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Acid digestion and symbiont: Proton sharing at the origin of mitochondriogenesis?

Proton production by a symbiotic bacterium may have been the origin of two hallmark eukaryotic features, acid digestion and mitochondria

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Abstract

The initial relationships between organisms leading to endosymbiosis and the first eukaryote are currently a topic of hot debate. Here, I present a theory that offers a gradual scenario in which the origins of phagocytosis and mitochondria are intertwined in such a way that the evolution of one would not be possible without the other. In this scenario, the premitochondrial bacterial symbiont became initially associated with a protophagocytic host on the basis of cooperation to kill prey with symbiont-produced toxins and reactive oxygen species (ROS). Subsequently, the cooperation was focused on the digestion stage, through the acidification of the protophagocytic cavities via exportation of protons produced by the aerobic respiration of the symbiont. The host gained an improved phagocytic capacity and the symbiont received organic compounds from prey. As the host gradually lost its membrane energetics to develop lysosomal digestion, respiration was centralized in the premitochondrial symbiont for energy production for the consortium.

KEYWORDS
acid digestion, eukaryogenesis, mitochondriogenesis, phagocytosis, proton gradient

INTRODUCTION

The origins of the amazing complexity common to all the eukaryotic organisms is one of the outstanding enigmas of biology. This complexity, in turn, is what appears to have enabled the evolution of the great variety of multicellular forms that we can see today. To imagine how such complexity was initially attained, begs many questions whose answers, for now, remain largely speculative. However, the acquisition of information from extant living systems is narrowing the possibility space in which those origins could have taken shape. Today, it is broadly accepted that eukaryotes were built as a mosaic in which one of the initial components was an archaeal-like organism that lived in the Proterozoic era.[1–3] In particular, the discovery of the asgard archaea[4] and the isolation of one of its members has put the focus on this clade for its possible affiliation with the eukaryotic archaeal-component (refs. [5, 6] see also ref. [7]). Also, it seems very probable that the organism that originated the mitochondria branched from the alphaproteobacterial class.[8,9]

However, from this point on, two main groups of theories diverge, in simplified terms, into the metabolism-based models and the phagocytosis-first theories (see [10, 11] and references therein). The models based on metabolism (syntrophy) propose combinations of

Abbreviations: ADMit, acid digestion-mitochondria; LECA, last eukaryotic common ancestor; ROS, reactive oxygen species.

host-symbiont/s that were coupled by the exchange of carbon sources and electron donors and acceptors,[12] ensuring that the consortium was not only stable, but that it would advance toward better integration.[13] Integration and functional adaptation of its members, with their corresponding genetic transactions, led to eukaryotic complexity. However, a potential problem with the syntrophic theories is that they need a mechanism for endogenization of the symbiont.[14] In addition to that, once a consortium is stable, it still must, i) advance to an endosymbiosis, and ii) transition to a phagocytic lifestyle and/or develop other eukaryotic features.

We may consider the evolution of eukaryotic metabolism as having two aspects; the acquisition of organic matter, and the conversion of that matter into energy for the consortium. While the syntrophic models focus on the second part, the phagocytosis-first family of theories focuses on the acquisition of organic matter, considering phagocytosis to be the key novelty of eukaryotes, since this process is extremely rare in prokaryotes, with only one reported case.[15,16] In general, this family of theories posits a late mitochondrial acquisition, and they prefigure an (asgard) archaeon, gradually incorporating bacterial genes by horizontal gene transfer (HGT), or new genes by *invention*. In this way, the endomembrane system and phagocytic capability took shape in this ancestral organism,[17] at some point acquiring the mitochondrial endosymbiont and making use of its energetic potential to evolve to LECA. For the premitochondrial merger, initial prey, parasitic, and pathogenic situations have been proposed, in addition to the mutualistic one.[18,19]

One of the early formulations of the phagocytic theory was proposed by T. Cavalier-Smith, based on a phagotrophic amitochondriate eukaryote ancestor.[15,16,20,21] Since evidence of this ancestor has never been found, this early formulation was abandoned. However, within this group of theories, a mito-intermediate scenario has also been proposed in which the archaeon host was first able to produce membrane protrusions and the acquisition of the symbiont enabled the rest of the evolutionary process.[22] In this line, the phagocytic archaeon theory (PhAT)[23] is based on the fact that many eukaryotic-specific proteins (ESPs) occur in archaea from the TACK superphylum, suggesting again that the archaeal pre-host had phagocytic capacity and that feature was eventually the key for the acquisition of mitochondria.

The phagocytosis first models, in general, are less specific on the metabolic exchange that would prompt the initial symbiosis to occur, rather they focus on the cellular features key for eukaryogenesis.[8,24] However, in this respect, even the energetic feasibility of an initial amitochondriate phagocytic lifestyle has been questioned.[25]

In the current state of the debate, it is strongly contested if LECA was actually a phagocytic organism.[26–28] And this despite the fact that, although extremely rare in prokaryotes,[29] phagocytosis is widespread in eukaryotes, being present in the earliest branching representatives,[30,31] and seemingly compatible with the deduced minimal protein set of LECA.[1,32,33] On the other hand, it appears that none of the extant representatives of asgard archaea are predicted to be phagocytic.[34]

Yet, protophagocytosis would represent a new way to obtain organic matter, and it would have put their first representatives at the top of the trophic chain. However, regarding its origin, we must admit that it is quite possible that protophagocytosis was initially developed for a different function, such as the elimination of parasites. Aerobic respiration, on the other hand, is the most efficient metabolism in the presence of oxygen and a source of organic matter. Could both features, protophagocytosis and aerobic respiration, have been combined almost from the beginning in a self-reinforcing cycle? And, what specific selective mechanisms would have been needed for that to occur, in light of both short prokaryotic generation-times and much more extended periods of evolutionary development?

