Mitochondria and the Rise of Eukaryotes

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There can be little doubt that mitochondria do not need much of an introduction. It is widely known that they are the "powerhouses" of the cell and that they produce all of the ATP (adenosine triphosphate) needed to sustain life. In addition, all eukaryotes (organisms with a cell nucleus) contain these important organelles—or so it was thought. Here lies an interesting paradox: Although it was generally believed that all eukaryotes did have mitochondria, it was also generally believed that the serial endosymbiosis theory was correct; the endosymbiosis theory required the existence of eukaryotes without mitochondria. This assumption was formalized with the now-disproven Archezoa hypothesis, which stated that several groups of "primitive" eukaryotes were of premitochondrial descent. This paradoxically defined group of amitochondriate eukaryotes has resulted in a spate of publications that have significantly changed the perception of the role of mitochondria in overall cellular metabolism and that have important ramifications for our understanding of the origin of eukaryotic life.

Keywords: anaerobic mitochondria, anoxia, evolution, cell biology, eukaryotes

Early history

Mitochondria (figure 1) were first described by the Swiss anatomist and physiologist Von Kölliker in 1856, when he was studying muscle tissue. These "sacrosomes" were later termed *mitochondria* by Benda in 1898, who observed the organelles during spermatogenesis. *Mitochondrion* is a combination of the Greek words *mitos* (thread) and *chondros* (granule). At the turn of the previous century, biologists were intrigued by the various cellular structures that they encountered, and they posited several ideas regarding the origin of these organelles. One of those ideas was that certain organelles were bacterial symbionts that had taken up residence inside eukaryotic cells. For example, in 1905, Mereschowsky suggested that the cell nucleus and chloroplasts were of bacterial origin, and Portier suggested the same for mitochondria in 1918 (reviewed in Martin 2007). However, serious opposition from the scientific community led to a nearly 50-year silence about the possibility of a bacterial origin of mitochondria. Scientific interest in mitochondria continued nonetheless, especially after it became possible to purify the organelles, opening the door to functional studies. The important role of mitochondria in early scientific research might be apparent from the famous names associated with these organelles. Arguably, it might be the organelle that has resulted in the most Nobel Prizes. Warburg (who won the Nobel Prize in 1931) realized that cellular respiration was associated with insoluble subcellular structures that we now know were mitochondria. Krebs (who won the Nobel Prize in 1953) localized the enzymes from the citric acid cycle to mitochondria. The ability to purify intact functional mitochondria greatly aided further work (by Palade, who won the Nobel Prize in 1974). Perhaps the most amazing discovery was that ATP (adenosine triphosphate) production in mitochondria has nothing to do with substrate-level phosphorylation. Mitchell's groundbreaking work to explain oxidative phosphorylation with his chemiosmotic hypothesis led to a Nobel Prize in 1978. Determining the composition and structures of the complexes involved in oxidative phosphorylation, in particular that of ATP synthase, resulted in a Nobel Prize for Walker and Boyer in 1997.

Interest in the evolutionary origin of mitochondria was reignited after the discovery that these organelles contained their own genomes (Nass and Nass 1963). This finding followed logically from earlier work that indicated that mitochondrial inheritance does not follow Mendelian rules (Mitchell and Mitchell 1952) and that mitochondria synthesize their own proteins (McLean et al. 1958). This renewed interest in mitochondrial evolution resulted in the seminal reformulation of the endosymbiont theory by Lynn Margulis (Sagan 1967). The serial endosymbiosis theory suggested that a bacterial endosymbiont established itself inside a protoeukaryote and became the mitochondrion. Although the concept was considered heretical some 40 years earlier, the scientific community was now ready to consider this "novel" idea. Schwartz and Dayhoff's (1978) phylogenetic analysis indeed suggested that mitochondrial-encoded cytochromes were of an alpha-proteobacterial nature. Comparison of eukaryotic mitochondrial 16S ribosomal RNA with that of alpha- and beta-proteobacteria clearly indicated the alpha-proteobacterial nature of mitochondrial RNA as well (Yang et al. 1985). Even nuclear-encoded chaperonins destined

