CellPress REVIEWS

Opinion

Was the Mitochondrion Necessary to Start Eukaryogenesis?

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Arguments based on cell energetics favour the view that a mitochondrion capable of oxidative phosphorylation was a prerequisite for the evolution of other features of the eukaryotic cell, including increased volume, genome size and, eventually, phagotrophy. Contrary to this we argue that: (i) extant amitochondriate eukaryotes possess voluminous phagotrophic cells with large genomes; (ii) picoeukaryotes demonstrate that phagotrophy is feasible at prokaryotic cell sizes; and (iii) the assumption that evolution of complex features requires extra ATP, often mentioned in this context, is unfounded and should not be used in such considerations. We claim that the diversity of cell organisations and functions observed today in eukaryotes gives no reason to postulate that a mitochondrion must have preceded phagocytosis in eukaryogenesis.

Highlights

The order in which novelties evolved during the process of eukaryogenesis is difficult to estimate because the fossil record is unhelpful in this case and organisms in which intermediate stages have been conserved are not known.

Energy-based arguments claim that the mitochondrion must have preceded the origin of phagocytosis because it allowed for reserves of ATP that were necessary for establishment of the phagocytic apparatus and the increase in cell size.

The discovery of an amitochondriate eukaryote (*Monocercomonoides exilis*) has shown that the mitochondrion is not essential for the process of phagocytosis.

Examples of picoeukaryotes (e.g., *Micromonas*) illustrate that bacterivores can have prokaryotic cell sizes.

There is neither theoretical nor experimental evidence that the process of evolution of complex cellular structures or systems requires extra ATP.

Eukaryotes and Mitochondria: A Chicken-and-Egg Dilemma?

Exactly how the eukaryotic cell originated will probably always remain a matter of discussion. Evolutionary scenarios explaining this major evolutionary transition differ in the order of events, in the forces driving the process, and in the number and identity of partners involved [1–6]. One of the most contentious questions concerns the timing and role of mitochondrial acquisition in **eukaryogenesis** (see Glossary). **Mitochondria** are semiautonomous organelles that evolved from a single common ancestor, an endosymbiont related to Alphaproteobacteria [7–9]. Mapping the presence of mitochondria onto a reconstructed eukaryote phylogeny [10,11] indicates that the mitochondrion was present in the **last eukaryotic common ancestor (LECA)** (i.e., at the root node) and this holds true for all plausible root positions [12–15]. Analogous to mitochondria, the presence of other cellular features of LECA can be inferred, depicting it as a complex cell possessing not only the nucleus and mitochondrion, but also the cytoskeleton and flagellum, the endomembrane system and the capability for **phagocytosis**, linear chromosomes with telomeres, introns and the spliceosome, and mitotic and meiotic division, as well as many other traits [16]. Unfortunately, this comparative approach does not allow us to see prior to LECA. Hence, the relative order of structural innovations associated with modern eukaryotic cells is effectively unknown and highly controversial [17,18].

Although we are confident that the mitochondrion had originated before LECA, and that its acquisition represents a very important eukaryote innovation, opinions sharply differ as to when this endosymbiosis occurred. One category of hypotheses proposes that this event occurred later in eukaryogenesis, when at least some eukaryotic features were already in place [2,3,5]. A common argument for these hypotheses is that the endosymbiosis event would become much more likely if the cell had already evolved an apparatus for engulfment of the endosymbiont. A later acquisition of the mitochondrion is supported by the finding that eukaryotic genes derived from Alphaproteobacteria have significantly shorter relative lengths of stem branches in their phylogenies than genes derived from other bacteria and archaea [19] (but see the discussion

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regarding the methodology [20,21]). Hypotheses of a second type propose that the mitochondrion originated early and that its origin was the actual trigger of eukaryogenesis [1,22,23]. The main argument for these mitochondrion-early hypotheses rests on energetics. Proponents of this view argue that, without the ATP reserve made possible by the presence of ATP-generating mitochondrial membranes, maintained by specialised small sets of genes (mitochondrial genomes), the cell would not have enough ATP to maintain its increased size and genome capacity and to evolve and perform energetically demanding tasks, such as phagocytosis.