This theory (ADMit, Acid Digestion-Mitochondria) offers a narrative to incorporate the premitochondrial symbiont to a protophagocytic host at a time when evolution was starting to shape the predatory capabilities of that host. The main features of this theory are as follows: (1) a series of steps for the association of the host with premitochondrial bacteria that would involve advantages for both sides, probably being easily selected in evolution, and many of which are observed in extant organisms; (2) incorporation of bottlenecks to explain the monophyly of eukaryotes; (3) compatibility with several of the theories now in discussion regarding metabolism and phagotrophy; and (4) being mito-early, it matches mitochondrial symbiosis with the origins of phagocytosis. For a glossary of terms used in this article, please see Box 1.

RESULTS

1. The beginning: A protophagocytic host

The starting point for this theory is a primitive protophagocytic host (Box 1), with a metabolism based on organic compounds, obtained through predation, as source of carbon and electron donors (see Figure 1, 1.). A number of genes for membrane processes would have already been acquired/evolved (potentially by pre-symbiosis), and digestive enzymes that would have also been incorporated early on. This would correspond to the beginning of phagocytic predation, although inefficient at this stage. The environment is appropriate for mesophiles and the oxygen concentration is moderate, albeit fluctuating, and increasing geologically. This host may be a single organism (similar to asgard archaea), a consortium, or a symbiotic organism, but other details of its metabolism are not defined in this theory.

The question of what was the host that associated the bacterium that eventually originated the mitochondria is very complex and intensely debated today. Such a protophagocyte, as I take as starting point, has previously been proposed as the initial stage for the development of a phagocytic eukaryotic cell.[16] From a metabolic perspective, there are several models that could fit the image of this host. Given that the host is feeding on organic compounds supplied by the digestion of prey, the models Reverse Flow (RF),[35] Entangle-Engulf-Endogenize (E3),[5] or hydrogen and sulfur-transfer-based model (HS Syntrophy)[36] would all be compatible with this theory, at least in its initial stages. This is because all three hypotheses postulate a host (archaeal in RF and E3 and a deltaproteobacterium in HS) that acquires organic compounds from the environment to be metabolized, and sub-

Box 1: Glossary of terms

Acid digestion: Biological process of enzymatic hydrolysis of organic matter in a low-pH microenvironment.

Protophagocytic host: Hypothetical initial host, from a time point before symbiosis to after the endosymbiosis event. Capable of predation on bacteria (bacterivory) as primary source of matter, although not necessarily being its only source. It would capture the prey by entanglement with membrane protrusions followed by digestion of the prey with extracellular enzymes. The acquisition of organic matter would be performed by transport of the digestion products (osmotrophy) after the enzymatic degradation of prey. This mode of predation is primitive and should not be considered to be under the modern definition of phagocytosis.

Symbiont: Premitochondrial alphaproteobacterium associated with the host.

Prey: Bacteria, of any species.

Protophagocytic cavities: Cavities formed by the membrane protrusions or membrane concavities of the host. They can contain prey, commensal bacteria, or symbionts.

Mito-early theories: Theories that postulate an early incorporation of the premitochondrial symbiont into the evolutionary lineage that gave rise to LECA. The incorporation happened earlier than modern-type phagocytosis, an endomembrane system or the nucleus, in case of ADmit.

Mito-late theories: Theories in which mitochondria are incorporated relatively late in the evolutionary line that gave rise to LECA. Traditionally, in these theories, the host was considered to already be phagocytic and having an endomembrane system before the acquisition of mitochondria.

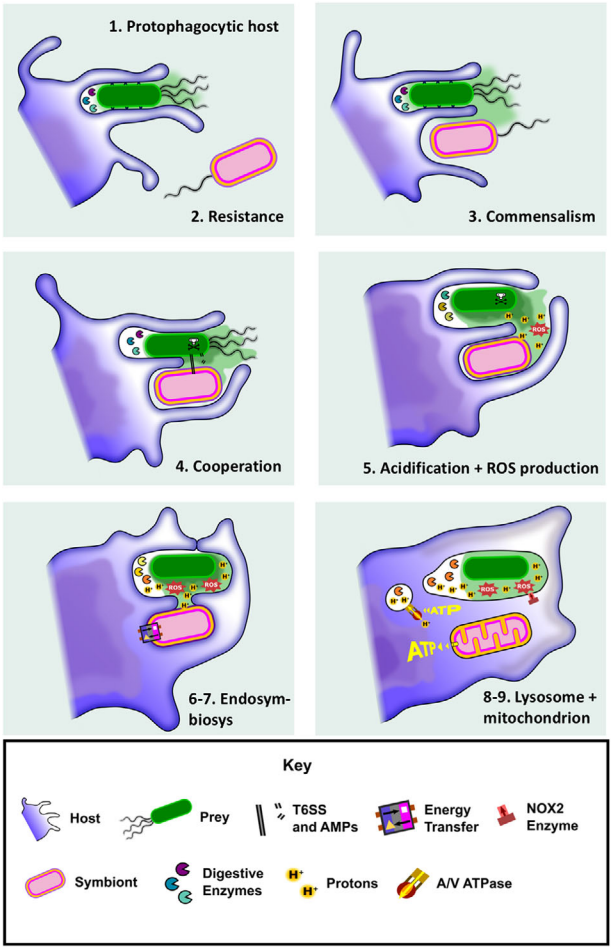

FIGURE 1 Stages in mitochondriogenesis. 1. Evolution of a protophagocytic host; 2. Appearance of resistant bacteria; 3. Certain bacteria become commensals; 4. Some commensals evolve a mutualistic relationship with the host based on their ability to help kill prey; 5. The mutualistic bacteria produce acidification of the phagocytic microenvironment; 6. The aerobic premitochondrial symbiont exports protons and ROS; 7. The bacterium becomes an endosymbiont while supporting evolution of acidic digestion; 8. Compartmentalization of acidic digestion in lysosomes and 9. Compartimentalization of endosymbiont as mitochondrion. Key: T6SS, type 6 secretion system; AMP, antimicrobial peptides

sequently transfer some of the resultant compounds to the symbionts. The organic matter, according to this theory, could be obtained by protophagocytosis.

