Energetics and evolution of anaerobic microbial eukaryotes

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Mitochondria and aerobic respiration have been suggested to be required for the evolution of eukaryotic cell complexity. Aerobic respiration is several times more energetically efficient than fermentation. Moreover, aerobic respiration occurs at internalized mitochondrial membranes that are not constrained by a sublinear scaling with cell volume. However, diverse and complex anaerobic eukaryotes (for example, free-living and parasitic unicellular, and even small multicellular, eukaryotes) that exclusively rely on fermentation for energy generation have evolved repeatedly from aerobic ancestors. How do fermenting eukaryotes maintain their cell volumes and complexity while relying on such a low energy-yielding process? Here I propose that reduced rates of ATP generation in fermenting versus respiring eukaryotes are compensated for by longer cell cycles that satisfy lifetime energy demands. A literature survey and growth efficiency calculations show that fermenting eukaryotes divide approximately four to six times slower than aerobically respiring counterparts with similar cell volumes. Although ecological advantages such as competition avoidance offset lower growth rates and yields in the short term, fermenting eukaryotes inevitably have fewer physiological and ecological possibilities, which ultimately constrain their long-term evolutionary trajectories.

The ancestor of extant eukaryotes was capable of harvesting energy by aerobic respiration in mitochondria. Aerobic respiration yields ~32 molecules of ATP per hexose, whereas fermentation and fumarate respiration yield ~2–4 ATPs. High energy-yielding aerobic respiration[1] and mitochondria[2,3] are often considered to have been a prerequisite for the origin of complex eukaryotic cells from much simpler prokaryotic cells. However, phylogenetically diverse eukaryotes, predominantly unicellular but also a few small multicellular metazoans, have adapted to anaerobic environments, including stratified lakes, aquatic sediments and animal guts[4]. Many of these anaerobic eukaryotes have either lost the ability to respire aerobically[5,6], or lost mitochondria altogether[7,8], and rely on fermentation to satisfy their energy demands. How do fermenting eukaryotes evolve and maintain complexity while relying on a less-efficient energy-yielding metabolism? In this Perspective, I assess the energetics of fermenting eukaryotes and explore the ecological and evolutionary consequences of adopting an obligately anaerobic lifestyle.

Diversity of anaerobic eukaryotes

Extant anaerobic eukaryotes are morphologically, physiologically and phylogenetically diverse. This is in part because of their multiple independent evolutionary origins. Some are facultatively anaerobic (that is, they can also perform aerobic respiration) and prefer hypoxic environments. Others have become obligately anaerobic by losing parts of or the whole respiratory electron transport chain from their mitochondria. Anaerobic eukaryotes thus display varying levels of oxygen sensitivity, and this is largely a consequence of both their reduced energy metabolism and repertoire of oxygen detoxifying enzymes[9].

In this Perspective, unicellular eukaryotes are defined as anaerobic if they have any energy metabolism independent of oxygen, regardless of their oxygen sensitivity. To satisfy their energy demands, anaerobic eukaryotes can respire fumarate (for example, some parasitic worms, ciliates and many other eukaryotes[5,10]) or nitrate (rotaliid foraminifera[11], the fungus *Fusarium*[12] and the ciliate *Loxodes*[13]), or perform different types of fermentations that produce a myriad of end-products, for

