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*William F. Martin, Aloysius G. M. Tielens,
Marek Mentel*

MITOCHONDRIA AND ANAEROBIC ENERGY METABOLISM IN EUKARYOTES

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Preface

Views of eukaryote evolution continue to undergo significant change. The cell nucleus is the defining organelle of eukaryotes, the compartment that gave them their name. But in addition to the nucleus, mitochondria have become recognized as organelles ancestral to eukaryotic cells. The family of mitochondria now includes, however, reduced forms of the organelle that do not respire oxygen: hydrogenosomes and mitosomes. The discovery of hydrogenosomes in the 1970s, and the subsequent recognition that they are anaerobic forms of mitochondria, led to the finding that mitochondria – once synonymous with oxygen respiration – have a key role both in the aerobic and in the anaerobic energy metabolism of eukaryotes. Genome data have also impacted our views of phylogenetic relationships of eukaryotes and this has led to an improved understanding of the evolutionary significance of anaerobic metabolism in eukaryotes. In parallel, geochemical evidence has uncovered revolutionary new findings about the rise of oxygen in the Earth's history. The new insights into Earth's ancient habitats reveal that from the time of eukaryote origin roughly 1,600 million years ago and early lineage diversification of eukaryotes, up until about 500 million years ago, the Earth's atmosphere contained a very low amount of oxygen corresponding roughly to 1% of the present atmospheric level, an oxygen level known as the Pasteur point. The Pasteur point is the level of oxygen where cells that are able to switch from oxygen respiration to anaerobic ATP synthesis and an anaerobic lifestyle, make that switch. Throughout much of that low oxygen past, the oceans were to a large extent anoxic and locally even rich in hydrogen sulfide, which is a strong inhibitor of oxygen respiration in mitochondria, hence a poison for cells that rely solely upon O₂ for their ATP synthesis and redox balance. Up until about 450 million years ago, there was no appreciable life on land; all life was in the oceans, in the sediment, and in the Earth's crust. Eukaryotes arose and diversified in anaerobic oceans. In the new view of Earth's oxygen history, oxygen-independent pathways of eukaryotic energy metabolism in mitochondria reflect environmental conditions that dominated Earth's history during eukaryote evolution. Those conditions were low oxygen or anaerobic. The mitochondria of eukaryotes have preserved the trace of that anaerobic past.

We have many people to thank, too many to list, so we will make the list very short and specific. We wish to thank Miklós Müller for many years of friendship and dialogue on the physiology of eukaryotic anaerobes, Fred Opperdoes for many years of discussions about the biochemistry of mammalian parasites, John F. Allen for many years of discussions about oxygen and energy in evolution, Jaap van Hellemond, Sven Gould, and Sriram Garg for daily discussions on biochemistry and eukaryote evolution, and Rebecca Gerhards and Verena Zimorski for their invaluable help in preparing the manuscript.

This book aims to provide an overview of the biochemistry and evolution of anaerobic energy metabolism in eukaryotes and, at the same time, strives to link the latest findings from biology, biochemistry, geochemistry, and biogeochemistry to form a general evolutionary picture. The work should serve as a source of information on this topic for students of biology and for faculty from various fields, including the earth sciences.

William F. Martin, Aloysius G.M. Tielens, and Marek Mentel
Düsseldorf, Rotterdam, and Bratislava, April 2020

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Part III: **Evolution**

Four main observations bear on our understanding of the evolution of anaerobic energy metabolism in eukaryotes. The first is the distribution of organelles of mitochondrial origin among eukaryotic lineages. The second is the biogeochemical origin of conditions on the Earth and detailed description of environments that, most likely, gave rise to eukaryotes and that they had to deal with in early stages of their evolution. The third is the uniformity of eukaryotic energy metabolism and the distribution of the underlying enzymes and their genes, that is, we encounter the same pathways and enzymes in different lineages. The fourth is the lack of lineage-specific evolution of genuinely novel routes in eukaryotic energy metabolism (plastids aside, which are a genuine lineage-specific novelty).