Thus, if the mitochondrion could not evolve without phagocytosis, and if phagocytosis could not evolve without the mitochondrion, we are facing a chicken-and-egg dilemma. How can this be solved? In the following text, we question the necessity of mitochondria for the evolution of complex cellular traits. We make no attempt to revisit the energetic calculations of Martin and Lane [22]. These have already been challenged by Lynch and Marinov [24] who showed that – compared to the cell surface – mitochondria do not provide a much higher membrane area for ATP synthesis; this, together with the nonnegligible cost of mitochondria, gives no reason to assume that eukaryotes generate substantially more ATP (per unit of cell volume) than do prokaryotes. Furthermore, they argue that the bloated eukaryotic genomes might have evolved not thanks to an extra reserve of ATP, but thanks to an inability of purifying selection to eliminate – from small populations of large eukaryotic cells – long chunks of nontranslated DNA which are relatively inexpensive for the large cell [25]. Please see [26–29] for comments and further debate on this topic.

Some of the ideas and arguments presented in our text are not new [30–32], but we believe that we can offer a new perspective on the topic based on our long-term interest in anaerobic eukaryotes. We argue that examples of extant eukaryotes attest to the plausibility of amitochondrial intermediates in eukaryogenesis.

Eukaryotic Life without Mitochondria Is Possible

Despite the seeming logic of the energetic argument, nature provides numerous examples of eukaryotes that thrive without ATP generated on mitochondrial membranes (i.e., without **oxidative phosphorylation**; **OxPhos**) and even without the mitochondrion itself (Figure 1). The most obvious example was recently revealed in a paper by Karnkowska *et al*. [33], which presented robust evidence for the absence of a mitochondrion in an oxymonad flagellate, now classified as *Monocercomonoides exilis* [34]. Regardless of its amitochondrial status, this organism behaves as a rather ordinary eukaryote. It moves actively using four flagella, has a well-developed cytoskeleton and endomembrane system, and is capable of massive phagocytosis of bacteria. The size of *M. exilis* cells (ca. 8 μm in length) and its genome (~75 Mb) fall well within the range of typical eukaryotic values. This species is, in fact, one of the smallest oxymonads (Figure 1D), and some of its relatives reach remarkable sizes (more than 100 μm in *Pyrsonympha*, Figure 1B, *Oxymonas*, or *Saccinobaculus*) and contain large or multiple nuclei, indicating that their DNA content is also large [35]. Yet, not only *M. exilis* but oxymonads as a group, probably lack mitochondria as there is no convincing evidence for the presence of this organelle.

Although oxymonads may be the only truly amitochondriate eukaryotes, many other protists pay all their energy costs using ATP produced by **substrate-level phosphorylation** (without OxPhos). These protists are represented by groups containing mitosomes (e.g., *Giardia*, *Entamoeba*), hydrogenosomes (e.g., parabasalids, some chytrid fungi), and hydrogen-producing mitochondria (e.g., *Blastocystis*, *Nyctotherus*) [36]. The examples mentioned above are all endobiotic parasites, commensals, or mutualistic symbionts, which arguably live in

Glossary

Eukaryogenesis: evolutionary transition in which the eukaryotic cell evolved from prokaryotes.

