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REVIEW

The Physiology of Phagocytosis in the Context of Mitochondrial Origin

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SUMMARY How mitochondria came to reside within the cytosol of their host has been debated for 50 years. Though current data indicate that the last eukaryote common ancestor possessed mitochondria and was a complex cell, whether mitochondria or complexity came first in eukaryotic evolution is still discussed. In autogenous models (complexity first), the origin of phagocytosis poses the limiting step at eukaryote origin, with mitochondria coming late as an undigested growth substrate. In symbiosis-based models (mitochondria first), the host was an archaeon, and the origin of mitochondria was the limiting step at eukaryote origin, with mitochondria providing bacterial genes, ATP synthesis on internalized bioenergetic membranes, and mitochondrion-derived vesicles as the seed of the eukaryote endomembrane system. Metagenomic studies are uncovering new host-related archaeal lineages that are reported as complex or phagocytosing, although images of such cells are lacking. Here we review the physiology and components of phagocytosis in eukaryotes, critically inspecting the concept of a phagotrophic host. From ATP supply and demand, a mitochondrion-lacking phagotrophic archaeal fermenter would have to ingest about 34 times its body weight in prokaryotic prey to obtain enough ATP to support one cell division. It would lack chemiosmotic ATP synthesis at the plasma membrane, because phagocytosis and chemiosmosis in the same membrane are incompatible. It would have lived from amino acid fermentations, because prokaryotes are mainly protein. Its ATP yield would have been impaired relative to typical archaeal amino acid fermentations, which involve chemiosmosis. In contrast, phagocytosis would have had great physiological benefit for a mitochondrion-bearing cell.

KEYWORDS eukaryogenesis, endocytic pathway, eukaryote evolution, metagenomics, mitochondria, phagocytosis

INTRODUCTION

The origin of eukaryotes remains one of biology's most formidable puzzles, an issue of the broadest evolutionary interest. A recent report of a new metagenomic lineage of archaea, first described by Christa Schleper and colleagues as the deep-sea archaeal group (1, 2), later renamed *Lokiarchaea* based on a metagenome assembled from marine sediment (3) and now included within the "Asgard" superphylum (4), sparked much interest in eukaryote origin. In the foreground of discussions about *Lokiarchaea* and relatives is the idea that the host that acquired the mitochondrion might have been a "phagocytosing archaeon" (5) and that the host was a complex archaeon that had some kind of "primitive phagocytic capability" (6, 7) or "rudimentary phagocytic capability" (3, 8, 9). Implications of alleged (primitive) phagocytic abilities for the new archaeal group have been discussed in several papers (3, 4, 10–15). The 2-fold message conveyed by those papers is that the host for the origin of mitochondria had a phagocytic lifestyle and that phagocytosis was required for it to acquire the organelle. In that view, the search for the host is a search for a phagotrophic archaeon or a primitively amitochondriate eukaryote.

Recent discussions surrounding a phagocytic origin of mitochondria, a scenario that can be called "mitochondria late" (Fig. 1A), resurrect the idea of archezoa, a hypothetical lineage of prokaryotes that supposedly evolved into nucleated eukaryotes and became phagocytic via point mutation and that served as the host for the origin of mitochondria (16). The idea of archezoa was attractive in its day, and it was rigorously tested during the 1990s and the 2000s. However, all of the predictions of the archezoa hypothesis failed, whether concerning phylogeny (17, 18), the anaerobic capabilities of mitochondria (19–21), or the identification of mitochondrion-derived organelles in what were thought to be "amitochondriate" eukaryote lineages (22–24). The archezoa hypothesis was found to lack molecular support in every investigation that ever set out to test it. Archezoa are, however, now alive and well again albeit living under the new name of (primitively) phagocytosing archaea. Although classical formulations of the endosymbiotic theory, starting with that of Margulis (25), assume that mitochondria were acquired by a host cell that was able to ingest food bacteria with the help of phagocytosis, the premise that the host was phagocytic has never received any experimental backing. Few key concepts in microbial evolution have enjoyed greater inertia in the face of less support in data than the idea of a phagocytic origin of mitochondria. At the same time, there are a number of theories out there for the origin of mitochondria that do not involve phagocytosis first (26); among those most widely discussed at present are models based on anaerobic syntrophy that account for the common ancestry of mitochondria and hydrogenosomes (Fig. 1B).

With new archaeal lineages being discovered, now is a good time to reinspect the phagocytosing-host theory. In the first three sections, we consider (i) where, historically, the idea of a phagotrophic host comes from in the first place; (ii) the physiological and energy metabolic implications of a phagocytosing archaeon; and (iii) which proteins are involved in phagocytosis and whether any metagenomic data suggest the possibility of phagocytic capabilities in the archaeal lineages now being discussed as relatives of the host. The new host-related archaeal lineages are being identified in anaerobic sediment environments where the energy supply is generally low (27, 28), where H_2-based syntrophic interactions are commonplace (29–32), and where direct interspecies electron transfer (33, 34) in archaeon-bacterium ecological interactions is being discussed (35, 36). In the final section, we present examples of observable archaeon-bacterium interactions that offer physiologically founded alternative models of phagocytosis for archaeon-bacterium (host-symbiont) associations at eukaryote origin.

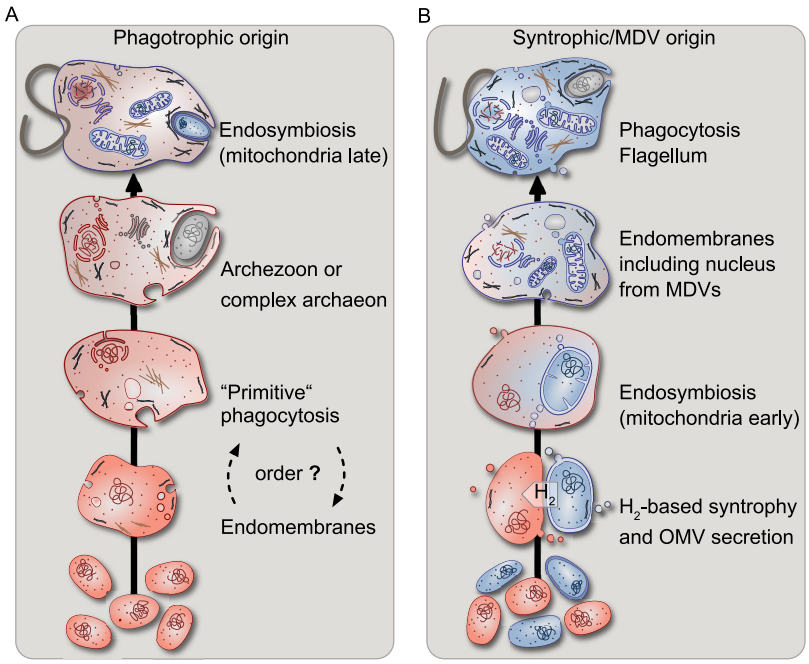

FIG 1 Mitochondrion-late and mitochondrion-early models for the origin of eukaryotes. Fossil evidence has it that eukaryotes are 1.5 billion to 1.8 billion years old (329, 330). All current models for the origin of eukaryotes have mitochondria in the eukaryote common ancestor. (A) In mitochondrion-late models, an archaeon (red) becomes a complex protoeukaryote, evolves phagocytosis, and acquires the proteobacterium (blue). The sequence of the emergence of compartments differs substantially across phagotrophic origin models: some have the nucleus first, and others have primitive phagocytosis or undefined endomembranes. Some mitochondrion-late models posit the participation of additional prokaryotic partners at eukaryote origin, for example, for the origin of the nucleus or the origin of the flagellum (26, 331). (B) In mitochondrion-early models, phagocytosis came after the mitochondrion. Mitochondrion-early models typically start with metabolic interactions between an archaeon and the proteobacterial ancestor of mitochondria (26). Models that entail anaerobic syntrophy to account for the origin of mitochondria simultaneously account for the common ancestry of mitochondria and hydrogenosomes (18, 90). In mitochondrion-early models, the origin of eukaryote-specific structures like the nucleus, the endomembrane system, and flagella, but also the origin of eukaryote-specific processes like phagocytosis, mitosis, meiosis, and sex (170), occurred after the phagocytosis-independent entry of the endosymbiont into the host's cytosol. In one formulation of mitochondrion-early models (199), outer membrane vesicles (OMVs) of the mitochondrial endosymbiont (mitochondrion-derived vesicles [MDVs]) physically gave rise to the first vesicles of the endomembrane system.

THE PHAGOCYTIC HOST: A *NOTLÖSUNG* TO THE ENDOSYMBIOSIS PROBLEM

In a historical context, the idea of a phagocytosing host was a *Notlösung*, a good German word with one specific meaning that has 10 translations ranging from its literal meaning (emergency solution) to "stopgap," "less-than-ideal solution," or "Band-Aid." In order to understand why the concept of phagocytosing archaea is so deeply engrained in thoughts about endosymbiotic theory, we need to briefly consider the historical context so as to see where concepts about the nature of the host come from in the first place. The concept of a host comes from endosymbiotic theory. In its most general form, endosymbiotic theory posits that some compartments of eukaryotic cells were once free-living bacteria. Endosymbiotic theory probably took its first step into the literature in 1883 with Schimper (37), who delivered a two-sentence comment in a footnote (in German) that if plastids really are transmitted from generation to generation through egg cells, their relationship to the plant cell that harbors them would be reminiscent of a symbiosis and that maybe the plant kingdom owed its origin to a symbiosis of a chlorophyll-containing cell with a colorless one. Mereschkowsky (38, 39) probably delivered the first full formulation of endosymbiotic theory for the origin of plastids in 1905. His paper was based on comparative physiology (photosynthesis, autotrophy, and even protein synthesis in plastids). It is astoundingly modern in many respects, and the English translation is a worthwhile read. Endosymbiotic theory for

mitochondria had a more problematic start, as mitochondria were not part of Mereschkowsky's theory at all, with Portier in 1918 (40) and Wallin in 1927 (41) getting the idea into print but in rather general terms and with a number of erroneous inferences along the way, especially concerning the ability of mitochondria to be cultivated outside the cell. Altmann (42) is sometimes credited with suggesting an endosymbiotic origin of mitochondria in his 1890 contribution, but that is a misattribution, as Altmann made no such suggestion (26).

The basic concept that chloroplasts (38) and mitochondria (40) were once endosymbionts long preceded the general recognition among biologists that there is a fundamental divide in the living world between prokaryotic and eukaryotic cells (43). This is important because without endosymbionts, there is no need to entertain ideas about the possible nature of the host that acquired those endosymbionts in the first place. The concept of the host was born entirely from the very robust idea that chloroplasts and mitochondria were once free-living bacteria, for if one does not think that chloroplasts and mitochondria arose from endosymbionts, there is no need to even entertain the concept of a host. Wilson (44), Lederberg (45), and Buchner (46) were sure that endosymbiotic theory was wrong; hence, they never devoted a word to the concept of a host. Even well into the 1970s, it was popular to think that chloroplasts and mitochondria did not stem from endosymbionts at all (47–49). Without endosymbionts, there is no need for a host. This general development in endosymbiotic theory has been amply reviewed (26, 50, 51). Mereschkowsky's (38, 39) *Amoeboplasma* probably corresponds to the first concept of a host (albeit not for the origin of mitochondria) (26), which, in his original proposal, arose as the hot water on early Earth had cooled down and as "organic substrates had become available; here a very different, amoeboid kind of plasm arose (probably in the form of small Monera) that is homologous to the cytoplasm [als das Wasser schon abgekühlt war und bereits organische Nahrung vorhanden war; da entstand ein ganz verschiedenes, amoebenartiges Plasma (wohl in Form von kleinsten Moneren), das dem Cytoplasma homolog ist]. The latter was invaded by small micrococci which lived as symbionts and ultimately gave rise to the nucleus (the chromosomes?)" (38, 39). Thus, Mereschkowsky spoke of invasion and not of phagocytosis.

Hans Ris was one of the few who dared to mention endosymbiotic theory in the classroom or in print during the early 1960s (52). Lynn Margulis sat next to Jonathan Gressel (personal communication) in Hans Ris's genetics class at the University of Wisconsin, where she learned about endosymbiotic theory. To her great credit, when she reported her version of symbiotic theory as Lynn Sagan (25), it was (probably) the first formulation to have mitochondria and plastids arising from different endosymbioses (Mereschkowsky rejected the notion that mitochondria were endosymbionts, while Wallin thought that plastids were transformed mitochondria of plants). Lynn Sagan's 1967 version of symbiotic theory always had a spirochete origin of flagella and, though seldom stated, 20 independent origins of plastids. Margulis's first host (25, 53) was initially described as an anaerobe that "ingested" the mitochondrion and then acquired the spirochete as a flagellum. Her later versions (54–56) had the flagellum first and the mitochondrion next; that is, the host was a merger of a spirochete and another prokaryote giving rise to a flagellated cell that acquired the mitochondrion. Before the discovery of archaea by Woese and Fox (57), Margulis's host (i.e., cytoplasm) prokaryote was proposed to be *Mycoplasma*-like (53); later, she proposed that it was *Thermoplasma*-like (55).

De Duve (58) and Stanier (59) can possibly be credited with first suggesting that the host for the origin of mitochondria was a bona fide phagotroph (although Sagan did say "ingesting" in 1967). Ideas about the host were spawned at a time when evolutionary biologists were still not at all comfortable with the idea of endosymbiosis. De Duve (58) had peroxisomes arising before mitochondria; he wrote, "What I am proposing, therefore, is that the peroxisome developed progressively to become the main respiratory organelle of a primitive phagocytic organism of relatively large size, which later became the host of the symbiotic ancestors of mitochondria and eventually

evolved to form eukaryotic cells." Stanier (59) saw it similarly, writing, "I should now like to suggest (. . .) that the progressive structural evolution of the eukaryotic cell received its initial impetus from the acquisition of a novel cellular property, the capacity to perform endocytosis." Modern data have it that peroxisomes arose in evolution subsequent to mitochondria (60–62) and that their biogenesis in modern cells requires blebbing of the outer mitochondrial membrane to give rise to the compartment (63), contrary to the views of De Duve (58) on the issue. In his paper, Stanier (59) went on to explain the reasons why plastids arose before mitochondria in evolution. Soon thereafter, Cavalier-Smith modified the phagocytosis idea to suggest that phagocytosis was indeed the key innovation for eukaryote origin but that plastids and mitochondria did not arise from endosymbionts (they arose from invaginations of the phagocytic plasma membrane) (49); he later modified the theory again such that phagocytosis was still the key innovation of eukaryote origin but that mitochondria and plastids arose from endosymbionts after all (64). People became accustomed to seeing drawings in evolutionary literature that depicted the host for the origin of mitochondria as a nucleated mitochondrion-lacking cell (65) and mitochondrial origin as a process of phagocytic engulfment by a nucleated, mitochondrion-lacking cell (66, 67).

The Origin of the Archezoan Concept

With the advent of the first sequencing technologies, data from organelle genomes started coming in to test the predictions of endosymbiotic theory (68, 69). Those data left no room for doubt: plastids and mitochondria turned out to be endosymbionts indeed, but where did that host come from? At a time when biologists were still not fully prepared to incorporate the endosymbiotic origin of organelles into the larger picture of cell evolution, ideas about the host arose not to explain the prokaryote-to-eukaryote transition but to satisfy the demands of endosymbiotic theory. The notional contours of the host thus took shape in a conceptual vacuum; as such, they had to be pieced together to accommodate the unexpected truth that mitochondria were of endosymbiotic origin after all. Three things came to bear on this issue.

First, there came the realization that some anaerobic eukaryotes, such as *Giardia* and *Trichomonas*, indeed lack typical mitochondria, although it was known that at least trichomonads possessed hydrogenosomes (70), whose evolutionary significance had not been recognized at that time. Second, people started making phylogenetic trees for eukaryotes, and those seemingly amitochondriate protists branched deep in the rRNA tree (71). This gave rise to the concept of archezoa: early-branching nucleated (eukaryotic) cells capable of phagocytosis that could take up a mitochondrial endosymbiont (16). If the mitochondrion indeed comes from a bacterial endosymbiont, which comparative physiology was indicating (72), then there had better be a host out there to acquire it. Third and most importantly, of course, was the discovery of archaebacteria (archaea) and the recognition that their ribosomes (73) and RNA polymerase (74) were like those of eukaryotes, which made them the natural ancestors of archezoa. A nucleated, phagocytosing, mitochondrion-lacking host (archezoan) looked like a natural fit for the endosymbiont hypothesis, on paper anyway (75–77).

However, when people looked closer at the cells that were thought to be archezoa, they all had mitochondria after all, but they were reduced mitochondria. Hydrogenosomes, H_2-producing organelles of fermentative ATP synthesis among eukaryotic anaerobes (78), had long been known among protists called archezoa, but it was not until the late 1990s that hydrogenosomes were identified as mitochondria (79–83). Mitosomes (reduced forms of mitochondria that do not generate ATP) started turning up among the remaining archezoa (22, 84–86), and when phylogeneticists started scrutinizing the lineage phylogenies, it turned out that those lineages once labeled as archezoa were not early branching either (17, 18). Thus, archezoa could hardly be direct descendants of the host.

Ideas designed to derive a phagocytic host were not based on data or observations in nature but rather from expectations generated from endosymbiotic theory, which suddenly needed such a cell for the sole purpose of acquiring mitochondria. Zillig et

al.'s premitochondrial endosymbiont (87) was one idea, Doolittle's idea of a phagocytosing cell vaguely related to archaea that acquired genes by eating bacteria (67) was another, and Gray's premitochondrial hypothesis (88) that had something like a mitochondrion in a cell that lacked a mitochondrion was another. As archezoa fell out of grace, so did discussion of early-branching eukaryotes. *Giardia*, *Entamoeba*, and *Trichomonas* assumed positions alongside mitochondriate sister clades within the commonly accepted eukaryotic supergroups (89) and were no longer part of discussions about the host. The rise and fall of archezoa prompted exploration of alternative symbiotic models for eukaryote origin using a prokaryotic host that did not phagocytose (18, 90–93), models in which mitochondria appear early (Fig. 1B) and trigger eukaryogenesis.

Supporters of the idea that phagotrophy came before mitochondria might cite the recent report of a highly reduced eukaryote, *Monocercomonoides* sp., which apparently lacks mitochondria altogether (94), as evidence in support of their case. However, this amoeba branches within a eukaryotic group that possesses mitochondria, such that the lack of mitochondria in *Monocercomonoides* is a secondarily derived trait. Clearly, the ancestral eukaryote was a very complex organism with the full set of traits that distinguish eukaryotes from prokaryotes: mitochondria, an endomembrane system, a nucleus, meiosis, mitosis, a cell cycle, and the like. Reductive evolution is very common in both prokaryotes (95) and eukaryotes (96). Phagocytosis-first theories predicted that eukaryotes lacking mitochondria should be primitively amitochondriate (Fig. 1A), evidence of which was never more lacking than now. In contrast, the hydrogen hypothesis (Fig. 1B), a mitochondrion-first theory, explicitly predicted that any eukaryotes lacking mitochondria should be the result of secondary mitochondrion loss (90), in agreement with current observations.

Genomic investigations of eukaryote origin have uncovered that nuclear genomes of eukaryotes harbor far more genes of bacterial origin than genes of archaeal origin (97–102). Roughly 75% of the genes in eukaryote genomes that have prokaryotic homologues can be traced to a bacterial rather than an archaeal origin (97–102). This is one of the strongest lines of evidence indicating a symbiogenic origin of eukaryotes (103–105). After all, if eukaryotes were of "archaeal origin," as their ribosomes suggest (3, 4, 106, 107), then the phylogenetic signals in eukaryote genomes would reflect an archaeal origin of eukaryotic genes in general. However, the converse is observed. This is especially true in the plant lineage, where a major influx of genes entered the eukaryotic lineage from cyanobacteria via the origin of plastids (108, 109). Traditional molecular systematic and taxonomic approaches to microbial evolution strive to place eukaryotes on "a branch" (that is, one branch) in the overall scheme of things, with a focus on "which branch" (110, 111). At the quantitative level, however, eukaryote genes that are not eukaryote-specific inventions are mainly bacterial in origin. The acquisition of these genes can be traced to the origin of plastids in the plant lineage and to the origin of mitochondria in the eukaryote common ancestor, and both the distribution and the phylogeny of genes in eukaryote genomes are primarily the result of a gain at endosymbiotic events followed by differential loss (102).

With improved sampling, archaeal genomes have turned out to encode homologues of a few proteins once thought to be eukaryote specific. These proteins included archaeal cell division proteins that show sequence similarity to proteins of the eukaryotic ESCRT (endosomal sorting complex required for transport) complex, certain ribosomal proteins, the ubiquitin system, longin-like domains, ribophorin1, and, more recently, Sec23/24 (3, 4, 112–119). Such findings rekindled the idea of a hypothetical archaeon either having primitive phagocytic capabilities or being on its way to becoming phagocytic (6, 114, 115). Stronger formulations entailed the idea of a bona fide phagocytosing archaeon (5). The notion of archezoa (phagocytosing hosts that lacked mitochondria) (Fig. 1B) was brought back to life, but they were no longer called archezoa; they were called primitive phagocytosing archaea.

PUTTING THE HYPOTHETICAL PHAGOCYTIC HOST TO A PHYSIOLOGICAL TEST

Current genome-wide analyses have it that the mitochondrion stems from the alphaproteobacteria (102), although metagenomic data for a mitochondrial origin are only beginning to be tapped (120, 121). The host lineage has been more difficult to identify. Newer phylogenetic data trace the origin of the host lineage to within the archaea (3, 4, 106, 107, 111), but nobody knows how these new archaeal lineages grow. They all have traces of a methanogenic ancestry. They might be H_2 dependent (122), they might even be acetogenic (123, 124), they might be anaerobic methane oxidizers (125), or they might have an anaerobic alkane-oxidizing ability (126). They are definitely anaerobes, and so far, no clear hints that they might have bacterial-type respiratory chains, like haloarchaea do, have come forth (127). One aspect is very striking, however, in the context of the *Lokiarchaea* and the Asgard data: if these lineages really are close relatives of the host, whatever energy metabolism the host had, it was replaced during mitochondrial endosymbiosis, as some models predict (90), because there are neither hints of archaeal energy metabolism in eukaryotes (19) nor hints of eukaryotic energy metabolism in either the *Lokiarchaea* (3) or Asgard (4) lineages detectable so far. The replacement of preexisting energy metabolic enzymes or abilities is a common principle in endosymbiotic associations that give rise to organelles: functionally redundant pathways sort themselves out through the differential loss of unneeded genes (128).

Eukaryotes are far better characterized with respect to physiology and energy metabolism than are the new archaeal lineages. Many lineages of eukaryotes are indeed phagotrophic. There can thus be little question that an archaeon, provided that it had already undergone the endosymbiosis with the bacterium that became the mitochondrion, can evolve eukaryotic traits such as phagocytosis, but how likely is it that an archaeon could become phagocytic before acquiring a mitochondrion? A look from the physiological standpoint is instructive.

Phagotrophy is a feeding habit. It entails the oxidation of ingested food particles for the purpose of energy metabolism (ATP synthesis). In literature on the phagotrophic origin of eukaryotes (129) or (primitively) phagotrophic hosts for the origin of mitochondria (3, 5–7, 77), the emphasis until now has always been on the "ingested food particles" part of phagotrophy. Here we emphasize the "oxidation" part. Oxidation is the removal of electrons from a chemical compound; the deposition of these electrons on an acceptor is reduction. Reactions that redistribute electrons among molecules or atoms are called redox reactions. With very rare exceptions (130), the source of electrons for energy metabolism in eukaryotes that lack plastids is organic matter (organotrophy); the source of carbon is always organic matter (organoheterotrophy). In order for an organism to survive from the oxidation of organic substrates in ingested food particles, it has to harness energy from that oxidation. There are only two ways to harness energy as ATP by oxidizing organic substrates: respiration and fermentation (131).