The protophagocytotic mechanism would not, at this point, imply an actual endosome. More likely, it would be based on the entanglement of prey with membrane processes, as proposed,[15,16] followed by hydrolysis and import of the resultant monomers. A more complex and efficient engulfment process would not be developed until later on, which means that, at this stage, simple diffusion to the surrounding environment during the digestion process would lead to an important loss of organic matter. Additionally, prey would predictably develop resistance mechanisms, prompting the protophagocyte to optimize its capture and digestion mechanisms.

We can imagine the exterior of the protophagocyte as something similar to that of the asgard archaeon *Candidatus Prometheoarchaeum* syntrophycum. This organism degrades amino acids by means of syntrophy with a sulfate-reducing bacteria and a methanogenic archaeon, and, conspicuously, has long membrane protrusions.[5] Along the eukaryotic evolutionary path, similar protrusions in a related host organism could have served to capture the prey by entanglement (E3 model).

For an asgard archaeal-like organism to become protophagocytic, it still would probably have had to integrate many additional genes within its genome. We can hypothesize that the capture and digestion of bacteria would require multiple genes: to direct the membrane processes for entanglement[22,21]; receptors to latch onto the surface of bacteria[37,38]; and exportable hydrolytic enzymes directed against bacterial polymers and components.[39] Interestingly, recent surveys of common eukaryotic ancestral gene families suggest that before the acquisition of mitochondria, substantial incorporation of bacterial

genes into the genome of the host organism had already occurred [refs. [40, 41] see also ref. [42]]. These genes apparently originated not only from alphaproteobacteria, but also from many other bacterial groups with possible preference for some specific clades (Deltaproteobacteria, Planctomycetes, and others). While true that this gene transfer could have occurred by standard HGT, in the case of certain groups of genes with a clear affinity such as Deltaproteobacteria, a pre-symbiotic origin is also possible, as has been proposed.[36]

Advantageously, an archaeal-like membrane and cell envelope in a protophagocyte would not be easily attacked by the hydrolytic enzymes required to digest its bacterial prey. This is because the lipids and the carbohydrate coatings of the archaeal envelopes are generally different from those of bacteria (e.g., archaea do not contain peptidoglycan), and the enzymes that degrade bacterial membranes and walls do not hydrolyze the archaeal counterparts.[43] Interestingly, the asgard *Prometheoarchaeum* contains four genes for S-layer synthesis,[5] suggesting that the most common archaeal wall would be adequate to support membrane protrusions. Notwithstanding, a bacterial-type exterior could also be compatible with extracellular digestion, given that some bacteria are capable of exporting peptidoglycan hydrolyzing enzymes, as in the case of *Myxococcus xanthus*, a deltaproteobacterium.[44]

In this sense, if the host membrane were formed by archaeal lipids, at some point in eukaryogenesis they should have been replaced by bacterial ones, and this, at the level of whole organism is far from a straightforward evolutionary change, with no other example known in biology. However, there exists at least one natural example of mixed bacterial-archaeal lipid membranes.[45]

2. Some bacteria evolve resistance to the primitive form of predation

Evolution of resistance to threats is a very common event for present-day bacteria. When some form of protophagocytic predation first appeared and got extended, it would have prompted their bacterial prey to evolve resistance strategies (Figure 1, 2.). Among these, several including, high motility,[46] filamentation,[47] change of surface receptors,[48] and others, can be seen in many bacterial species today.[49,50] Furthermore, a number of bacterial species have been reported to show resistance to digestion by protozoa.[51,52]

During the early stages of phagocytosis evolution, elaborate resistance mechanisms would probably not have been needed. From this theoryt's point of view, simple resistance to the external digestive enzymes (operating at an environmental pH near neutral) would have been enough, of which there are many present-day examples.[53]

3. Resistant bacteria become associated with the host as commensals

Commensalism by bacteria is ubiquitous in today's world. Our gastrointestinal microbiota, composed of a host of microorganisms, springs to mind.[54,55] There is also evidence of widespread non-pathogenic bacteria associated with unicellular phagocytes,[56,57] although the term commensal is more difficult to ascertain in this case.

As mentioned above, it is conceivable that primitive phagocytosis would not have been very efficient. Still, a nutritional mode based on extracellular digestion of prey could, in theory, allow survival of the host much sooner than evolution of the extremely efficient and complex eukaryotic phagocytosis, as proposed in earlier theories.[15] In this case, a proportion of the hydrolyzed or partially hydrolyzed organic matter from the prey would not be absorbed by the host, at least not quickly. That organic matter could be available for some commensal organisms to feed on (Figure 1, 3.). Initially these commensal bacteria would not have entailed a benefit for the host, but the physiological burden would not have been substantial, either.[58]

The commensal bacteria could be of several different types at this stage, but at least one would be an oxygenic respirator and facultative anaerobe, branching from the stem of alphaproteobacteria.[9] Let us remember that some groups of extant alphaproteobacteria are specialized at modes of living inside eukaryotic hosts.[59] The preferred location for the commensals would be near the protophagocytic cavities (Box 1). The bacteria could home to the phagosome by chemotaxis followed by attachment to the host exterior.[60]

4. Soon, the bacterium starts to help the host on prey-killing, with injectable toxins and/or bactericidal molecules

This step would represent the beginning of true symbiosis, since a more efficient predation leads to a greater amount of nutrients available for the consortium (Figure 1, 4.). This scenario is also compatible with an initial metabolic exchange.