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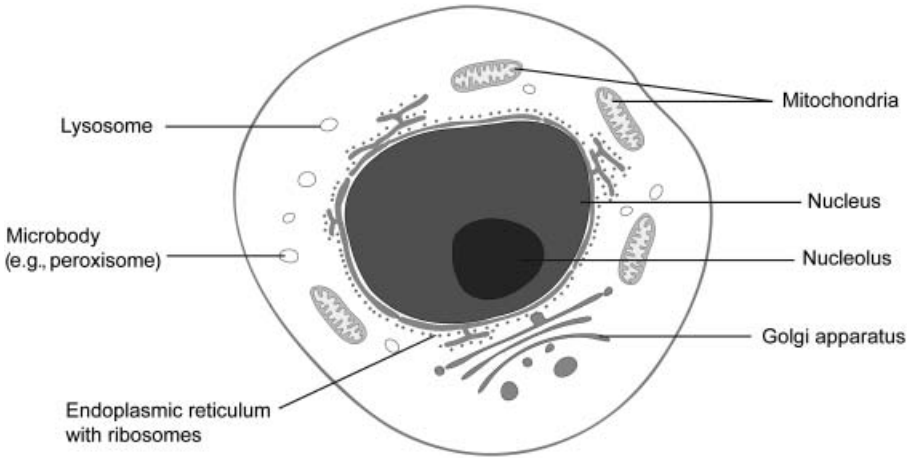

Figure 1. A typical eukaryotic cell. A schematic representation of a classic eukaryotic cell showing the group-defining nucleus and mitochondria among other eukaryotic organelles. Source: Bryony Williams, University of Exeter.

for the mitochondria were shown to provide evidence of a proteobacterial origin (Gupta et al. 1989). The crowning achievement in the hunt for the endosymbiont's origin was the completion of the sequencing of the genome of the obligate intracellular bacterium *Rickettsia prowazekii* (Andersson et al. 1998). The proteins encoded by the genome of this bacterial pathogen showed many similarities to mitochondrial proteins, which strongly suggests that the endosymbiont that gave rise to mitochondria must have been related to an organism similar to *R. prowazekii* (Kurland and Andersson 2000).

The confusion

During 1990s, there seemed to be a convincing argument to explain the origin of eukaryotes and their mitochondria: The protoeukaryote must have evolved from an archaebacterial ancestor, because most eukaryotic informational genes (i.e., those involved in the genetic machinery) are of archaebacterial origin (Rivera et al. 1998). Indeed, phylogenies showed that eukaryotes and archaebacteria are sister groups (Woese et al. 1990). In addition, growing amounts of data clearly indicated that the mitochondrial endosymbiont was of alpha-proteobacterial origin (Gray et al. 1999). Earlier, with the Archezoa hypothesis, Cavalier-Smith (1983) had postulated the existence of primitive amitochondriate eukaryotes whose descendents could now be found among simple eukaryotes such as *Giardia*, *Entamoeba*, *Trichomonas*, and microsporidia. These amitochondriates would therefore have been ideal candidates for the host that took up an alpha-proteobacterium that ultimately became the mitochondrion. Molecular phylogenies had indeed shown the deep positions of these Archezoa on the tree of life (Vossbrinck et al. 1987); thus, all of these observations were in agreement with the serial endosymbiosis theory (Sagan 1967). To summarize, eukaryotes evolved from archaebacteria, and the subsequent protoeukaryote took up an alpha-proteobacterium, which became the mitochondrion, which in turn led to the evolution of mitochondria-containing eukaryotes. This narrative can still be found in many textbooks.

However, a finding reported by Clark and Roger in 1995 was not compatible with this story. They demonstrated that one of the supposedly amitochondriate Archezoa (*Entamoeba histolytica*) contained two genes in its genome that encode mitochondrial-targeted proteins in other eukaryotes. Their publication was the first of many similar studies to indicate that the Archezoa did contain genes with mitochondrial ancestry in their genomes (more extensively reviewed in van der Giezen 2009). This could perhaps be explained by saying that the Archezoa did once contain mitochondria but subsequently lost them, and the identified mitochondrial proteins would be the last remaining evidence of this evolutionary past. However, this explanation would mean that the Archezoa were not primitively amitochondriate and could therefore not be taken as examples of eukaryotes that were related to the protoeukaryote that took up the mitochondrial endosymbiont. The final blow for the Archezoa hypothesis was that the laboratories that identified these mitochondrial proteins subsequently localized these proteins to small organelles (Tovar et al. 1999, 2003, Williams et al. 2002). The organelles were all surrounded by two membranes, suggesting that they might be previously undetected mitochondria, which would falsify the Archezoa hypothesis.