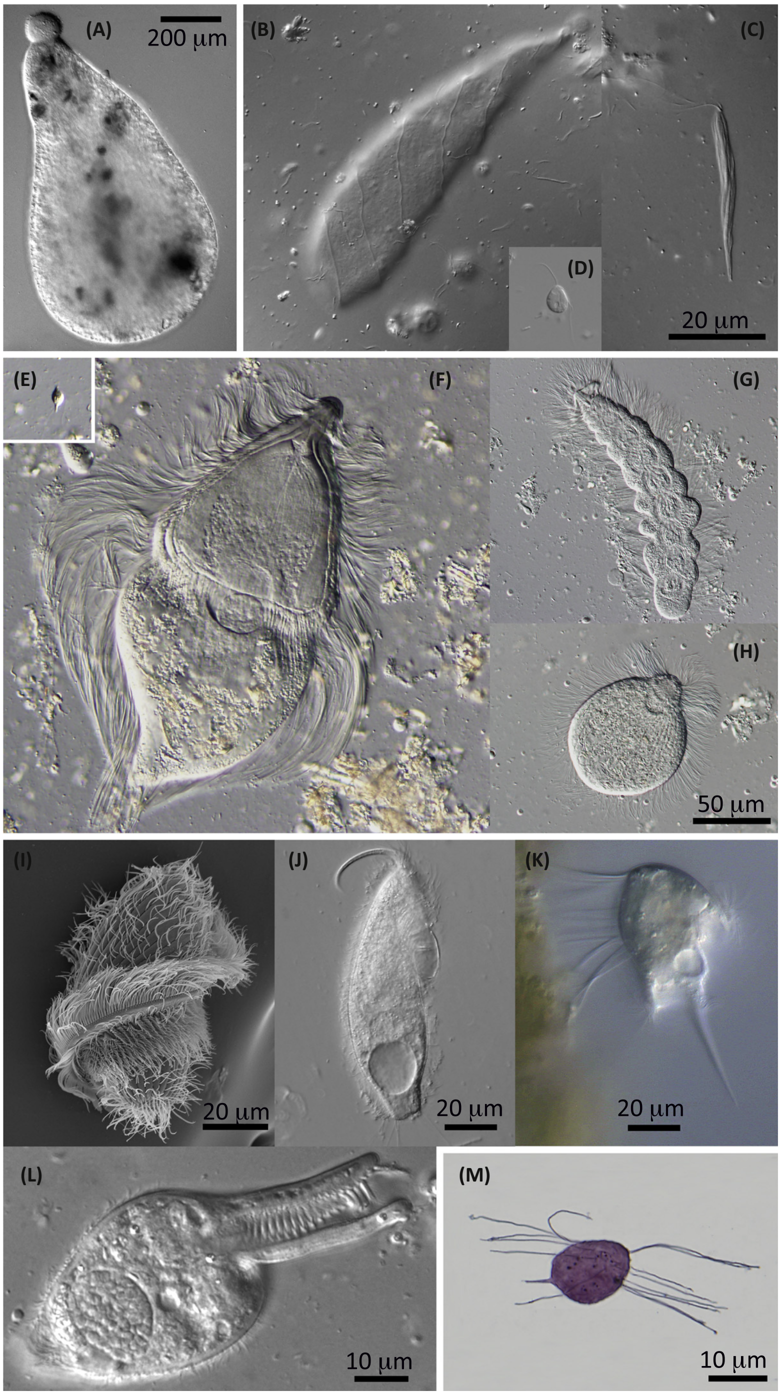
Last eukaryotic common ancestor (LECA): the most recent ancestor of all extant eukaryotes.

Mitochondrion: double-membrane-bound semiautonomous organelle that originated from an endosymbiotic alphaproteobacterium. Aerobic mitochondrion generates ATP by oxidative phosphorylation.

Oxidative phosphorylation (OxPhos): a process in which ATP is produced by an F_oF_1 ATPase at the expense of a proton gradient formed across the inner membrane of a mitochondrion.

Phagocytosis: the capability of engulfing (eating) solid food particles by the concerted action of the cytoskeleton and the endomembrane system.

Substrate-level phosphorylation: a metabolic reaction in which a molecule of ATP or GTP is formed by direct transfer of the phosphate group from another phosphorylated compound.



Trends in Microbiology

Figure 1. Examples of Morphological Diversity in Eukaryotes That Do Not Perform Oxidative Phosphorylation. Giant free-living amoeba *Pelomyxa palustris*, adapted from [64] (A); three oxymonads shown to the same scale – *Pyrsonympha major* (B) from the intestine of *Reticulitermes flavipes*, *Streblomastix strix* (C) from the intestine of *Zootermopsis angusticollis* and *Monocercomonoides exilis* (D) from culture; four parabasalids shown to the same scale – unidentified trichomonad (E), *Trichonympha* sp. (F), *Leptospironympha* sp. (G), and *Eucomonympha* sp. (H), all from the intestine of *Cryptocercus* sp., photos taken by Michael Kotyk; ciliates from the class Armophorea – *Brachonella contorta* (I) by Johana Rotterová, *Metopus nasutus* (J) by Johana Rotterová, *Caenomorpha* sp. (K) by Johana Rotterová, and *Clevelandella* sp. from the intestine of *Panesthia* sp. (L) by Michael Kotyk; heterolobosean *Creneis carolina* (M).