When protophagocytosis originated, in order to be effective, it would have had to contend with a series of problems. Once the bacterial prey was entangled, it still had to be killed and digested, which would eventually happen by exposure to hydrolytic enzymes. However, this process takes time, thus, present-day phagocytes are equipped with specific and rapid mechanisms to deactivate bacteria.[61] Bacteria themselves are specialists in producing agents to kill or thwart other bacteria, including antibiotics, bacteriocins, or toxins delivered through injection structures, among others (see Box 2). Thus, if the bacterial protosymbiont could have produced any of these bacterial-inactivating agents (AMPs, T6SS), it would have aided in the immobilization/killing of prey and, therefore, increased the predation efficiency of the host. This would constitute an initial, quick, and short-term selective advantage for the host-symbiont consortium that was independent of other exchanges that could be established

While, in principle, there could have been many species capable of establishing an association with the host, only the most beneficial combination would remain over the long term. Parasitic or even neutral associations would have been purged with time. AMPs and T6SS (Box 2) are bacterial traits, such that the genes conferring these traits could not have been easily transferred to the host by HGT.

Box 2: Possible bactericidal weapons of premitochondria

Bacteriocins or AMPs (antimicrobial peptides).[62] These are small, ribosomally produced, peptidic toxins that many bacteria secrete to eliminate other, potentially competitor, bacterial species. While more frequent in Gram-positive bacteria and considered to have narrow killing spectrum, it has been found that most bacterial species can produce these agents, and some bacteriocins are of wide spectrum.[63] The most common mode of bacteriocin action is through insertion and destabilization of the bacterial cytoplasmic membrane. Interestingly, it has been found that pore-forming peptides represent a broad killing mechanism not only in phagocytic cells, but in the majority of eukaryotes,[64–66] although these peptides do not show a clear relationship to bacterial ones.

T6SS (type 6 secretion system). A contact-dependent toxin injection mechanism is another type of weapon that could have been used by the premitochondrial symbiont, among which a typical example is the type VI secretion system (T6SS).[67,68] Widely extended in Gram-negative bacteria, this system injects one or several of a series of protein effectors directly into the cytoplasm of adjacent bacteria. Toxicity is produced in recipient cells through activities with wide killing spectrum such as DNases, lipases, lysozymes, or NAD(P) hydrolases. Of course, the use of this system in the context of premitochondrial symbiosis would have involved the non-activation of injection against the host, something that must happen in extant communities.

Other associations would not have been stable in the long run, leading to symbiont integration or loss.[69] Probably just one symbiont would be finally selected because the set of traits and adaptations to resist the harsh microenvironment (residence in protophagocytic cavities, resistance to digestion, and bacteria-killing mechanisms) would have been uncommon and forbidding to other bacteria. The niche offered by the host would be limiting, given that the hostt's growth rate most probably would be slower than that of the potential symbionts, and, therefore, the symbionts would be competing for that niche.

5. Under anoxic conditions, fermentation by the symbiont contributes to acidification of the protophagocytic cavities

Under local anoxic conditions, the symbiont would engage its facultative fermentative metabolism, consequently exporting acid metabolites outside of the bacterial cell, this is, to the protophagocytic cavities of the host (Figure 1, 5.). This would lead to the acidification of these cavities as the process of digestion proceeds. It is important to note here that it is not difficult that the host itself could also produce and export acidic molecules, as is proposed by the hydrogen hypothesis,[70] the RF or E3 models. However, the production of acidic metabolites by the bacterial symbiont could yield higher concentrations of them, given that the entire symbiont would be placed within the protophagocytic cavity, whereas in principle, the host would export the metabolites across all its surface. Still, the host could have developed some metabolite transport mechanism directed specifically to the protophagocytic cavities. However, a possible evolution and maintenance of that transport of acid metabolites to the cavities would be anti-economic for the host, since the cavities would be acidified in any case due to the symbiont's fermentation.

The routine accumulation of acidic metabolites in the protophagocytic cavities would have driven host and symbiont to develop adaptations to that acidic microenvironment.

6. Under aerobiosis, symbiont respiration produces protons and reactive oxygen species (ROS) that are exported to the protophagocytic cavities

Oxygenic respiration of organic matter is much more efficient than fermentation for ATP generation. Respiration is based on pumping protons to the outer side of the cytoplasmic membrane, building a proton motive force that drives the ATP production, as the protons reenter into the cytoplasm via the ATP synthase [see for recent reviews[71,72]]. Respiration is also more efficient than fermentation at converting organic compounds to protons outside the cell. Starting with a glucose molecule, only two acidic molecules would be exported by the most common fermentative metabolisms; whereas respiration of the same molecule with oxygen as final electron acceptor, in theory, transports 93 protons.[73] Under normal conditions, respiration does not acidify the surrounding media because the protons are confined to specific compartments (bacterial periplasm or intermembrane space of mitochondria) and because they continuously reenter the cytoplasm/matrix.

Now, let us imagine that the premitochondrial symbiont, due to the fact that is partially shielded from the outside, gradually acquires the ability of leaking protons to the external microenvironment (i.e., the protophagocytic cavities), this permeability being constitutive or regulated. These protons would have served to acidify the digestive microenvironment in aerobic conditions, similar to that seen in anoxia, and this acidification would have been adaptive, as I discussed below (Figure 1, 6.). For the bacterial premitochondrion, the loss of protons would not have been critical, (i) because bacteria have mechanisms to compensate pH shifts in any of the two directions in the environment,[74] (ii) the energetic loss involved would be compensated by the more efficient digestion, and (iii) a published hypothesis points to a special, more protected, proton route within the bacterial membrane.[75] In this scenario, acidification of the phagocytic microenvironment could potentially be more intense as it is driven by oxidative respiration.

Other respiration products exported by the symbiont to the protophagocytic environment would have been ROS, which could have helped to kill the prey. It is true that ROS are indiscriminately toxic molecules, so it follows that host and symbiont would have to get adapted to certain levels of ROS. The prey-killing ability of ROS has been well demonstrated in today's phagocytes.[76] In fact, mitochondrially produced ROS have been shown to have bactericidal activity within macrophages.[77,78] ROS have also been proposed to be a driver for eukaryogenesis since they would have prompted the evolution of the nucleus for protection of genomic DNA.[11]

In agreement with the ROS production idea, it has been shown that bacteria experimentally devoid of their peptidoglycan (L-forms) experience an increase in ROS production due to the stimulation of the electron transport chain, and the cells must compensate this imbalance in order to grow.[79,80] Thus, if the premitochondrial symbiont had undergone a reduction in its peptidoglycan wall, as an adaptation to live in association with the host,[81] it could have resulted in leakage of protons and ROS, with the adaptive consequences mentioned above.