Eukaryotic anaerobes are everywhere. But they are not always visible to the naked eye. If we focus on large, conspicuous life forms that inhabit land above the soil line, we are looking at organisms that have been selected to survive in, and depend on, an atmosphere containing 21% oxygen. Moreover, most of their ancestors went through a phase in Earth's history (the Carboniferous and Permian) when there was ~30% oxygen in the atmosphere. Anaerobic habitats harbor many life forms that never made the transition to fully oxygenated environments. The evolutionary position and significance of eukaryotic anaerobes is linked to views on the origin of mitochondria. In the literature dealing with the evolution of oxygen respiring mitochondria, eukaryotic anaerobes never really fit into the picture at all (Gray et al. 1999; Gray 2005; Gray 2014). Accordingly, views on the evolution of mitochondria have undergone some change in recent years in order to incorporate newer findings and to incorporate the mitochondria of anaerobes into the bigger picture of eukaryote evolution.

At the same time as views on the evolution of mitochondria have been changing, views on the evolution of O₂ in the atmosphere have undergone their own dramatic changes over the last 20 years as well. In newer views of atmospheric evolution, our present oxic atmosphere is a true latecomer in evolution, arising only about 500 million years ago (Lyons et al. 2014; Fischer et al. 2016; Catling and Zahnle 2020). In the modern context of late oxygenation in atmospheric evolution, it is now the strictly O₂-dependent mitochondria that do not fit into the bigger picture, because they were only useful for the last third of eukaryotic evolution, placing the first two thirds of eukaryotic history into the era of anaerobes. Reconciling anaerobes in mitochondrial evolution with modern views of oxygen in Earth's history was a main motivation to write this book.

29 Why did mitochondria become synonymous with O₂?

In order to appreciate the impact that eukaryotic anaerobes have had upon views of eukaryotic evolution, it is helpful to briefly retrace the steps to see how it came to be that the physiology of eukaryotic anaerobes became an evolutionarily important issue in the first place. If we do not delve deeply into different theories about mitochondrial evolution here – reviewed in (Zimorski et al. 2014) and (Martin et al. 2015) – it is because there are just too many of them to cover succinctly and (almost) none of them addressed the evolution of eukaryotic anaerobes, anaerobic mitochondria in particular. Historical developments in the field concerning the evolutionary significance of anaerobic mitochondria start with the idea that mitochondria were endosymbionts in the first place. A number of authors, including O'Rourke (2010) and many easily searchable internet sites, attribute the idea that mitochondria arose through symbiosis to Richard Altmann in his book *Die Elementarorganismen und ihre Beziehung zu den Zellen* (1890). That is however not correct because in that book, Altmann writes about “bioblasts” (*Bioblasten*), which he described as granules, visible in fixed material under the light microscope, that represent an organizational state of matter intermediate between that of a molecule and that of an organelle. For Altmann, everything in the cell was made of bioblasts, including metaphase chromosomes. He was not suggesting that chromosomes are made of mitochondria, he was suggesting that chromosomes were made of bioblasts, which in today's terms might equate to macromolecular complexes. Nowhere in the 1890 book does Altmann mention mitochondria, obviously, because the term mitochondria was introduced well after 1890, by Benda (1898). Nor does Altmann mention their older name, chondriosomes, nor does he make a suggestion about their possible bacterial nature. Altmann's *Bioblasten* are not mitochondria.

