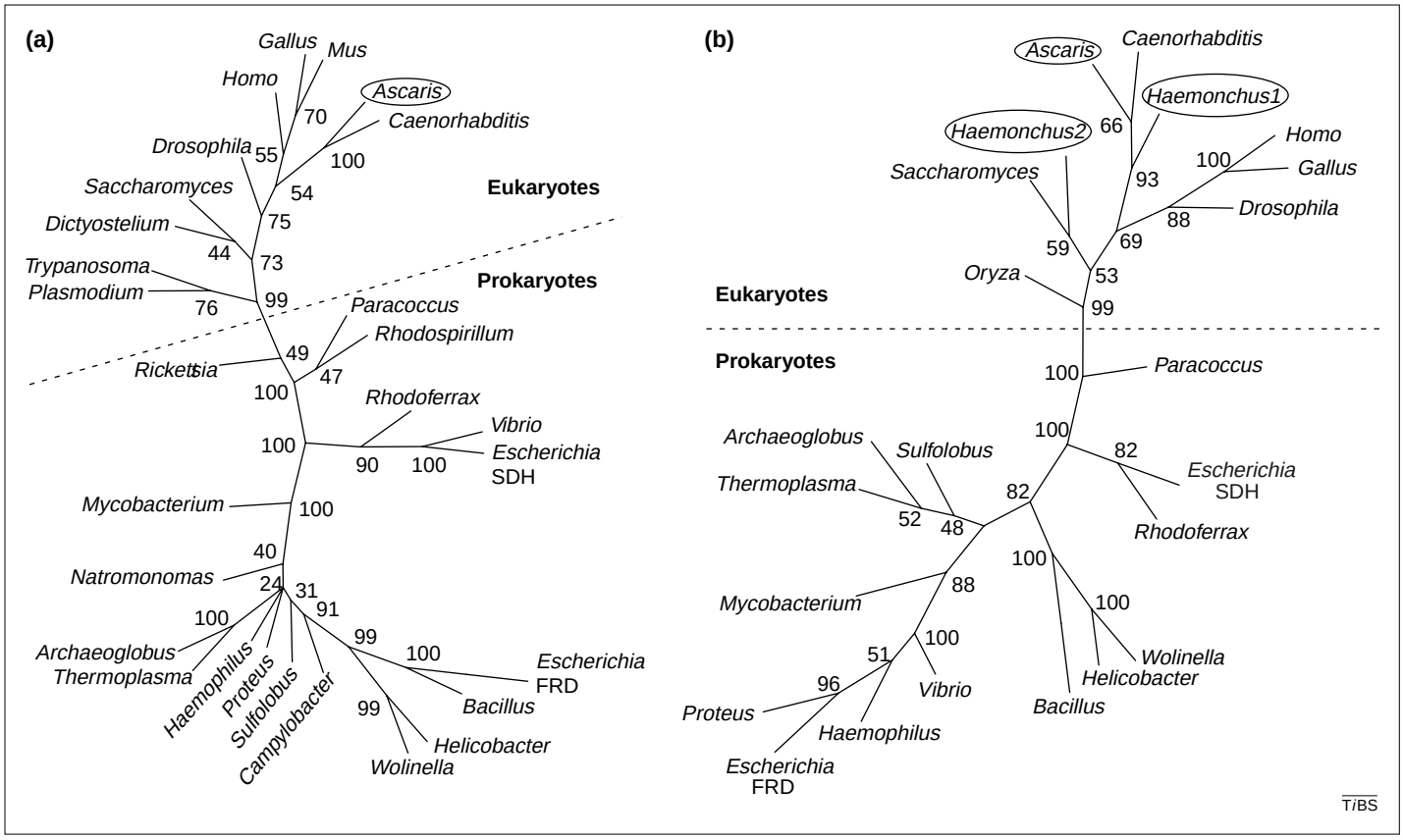

Fig. 3. Unrooted maximal parsimony phylogenetic tree of the Fp (a) and Ip (b) subunits of succinate dehydrogenases and fumarate reductases. Amino acid sequences were aligned by Clustal X and phylogenetic trees were prepared by the Phylip package. Bootstrap values of 100 random order samples are shown. Similar trees were found by neighbour joining analyses. Subunits of eukaryotes containing anaerobically functioning mitochondria are shown in ovals.

to fumarate (Fig. 1). Succinate oxidation and fumarate reduction are generally carried out by separate enzymes *in vivo*. Accordingly, in *Escherichia coli* the change between aerobic and anaerobic metabolism is accompanied by differential expression of two different enzymes for these reactions [18,23,24]. Succinate dehydrogenase (SDH, complex II) is expressed for succinate oxidation under aerobic conditions, whereas FRD is expressed for fumarate reduction under anaerobic conditions. Differential expression of two different, stage-specific forms of SDH/FRD during development also occurs in the parasitic helminths *Haemonchus contortus* and *A. suum* [25,26].