special niches unavailable at the time of eukaryogenesis. However, the list of free-living eukaryotes with the same capabilities is growing fast: the amoebozoan *Mastigamoeba balamuthi* [37], breviates *Pygsuia biforma* [38] and *Lenisia limosa* [39], the heterolobosean *Sawyeria marylandensis* [40], the stramenopile *Cantina marsupialis* [41], the jakobid *Stygiella incarcerata* [42], the metamonad *Paratrimastix pyriformis* [43], and a spectrum of flagellates related to diplomonads [44]. Apart from genomic evidence, a reliable indication for the absence of ATP generation on mitochondrial membranes is a reduction of mitochondrial cristae demonstrated by transmission electron microscopy. We can find quite a few additional examples of free-living protists with this characteristic among ciliates from the Armophorea lineage (Figure 1I–L). In fact, anaerobic ciliates may serve as excellent examples that energy generated in the mitochondrion is not necessary for sustaining large and highly complex eukaryotic cells with a large genome size.

Observations of anaerobic hypermastigotes (with thousands of flagella), fast-moving ciliates, or of oxymonads from the genus *Saccinobaculus* (vigorously beating their huge axostyle composed of thousands of microtubules [45]) provide striking examples that substrate-level phosphorylation alone can supply cells with sufficient ATP, without appreciably limiting energetically demanding functions.

Evolution of Complex Cellular Features Does Not Require Mitochondrial ATP

A frequent argument of advocates for mitochondrion-early hypotheses is that extra ATP supplied by OxPhos was needed for the evolution of complex features, such as the cytoskeleton, endomembrane system, and phagocytosis. As the argument goes, once these features were established, their maintenance did not require as much energy, and hence some eukaryotes might then have lost OxPhos. A comparison is drawn to the large primary investment of building a bridge and the lower cost of maintaining it [31]. This analogy is, however, not very appropriate for an obvious reason – organisms do not only maintain the structures built by their ancestors, rather they build them again and again in every daughter cell with a similar cost as the first structure of its kind. A modification of the energetic argument states that the process by which novel genes/structures evolve is energetically demanding. The idea being that while the new feature is evolving, the organismal lineage explores a broad protein space. During such explorations, many variants of proteins are produced, most of them nonfunctional, and this is argued to be very costly [22].

We are not aware of any theoretical or experimental evidence for the evolutionary process that generates new structures being costly in energetic terms, so this argument deserves more detailed scrutiny and is, in our opinion, incorrect. An organism does not invest energy in the evolution of new structures as human society invests in the engineering of a bridge. This is because evolution of a feature is not happening in a single cell but in an organismal lineage – a branch of the evolutionary tree. This lineage consists of populations of organisms changing over generations following the rules of microevolution. Both dimensions of the lineage – that is, the size of the population at a certain point in time and the number of generations required to evolve a new feature – can be very large, so large it is difficult to comprehend. Therefore, we cannot know how broad protein space the eukaryotic stem lineage was able to explore either with the luxury of mitochondrial ATP or without it.

A careful look at the diversity of protistan groups that are amitochondriate, or that possess mitochondrion-related organelles without OxPhos, reveals that their potential to diversify and evolve new (or modify existing) structural or molecular features is not visibly reduced (Boxes 1 and 2 and Table 1). In addition to the striking amount of evolutionary change in the cell systems