In respiration, ATP is synthesized via electron transfer phosphorylation (ETP), which entails the transfer of the electrons from the substrate to a suitable electron acceptor. The electron acceptor in ETP can be a compound that is available in the environment, such as oxygen, sulfate, Fe^{3+}, or the like, or it can be a compound that is produced during metabolism, such as fumarate or protons. In ETP, electron transfer is coupled to the formation of ion gradients across the plasma membrane (prokaryotes) or across the mitochondrial inner membrane (eukaryotes) via the pumping of ions across the membrane from inside to out and the harnessing of these gradients via a rotor-stator-type ATPase, which transfers phosphate to ADP, yielding ATP (Fig. 2). The ion gradient is used for not only ATP synthesis but also protein translocation at the plasma membrane (prokaryotes) and into the mitochondrial matrix (eukaryotes) (Fig. 2). The principle of generating ion gradients via redox chemistry and harnessing of energy stored in the ion gradient via a coupling factor is called chemiosmotic coupling (131–133).

The second way to conserve energy from the oxidation of organic compounds is fermentation. In fermentations, the electrons from the oxidation of the organic food

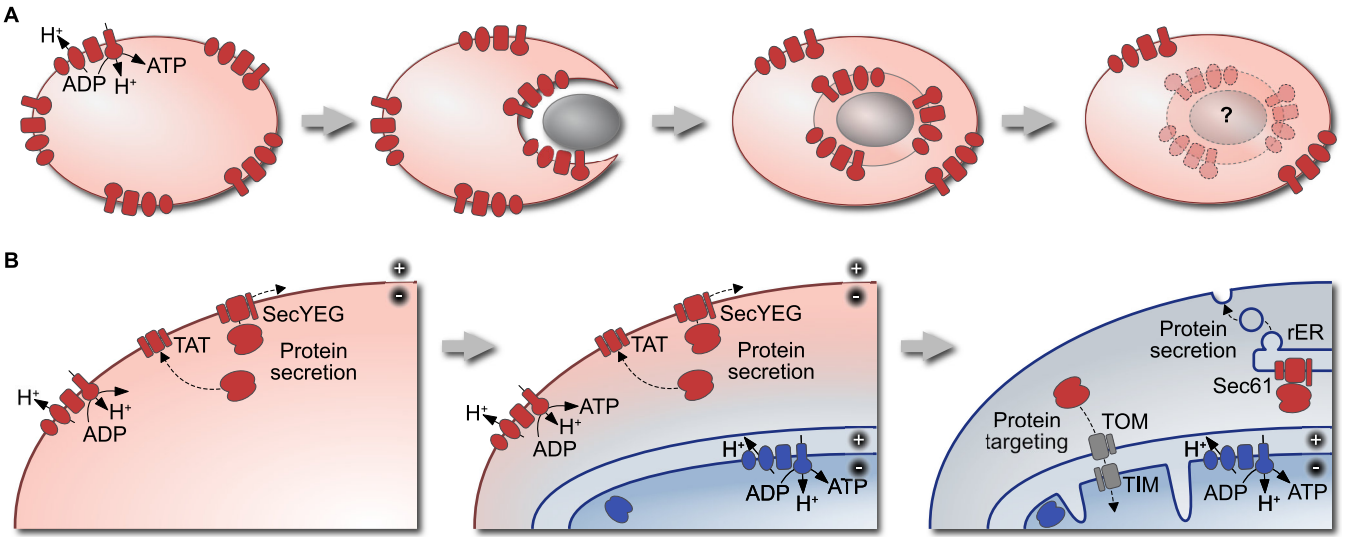

FIG 2 Bioenergetic membranes in phagocytosis and protein translocation. Eukaryogenesis models rarely consider the role and fate of bioenergetic membranes. (A) The vacuolar ATPase of eukaryotes is of archaeal origin, which suggests that the archaeal host synthesized ATP at its plasma membrane. This poses a problem concerning the phagocytic origin of mitochondria. A phagocytosing archaeon would digest its own ATP synthesis machinery, an energetically unfavorable condition. (B) Bioenergetic membranes are characterized by a proton motive force (PMF), here symbolized by plus and minus signs. The proton motive force influences protein secretion through the SecYEG and TAT machineries at the prokaryotic plasma membrane via electrophoretic properties but also in eukaryotes at the inner (bioenergetic) membrane of the mitochondrion (155, 332). Both prokaryotic signal peptides and targeting peptides of mitochondrial matrix proteins carry positively charged amino acid residues that respond to the proton motive force. Upon endosymbiosis, the presence of two independent bioenergetic membranes (and a proton motive force) likely would have fostered false targeting. Eukaryotic cells today have retained one bioenergetic membrane, the inner mitochondrial membrane, which also has retained the use of the proton motive force for protein translocation. Targeting to the eukaryotic plasma membrane commences with cotranslational import into the rough endoplasmic reticulum (rER) through the Sec61 translocon (which stems from archaeal SecYEG) that is targeted by eukaryotic signal peptides that have lost their positively charged character (in contrast to their prokaryotic signal peptides). TOM, translocase of the outer mitochondrial membrane; TIM, translocase of the inner mitochondrial membrane; TAT, twin arginine translocon.

source are transferred to compounds generated from the food source itself, chemically a disproportionation. In yeast-type glucose fermentation, for example, the end products are CO_2 (more oxidized than glucose) and ethanol (more reduced than glucose), with the overall energetics of the reaction being exergonic (131, 134) so that it can support ATP synthesis and life. In fermentative metabolism of human muscles, lactate is produced, which has forms of carbon in the same molecule that are more reduced (methyl group) and more oxidized (carboxyl group) than the carbon in glucose. Neither yeast-type ethanol fermentation nor human muscle lactate fermentation involves chemiosmotic coupling. Fermentations involve energy conservation via substrate-level phosphorylation (SLP), the direct phosphorylation of ADP by a small "energy-rich" organic compound like phosphoenolpyruvate, 1,3-bisphosphoglycerate, creatine phosphate, or the like (135). In prokaryotes, fermentations typically entail chemiosmotic coupling (136, 137). They can do so in eukaryotes as well, for example, in the case of fumarate reduction during malate dismutation in anaerobic mitochondria of liver fluke and other invertebrates (19).

In archaea (to which the *Lokiarchaeota*, as a proxy for the host lineage, belong by the current measure of ribosome phylogeny), fermentation substrates are typically peptides, but carbohydrates can also be fermented by many species (138). Archaeal amino acid fermentations involve chemiosmotic coupling (139); the amino acid is typically converted to ammonia and the corresponding 2-oxoacid, which is then converted into the corresponding thioester by ferredoxin-dependent 2-oxoacid oxidoreductases (140–142). Energy in the thioester bond is conserved as ATP via SLP by members of the ACD family (acetyl coenzyme A [acetyl-CoA] synthetases [ADP forming]), enzymes typical of archaeal fermenters (143–149) that generate the resulting organic acid, shortened by one carbon. For example, in alanine fermentation, alanine is first converted to ammonia and pyruvate, with the latter generating acetyl-CoA, and is then converted to acetate and ATP (from ADP and P_i). The electrons in reduced ferredoxin generated during amino acid oxidation are transferred either to protons to generate H_2 as the reduced

end product or to elemental sulfur (139). In the latter case, the process is sometimes called "facilitated fermentation" (150), which is another way of saying that they are not really fermentations at all; rather, they are basically respirations of elemental sulfur, which is often required as an environmental electron acceptor for growth or which can be used as an alternative to H_2 production (139).

The use of either protons or sulfur as the electron acceptor in the archaea studied so far entails chemiosmotic coupling, follows the same basic route, involves related proteins, and can depend upon environmental sulfur (S^0) availability (139). When protons are the acceptor, H_2 is generated by a membrane-bound hydrogenase called Mbh that couples the oxidation of reduced ferredoxin to the generation of an ion gradient that is harnessed via the rotor-stator ATPase (136). When sulfur is the acceptor, H_2S is generated, involving a membrane-bound oxidoreductase called Mbx that generates an ion gradient during the transfer of electrons from reduced ferredoxin to $S^{\pm 0}$ (139). The Mbh and Mbx complexes are related, they can have similar gene clusters, and both belong to the larger family of Nuo-like ("complex I"-like) proteins with Fe-Ni hydrogenase catalytic subunits (139).

Archaeal carbohydrate fermentations (133, 138) also generate reduced ferredoxin and involve ACD for SLP (143–149), with the use of Mbh or Mbx for chemiosmotic coupling and ATP synthesis via the rotor-stator ATPase (139). The contribution of chemiosmotic coupling to energy conservation in archaeal fermentation is quantitatively significant. In *Pyrococcus furiosus* carbohydrate fermentation, 1 mol glucose was estimated to yield 2 mol ATP via SLP and ca. 1.2 mol ATP via chemiosmotic coupling (136). Buckel and Thauer (137) estimate that *P. furiosus* obtains, per glucose, 2 mol ATP via SLP and 2 mol ATP via pumping at Mbh, also called Ech, for energy-converting hydrogenase (151), which would thus appear to yield 4 ATP per glucose, but each glucose costs 1 ATP for substrate import, yielding a net balance of 3 ATP per glucose, with equal contributions from SLP and chemiosmotic coupling (137).

A Respiring or Fermenting Host at Mitochondrial Origin?

To discuss the idea of primitively phagocytosing archaea, we consider the ancestral trophic state of an archaeon (seldom if ever explicitly specified in phagotrophic theories) that is aspiring to undergo the evolutionary transition to phagotrophy so that it can acquire a mitochondrion. The archaeon has to be heterotrophic, living from the oxidation of reduced organic compounds, for phagotrophy to have any benefit or value. There are two possibilities: it is a respirer, or it is a fermenter.

If it is a respirer, then the origin of phagotrophy would have it engulfing food particles at the plasma membrane and, in essence, converting internalized vesicles into something topologically equivalent to a food vacuole, where the ingested loot is oxidized. This means that a section of the bioenergetic membrane underpinning the archaeon's chemiosmotic ATP synthesis large enough to surround a food particle (a bacterium at some point) would also be digested (Fig. 2A) and have to be resynthesized. The digestion of one's own proteinaceous ATP synthesis machinery to obtain ATP is not a very good strategy, especially since protein synthesis is the most energetically expensive thing a cell does, with 75% of the energy budget being devoted to protein synthesis (152). For every protein of the archaeal plasma membrane that is digested, a 4-fold penalty is incurred (at least), because the cost of synthesizing proteins from amino acids is 4 ATP per peptide bond (153). Digested proteins of the plasma membrane have to be replaced; otherwise, bioenergetics comes to a halt for respirers. Every protein that the primitive phagocyte digests has to be resynthesized at ribosomes, at a cost of 4 ATP per amino acid. We know of no archaea that have mechanisms for recycling plasma membrane proteins. In contrast, all modern eukaryotes possess elaborate recycling systems for plasma membrane proteins, in some cases even being able to turn over the entire plasma membrane 1 to 5 times per h (154), but eukaryotes do not synthesize ATP at the plasma membrane. The absence of chemiosmotic ATP synthesis at the plasma membrane in eukaryotes impacted the evolution of SEC61-dependent translocation at the endoplasmic reticulum (ER) and ion gradient-

dependent protein translocation into the mitochondrial matrix (155) (Fig. 2B). The point of phagocytosis is the digestion of environmental organics and other organisms and not the digestion of one's energy-harnessing system.

Among prokaryotes, the strategy of heterotrophs is to access low-molecular-weight compounds from the environment via membrane importers (156), sometimes with the help of enzymatically active secreted hydrolases to break down substrates for import (157–159). In a world where substrate resources are at a premium (the densely populated microbial environment), the secretion of hydrolases or peptidases to externally digest polymers and mobilize carbon substrates for ingestion as lower-molecular-weight metabolites via membrane importers, as opposed to the digestion of one's own bioenergetic membrane, has distinct advantages. This perhaps helps to explain why no respiring prokaryotes that phagocytose have been found so far.

Alternatively, if the aspiring phagocytic archaeon is initially a fermenter, then it is probably performing the typical archaeal peptide or carbohydrate fermentations outlined above; hence, it is chemiosmotic just like the respirer and is also digesting its own bioenergetic membrane as a phagotroph. Therefore, fermentation does not seem to help an archaeon become phagocytic either. Of course, the archaeal fermenter could use cytosolic hydrogenases and give up respiration at the plasma membrane altogether. This is an option, but it would mean sacrificing 40 to 50% of the ATP yield (the chemiosmotic component) from amino acid or carbohydrate fermentation in order to acquire substrates via engulfment, as opposed to the standard prokaryotic small-molecule substrate import mechanism. The archaeal fermenters characterized so far seem to employ membrane-bound ion-pumping Fe-Ni hydrogenases of the Mbh/Ech type (138, 139), the subunits of which are related to the complex I subunits NuoDIBLH (151) rather than the soluble trimeric, electron-confurcating Fe-Fe hydrogenases that are typical of bacterial fermenters (160), syntrophic H_2 producers (31), and eukaryotes (19), two subunits of which are related to the complex I subunits NuoEF (19).

How Much Prey for One Phagocytosing Archaeon To Divide?

Thus, there are number of problems associated with the notions that either a respiring archaeon or a fermenting archaeon evolved phagocytosis (as a means to acquire the mitochondrion), but we are not done yet. Phagocytes eat other cells. For our hypothetical phagocytosing archaeon, eating other prokaryotes is not a matter of just practicing to get ready for the acquisition of that mitochondrion, it is a way of survival in the wild. What is on the menu for bacterium-gobbling phagocytes? Prokaryotic cells. By dry weight, a typical bacterium (*Escherichia coli*) is roughly 55% protein, 20% RNA, 3% DNA, 9% lipids, 6% mixed saccharides, 3% cell wall, and 4% metabolites (150, 161). Prokaryotes are mostly protein.

As we describe above, if the phagocytic archaeon is a respirer, phagocytosis brings bioenergetics to a halt. If it is a sugar fermenter, 50% of the energy yield (the chemiosmotic component) from about 6% (wt/wt) of the prey (mixed saccharides) has to be sacrificed to begin with, because chemiosmosis at a phagocytic membrane is not going to work. If our archaeon had mitochondria, there would be plenty of energy to be gleaned from lipids and other cell components via oxidations using the internalized bioenergetic membranes that mitochondria afford (93), but it does not have mitochondria. It is trying to get ready to acquire some. So what's for lunch? Lipids are nonfermentable, amino acids are the main course, and the free energy change, and, hence, the energy yield, from amino acid fermentations is modest.

The best-studied amino acid fermentations are those of the firmicutes (162). For example, in *Clostridium tetanomorphum* and *Acidaminococcus fermentans*, glutamate fermentation involves SLP and chemiosmotic coupling, as in archaeal sugar fermenters, and delivers 0.95 ATP per glutamate, with a change in free energy on the order of -63 kJ $\cdot$ mol^{-1} per glutamate (137). Similar to the case of glucose fermentation in *Pyrococcus*, about 60% of the ATP in these glutamate fermentations stems from SLP, and about 40% stems from chemiosmosis (137). The ability to phagocytose is not going to improve the ATP yield per amino acid, with the loss of chemiosmosis at the plasma

membrane costing about half of the ATP yield per amino acid to start with. This means that the aspiring phagocytic fermenter, assuming that it has soluble enzymes for redox balance, might be able to scratch about 0.5 ATP out of each ingested fermentable amino acid, import included, but assuming no costs for breaking down ingested cells. The energy yield from other minor components of ingested cells, such as purine fermentation from RNA (163–165) or carbohydrate monomers, which might comprise as much as 15% by weight if we count the ribose in RNA (150), can probably just be neglected, but we account it against the cost of excreting the undigested or nonfermentable components of the ingested cell (exocytotic secretion requires a very sophisticated apparatus [see below]).

Thus, if we are fairly generous in our accounting, the hypothetical amitochondriate phagocyte can possibly gain a net of 0.5 ATP per amino acid ingested. How much is that? How much ATP does a prokaryote need to divide? On oxygen and glucose minimal medium, *E. coli* generates about 13.9 g of cells per mol ATP (or ATP equivalent) that it synthesizes (166), which is converted to 0.072 mol ATP per g of *E. coli* cells (167). One *E. coli* cell weighs about 2.8×10^{-13} g (161), yielding 0.2×10^{-13} mol ATP per cell, which is 1.2×10^{10} molecules of ATP per cell or roughly 12 billion ATP to make two *E. coli* cells from one. We will work with the value of 12 billion ATP, even though slightly higher values (20 billion to 60 billion ATP per cell) can be found in the literature (153, 168). Based upon similar values of growth yields (grams of cell per mole ATP) for archaea (169), we can assume the value to be similar to that for *E. coli*.

How much energy can a hypothetical archaeal fermenter obtain from a hypothetically phagocytosed cell? By dry weight, our microbial schnitzel, *E. coli*, is 55% protein and has about 2.4 million proteins per cell, with an average of 300 amino acids each (161, 167), or potentially 0.7 billion amino acids per ingested cell. At about 0.5 ATP per amino acid, this generates roughly 0.35 billion ATP per ingested prokaryotic cell as food. Thus, in order to obtain enough ATP to fuel one cell division, our phagotroph needs to consume about 34 times its weight (12/0.35) in prokaryotic prey. If the phagotroph or the prey is larger or smaller, the nature of the problem does not change; the ratio remains ~34:1 for the hypothetical archaeal phagotroph. If we assume an energy requirement of 60 billion ATP to make one cell (168), the ratio becomes ~170:1. Slower doubling times generally entail higher ATP requirements to generate a new cell because of increasing demands for maintenance energy (28).

The primitive, fermenting phagotroph thus needs to consume 34 times its body weight in order to divide if, that is, its prey swims up to it to be eaten. If it needs to expend energy to chase down prey, or if it has to expend maintenance energy, which can consume about half of all ATP synthesized by a cell (166), for long periods of time without feeding, the value of 34 is not enough. The issue of how it is going to physically divide, as a prokaryote, while ingesting ~30 prey per division at the same time poses serious problems into which we do not delve here (see reference 170 for a discussion of early eukaryote cell division). Is the 34-fold estimate completely off target?

As a reality check on the conservative 34:1 ratio, in *Clostridium thermoaceticum* growing on H_2 and CO_2, about 24 molecules of CO_2 (food) pass through the cell as acetate for every molecule of CO_2 that is fixed and retained as cell carbon (171), a molar ratio of 24:1. Acetogens grow from low free energy changes (135), as do methanogens, for which a similar number can be calculated. Schönheit et al. (172) reported a growth yield of 1.6 g dry cell mass per mol of methane produced (per 12 g C passing through the cell) for *Methanobacterium thermoautotrophicum*. The dry methanogen cell mass is roughly 40% C by weight (173), such that roughly 19 molecules of CO_2 exit the cell as methane for each CO_2 molecule incorporated into dry weight. One might wonder why amino acid fermentation comes up looking even less efficient than the situation for the chemolithoautotrophs. First, phagocytosis required the relinquishing of chemiosmosis at the plasma membrane (almost half of the energy yield). Second, chemolithoautotrophs live from gasses, which diffuse freely across membranes, as opposed to amino acids. Thus, the 34:1 estimate might not be way off the mark for the energetic situation that the hypothetical phagocytosing archaeon faced, and the ratio of 170:1 remains a

possibility. For a phagocytosing archaeon, the energetic picture becomes bleak, much worse than that without phagocytosis.

With Mitochondria, Phagocytosis Has Energetic Benefit

If, however, the cell that evolved phagocytosis already had mitochondria, everything works a lot better, just splendidly in fact, because then the physiology of phagocytic feeding works just like it does in real eukaryotes: with membrane flux, with acidified food vacuoles, and with ATP synthesis via chemiosmosis through oxidation in mitochondria.

For a direct comparison of a phagotroph with mitochondria versus one without mitochondria, we can estimate how much ATP a phagotroph can gain from the main component (protein) of ingested cells. Bender (174) recently recalculated the ATP yield from amino acid oxidation using mitochondria and oxygen as the terminal acceptor in mammalian cells and separated the calculation into two components that are very convenient for our purpose: (i) the net ATP gain per amino acid via SLP and (ii) the net ATP gain per amino acid using the mitochondrial respiratory chain with O_2 as the terminal acceptor for the reoxidation of NAD(P)H and reduced flavin adenine dinucleotide ($FADH_2$).

Using the very conveniently tabulated values reported by Bender (174) and assuming for simplicity that all amino acids are equally abundant in a protein, the net ATP gain via SLP alone turns out to be roughly 1.4 ATP per amino acid. This is ~3-fold better than that without mitochondria and represents a rough lower bound for the ATP yield for the mitochondrion-possessing phagotroph, because it assumes that all reducing equivalents are reoxidized via the excretion of ethanol or H_2 as a metabolic end product. If we assume that the mitochondriate phagotroph was subjecting all amino acids to complete oxidation through the Krebs cycle, the net ATP yield (including the SLP component) comes in at 21.8 ATP per amino acid (174), which is more than 40-fold better than that without mitochondria (and without oxygen). Thus, with mitochondria and in the presence of O_2, amino acids are roughly two-thirds as good as glucose as a source of ATP (174).

An estimate for the ATP yield per amino acid with mitochondria, but without O_2, is more challenging because anaerobic mitochondria can use different pathways for ATP synthesis (19, 175). For glucose, the ATP yields are well known. Organisms with anaerobic mitochondria obtain about 5 ATP per glucose (19, 176), and organisms with hydrogenosomes obtain 4 ATP per glucose, while organisms with mitosomes that are not energy parasites obtain between 2 and 4 ATP per glucose, depending on whether they metabolize acetyl-CoA to ethanol or to acetate (19, 90).

If, however, we look to the literature for estimates of the ATP yield from anaerobic amino acid metabolism in eukaryotes, a deficit of investigation becomes evident. With two exceptions, we cannot find reports where anaerobic amino acid breakdown has been surveyed in any depth for eukaryotes. One exception is the arginine dihydrolase pathway, which generates 1 ATP per arginine (177, 178) and involves mitochondrial enzymes (179, 180); the other exception is the synthesis, intracellular accumulation, and breakdown of opines in marine invertebrates such as the mussel *Mytilus* (181). We also find no reports where the end products of anaerobic energy metabolism from peptide breakdown have been extensively measured in mitochondrion-bearing organisms. However, we can survey the basic routes that are possible for anaerobic peptide breakdown to provide some upper and lower bounds for ATP yields based on known routes of ATP synthesis in eukaryotic anaerobes (19, 176) and based on known eukaryotic biochemical conversions of amino acids that do not require oxygen as a cosubstrate (174). An outline of expected intermediate amino acid breakdown products in eukaryotes that could yield ATP in the absence of O_2 is given in Fig. 3.