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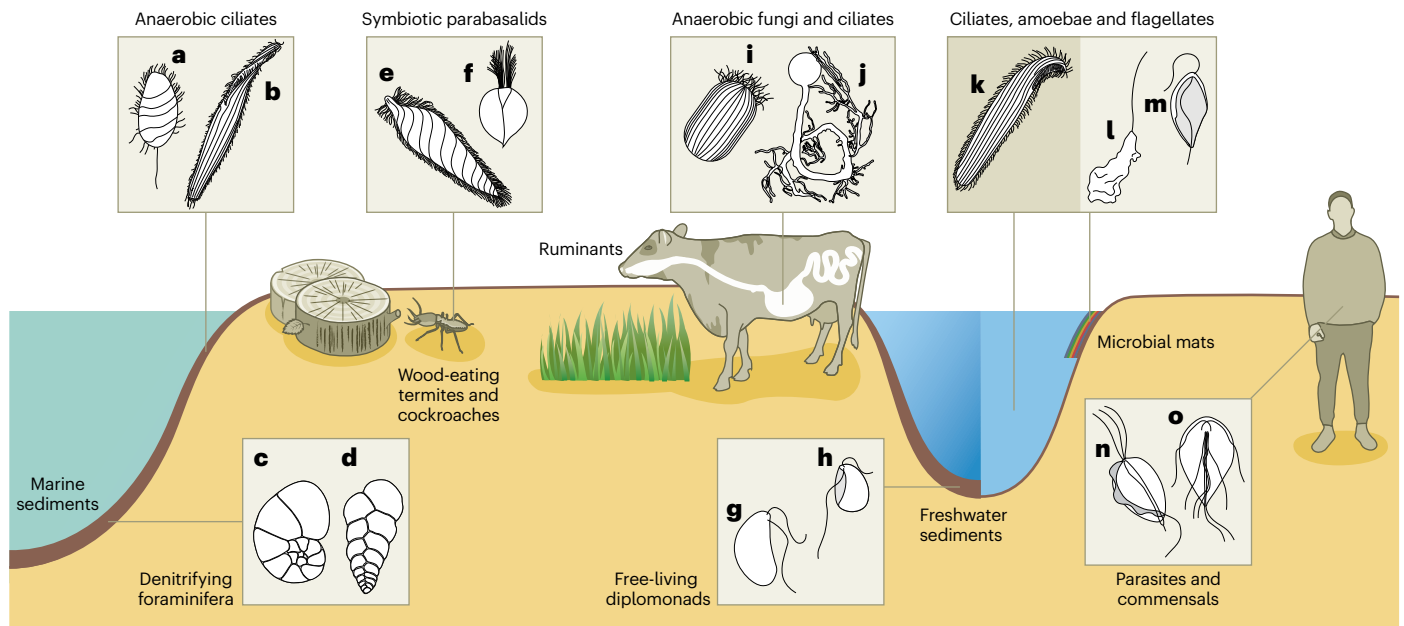
Fig. 1 | Environmental and phylogenetic diversity of anaerobic eukaryotes. Anaerobic eukaryotes are phylogenetically diverse and have evolved multiple times from aerobic ancestors. They are found in almost every major eukaryotic lineage. Some of the most well-studied eukaryotic anaerobes are parasites or symbionts of animals (for example, *Giardia*, *Trichomonas*, parabasalids). However, free-living anaerobes are found in many hypoxic and anoxic environments. **a**–**o**, The figure shows ciliates *Trimyema* sp. (**a**) and *Parablepharisma* sp. (**b**), the denitrifying foraminifera *Valvulineria* sp. (**c**) and *Bolivina* sp. (**d**), the parabasalids *Spirotrichonympha flagellata* (**e**) and *Lophomonas blattarum* (**f**), the free-living diplomonads *Aduncisulcus paluster* (**g**) and *Ergobibamus cyprinoides* (**h**), the endobiotic ciliate *Entodinium caudatum* (**i**), the chytridiomycete *Neocallimastix frontalis* (**j**), the nitrate-respiring ciliate *Loxodes* sp. (**k**), the archamoeba *Mastigamoeba* sp. (**l**), the jakobid *Stygiella incarcerata* (**m**), the parasitic parabasalid *Trichomonas* sp. (**n**) and the intestinal parasite *Giardia* sp. (**o**).

example, ethanol, lactate, acetate, propionate, butyrate, succinate and so on[5,6]. Fumarate and nitrate respiration, and hydrogen-releasing fermentation, often occur within mitochondria or hydrogenosomes (derived mitochondria). Many reviews in the past decade have summarized the known energy metabolic pathways of anaerobic eukaryotes[5,6]. Here I primarily focus on the energetics of fermenting eukaryotes that have the lowest energy yield. Other types of anaerobic energy metabolism, for example, nitrate respiration, have energy yields intermediate between those of fermentation and aerobic respiration and are not considered here.