Box 1. Morphological Creativity of Eukaryotic Anaerobes

Morphological diversity within protistan lineages lacking OxPhos indicates that its absence does not hinder evolvability. The free-living amoeba *Pelomyxa palustris* (Figure 1A), a member of Archamoebae, does not contain a recognisable mitochondrion, yet it has grown into one of the largest unicellular organisms, reaching millimetres in size. An explosion of morphological variability can be observed in amitochondriate oxymonads (Figure 1B–D) and hydrogenosome-containing parabasalids (Figure 1E–H). These two groups, which rely solely on substrate-level phosphorylation, have diversified into hundreds of described species. Although the ancestral morphotype of both groups is a small cell (less than 10 μm long) with four flagella (Figure 1D,E), some parabasalids and oxymonads (e.g., genera *Trichonympha*, *Spirotrichonympha*, *Mixotricha*, *Pyrsonympha*, *Saccinobaculus*) have evolved into cells more than 100 μm in diameter, expanding their volume by more than three orders of magnitude (Figure 1B,F–H). They also have rather complicated cytoskeletons and large nuclei, likely with high DNA content. Some of them have evolved elaborate structures used for attachment to the insect gut epithelium, and they probably undergo complex cell cycles not observed in their simple relatives such as *Trichomonas* and *Monocercomonoides*. Importantly, some of these complex cells have increased the number of actively beating and ATP-consuming flagella to thousands. Both groups inhabit the intestines of termites and wood-eating cockroaches, which probably stimulate this burst of morphological variability. Still, if the absence of oxidative phosphorylation hindered the evolution of complex structures, such creativity would not be possible. The existence of the parabasalid *Trichonympha,* capable of supporting a large volume of cytoplasm and hundreds of flagella by fermentative metabolism, embodies living evidence that fermentation is a powerful source of energy and that it does not represent a principal energetic constraint. Ciliates from the class Armophorea represent another example of the morphological evolvability of anaerobes (Figure 1I–L). The absence of OxPhos in *Nyctotherus ovalis* is suggested from sequencing of the mitochondrial genome [55]. The situation in its free-living relatives, *Brachonella* (I), *Metopus* (J), and *Caenomorpha* (K), as well as the endobiotic *Clevelandella* (L), is unknown, but the reduction of cristae in their mitochondria suggests that OxPhos is not present. *Creneis carolina* (Figure 1M) is an anaerobic free-living heterolobosean with unusual morphology and life cycle and highly derived gene sequences [47], indicating that the evolution of novel structures in this anaerobic lineage has also not been hindered by a lack of OxPhos.

Box 2. Creativity of Anaerobic Eukaryotes at the Molecular Level

Genomic and biochemical studies have provided ample evidence that eukaryotes lacking an ATP supply from OxPhos are no less creative at the molecular level than those without this deficiency. Quite the contrary, these organisms have proven to be a goldmine of unusual features concerning genomes, proteins, and functional molecular pathways. It is, for example, noteworthy that some of the most gene-rich eukaryotic genomes are found among anaerobic eukaryotes. The genome of *Trichomonas vaginalis* harbours ~60 000 protein-coding genes [56], and a similar expansion of coding capacity seems to be shared by other parabasalids [57]. This high gene content in parabasalid genomes can primarily be accounted for by gene family expansions, as exemplified by Rab GTPases (key mediators of membrane trafficking). The number of Rab loci detectable in the *T. vaginalis* genome approaches ~300, a truly impressive value dwarfing the number of Rab genes in the human genome (~70) and all other eukaryotes investigated so far [56]. As noted in an initial study of a smaller subset of *T. vaginalis* Rabs (before completion of the genome sequencing), the different paralogs tend to be divergent from each other and from Rabs of other species, many of them constituting novel subfamilies without obvious orthologs in other eukaryotes [58]. This indicates an unprecedented pace of evolutionary exploration of the Rab family ‘sequence space’ and presumably much novelty at the level of the organization and function of the endomembrane system. Other examples of evolutionary creativity of anaerobic eukaryotes include: departure from the standard genetic code in three independent lineages of metamonads [59–61], emergence of *trans*-spliced split introns (splintrons) in the *Giardia* lineage [62], and novel proteins contributing to unique cytoskeletal structures [63]. The existence of an unexplored universe of novel biological phenomena at the molecular level is further attested to by large amounts of uncharacterized proteins specific for different lineages of anaerobic eukaryotes (i.e., proteins lacking readily discernible homologs elsewhere). In the genome project of the amitochondriate oxymonad *M. exilis*, approximately 60% of predicted genes remained unannotated because they lack significant similarity to proteins in the database [33]. Why should the evolutionary creativity of primarily amitochondriate eukaryotes differ from that of secondarily amitochondriate metamonads?

observed in these examples, gene and genome sequencing has revealed that the structures (from primary to tertiary) of their rRNA molecules and proteins change relatively quickly, as reflected by the increased lengths of their branches in phylogenetic trees [33,46,47]. This indicates that these lineages move through the protein space actually faster than many eukaryotes that generate ATP on mitochondrial membranes. Clear illustrations of this are the free-living heterolobosean *Creneis carolina,* or the parasitic groups Microsporidia and