Except for the pathways involving malate dismutation, where rhodoquinone-dependent proton pumping and chemiosmotic coupling can occur (19, 176), the following estimates for anaerobic ATP synthesis from amino acids in mitochondriate eukaryotes assume SLP only and, importantly, do not imply redox balance; that is, the

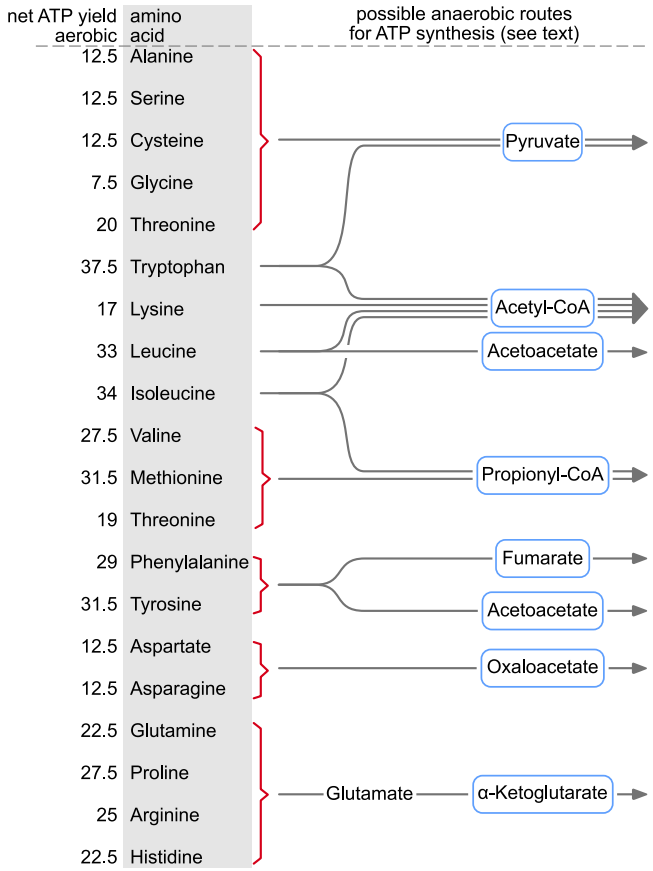

FIG 3 Amino acids as a source of energy. Aerobic ATP yields (174) are shown on the left and correspond to the net ATP yield for the complete oxidation of the amino acids to carbon dioxide by mammals via the intermediates shown on the right. The end products of anaerobic amino acid breakdown pathways in eukaryotes have not been determined. The expected degradation pathways of amino acids to intermediates that can be broken down further to fermentation products by anaerobically functioning mitochondria to generate ATP are shown. Branched arrows are used to show that the degradation of one molecule of the amino acid in question results in the two products. The possible yields of ATP of these anaerobic pathways are discussed in the text.

reoxidation of NADH or $FADH_2$ generated during amino acid breakdown (by any of the pathways that eukaryotes possess) is not accounted for. Pyruvate can enter malate dismutation, through which 2 pyruvates can yield acetate, propionate, and 3 ATP. Acetyl-CoA can enter the acetate:succinate CoA-transferase (ASCT) cycle to yield 1 ATP. Acetoacetate can be activated to acetoacetyl-CoA, and acetoacetyl-CoA can generate 2 acetyl-CoAs, which yield 1 ATP each, but activation expends either a thioester or an ATP, leaving a net yield of 1 ATP from acetoacetate. Propionyl-CoA can enter the ASCT cycle to yield 1 ATP. Fumarate can enter malate dismutation, where 3 fumarates yield 1 acetate, 2 propionates, and 5 ATP. Oxaloacetate can enter malate dismutation, where 2 oxaloacetates yield 1 acetate, 1 propionate, and 3 ATP. Alpha-ketoglutarate can be oxidized via succinyl-CoA to succinate, which is accompanied by the production of 1 ATP, if the NADH formed is reoxidized in another process, for example, the reduction of fumarate, the intermediate of phenylalanine and tyrosine breakdown. This brings us to the issue of redox balance.

In amino acid breakdown, NADH and $FADH_2$ are generated at many steps (163), for example, the removal of the α-amino group or the oxidation of the branched amino acids Val, Ile, and Leu. This demands the presence of electron acceptors to maintain redox balance. Anaerobic eukaryotes have a variety of biochemically characterized enzymes (and electron acceptors plus end products) for reoxidizing reduced ferredoxin, NADH, NADPH, and $FADH_2$ to maintain redox balance. These enzymes include Fe-Fe

hydrogenase (protons; H_2) as in *Trichomonas* (182) or *Chlamydomonas* (183), glycerol-P dehydrogenase (triose phosphate; glycerol) (184) or lactate dehydrogenase (pyruvate; lactate) as in *Trichomonas* (185), the bifunctional alcohol/aldehyde dehydrogenase (ADHE) (acetyl-CoA; ethanol) as in *Entamoeba* (186) or algae (187), volatile fatty acid synthesis as in *Ascaris* (acetyl-CoA; 2-methylbutanoate) (188), wax esters as in *Euglena* (acetyl-CoA; fatty acids) (189), rhodoquinone-dependent fumarate reduction during malate dismutation (fumarate; succinate and/or propionate) as in *Fasciola* (190), or soluble fumarate reductase (fumarate; succinate) as in *Trypanosoma* (191) (reviewed in reference 19). The problem is that the end products of amino acid breakdown in anaerobic eukaryotes have not been studied to any extent.

There are other possible alternatives for maintaining redox balance; these include lipid synthesis via glutamine flux through the Krebs cycle running in reverse (192) or Stickland reactions, in which one amino serves as the energy source and electron donor while another amino acid serves as the electron acceptor (193, 194). Despite the need for end product studies of amino acid breakdown on eukaryotic anaerobes using labeled substrates, we can still estimate, based on known pathways, the range: anaerobic amino acid breakdown in mitochondrion-bearing cells yields less ATP per monomer than in the case of glucose (5 ATP per monomer), mainly because of the lack of a contribution from glycolysis, but more ATP than in the case of a phagocytosing archaeon without mitochondria (~0.5 ATP per amino acid), because pathways of SLP are widespread in anaerobic mitochondria, in particular the ASCT cycle (19, 195, 196), and because NADH reoxidation via rhodoquinone-dependent pumping at mitochondrial complex I generates roughly one extra ATP per NADH in addition to SLP (19, 176).

Without Mitochondria, Phagocytosis Depletes the ATP Yield

Of course, not all amino acids yield equal amounts of ATP (174), and amino acids are not equally abundant in a protein, but the above-described data reveal that compared to amino acid oxidation for a hypothetical phagotroph without mitochondria, amino acid oxidation in a phagotroph with mitochondria yields a good energetic situation for amino acid oxidation without oxygen as the terminal acceptor and roughly a 40-fold improvement with oxygen as the terminal acceptor. The bottom line is this: despite numerous assumptions about potential advantages of phagocytic feeding, phagocytosis without mitochondria brings no feeding (energetic) benefit to the cell. On the contrary, the data described above reveal that phagocytosis in an archaeal cell would cut the energetic yield roughly in half relative to the uptake of low-molecular-weight metabolites, due to abolished chemiosmosis at the plasma membrane.

The above-described calculation did not enter costs for the resynthesis of chemiosmotic coupling proteins at the plasma membrane or for the synthesis of the many structural proteins that are required to make phagocytosis work. For comparison, roughly as much ATP (5 billion to 50 billion molecules) is required to synthesize the microtubules needed to separate a single eukaryotic chromosome pair as is required to generate two *E. coli* cells from one (170). The cost of making protein is high. This makes it exceedingly unlikely that any phagocytic lifestyle ever arose in a mitochondrion-lacking cell, which would directly account for the observation that the cells that evolved phagocytosis (eukaryotes) had mitochondrion-bearing ancestors. If, on the other hand, phagocytosis arose in a cell that already possessed mitochondria, it could have been seamlessly integrated into eukaryotic energy metabolism via mitochondrial amino acid oxidation. This is true either with or without O_2 as the terminal acceptor, because ancestral mitochondria had facultative anaerobic capabilities (19), and eukaryotes arose and diversified during low-oxygen phases of Earth's history (19, 197, 198).

Thus, the ability to genuinely realize energetic benefit from phagocytosis requires the preexistence of mitochondria in the cell. The reason stems from the simple circumstance that mitochondria bestowed upon eukaryotes the ability to perform chemiosmotic ATP synthesis on a system of internalized bioenergetic membranes—the mitochondrial inner membrane—that was functionally independent of the plasma

membrane (Fig. 2) (93, 199), which most prokaryotes use for bioenergetics (cyanobacteria evolved thylakoids in which the photosystems and ATP synthase reside).

Of course, for phagocytosis to operate at all, a functional endomembrane system and endomembrane flux are also required. In the next sections, we address the issue that mitochondria not only were required for phagocytosis to bear benefit for the cell but also were quite likely physically involved in the origin of the eukaryotic endomembrane system to begin with.

PHAGOCYTOSIS: COMPONENTS UNDERLYING FUNCTION

In contrast to prokaryotes, which import substrates for growth via a range of different importers for low-molecular-weight compounds in the plasma membrane (156), eukaryotes can assimilate substrates via invagination of the plasma membrane and extraction of substrates from internalized membrane vesicles (154, 200). There are several different manifestations of this feeding habit, which are commonly divided into categories such as pinocytosis, endocytosis, and phagocytosis (reviewed in reference 200). The list can be expanded to include the caveola/caveolin1-dependent pathway, the Arf6-dependent pathway, CLIC/GEEC-type endocytosis (clathrin-independent carriers/glycosylphosphatidylinositol-anchored protein [GPI-AP]-enriched compartments), the IL2Rbeta pathway, circular dorsal ruffles, and entosis (reviewed in reference 200). Here we refer to these pathways collectively as endocytic processes.

Caveola-dependent endocytosis through caveolins provides an example of a lineage-specific endocytic mechanism that arose comparatively late in evolution, within the metazoan lineage (201). Moreover, the presence of a single caveolin gene alone does not mean that the organism possessing it is able to form caveolae (202). The different endocytic processes differ with regard to cargo size (203), the recognition of cargo through associated motifs (154), and alleys of downstream processing (154). In some cases, the different endocytic pathways can overlap and can involve many of the same proteins. Only a subset of endocytic pathways is involved in feeding; many are solely involved in signaling. All endocytic processes serve the uptake of extracellular molecules and fluids (and solutes dissolved therein) and the recycling of plasma membrane proteins and lipids. Many of the functions involving membrane traffic that have been studied in eukaryotes have to do with signaling and differentiation via receptor processing or the evasion of host defense but not feeding (204–208).

Of the various endocytic processes, only phagocytosis serves the uptake of entire cells, whether as a mechanism to secure tissue homeostasis in the case of human macrophages (209) or for feeding, as observed for many different protists. A comparison of the main endocytic pathways underscores the difference in scale among them (Fig. 4). Arguably, phagocytosis is the most complex, elaborate, and energetically and physically demanding endocytic pathway and might be seen as the most highly evolved of all of them; it is an endpoint in endocytosis evolution and not the starting point. It was suggested that the "incidental capture" of the mitochondrion through endocytosis allowed the host to evolve phagocytosis (210). This by itself presents a conflict, because in endocytic processes, cargo recognition is receptor mediated, and cargo size matters. A vesicle of 100 nm in diameter, the average diameter of a clathrin-coated endocytic vesicle, can pack something around 20 prokaryotic ribosomes. For comparison, an *Escherichia coli* cell contains tens of thousands of ribosomes (211). In the context of mitochondrial origin, it has become popular to talk about "primitive" phagocytosis as if there was a form of it that did not require membrane traffic. What does phagocytosis entail?

Phagocytosis Demands the Full Complexity of a Eukaryotic Cell

Phagocytosis is a sophisticated endocytic process and not a universal feature of eukaryotic biology. It is actin cytoskeleton dependent, serves the absorption of particles larger than about 0.5 μm (212, 213) (effectively, the uptake of other cells), and consists of roughly six interwoven steps. In the main, these steps are (i) cargo-driven recognition at the plasma membrane through a diverse range of receptors (214, 215), (ii) the

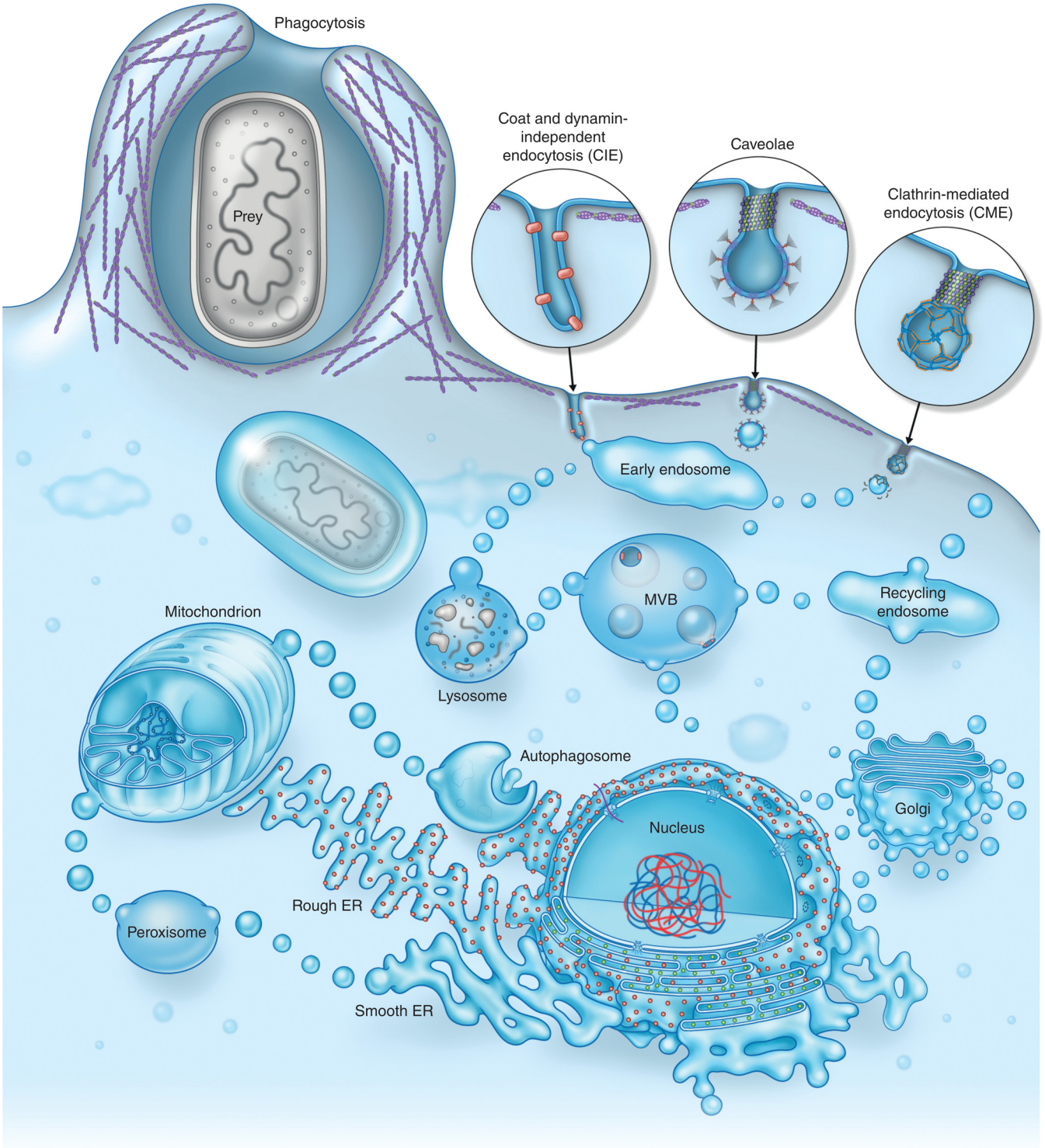

FIG 4 Endocytic processes to scale. There are many separate endocytic processes that can occur at the eukaryotic plasma membrane. Among all eukaryotic supergroups, three main types are found: phagocytosis, clathrin-independent endocytosis (CIE), and clathrin-mediated endocytosis, while caveolae are restricted to metazoans and appear to have evolved later in evolution. All of these processes have in common that they require an elaborate set of proteins, downstream processing through the endomembrane system that includes the early endosome and multivesicular bodies (MVB), and recycling of certain protein components and membranes back to the plasma membrane through vesicles. The different processes are shown roughly to scale for comparison at the plasma membrane. Clathrin-independent endocytosis, caveolae, and clathrin-mediated endocytosis are also enlarged to highlight a few details, such as the absence or presence of a coat. Both phagocytosis and clathrin-mediated endocytosis depend on dynamin for terminal membrane scission. Dynamin is likely of mitochondrial origin and absent from archaeal genomes.

triggering of a signaling pathway that induces the temporal and spatial reorganization of the actin cytoskeleton (216, 217), (iii) eversion of the plasma membrane and wrapping of the cargo, (iv) scission of the phagosomal vacuole from the plasma membrane through dynamin and depolymerization of the actin cytoskeleton (218, 219), (v) fusion with V-type ATPase-acidified lysosomal vesicles that are loaded with digestive enzymes to ultimately form the phagolysosome (220), and, finally, (vi) recycling of components to the plasma membrane and fusion with multivesicular bodies for further degradation (221). For the individual steps of phagocytosis to function, not only are hundreds of proteins required, a fully functional endomembrane system is required as well.

The endomembrane system is unique to eukaryotes. Dozens of attempts have been made to explain its origin. Both of the two most recent models rest on the premise that phagocytosing archaea do not exist. The inside-out model proposes a gradual increase of host complexity through actin- and tubulin-driven extrusions of the plasma membrane (a process similar to phagocytosis, we point out) and the formation of a protonucleus in the absence of endosymbionts (222). The other proposal has it that the endomembrane system, including the nucleus, originated from outer membrane vesicles (OMVs) that the bacterial endosymbiont was shedding from its outer membrane in the cytosol of the host (199). The secretion of OMVs by prokaryotes is very common; it is a part of their routine biology (223, 224). The inside-out model requires archaeal biology that has not been observed, while mitochondria have been observed to secrete vesicles (the mechanism homologous to OMV secretion) in both animals (63, 225) and plants (226). In contrast to the inside-out model, the OMV-based model for the origin of the endomembrane system accounts for (i) the functional homology of the ER and mitochondrial intermembrane space regarding Ca^{2+} storage (227–229) and the disulfide relay system (230, 231), (ii) the formation of the newly forming nucleus from the ER in cells with open mitosis (an issue regarding the inside-out model, in which the nuclear envelope is homologous to the plasma membrane of the archaeal host) (232–234), (iii) how bacterial lipids replaced archaeal lipids from the inside in the first place (11), and (iv) why eukaryotic lipid synthesis is now the joint work of the ER and mitochondria (235), as opposed to occurring at the plasma membrane as in all prokaryotes.

Empirical evidence for the origin of eukaryotic compartments from mitochondrion-derived vesicles comes from the recent observation that the *de novo* biogenesis of peroxisomes commences with the blebbing of the outer mitochondrial membrane induced through Pex3/Pex14, which fuse with endoplasmic reticulum-derived vesicles to mature (63). The OMV-based model (199) predicts that phagocytosing archaea do not exist, because phagocytosis depends on the endomembrane system, whose origin was a result of—not a prerequisite for—mitochondrial origin.

The processes and compartments involved in phagocytosis require structural proteins in abundance. Protein synthesis consumes 75% of a cell's energy budget, and the reason why eukaryotes have structural proteins in abundance is because they have mitochondria (93), which can pay the energetic price of the massive (over)expression of proteins that do not directly serve ATP synthesis, biosynthetic pathways, or cell division but serve other functions that have nothing to do with core metabolic substrate conversions (170). There are substantial differences in feeding habits across the recognized eukaryotic supergroups (Fig. 5), and entire phyla can thrive without phagocytosis-based feeding, with fungi being the best-known example.

Prevalence and Origin of Phagocytosis among Eukaryotic Supergroups

Most of what we know about membrane traffic and phagocytosis stems from work with the opisthokonts, the group that contains fungi and metazoa (including humans), but there, feeding modes differ substantially. In the fungi, there are no phagocytic forms, a trait that helps define the fungi as a group (89). Feeding in fungi can involve endocytic vesicles (236, 237) and is based on the secretion of digestive enzymes with the resorption of low-molecular-weight metabolites. Basal to the metazoans are the choanoflagellates. They are characterized by a peculiar collar that carries a flagellum,

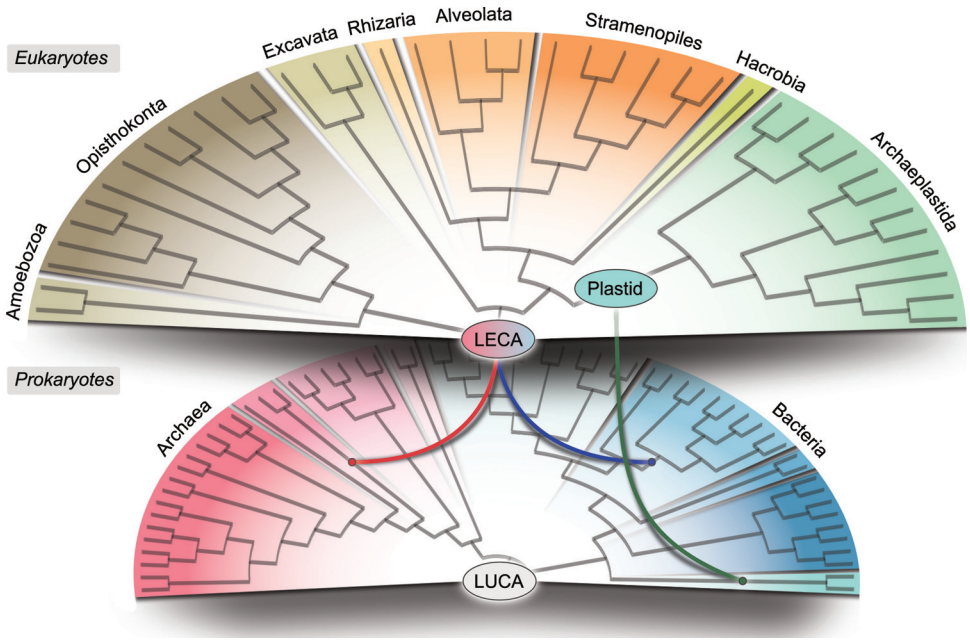

FIG 5 From prokaryotes to eukaryotes through endosymbiosis. Prokaryotic life belongs to either the bacterial or archaeal group. Prokaryotes are descended from the last universal common ancestor (LUCA) that marks the origin of life. Eukaryogenesis and the origin of the last eukaryotic common ancestor (LECA) hinge upon the endosymbiotic acquisition of a bacterial endosymbiont (the mitochondrion) by an archaeal host. The pivotal role of mitochondrial acquisition for the emergence of eukaryotes is evident through the existence of mitochondria in all eukaryotic supergroups (and, hence, the eukaryote common ancestor), among which no family that lacks mitochondria or mitochondrion-derived organelles is known. The Archaeplastida, uniting algae and plants, emerged after the endosymbiotic origin of the plastid from a cyanobacterium.

which generates a current to direct the bacterial prey to the feeding aperture, where it is phagocytosed (238). Among the metazoans, feeding typically occurs through the digestive tract, the epithelial cells of which resorb low-molecular-weight compounds generated by the combined actions of digestive enzymes and the end products of microbial breakdown by the intestinal flora (usually small volatile fatty acids) through pino- and endocytosis but not phagocytosis.

Among the Amoebozoa, uniting the Tubulinea and Mycetozoa, phagocytosis as a feeding habit is common and well studied in some species. Examples include the parasite *Entamoeba histolytica*, whose motile trophozoites phagocytose a wide range of different cells. The degree to which they phagocytose human cells is an indicator of pathogenesis and strain-specific virulence (239). Phagocytosis in *E. histolytica* is triggered by external stimuli such as ligands recognized by a galactose/*N*-acetylgalactosamine-specific lectin (240) and phosphatidyl serine exposure (241).

The Archaeplastida comprise the three lineages of photosynthetic eukaryotes (the glaucophytes, rhodophytes, and Chloroplastida; the latter include land plants) that can be traced back to the monophyletic acquisition of a cyanobacterium (242)—possibly through phagocytosis, possibly not—more than 1.2 billion years ago (51) and possibly 1.6 billion years ago (243). Among the Archaeplastida, there are a few very versatile heterotrophs, such as *Galdieria* (244), but the ability to phagocytose is utterly rare. It occurs in neither rhodophytes nor glaucophytes (two of the three archaeplastidal lineages) (242), and in the third lineage, the Chloroplastida, it occurs in only a few basal-branching species such as *Micromonas* (245) and *Cymbomonas* (246). Organisms such as the parasitic alga *Helicosporidium* (247), the free-living alga *Polytomella* (248), and apicomplexan parasites (249), all of which have lost the ability to photosynthesize, did not revert to phagocytosis for feeding. Endocytosis, however, is common and also occurs in plants (250), where it is regulated by osmotic stress and turgor pressure (251), using a canonical set of proteins, including those of the AP2 complex, clathrin, and dynamin for scission (252).