7. A self-reinforcing cycle is established: acid enzymes evolve in response to the lowering of the pH of the cavities, which facilitates the digestion of prey. The premitochondrion becomes an endosymbiont

Acid digestion, a hallmark eukaryotic capability, has received little attention from the point of view of its evolutionary origins. Commonly, it is associated with the appearance of the lysosome and the endomembrane system,[82] yet, acid digestion may have been key to the process of eukaryogenesis.

As this theory posits, whether by fermentation or aerobic metabolism, the protoendosome would have been acidified, leading the digestive enzymes to evolve to work optimally at lower pH (Figure 1, 7.). From that point, a self-reinforced cycle would have been established: as enzymes adapted to withstand acidic conditions, it would have allowed the protoendosome to become progressively more acidic. This would entail an evolutionary gain, given that strong acid digestion would decisively facilitate the digestion of prey, increasing nutrient yield, and probably avoid the rise of resistance to digestion.

This cycle can be considered as a point of no return toward the mitochondrial endosymbiosis since the most successful symbiosis would have closed the window of opportunity for other associations to take place or survive. An inefficient protophagocyte would disappear upon the evolution of optimized hosts able to exploit the symbiosis, and, reciprocally, no other bacterial species would be able to outcompete the symbiont (now probably obligate) that became so closely associated and adapted to the host. The least efficient phagocytes would be counterselected by the evolution of prey and competitors, and by predation itself. Eventually, the symbiont would become obligate because the export of protons would require outer membrane and peptidoglycan modifications that would finally render the symbiont unfit to survive outside the host, while, at the same time, being well adapted not only to withstand but also to generate, the protophagosomal acid digestion conditions.

An acidic milieu denatures biopolymers (polysaccharides, proteins), making them more susceptible to the action of hydrolytic enzymes. For example, DNA itself is easily degraded under acid conditions. Evolution of resistance to acid digestion is not easy, and many current cell-invading pathogens block the formation of the late endosome or its fusion to the lysosome rather than withstanding the acid digestion.[74] Other bacteria can live in the mammalian stomach,[84] or can survive digestion by protozoa,[52] for instance, but they usually are specially adapted.

Why would the host need the symbiont to develop acid digestion capability? As mentioned above, the acidic species would concentrate around the bacterial volume while the much larger host cell area would not easily favor accumulation in specific regions. More importantly, rising environmental oxygen concentrations gradually drove aerobic respiration to take the place of other metabolisms, and, according to this theory, respiration would function as a much better proton source for acidification. Thus, it would be adaptive for the symbiont to harbor and maintain the oxygenic respiratory metabolism.

By this stage, probably, the premitochondrion was totally adapted to the protophagocytic compartment, with a metabolism complementary to that of the host. In this sense, the membrane energetics would be gradually reduced in the host, and the energy production by respiration dependent mostly or totally on the symbiont. An energy transfer from symbiont to host in the form of high-energy molecules is likely at this point. It would probably be based on fermentable sugars or membrane vesicles, (possibly contributing to the process of membrane lipid substitution), and, eventually, of ATP. If we assume that the growth rate of the larger host would have been slower than that of the symbiont, then the transfer of energy equivalents would have served to channel excess matter or energy from symbiont to host. Thus, the growth of the symbiont would be slowed and the cycles of the two partners would be synchronized.

Thus, at this advanced stage, the benefits contributed by the symbiont would have been:

a. Killing of prey by bacterial-specific mechanisms (AMPs and T6SS).
b. Acidification of the digestive compartment by fermentation or respiration.
c. Killing of prey with exported ROS.
d. Transfer of high-energy molecules.

The contribution of the host would have been to perform the protophagocytosis itself, and the symbiont would feed on the organic matter acquired in this particular way.

From the point of view of this theory, it is possible that archaeal and bacterial membranes could have still coexisted as late as this stage. However, in my opinion, from this point on, it would have been an evolutionary benefit to change to a bacterial membrane (for reasons of membrane homogeneity), in case that event hadnt't happened

yet. The bacterial membrane should remain in any case for efficient respiration.[75]

8. As the acid digestion becomes more aggressive, the host is prompted to compartmentalize the process

Although the protophagocyte's cell membrane would need to operate at environmental pH to capture and engulf the prey, it would have to withstand the acid digestion too. Thus, the endosome-lysosome system would evolve to separate the acid-adapted membranes from the external neutralophilic membranes of the cell (Figure 1, 8.). The protolysosome evolves as part of the anterograde membrane transport developed in the protoeukaryote, carrying the digestive enzymes within an acidic milieu for the targeted delivery to the phagocytic endosome by membrane fusion. Upon development of the endosome-lysosome, the archaeal ATPase would probably have been repurposed to acidify this compartment.[85,86] The endosome also acquired the enzyme NOX2[87] to produce ROS to kill prey independently of the symbiont. In this sense, phylogenesis shows that there are two large, related clusters of NOX gene sequences, one formed by the bacterial (NADPH) NOXs, and the other being the eukaryotic NOXs.[88] The simplest explanation would be that eukaryotes originally acquired NOX from bacteria, and one of the variants was incorporated as a way to generate ROS for use in the phagosome.

9. The respiring endosymbiont is also compartmentalized becoming the cell power plant in the form of the mitochondrion

Simultaneous to the compartmentalization of the digestion, the protomitochondrion probably lost its association with the digestive function and fully assumed its role as cellular power plant, its membrane energetics serving as the main source of ATP for the consortium (Figure 1, 9.). The appearance of the mitochondrial ATP carrier enabled the ATP-based energy exchange between host and endosymbiont.