Obligately anaerobic eukaryotes that rely on fermentation as their sole energy source are found in almost every major phylogenetic group. These include animals (for example, some species of Loricifera and Myxozoa), fungi (for example, Neocallimastigomycota), breviates (for example, *Pygsuia*, *Lenisia*), amoebozoans (for example, Archamoebae), stramenopiles (for example, *Suigetsumonas*, *Blastocystis*), alveolates (for example, the apicomplexan *Cryptosporidium* and the ciliates *Metopus* and *Plagiopyla*), rhizarians (for example, the cercozoan *Brevimastigomonas*), heteroloboseans (for example, *Psalteriomonas*) and jakobids (for example, *Stygiella*), among others[5,6,14–16]. The Metamonada, which encompasses the Anaeramoebae, Parabasalia, Preaxostyla and Fornicata, is a major lineage of eukaryotes that exclusively comprises anaerobic species[17] (Fig. 1). Many fermenting eukaryotes that release hydrogen as an end-product engage in ecto- or endosymbiotic relationships with hydrogenotrophic prokaryotes (for example, methanogens and sulfate reducers) that act as syntrophic partners. It is thought that these symbioses facilitated the evolutionary transition of facultatively anaerobic eukaryotes adapted to low oxygen tensions to an obligately anaerobic lifestyle[9,18].

Anaerobic eukaryotes have adapted to diverse ecological niches. The most studied ones are symbionts found in the guts of animals (for example, the hindguts of wood-eating termite and cockroaches or the foregut of herbivorous mammals). Free-living anaerobes, on the other hand, are found in diverse environments such as temporarily or permanently stratified water bodies (for example, the Black Sea, fjords, salt marshes, lakes and ponds), marine and freshwater sediments, microbial mats, sewage treatment plants and landfill sites[9] (Fig. 1). In some of these environments, anoxia emerges because the accumulation and degradation of dead organic matter are faster than the supply of oxygen through diffusion. It is conceivable that the increased pollution of water bodies and the loss of dissolved oxygen due to warming will expand the breadth of anaerobic ecosystems and promote the evolution of new anaerobic lineages.

Views on the origin and maintenance of cellular complexity

It has been argued that the origin of eukaryotic cell complexity required an increase in available energy that was enabled by acquisition of mitochondria[2]. The repeated loss of aerobic respiration, or even mitochondria, throughout eukaryotic diversification is inconsistent with this proposal. If cellular complexity requires substantial energy, such as that generated by aerobic respiration, how are complex eukaryotic cells that rely on fermentation physiologically possible? To solve this conundrum, Lane and Martin argued that the origin of cellular complexity is energetically much more expensive than its evolutionary maintenance[2,19]. In their view, once the earliest ancestors of eukaryotes evolved most of their cellular complexity, less energetically efficient forms could evolve without major disadvantages. Lane further proposed that because extant anaerobic eukaryotes evolved reductively from more complex ancestors, they may have lower energy demands that are met by fermentation alone in nutrient-rich environments[20]. This implies that anaerobic eukaryotes are constrained and may only evolve under specific circumstances.

In contrast, Hampl and colleagues argued that the origin of cellular complexity does not require a higher energetic investment than its evolutionary maintenance, as cells must synthesize all of their components
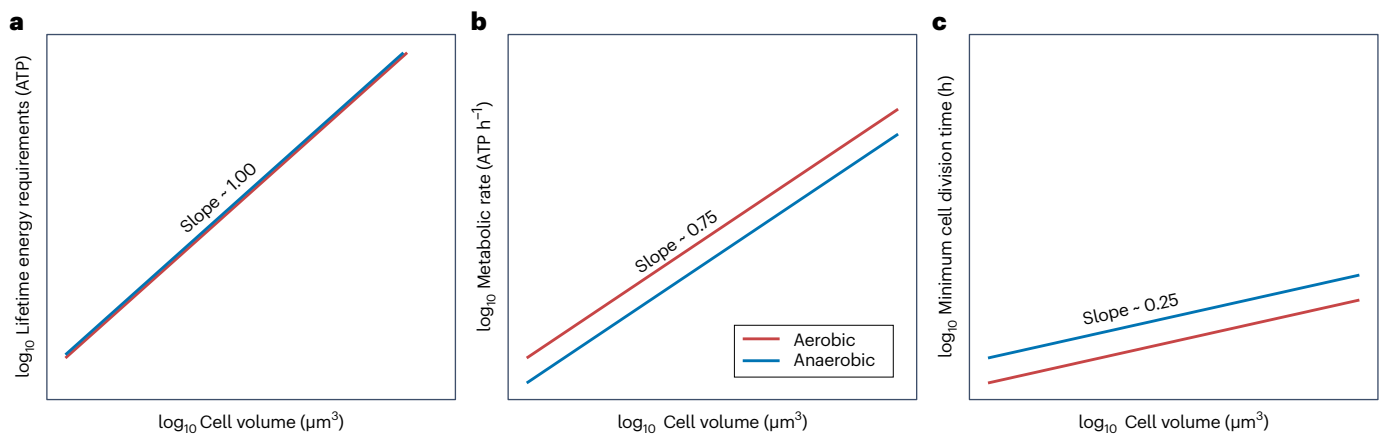
Fig. 2 | The lifetime energy requirements of both aerobic and anaerobic eukaryotic cells are a function of their metabolic rates and division times. a, The scaling of energy demands or lifetime energy requirements (E) with cell volume. **b**, The scaling of metabolic rate expressed as ATP h^{-1} (R) with cell volume. **c**, The scaling of minimum cell division time (t_d) with cell volume. The cross-species relationship between metabolic rate and minimum cell division time (inverse of growth rate) is expected to hold regardless of the constancy and precise values taken by the scaling exponents across the wide range of cell volumes covered by unicellular eukaryotes. Metabolic rate scales with an exponent of ~0.75 and minimum cell division time scales with an exponent of ~0.25 (refs. [27–30]), so that the lifetime energy requirements of cells are approximately linearly proportional to cell volumes as $E = R \times t_d$ and thus, $aV_{cell}^{1.0} = bV_{cell}^{0.75} \times cV_{cell}^{0.25}$. This is an equivalence relationship that implies that all cells regardless of size, physiology and organization invest about the same amount of energy per life cycle. Anaerobes that rely on fermentation are predicted to have slightly higher life energy requirements due to a higher investment in maintenance functions owing to longer cell division times.