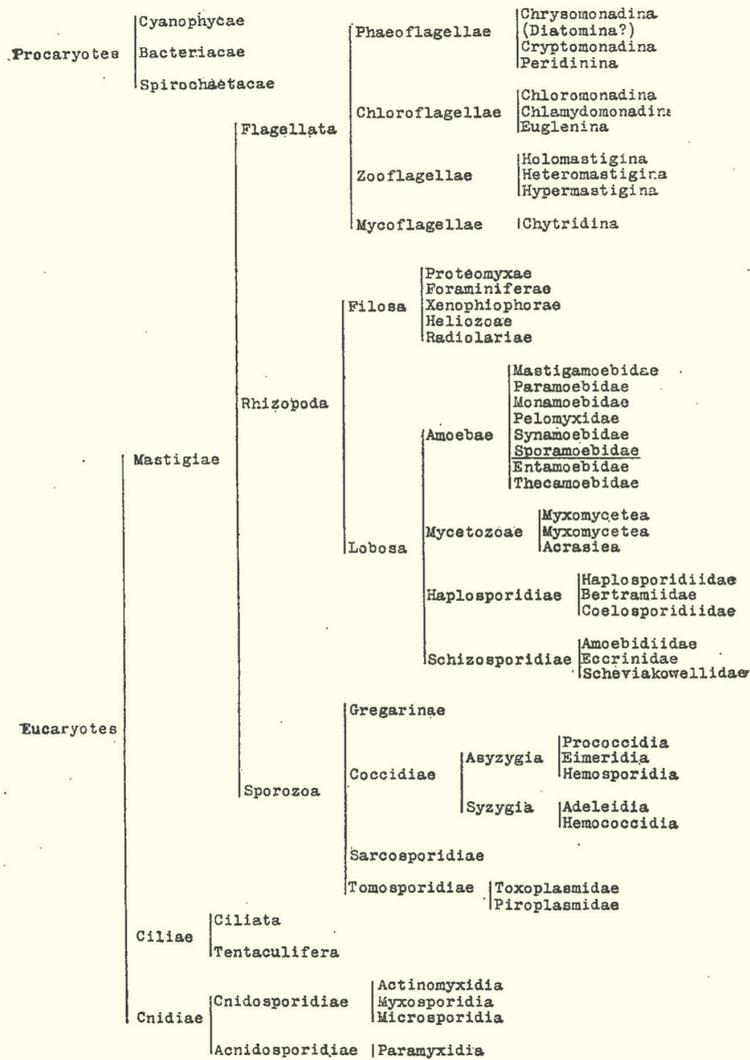
Endosymbiotic theory generally starts with Mereschkowsky's theory for the origin of plastids (1905). Endosymbiotic theories for the origin of mitochondria do not, however, start with Mereschkowsky, because Mereschkowsky neither entertained nor proposed a symbiotic origin of mitochondria. He did think that there was a symbiosis that preceded the plastid in the evolution of the plant and animal lineages, and he was convinced that symbiosis marked the origin of the physiological attributes of cells that we today attribute to mitochondria, but he thought that the organelle responsible for those traits was the nucleus, which in his view was derived from an endosymbiotic bacterium (Mereschkowsky 1905). He furthermore thought that the nucleus in fungi arose autogenously (not from an endosymbiont) such that the nucleus in fungi and other eukaryotes had separate origins, one endosymbiotic in origin, the other not, a line of reasoning that caused him to miss the prokaryote eukaryote dichotomy. The prokaryote eukaryote dichotomy is, in turn, usually attributed to a 407-page 1937 book by Edouard Chatton that is extremely rare, of which we have no

copy and hence will not cite. Indeed, as beautifully explained by Katscher (2004), the situation is more complicated because Chatton (1925) introduced the terms *Eucaryotes* and *Procaryotes*, but only in diagrams in the very final pages of the article, the initial occurrence being shown in Figure 31, while the 1937 book uses the term *Eucaryotes* and *Procaryotes* only once (Katscher 2004), in the text. The broader meaning that became attached to the terms that Chatton introduced, namely the prokaryote eukaryote divide that is now recognized, was provided much later by Stanier and van Niel (1962). A look at the 1925 scheme by Chatton (Figure 31), the page on which the term *Eucaryotes* entered the literature, reveals a number of protists with anaerobic physiology. The later, modern spelling (eukaryotes with a k) is justified because the transcription of the original ancient Greek *karyon* (for “nut”) prescribes a k.

There were endosymbiotic ideas about the evolution of mitochondria before Margulis’s first paper on the topic, which she published under the name Sagan (Sagan 1967). In his 1918 book *Les Symbiotes*, Paul Portier published ideas on the symbiotic origin of mitochondria in French, as discussed in Sapp (1994) and Archibald (2014). The contributions by Ivan Wallin (1925) should be mentioned, as he was explicit about the bacterial nature of mitochondria and even went so far as to suggest that genes would be transferred from the endosymbiont to the host, at a time when it was not known what genes were or what their chemical composition was. We recall that people did not know how ATP was made in mitochondria until the 1970s, just after DNA was being discovered in organelles (Nass and Nass 1963). We also recall that endosymbiotic theory was not widely accepted until the 1980s. An in-depth survey of the very early history of symbiosis is given in a book by Geus and Höxtermann (2007), but published in German. It was there that we found mention of Katscher (2004), which provides valuable and thorough coverage of the terms prokaryote and eukaryote but is almost never cited.