Available evidence suggests a symbiont entry event in which the outer membrane of the symbiont is key; this membrane was subsequently maintained, and probably played an important role in the evolution of the host endomembrane system.[82,89] As the integration with the host advanced more and more, most of the bacterial genes were lost from its genome.

The evolutionary drive pushes in the direction of more integration with the host because this permits better coordination and regulation. The hallmark of the integration for the premitochondrion would be the influx of alphaproteobacterial genes to the host genome. From recent phylogenetic data, it is interesting to note that the evolutionary appearance of the lysosome occurred at approximately the same time as and the peak of alphaproteobacterial gene acquisition in the host genome.[41]

10. Postmitochondrial evolution to LECA

After the appearance of the mitochondrion as an organelle, the evolution of the protoeukaryote would probably have continued, adding, among other things, functions and pathways that are now typical or depend on the mitochondria, such as calcium regulation,[90] apoptosis pathways,[91] and others. Simultaneously, higher levels of optimization and integration between the different cell compartments would have been grown, up to the appearance of the organism that we call LECA.

DISCUSSION

The ADMit theory offers an alternative to the current "mito-early, mito- late" discussion. By fusing the phagocytosis and mitochondria origins, we can better explain observations suggesting that the origin of mitochondria is deeply ingrained in the evolution of the eukaryotic cell. Not only that, this theory provides a basis for the initial symbiosis and suggests the possible gains for both partners. Additionally, this theory could help to explain the monophyly and uniqueness of the eukaryotic domain. After the initial window starting with a protophagocytic organism as the host, there likely would not exist the opportunity for the right type of symbiosis to occur again. After a relatively short period, a point would be reached where it would be extremely difficult for a new candidate symbiont to outcompete the one that had already accumulated specific adaptations and significant mutual advantages with the host.

This theory connects the optimization of predation as the primary matter/energy source with a more efficient utilization of that income by respiratory metabolism, embodied by the symbiont, whose properties in turn helped to perfect predation. In this sense, ADMit theory would explain the extant amitochondriate phagocytes (see ref. [17]); while mitochondrial symbiosis was required to evolve the eukaryotic complexity, once evolved, it can operate without mitochondria under certain conditions.

This theory has also several issues to contend with. First, no symbiosis based on sharing protons has, so far, been described. However, this does not necessarily mean that such mechanism does not exist or has not in the past. In order to demonstrate its existence, we would have to search specifically for it, applying techniques that are not the usual ones to characterize microorganisms. Also, this theory is based on other symbiotic exchanges (prey killing with bacteriocins and ROS; and metabolite exchange) and it is compatible with at least three of the metabolic models proposed. So, even if some aspect of this theory is falsified, the central narrative of this model could still hold. It is important to note here that to the best of my knowledge there is no actual clue that would allow us to connect mitochondria with acidification of the phagosomal compartment. Still, we have to keep in mind that more than one eon has passed since the proposed events and possible traces could have been simply lost in evolution.

The fact that phagocytotic asgard archaea have not been found should not be an important obstacle, because it is apparent that the vast majority of transitional forms in the eukaryotic stem have been

lost. And, more generally, to judge the Proterozoic earth biosphere using as measure the extant biodiversity may lead us to discard plausible scenarios. Genomes, molecular characteristics and metabolisms, commonly, do not fossilize, and it is prudent to imagine that many lifeforms of entire eras remain lost to modern scientific consideration.

As commented in the Introduction, several recent articles have pointed to a non-phagocytic LECA.[26,27,28] While the most straightforward course for the ADMit theory would end up with a phagocytic LECA, we are considering phagocytosis as defined in extant organisms. This theory describes a protophagocytic organism, and ends not in LECA, but in an intermediate form (see ref. [26]), that probably had lysosomes, and by extension Golgi apparatus, endoplasmic reticulum, and mitochondria which are considered features common to all eukaryotes.[92,93] From that point to LECA, many events could have occurred including the secondary loss of protophagocytosis.

A recent report of a planctomycete bacterium with phagocytic-like capacity can provide a counterexample for ADMit theory.[29] The "*Candidatus* Uab amorphum" can effectively prey on other bacteria and picoeukaryotes by engulfing them with invaginations that comprise both the outer and the inner membrane of the planctomycete to form phagocytic-like vacuoles. It was not ascertained whether these vacuoles completely close, or if they maintain a connection with the outer surface instead. Interestingly, this organism carries few genes with similarity to eukaryotic phagocytosis genes, and acidic species or ROS were not detected. This finding would suggest that an alternative route to phagocytosis exists. However, at the moment, it appears that such a route did not result in a symbiotic process or bona fide eukaryotic phagocytosis.

How to prove or falsify this theory

Although the ADMit theory includes an explanation for the monophyly of eukaryotes, the same evolutionary bottlenecks would make it difficult to find extant examples close to the lines proposed by this theory. In any case, possible ways to falsify the ADMit theory would be the demonstration of several independent origins of phagocytosis or acid digestion in eukaryotes, the bona fide temporal dissociation between acquisition of phagocytosis and that of mitochondria in any of the two possible ways (first mitochondria then phagocytosis or the reverse), or even the discovery of a phagocytic organism that evolved acid digestion in a way not connected to eukaryotes, or symbiosis.

A special characteristic of this theory is that it would be possible to generate an experimental narrative based on the biological simulation of protophagocyte, symbiont and prey, and their evolution, and then observe whether and how, we can recapitulate the key steps put forth by the ADMit theory. In this sense, the main prediction would be that, without the right symbiont, acid digestion would not appear (see the planctomycete case[29]) or it would represent an evolutive dead-end for the protophagocyte. By mutualistic association with the symbiont, on the other hand, acid digestion would be developed, followed by metabolic specialization and a gradual increase in the complexity of the host (i.e., primitive endomembrane system).