Mitochondrial remnants

There are two classes of organelles that are surrounded by two membranes: plastids and mitochondria. Both of these organelles are of endosymbiotic origin. The discovery of organelles that were surrounded by two membranes in the Archezoa clearly suggested that those were endosymbiotically derived as well. Antibodies raised against mitochondrial proteins were used to identify and localize these organelles, which constitutes a strong case that these organelles are mitochondria related. Various names have been given to these newly discovered organelles, which in principle can be divided into two groups: Those that do play a role in ATP production (also called metabolic "type II" after Martin and Müller [1998]) are called *hydrogenosomes* and those that do not ("type I") are called *mitosomes*.

Hydrogenosomes

The discovery of hydrogenosomes predates the Archezoa hypothesis, since these organelles were first described by Cerkasovová and colleagues (1973) and Lindmark and Müller (1973). Early studies were focused on clarifying the carbon

fluxes through this organelle, and such work provided a clear comparative framework for further studies in the field. Similar to mitochondria, hydrogenosomes convert pyruvate into carbon dioxide and ATP, but they use different enzymes to do so (Müller 1993). The evolutionary origin of hydrogenosomes was not clear and was hotly debated at the time (for a historical overview, see Müller 2007). The first hydrogenosomes were discovered in sexually transmitted trichomonads and more recently have been found in many different eukaryotes (van der Giezen 2009). It was, however, the start of molecular work that revealed the evolutionary links of these organelles. The gene encoding the trichomonad hydrogenosomal ferredoxin was the first to be sequenced and, surprisingly, seemed to contain a short mitochondrial presequence that was missing from the mature protein (Johnson et al. 1990). Further studies revealed more of these presequences on hydrogenosomal proteins, and not only on those from trichomonads, but also on hydrogenosomal proteins from ciliates and fungi. The presequences resembled mitochondrial targeting signals that are normally needed to correctly target and import proteins into the mitochondrial matrix. Studies using heterologous hosts demonstrated the sufficiency and essentiality of these hydrogenosomal presequences to target hydrogenosomal and reporter proteins to mitochondria (Bradley et al. 1997, van der Giezen et al. 1998). Thus, hydrogenosomes and mitochondria were found to share the same protein import machinery. The discovery of mitochondrial chaperones with clear mitochondrial phylogenies and mitochondrial-like targeting signals more or less sealed the deal with respect to the mitochondrial origin of hydrogenosomes.

Mitosomes

The second class of mitochondrial organelles—the mitosomes—was discovered only at the end of the previous century (Tovar et al. 1999). Antibodies raised against the previously discovered mitochondrial Hsp60 (Clark and Roger 1995) showed a discrete localization, suggesting the presence of an organelle in *E. histolytica*. When the putative organellar targeting signal was removed from the *E. histolytica* Hsp60, the protein accumulated in the cytosol, a phenotypical trait that could be reversed by the addition of a genuine mitochondrial targeting signal from another protein from a completely different organism (Tovar et al. 1999). Later, using antibodies raised against mitochondrial chaperones, researchers discovered mitosomes in the apicomplexan *Cryptosporidium parvum* (Riordan et al. 1999) and the microsporidian *Trachipleistophora hominis* (Williams et al. 2002). In the case of the excavate *Giardia intestinalis*, antibodies raised against proteins involved in iron–sulfur cluster synthesis were used (Tovar et al. 2003), because it had become clear that mitochondria play essential roles in the production of such clusters as cofactors for many enzymes (Lill et al. 1999). Genes encoding enzymes involved in iron–sulfur cluster assembly had indeed been identified in *Trichomonas vaginalis* and *G. intestinalis* (Tachezy et al. 2001), and their phylogenetic history suggested a mitochondrial origin for their proteins. The discovery of the involvement of the proteins in iron–sulfur cluster assembly and their localization to tiny organelles surrounded by two membranes in *Giardia* convincingly demonstrated the mitochondrial nature of these organelles (Tovar et al. 2003). Similar studies on other mitosome-containing organisms confirmed these findings: All such organisms have proteins involved in iron–sulfur cluster assembly that are localized to their mitosomes (LaGier et al. 2003, Ali et al. 2004, van der Giezen et al. 2004, Goldberg et al. 2008, Maralikova et al. 2010). Interestingly, two amoebae, *E. histolytica* and *Mastigamoeba balamuthi*, seem to have replaced their mitochondrial iron–sulfur cluster-assembly proteins with a simpler system (Ali et al. 2004, van der Giezen et al. 2004, Gill et al. 2007). In neither of these organisms do the proteins contain targeting signals, which suggests that they might not be targeted to the organelles (although in the case of *Entamoeba*, the discussion is ongoing) (Mi-ichi et al. 2009, Maralikova et al. 2010). Many mitosomal proteins contain (as do hydrogenosomal proteins) presequences that resemble mitochondrial targeting signals, suggesting the presence of similar protein import mechanisms, and these have indeed been identified (reviewed in Lithgow and Schneider 2010).