Table 1. Examples of the Creativity of Anaerobic Eukaryotes at the Molecular Level

Organism	Feature	Brief description	Refs
Trichomonas vaginalis	Gene-rich genome	*T. vaginalis* genome harbours ~60 000 protein-coding genes	[56]
Trichomonas vaginalis	Gene family expansion	Number of Rab GTPase loci detectable in the *T. vaginalis* genome approaches ~300	[56]
Giardia intestinalis	New intron type	*Trans*-spliced split introns (splintrons)	[62]
Streblomastix strix	New genetic code variant	UAG and UAA encode glutamine (novel decoding tRNAs)	[60]
Hexamitinae	New genetic code variant	UAG and UAA encode glutamine (novel decoding tRNAs)	[59]
Iotanema spirale	New genetic code variant	UAG encodes glutamine (novel decoding tRNA)	[61]
Parabasalia	New cytoskeletal proteins	Group specific fibre (costa) is composed of novel proteins	[63]

Mikrocytida, which have all evolved unique morphology dissimilar to their relatives with gene sequences that are typically highly divergent [47–49].

The examples presented above clearly show that lineages without OxPhos indeed undergo microevolutionary changes leading to complex structures and functions. As long as the majority of individuals in the lineage are able to maintain their own processes of living, growth, and replication, the lineage evolves and does not need any extra energy to push its evolution forward. We do not see any reason why a character that is being operated and maintained by an amitochondriate cell (e.g., the toolkit for phagocytosis in oxymonads) could not evolve in an amitochondriate lineage. Evolution of new characters does not require extra energy, and other factors – characteristics of the population and width of the niche – affect it more than cell energetics. It is difficult to reconstruct the ecological factors under which the eukaryotic stem lineage evolved, as well as its population characteristics. However, it is possible that the population size of the eukaryotic stem lineage was small in some periods, diminishing the power of selection. Moreover, as will be discussed further, the new niche that the eukaryotic lineage entered – predation on prokaryotes – was empty and wide, thus potentially facilitating fast character evolution and diversification of eukaryotes.

What Came First: A Mitochondrion or Phagocytosis?

It should be mentioned that many extant eukaryotes that use substrate-level phosphorylation as their sole source of energy have the advantage of phagocytosis. Swallowing lumps of energy-rich food may provide a more efficient supply of energy sources than osmotrophy, especially in environments with low concentrations of soluble nutrients. Possibly, it is this capability for phagocytosis that enables large amitochondriate cells to generate, at sufficient speed, the ATP required to run their large volume of cytoplasm. But we still do not know what the eukaryotic ancestors were like before the invention of phagocytosis. In the field, an ongoing bone of contention is whether these ancestors contained mitochondria, which would greatly increase the efficiency of ATP generation, or whether they managed without it [1–3,5,22].

As we argued above, performing phagocytosis without mitochondrial ATP is possible, and evolution of the protein apparatus necessary for phagocytosis did not require any extra cost. Moreover, elements of the cytoskeleton (tubulins, actin, and actin-interacting proteins) and genes for proteins putatively involved in endomembrane sorting and trafficking (ESCRT proteins, an expanded family of small GTPases, TRAPP domain proteins, Sec23/24 family proteins) were found in the metagenomes of the recently discovered archaeal superphylum Asgardarchaeota [50,51], and some are known also from other archaeal lineages [5].

Asgardarchaeotes are currently considered the closest known archaeal relatives of eukaryotes. However, it should be stressed that these organisms have not been isolated and their cell biology has not been studied, so it cannot be established whether they are capable of endocytosis of any kind, and prediction based on their gene content does not support the presence of phagocytosis in this group [52].