CONCLUDING REMARKS

The ADMit theory offers a series of plausible stepping stones that allow the progressive selection of a more and more integrated consortium ending up with mitochondria in the cytoplasm of the host around the same time as the phagocytic ability took shape. This would help to explain the deep integration of mitochondria as a key actor in many functions of the eukaryotic cell. From the beginning, the mitochondrial precursor functionally participated in the evolution of eukaryotic complexity beyond the key role in energy production for which it is recognized.

With its main thread the evolution of acid digestion, the ADMit theory provides immediate benefits for both, host and symbiont, through optimized capture and digestion of prey, via bacteria-specific mechanisms. Then, the respiratory metabolism of the symbiont would be channeled to produce ROS to kill the prey and protons for the acid digestion. Higher levels of integration are incrementally gained as the protophagocytosis is continuously optimized and the energetic function is centralized in the symbiont to eventually yield a pre-LECA, phagocytic host, home to mitochondria. This path to eukaryogenesis is connected to ecology and prokaryotic physiology, and it may accommodate proposed metabolism-based models. New findings on the extant diversity of organisms and symbiosis, along with refinement of molecular phylogenies should provide ways to put this theory to the test in the future.

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CONFLICT OF INTEREST

The authors declare no competing interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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REFERENCES

1. Zaremba-Niedzwiedzka, K., Caceres, E. F., Saw, J. H., Bäckström, D., Juzokaite, L., Vancaester, E., Seitz, K. W., Anantharaman, K., Starnawski, P., Kjeldsen, K. U., Stott, M. B., Nunoura, T., Banfield, J. F., Schramm, A., Baker, B. J., Spang, A., & Ettema, T. J. G. (2017). Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature*, *541*(7637), 353–358. https://doi.org/10.1038/nature21031
2. Betts, H. C., Puttick, M. N., Clark, J. W., Williams, T. A., Donoghue, P. C. J., & Pisani, D. (2018). Integrated genomic and fossil evidence illuminates life's early evolution and eukaryote origin. *Nature Ecology & Evolution*, *2*(10), 1556–1562. https://doi.org/10.1038/s41559-018-0644-x