Mitochondrial genomes

The assumption that bacteria, at the time of the mitochondrial endosymbiotic event, were as complex as current bacteria can be justified on the basis of biogeochemical evidence (Nisbet and Sleep 2001). We can therefore assume that their genomes were probably as large as those of modern bacteria. Remnants of these bacterial genomes are still visible in modern-day mitochondria. Human mitochondria, for example, contain a small, circular genome nearly 17 kilobases (kb) in size that encodes various mitochondrial proteins. However, bacterial genomes range from 160 kb to 13 megabases (Koonin 2009); some DNA was lost during the transition from endosymbiont to mitochondrion. Such DNA has either been lost entirely or has been transferred to the host nucleus through a process known as *endosymbiotic gene transfer* (Timmis et al. 2004). Phylogenetic analysis of genes still encoded on mitochondrial genomes has been one of the strongest pieces of evidence in support of the alpha-proteobacterial origin of mitochondria (Gray et al. 1999). However, the first hydrogenosomes and mitosomes discovered had lost their organellar genomes completely. Although this finding was perfectly aligned with ideas of why mitochondria retain genomes (Allen 2003), it implied that the evolutionary relationship of hydrogenosomes and mitosomes could only indirectly be determined. The recent discovery of hydrogenosomes with genomes has changed this, and analyses of the organellar genomes of the cockroach intestinal ciliate *Nyctotherus ovalis* (de Graaf et al. 2011) and the human intestinal pathogen *Blastocystis* (Pérez-Brocal and Clark 2008) clearly show the mitochondrial nature of these organelles. The organellar genome from another intestinal anaerobe, *Proteromonas lacertae* (Pérez-Brocal et al. 2010), which is predicted to contain hydrogenosomes, also supports this notion, although the nature of this organelle has not been completely determined.

Better sampling?