anew every generation[7]. Moreover, Hampl and colleagues compiled examples of anaerobic eukaryotes that display high degrees of cellular complexity (genomic, ultrastructural and morphological) and large cell volumes, and belong to diversified anaerobic lineages of both parasitic and free-living eukaryotes[7]. These observations suggest that relative to their aerobic counterparts, anaerobic eukaryotes are not evolutionarily or energetically constrained by fermentation to evolve greater cellular complexity and cell volumes[7,21]. Many anaerobes are also just as active and swim as fast as their aerobically respiring relatives. This is, however, not surprising because the amount of energy required for such maintenance functions represents a very small fraction of a cell's energy budget[22,23]. Given the diversity of extant anaerobic eukaryotes, do fermenting eukaryotes face any relative disadvantages at all?

Satisfying the energy demands of cells

Mitochondria give aerobic eukaryotes an energetic advantage by providing internalized respiratory membranes that, instead of expanding sub-linearly with cell volume as the cytoplasmic membrane does, expand nearly linearly with cell volume (or super-linearly with surface area)[24]. Empirical estimates show that both the number of ATP synthase complexes (N_{FoF1}) and the surface area of the mitochondrial inner membrane (S_{MIM}) scale super-linearly with the surface area of the cell ($N_{FoF1} = 83S^{1.31}$ and $S_{MIM} = 0.4S^{1.30}$, refs. [24,25]). This suggests that many eukaryotic cells would not be able to support their own volumes while respiring at the cell surface, that is, if the respiratory chain were placed at the cytoplasmic membrane. For example, whereas a small eukaryotic cell (~10 μm^3) would require ~30% of their surface area devoted to aerobic respiration, a slightly larger eukaryotic cell (~1,000 μm^3) would require ~500% of its surface area to support its own volume. Recent theoretical predictions agree with these empirical estimates[24]. Mitochondria thus allow extant eukaryotes to scale up energy supply to match energy demands at larger cell volumes and relatively fast growth rates[24]. However, these theoretical analyses also suggest that a respiratory deficit (for example, insufficient amounts of respiratory membranes to support cell volumes, or lower rates of energy supply) can be compensated for by longer cell division times[24]. This is because longer cell division times reduce the rate of ATP consumption since only maintenance costs (which are about two orders of magnitude

lower than growth costs) increase with time – growth costs are, in contrast, strictly volume-dependent and a one-time investment. This prediction suggests a strategy by which anaerobic eukaryotes, which rely on a much less-efficient energy-harnessing process, may be able to support their large cell volumes.