Coming back to oxygen, Margulis’s revival of endosymbiotic theory had it that eukaryotes are ancestrally aerobes, it matched with the view of atmospheric evolution that was current at the time (Cloud 1968). Her version of endosymbiotic theory ended up in college microbiology classrooms (including our own). It said that eukaryotes were ancestrally bacterium-eating (phagotrophic) and their mitochondria arose with rising O₂ levels 2 billion years ago at a time when oxygen started accumulating in the atmosphere (later known as the great oxidation event): “It is suggested that the first step in the origin of eukaryotes from prokaryotes was related to survival in the new oxygen-containing atmosphere: an aerobic prokaryotic microbe (i.e. the protomitochondrion) was ingested into the cytoplasm of a heterotrophic anaerobe.” (Sagan 1967, p. 228). This basic idea is found in many other contributions on the topic (Andersson et al. 1998; Andersson and Kurland 1999; Cavalier-Smith 2002). It is implicit in many others, so implicit that it is often not even spelled out as an assumption. Biologists familiar with anaerobic physiology in specific groups of eukaryotes steered clear of the topic of endosymbiotic origin of mitochondria (Fenchel and Finlay 1995; Hochachka and Somero 2002), probably because

ESSAI DE CLASSIFICATION DES PROTISTES(1)



(1) La subdivision n'a été poussée jusqu'aux familles que dans quelques groupes. Il ne s'agissait pas de dresser ici un tableau complet et détaillé de la classification de Protistes, mais de résumer et d'illustrer les vues exposées dans ce mémoire et de montrer en quoi elles diffèrent des notions classiques.

anaerobic mitochondria did not fit into the mold that Margulis' version of endosymbiotic theory had cast and because there were no physiologically founded alternatives available that would account for anaerobic mitochondria.

The host in Margulis's version of endosymbiotic theory, and variants descended from it, was an anaerobic, phagocytosing microbe that could gain protection from toxic oxygen by acquiring an oxygen respiring symbiont. As time passed, so the simplistic version, the symbiotic association would lead to internalization of the aerobic bacteria and direct detoxification of the host's cytoplasm before eventually the endosymbionts became aerobic, respiring mitochondria, which currently generate ATP for eukaryotic cells (Andersson and Kurland 1999). From this traditional line of reasoning came the premise that the endosymbiotic event and formation of aerobic mitochondria represented the evolutionary separation of primitive mitochondrion lacking anaerobic eukaryotes (ancestral) from aerobic eukaryotes with mitochondria (derived). The most explicit and consistent forms of that theory were presented by Margulis (Margulis et al. 2006), although her version of endosymbiosis always had spirochaetes as additional symbionts that, in her view, gave rise to eukaryotic flagella. The flagella part of the theory had no physiological basis and never gained much footing as an explanatory tool in the literature. Though the spirochaete part of her proposal was not widely adopted, her basic idea that the entry of aerobic mitochondria into a phagocytosing fermenter coincided with the advent of an oxygenated atmosphere, and that mitochondria separated anaerobic eukaryotes (ancestral) from aerobic eukaryotes with mitochondria (derived) became the standard model.

Figure 31: Coinage of the terms Procaryotes and Eucaryotes in 1925 on page 76 of Chatton (1925). Source gallica.bnf.fr / Bibliothèque nationale de France. Chlamydomonads, euglenids, chytrids (as flagellates), entamoebids, apicomplexans (toxoplasma), ciliates and microporida are included in the scheme. According to Katscher (2004), page 76 of Chatton (1925) marks the first appearance of the terms in the literature. As pointed out by Katscher, Chatton was concerned only with protists and did not include higher plants or animals in his scheme, hence one could discuss whether Chatton, like Mereschkowsky, actually missed the prokaryote eukaryote divide that we now recognize, but for a different reason, despite introducing the terms.