In the Excavata, feeding usually occurs via a specialized flagellated feeding groove that characterizes this group (89, 253). In some cases, such as in the parasite *Trichomonas*, the feeding groove has been secondarily lost. *Trichomonas* can, however, phagocytose independent of a feeding groove (254), for which the parasite also makes use of the actin cytoskeleton and a conserved set of more than a dozen accessory proteins (255).

In some of the nonexcavate taxa, phagocytosis is supported by an elaborate cytoskeletal structure known as the feeding groove or oral apparatus (e.g., the above-mentioned basal-branching chlorophyte algae and ciliates), which is maintained by a network of cytoskeletal proteins, mainly by a set of specifically arranged microtubules. It is hard to say whether these structures are truly homologous to the feeding groove of the Excavata. In some, but not all, recent phylogenetic analyses, excavates branch basally, close to the presumed root of eukaryotes (256, 257), which prompts us to speculate that the origin of phagocytosis might be associated with the origin of the excavate(-like) feeding groove.

Vesicle and Vacuole Function in Phagocytic Feeding

Theories that assume a role for some form of phagocytosis at the origin of mitochondria posit that the host was phagocytic for the purpose of feeding. That assumption entails several other corollary assumptions, both general and specific, that are rarely if ever spelled out. From a general perspective, when eukaryotes feed from internalized vesicles or food vacuoles, nutrients reach the cytosol via substrate importers present in the vesicle or vacuole membrane. For this, ingested food has to be digested by enzymes, which, in the case of food vacuoles, are activated by acidification through the vacuolar ATPase (V-ATPase) (258, 259). The eukaryotic V-ATPase is evolutionarily derived from the archaeal A-type ATPase, which was present in the host's plasma membrane. However, instead of synthesizing ATP from ADP and P_i via chemiosmotic gradients generated by coupling at the plasma membrane (its ancestral archaeal function in the host), the V-ATPase pumps protons into the food vacuole to acidify it. Clearly, in the ancestral eukaryote, this requires a cytosolic source of ATP that is independent of chemiosmosis at the plasma membrane; for phagocytosis-first theories, this would be meager amino acid fermentation (see Putting the Hypothetical Phagocytic Host to a Physiological Test, above), and for mitochondrion-first theories, this would naturally be the mitochondrial ATP supply (199).

Phagocytosis of the type that would feed a cell—the central, nonnegotiable premise of all phagotrophy-first theories for the origin of mitochondria—has a lot of moving parts (Fig. 4 and Table 1). First and foremost, there has to be 1:1 correspondence between the amount of membrane entering the cytosol and the amount exiting the cytosol (154); otherwise, the cell consumes its own plasma membrane, and the cytosol spills into the environment. In other words, before phagocytosis is possible as a feeding mechanism, the cell has to have a fully functional system of membrane flux with (i) vesicles incoming and outgoing, (ii) vesicles specifically addressed to their proper destination, (iii) the cytoskeletal components needed to guide them from point A to point B, and (iv) the energy required not only to run those processes but also to have evolved them (90). Tabulated for human cells, the membrane traffic and endomembrane system repertoire encompasses easily a hundred or more proteins, representing a dozen or more gene families (Table 1). For yeast and higher plants, which are not phagocytic, the endomembrane traffic system encompasses an equally comprehensive number of components (260–262).

At the cellular level, substrate uptake from the environment by endocytic mechanisms requires tight spatiotemporal regulation of numerous proteins, protein complexes, vesicular compartments, and the cytoskeleton (209, 212–220), the components of which can differ across eukaryotic lineages, with one report suggesting that phagocytosis even evolved several times independently in eukaryotes (6). The list of essential phagocytosis components is still growing in some lineages (263). Like mitochondria, phagocytosis occurs among free-living forms in all eukaryotic supergroups, but in

TABLE 1 Comparison of various endocytic and phagocytic processes in eukaryotes[a]

Type of process	Morphology	Size (nm)	Cargo	Major components involved	Estimated no. of components	References
Clathrin-dependent endocytosis	Vesicular	~30–200	Transferrin, EGF, LDL, Semliki Forest virus, etc.	Clathrin, FCHO, AP2, intersectin, AP180, CALM, epsin, amphiphysin, dynamin, SNX9, endophilin, respective cargo receptors, etc.	~95	200, 333, 345
Caveola-dependent endocytosis	Vesicular	~60–80	Cholera toxin B subunit, SV40 virions, GPI-linked proteins, integrins, albumin, glycosphingolipids, etc.	Caveolins (e.g., Cav1/VIP21), PTRF/cavin-1, SDR/cavin-2, SRBC/cavin-3, MURC/cavin-4, dynamin, Src, Rab5, etc.	~85	335, 336
Coat- and dynamin-independent endocytic pathways	Vesicular/tubular	~100	Shiga toxin, GPI-anchored proteins, Glut1, CD98, Lat1, MHC class I, CD44, CD59, etc.	GBF1, Arf1, ARHGAP10/21, Cdc42, actin, GRAF1, Arf6, flotillins, Rac1, etc.	~78	337, 334
Pinocytosis	Vesicular	~100	Fluid-phase uptake, membrane receptors, etc.	Actin, Ras proteins, small GTPase Rac proteins, Rab proteins, PI 3-kinases, etc.	~79	212, 338, 339, 340
Phagocytosis	Shaped by cargo	~500–9,000	Apoptotic cells and vesicles, environmental debris, prey cells, diverse particulate targets, etc.	Actin, dynamin, IQGAP1, formin Dia1, Amphiphysin1, small GTPases (different families), Rho kinases, SWAP70 V-ATPase, NADPH oxidase and its activating factors, VPS34, EEA1, respective cargo receptors, etc.	~127	200, 212, 263, 340, 341, 342, 343

[a]The estimated number of components includes proteins involved in endosomal processing (early/late and recycling endosomes) and phagosomal degradation available from the literature and the KEGG database (344). EGF, epidermal growth factor; LDL, low-density lipoprotein; SV40, simian virus 40; GPI, glycosylphosphatidylinositol; MHC, major histocompatibility complex; PI, phosphatidylinositol.

contrast to mitochondria, it is not essential for cell function in free-living eukaryotic cells.

New Archaeal Genomes and Inferring Phenotype from Single Genes

In recent metagenomic studies, the additional presence of a few proteins such as archaeal cell division (Cdv) proteins, which are related to some of the eukaryotic endomembrane system components (ESCRT proteins), or the presence of crenactin and gelsolin has been interpreted as evidence for the existence of complex or primitively phagocytosing archaea (3, 9, 264). This is a curious development. The expectation that a phagocytosing host for the origin of mitochondria must be out there makes the inference of phagocytosis from an archaeal cell division protein somehow palatable. With metagenomic data being interpreted as evidence documenting the existence of phagocytosing archaea that possess eukaryote-like membrane-trafficking processes (9), it is important to see what the new archaeal metagenome data actually say.

The metagenome assemblies (3, 4) report (i) a large family of small GTPases, many with homology to the Ras superfamily; (ii) crenactin and gelsolin domain-containing proteins; (iii) an extended suite of archaeal Cdv proteins; which are related to eukaryotic ESCRT proteins; and (iv) some proteins with homology to eukaryotic proteins associated with vesicle formation and trafficking that harbor longin, BAR/IMD (Bin/amphiphysin/Rvs/IRSp53 and MIM [missing in metastases] homology domain), or MON1 (monensin sensitivity) domains or that are Sec23/24-like. Due to a lack of enriched cultures, it is still not yet clear whether these metagenomic proteins and gene families all belong to one organism or several. Some of the reported protein family distributions are patchy among the Asgard superphylum, which complicates speculations regarding their overall function (265). More importantly, these few proteins and domains fall short of the long list of components that are required to underpin endomembrane flux and phagocytosis (118, 119) (Table 1).

What do the small GTPases indicate? GTPases of the Rab family are important components of eukaryotic vesicle trafficking through mediating membrane, and, hence, also vesicle, identity (266). The Asgard phylum was reported to encode an extended family of small GTPases (4). It is currently not possible to know how many small GTPases a single archaeal genome encodes, but quantity is perhaps not the main issue. One of the first studies analyzing lokiarchaean data concluded that in terms of domains defined at the fold superfamily level, *Lokiarchaea* were no more special than other microbial dark matter, including giant viruses (267). Small GTPases are not unique to these archaea, nor do they encode any membrane association signals or regions (such as prenylatable C-terminal cysteine residues) conserved among eukaryotic Rabs and essential for their function (119). The archaeal proteins are likely not membrane-bound Rabs but cytosolic small GTPases (119). GTPase-activating proteins and exchange factors are also absent from the *Lokiarchaeota*, as are domain fusions characteristic of proteins associated with eukaryotic membrane trafficking (118). Without membrane-bound small GTPases plus GTPase-activating proteins and exchange factors, it is hardly possible that the organisms behind the metagenome data have signaling and membrane flux pathways approaching those of eukaryotes.

Then come crenactin and gelsolin domains. The presence of genes encoding crenactin and gelsolin domains prompted speculation about a "dynamic" actin cytoskeleton in the *Lokiarchaea* or the ancestor of the last eukaryotic common ancestor (LECA) host cell more generally (3, 9). Crenactin has been shown to associate with morphology-determining structures in *Pyrobaculum*, and its phylogenetic distribution correlates to a degree with rod-shaped and filamentous cell morphologies (268). While the bacterial homologue, MreB, has been discussed as being associated with morphology-determining structures in bacteria (269), some results have been challenged. MreB-based filaments observed in *E. coli* were shown to be an artifact caused by the yellow fluorescent protein tag (270), and electron cryotomography did not find any evidence for long helical filaments in five additional prokaryotes analyzed (271). Prokaryotic actin homologues appear to associate with morphology-determining struc-

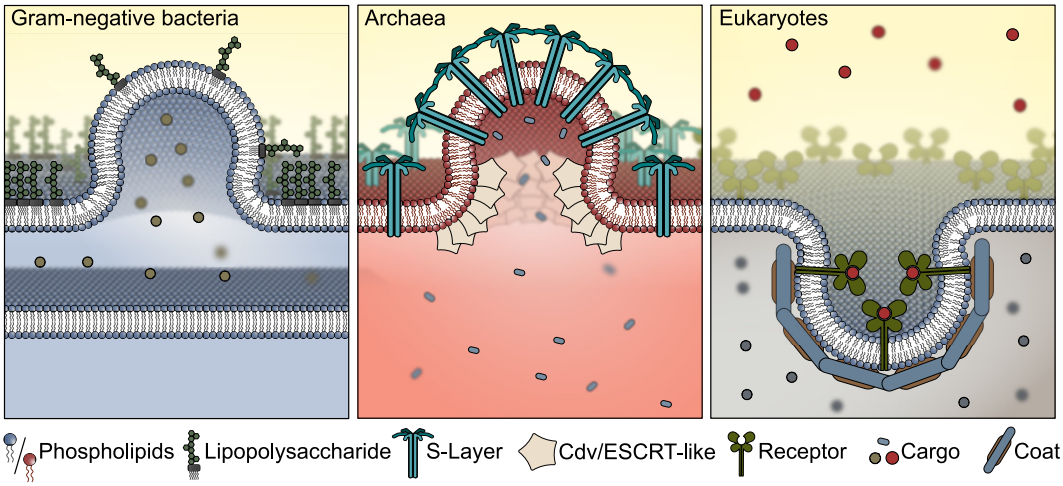

FIG 6 The in and out of vesicles. Vesicles form at both prokaryotic and eukaryotic plasma membranes. There are, however, crucial differences. Prokaryotic vesicles are secreted into the environment only through blebbing of the plasma membrane away from the cytosol. These types of vesicles are known as OMVs, and they are found among all types of prokaryotes. Archaeal OMVs are also released with the help of proteins of the CDV family (cell division) that are homologous to eukaryotic ESCRT proteins. The topology in the function of the ESCRT (endosomal sorting complex required for transport) machinery in eukaryotes has been conserved, as the eukaryotic ESCRT machinery, like the archaeal CDV machinery, works on membranes that are bending away from the cytosol. Eukaryotic vesicles can be secreted into the environment (exosomes) or into the cytosol through endocytic mechanisms. Endocytic vesicle maturation often requires the formation of a complex coat that is usually composed of a combination of membrane proteins and peripheral proteins that interact with each other (Table 1). Endocytic mechanisms are receptor mediated, like, for example, in the case of iron uptake from the environment by the transferrin receptor that is internalized through the formation of clathrin-coated vesicles.

tures, but they are more stiff than dynamic, and those analyzed so far form single- but not double-stranded filaments that are characteristic of eukaryotic actin (272). The questions of whether potential crenactin-based structures in the *Lokiarchaea* or the Asgard archaea are dynamic (3, 9) and whether they even exist remain very open, recalling that no one has ever seen a cell of this new archaeal phylum.

Some might consider the presence of a gelsolin-like protein an indicator of eukaryote complexity, but actin dynamics such as those associated with eukaryotic phagocytosis require dozens of proteins, not just gelsolin, and the interplay of large parts of the endomembrane system (209, 212–220, 255, 273). Furthermore, the functions of gelsolin are by no means limited to actin binding. They include signal transduction (274) and, intriguingly, the stabilization of mitochondria and the inhibition of apoptosis through the suppression of cytochrome *c* secretion (275). A gelsolin-like protein in the archaeal host could have just as easily stabilized the early mitochondrial endosymbiont rather that enabling its uptake through phagocytosis.

Archaea divide with the help of Cdv proteins (276, 277), of which CdvB and CdvC are homologous to proteins of the eukaryotic ESCRT III complex (278). What is special about the Asgard data is the presence of proteins homologous to components of all three ESCRT complexes I to III. In eukaryotes, ESCRT III complexes carry out the scission of many different membranes. Importantly, ESCRT complexes in eukaryotes excise membranes that bend away from the cytosol (279, 280), and membrane topology here matters (Fig. 6). At the eukaryotic plasma membrane, ESCRTs aid in neuron pruning, wound repair, and vesicle scission into the environment, with the latter being a process that HIV particles highjack (279). ESCRT complexes have not been reported to be involved in the scission of any incoming endosomal or phagosomal membranes. In this light, it is therefore all the more surprising that the presence of proteins in metagenomic data with sequence homology to eukaryotic ESCRT proteins was interpreted as evidence for the plausibility of phagocytic archaea.

In eukaryotes, the scission of the phagocytic cavity, which bends inward toward the cytosol (Fig. 4 and 6), is the job carried out by large GTPases of the dynamin family (219, 281). These large GTPases have not been identified from data available for the *Lokiar-*

chaeota and relatives; the eukaryotic proteins are bacterium derived and likely of mitochondrial origin (282). While scission can also be mediated by the actin machinery (although not through actin alone), it requires the active protrusion of the membrane through an extracellular force such as the Shiga toxin that forms a tubule (283).

What about the remaining proteins identified with homology to components of the eukaryotic endomembrane system and vesicle trafficking machinery? The presence of genes encoding BAR-, longin-, TRAPP-, or Sec23/24-like domains in the new archaeal lineages is intriguing. Zaremba-Niedzwiedzka et al. (4) speculate that these genes might support some kind of vesicle trafficking as it occurs in eukaryotes, namely, in the cytosol. Those authors infer that Asgard archaea might "have (had) the ability to bend membranes and to form and transport internal vesicles, albeit at a much more primitive level than observed in modern eukaryotes," furthermore concluding that the increase in eukaryotic complexity depended on the presence of the mitochondrial endosymbiont (4). Those authors also mention that some archaea such as *Ignicoccus hospitalis* are surrounded by two membranes. Intriguingly, *I. hospitalis* indeed forms vesicles, but the vesicles are not found in the cytosol; they instead occur between the two membranes that surround the cytosol (284). This indicates that the *I. hospitalis* vesicles reflect typical archaeal (outward) (Fig. 6) OMV secretion and not inwardly directed membrane flux as in eukaryotes. Hence, if archaeal proteins with the above-mentioned domains really are associated with vesicle formation, the simplest interpretation is that they are associated with the formation of outwardly secreted vesicles, which are observed in archaea (223, 285). In the case of *Ignicoccus*, a simple interpretation is that OMVs are involved in the biogenesis and origin of its additional outer membrane, which similarly could apply to the lipopolysaccharide layer of Gram-negative bacteria (sometimes called diderm prokaryotes [286]) as well. In any case, the eukaryotic homologues of these identified archaeal domains are not linked to the recognition of cellular cargo and the formation of a phagocytic cavity and hence lend no support regarding the phagocytotic acquisition of the mitochondrion.

The finding that the new archaeal lineages encode additional proteins or domains previously considered unique to eukaryotes is important, but the pitfalls of extrapolation from a few metagenomic genes to a biological trait as complex as phagocytosis remain. This can be illustrated with the example of tubulins. Eukaryotic tubulins are the building blocks of microtubules, whose functions are manifold and include providing the tracks along which cellular compartments migrate (287, 288) as well as forming the spindle apparatus required for chromosome segregation during mitosis and meiosis (289, 290). Does the presence of FtsZ, the bacterial homologue of tubulin, provide evidence for microtubule-dependent chromosome division in bacteria or the presence of prokaryotic 9+2 flagella? Hardly. What is more, FtsZ is part of the Z ring that mediates prokaryotic cell division, while eukaryotes make no use of tubulins for this purpose but make use of the actin-myosin machinery instead (291). Inferring phenotype from genotype is a problematic undertaking (292), especially across the prokaryote-to-eukaryote divide. Metagenomic interpretations about the host (3, 4) have been guided by the expectation that an archezoan—a phagocytosing host aspiring to acquire mitochondria—is out there to be discovered.

GETTING ENDOSYMBIONTS INSIDE: ALTERNATIVES TO PHAGOCYTOSIS

From the standpoint of comparative genomics, the first eukaryotic cell was a merger of two prokaryotes, an alphaproteobacterium and an archaeon, that became the mitochondrion and its archaeal host (97, 98, 102, 105). The concept of primitively phagocytosing archaea is problematic from the standpoint of physiology (energy metabolism and ATP synthesis) and as it concerns the nature of proteins and vesicles required for phagocytosis to operate. This forces the issue of mechanisms. If the mitochondrion was not acquired through phagocytosis, how could the mitochondrial ancestor have ended up as an endosymbiont in the cytosol of its archaeal host?

There are examples of prokaryotes that reside in other prokaryotes (Fig. 7A to D). They are rare, but at least they have been observed in nature, in contrast to archezoa

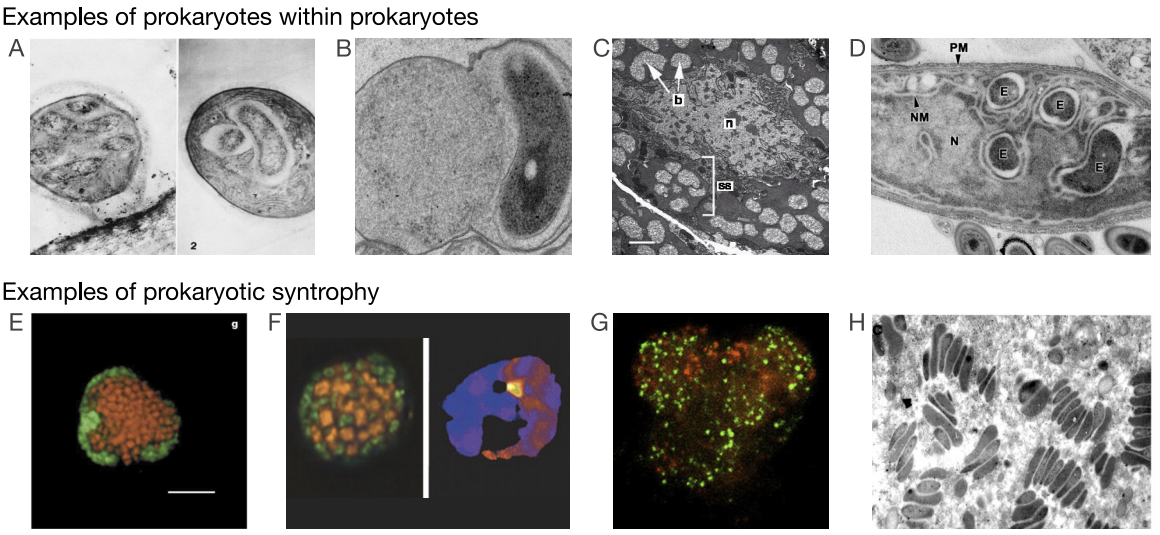

FIG 7 Prokaryotic partnerships: alternatives to phagocytosis. There are many examples of syntrophic interactions among prokaryotes and a few rare cases of one prokaryote residing within another. (A) Transmission electron micrographs of longitudinal sections of *Pleurocapsa minor* showing intracellular bacteria. (Reproduced from reference 302 with permission of John Wiley and Sons.) (B) Infection of the *Pseudomonas fluorescens* periplasm by *Bdellovibrio bacteriovorus*. (Courtesy of Edouard Jurkevitch; reproduced with permission.) (C) Transmission electron micrograph showing nested, multilayer endosymbiosis inside the bacteriome of a mealybug (Pseudococcidae). Bacteriomes carry betaproteobacterial *Trembleya* endosymbionts, which themselves carry gammaproteobacterial *Morganella* endosymbionts. b, bacteria; n, nucleus; ss, symbiotic sphere. (Reproduced from reference 309 by permission from Macmillan Publishers Ltd.) (D) Transmission electron micrograph of *Parakaryon myojinensis* harboring endosymbionts of an unknown nature (marked with "E"). NM, nuclear membrane; PM, plasma membrane; N, nucleus. (Reproduced from reference 310 with permission [copyright the author 2012; published by Oxford University Press {on behalf of Japanese Society of Microscopy}].) (E) Confocal laser scanning micrograph of *in situ* hybridization of bacteria (green) and archaea (red). (Reproduced from reference 316 by permission from Macmillan Publishers Ltd.) (F) Confocal images of consortia of bacteria (green) and archaea (orange). The picture on the right side is a false-color image that highlights the nitrogen-fixing properties of archaeal cells. (Reproduced from reference 317 with permission from AAAS.) (G) Fluorescence *in situ* hybridization of HotSeep-1 bacteria (green) that receive reducing equivalents from their archaeal partner (red). (Reproduced from reference 126 by permission from Macmillan Publishers Ltd.) (H) Stacks of electron-dense hydrogenosomes (darker) in the cytosol of the ciliate *Plagiopyla frontata* sandwiched between methanogens (lighter gray structures). (Reproduced from reference 318 with permission of Springer.)

or phagocytosing archaea, for which there are no observable examples. Rarity is furthermore a desirable property of endosymbiosis, because mitochondria arose only once in 4 billion years, roughly the same rate at which life and the solar system arose. Bacterial endosymbionts in the cytosol of phagocytosing eukaryotic cells are nothing unusual; on the contrary, they are extremely common. Examples include the many known cases of proteobacterial endosymbionts of insects (293–295), the methanogenic endosymbionts of anaerobic ciliates (296), the purple endosymbionts of the ciliate *Strombidium* (297), the sulfur-metabolizing symbionts of clam gills (298), the chemosynthetic endosymbiont consortia of gutless tubeworms (299), endosymbionts that live within the endoplasmic reticulum of diatoms (300), or the cyanobacterial endosymbionts of sponges (301), to name just a few. The commonplace occurrence of bacterial endosymbionts in phagocytic cells stands in diametric contrast to the very rare origin of mitochondria (a singular event among the ancestors of all microbes that have left known descendants in 4 billion years of evolution). Thus, we can safely say that phagocytosis promotes the frequency with which endosymbionts can come to reside within the eukaryotic cytosol, but it has no bearing whatsoever on the rate at which mitochondria arise from endosymbionts. This is one more (strong) reason why phagocytosis is unlikely to have anything to do with mitochondrial origin.