An important question remains whether the putative amitochondriate eukaryotic ancestor could reach a sufficient size to become an effective bacterivorous predator. First we should ask, how big does a bacterivorous eukaryote need to be? Extant picoplankton show that bacterivorous predators may be as small as 2 μm. *Micromonas pusilla* ($<$2 μm) is able to digest microspheres of 0.9 μm in diameter [53]. Furthermore, an interesting form of cell feeding, pomacytosis, which is described as semiextracellular phagocytosis, was recently reported from a 1.3 μm haptophyte alga preying on a tiny cyanobacterium [54]. The body sizes of these predators fall safely into the range of prokaryotic cell sizes and demonstrate that prokaryotes reached dimensions where bacterivory becomes feasible. The hypothetical first amitochondriate bacterivore might thus have been only slightly larger than the bacteria it preyed on. Such a cell could, in our opinion, evolve without OxPhos on internal membranes of an endosymbiont, or even without OxPhos at all, if the prokaryotic ancestor was an anaerobic fermenting prokaryote. After the invention of phagocytosis, possibly through a pomacytosis-like stage, the road to an increased cell size and evolution of novel cellular features would be wide open, as demonstrated by the ability of different extant lineages of anaerobic phagotrophs to increase cell size and diversify in structure and function.

We can envisage that the emergence of phagocytosis could have played the igniting role in eukaryogenesis that Lane and Martin ascribe to the mitochondrion [22]. Phagocytosis might have not only boosted cell energetics but also, and maybe more importantly, it may have opened a brand new niche. Phagotrophs suddenly escaped from competition in the effectiveness of osmotrophy and started to feed on their former competitors. Evolution of the phagocytic apparatus should have been possible without OxPhos on internal mitochondrial membranes, considering that other complex prokaryotic structures (e.g., secretion systems, flagellum, oxygenic photosynthesis, and magnetosomes) did evolve without it. Is there a reason to think that the evolutionary journey from an actin-containing prokaryote to a fully-fledged prokaryotic phagotroph costs more ATP than the journey from an archaeon containing hydrogen-producing bacterial endosymbionts to the organism with an established respiring mitochondrion that is able to provide ATP to the cell? We do not see a reason to believe so. Maintaining and replicating cells is costly, but the evolution of structures does not require extra energy; therefore, it is not obvious why the mitochondrion must have preceded phagocytosis.

Concluding Remarks

Inferring the order of steps in the process of eukaryogenesis is a daunting task. This is particularly true in the absence of informative fossils and lineages preserving intermediary character states of stem eukaryotes. Energetic arguments brought up by Lane and Martin [22] claim that mitochondrial endosymbiosis preceded other crucial events in eukaryogenesis, including the invention of phagocytosis. They postulate that, without the ATP reserve associated with possession of mitochondria, evolution of complex cells – including the ATP-costly process of phagocytosis – would not be possible. In this article, we question their reasoning and show, using examples of extant eukaryotes, that their assumptions do not hold. Firstly, eukaryotes without mitochondria are capable of phagocytosis. Secondly, we are neither aware of any evidence for the evolution of complex features having a certain ATP requirement, nor have we observed indications that eukaryotes without mitochondrial respiration have a lower

Outstanding Questions

Genomic data of the Asgardarchaeota superphylum of Archaea obtained from deep-sea environmental samples show extraordinarily rich sets of genes from protein families that are, in eukaryotes, involved in membrane trafficking and cytoskeleton construction. How does their gene complement manifest in cell capabilities? In particular, if these archaea have a cytoskeleton, how is it organised, and are they capable of vesicular transport or even phagocytosis?

Truly amitochondriate eukaryotes (oxymonads) should be studied in detail, namely their cell biology and energetics, as they represent a model of a putative intermediate stage of eukaryogenesis.

How does the substitution rate of protein sequences in anaerobic eukaryotes differ from that of their relatives capable of oxidative phosphorylation?

The assumption that the process of evolution of structures and functions costs extra ATP (on top of cell maintenance, growth, and replication) should be carefully scrutinised theoretically and experimentally, because it represents an important premise in the formulation of evolutionary hypotheses.

capacity to evolve structures and functions and to explore the protein space. Finally, many extant prokaryotes have dimensions that would theoretically enable them to perform phagocytosis. We do not refute the claim that the invention of mitochondria greatly boosted the energetic metabolism of eukaryotes, providing a very significant competitive advantage. At the same time, we remain agnostic regarding the order of evolutionary events because we think that the invention of phagocytosis could have been as good a trigger for eukaryogenesis as mitochondrial endosymbiosis.

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