30 Ubiquitous mitochondria among anaerobes

The discovery of hydrogenosomes (Lindmark and Müller 1973) initially had no impact on endosymbiotic theories for the origin of mitochondria, except for the occasional suggestion that hydrogenosomes might have descended from a different endosymbiont than the mitochondrion did (Whatley et al. 1979). The RNA-based revolution in microbial taxonomy spearheaded by Woese and colleagues had *Giardia*, *Trichomonas*, *Entamoeba*, and microsporidians branching early in eukaryote evolution, basal to mitochondrial lineages. At the time, this notion matched with cytological observations that these parasites were structurally simple eukaryotic species, intermediate forms from the prokaryote-to-eukaryote transition. That resulted in the creation of a paraphyletic group following the idea of primitive eukaryotes under a common formal name Archezoa (Cavalier-Smith 1983a). Archezoa gave the presumed primitive anaerobes in the standard model a formal name and rank. The representatives of archezoa were thought to lack mitochondria because they had branched off from the mainstream of eukaryotic evolution before the endosymbiotic event with the α -proteobacterium occurred that gave rise to the mitochondrion. Note that the term α -proteobacteria was not introduced until the late 1980s (Stackebrandt et al. 1988), before that they were called purple nonsulfur bacteria (John and Whatley 1975). The species of archezoa were considered as contemporary descendants of phagocytosing primitively amitochondriate cells with nuclei, direct descendants of the host that acquired the α -proteobacterial endosymbiont (Cavalier-Smith 1983b). Margulis' version of the standard model had the phagotroph arising from an archaeal-spirochaete symbiosis (Margulis et al. 2000). Doolittle's (1998) version of the standard model did not specify the mechanism by which the phagotroph arose. There was a time when people were quite confident in the standard model that had eukaryotes arising via point mutation from archaea, becoming phagocytotic for some reason. Only recently has anyone even inspected, from the standpoint of physiology, the assumption that a prokaryote could become phagocytotic via point mutation. The idea does not work because a phagocytotic prokaryote would be digesting its bioenergetic membrane in an attempt to gain energy (Martin et al. 2017), the origin of phagocytosis only works if a cell already has mitochondria.

The problems with the archezoa idea were severalfold. First, the trees were wrong. The lineages thought to be archezoa were branching in the wrong place, artifactually deep (Stiller and Hall 1999; Philippe et al. 2000a). Second, the archezoa all turned out to have organelles of mitochondrial origin after all (van der Giezen 2009). Third, aerobes and anaerobes interleaved in phylogenies (Embley and Martin 2006). Aerobic, anaerobic, and facultative anaerobic eukaryotic species, and their diverse forms of organelles of mitochondrial origin, were found to result from multiple independent specialization events to ecological niches with variable oxygen availabilities, within different evolutionary lineages (Müller et al. 2012). The defining trait of the

prokaryote eukaryote divide no longer boiled down to the presence or absence of nuclei in cells; it had to be expanded by the presence of mitochondria.

The microsporidian fungi exemplify the phylogenetic problem. Using traditional phylogeny models, which presumed identical rate of evolution for all regions of compared sequences, they branched relatively early compared to other eukaryotes and their long branches split close to the long branches of prokaryotic outgroups (Leipe et al. 1993; Hashimoto et al. 1997). However, inclusion of larger amounts of sequence data in the comparison and the use of more modern phylogenetic methods led to completely different results, putting microsporidia into the kingdom of fungi within phylogenetic trees (Hirt et al. 1999; Keeling et al. 2000; James et al. 2006). The early branching of microsporidia in older phylogenetic trees of eukaryotes was, in hindsight, a phylogenetic artifact.

New genome sequences, the sequencing of large numbers of representatives of many eukaryotic lineages, including newly discovered ones, and the development of more complicated phylogenetic methods has led to a thorough reorganization of evolutionary relations among eukaryotes (Caron et al. 2017). Although the concept of archezoa fell apart, Margulis's phagocytotic mitochondrial acquisition idea at its heart – mitochondria as evolutionary indigestion – remained inertial to much literature on the topic, even as it was becoming evident that none of the organisms people had thought were archezoa were what the theory had predicted them to be (Doolittle 1998). Additional information was also provided by less apparent molecular characteristics, such as gene insertions and deletions at selected loci or fusions of particular genes (Burki 2014).