The energetics of anaerobic eukaryotes

The energy demands of both aerobic and anaerobic cells are expected to primarily be a function of their volumes. This is because energy demands per unit volume are approximately constant across both prokaryotic and eukaryotic cells[26] (Fig. 2) – note that this observation further implies that cellular complexity does not impinge upon energy demands as these are proportional to volume regardless of cellular organization (in contrast to ref. 2; see ref. 24). There is also no reason to assume that biosynthesis costs differ considerably between aerobes and anaerobes as both have similar anabolic pathways. To remain viable, both aerobes and anaerobes must ensure that their energy demands, or lifetime energy requirements, are met by energy supply[24]. To achieve this, the respiratory membrane area and mitochondrial volume of aerobes scale linearly with cell volume[24,27]. In a similar way, the cytoplasmic (or hydrogenosomal) volume devoted to fermentation enzymes in anaerobes is expected to scale at least linearly with cell volume as to not decrease the rate of ATP supply relative to the rate of ATP consumption.

The lifetime energy requirements of cells (as ATPs per cell cycle) scale approximately linearly across a wide cell volume range (Fig. 2a) and can be expressed as the product of metabolic rate (ATPs h^{-1}) and cell division time (h). Across unicellular aerobic eukaryotes, respiratory (metabolic) rates have been reported to scale with an exponent of ~0.75 and minimum cell division times with an exponent of ~0.25 (or ~−0.25 for maximum growth rates)[28–30] (Fig. 2b,c). This suggests that growth rates respond to metabolic rates regardless of a possible change in their scaling exponents across different phylogenetic or size groups[27,29,30]. This notion further predicts that aerobic eukaryotes, which have high ATP synthesis rates due to aerobic respiration (oxidative phosphorylation), have relatively short cell division times (or fast growth rates; Fig. 2b,c). On the other hand, anaerobic eukaryotes, which have low ATP synthesis rates due to a much less-efficient fermentation
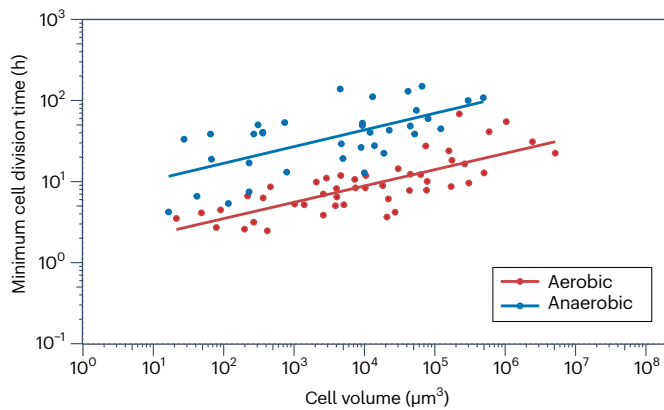
Fig. 3 | Anaerobic eukaryotes divide several times more slowly than their aerobic counterparts. The scaling of minimum cell division times (h) with cell volumes (µm³) in aerobic and anaerobic eukaryotes. Anaerobic eukaryotes for which minimum cell division times (inverse of growth rate) were compiled are phylogenetically diverse (for example, discobans, metamonads, amoebozoans, breviates, cercozoans and ciliates) and comprise facultative anaerobes grown in strict anoxic conditions[38], obligate free-living anaerobes and some parasitic anaerobes. Thus, the data correspond to both osmotrophs and phagotrophs. Aerobic eukaryotes include flagellates, ciliates and amoebae[28]. Regression equations for aerobic and anaerobic eukaryotes are $y = 1.4x^{0.20}$ and $y = 6.5x^{0.20}$, respectively. According to the regression equations, aerobes divide ~4–6 times faster than anaerobes. The scatter in the data is probably caused by several confounding factors such as higher anaerobic growth efficiencies for osmotrophic symbionts of animals, diversity in the energy yield of fermentation pathways (for example, pyrophosphate-dependent glycolysis), some degree of aerobic respiration at very low partial oxygen pressures by facultative anaerobes, non-optimal growth medium and so on. See Supplementary Table 1 for data sources.

Fig. 4 | Anaerobes dissimilate most of their ingested carbon. Relationship between the amount of carbon assimilated or dissimilated as a function of the efficiency of energy harnessing. Energy-harnessing efficiencies are >28 ATP per hexose (red area) for aerobic respiration and >1–5 ATP per hexose for fermentation (blue area). See main text for more explanation.

(substrate-level phosphorylation), require relatively longer cell division times (Fig. 2b,c). Figure 2 diagrams the hypothesized cross-species relationship between metabolic rate and minimum cell division time.