When the distribution of mitosomes and hydrogenosomes is plotted on a eukaryotic phylogenetic tree, it becomes clear that they are omnipresent in eukaryotes and that this distribution has important implications (figure 2). A theory favored by some is that these organelles are evolutionary "dead ends" that are the consequence of adaptations to anoxic habitats. The distribution of hydrogenosomes in various ciliates' lineages (Embley et al. 1995) suggests that hydrogenosomes are some sort of evolutionary stable state that occurs as a result of similar evolutionary pressures (van der Giezen 2009). When an organism ventures into an anaerobic niche, it discards most of the classical mitochondrial features, such as oxidative phosphorylation and a genome, and borrows anaerobic enzymes, such as hydrogenase, from bacteria that are prevalent in that niche. However, considering the wealth of biochemical possibilities that bacteria have when compared with eukaryotes, why do eukaryotes always select a similar gene set for the manufacture of hydrogenosomes (Lane 2010)? An explanation that requires the invocation of fewer evolutionary events would be that anaerobic biochemistry is hardwired in eukaryotes from a much earlier time. This might also explain the relatedness of hydrogenosomal hydrogenases and essential and universal eukaryotic Nar1 proteins (Balk et al. 2004) or complex I components found in all classic mitochondria (Vignais 2008). One of the problems here lies in the generally poor sampling of model organisms. Most founding mitochondrial biochemical work has been done on animal mitochondria. In addition, studies in which yeast was used have provided a wealth of information about mitochondrial function; however, yeast and animals are part of the same eukaryotic branch—that of the opisthokonts (see figure 2). Extrapolating the wealth of information on opisthokont mitochondria to all other eukaryotes might be a tempting approach, but it is one fraught with danger. Flowering plants and their pollinators are obviously different, and some see this as a clear consequence of their long independent evolutionary histories. If this is the case, why is it generally assumed that their mitochondria, which have a much longer independent evolutionary history, are identical, either in form or function? An amazing morphological variety of mitochondria can be seen in the classic book by the great electron microscopist Don Fawcett, clearly showing that mitochondria are much more than the sausage-shaped organelles depicted in textbooks (Fawcett 1981). Current efforts aimed at increasing sampling by sequencing and studying "nonmodel" organisms have revealed that there is no standard mitochondrion. Several recent reviews have discussed the extended metabolic repertoire discovered in the various mitochondrial forms (Mentel and Martin 2008, van der Giezen 2009, Ginger et al. 2010). In summary, it seems that there is an extensive range of anaerobic mitochondrial biochemistry present across all six eukaryotic supergroups (van der Giezen 2009, Ginger et al. 2010).

But mitochondria were aerobic?

The general perception is that mitochondria are rich in oxygen as a result of extensive oxidative phosphorylation