One of the earliest-documented examples of a prokaryote harboring a prokaryotic endosymbiont was that of rod-shaped structures characteristic of bacteria observed within the cyanobacterium *Pleurocapsa minor* (Fig. 7A) by Wujek (302). He also noted that the 1.5-μm- by 0.2-μm-long bacteria were not surrounded by an extra membrane. The identity of these intracellular bacteria remains unknown.

Another example is that of the predatory bacteria of the *Bdellovibrio* family (Fig. 7B).

These bacteria cross a single membrane and invade the periplasm of other Gram-negative bacteria, where they are known to proliferate (303). Since their discovery in 1963 (304), the infection mechanism of *Bdellovibrio* and relatives has been in the focus of research, as they offer a rare example of prokaryotes infecting other prokaryotes (303, 305, 306). It has also been proposed that a *Bdellovibrio*-like alphaproteobacterium could have been the ancestor of the mitochondrion (307, 308).

Examples of prokaryotes residing within other prokaryotes are also found among the bacterial endosymbionts of insects. In mealybugs, a betaproteobacterial bacterium lives within a specialized organ of the insect called the bacteriome (294, 309). The betaproteobacterium harbors its own endosymbiont, a gammaproteobacterium (Fig. 7C). Notably, it was recently shown that the gammaproteobacterial symbiont living within the betaproteobacterium has been replaced on five different occasions during evolution (295).

The deep-sea microbe "*Parakaryon myojinensis*" presents another special case (310). The host cell has as an ~50-nm-thick cell wall, a single membrane-bound nucleoid that contains naked DNA, and endosymbionts with a hydrogenosome-like appearance (Fig. 7D). It was speculated that this organism might represent an intermediate between a prokaryote-like and a eukaryote-like cell (310). While the exact nature of the organism in the reported images (310) remains to be elucidated, it might present another example of a prokaryote residing inside another prokaryote.

There are also bacteria that invade mitochondria (311) and plastids (300) of eukaryotes, whereby neither mitochondria nor plastids are able to phagocytose, providing additional albeit derived examples of prokaryotes living within other prokaryotes. The mechanisms that mediate the process during which one prokaryote enters another remain unknown. Similarly, it is not known whether there are bacteria that live within modern archaea. At eukaryote origin, a bacterium came to reside within an archaeal host. Phagocytosing prokaryotes are intellectual constructs, while the phagocytosis-independent residence of one prokaryote within another occurs in nature (Fig. 7A to D).

Some archaea have the ability to fuse. In this process, which occurs in both crenarchaeotes and euryarchaeotes, the walls and plasma membrane of two adjacent cells merge to surround one cytoplasm containing the genetic material of the two starting individuals. Following fusion and subsequent divisions, new combinations of genes can ensue (312–315). Archaeal fusion is strikingly similar in its basic biology to gamete fusion in eukaryotes (170).

In syntrophic consortia, bacterial and archaeal cells are intimately connected; several examples of such close interactions are known. Syntrophic aggregates of methane-oxidizing archaea and sulfate-reducing bacteria are common in deep-sea sediments and have recently been shown to be crucial for marine carbon and nitrogen cycling (Fig. 7E and F) (316, 317). One such consortium of methanogens and sulfate reducers was shown to be capable of alkane oxidations, extending the metabolic capabilities of archaeal/bacterial syntrophy (Fig. 7G) (126). Syntrophic interactions even occur in the cytosol of eukaryotic cells: in the anaerobic ciliate *Plagiopyla frontata*, the H_2-producing hydrogenosomes are usually decorated with H_2-dependent methanogenic endosymbionts (Fig. 7H) (318). The only model of host-endosymbiont interactions at mitochondrial origin that accounts for the presence of anaerobic physiology in mitochondria and hydrogenosomes is based on such anaerobic syntrophic interactions (90).

If modern archaea can fuse, ancient ones might have been able to as well, and there is the possibility that fusion could lead to the entrapment or inclusion of a foreign cell so as to render a syntrophic partner a surrounded endosymbiont. If we knew of factors that promote archaeal fusion, it might even be possible to induce such events in the laboratory.

Of course, microbial cells snuggling up to one another should not evoke the impression that they are only one step away from one getting inside and becoming a mitochondrion. The endosymbiosis that gave rise to eukaryotes was rare. How rare? Whitman et al. (319) estimated that roughly 10^{30} prokaryotic cells exist on Earth today. If we are granted a simplifying assumption, namely, that the environment has harbored

roughly the same number of cells over the last 2 billion years, and furthermore granted a pure guess that an average cell has a doubling time of about 2 months in nature (some are slower, and some are faster [27]), we obtain a rough but round estimate of about 10^{40} prokaryotic cells that have lived in the last 2 billion years. Most or all of them had a partner from the other domain nearby. This represents a very large number of opportunities to create eukaryotes, opportunities where nothing other than metabolic interactions and occasional interdomain gene transfer (127, 320) ever happened, except once during a fateful encounter at eukaryote origin. Eukaryote origin was a very rare event.

Association of Host and Symbiont: Syntrophy and Gene Transfer

The new archaeal lineages that, by the measure of metagenomic ribosomal protein concatenation, are more closely related to the host than other previously known archaea live in anaerobic marine sediments. The microbial communities in anaerobic marine sediments have been studied to some degree. They harbor widespread syntrophic interactions involving interspecies hydrogen transfer (27, 29–31, 321). A characteristic of syntrophic hydrogen-producing bacteria is the trimeric confurcating Fe-Fe hydrogenase (31), which contains the 24- and 51-kDa subunits of complex I (NuoE and NuoF) in addition to the catalytic subunit harboring the H cluster (160). These additional subunits permit H_2-producing organisms to reoxidize NADH with the help of reduced ferredoxin via electron confurcation (160), a special case of electron bifurcation (137). The confurcating Fe-Fe hydrogenase is used because the midpoint potential of NADH ($E_0' = -320$ mV) is not sufficiently negative to generate H_2 ($E_0' = -420$ mV) from NADH under physiological conditions (160). If one electron is donated to Fe-Fe hydrogenase from NADH and one is donated from low-potential reduced ferredoxin ($E_0' = -453$ mV), the overall reaction can become exergonic under physiological conditions (31, 160), allowing organisms to reoxidize NADH using protons as the terminal acceptor. Eukaryotes that express the long form of Fe-Fe hydrogenases typically possess the NuoE (24-kDa) and NuoF (51-kDa) subunits of the trimeric confurcating hydrogenase (19–21, 120, 322).

The findings that the trimeric confurcating Fe-Fe hydrogenase is typical of syntrophic hydrogen production in bacteria (29) and that a trimeric confurcating Fe-Fe hydrogenase of alphaproteobacterial ancestry (121) occurs in mitochondria and in the common ancestor of eukaryotes (19, 120) point to H_2 production in the ancestral mitochondrion and H_2-based syntrophic interactions (90), not failed phagocytosis, as the ecological basis of mitochondrial origin. Two other sets of findings from phylogeny and physiology bear on eukaryote origin and point in the same direction.

The first set of findings concerns the physiology of the host. Methanogens are the main H_2-dependent syntrophic partners when it comes to H_2-based syntrophic interactions. The new ribosomal protein-based trees of life have methanogens branching basally among archaea; hence, all other archaea ultimately appear to be derived phylogenetically from methanogens (107, 111, 323), which means in turn that all nonmethanogenic archaea are physiologically derived from methanogens (26). Indeed, the new lineages of archaea that are being uncovered in marine sediments all possess basic components of methanogenesis, such as the archaeal version of the acetyl-CoA pathway of methyl synthesis, heterodisulfide reductase, methyl coenzyme M (methyl-CoM) reductase, and genes involved in the synthesis of the cofactors specific for these pathways (123, 125, 324). This places methanogenesis (H_2-dependent chemolithoautotrophy) at the base of the host lineage, regardless of where, specifically, it branches within the archaea, in line with the predictions of models entailing anaerobic syntrophy for mitochondrion-host interactions at eukaryote origin (19, 90).

The second set of findings concerns the physiology of eukaryotes. Not only are eukaryotes ancestrally mitochondrion bearing, they are also ancestrally facultatively anaerobic heterotrophs (19, 90). In light of the antiquity of methanogenesis (325) and the new methanogenic root of archaea, this means that an H_2-dependent chemoautotroph underwent the physiological transition to facultatively anaerobic heterotrophy

in the eukaryotic lineage. Is this likely, and is it even possible? The example of the haloarchaea is very instructive. Haloarchaea are facultative anaerobic heterotrophs that have always branched within methanogens in ribosomal and rRNA trees. They have a cytochrome- and menaquinone-dependent respiratory chain consisting of complexes I, II, II, and IV in their archaeal plasma membrane (127). The haloarchaea acquired their O_2-respiring respiratory chain, in addition to 1,000 other genes, including those underpinning menaquinone synthesis, from bacteria (127). A single subunit of a respiratory chain is useless if acquired by itself as a result of single-gene transfer; this indicates that a single event of mass gene transfer underpinned the origin of the haloarchaeal respiratory chain.

Such large-scale gene transfer events with "quantum" physiological transformation are not unprecedented in prokaryote evolution: they happened with photosynthesis in six different prokaryotic lineages (326), with each transfer of the photosynthetic machinery entailing the transfer of up to 100 genes or more for photosystem biogenesis, chlorophyll, and carotenoids (327) and with each event founding a new photosynthetic lineage. The acquisition of bacterial genes also corresponds to the origin of several archaeal lineages (127). Mass gene transfer, but involving more than 1,000 genes, also occurred during the acquisition of photosynthesis by eukaryotes via plastids at the origin of the Archaeplastida lineage (108). Thus, haloarchaea underwent the same kind of physiological transformation as syntrophic models for the origin of mitochondria predict, the main difference being that haloarchaea express their respiratory chain in their archaeal plasma membrane, while eukaryotes express it in the mitochondrial inner membrane (127). The mitochondrial configuration of bioenergetic membranes is key to eukaryote cell complexity (93), because it frees the eukaryotic cell from the constraints imposed by chemiosmotic coupling at the plasma membrane. Mitochondria thus freed the incipient eukaryotic cell to use its plasma membrane for other purposes such as endocytosis (199). A mass acquisition of bacterial genes also corresponds to the origin of major archaeal clades (320) and to the origin of the mitochondrion in the eukaryotic lineage itself (100, 102).

There have been some distinct discontinuities in prokaryotic evolution, and many of them have to do with bacterial-to-archaeal gene transfers. Why from bacteria to archaea? If archaea started out as methanogens (107, 325, 328), they ancestrally lacked cytochromes and quinone-based respiratory chains. Cytochrome- and quinone-dependent respiratory chains are very prevalent among bacteria and were apparently invented by bacteria. Like photosynthesis (326), they are very useful if transferred but not generally easy to donate in a fully functional form because of the number of genes for subunits and cofactor biosynthesis involved. Physiology like that of mitochondria would not have easily been transferred to eukaryotes one gene at a time, because one subunit of complex I, for example, is useless and will be lost (127). For such a dramatic change in physiology as the common ancestor of eukaryotes underwent, gene transfer in large chunks harbors far greater utility and probability of successful physiological transformation than single-gene transfers. The greatest discontinuity in prokaryotic evolution was clearly the one that gave rise to eukaryotes; the main evolutionary mechanisms involved were endosymbiosis and gene transfer during endosymbiosis, it would seem.

CONCLUSION

For as long as the endosymbiont hypothesis has been around, so has the notion that the host that acquired the mitochondrion was a phagocytosing cell. Here we have explored the bioenergetic implications of that assumption. We found that an archaeon would have no benefit from phagotrophy; on the contrary, its bioenergetic situation would deteriorate for the consumption of its bioenergetic membrane. Phagotrophy is a highly complex process that requires the coordinated interactions of hundreds of different proteins and dozens of protein families. Moreover, it requires outward and inward membrane flux within a preexisting endomembrane system that can direct food particles to digestion and recycle proteins and membrane back to the plasma mem-

brane. A fully functional endomembrane system was thus a prerequisite for the origin of phagocytosis, even primitive forms thereof. Because the evolution of cellular complexity comes at an energetic price, mitochondria were involved in the origins of the many eukaryote-specific protein families that underpin both vesicle flux and phagocytosis. Furthermore, newer findings implicate bacterial outer membrane vesicles and mitochondrion-derived vesicles in the origin of the eukaryotic endomembrane itself. For a cell that had already evolved endomembrane flux for the transport of food particles to the digestive vacuole and that already possessed internalized bioenergetic membranes in mitochondria for the breakdown of an ingested substrate (with or without oxygen as the terminal acceptor), phagocytosis could be useful, but not superior to extracellular digestion and endosomal feeding, as the example of two very successful eukaryotic lineages, fungi and animals, underscores.

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REFERENCES

1. Jorgensen SL, Hannisdal B, Lanzén A, Baumberger T, Flesland K, Fonseca R, Øvreås L, Steen IH, Thorseth IH, Pedersen RB, Schleper C. 2012. Correlating microbial community profiles with geochemical data in highly stratified sediments from the Arctic Mid-Ocean Ridge. Proc Natl Acad Sci U S A 109:E2846–E2855. https://doi.org/10.1073/pnas.1207574109.
2. Jorgensen SL, Thorseth IH, Pedersen RB, Baumberger T, Schleper C. 2013. Quantitative and phylogenetic study of the deep sea archaeal group in sediments of the Arctic mid-ocean spreading ridge. Front Microbiol 4:299. https://doi.org/10.3389/fmicb.2013.00299.
3. Spang A, Saw JH, Jørgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, Schleper C, Guy L, Ettema TJG. 2015. Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521:173–179. https://doi.org/10.1038/nature14447.
4. Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Bäckström D, Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU, Stott MB, Nunoura T, Banfield JF, Schramm A, Baker BJ, Spang A, Ettema TJG. 2017. Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature 541:353–358. https://doi.org/10.1038/nature21031.
5. Martijn J, Ettema TJG. 2013. From archaeon to eukaryote: the evolutionary dark ages of the eukaryotic cell. Biochem Soc Trans 41:451–457. https://doi.org/10.1042/BST20120292.
6. Yutin N, Wolf MY, Wolf YI, Koonin EV. 2009. The origins of phagocytosis and eukaryogenesis. Biol Direct 4:9. https://doi.org/10.1186/1745-6150-4-9.
7. Koonin EV, Yutin N. 2014. The dispersed archaeal eukaryome and the complex archaeal ancestor of eukaryotes. Cold Spring Harb Perspect Biol 6:a016188. https://doi.org/10.1101/cshperspect.a016188.
8. Guy L, Ettema TJG. 2011. The archaeal "TACK" superphylum and the origin of eukaryotes. Trends Microbiol 19:580–587. https://doi.org/10.1016/j.tim.2011.09.002.
9. Koonin EV. 2015. Origin of eukaryotes from within archaea, archaeal eukaryome and bursts of gene gain: eukaryogenesis just made easier? Philos Trans R Soc Lond B Biol Sci 370:20140333. https://doi.org/10.1098/rstb.2014.0333.
10. Embley TM, Williams TA. 2015. Evolution: steps on the road to eukaryotes. Nature 521:169–170. https://doi.org/10.1038/nature14522.
11. López-García P, Moreira D. 2015. Open questions on the origin of eukaryotes. Trends Ecol Evol 30:697–708. https://doi.org/10.1016/j.tree.2015.09.005.
12. Rout MP, Field MC. 3 May 2017. The evolution of organellar coat complexes and organization of the eukaryotic cell. Annu Rev Biochem https://doi.org/10.1146/annurev-biochem-061516-044643.
13. Pittis AA, Gabaldón T. 2016. Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry. Nature 531:101–104. https://doi.org/10.1038/nature16941.
14. McCutcheon JP. 2016. From microbiology to cell biology: when an intracellular bacterium becomes part of its host cell. Curr Opin Cell Biol 41:132–136. https://doi.org/10.1016/j.ceb.2016.05.008.
15. Dacks JB, Field MC, Buick R, Eme L, Gribaldo S, Roger AJ, Brochier-Armanet C, Devos DP. 2016. The changing view of eukaryogenesis—fossils, cells, lineages and how they all come together. J Cell Sci 129:3695–3703. https://doi.org/10.1242/jcs.178566.
16. Cavalier-Smith T. 1987. The origin of eukaryote and archaebacterial cells. Ann N Y Acad Sci 503:17–54. https://doi.org/10.1111/j.1749-6632.1987.tb40596.x.
17. Embley TM, Hirt RP. 1998. Early branching eukaryotes? Curr Opin Genet Dev 8:624–629. https://doi.org/10.1016/S0959-437X(98)80029-4.
18. Embley TM, Martin W. 2006. Eukaryotic evolution, changes and challenges. Nature 440:623–630. https://doi.org/10.1038/nature04546.
19. Müller M, Mentel M, van Hellemond JJ, Henze K, Woehle C, Gould SB, Yu R-Y, Van der Giezen M, Tielens AGM, Martin WF. 2012. Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol Mol Biol Rev 76:444–495. https://doi.org/10.1128/MMBR.05024-11.
20. Atteia A, van Lis R, Tielens AGM, Martin WF. 2013. Anaerobic energy metabolism in unicellular photosynthetic eukaryotes. Biochim Biophys Acta 1827:210–223. https://doi.org/10.1016/j.bbabio.2012.08.002.
21. Stairs CW, Leger MM, Roger AJ. 2015. Diversity and origins of anaerobic metabolism in mitochondria and related organelles. Philos Trans R Soc Lond B Biol Sci 370:20140326. https://doi.org/10.1098/rstb.2014.0326.
22. Tovar J, Fischer A, Clark CG. 1999. The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. Mol Microbiol 32:1013–1021. https://doi.org/10.1046/j.1365-2958.1999.01414.x.
23. Van der Giezen M. 2009. Hydrogenosomes and mitosomes: conservation and evolution of functions. J Eukaryot Microbiol 56:221–231. https://doi.org/10.1111/j.1550-7408.2009.00407.x.
24. Embley TM, Van der Giezen M, Horner DS, Dyal PL, Foster P. 2003. Mitochondria and hydrogenosomes are two forms of the same fundamental organelle. Philos Trans R Soc Lond B Biol Sci 358:191–201. https://doi.org/10.1098/rstb.2002.1190.
25. Sagan L. 1967. On the origin of mitosing cells. J Theor Biol 14:255–274. https://doi.org/10.1016/0022-5193(67)90079-3.

26. Martin WF, Garg S, Zimorski V. 2015. Endosymbiotic theories for eukaryote origin. Philos Trans R Soc Lond B Biol Sci 370:20140330. https://doi.org/10.1098/rstb.2014.0330.
27. Hoehler TM, Jørgensen BB. 2013. Microbial life under extreme energy limitation. Nat Rev Microbiol 11:83–94. https://doi.org/10.1038/nrmicro2939.
28. LaRowe DE, Amend JP. 2015. Catabolic rates, population sizes and doubling/replacement times of microorganisms in natural settings. Am J Sci 315:167–203. https://doi.org/10.2475/03.2015.01.
29. Schink B. 1997. Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61:262–280.
30. Stams AJM, Plugge CM. 2009. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat Rev Microbiol 7:568–577. https://doi.org/10.1038/nrmicro2166.
31. Sieber JR, McInerney MJ, Gunsalus RP. 2012. Genomic insights into syntrophy. The paradigm for anaerobic metabolic cooperation. Annu Rev Microbiol 66:429–452. https://doi.org/10.1146/annurev-micro-090110-102844.
32. Morris BEL, Henneberger R, Huber H, Moissl-Eichinger C. 2013. Microbial syntrophy: interaction for the common good. FEMS Microbiol Rev 37:384–406. https://doi.org/10.1111/1574-6976.12019.
33. Rotaru A-E, Shrestha PM, Liu F, Shrestha M, Shrestha D, Embree M, Zengler K, Wardman C, Nevin KP, Lovely DR. 2014. A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to *Methanosaeta* for the reduction of carbon dioxide to methane. Energy Environ Sci 7:408–415. https://doi.org/10.1039/C3EE42189A.
34. Rotaru A-E, Shrestha PM, Lui F, Markovaite B, Chen S, Nevin KP, Lovley DR. 2014. Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. Appl Environ Microbiol 80:4599–4605. https://doi.org/10.1128/AEM.00895-14.
35. Wegener G, Krukenberg V, Riedel D, Tegtmeyer HE, Boetius A. 2015. Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. Nature 526:587–590. https://doi.org/10.1038/nature15733.
36. McGlynn SE, Chadwick GL, Kempes CP, Orphan VJ. 2015. Single cell activity reveals direct electron transfer in methanotrophic consortia. Nature 526:531–535. https://doi.org/10.1038/nature15512.
37. Schimper AFW. 1883. Ueber die Entwickelung der Chlorophyllkörner und Farbkörper. Bot Z 41:105–114.
38. Mereschkowsky C. 1905. Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. Biol Centralbl 25:593–604.
39. Martin W, Kowallik K. 1999. Annotated English translation of Mereschkowsky's 1905 paper 'Über Natur und Ursprung der Chromatophoren im Pflanzenreiche'. Eur J Phycol 34:287–295. https://doi.org/10.1080/09670269910001736342.
40. Portier P. 1918. Les symbiotes. Masson, Paris, France.
41. Wallin IE. 1927. On the nature of mitochondria. IX. Demonstration of the bacterial nature of mitochondria. Am J Anat 36:131–149. https://doi.org/10.1002/aja.1000360106.
42. Altmann R. 1890. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Verlag von Veit & Co, Leipzig, Germany.
43. Stanier RY, van Niel CB. 1962. The concept of a bacterium. Archiv Mikrobiol 42:17–35. https://doi.org/10.1007/BF00425185.
44. Wilson EB. 1925. The cell in development and heredity. Macmillan, New York, NY.
45. Lederberg J. 1952. Cell genetics and hereditary symbiosis. Physiol Rev 32:403–430.
46. Buchner P. 1953. Endosymbiose der Tiere mit Pflanzlichen Mikroorganismen. Springer-Verlag, Berlin, Germany.
47. Bogorad L. 1975. Evolution of organelles and eukaryotic genomes. Science 188:891–898. https://doi.org/10.1126/science.1138359.
48. Raff RA, Mahler HR. 1972. The non symbiotic origin of mitochondria. Science 177:575–582. https://doi.org/10.1126/science.177.4049.575.
49. Cavalier-Smith T. 1975. The origin of nuclei and of eukaryotic cells. Nature 256:463–468. https://doi.org/10.1038/256463a0.
50. Zimorski V, Ku C, Martin WF, Gould SB. 2014. Endosymbiotic theory for organelle origins. Curr Opin Microbiol 22:38–48. https://doi.org/10.1016/j.mib.2014.09.008.
51. Archibald JM. 2015. Endosymbiosis and eukaryotic cell evolution. Curr Biol 25:R911–R921. https://doi.org/10.1016/j.cub.2015.07.055.
52. Ris H, Plaut W. 1962. Ultrastructure of DNA-containing areas in the chloroplast of chlamydomonas. J Cell Biol 13:383–391. https://doi.org/10.1083/jcb.13.3.383.
53. Margulis L. 1970. Origin of eukaryotic cells. Yale University Press, New Haven, CT.
54. Margulis L. 1996. Archaeal-eubacterial mergers in the origin of Eukarya: phylogenetic classification of life. Proc Natl Acad Sci U S A 93:1071–1076. https://doi.org/10.1073/pnas.93.3.1071.
55. Margulis L, Dolan MF, Guerrero R. 2000. The chimeric eukaryote: origin of the nucleus from the karyomastigont in amitochondriate protists. Proc Natl Acad Sci U S A 97:6954–6959. https://doi.org/10.1073/pnas.97.13.6954.
56. Margulis L, Chapman M, Guerrero R, Hall J. 2006. The last eukaryotic common ancestor (LECA): acquisition of cytoskeletal motility from aerotolerant spirochetes in the Proterozoic Eon. Proc Natl Acad Sci U S A 103:13080–13085. https://doi.org/10.1073/pnas.0604985103.
57. Woese CR, Fox GE. 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc Natl Acad Sci U S A 74:5088–5090. https://doi.org/10.1073/pnas.74.11.5088.
58. De Duve C. 1969. Evolution of the peroxisome. Ann N Y Acad Sci 168:369–381. https://doi.org/10.1111/j.1749-6632.1969.tb43124.x.
59. Stanier RY. 1970. Some aspects of the biology of cells and their possible evolutionary significance, p 1–38. *In* Charles HP, Knight BCJG (ed), Organisation and control in prokaryotic and eukaryotic cells. Cambridge University Press, Cambridge, United Kingdom.
60. Speijer D. 2015. Birth of the eukaryotes by a set of reactive innovations: new insights force us to relinquish gradual models. Bioessays 37:1268–1276. https://doi.org/10.1002/bies.201500107.
61. Bolte K, Rensing SA, Maier UG. 2015. The evolution of eukaryotic cells from the perspective of peroxisomes: phylogenetic analyses of peroxisomal beta-oxidation enzymes support mitochondria-first models of eukaryotic cell evolution. Bioessays 37:195–203. https://doi.org/10.1002/bies.201400151.
62. Speijer D. 2011. Oxygen radicals shaping evolution: why fatty acid catabolism leads to peroxisomes while neurons do without it. Bioessays 33:88–94. https://doi.org/10.1002/bies.201000097.
63. Sugiura A, Mattie S, Prudent J, McBride HM. 2017. Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. Nature 542:251–254. https://doi.org/10.1038/nature21375.
64. Cavalier-Smith T. 2002. The phagotrophic origin of eukaryotes and phylogenetic classification of protozoa. Int J Syst Evol Microbiol 52:297–354. https://doi.org/10.1099/00207713-52-2-297.
65. Taylor FJR. 1974. Implications and extensions of the serial endosymbiosis theory of the origin of eukaryotes. Taxon 23:229–258. https://doi.org/10.2307/1218702.
66. Doolittle WF. 1980. Revolutionary concepts in evolutionary cell biology. Trends Biochem Sci 5:146–149. https://doi.org/10.1016/0968-0004(80)90010-9.
67. Doolittle WF. 1998. You are what you eat: a gene transfer ratchet could account for bacterial genes in eukaryotic nuclear genomes. Trends Genet 14:307–311. https://doi.org/10.1016/S0168-9525(98)01494-2.
68. Bonen L, Doolittle WF. 1975. On the prokaryotic nature of red algal chloroplasts. Proc Natl Acad Sci U S A 72:2310–2314. https://doi.org/10.1073/pnas.72.6.2310.
69. Schwartz RM, Dayhoff MO. 1978. Origins of prokaryotes, eukaryotes, mitochondria, and chloroplasts. Science 199:395–403. https://doi.org/10.1126/science.202030.
70. Lindmark DG, Müller M. 1973. Hydrogenosome, a cytoplasmic organelle of the anaerobic flagellate *Tritrichomonas foetus*, and its role in pyruvate metabolism. J Biol Chem 248:7724–7728.
71. Vossbrinck CR, Maddox JV, Friedman S, Debrunner-Vossbrinck BA, Woese CR. 1987. Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. Nature 326:411–414. https://doi.org/10.1038/326411a0.
72. John P, Whatley FR. 1975. *Paracoccus denitrificans* and the evolutionary origin of the mitochondrion. Nature 254:495–498. https://doi.org/10.1038/254495a0.
73. Fox GE, Stackebrandt E, Hespell RB, Gibson J, Maniloff J, Dyer TA, Wolfe RS, Balch WE, Tanner RS, Magrum LJ, Zablen LB, Blakemore R, Gupta R, Bonen L, Lewis BJ, Stahl DA, Luehrsen KR, Chen KN, Woese CR. 1980. The phylogeny of prokaryotes. Science 209:457–463. https://doi.org/10.1126/science.6771870.
74. Zillig W, Stetter KO, Tobien M. 1978. DNA-dependent RNA polymerase from *Halobacterium halobium*. Eur J Biochem 91:193–199. https://doi.org/10.1111/j.1432-1033.1978.tb20951.x.
75. Keeling PJ. 1998. A kingdom's progress: archezoa and the origin of eukaryotes. Bioessays 20:87–95.