Some facultatively anaerobic eukaryotes, such as the yeast *Saccharomyces cerevisiae*, prefer to ferment aerobically (in the presence of oxygen) under nutrient-rich environments (that is, the Crabtree effect). Several competing hypotheses have been proposed to explain this paradoxical physiological strategy (see ref. 31 and references therein). A common explanation is that this occurs primarily because fermentation is more proteome efficient than respiration, that is, it uses a smaller fraction of the proteome. Under such conditions, switching to fermentation releases resources for biosynthesis (for example, ribosomes) which may increase growth rate at the expense of yield[31,32]. Other yeasts for example, *Kluyveromyces marxianus* and *Scheffersomyces stipitis*, prefer to respire aerobically under nutrient-rich conditions and achieve higher growth rates and yields[33]. In support of this, the physiological study of respiration-competent and -incompetent strains of *S. cerevisiae* ('grande' and 'petit' strains, respectively) showed that the former attains faster growth rates and yields due to the higher rates of ATP synthesis through aerobic respiration[34].

A survey of the literature was done to compile the cell volumes and minimum cell division times for 34 phylogenetically diverse anaerobic eukaryotes with different lifestyles and physiologies (Supplementary Table 1). The data show that anaerobic eukaryotes have, on average, minimum cell division times that are ~4–6 times longer than those of their aerobic counterparts (Fig. 3 and Supplementary Table 1). This difference in cell division times is consistent with calculations of gross growth efficiency, which show that aerobes are ~3.4–6.4 times more efficient at assimilating carbon (C) from ingested food than anaerobes (see ref. 35 and below). Growth efficiency measures how much ingested food is successfully transformed into biomass (assimilated C).
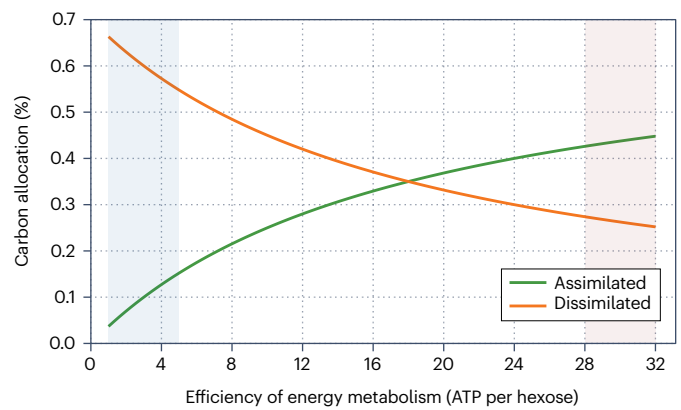
Compared with an efficient energy metabolism (~32 ATPs per glucose), an inefficient energy metabolism such as fermentation (~2–4 ATPs per glucose) leads to a lower growth efficiency as more food needs to be dissimilated (or burned for ATP synthesis) to assimilate the same amount of C. The gross growth efficiencies of aerobes and anaerobes are ~45% and 7–13%, respectively:

Gross growth efficiency

$$= \frac{\text{Assimilated C}}{\text{Consumed C}} = \frac{\text{Assimilated C}}{\text{Assimilated C} + \text{Dissimilated C} + \text{Lost C}},$$

Gross growth efficiency (aerobe)

$$= \frac{(32 \times 4\,\text{g C})}{(32 \times 4\,\text{g C}) + 72\,\text{g C} + 85.7\,\text{g C}} = 0.45,$$

Gross growth efficiency (anaerobe)

$$= \frac{(2 \times 4\,\text{g C})}{(2 \times 4\,\text{g C}) + 72\,\text{g C} + 34.3\,\text{g C}} = 0.07,$$

Gross growth efficiency (H$_2$-releasing anaerobe)

$$= \frac{(4 \times 4\,\text{g C})}{(4 \times 4\,\text{g C}) + 72\,\text{g C} + 37.7\,\text{g C}} = 0.13.$$

These calculations are based on the dissimilation of 1 mole of glucose (72 g C). Dissimilating this amount of glucose allows for the synthesis of ~32 moles of ATP in aerobes and ~2–4 in anaerobes. This, in turn, allows for the assimilation of either ~32 × 4 or ~4 × 4 g of C from ingested food — it has been estimated that assimilating 4 g of C requires 1 mole of ATP in heterotrophs[36]. Furthermore, it is assumed that both aerobic and anaerobic phagotrophs have similar food ingestion rates (which appear to be a function of cell volume[37]) and digestion efficiencies (that is, they lose ~30% of C consumed as undigested food[28]). While aerobes synthesize ~8–16 times more ATP per hexose than anaerobes, their growth efficiencies are ~4–8 times those of anaerobes. The reason for this is that aerobes have to ingest about twice as much food as anaerobes to assimilate the amount of C that is possible by the dissimilation of one mole of glucose (Fig. 4). Aerobes incorporate into biomass (or assimilate) 45% and use for energy harnessing (or dissimilate) 25% of the C consumed. In contrast, anaerobes assimilate 7–13% and dissimilate 63–57% of the C consumed. Thus, most of the C consumed by anaerobes is used for energy harnessing rather than growth. The opposite is true

BOX 1

Did eukaryotes evolve their complexity from an anaerobic fermentative ancestor?