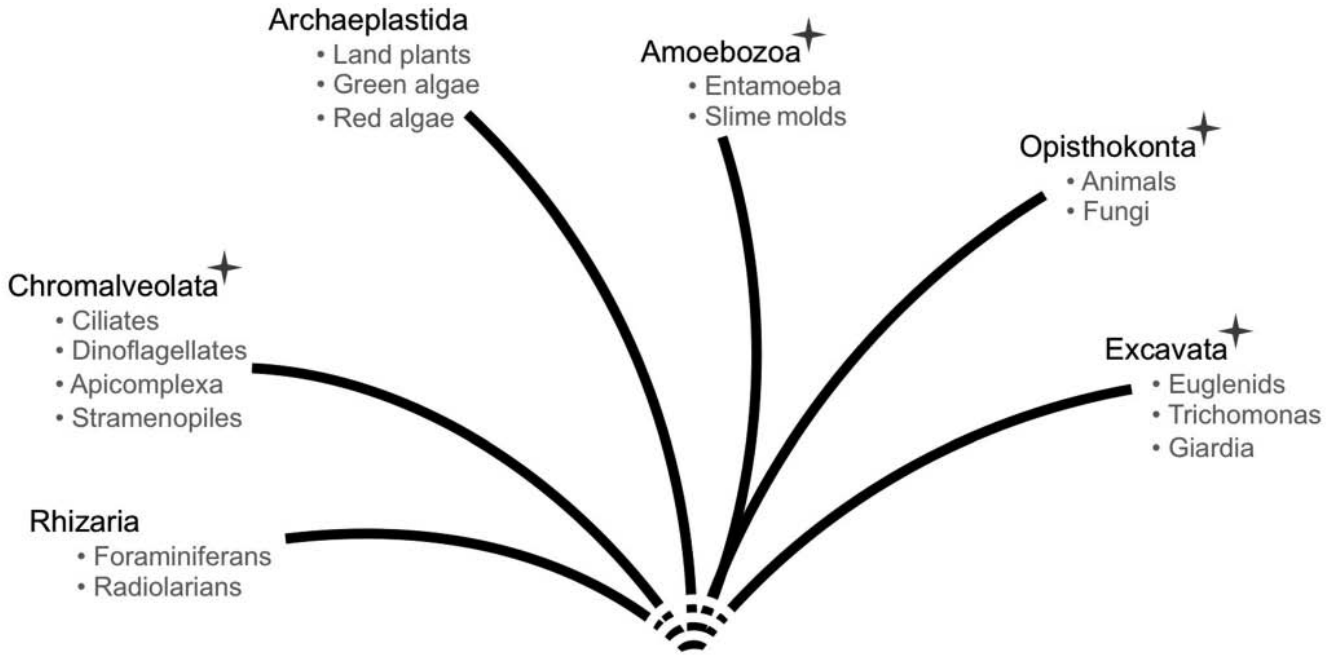

Figure 2. The six eukaryotic supergroups. Until recently, eukaryotic life was distributed into a few "kingdoms." However, recent advances in molecular phylogenetics coupled with an increased interest in traditionally understudied taxa have revealed a completely different classification of the eukaryotes that actually better reflects the true relationships among the eukaryotes (Simpson and Roger 2004). The dashed "root" indicates uncertainty about branching order at the base. The presence of mitosomes or hydrogenosomes is indicated by a star (absence of a star by the Rhizaria is likely due to lack of data, but hydrogenosomes and mitosomes are expected).

activities. However, the extensively folded inner membrane is more likely to act as an oxygen scrubber and the mitochondrial matrix arguably has the lowest oxygen tension of the cell. Oxygen itself is not very reactive, but the free radicals that are generated by the electron transport chain will react with oxygen to produce relatively long-lived oxygen radicals. Because of the high-energy nature of these oxygen radicals, they react with anything in their vicinity, thereby destroying enzymes and nucleic acids, which ultimately results in the aging of the cell. It has been suggested that the role of mitochondria is to protect the cell (the host of the endosymbiont) from the dangers of molecular oxygen (Kurland and Andersson 2000). However, it is the ability to use molecular oxygen that actually causes the danger; the host cell would have been much better off without this oxygen radical producer inside its cytoplasm. The "strategy" has been compared to trying to save oneself from drowning by drinking the ocean (Lane 2010). But if many—if not all—eukaryotes have the ability to perform anaerobic biochemistry in their mitochondria, why would this be? Again, the general perception is that mitochondria arose at the time when oxygen levels were rising (Kurland and Andersson 2000), some 2.4 billion to 2 billion years ago, as is evidenced by the disappearance of mass-independent fractionation of sulfur (Dietrich et al. 2006). However, this first oxygenation event did not result in a complete oxygenation of every niche—far from it. Rather, the oxygenation event resulted in an unexpected ocean chemistry: Oxidatively weathered sulfate ended up in the oceans, where it reacted with molecular hydrogen, resulting in hydrogen sulfide (Anbar and Knoll 2002). These reducing sulfidic oceans are called *Canfield oceans* (Dietrich et al. 2006) and would have been a shelter for anaerobic life for several billion years (Martin et al. 2003). If the planet's oceans had been anaerobic for several billion years (Dietrich et al. 2006) and had reached the current oxygen levels only half a billion years ago, all major eukaryotic branches would have evolved by this time, which would explain the widespread anaerobic biochemistry in every eukaryotic branch.

It now seems that even some true multicellular animals can live under continuous anoxic conditions. Last year, Danovaro and colleagues (2010) reported the first metazoan (figure 3) from a completely anoxic habitat (Mentel and Martin 2010). Currently, the tendency is to dismiss ancestral eukaryotic anaerobic biochemistry by suggesting that all of these eukaryotes are secondarily anaerobic and that they are derived from "normal" aerobic eukaryotes. However, invoking continuous and repeated secondary events might be scientifically questionable when a more simple explanation exists: All eukaryotes may have been capable of anaerobic biochemistry from the start.

A different scenario

Various theories have been proposed to explain the origin of eukaryotes. The fundamental differences among these theories were recently reviewed by O'Malley (2010). In the present article, I will discuss how all the findings from the last 10 to 15 years fit into our current understanding of eukaryotic evolution. The serial endosymbiosis theory relied on the existence of a primitive eukaryotic host without mitochondria. All of the proposed lineages of primitive amitochondriates have now been shown to contain mitochondria. The absence of such a primarily amitochondriate lineage could be explained by a mass extinction event, but there is no evidence for this. Similarly, the force that forged the endosymbiosis has been suggested to be ATP or oxygen removal. As has been argued elsewhere (e.g., Martin and Müller 1998, Lane 2010), no known organism leaks ATP to the environment; therefore, it seems highly unlikely that the free transfer of ATP from endosymbiont to host was the currency that resulted in the establishment of mitochondriate eukaryotes. Oxygen removal, as was discussed above, seems unlikely because as recently as a half billion years ago, many parts of the planet were still anaerobic, whereas we know that eukaryotes arose