In vitro, the interconversion of succinate and fumarate is reversible for both SDH and FRD. However, *in vivo* these oxidation and reduction reactions run efficiently only when electrons are transferred to an acceptor with a higher standard redox potential. Accordingly, FRD complexes *in vivo* interact with lower redox potential quinones, whereas SDH complexes generally interact with quinones having a higher redox potential [27] (Fig. 2).

The FRD and SDH complexes are structurally very similar and each is usually composed of four non-identical subunits: a flavin-containing A subunit (Fp subunit), a B subunit containing three iron–sulphur clusters (Ip subunit) and one or two hydrophobic, often cytochrome *b*-containing, subunits that are essential for the attachment of the catalytic subunits A and B to the membrane and for the interaction of the catalytic subunits with the quinones [13,18,24,27]. The Fp and the Ip subunits of SDH are highly conserved and are closely related to the Fp and Ip subunits of FRD [18,20]. High resolution analyses show that the various membrane-anchor domains also show a conserved structure, despite their low primary-sequence similarity [28–30].

Analyses of enzyme kinetics, as well as the known differences in primary structures of prokaryotic and eukaryotic fumarate reducing complexes, suggests that FRD from anaerobic mitochondria is structurally related to SDH-type complex II, but has the functional characteristics of the FRD complexes of prokaryotes [20,21]. The phylogenetic relationships between the amino acid sequences of Fp and Ip subunits of SDH and FRD are shown in Fig. 3. Despite the limited amount of data available, the sequences of eukaryotic FRDs are clearly more closely related to SDH than to the bacterial FRDs, indicating that, during the evolution of anaerobic mitochondria, their SDH has been rebuilt by natural selection to run backwards preferentially and to use quinones with lower redox potential.

Other oddball mitochondria

The facultatively anaerobic mitochondria of *Euglena gracilis* have a unique ATP-producing biochemistry [31–33]. They perform the oxidative decarboxylation of pyruvate using an oxygen-sensitive enzyme – pyruvate:$NADP^+$ oxidoreductase (PNO) – which is closely related to pyruvate:ferredoxin oxidoreductase (PFO) from hydrogenosomes [34]. Mitochondria from aerobically grown *Euglena* cells also have PDH activity [35], but PNO is active [33]

and expressed [34] under both aerobic and anaerobic conditions.

When oxygen is present, *Euglena* respires normally, but uses a modified Krebs cycle in which succinate semialdehyde, rather than succinyl-CoA, is formed as an intermediate [36]. Notably, the α-proteobacterium *Bradyrhizobium japonicum* possesses the same succinate semialdehyde shunt [37].

Under anaerobiosis, PNO is the key enzyme of the unique wax ester fermentation of *Euglena*, where fatty acid biosynthesis serves as the main final acceptor for the electrons resulting from glucose breakdown [33,38] (Table 1). Acetyl-CoA is used both as a primer and C2-donor for synthesis of fatty acids, most of which have an even number of carbons. Chains with an odd number of carbons are synthesized through an alternative pathway involving RQ and FRD [32]. Half of the fatty acids are reduced to alcohols, which are esterified to the other half, yielding waxes that accumulate in the cytosol [31]. On return to aerobiosis, the waxes undergo β-oxidation for oxidative phosphorylation.

As well as the classical aerobic mitochondria and the unconventional anaerobically functioning mitochondria, other eccentric mitochondria exist that could shed light on the evolutionary relationships between the various types of energy-producing organelles. The single mitochondrion of *Trypanosoma brucei* in its insect stage is another interesting example. These mitochondria are obligate aerobic, but they do not completely oxidize the substrates to carbon dioxide, producing fermentation products instead (Table 1) [39]. Acetate is a major end product of their carbohydrate breakdown. This involves ASCT [16], an enzyme typical of hydrogenosomes, but which also occurs in the anaerobically functioning mitochondria of parasitic helminths. The insect stage of *T. brucei* contains a branched electron-transport chain: next to an NADH dehydrogenase and the complexes III (ubiquinone:cytochrome *c* oxidoreductase) and IV (cytochrome *c* oxidase), it also contains a plant-like cyanide-insensitive alternative oxidase [39,40] (Fig. 2). The reason why these trypanosomal mitochondria produce fermentation products such as acetate and succinate instead of fully oxidizing pyruvate to carbon dioxide, like classical mitochondria do, is still unknown.