One popular set of hypotheses for the origin of eukaryotes proposes that the first eukaryote comprised a bacterial symbiont inside an archaeal host[45]. This configuration, that is, a prokaryote inside another prokaryote, was a prerequisite and trigger for the evolution of eukaryotic cellular complexity[3]. In this context, the energetic hypothesis of Lane and Martin[2] argues that prokaryotes are energetically constrained, and only the acquisition of an aerobically respiring symbiont released these energetic constraints and allowed for the evolution of cellular complexity in ancestral eukaryotes[2]. Another group of hypotheses propose that the first eukaryotes evolved largely autogenously from a prokaryote, today largely viewed as an Asgard archaeon by most, without the aid of an intracellular symbiont. These hypotheses often envision a proto-eukaryotic lineage that evolved some degree of cellular complexity before the acquisition of the mitochondrial ancestor and its transformation into a respiratory organelle[45]. Some of these cellular innovations, such as a primitive cytoskeleton or endomembranes, made the acquisition of the mitochondrial ancestor possible through some sort of phagocytosis.

Could the ancestor of eukaryotes have evolved greater complexity without the aid of a respiring symbiont? There is no direct relationship between cellular complexity and energy demands or requirements (see above). However, the evolution of larger cell volumes, and the higher complexity that might ensue, are arguably constrained in simpler prokaryotic cells[24]. At least three major ancestral adaptations enabled eukaryotes to achieve large cell volumes. Mitochondria allowed for expanded respiratory membranes, the endomembrane system for expanded nutritional membranes and the cytoskeleton for active cytoplasmic transport. As shown above, larger cell volumes can be achieved while having energy deficiencies at the cost of

longer cell division times. Thus, because higher complexity does not demand more energy (see above), a primitive cytoskeleton and endomembrane system may have evolved in a slow-growing archaeon in the absence of respiring symbionts. This notion is compatible with observations on modern Asgard archaea, the closest relatives to the eukaryotic nucleocytoplasm, most of which ferment in anoxic sediments[46], display slow growth rates[47] and have relatively larger archaeal genomes with genes homologous to those of the eukaryotic cytoskeleton (for example, actin and actin-related proteins) and endomembrane system (for example, TRAPP-domain and Sec23/24-like proteins)[48]. Furthermore, the discovery of 'Candidatus Uab amorphum', a planctomycete that preys on smaller prokaryotes and eukaryotes through a process analogous to eukaryotic phagocytosis[49], demonstrates that complex cellular structures and processes can evolve in a prokaryote without respiring symbionts. Both a primitive cytoskeleton and endomembrane system may have subsequently allowed an Asgard archaeon to evolve larger volumes, and possibly, phagotrophy. However, if early proto-eukaryotes were fermentative as many Asgard archaea are[50], they may have been macroevolutionarily constrained (see main text). (Note, however, that some Asgard archaeal phyla possess short, presumably less-efficient aerobic and anaerobic respiratory chains[51].) The acquisition of an aerobic symbiont and its specialization as a respiratory organelle allowed eukaryotes to colonize aerobic environments and further diversify[9,24]. Although this hypothetical sequence of events may not be true, it does show that prokaryotes are not necessarily constrained to become more complex, and that there are different evolutionary pathways by which eukaryotic cell complexity may have evolved without the need for a respiratory organelle.

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for aerobes. These gross calculations provide a basis for future and more detailed analyses.