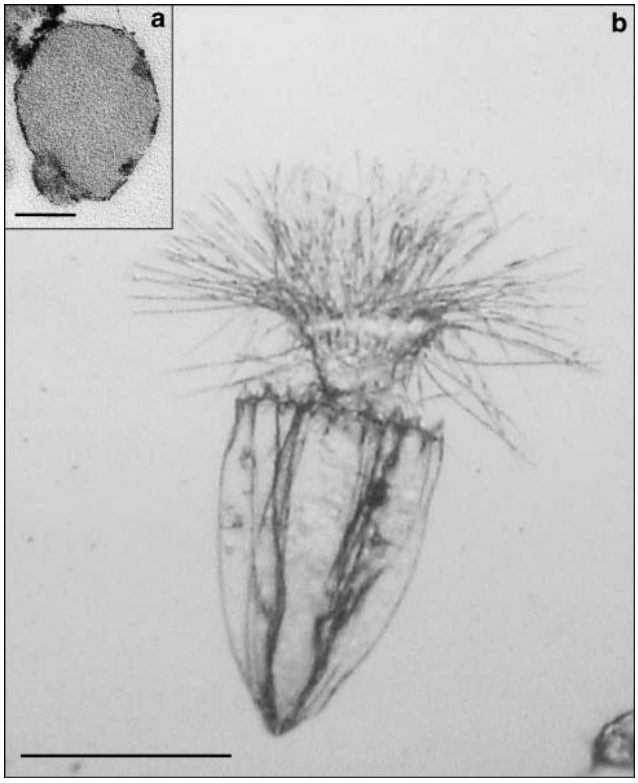

Figure 3. Dogma-changing animals? It is generally believed that multicellular organisms cannot complete their life cycles in the absence of oxygen. Recently, metabolically active animals from the phylum Loricifera have been discovered in the permanently anoxic marine sediments of the Mediterranean Sea (Danovaro et al. 2010). Interestingly, electron microscopy suggests that these animals contain cellular structures similar to anaerobic mitochondria called* hydrogenosomes *(inset). Bars: 0.2 micrometer (a) and 50 micrometers (b). Photograph: Antonio Pusceddu, Ancona, Italy.

nearly two billion years ago. In addition, having an "oxygen magnet" inside an oxygen-sensitive organism seems, from an engineering point of view, absurd, to say the least. There are serious issues relating to the origin and nature of both the host and the endosymbiont.

Let's first have a look at the host. Ever since Woese and colleagues (1990) described the existence of a third domain of life, the Archaea (not Archezoa) have been put forward as the closest relatives of the eukaryotes. Many single-gene trees have resolved this three-domain-of-life tree with eukaryotes sister to the Archaea (Harris et al. 2003). However, methods aimed at identifying the root of the universal tree of life have not been able to reveal this three-domain relationship (e.g., Baldauf et al. 1996). These studies suggest that the host lineage of eukaryotes arose from within the Archaea—more particularly, from within the Crenarchaeota or eocytes (Lake et al. 1984). All recent large-scale analyses show strong support for this within-the-Archaea hypothesis (Pisani et al. 2007, Cox et al. 2008, Kelly et al. 2011).