A new chapter in mitochondrial ATP synthesis: chemolithoheterotrophy

Despite their differences, all the various mitochondria described above have one thing in common, which they also share with aerobic mitochondria: all the electrons involved in mitochondrial ATP synthesis always stem from organic compounds. This rule is so general that almost nobody would think of looking for an exception. But there is an exception, an exciting one.

Many aquatic invertebrates inhabit oxygen-poor, sulfide-rich sediments [41–43]. The mitochondria of these organisms, for example the mussel *Geukensia demissa* and the lugworm *A. marina*, can respire O_2 when it is available, but they switch to fumarate respiration under hypoxic conditions or when sulfide levels become so high that cytochrome oxidase will be inhibited (Table 1) [44]. Notably, their mitochondrial respiration (whether O_2 or fumarate) is linked to sulfide oxidation (Fig. 2), whereby the sulfide, S_2^-, is oxidized to thiosulphate, $S_2O_3^{2-}$ [44]. Although this was long thought to be simply a sulfide detoxification process, Doeller *et al.* [44,45] recently showed that isolated *G. demissa* gills use sulfide as an electron donor for respiratory electron transport and coupled ATP production to support cellular work. The finding of an inorganic donor for ATP-producing respiratory electron transport in *Geukensia* (and *Mytilus*) mitochondria expands the family of mitochondria to include chemolithoheterotrophs (Table 1); that is, mitochondria that gain their ATP from redox reactions involving inorganic electron donors [44]. The evolutionary origin of this truly surprising mitochondrial biochemistry remains to be elucidated.

Evolutionary origin of anaerobic mitochondria

The diversity of anaerobic mitochondria (and hydrogenosomes) raises the question of the diversity of the underlying evolutionary routes. Two explanatory principles appear to account for the origin of anaerobic mitochondria and hydrogenosomes: 1) inheritance and 2) lateral acquisition [46,47]. Both views include the possibility of evolutionary modification of either inherited or acquired genes.

In the inheritance model, the genes for the enzymes specific to anaerobic mitochondria (and hydrogenosomes) are viewed as originating from the same endosymbiont that gave rise to mitochondria [48], which must have been a facultatively anaerobic eubacterium [49]. This would predict the genes either to stem directly from the genome of the ancestral mitochondrion or to be evolutionary modifications of its pre-existing biochemical repertoire. This model accounts, for example, for the relationships of enzymes such as PFO, which is shared by *Euglena* mitochondria and hydrogenosomes [34], and for the occurrence of ASCT, which is shared among *Trypanosoma* mitochondria, hydrogenosomes and metazoan anaerobic mitochondria [16] (Table 1). Of course, inheritance can be followed by modification, examples of which are found in domain fusions of PFO in *Euglena* [34], in domain fusions of hydrogenase in *Nyctotherus* [50], in the divergence of hydrogenase to become a nuclear-associated protein called Narf [51], or most importantly for this review, in the evolutionary transition from SDH to FRD in anaerobic helminths (Fig. 3). Yet not only do the structural proteins themselves need explanation, but so does the synthesis of their cofactors. To date, all eukaryotes investigated that use FRD also use RQ [21] (a benzoquinone), which is structurally more similar to the ubiquinone found in classical