New views of phylogenetic relations among eukaryotes have emerged and continue to emerge (Caron et al. 2017). Most eukaryotic species are now classified into novel utilitarian taxa called supergroups (Baldauf 2003; Simpson and Roger 2004; Adl et al. 2005; Keeling et al. 2005; Parfrey et al. 2006). In newer phylogenetic trees of eukaryotes, amitochondriate lineages and anaerobes are interspersed among roughly six major branches, each representing one supergroup: Opisthokonta, Amoebozoa, Archaeplastida, Excavata, Chromalveolata, and Rhizaria, the terminology we use here. However, relationships between individual supergroups have not been completely resolved to date (Caron et al. 2017), and new lineages continue to be discovered. These new trees sometimes contain polytomies (multifurcations) at their base. This is due to different reasons. Some eukaryotic lineages are represented by insufficient numbers of species, which presents an obstacle for phylogenetics when the identification of very early branching lineages is the goal (Graybeal 1998). In addition, the problem of resolving deep branches in phylogenetic trees is generally severe (Penny et al. 2001; Ho and Jermiin 2004). But worse, even if molecular phylogeny worked perfectly, a substantial amount (perhaps all?) of the phylogenetic signal needed to solve the early branches in the diversification of eukaryotes might have been lost from sequence data (Embley and Martin 2006). Were that not bad enough, the genome of the last eukaryote common ancestor was replete with gene duplications (Tria et al. 2019), and such duplications

are well known to confound attempts to get phylogenetic relationships sorted out. Note that some robust characters like the presence of plastids and mechanisms of protein import (Gould et al. 2015) are in direct conflict with molecular phylogenies. The only thing we can say for sure about molecular phylogenies is that they change in their most important aspects on a very regular basis as new data and new methods accrue.

As mentioned previously, the classification of ciliates is difficult, and one generally accepted important change has actually taken place in eukaryotic supergroups, in spite of numerous controversies and ambiguities, it concerns the Chromalveolata supergroup. Originally, it included four species-rich lineages of eukaryotes: alveolates, stramenopiles, haptophytes, and cryptomonads (Keeling 2004; Reyes-Prieto et al. 2007). However, as more and more phylogenetic data accumulated, support for the monophyletic origin of the four groups vanished (Burki et al. 2007; Rodríguez-Ezpeleta et al. 2007; Burki et al. 2008). The phylogenetic position of haptophytes and cryptomonads became unclear and, conversely, the alveolates and stramenopiles were joined by the supergroup Rhizaria. This grouping created, on the rubble of the Chromalveolata supergroup, a new monophyletic SAR supergroup named after the included clades (the acronym contains the first letters of “*s*tramenopiles,” “*a*lveolates,” and the name of the original eukaryotic supergroup *R*hizaria) (Burki et al. 2007; Burki 2014). However, the mechanism and protein machinery that the photosynthetic members of the chromalveolates (or SAR) use to import proteins into their red secondary plastids are the same (Gould et al. 2015), an observation that argues in favor of chromalveolates being correct after all and that is not subject to the vagaries of phylogenetic tree parameters and uncertainty. As a side note, we point out that the loss of support for the chromalveolates with larger amounts of data could be due to additive effects of undetected paralogy (gene duplications). The plastids of the chromalveolates are an example where endosymbiosis as an organelle-generating process produced higher level cytological structures (plastids) that have a very clear phylogenetic interpretation (single origin, recurrent reduction, occasional loss), while individual gene trees generate massively conflicting results.

Coming back to the mitochondrial family of organelles and anaerobic energy metabolism in eukaryotes, the current phylogenetic tree with five (or six) supergroups provides a completely different view than the one described in the 1980s and 1990s by the concept of archezoa. Hydrogenosomes, mitosomes, and various transition forms of the organelles of mitochondrial origin are found on different branches located throughout the phylogenetic tree representing the full breadth of eukaryotic diversity (Figure 32). Eukaryotic anaerobes are not rare, unusual, or phylogenetically primitive species occupying obscure early branches of the tree. On the contrary, their numerous representatives overlap, evolutionarily, with aerobic relatives, which indicates that aerobic and anaerobic species of eukaryotes evolved side by side, at least since ocean oxygenation at the end of Proterozoic (Mentel and Martin 2008; Lyons et al. 2014).