76. Roger AJ. 1999. Reconstructing early events in eukaryotic evolution. Am Nat 154:S146–S163. https://doi.org/10.1086/303290.
77. Poole AM, Penny D. 2006. Evaluating hypotheses for the origin of eukaryotes. Bioessays 29:74–84. https://doi.org/10.1002/bies.20516.
78. Müller M. 1988. Energy metabolism of protozoa without mitochondria. Annu Rev Microbiol 42:465–488. https://doi.org/10.1146/annurev.mi.42.100188.002341.
79. Roger AJ, Clark CG, Ford Doolittle W. 1996. A possible mitochondrial gene in the early-branching amitochondriate protist *Trichomonas vaginalis*. Proc Natl Acad Sci U S A 93:14618–14622. https://doi.org/10.1073/pnas.93.25.14618.
80. Horner DS, Hirt RP, Kilvington S, Lloyd D, Embley TM. 1996. Molecular data suggest an early acquisition of the mitochondrion endosymbiont. Proc Biol Sci 263:1053–1059. https://doi.org/10.1098/rspb.1996.0155.
81. Germot A, Philippe H, Le Guyader H. 1996. Presence of a mitochondrial-type 70-kDa heat shock protein in *Trichomonas vaginalis* suggests a very early mitochondrial endosymbiosis in eukaryotes. Proc Natl Acad Sci U S A 93:14614–14617. https://doi.org/10.1073/pnas.93.25.14614.
82. Bui E, Bradley PJ, Johnson PJ. 1996. A common evolutionary origin for mitochondria and hydrogenosomes. Proc Natl Acad Sci U S A 93:9651–9656. https://doi.org/10.1073/pnas.93.18.9651.
83. Biagini GA, Finlay BJ, Lloyd D. 1997. Evolution of the hydrogenosome. FEMS Microbiol Lett 155:133–140. https://doi.org/10.1111/j.1574-6968.1997.tb13869.x.
84. Mai Z, Ghosh S, Frisardi M, Rosenthal B, Rogers R, Samuelson J. 1999. Hsp60 is targeted to a cryptic mitochondrion-derived organelle ("crypton") in the microaerophilic protozoan parasite *Entamoeba histolytica*. Mol Cell Biol 19:2198–2205. https://doi.org/10.1128/MCB.19.3.2198.
85. Williams BAP, Hirt RP, Lucocq JM, Embley TM. 2002. A mitochondrial remnant in the microsporidian *Trachipleistophora hominis*. Nature 418:865–869. https://doi.org/10.1038/nature00949.
86. Tovar J, León-Avila G, Sánchez LB, Sutak R, Tachezy J, Van der Giezen M, Hernández M, Müller M, Lucocq JM. 2003. Mitochondrial remnant organelles of Giardia function in iron-sulphur protein maturation. Nature 426:172–176. https://doi.org/10.1038/nature01945.
87. Zillig W, Klenk HP, Palm P. 1989. Did eukaryotes originate by a fusion event? Endocyt Cell Res 6:1–25.
88. Gray MW. 2014. The pre-endosymbiont hypothesis: a new perspective on the origin and evolution of mitochondria. Cold Spring Harb Perspect Biol 6:a016097. https://doi.org/10.1101/cshperspect.a016097.
89. Adl SM, Simpson AGB, Lane CE, Lukes J, Bass D, Bowser SS, Brown MW, Burki F, Dunthorn M, Hampl V, Heiss A, Hoppenrath M, Lara E, le Gall L, Lynn DH, McManus H, Mitchell EAD, Mozley-Stanridge SE, Parfrey LW, Pawlowski J, Rueckert S, Shadwick RS, Shadwick L, Schoch CL, Smirnov A, Spiegel FW. 2012. The revised classification of eukaryotes. J Eukaryot Microbiol 59:429–493. https://doi.org/10.1111/j.1550-7408.2012.00644.x.
90. Martin W, Müller M. 1998. The hydrogen hypothesis for the first eukaryote. Nature 392:37–41. https://doi.org/10.1038/32096.
91. Vellai T, Vida G. 1999. The origin of eukaryotes: the difference between prokaryotic and eukaryotic cells. Proc Biol Sci 266:1571–1577. https://doi.org/10.1098/rspb.1999.0817.
92. Lopez-Garcia P, Moreira D. 1999. Metabolic symbiosis at the origin of eukaryotes. Trends Biochem Sci 24:88–93. https://doi.org/10.1016/S0968-0004(98)01342-5.
93. Lane N, Martin W. 2010. The energetics of genome complexity. Nature 467:929–934. https://doi.org/10.1038/nature09486.
94. Karnkowska A, Vacek V, Zubáčová Z, Treitli SC, Petrželková R, Eme L, Novák L, Žárský V, Barlow LD, Herman EK, Soukal P, Hroudová M, Doležal P, Stairs CW, Roger AJ, Eliáš M, Dacks JB, Vlček Č, Hampl V. 2016. A eukaryote without a mitochondrial organelle. Curr Biol 26:1274–1284. https://doi.org/10.1016/j.cub.2016.03.053.
95. Martínez-Cano DJ, Reyes-Prieto M, Martínez-Romero E, Partida-Martínez LP, Latorre A, Moya A, Delaye L. 2015. Evolution of small prokaryotic genomes. Front Microbiol 5:742. https://doi.org/10.3389/fmicb.2014.00742.
96. Hjort K, Goldberg AV, Tsaousis AD, Hirt RP, Embley TM. 2010. Diversity and reductive evolution of mitochondria among microbial eukaryotes. Philos Trans R Soc Lond B Biol Sci 365:713–727. https://doi.org/10.1098/rstb.2009.0224.
97. Esser C, Ahmadinejad N, Wiegand C, Rotte C, Sebastiani F, Gelius-Dietrich G, Henze K, Kretschmann E, Richly E, Leister D, Bryant D, Steel MA, Lockhart PJ, Penny D, Martin W. 2004. A genome phylogeny for mitochondria among alpha-proteobacteria and a predominantly eubacterial ancestry of yeast nuclear genes. Mol Biol Evol 21:1643–1660. https://doi.org/10.1093/molbev/msh160.
98. Pisani D, Cotton JA, McInerney JO. 2007. Supertrees disentangle the chimerical origin of eukaryotic genomes. Mol Biol Evol 24:1752–1760. https://doi.org/10.1093/molbev/msm095.
99. Cotton JA, McInerney JO. 2010. Eukaryotic genes of archaebacterial origin are more important than the more numerous eubacterial genes, irrespective of function. Proc Natl Acad Sci U S A 107:17252–17255. https://doi.org/10.1073/pnas.1000265107.
100. Thiergart T, Landan G, Schenk M, Dagan T, Martin WF. 2012. An evolutionary network of genes present in the eukaryote common ancestor polls genomes on eukaryotic and mitochondrial origin. Genome Biol Evol 4:466–485. https://doi.org/10.1093/gbe/evs018.
101. Ku C, Nelson-Sathi S, Roettger M, Garg S, Hazkani-Covo E, Martin WF. 2015. Endosymbiotic gene transfer from prokaryotic pangenomes: inherited chimerism in eukaryotes. Proc Natl Acad Sci U S A 112:10139–10146. https://doi.org/10.1073/pnas.1421385112.
102. Ku C, Nelson-Sathi S, Roettger M, Sousa FL, Lockhart PJ, Bryant D, Hazkani-Covo E, McInerney JO, Landan G, Martin WF. 2015. Endosymbiotic origin and differential loss of eukaryotic genes. Nature 524:427–432. https://doi.org/10.1038/nature14963.
103. Rivera MC, Lake JA. 2004. The ring of life provides evidence for a genome fusion origin of eukaryotes. Nature 431:152–155. https://doi.org/10.1038/nature02848.
104. Dagan T, Martin W. 2007. Testing hypotheses without considering predictions. Bioessays 29:500–503. https://doi.org/10.1002/bies.20566.
105. McInerney JO, O'Connell MJ, Pisani D. 2014. The hybrid nature of the Eukaryota and a consilient view of life on Earth. Nat Rev Microbiol 12:449–455. https://doi.org/10.1038/nrmicro3271.
106. Cox CJ, Foster PG, Hirt RP, Harris SR, Embley TM. 2008. The archaebacterial origin of eukaryotes. Proc Natl Acad Sci U S A 105:20356–20361. https://doi.org/10.1073/pnas.0810647105.
107. Raymann K, Brochier-Armanet C, Gribaldo S. 2015. The two-domain tree of life is linked to a new root for the archaea. Proc Natl Acad Sci U S A 112:6670–6675. https://doi.org/10.1073/pnas.1420858112.
108. Martin W, Rujan T, Richly E, Hansen A, Cornelsen S, Lins T, Leister D, Stoebe B, Hasegawa M, Penny D. 2002. Evolutionary analysis of arabidopsis, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. Proc Natl Acad Sci U S A 99:12246–12251. https://doi.org/10.1073/pnas.182432999.
109. Dagan T, Roettger M, Stucken K, Landan G, Koch R, Major P, Gould SB, Goremykin VV, Rippka R, Tandeau de Marsac N, Gugger M, Lockhart PJ, Allen JF, Brune I, Maus I, Pühler A, Martin WF. 2013. Genomes of stigonematalean cyanobacteria (subsection V) and the evolution of oxygenic photosynthesis from prokaryotes to plastids. Genome Biol Evol 5:31–44. https://doi.org/10.1093/gbe/evs117.
110. Woese CR, Kandler O, Wheelis ML. 1990. Towards a natural system of organisms: proposal for the domains archaea, bacteria, and eucarya. Proc Natl Acad Sci U S A 87:4576–4579. https://doi.org/10.1073/pnas.87.12.4576.
111. Williams TA, Foster PG, Cox CJ, Embley TM. 2013. An archaeal origin of eukaryotes supports only two primary domains of life. Nature 504:231–236. https://doi.org/10.1038/nature12779.
112. Lindås A-C, Karlsson EA, Lindgren MT, Ettema TJG, Bernander R. 2008. A unique cell division machinery in the Archaea. Proc Natl Acad Sci U S A 105:18942–18946. https://doi.org/10.1073/pnas.0809467105.
113. Samson RY, Dobro MJ, Jensen GJ, Bell SD. 2017. The structure, function and roles of the archaeal ESCRT apparatus, p 357–377. *In* Löwe J, Amos LA (ed), Prokaryotic cytoskeletons, subcellular biochemistry, vol 84. Springer Publishing, New York, NY. https://doi.org/10.1007/978-3-319-53047-5_12.
114. Koonin EV. 2009. The origin and early evolution of eukaryotes in the light of phylogenomics. Genome Biol 11:209. https://doi.org/10.1186/gb-2010-11-5-209.
115. Makarova KS, Yutin N, Bell SD, Koonin EV. 2010. Evolution of diverse cell division and vesicle formation systems in archaea. Nat Rev Microbiol 8:731–741. https://doi.org/10.1038/nrmicro2406.
116. Yutin N, Koonin EV. 2012. Archaeal origin of tubulin. Biol Direct 7:10. https://doi.org/10.1186/1745-6150-7-10.
117. Saw JH, Spang A, Zaremba-Niedzwiedzka K, Juzokaite L, Dodsworth JA, Murugapiran SK, Colman DR, Takacs-Vesbach C, Hedlund BP, Guy L, Ettema TJG. 2015. Exploring microbial dark matter to resolve the deep

archaeal ancestry of eukaryotes. Philos Trans R Soc Lond B Biol Sci 370:20140328. https://doi.org/10.1098/rstb.2014.0328.
118. Klinger CM, Spang A, Dacks JB, Ettema TJG. 2016. Tracing the archaeal origins of eukaryotic membrane-trafficking system building blocks. Mol Biol Evol 33:1528–1541. https://doi.org/10.1093/molbev/msw034.
119. Surkont J, Pereira-Leal JB. 2016. Are there Rab GTPases in archaea? Mol Biol Evol 33:1833–1842. https://doi.org/10.1093/molbev/msw061.
120. Degli Esposti M. 2017. The long story of mitochondrial DNA and respiratory complex I. Front Biosci 22:722–731. https://doi.org/10.2741/4512.
121. Degli Esposti M, Cortez D, Lozano L, Rasmussen S, Nielsen HB, Martínez-Romero E. 2016. Alpha proteobacterial ancestry of the (Fe-Fe)-hydrogenases in anaerobic eukaryotes. Biol Direct 11:34. https://doi.org/10.1186/s13062-016-0136-3.
122. Sousa FL, Neukirchen S, Allen JF, Lane N, Martin WF. 2016. Lokiarchaeon is hydrogen dependent. Nat Microbiol 1:16034. https://doi.org/10.1038/nmicrobiol.2016.34.
123. He Y, Li M, Perumal V, Feng X, Fang J, Xie J, Sievert SM, Wang F. 2016. Genomic and enzymatic evidence for acetogenesis among multiple lineages of the archaeal phylum Bathyarchaeota widespread in marine sediments. Nat Microbiol 1:16035. https://doi.org/10.1038/nmicrobiol.2016.35.
124. Martin WF, Neukirchen S, Zimorski V, Gould SB, Sousa FL. 2016. Energy for two: new archaeal lineages and the origin of mitochondria. Bioessays 38:850–856. https://doi.org/10.1002/bies.201600089.
125. Evans PN, Parks DH, Chadwick GL, Robbins SJ, Orphan VJ, Golding SD, Tyson GW. 2015. Methane metabolism in the archaeal phylum Bathyarchaeota revealed by genome-centric metagenomics. Science 350:434–438. https://doi.org/10.1126/science.aac7745.
126. Laso-Pérez R, Wegener G, Knittel K, Widdel F, Harding KJ, Krukenberg V, Meier DV, Richter M, Tegetmeyer HE, Riedel D, Richnow H-H, Adrian L, Reemtsma T, Lechtenfeld O, Musat F. 2016. Thermophilic archaea activate butane via alkyl-coenzyme M formation. Nature 539:396–401. https://doi.org/10.1038/nature20152.
127. Nelson-Sathi S, Dagan T, Landan G, Janssen A, Steel M, McInerney JO, Deppenmeier U, Martin WF. 2012. Acquisition of 1,000 eubacterial genes physiologically transformed a methanogen at the origin of haloarchaea. Proc Natl Acad Sci U S A 109:20537–20542. https://doi.org/10.1073/pnas.1209119109.
128. Martin W, Schnarrenberger C. 1997. The evolution of the Calvin cycle from prokaryotic to eukaryotic chromosomes: a case study of functional redundancy in ancient pathways through endosymbiosis. Curr Genet 32:1–18. https://doi.org/10.1007/s002940050241.
129. Cavalier-Smith T. 2001. The neomuran origin of archaebacteria, the negibacterial root of the universal tree and bacterial megaclassification. Int J Syst Evol Microbiol 52:7–76. https://doi.org/10.1099/00207713-52-1-7.
130. Doeller JE, Gaschen BK, Parrino V, Kraus DW. 1999. Chemolithoheterotrophy in a metazoan tissue: sulfide supports cellular work in ciliated mussel gills. J Exp Biol 202:1953–1961.
131. Thauer RK, Jungermann K, Decker K. 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180.
132. Mitchell P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191:144–148. https://doi.org/10.1038/191144a0.
133. Schäfer G, Engelhard M, Müller V. 1999. Bioenergetics of the archaea. Microbiol Mol Biol Rev 63:570–620.
134. Decker K, Jungermann K, Thauer RK. 1970. Energy production in anaerobic organisms. Angew Chem Int Ed Engl 9:138–158. https://doi.org/10.1002/anie.197001381.
135. Schuchmann K, Müller V. 2014. Autotrophy at the thermodynamic limit of life: a model for energy conservation in acetogenic bacteria. Nat Rev Microbiol 12:809–821. https://doi.org/10.1038/nrmicro3365.
136. Sapra R, Bagramyan K, Adams MWW. 2003. A simple energy-conserving system: proton reduction coupled to proton translocation. Proc Natl Acad Sci U S A 100:7545–7550. https://doi.org/10.1073/pnas.1331436100.
137. Buckel W, Thauer RK. 2013. Energy conservation via electron bifurcating ferredoxin reduction and proton/Na+ translocating ferredoxin oxidation. Biochim Biophys Acta 1827:94–113. https://doi.org/10.1016/j.bbabio.2012.07.002.
138. Brasen C, Esser D, Rauch B, Siebers B. 2014. Carbohydrate metabolism in archaea: current insights into unusual enzymes and pathways and their regulation. Microbiol Mol Biol Rev 78:89–175. https://doi.org/10.1128/MMBR.00041-13.
139. Schut GJ, Boyd ES, Peters JW, Adams MWW. 2013. The modular respiratory complexes involved in hydrogen and sulfur metabolism by heterotrophic hyperthermophilic archaea and their evolutionary implications. FEMS Microbiol Rev 37:182–203. https://doi.org/10.1111/j.1574-6976.2012.00346.x.
140. Adams MWW, Holden JF, Menon AL, Schut GJ, Grunden AM, Hou C, Hutchins AM, Jenney FE, Jr, Kim C, Ma K, Pan G, Roy R, Sapra R, Story SV, Verhagen MFJM. 2001. Key role for sulfur in peptide metabolism and in regulation of three hydrogenases in the hyperthermophilic archaeon *Pyrococcus furiosus*. J Bacteriol 183:716–724. https://doi.org/10.1128/JB.183.2.716-724.2001.
141. Kletzin A, Urich T, Müller F, Bandeiras TM, Gomes CM. 2004. Dissimilatory oxidation and reduction of elemental sulfur in thermophilic archaea. J Bioenerg Biomembr 36:77–91. https://doi.org/10.1023/B:JOBB.0000019600.36757.8c.
142. Liu Y, Beer LL, Whitman WB. 2012. Sulfur metabolism in archaea reveals novel processes. Environ Microbiol 14:2632–2644. https://doi.org/10.1111/j.1462-2920.2012.02783.x.
143. Musfeldt M, Selig M, Schonheit P. 1999. Acetyl coenzyme A synthetase (ADP forming) from the hyperthermophilic archaeon *Pyrococcus furiosus*: identification, cloning, separate expression of the encoding genes, *acdAI* and *acdBI*, in *Escherichia coli*, and in vitro reconstitution of the active heterotetrameric enzyme from its recombinant subunits. J Bacteriol 181:5885–5888.
144. Musfeldt M, Schönheit P. 2002. Novel type of ADP-forming acetyl coenzyme A synthetase in hyperthermophilic archaea: heterologous expression and characterization of isoenzymes from the sulfate reducer *Archaeoglobus fulgidus* and the methanogen *Methanococcus jannaschii*. J Bacteriol 184:636–644. https://doi.org/10.1128/JB.184.3.636-644.2002.
145. Bräsen C, Schmidt M, Grötzinger J, Schönheit P. 2008. Reaction mechanism and structural model of ADP-forming acetyl-CoA synthetase from the hyperthermophilic archaeon *Pyrococcus furiosus*. J Biol Chem 283:15409–15418. https://doi.org/10.1074/jbc.M710218200.
146. Elkins JG, Podar M, Graham DE, Makarova KS, Wolf Y, Randau L, Hedlund BP, Brochier-Armanet C, Kunin V, Anderson I, Lapidus A, Goltsman E, Barry K, Koonin EV, Hugenholtz P, Kyrpides N, Wanner G, Richardson P, Keller M, Stetter KO. 2008. A korarchaeal genome reveals insights into the evolution of the Archaea. Proc Natl Acad Sci U S A 105:8102–8107. https://doi.org/10.1073/pnas.0801980105.
147. Schmidt M, Schönheit P. 2013. Acetate formation in the photoheterotrophic bacterium *Chloroflexus aurantiacus* involves an archaeal type ADP-forming acetyl-CoA synthetase isoenzyme I. FEMS Microbiol Lett 349:171–179. https://doi.org/10.1111/1574-6968.12312.
148. Scott JW, Poole FL, Adams MWW, Scott JW, Poole FL. 2014. Characterization of ten heterotetrameric NDP-dependent acyl-CoA synthetases of the hyperthermophilic archaeon *Pyrococcus furiosus*. Archaea 2014:176863. https://doi.org/10.1155/2014/176863.
149. Awano T, Wilming A, Tomita H, Yokooji Y, Fukui T, Imanaka T, Atomi H. 2014. Characterization of two members among the five ADP-forming acyl coenzyme A (acyl-CoA) synthetases reveals the presence of a 2-(imidazol-4-yl) acetyl-CoA synthetase in *Thermococcus kodakarensis*. J Bacteriol 196:140–147. https://doi.org/10.1128/JB.00877-13.
150. Schönheit P, Buckel W, Martin WF. 2016. On the origin of heterotrophy. Trends Microbiol 24:12–25. https://doi.org/10.1016/j.tim.2015.10.003.
151. Hedderich R. 2004. Energy-converting (NiFe) hydrogenases from archaea and extremophiles: ancestors of complex I. J Bioenerg Biomembr 36:65–75. https://doi.org/10.1023/B:JOBB.0000019599.43969.33.
152. Harold FM. 1986. The vital force: a study of bioenergetics. WH Freeman, New York, NY.
153. Stouthamer AH. 1973. A theoretical study on the amount of ATP required for synthesis of microbial cell material. Antonie Van Leeuwenhoek 39:545–565. https://doi.org/10.1007/BF02578899.
154. Grant BD, Donaldson JG. 2009. Pathways and mechanisms of endocytic recycling. Nat Rev Mol Cell Biol 10:597–608. https://doi.org/10.1038/nrm2755.
155. Garg SG, Gould SB. 2016. The role of charge in protein targeting evolution. Trends Cell Biol 26:894–905. https://doi.org/10.1016/j.tcb.2016.07.001.
156. Davidson AL, Dassa E, Orelle C, Chen J. 2008. Structure, function, and evolution of bacterial ATP-binding cassette systems. Microbiol Mol Biol Rev 72:316–364. https://doi.org/10.1128/MMBR.00031-07.