With regard to the endosymbiont, earlier single-gene phylogenies suggested that an ancestral relative of the obligate intracellular parasite *R. prowazekii* was related to the mitochondrial endosymbiont. Again, more recent, large-scale analyses do not demonstrate such a relationship, and facultative anaerobes such as *Rhodobacter*, an alpha-proteobacterium, do seem to be more closely related to the mitochondrial endosymbiont (Esser et al. 2004, Atteia et al. 2009). *Rhodobacter* species are free-living and facultative anaerobic photosynthesizers and are less derived than *Rickettsia*, which are obligate parasites. Therefore, *Rhodobacter* might be more similar to the bacterial symbiont at the time of the endosymbiosis. The anaerobic biochemistry of *Rhodobacter* species would account for the discovered anaerobic biochemistry of hydrogenosomes and mitosomes. Combining the ideas described above would suggest that a facultative anaerobe similar to the present-day *Rhodobacter* became the endosymbiont inside an archaea-bacterium, which resulted in a eukaryote with powerful mitochondria. Interestingly, just before the discovery of the first mitosome (Tovar et al. 1999), a novel theory had been postulated to explain the origin of both eukaryotes and mitochondria. The novelty was that this theory is not based on similarities among certain cell biological features, but on the biochemical capabilities of the host and endosymbiont. This distinction is important, because biochemical capabilities are the consequence of the presence (or absence) of enzymes that are encoded on genomes and that can be reconstructed using genomics studies. This hydrogen hypothesis states that a hydrogen-producing eubacterium (e.g., *Rhodobacter*) capable of both aerobic and anaerobic biochemistry was taken up by a hydrogen-dependent archaeabacterium (Martin and Müller 1998). This theory therefore explains the widespread presence of enzymes required for anaerobic biochemistry among the eukaryotes; these would have been present from the start. In addition, the hypothesis explains the presence of the two distinct gene classes in the eukaryotes (Rivera et al. 1998) and fits comfortably with more recent large-scale analyses that suggest that eukaryotes arose from within the Archaea (Pisani et al. 2007, Cox et al. 2008, Kelly et al. 2011). Criticism leveled against such a syntrophic model hinged on the idea that bacterial fusions have never been observed. This turned out to be incorrect: We know of two such cases—a cyanobacterium with an internal bacterium (Wujek 1979) and the double mealybug symbionts (Von Dohlen et al. 2001). Most important, the event that led to mitochondria was the same that led to the eukaryotes. Because of this single event, one need not invoke the presence of primitive amitochondriate eukaryotes, which have indeed never been found. An interesting thermodynamic explanation of why eukaryotes could never have evolved without mitochondria has recently appeared (Lane and Martin 2010). It goes without saying that the hypothesis that mitochondria and eukaryotes share their origins in a common event is controversial, and other theories have been put forward as well (see, e.g., de Duve 2007).

The significance

The resurrection and elaboration of the idea that mitochondria are of bacterial origin (Sagan 1967) has stood the test of time. However, the theory stating that a strictly aerobic bacterium related to modern-day *Rickettsia* took up residence inside a primitive eukaryote has not. Studies into the supposedly primitive amitochondriate lineages have clearly indicated that these lineages all have mitochondria of some sort. In addition, with the wealth of genomic information now available and the recent appearance of bioinformatics pipelines able to analyze such large data sets, it has become apparent that perhaps alpha-proteobacteria other than *Rickettsia* might be more similar to the bacterium that became the mitochondrion.

Why would this be important? Can all these obscure organelles in even more obscure protistan lineages not be assigned to the margins of scientific research? Certainly not; the large-scale genomics and cell biological studies resulting from the Archezoa hypothesis have revealed something unsuspected thus far: The evolutionary relationships among major eukaryotic groups are completely different from what was previously thought (Simpson and Roger 2004). Currently, it seems that the diversity of eukaryotic life is best described as six major supergroups (figure 2). This knowledge is important when one tries to understand overall cell biological and biochemical features, which would be difficult to grasp if one were studying only "model" organisms. For example, yeast is one such model organism from the Fungi, and a multitude of animal model organisms exist (e.g., mouse, fruit fly, *Caenorhabditis elegans*). However, we now know that these are relatively closely related opisthokonts and that comparative studies will not be good proxies for organisms outside the opisthokonts. When it comes to major human parasites, such as the malaria-causing chromalveolate *Plasmodium falciparum* or the sexually transmitted excavate

T. vaginalis, information gleaned from yeast or mice is of little use. We cannot extrapolate cell biological and biochemical processes over such expansive evolutionary distances. Better-sourced model organisms across all six eukaryotic supergroups will enable elucidation of core eukaryotic features and lineage-specific "accessories." The core features will be important for understanding the basics of eukaryotic life, whereas the accessories will allow for the development of specific drug targets for disease-causing organisms, for example. Current next-generation sequencing and bioinformatics efforts exist for many underrepresented eukaryotic groups, and the protist community is perhaps at the forefront of these fields. In addition, the presence of many eukaryotic genomes for each eukaryotic supergroup might allow for more holistic approaches to reveal hitherto hidden features of the eukaryotic domain, as did pangenomics for bacterial vaccine development (Medini et al. 2005).

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