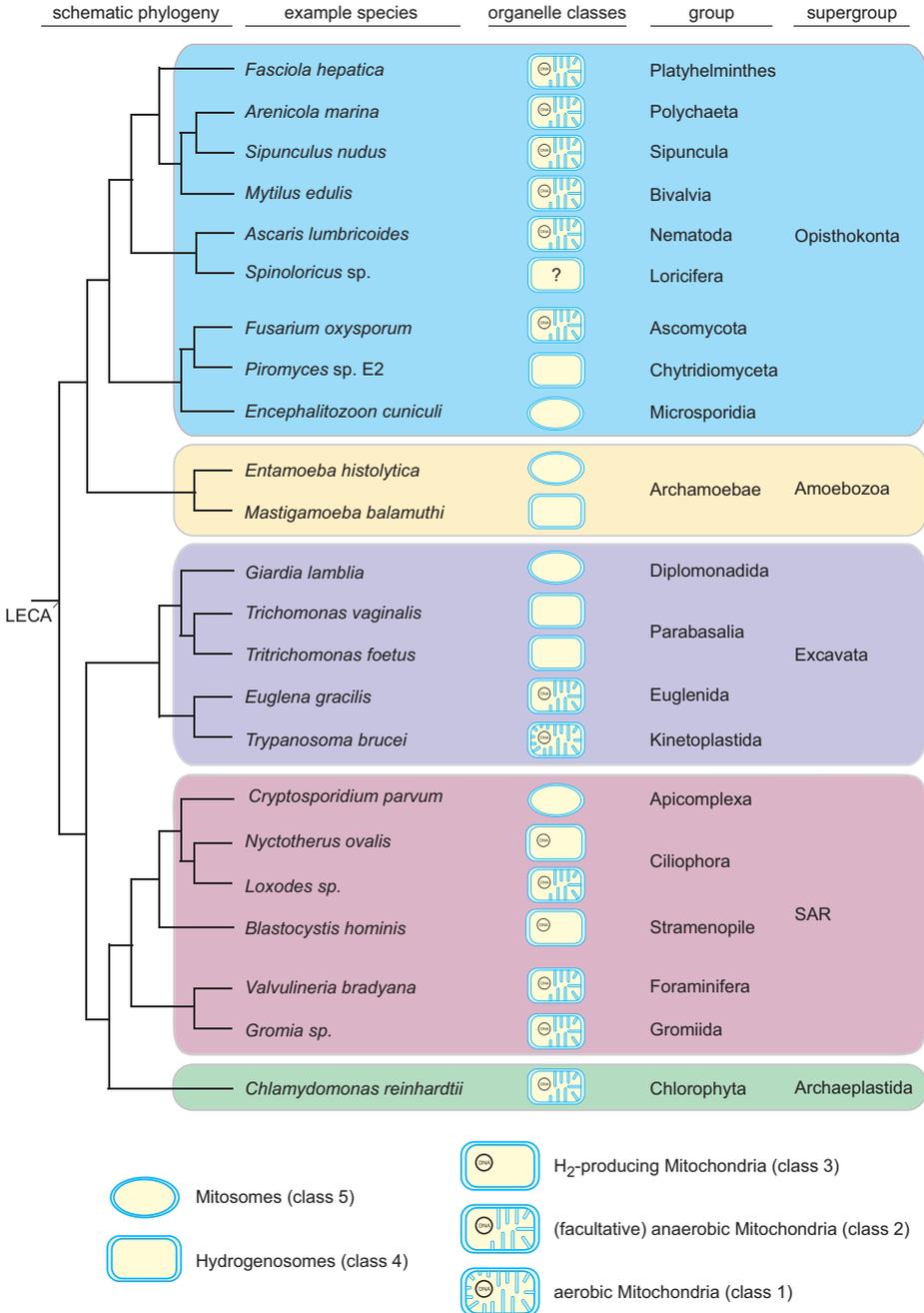


Figure 32: Anaerobic mitochondria across eukaryote supergroups. The topology of the tree reflects the five currently accepted eukaryotic supergroups (Burki 2014); the branching adheres to recent suggestions (Burki et al. 2016). The species shown are those model organisms whose anaerobic