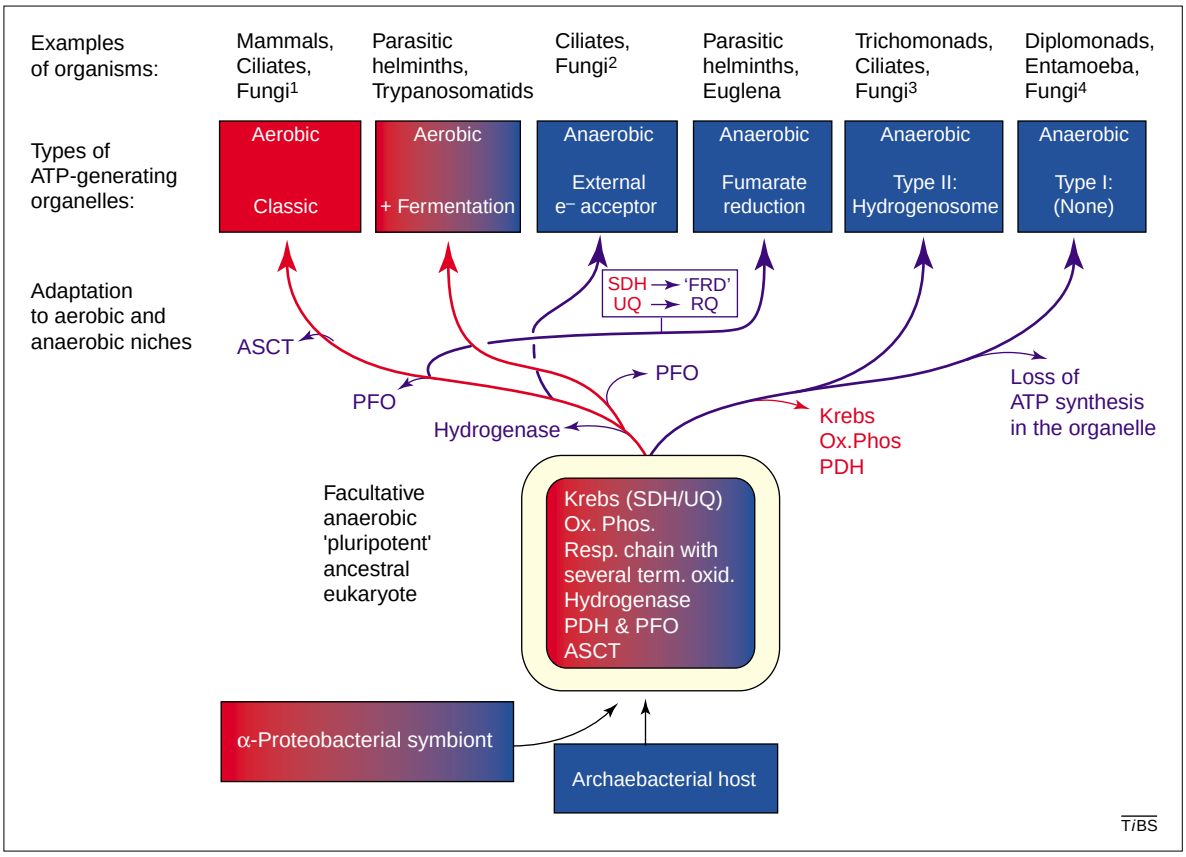

Fig. 4. Possible evolutionary relations between distinct energy generating organelles. Various adaptations in the metabolic machinery of the facultative anaerobic 'pluripotent' ancestral eukaryote to anaerobic and aerobic niches of unicellular eukaryotes as well as metazoans resulted in the various types of organelles (or even their disappearance). Examples of the various fungi: [1] *Saccharomyces*, [2] *Fusarium*, [3] Chytrids, [4] Microsporidia. Abbreviations: ASCT, acetate:succinate CoA-transferase; FRD, fumarate reductase; Ox.Phos., oxidative phosphorylation; PDH, pyruvate dehydrogenase; PFO, pyruvate:ferredoxin oxidoreductase; Resp., respiratory; RQ, rhodoquinone; SDH, succinate dehydrogenase; term. oxid., terminal oxidases; UQ, ubiquinone.

mitochondria (also a benzoquinone) than it is to the menaquinone (a naphthoquinone) that is used anaerobically by prokaryotes. RQ synthesis could be an inheritance from a mitochondrial endosymbiont that was similar to *Rhodospirillum rubrum* – a facultative anaerobe that possesses RQ – or it could have involved the modification of one or more pre-existing enzymes.

By contrast, the acquisition model presumes that the genes for anaerobic ATP synthesis in eukaryotes are the result of lateral gene transfers from prokaryotic donors other than the mitochondrial symbiont. Under this model, the nuclear-encoded proteins germane to anaerobic mitochondria (and hydrogenosomes) should reflect multiple, rather than single, evolutionary origins from anaerobic prokaryotes, but the proteins studied that address this issue indicate single, rather than multiple origins [34,51,52]. The evolutionary origins of genes that appear to be restricted to specific groups of eukaryotes, for example, the sulfide oxidase of mussel mitochondria, might yet reveal evidence for lateral gene transfer. Alternatively, they might reveal examples where the assembly of novel functions has resulted from the reshuffling of pre-existing domains, as was found for yeast sulfite reductase, which is not homologous to the prokaryotic enzyme but arose through recombination of PFO domains [34].