157. Beguin P, Aubert JP. 1994. The biological degradation of cellulose. FEMS Microbiol Rev 13:25–58. https://doi.org/10.1111/j.1574-6976.1994.tb00033.x.
158. Jaeger KE, Ransac S, Dijkstra BW, Colson C, Van Heuvel M, Misset O. 1994. Bacterial lipases. FEMS Microbiol Rev 15:29–63. https://doi.org/10.1111/j.1574-6976.1994.tb00121.x.
159. Law J, Haandrikman A. 1997. Proteolytic enzymes of lactic acid bacteria. Int Dairy J 7:1–11. https://doi.org/10.1016/0958-6946(95)00073-9.
160. Schut GJ, Adams MWW. 2009. The iron-hydrogenase of *Thermotoga maritima* utilizes ferredoxin and NADH synergistically: a new perspective on anaerobic hydrogen production. J Bacteriol 191:4451–4457. https://doi.org/10.1128/JB.01582-08.
161. Neidhardt FC, Ingraham JL, Schaechter M. 1990. Physiology of the bacterial cell. Sinauer Associates, Sunderland, MA.
162. Buckel W. 2001. Unusual enzymes involved in five pathways of glutamate fermentation. Appl Microbiol Biotechnol 57:263–273. https://doi.org/10.1007/s002530100773.
163. Barker HA. 1961. Fermentations of nitrogenous organic compounds, p 151–207. *In* Gunsalus IC, Stanier RY (ed), The bacteria: a treatise on structure and function, vol 2. Metabolism. Academic Press, New York, NY.
164. Hartwich K, Poehlein A, Daniel R. 2011. The purine-utilizing bacterium *Clostridium acidurici* 9a: a genome-guided metabolic reconsideration. PLoS One 7:e51662. https://doi.org/10.1371/journal.pone.0051662.
165. Wang S, Huang H, Kahnt J, Thauer RK. 2013. *Clostridium acidurici* electron-bifurcating formate dehydrogenase. Appl Environ Microbiol 79:6176–6179. https://doi.org/10.1128/AEM.02015-13.
166. Farmer IS, Jones CW. 1976. Energetics of *Escherichia coli* during aerobic growth in continuous culture. Eur J Biochem 67:115–122. https://doi.org/10.1111/j.1432-1033.1976.tb10639.x.
167. Milo R, Jorgensen P, Moran U, Weber G, Springer M. 2010. BioNumbers—the database of key numbers in molecular and cell biology. Nucleic Acids Res 38:D750–D753. https://doi.org/10.1093/nar/gkp889.
168. Akashi H, Gojobori T. 2002. Metabolic efficiency and amino acid composition in the proteomes of *Escherichia coli* and *Bacillus subtilis*. Proc Natl Acad Sci U S A 99:3695–3700. https://doi.org/10.1073/pnas.062526999.
169. Thauer RK, Kaster A-K, Seedorf H, Buckel W, Hedderich R. 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. Nat Rev Microbiol 6:579–591. https://doi.org/10.1038/nrmicro1931.
170. Garg SG, Martin WF. 2016. Mitochondria, the cell cycle, and the origin of sex via a syncytial eukaryote common ancestor. Genome Biol Evol 8:1950–1970. https://doi.org/10.1093/gbe/evw136.
171. Daniel SL, Hsu T, Dean SI, Drake HL. 1990. Characterization of the H_2-and CO-dependent chemolithotrophic potentials of the acetogens *Clostridium thermoaceticum* and *Acetogenium kivui*. J Bacteriol 172:4464–4471. https://doi.org/10.1128/jb.172.8.4464-4471.1990.
172. Schonheit P, Moll J, Thauer RK. 1980. Growth parameters (K_s, μ_{max}, Y_s) of *Methanobacterium thermoautotrophicum*. Arch Microbiol 127:59–65. https://doi.org/10.1007/BF00414356.
173. Scherer P, Lippert H, Wolff G. 1983. Composition of the major elements and trace elements of 10 methanogenic bacteria determined by inductively coupled plasma emission spectrometry. Biol Trace Elem Res 5:149–163. https://doi.org/10.1007/BF02916619.
174. Bender DA. 2012. The metabolism of "surplus" amino acids. Br J Nutr 108:S113–S121. https://doi.org/10.1017/S0007114512002292.
175. Tielens AGM, van Hellemond JJ. 2007. Anaerobic mitochondria: properties and origins, p 85–103. *In* Martin WF, Muller M (ed), Origin of mitochondria and hydrogenosomes. Springer, Berlin, Germany.
176. Tielens AGM, Rotte C, van Hellemond JJ, Martin W. 2002. Mitochondria as we don't know them. Trends Biochem Sci 27:564–572. https://doi.org/10.1016/S0968-0004(02)02193-X.
177. Yarlett N, Martinez MP, Ali Moharrami M, Tachezy J. 1996. The contribution of the arginine dihydrolase pathway to energy metabolism by *Trichomonas vaginalis*. Mol Biochem Parasitol 78:117–125. https://doi.org/10.1016/S0166-6851(96)02616-3.
178. Kleydman Y, Yarlett N, Gorrell TE. 2004. Production of ammonia by *Tritrichomonas foetus* and *Trichomonas vaginalis*. Microbiology 150:1139–1145. https://doi.org/10.1099/mic.0.26939-0.
179. Gelius-Dietrich G, Ter Braak M, Henze K. 2007. Mitochondrial steps of arginine biosynthesis are conserved in the hydrogenosomes of the chytridiomycete *Neocallimastix frontalis*. J Eukaryot Microbiol 54:42–44. https://doi.org/10.1111/j.1550-7408.2006.00146.x.
180. Morada M, Šmíd O, Hampl V, Sutak R, Lam B, Rappelli P, Dessì D, Fiori PL, Tachezy J, Yarlett N. 2011. Hydrogenosome-localization of arginine deiminase in *Trichomonas vaginalis*. Mol Biochem Parasitol 176:51–54. https://doi.org/10.1016/j.molbiopara.2010.10.004.
181. Grieshaber MK, Hardewig I, Kreutzer U. 1994. Physiological and metabolic responses to hypoxia in invertebrates. Rev Physiol Biochem Pharmacol 125:43–147. https://doi.org/10.1007/BFb0030909.
182. Bui ET, Johnson PJ. 1996. Identification and characterization of (Fe)-hydrogenases in the hydrogenosome of *Trichomonas vaginalis*. Mol Biochem Parasitol 76:305–310. https://doi.org/10.1016/0166-6851(96)02567-4.
183. Happe T, Naber JD. 1993. Isolation, characterization and N-terminal amino-acid-sequence of hydrogenase from the green-alga *Chlamydomonas reinhardtii*. Eur J Biochem 214:475–481. https://doi.org/10.1111/j.1432-1033.1993.tb17944.x.
184. Steinbüchel A, Müller M. 1986. Glycerol, a metabolic end product of *Trichomonas vaginalis* and *Tritrichomonas foetus*. Mol Biochem Parasitol 20:45–55. https://doi.org/10.1016/0166-6851(86)90141-6.
185. Wu G, Fiser A, ter Kuile B, Sali A, Müller M. 1999. Convergent evolution of *Trichomonas vaginalis* lactate dehydrogenase from malate dehydrogenase. Proc Natl Acad Sci U S A 96:6285–6290. https://doi.org/10.1073/pnas.96.11.6285.
186. Bruchhaus I, Tannich E. 1994. Purification and molecular characterization of the NAD^+-dependent acetaldehyde/alcohol dehydrogenase from *Entamoeba histolytica*. Biochem J 303:743–748. https://doi.org/10.1042/bj3030743.
187. Atteia A, van Lis R, Mendoza-Hernandez G, Henze K, Martin W, Riveros-Rosas H, Gonzalez-Halphen D. 2003. Bifunctional aldehyde/alcohol dehydrogenase (ADHE) in chlorophyte algal mitochondria. Plant Mol Biol 53:175–188. https://doi.org/10.1023/B:PLAN.0000009274.19340.36.
188. Komuniecki R, McCrury J, Thissen J, Rubin N. 1989. Electron-transfer flavoprotein from anaerobic *Ascaris suum* mitochondria and its role in NADH-dependent 2-methyl branched-chain enoyl-CoA reduction. Biochim Biophys Acta 975:127–131. https://doi.org/10.1016/S0005-2728(89)80210-5.
189. Hoffmeister M, Piotrowski M, Nowitzki U, Martin W. 2005. Mitochondrial trans-2-enoyl-CoA reductase of wax ester fermentation from *Euglena gracilis* defines a new family of enzymes involved in lipid synthesis. J Biol Chem 280:4329–4338. https://doi.org/10.1074/jbc.M411010200.
190. Tielens A, van Hellemond JJ. 1998. The electron transport chain in anaerobically functioning eukaryotes. Biochim Biophys Acta 1365:71–78. https://doi.org/10.1016/S0005-2728(98)00045-0.
191. Besteiro S, Biran M, Biteau N, Coustou V, Baltz T, Canioni P, Bringaud F. 2002. Succinate secreted by *Trypanosoma brucei* is produced by a novel and unique glycosomal enzyme, NADH-dependent fumarate reductase. J Biol Chem 277:38001–38012. https://doi.org/10.1074/jbc.M201759200.
192. Altman BJ, Stine ZE, Dang CV. 2016. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer 16:619–634. https://doi.org/10.1038/nrc.2016.71.
193. Andreesen JR. 1994. Glycine metabolism in anaerobes. Antonie Van Leeuwenhoek 66:223–237. https://doi.org/10.1007/BF00871641.
194. Fonknechten N, Chaussonnerie S, Tricot S, Lajus A, Andreesen JR, Perchat N, Pelletier E, Gouyvenoux M, Barbe V, Salanoubat M, Le Paslier D, Weissenbach J, Cohen GN, Kreimeyer A. 2009. *Clostridium sticklandii*, a specialist in amino acid degradation: revisiting its metabolism through its genome sequence. BMC Genomics 11:555. https://doi.org/10.1186/1471-2164-11-555.
195. van Grinsven KWA, Rosnowsky S, van Weelden SWH, Pütz S, Van der Giezen M, Martin W, van Hellemond JJ, Tielens AGM, Henze K. 2008. Acetate:succinate CoA-transferase in the hydrogenosomes of *Trichomonas vaginalis*: identification and characterization. J Biol Chem 283:1411–1418. https://doi.org/10.1074/jbc.M702528200.
196. Tielens AGM, van Grinsven KWA, Henze K, van Hellemond JJ, Martin W. 2010. Acetate formation in the energy metabolism of parasitic helminths and protists. Int J Parasitol 40:387–397. https://doi.org/10.1016/j.ijpara.2009.12.006.
197. Mentel M, Martin W. 2008. Energy metabolism among eukaryotic anaerobes in light of Proterozoic ocean chemistry. Philos Trans R Soc Lond B Biol Sci 363:2717–2729. https://doi.org/10.1098/rstb.2008.0031.
198. Reinhard CT, Planavsky NJ, Olson SL, Lyons TW, Erwin DH. 2016. Earth's oxygen cycle and the evolution of animal life. Proc Natl Acad Sci U S A 113:8933–8938. https://doi.org/10.1073/pnas.1521544113.
199. Gould SB, Garg SG, Martin WF. 2016. Bacterial vesicle secretion and the

evolutionary origin of the eukaryotic endomembrane system. Trends Microbiol 24:525–534. https://doi.org/10.1016/j.tim.2016.03.005.
200. Doherty GJ, McMahon HT. 2009. Mechanisms of endocytosis. Annu Rev Biochem 78:857–902. https://doi.org/10.1146/annurev.biochem.78.081307.110540.
201. Field MC, Gabernet-Castello C, Dacks JB. 2008. Reconstructing the evolution of the endocytic system: insights from genomics and molecular cell biology, p 84–96. *In* Jékely G (ed), Eukaryotic membranes and cytoskeleton: origins and evolution. Advances in experimental medicine and biology, vol 607. Springer, Berlin, Germany.
202. Kirkham M, Nixon SJ, Howes MT, Abi-Rached L, Wakeham DE, Hanzal-Bayer M, Ferguson C, Hill MM, Fernandez-Rojo M, Brown DA, Hancock JF, Brodsky FM, Parton RG. 2008. Evolutionary analysis and molecular dissection of caveola biogenesis. J Cell Sci 121:2075–2086. https://doi.org/10.1242/jcs.024588.
203. Kumari S, Swetha MG, Mayor S. 2010. Endocytosis unplugged: multiple ways to enter the cell. Cell Res 20:256–275. https://doi.org/10.1038/cr.2010.19.
204. Bonifacino JS, Traub LM. 2003. Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu Rev Biochem 72:395–447. https://doi.org/10.1146/annurev.biochem.72.121801.161800.
205. Engstler M, Boshart M. 2004. Cold shock and regulation of surface protein trafficking convey sensitization to inducers of stage differentiation in *Trypanosoma brucei*. Genes Dev 18:2798–2811. https://doi.org/10.1101/gad.323404.
206. Engstler M, Thilo L, Weise F, Grunfelder CG, Schwarz H, Boshart M, Overath P. 2004. Kinetics of endocytosis and recycling of the GPI-anchored variant surface glycoprotein in *Trypanosoma brucei*. J Cell Sci 117:1105–1115. https://doi.org/10.1242/jcs.00938.
207. Hoeller D, Volarevic S, Dikic I. 2005. Compartmentalization of growth factor receptor signalling. Curr Opin Cell Biol 17:107–111. https://doi.org/10.1016/j.ceb.2005.01.001.
208. Gow NAR, van de Veerdonk FL, Brown AJP, Netea MG. 2011. *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. Nat Rev Microbiol 10:112–122. https://doi.org/10.1038/nrmicro2711.
209. Gordon S. 2016. Phagocytosis: an immunobiologic process. Immunity 44:463–475. https://doi.org/10.1016/j.immuni.2016.02.026.
210. Ball SG, Bhattacharya D, Weber APM. 2016. Pathogen to powerhouse. Science 351:659–660. https://doi.org/10.1126/science.aad8864.
211. Bremer H, Dennis PP. 7 October 2008, posting date. Modulation of chemical composition and other parameters of the cell at different exponential growth rates. EcoSal Plus 2008. https://doi.org/10.1128/ecosal.5.2.3.
212. Aderem A, Underhill DM. 1999. Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 17:593–623. https://doi.org/10.1146/annurev.immunol.17.1.593.
213. Haas A. 2007. The phagosome: compartment with a license to kill. Traffic 8:311–330. https://doi.org/10.1111/j.1600-0854.2006.00531.x.
214. Brown GC, Neher JJ. 2014. Microglial phagocytosis of live neurons. Nat Rev Neurosci 15:209–216. https://doi.org/10.1038/nrn3710.
215. Freeman SA, Grinstein S. 2014. Phagocytosis: receptors, signal integration, and the cytoskeleton. Immunol Rev 262:193–215. https://doi.org/10.1111/imr.12212.
216. Chimini G, Chavrier P. 2000. Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. Nat Cell Biol 2:E191–E196. https://doi.org/10.1038/35036454.
217. Rougerie P, Miskolci V, Cox D. 2013. Generation of membrane structures during phagocytosis and chemotaxis of macrophages: role and regulation of the actin cytoskeleton. Immunol Rev 256:222–239. https://doi.org/10.1111/imr.12118.
218. Gu C, Yaddanapudi S, Weins A, Osborn T, Reiser J, Pollak M, Hartwig J, Sever S. 2010. Direct dynamin-actin interactions regulate the actin cytoskeleton. EMBO J 29:3593–3606. https://doi.org/10.1038/emboj.2010.249.
219. Marie-Anaïs F, Mazzolini J, Herit F, Niedergang F. 2016. Dynamin-actin cross talk contributes to phagosome formation and closure. Traffic 17:487–499. https://doi.org/10.1111/tra.12386.
220. Levin R, Grinstein S, Canton J. 2016. The life cycle of phagosomes: formation, maturation, and resolution. Immunol Rev 273:156–179. https://doi.org/10.1111/imr.12439.
221. Wu L-G, Hamid E, Shin W, Chiang H-C. 2013. Exocytosis and endocytosis: modes, functions, and coupling mechanisms. Annu Rev Physiol 76:301–331. https://doi.org/10.1146/annurev-physiol-021113-170305.
222. Baum DA, Baum B. 2014. An inside-out origin for the eukaryotic cell. BMC Biol 12:76. https://doi.org/10.1186/s12915-014-0076-2.
223. Deatherage BL, Cookson BT. 2012. Membrane vesicle release in bacteria, eukaryotes, and archaea: a conserved yet underappreciated aspect of microbial life. Infect Immun 80:1948–1957. https://doi.org/10.1128/IAI.06014-11.
224. Schwechheimer C, Kuehn MJ. 2015. Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. Nat Rev Microbiol 13:605–619. https://doi.org/10.1038/nrmicro3525.
225. Sugiura A, McLelland GL, Fon EA, McBride HM. 2014. A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. EMBO J 33:2142–2156. https://doi.org/10.15252/embj.201488104.
226. Yamashita A, Fujimoto M, Katayama K, Yamaoka S, Tsutsumi N, Arimura S-I. 2016. Formation of mitochondrial outer membrane derived protrusions and vesicles in *Arabidopsis thaliana*. PLoS One 11:e0146717. https://doi.org/10.1371/journal.pone.0146717.
227. Koch GL. 1990. The endoplasmic reticulum and calcium storage. Bioessays 12:527–531. https://doi.org/10.1002/bies.950121105.
228. Kaufman RJ, Malhotra JD. 2014. Calcium trafficking integrates endoplasmic reticulum function with mitochondrial bioenergetics. Biochim Biophys Acta 1843:2233–2239. https://doi.org/10.1016/j.bbamcr.2014.03.022.
229. Marchi S, Patergnani S, Pinton P. 2014. The endoplasmic reticulum-mitochondria connection: one touch, multiple functions. Biochim Biophys Acta 1837:461–469. https://doi.org/10.1016/j.bbabio.2013.10.015.
230. Hell K. 2008. The Erv1-Mia40 disulfide relay system in the intermembrane space of mitochondria. Biochim Biophys Acta 1783:601–609. https://doi.org/10.1016/j.bbamcr.2007.12.005.
231. Herrmann JM, Riemer J. 2014. Three approaches to one problem: protein folding in the periplasm, the endoplasmic reticulum, and the intermembrane space. Antioxid Redox Signal 21:438–456. https://doi.org/10.1089/ars.2014.5841.
232. Güttinger S, Laurell E, Kutay U. 2009. Orchestrating nuclear envelope disassembly and reassembly during mitosis. Nat Rev Mol Cell Biol 10:178–191. https://doi.org/10.1038/nrm2641.
233. Wandke C, Kutay U. 2013. Enclosing chromatin: reassembly of the nucleus after open mitosis. Cell 152:1222–1225. https://doi.org/10.1016/j.cell.2013.02.046.
234. Olmos Y, Hodgson L, Mantell J, Verkade P, Carlton JG. 2015. ESCRT-III controls nuclear envelope reformation. Nature 522:236–239. https://doi.org/10.1038/nature14503.
235. van Meer G, de Kroon AIPM. 2011. Lipid map of the mammalian cell. J Cell Sci 124:5–8. https://doi.org/10.1242/jcs.071233.
236. Weber RWS. 2002. Vacuoles and the fungal lifestyle. Mycologist 16:10–20. https://doi.org/10.1017/S0269915X02006110.
237. Webster J, Weber R. 2007. Introduction to Fungi. Cambridge University Press, Cambridge, United Kingdom.
238. Dayel MJ, King N. 2014. Prey capture and phagocytosis in the choanoflagellate *Salpingoeca rosetta*. PLoS One 9:e95577. https://doi.org/10.1371/journal.pone.0095577.
239. Sateriale A, Huston CD. 2011. A sequential model of host cell killing and phagocytosis by *Entamoeba histolytica*. J Parasitol Res 2011:926706. https://doi.org/10.1155/2011/926706.
240. Okada M, Huston CD, Oue M, Mann BJ, Petri WA, Jr, Kita K, Nozaki T. 2006. Kinetics and strain variation of phagosome proteins of *Entamoeba histolytica* by proteomic analysis. Mol Biochem Parasitol 145:171–183. https://doi.org/10.1016/j.molbiopara.2005.10.001.
241. Boettner DR, Huston CD, Sullivan JA, Petri WA. 2005. *Entamoeba histolytica* and *Entamoeba dispar* utilize externalized phosphatidylserine for recognition and phagocytosis of erythrocytes. Infect Immun 73:3422–3430. https://doi.org/10.1128/IAI.73.6.3422-3430.2005.
242. de Vries J, Stanton A, Archibald JM, Gould SB. 2016. Streptophyte terrestrialization in light of plastid evolution. Trends Plant Sci 21:467–476. https://doi.org/10.1016/j.tplants.2016.01.021.
243. Bengtson S, Sallstedt T, Belivanova V, Whitehouse M. 2017. Three-dimensional preservation of cellular and subcellular structures suggests 1.6 billion-year-old crown-group red algae. PLoS Biol 15:e2000735. https://doi.org/10.1371/journal.pbio.2000735.
244. Tischendorf G, Oesterhelt C, Hoffmann S, Girnus J, Schnarrenberger C, Gross W. 2007. Ultrastructure and enzyme complement of proplastids from heterotrophically grown cells of the red alga *Galdieria sulphuraria*. Eur J Phycol 42:243–251. https://doi.org/10.1080/09670260701437642.