The changes in views about taxonomic relations among eukaryotes will continue. There has been a proposal, for instance, that the drop-outs from the Chromalveolata supergroup, haptophytes and cryptomonads, together with several minor lineages, form a new supergroup Hacrobia or clade CCTH (abbreviation contains the first letters of “*cryptophyta*,” “*centrohelida*,” “*telonemia*,” and “*haptophytes*”) (Okamoto et al. 2009). However, same as with the Chromalveolata supergroup, the monophyletic origin of all members of the club has never been confirmed; therefore, it is simpler to refer to individual lineages with unclear phylogenetic affinity. The same applies to representatives of the lineages “*Picozoa*,” “*Microhelida*,” or photosynthesizing rappemonads (lat. “*Rappemonada*”) (Speijer et al. 2015). The only constant in molecular phylogenetics is that the trees always change; the organisms stay as they are, regardless of where they branch in phylogenetic trees.

An ever-growing group of small but evolutionarily relevant eukaryotic lineages has been uncovered, consisting of amoebas, amoeboflagellates, and flagellates, which have been assigned the rank of microkingdoms and cannot be included in any of the accepted supergroups (Pawlowski 2013). They are, for instance, microkingdoms of apusomonadids (lat. “*Apusomonadida*”) and breviateids (lat. “*Breviatea*”), which were proposed to fuse with the Opisthokonta supergroup and form an even larger group under a new name Obazoa (Brown et al. 2013). The legitimacy of such grouping depends, though, on the root position of the eukaryotic tree, which is another phylogenetic issue that has not yet been resolved (Burki 2014). Historically, during the division of eukaryotes into six supergroups, the root of the phylogenetic tree including all eukaryotes was placed between two large groups: (1) Unikonta, which included supergroups Opisthokonta and Amoebozoa and (2) Bikonta with supergroups Archaeplastida, Excavata, Chromalveolata, and Rhizaria (later Archaeplastida, Excavata, and SAR) (Cavalier-Smith 2002). The group Unikonta was later renamed to Amorphea (Adl et al. 2012) and the position of the tree root between Unikonta and Bikonta (Embley and Martin 2006) indicated in Figure 32 is still the most popular (Burki 2014).

Multiple hypotheses have appeared, though, with an alternative positioning of the root, one that splits eukaryotic life into two groups named Opimoda and Diphoda (Derelle et al. 2015). However, that division of eukaryotes would dissolve the Excavata as a supergroup because one of its three main groups, Discoba, would

Figure 32 (continued)

energy metabolism has been described in detail, in most cases. Their distribution in the phylogenetic tree indicates that anaerobic eukaryotic species are not isolated from aerobic eukaryotes, as they are found in all supergroups and cannot be considered to be evolutionarily primitive or descendants of primitive eukaryotes. As the only obligate aerobic species, the protist *Trypanosoma brucei* is shown. Presence of a genome in individual organelles is indicated by a circle inscribed “DNA.” Sizes of the different organelles of mitochondrial origin are not shown to scale. Identity of mitochondria of the animal phylum Loricifera has not been clarified so far. Abbreviation: LECA, last eukaryotic common ancestor.

be classified to the group Diphoda, while another main group Malawimonadida would belong to the group Opimoda. The phylogenetic position of the third main group, Metamonada, has not been resolved convincingly (Speijer et al. 2015). These groupings change the classification of the organisms, but not their physiology. The last eukaryote common ancestor, LECA, had hundreds of gene duplications in its genome (Tria et al. 2019). Duplications generate differential gene loss in different lineages, which generates paralogy, which in turn makes the identification of the root in the eukaryote tree with gene phylogenies difficult at best. The phylogenetic distribution of the duplications themselves places the eukaryotic root on the branch bearing the Excavata (Tria et al. 2019). In any case, topological adjustments of the eukaryotic phylogenetic tree do not change the central finding that anaerobic eukaryotes are found throughout the breadth of eukaryotic diversity on the Earth, mutually interlacing with their aerobic relative species, from which they are not distinguished in a phylogenetic manner, regardless of where the root lies.