Ecology and evolution of anaerobic eukaryotes

What are the ecological and evolutionary consequences of an anaerobic lifestyle? Specialization to anaerobic environments confers ecological advantages. By switching to an anaerobic lifestyle, a facultatively anaerobic eukaryote may escape larger aerobic predators and face less competition for food from fast-growing aerobic grazers[18,38]. Many anaerobic environments exclude aerobic eukaryotes due to high levels of sulfide which is poisonous to their respiratory chains[39]. The selective costs associated with slower growth rates caused by an inefficient energy metabolism are thus compensated for by an ecological advantage[24]. Even though the relationship between growth rate and yield remains unresolved[40,41], theory predicts that microbes with lower growth rates devote larger fractions of their energy budgets to maintenance and will thus have lower growth yields[42]. (Many of the anaerobic eukaryotes shown in Fig. 3 allocate ~10–50% of their energy budgets to maintenance, whereas most of their aerobic counterparts devote <10%; Supplementary Table 1.) The lower growth rates and yields observed in facultatively anaerobic eukaryotes when grown in anoxic conditions are consistent with this expectation[38]. Similarly, when the anaerobic ciliates *Plagiopyla frontata* and *Metopus contortus* are cured of their symbiotic methanogens, the efficiency of their hydrogen-releasing fermentation is presumably reduced, leading to a proportional decrease in both

growth rate and yield[43]. This might imply that everything else being equal, anaerobic eukaryotes will have lower population sizes on average. The power of random genetic drift is often assumed to be stronger in smaller populations and this may thus explain the fast evolutionary rates observed in anaerobic lineages (for example, see Diplomonada[17]) and many of their unnecessarily complex cellular features[7,44].

At a macroevolutionary scale, the lower growth rates and efficiencies of anaerobic eukaryotes constrain their evolutionary potential and diversification. Aerobic ecosystems are known to support up to six trophic levels. Assuming a growth efficiency of ~40% for aerobic consumers (see above), only about 1% of the carbon and energy of primary producers (oxygenic photosynthesizers) is preserved at the sixth trophic level. On the other hand, there are steeper energy losses across trophic levels in anaerobic ecosystems due to inefficient energy conservation and growth. Assuming a growth efficiency of ~10% for anaerobic phagotrophs, secondary consumers would only preserve about 1% of the carbon available in the detritus that comes from surrounding aerobic environments. Thus, the same degree of productivity is found at the sixth and second trophic levels of aerobic and anaerobic ecosystems, respectively (see refs. 9,35). If primary production is represented by chemoautotrophs or anoxygenic photoautotrophs instead, one more trophic level of anaerobic eukaryovores may be supported, especially if these predators are omnivorous (that is, they also prey on bacteria). In agreement with this notion, the biomass ratio between eukaryotic predators and prokaryotic prey in anaerobic environments

is lower, ~25% of that in aerobic environments[35]. Altogether, this implies that even though evolutionary diversification may happen within a single trophic level, macroevolutionary diversification across trophic levels is constrained in anaerobic environments. The evolution of more complex predators (for example, eukaryovorous phagotrophs and small metazoans) and the arms-race that ensue are thus unlikely in anaerobic ecosystems; eukaryotes in anaerobic communities have been reported to predominantly comprise bacterivores[4]. Aerobic environments, on the other hand, inevitably support much more complex food webs and ecosystems.

Conclusion

Obligate anaerobic microbial eukaryotes have evolved repeatedly from aerobic ancestors throughout the evolutionary history of eukaryotes. They are not morphologically simpler or smaller in size (except for some parasites) and inhabit both nutrient-rich and nutrient-poor anoxic environments (see Fig. 1). However, as many of them rely on much lower energy-yielding processes than aerobic respiration, they display lower growth rates and potentially yields. Fermenting microbial eukaryotes thus have fewer physiological and ecological possibilities, and this ultimately constrains their long-term evolutionary trajectories. Such evolutionary constraints further underscore the macroevolutionary advantages afforded by the acquisition of mitochondria, for example, larger cell volumes coupled to faster growth rates[24], longer food chains and more complex communities[9]. Future research on the physiology of anaerobic eukaryotes will complement our current genome-based catalogues of anaerobic energy enzyme repertoires. For example, measuring fermentation rates and minimum cell division times in phylogenetically disparate anaerobes will add valuable data to dissect the cross-species relationship between these two physiological parameters. In addition, estimating the proteome or volume fractions devoted to energy metabolism will shed light on the proteome allocation strategies of anaerobic eukaryotes. The eukaryotic composition and trophic interactions in anaerobic ecosystems are other topics that deserve future attention. A renewed focus on physiology and energetics will refine our understanding of the ecological role of anaerobic eukaryotes and how their evolution might differ from that of their aerobic relatives (Box 1).

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Competing interests

The author declares no competing interests.

Additional information

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