245. McKie-Krisberg ZM, Sanders RW. 2014. Phagotrophy by the picoeukaryotic green alga Micromonas: implications for Arctic oceans. ISME J 8:1953–1961. https://doi.org/10.1038/ismej.2014.16.
246. Maruyama S, Kim E. 2013. A modern descendant of early green algal phagotrophs. Curr Biol 23:1081–1084. https://doi.org/10.1016/j.cub.2013.04.063.
247. Tartar A, Boucias DG. 2004. The non-photosynthetic, pathogenic green alga *Helicosporidium* sp. has retained a modified, functional plastid genome. FEMS Microbiol Lett 233:153–157. https://doi.org/10.1016/j.femsle.2004.02.006.
248. Smith DR, Lee RW. 2014. A plastid without a genome: evidence from the nonphotosynthetic green algal genus *Polytomella*. Plant Physiol 164:1812–1819. https://doi.org/10.1104/pp.113.233718.
249. McFadden GI, Yeh E. 2016. The apicoplast: now you see it, now you don't. Int J Parasitol 47:137–144. https://doi.org/10.1016/j.ijpara.2016.08.005.
250. Fan L, Li R, Pan J, Ding Z, Lin J. 2015. Endocytosis and its regulation in plants. Trends Plant Sci 20:388–397. https://doi.org/10.1016/j.tplants.2015.03.014.
251. Zwiewka M, Nodzyński T, Robert S, Vanneste S, Friml J. 2015. Osmotic stress modulates the balance between exocytosis and clathrin-mediated endocytosis in *Arabidopsis thaliana*. Mol Plant 8:1175–1187. https://doi.org/10.1016/j.molp.2015.03.007.
252. Fujimoto M, Arimura S-I, Ueda T, Takanashi H, Hayashi Y, Nakano A, Tsutsumi N. 2010. Arabidopsis dynamin-related proteins DRP2B and DRP1A participate together in clathrin-coated vesicle formation during endocytosis. Proc Natl Acad Sci U S A 107:6094–6099. https://doi.org/10.1073/pnas.0913562107.
253. Simpson AGB. 2003. Cytoskeletal organization, phylogenetic affinities and systematics in the contentious taxon Excavata (Eukaryota). Int J Syst Evol Microbiol 53:1759–1777. https://doi.org/10.1099/ijs.0.02578-0.
254. Pereira-Neves AA, Benchimol MM. 2007. Phagocytosis by *Trichomonas vaginalis*: new insights. Biol Cell 99:87–101. https://doi.org/10.1042/BC20060084.
255. Kusdian G, Woehle C, Martin WF, Gould SB. 2013. The actin-based machinery of *Trichomonas vaginalis* mediates flagellate-amoeboid transition and migration across host tissue. Cell Microbiol 15:1707–1721. https://doi.org/10.1111/cmi.12144.
256. Wideman JG, Gawryluk RMR, Gray MW, Dacks JB. 2013. The ancient and widespread nature of the ER-mitochondria encounter structure. Mol Biol Evol 30:120–2049. https://doi.org/10.1093/molbev/mst120.
257. He D, Fiz-Palacios O, Fu C-J, Fehling J, Tsai C-C, Baldauf SL. 2014. An alternative root for the eukaryote tree of life. Curr Biol 24:465–470. https://doi.org/10.1016/j.cub.2014.01.036.
258. Yamashiro CT, Kane PM, Wolczyk DF, Preston RA, Stevens TH. 1990. Role of vacuolar acidification in protein sorting and zymogen activation: a genetic analysis of the yeast vacuolar proton-translocating ATPase. Mol Cell Biol 10:3737–3749. https://doi.org/10.1128/MCB.10.7.3737.
259. Kane PM. 2006. The where, when, and how of organelle acidification by the yeast vacuolar H^+-ATPase. Microbiol Mol Biol Rev 70:177–191. https://doi.org/10.1128/MMBR.70.1.177-191.2006.
260. Goode BL, Eskin JA, Wendland B. 2015. Actin and endocytosis in budding yeast. Genetics 199:315–358. https://doi.org/10.1534/genetics.112.145540.
261. Lu R, Drubin DG, Sun Y. 2016. Clathrin-mediated endocytosis in budding yeast at a glance. J Cell Sci 129:1531–1536. https://doi.org/10.1242/jcs.182303.
262. Nebenführ A. 2002. Vesicle traffic in the endomembrane system: a tale of COPs, Rabs and SNAREs. Curr Opin Plant Biol 5:507–512. https://doi.org/10.1016/S1369-5266(02)00303-5.
263. Baranov MV, Revelo NH, Dingjan I, Maraspini R, Ter Beest M, Honigmann A, van den Bogaart G. 2016. SWAP70 organizes the actin cytoskeleton and is essential for phagocytosis. Cell Rep 17:1518–1531. https://doi.org/10.1016/j.celrep.2016.10.021.
264. Williams TA, Embley TM. 2015. Changing ideas about eukaryotic origins. Philos Trans R Soc Lond B Biol Sci 370:20140318. https://doi.org/10.1098/rstb.2014.0318.
265. McInerney JO, O'Connell MJ. 2017. Microbiology: mind the gaps in cellular evolution. Nature 541:297–299. https://doi.org/10.1038/nature21113.
266. Barr FA. 2013. Rab GTPases and membrane identity: causal or inconsequential? J Cell Biol 202:191–199. https://doi.org/10.1083/jcb.201306010.
267. Nasir A, Kim KM, Caetano-Anolles G. 2015. Lokiarchaeota: eukaryote-like missing links from microbial dark matter? Trends Microbiol 23:448–450. https://doi.org/10.1016/j.tim.2015.06.001.
268. Ettema TJG, Lindås A-C, Bernander R. 2011. An actin-based cytoskeleton in archaea. Mol Microbiol 80:1052–1061. https://doi.org/10.1111/j.1365-2958.2011.07635.x.
269. Carballido-Lopez R. 2006. The bacterial actin-like cytoskeleton. Microbiol Mol Biol Rev 70:888–909. https://doi.org/10.1128/MMBR.00014-06.
270. Swulius MT, Jensen GJ. 2012. The helical MreB cytoskeleton in Escherichia coli MC1000/pLE7 is an artifact of the N-terminal yellow fluorescent protein tag. J Bacteriol 194:6382–6386. https://doi.org/10.1128/JB.00505-12.
271. Swulius MT, Chen S, Ding HJ, Li Z, Briegel A, Pilhofer M, Tocheva EI, Lybarger SR, Johnson TL, Sandkvist M, Jensen GJ. 2011. Long helical filaments are not seen encircling cells in electron cryotomograms of rod-shaped bacteria. Biochem Biophys Res Commun 407:650–655. https://doi.org/10.1016/j.bbrc.2011.03.062.
272. Ghoshdastider U, Jiang S, Popp D, Robinson RC. 2015. In search of the primordial actin filament. Proc Natl Acad Sci U S A 112:9150–9151. https://doi.org/10.1073/pnas.1511568112.
273. Flannagan RS, Jaumouillé V, Grinstein S. 2012. The cell biology of phagocytosis. Annu Rev Pathol 7:61–98. https://doi.org/10.1146/annurev-pathol-011811-132445.
274. Li GH, Arora PD, Chen Y, McCulloch CA, Liu P. 2012. Multifunctional roles of gelsolin in health and diseases. Med Res Rev 32:999–1025. https://doi.org/10.1002/med.20231.
275. Kusano H, Shimizu S, Koya RC, Fujita H, Kamada S, Kuzumaki N, Tsujimoto Y. 2000. Human gelsolin prevents apoptosis by inhibiting apoptotic mitochondrial changes via closing VDAC. Oncogene 19:4807–4814. https://doi.org/10.1038/sj.onc.1203868.
276. Samson RY, Obita T, Freund SM, Williams RL, Bell SD. 2008. A role for the ESCRT system in cell division in archaea. Science 322:1710–1713. https://doi.org/10.1126/science.1165322.
277. Samson RY, Bell SD. 2011. Cell cycles and cell division in the archaea. Curr Opin Microbiol 14:350–356. https://doi.org/10.1016/j.mib.2011.04.005.
278. Lindås A-C, Bernander R. 2013. The cell cycle of archaea. Nat Rev Microbiol 11:627–638. https://doi.org/10.1038/nrmicro3077.
279. Hurley JH. 2015. ESCRTs are everywhere. EMBO J 34:2398–2407. https://doi.org/10.15252/embj.201592484.
280. Christ L, Raiborg C, Wenzel EM, Campsteijn C, Stenmark H. 2016. Cellular functions and molecular mechanisms of the ESCRT membrane-scission machinery. Trends Biochem Sci 42:42–56. https://doi.org/10.1016/j.tibs.2016.08.016.
281. Praefcke GJK, McMahon HT. 2004. The dynamin superfamily: universal membrane tubulation and fission molecules? Nat Rev Mol Cell Biol 5:133–147. https://doi.org/10.1038/nrm1313.
282. Purkanti R, Thattai M. 2015. Ancient dynamin segments capture early stages of host-mitochondrial integration. Proc Natl Acad Sci U S A 112:2800–2805. https://doi.org/10.1073/pnas.1407163112.
283. Römer W, Pontani L-L, Sorre B, Rentero C, Berland L, Chambon V, Lamaze C, Bassereau P, Sykes C, Gaus K, Johannes L. 2010. Actin dynamics drive membrane reorganization and scission in clathrin-independent endocytosis. Cell 140:540–553. https://doi.org/10.1016/j.cell.2010.01.010.
284. Rachel R, Wyschkony I, Riehl S, Huber H. 2002. The ultrastructure of *Ignicoccus*: evidence for a novel outer membrane and for intracellular vesicle budding in an archaeon. Archaea 1:9–18. https://doi.org/10.1155/2002/307480.
285. Ellen AF, Zolghadr B, Driessen AMJ, Albers S-V. 2010. Shaping the archaeal cell envelope. Archaea 2010:608243. https://doi.org/10.1155/2010/608243.
286. Gupta RS. 2011. Origin of diderm (Gram-negative) bacteria: antibiotic selection pressure rather than endosymbiosis likely led to the evolution of bacterial cells with two membranes. Antonie Van Leeuwenhoek 100:171–182. https://doi.org/10.1007/s10482-011-9616-8.
287. Vale RD. 1987. Intracellular transport using microtubule-based motors. Annu Rev Cell Biol 3:347–378. https://doi.org/10.1146/annurev.cb.03.110187.002023.
288. Apodaca G. 2001. Endocytic traffic in polarized epithelial cells: role of the actin and microtubule cytoskeleton. Traffic 2:149–159. https://doi.org/10.1034/j.1600-0854.2001.020301.x.

289. Higuchi T, Uhlmann F. 2005. Stabilization of microtubule dynamics at anaphase onset promotes chromosome segregation. Nature 433:171–176. https://doi.org/10.1038/nature03240.
290. Meunier S, Vernos I. 2016. Acentrosomal microtubule assembly in mitosis: the where, when, and how. Trends Cell Biol 26:80–87. https://doi.org/10.1016/j.tcb.2015.09.001.
291. Miller AL. 2011. The contractile ring. Curr Biol 21:R976–R978. https://doi.org/10.1016/j.cub.2011.10.044.
292. Dey G, Thattai M, Baum B. 2016. On the archaeal origins of eukaryotes and the challenges of inferring phenotype from genotype. Trends Cell Biol 26:476–485. https://doi.org/10.1016/j.tcb.2016.03.009.
293. Zientz E, Dandekar T, Gross R. 2004. Metabolic interdependence of obligate intracellular bacteria and their insect hosts. Microbiol Mol Biol Rev 68:745–770. https://doi.org/10.1128/MMBR.68.4.745-770.2004.
294. Husnik F, Nikoh N, Koga R, Ross L, Duncan RP, Fujie M, Tanaka M, Satoh N, Bachtrog D, Wilson ACC, von Dohlen CD, Fukatsu T, McCutcheon JP. 2013. Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis. Cell 153:1567–1578. https://doi.org/10.1016/j.cell.2013.05.040.
295. Husnik F, McCutcheon JP. 2016. Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. Proc Natl Acad Sci U S A 113:E5416–E5424. https://doi.org/10.1073/pnas.1603910113.
296. Embley TM, Finlay BJ. 1994. Systematic and morphological diversity of endosymbiotic methanogens in anaerobic ciliates. Antonie Van Leeuwenhoek 64:261–271. https://doi.org/10.1007/BF00873086.
297. Fenchel T, Bernard C. 1993. A purple protist. Nature 362:300. https://doi.org/10.1038/362300a0.
298. Duplessis MR, Ziebis W, Gros O, Caro A, Robidart J, Felbeck H. 2004. Respiration strategies utilized by the gill endosymbiont from the host lucinid Codakia orbicularis (Bivalvia: Lucinidae). Appl Environ Microbiol 70:4144–4150. https://doi.org/10.1128/AEM.70.7.4144-4150.2004.
299. Woyke T, Teeling H, Ivanova NN, Huntemann M, Richter M, Gloeckner FO, Boffelli D, Anderson IJ, Barry KW, Shapiro HJ, Szeto E, Kyrpides NC, Mussmann M, Amann R, Bergin C, Ruehland C, Rubin EM, Dubilier N. 2006. Symbiosis insights through metagenomic analysis of a microbial consortium. Nature 443:950–955. https://doi.org/10.1038/nature05192.
300. Schmid AMM. 2003. Endobacteria in the diatom *Pinnularia* (Bacillariophyceae). I. "Scattered ct-nucleoids" explained: DAPI-DNA complexes stem from exoplastidial bacteria boring into the chloroplasts. J Phycol 39:122–138. https://doi.org/10.1046/j.1529-8817.2003.02084.x.
301. Thacker RW. 2005. Impacts of shading on sponge-Cyanobacteria symbioses: a comparison between host-specific and generalist associations. Integr Comp Biol 45:369–376. https://doi.org/10.1093/icb/45.2.369.
302. Wujek DE. 1979. Intracellular bacteria in the blue-green alga *Pleurocapsa minor*. Trans Am Microsc Soc 98:143–145. https://doi.org/10.2307/3225953.
303. Sockett RE. 2009. Predatory lifestyle of *Bdellovibrio bacteriovorus*. Annu Rev Microbiol 63:523–539. https://doi.org/10.1146/annurev.micro.091208.073346.
304. Stolp H, Starr MP. 1963. *Bdellovibrio bacteriovorus* gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie Van Leeuwenhoek 29:217–248. https://doi.org/10.1007/BF02046064.
305. Starr MP, Seidler RJ. 1971. The bdellovibrios. Annu Rev Microbiol 25:649–678. https://doi.org/10.1146/annurev.mi.25.100171.003245.
306. Jurkevitch E. 2006. Predatory prokaryotes. Springer, Berlin, Germany.
307. Davidov Y, Huchon D, Koval SF, Jurkevitch E. 2006. A new alpha-proteobacterial clade of Bdellovibrio-like predators: implications for the mitochondrial endosymbiotic theory. Environ Microbiol 8:2179–2188. https://doi.org/10.1111/j.1462-2920.2006.01101.x.
308. Davidov Y, Jurkevitch E. 2009. Predation between prokaryotes and the origin of eukaryotes. Bioessays 31:748–757. https://doi.org/10.1002/bies.200900018.
309. von Dohlen CD, Kohler S, Alsop ST, McManus WR. 2001. Mealybug beta-proteobacterial endosymbionts contain gamma-proteobacterial symbionts. Nature 412:433–436. https://doi.org/10.1038/35086563.
310. Yamaguchi M, Mori Y, Kozuka Y, Okada H, Uematsu K, Tame A, Furukawa H, Maruyama T, Worman CO, Yokoyama K. 2012. Prokaryote or eukaryote? A unique microorganism from the deep sea. J Electron Microsc (Tokyo) 61:423–431. https://doi.org/10.1093/jmicro/dfs062.
311. Mariconti M, Epis S, Gaibani P, Dalla Valle C, Sassera D, Tomao P, Fabbi M, Castelli F, Marone P, Sambri V, Bazzocchi C, Bandi C. 2012. Humans parasitized by the hard tick *Ixodes ricinus* are seropositive to *Midichloria mitochondrii*: is *Midichloria* a novel pathogen, or just a marker of tick bite? Pathog Glob Health 106:391–396. https://doi.org/10.1179/2047773212Y.0000000050.
312. Schleper C, Holz I, Janekovic D, Murphy J, Zillig W. 1995. A multicopy plasmid of the extremely thermophilic archaeon Sulfolobus effects its transfer to recipients by mating. J Bacteriol 177:4417–4426. https://doi.org/10.1128/jb.177.15.4417-4426.1995.
313. Kuwabara T, Minaba M, Iwayama Y, Inouye I, Nakashima M, Marumo K, Maruyama A, Sugai A, Itoh T, Ishibashi J-I, Urabe T, Kamekura M. 2005. *Thermococcus coalescens* sp. nov., a cell-fusing hyperthermophilic archaeon from Suiyo Seamount. Int J Syst Evol Microbiol 55:2507–2514. https://doi.org/10.1099/ijs.0.63432-0.
314. Naor AA, Lapierre PP, Mevarech MM, Papke RTR, Gophna UU. 2012. Low species barriers in halophilic archaea and the formation of recombinant hybrids. Curr Biol 22:1444–1448. https://doi.org/10.1016/j.cub.2012.05.056.
315. Naor A, Gophna U. 2014. Cell fusion and hybrids in archaea. Bioengineered 4:126–129. https://doi.org/10.4161/bioe.22649.
316. Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jørgensen BB, Witte U, Pfannkuche O. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626. https://doi.org/10.1038/35036572.
317. Dekas AE, Poretsky RS, Orphan VJ. 2009. Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. Science 326:422–426. https://doi.org/10.1126/science.1178223.
318. Fenchel T, Finlay BJ. 2010. Free-living protozoa with endosymbiotic methanogens, p 2–9. *In* Hackstein J (ed), (Endo)symbiotic methanogenic archaea. Springer, Berlin, Germany.
319. Whitman WB, Coleman DC, Wiebe WJ. 1998. Prokaryotes: the unseen majority. Proc Natl Acad Sci U S A 95:6578–6583. https://doi.org/10.1073/pnas.95.12.6578.
320. Nelson-Sathi S, Sousa FL, Roettger M, Lozada-Chávez N, Thiergart T, Janssen A, Bryant D, Landan G, Schönheit P, Siebers B, McInerney JO, Martin WF. 2015. Origins of major archaeal clades correspond to gene acquisitions from bacteria. Nature 517:77–80. https://doi.org/10.1038/nature13805.
321. McInerney JO, Cotton JA, Pisani D. 2008. The prokaryotic tree of life: past, present. . .and future? Trends Ecol Evol 23:276–281. https://doi.org/10.1016/j.tree.2008.01.008.
322. Hrdý I, Hirt RP, Doležal P, Bardonová L, Foster PG, Tachezy J, Embley TM. 2004. Trichomonas hydrogenosomes contain the NADH dehydrogenase module of mitochondrial complex I. Nature 432:618–622. https://doi.org/10.1038/nature03149.
323. Kelly S, Wickstead B, Gull K. 2011. Archaeal phylogenomics provides evidence in support of a methanogenic origin of the Archaea and a thaumarchaeal origin for the eukaryotes. Proc Biol Sci 278:1009–1018. https://doi.org/10.1098/rspb.2010.1427.
324. Sousa FL, Martin WF. 2014. Biochemical fossils of the ancient transition from geoenergetics to bioenergetics in prokaryotic one carbon compound metabolism. Biochim Biophys Acta 1837:964–981. https://doi.org/10.1016/j.bbabio.2014.02.001.
325. Liu Y, Beer LL, Whitman WB. 2012. Methanogens: a window into ancient sulfur metabolism. Trends Microbiol 20:251–258. https://doi.org/10.1016/j.tim.2012.02.002.
326. Fischer WW, Hemp J, Johnson JE. 2016. Evolution of oxygenic photosynthesis. Annu Rev Earth Planet 44:647–683. https://doi.org/10.1146/annurev-earth-060313-054810.
327. Petersen J, Brinkmann H, Bunk B, Michael V, Paeuker O, Pradella S. 2012. Think pink: photosynthesis, plasmids and the Roseobacter clade. Environ Microbiol 14:2661–2672. https://doi.org/10.1111/j.1462-2920.2012.02806.x.
328. Weiss MC, Sousa FL, Mrnjavac N, Neukirchen S, Roettger M, Nelson-Sathi S, Martin WF. 2016. The physiology and habitat of the last universal common ancestor. Nat Microbiol 1:16116. https://doi.org/10.1038/nmicrobiol.2016.116.
329. Javaux EJ, Knoll AH, Walter MR. 2001. Morphological and ecological complexity in early eukaryotic ecosystems. Nature 412:66–69. https://doi.org/10.1038/35083562.
330. Parfrey LW, Lahr DJG, Knoll AH, Katz LA. 2011. Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proc Natl Acad Sci U S A 108:13624–13629. https://doi.org/10.1073/pnas.1110633108.
331. Baum DA. 2015. A comparison of autogenous theories for the origin of

eukaryotic cells. Am J Bot 102:1954–1965. https://doi.org/10.3732/ajb.1500196.
332. Garg S, Stölting J, Zimorski V, Rada P, Tachezy J, Martin WF, Gould SB. 2015. Conservation of transit peptide-independent protein import into the mitochondrial and hydrogenosomal matrix. Genome Biol Evol 7:2716–2726. https://doi.org/10.1093/gbe/evv175.
333. McMahon HT, Boucrot E. 2011. Molecular mechanism and physiological functions of clathrin-mediated endocytosis. Nat Rev Mol Cell Biol 12:517–533. https://doi.org/10.1038/nrm3151.
334. Mayor S, Parton RG, Donaldson JG. 2014. Clathrin-independent pathways of endocytosis. Cold Spring Harb Perspect Biol 6:a016758. https://doi.org/10.1101/cshperspect.a016758.
335. Lajoie P, Nabi IR. 2010. Lipid rafts, caveolae, and their endocytosis. Int Rev Cell Mol Biol 282:135–163. https://doi.org/10.1016/S1937-6448(10)82003-9.
336. Bastiani M, Parton RG. 2010. Caveolae at a glance. J Cell Sci 123:3831–3836. https://doi.org/10.1242/jcs.070102.
337. El-Sayed A, Harashima H. 2013. Endocytosis of gene delivery vectors: from clathrin-dependent to lipid raft-mediated endocytosis. Mol Ther 21:1118–1130. https://doi.org/10.1038/mt.2013.54.
338. Damke H, Baba T, Vanderbliek AM, Schmid SL. 1995. Clathrin-independent pinocytosis is induced in cells overexpressing a temperature-sensitive mutant of dynamin. J Cell Biol 131:69–80. https://doi.org/10.1083/jcb.131.1.69.
339. Li G, D'Souza-Schorey C, Barbieri MA, Cooper JA, Stahl PD. 1997. Uncoupling of membrane ruffling and pinocytosis during Ras signal transduction. J Biol Chem 272:10337–10340. https://doi.org/10.1074/jbc.272.16.10337.
340. Underhill DM, Goodridge HS. 2012. Information processing during phagocytosis. Nat Rev Immunol 12:492–502. https://doi.org/10.1038/nri3244.
341. Pratten MK, Lloyd JB. 1986. Pinocytosis and phagocytosis: the effect of size of a particulate substrate on its mode of capture by rat peritoneal macrophages cultured in vitro. Biochim Biophys Acta 881:307–313. https://doi.org/10.1016/0304-4165(86)90020-6.
342. Champion JA, Walker A, Mitragotri S. 2008. Role of particle size in phagocytosis of polymeric microspheres. Pharm Res 25:1815–1821. https://doi.org/10.1007/s11095-008-9562-y.
343. Paul D, Achouri S, Yoon Y-Z, Herre J, Bryant CE, Cicuta P. 2013. Phagocytosis dynamics depends on target shape. Biophys J 105:1143–1150. https://doi.org/10.1016/j.bpj.2013.07.036.
344. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 45:D353–D361. https://doi.org/10.1093/nar/gkw1092.
345. Rejman J, Oberle V, Zuhorn IS, Hoekstra D. 2004. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem J 377:159–169. https://doi.org/10.1042/bj20031253.