A simplified scheme of the inheritance model is depicted in Fig. 4 (although some additional lateral gene transfer should not be excluded, see above). Mitochondria probably descend from facultative anaerobic α-proteobacteria [49,53]. It is proposed that, in aerobic environments, the anaerobic capacities of the original symbiont were lost (or modified), giving rise to classical mitochondria, whereas in anaerobic environments, the aerobic capacities were lost [49]. In various independent lineages this resulted in the formation of two types of amitochondriate organisms: Type II, where the symbiont evolved into anaerobically functioning organelles – the various types of hydrogenosomes – and Type I amitochondriates, where much of the biochemistry of the organelle was retained, but the organelle as such was no longer involved in ATP synthesis, for example in *Entamoeba* [54] or *Giardia* (Fig. 4). For those concerned with anaerobic mitochondria and hydrogenosomes, a facultatively anaerobic eubacterium as the ancestor of mitochondria fits this picture better than a strictly O_2-dependent eubacterium such as *Rickettsia*. A facultative anaerobe, as we suggest, would have brought along a broader spectrum of biochemical starting material that could generate the diversity of metabolic pathways seen in today's wide variety of ATP-generating organelles.

However, the anaerobic biochemistry found in some mitochondria, such as those of parasitic helminths (which are metazoans), probably did not exist in its present form in the pluripotent ancestral mitochondrion. In our view, it arose later in evolution from the more conventional aerobic type of

mitochondrial biochemistry, at a time after these organelles had already specialized to an aerobic way of life by losing their anaerobic capacities in some lineages (Fig. 4). This later adaptation of the conventional aerobic mitochondria to anaerobic functioning must have been accompanied by minor modifications of the SDH enzyme, making it more suitable for the reduction of fumarate, resulting in an enzyme that is structurally more related to the original SDH, but functionally more related to the FRDs of prokaryotes (Fig. 3). Such adaptation also required the biosynthesis of the quinone involved in fumarate reduction. The enzymes specific to RQ biosynthesis are still uncharacterized, although work on this is in progress (A.G.M. Tielens and J.J. van Hellemond, unpublished); a comparison of these enzymes to their prokaryotic homologues should reveal their evolutionary origin. RQ synthesis probably differs only in the last step from ubiquinone synthesis, suggesting modification of pre-existing components during the adaptation to anaerobic niches. This is consistent with the finding that parasitic helminths have evolved from free-living worms, which generally are aerobic specialists [55].

Although anaerobic metabolism is usually considered to be a primitive and, hence, ancient way of life in eukaryotes, the emerging picture reveals that there are multiple origins of anaerobic metabolism. Not only have various lineages of eukaryotes undergone independent adaptations to aerobic and anaerobic lifestyles, but some of them have made these transitions early in evolution, for example among the protists, and some of them have made these transitions late, for example parasitic helminths which are derived from their more primitive aerobic ancestors. Eukaryotes and their characteristic ATP-producing organelles (mitochondria and hydrogenosomes) reveal a previously underestimated 'anaerobic plasticity', the origin of which is an intriguing evolutionary puzzle.

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Telomerase: biochemical considerations for enzyme and substrate

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Telomerase extends chromosome ends by iterative reverse transcription of its RNA template. Following the addition of each telomeric repeat, the RNA template and the telomeric substrate reset their relative position in the active site provided by the telomerase reverse transcriptase (TERT). This step might require the formation of guanine-rich secondary structures in the nascent product. Results from numerous studies begin to delineate TERT sub-domains that orchestrate these events and support the model of cooperative action between distinct active sites within telomerase multimers. Natural telomere substrates are protein–DNA complexes that show an asymmetry between the two ends of a chromosome, possibly reflecting their differential mode of replication.

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Telomeres are the nucleoprotein complexes that protect eukaryotic chromosome ends from degradation and fusion [1]. The DNA component of telomeres is typically made up of tandem repeats of simple sequences that are rich in guanosine residues in the strand containing the protruding 3′ end. Conventional DNA polymerases cannot synthesize the G-rich 3′ overhang because the parental C-rich strand template is recessed. Telomere length maintenance requires the telomerase enzyme whose RNA moiety (TER or TERC) contains the template for tandem telomere repeat synthesis. The telomerase reverse transcriptase subunit (TERT) bears structural similarity to reverse transcriptases from retroelements and provides the active site [2]. Here we examine the mechanism of this unusual reverse transcriptase and discuss its reaction cycle and structure, limiting the scope to TERT and its multimerization. We also discuss telomerase activity in the context of the end replication problem, its coordination with semiconservative DNA replication and the role of the Est1 protein that either recruits telomerase to the telomere or activates it while bound at the telomere. For a comprehensive overview on the function of other telomere end replication factors, including telomerase-associated proteins, telomere-binding proteins, nucleases and kinases, see Refs [3–6].

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