3. Javaux, E. J., & Lepot, K. (2018). The Paleoproterozoic fossil record: Implications for the evolution of the biosphere during Earth's middle-age. *Earth-Science Reviews*, *176*, 68–86. https://doi.org/10.1016/j.earscirev.2017.10.001
4. Spang, A., Saw, J. H., Jørgensen, S. L., Zaremba-Niedzwiedzka, K., Martijn, J., Lind, A. E., van Eijk, R., Schleper, C., Guy, L., & Ettema, T. J. G. (2015). Complex archaea that bridge the gap between prokaryotes and eukaryotes. *Nature*, *521*(7551), 173–179. https://doi.org/10.1038/nature14447
5. Imachi, H., Nobu, M. K., Nakahara, N., Morono, Y., Ogawara, M., Takaki, Y., Takano, Y., Uematsu, K., Ikuta, T., Ito, M., Matsui, Y., Miyazaki, M., Murata, K., Saito, Y., Sakai, S., Song, C., Tasumi, E., Yamanaka, Y., Yamaguchi, T., … Takai, K. (2020). Isolation of an archaeon at the prokaryote–eukaryote interface. *Nature*, *577*(7791), 519–525. https://doi.org/10.1038/s41586-019-1916-6
6. Wu, F., Speth, D. R., Philosof, A., Crémière, A., Narayanan, A., Barco, R. A., Connon, S. A., Amend, J. P., Antoshechkin, I. A., & Orphan, V. J. (2022). Unique mobile elements and scalable gene flow at the prokaryote–eukaryote boundary revealed by circularized Asgard archaea genomes. *Nature Microbiology*, *7*(2), 200–212. https://doi.org/10.1038/s41564-021-01039-y
7. Liu, Y., Makarova, K. S., Huang, W.-C., Wolf, Y. I., Nikolskaya, A. N., Zhang, X., Cai, M., Zhang, C.-J., Xu, W., Luo, Z., Cheng, L., Koonin, E. V., & Li, M. (2021). Expanded diversity of Asgard archaea and their relationships with eukaryotes. *Nature*, 553–557. https://doi.org/10.1038/s41586-021-03494-3
8. Roger, A. J., Muñoz-Gómez, S. A., & Kamikawa, R. (2017). The origin and diversification of mitochondria. *Current Biology*, *27*(21), R1177–R1192. https://doi.org/10.1016/j.cub.2017.09.015
9. Martijn, J., Vosseberg, J., Guy, L., Offre, P., & Ettema, T. J. G. (2018). Deep mitochondrial origin outside the sampled alphaproteobacteria. *Nature*, *557*(7703), 101–105. https://doi.org/10.1038/s41586-018-0059-5
10. O'Malley, M. A. (2010). The first eukaryote cell: An unfinished history of contestation. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, *41*(3), 212–224. https://doi.org/10.1016/j.shpsc.2010.07.010
11. Speijer, D. (2020). Debating eukaryogenesis—Part 1: Does eukaryogenesis presuppose symbiosis before uptake? *BioEssays*, *42*(4), 1900157. https://doi.org/10.1002/bies.201900157
12. Martin, W. F., Garg, S., & Zimorski, V. (2015). Endosymbiotic theories for eukaryote origin. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1678), 20140330. https://doi.org/10.1098/rstb.2014.0330
13. López-García, P., & Moreira, D. (2019). Eukaryogenesis, a syntrophy affair. *Nature Microbiology*, *4*(7), 1068–1070. https://doi.org/10.1038/s41564-019-0495-5
14. Poole, A. M., & Gribaldo, S. (2014). Eukaryotic origins: How and when was the mitochondrion acquired? *Cold Spring Harbor Perspectives in Biology*, *6*(12), a015990–a015990. https://doi.org/10.1101/cshperspect.a015990
15. Cavalier-Smith, T. (2002). The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa. *International Journal of Systematic and Evolutionary Microbiology*, *52*(2), 297–354. https://doi.org/10.1099/00207713-52-2-297
16. Cavalier-Smith, T. (2009). Predation and eukaryote cell origins: A coevolutionary perspective. *The International Journal of Biochemistry & Cell Biology*, *41*(2), 307–322. https://doi.org/10.1016/j.biocel.2008.10.002
17. Hampl, V., Čepička, I., & Eliáš, M. (2019). Was the mitochondrion necessary to start eukaryogenesis? *Trends in Microbiology*, *27*(2), 96–104. https://doi.org/10.1016/j.tim.2018.10.005
18. Davidov, Y., & Jurkevitch, E. (2009). Predation between prokaryotes and the origin of eukaryotes. *BioEssays*, *31*(7), 748–757. https://doi.org/10.1002/bies.200900018
19. Zachar, I., & Boza, G. (2020). Endosymbiosis before eukaryotes: Mitochondrial establishment in protoeukaryotes. *Cellular and Molecular Life Sciences*, *77*(18), 3503–3523. https://doi.org/10.1007/s00018-020-03462-6
20. Cavalier-Smith, T. (1987). The origin of eukaryote and archaebacterial cells. *Annals of the New York Academy of Sciences*, *503*(1 Endocytobiolo), 17–54. https://doi.org/10.1111/j.1749-6632.1987.tb40596.x
21. Cavalier-Smith, T. (2014). The Neomuran Revolution and phagotrophic origin of eukaryotes and cilia in the light of intracellular coevolution and a revised tree of life. *Cold Spring Harbor Perspectives in Biology*, *6*(9), a016006–a016006. https://doi.org/10.1101/cshperspect.a016006
22. Yutin, N., Wolf, M. Y., Wolf, Y. I., & Koonin, E. V. (2009). The origins of phagocytosis and eukaryogenesis. *Biology Direct*, *4*(1), 9. https://doi.org/10.1186/1745-6150-4-9
23. Martijn, J., & Ettema, T. J. G. (2013). From archaeon to eukaryote: The evolutionary dark ages of the eukaryotic cell. *Biochemical Society Transactions*, *41*(1), 451–457. https://doi.org/10.1042/BST20120292
24. Dacks, J. B., & Field, M. C. (2007). Evolution of the eukaryotic membrane-trafficking system: Origin, tempo and mode. *Journal of Cell Science*, *120*(17), 2977–2985. https://doi.org/10.1242/jcs.013250
25. Martin, W. F., Tielens, A. G. M., Mentel, M., Garg, S. G., & Gould, S. B. (2017). The Physiology of Phagocytosis in the Context of Mitochondrial Origin. *Microbiology and Molecular Biology Reviews*, *81*(3), e00008–17. https://doi.org/10.1128/MMBR.00008-17
26. Mills, D. B. (2020). The origin of phagocytosis in Earth history. *Interface Focus*, *10*(4), 20200019. https://doi.org/10.1098/rsfs.2020.0019
27. Bremer, N., Tria, F. D. K., Skejo, J., Garg, S. G., & Martin, W. F. (2022). Ancestral State Reconstructions Trace Mitochondria But Not Phagocytosis to the Last Eukaryotic Common Ancestor. *Genome Biology and Evolution*, *14*(6), evac079. https://doi.org/10.1093/gbe/evac079
28. Speijer, D. (2022). Eukaryotes were shaped by Oxygen. *Nature Ecology & Evolution*, *6*(9), 1242–1242. https://doi.org/10.1038/s41559-022-01819-7
29. Shiratori, T., Suzuki, S., Kakizawa, Y., & Ishida, K. (2019). Phagocytosis-like cell engulfment by a planctomycete bacterium. *Nature Communications*, *10*(1), 5529. https://doi.org/10.1038/s41467-019-13499-2
30. Burger, G., Gray, M. W., Forget, L., & Lang, B. F. (2013). Strikingly bacteria-like and gene-rich mitochondrial genomes throughout jakobid protists. *Genome Biology and Evolution*, *5*(2), 418–438. https://doi.org/10.1093/gbe/evt008
31. Cavalier-Smith, T. (2013). Early evolution of eukaryote feeding modes, cell structural diversity, and classification of the protozoan phyla Loukozoa, Sulcozoa, and Choanozoa. *European Journal of Protistology*, *49*(2), 115–178. https://doi.org/10.1016/j.ejop.2012.06.001
32. Hartman, H., & Fedorov, A. (2002). The origin of the eukaryotic cell: A genomic investigation. *Proceedings of the National Academy of Sciences*, *99*(3), 1420–1425. https://doi.org/10.1073/pnas.032658599
33. Koumandou, V. L., Wickstead, B., Ginger, M. L., van der Giezen, M., Dacks, J. B., & Field, M. C. (2013). Molecular paleontology and complexity in the last eukaryotic common ancestor. *Critical Reviews in Biochemistry and Molecular Biology*, *48*(4), 373–396. https://doi.org/10.3109/10409238.2013.821444
34. Burns, J. A., Pittis, A. A., & Kim, E. (2018). Gene-based predictive models of trophic modes suggest Asgard archaea are not phagocytotic. *Nature Ecology & Evolution*, *2*(4), 697–704. https://doi.org/10.1038/s41559-018-0477-7
35. Spang, A., Stairs, C. W., Dombrowski, N., Eme, L., Lombard, J., Caceres, E. F., Greening, C., Baker, B. J., & Ettema, T. J. G. (2019). Proposal of the reverse flow model for the origin of the eukaryotic cell based on comparative analyses of Asgard archaeal metabolism. *Nature Microbiology*, *4*(7), 1138–1148. https://doi.org/10.1038/s41564-019-0406-9
36. López-García, P., & Moreira, D. (2020). The Syntrophy hypothesis for the origin of eukaryotes revisited. *Nature Microbiology*, *5*(5), 655–667. https://doi.org/10.1038/s41564-020-0710-4

37. Wootton, E. C., Zubkov, M. V., Jones, D. H., Jones, R. H., Martel, C. M., Thornton, C. A., & Roberts, E. C. (2007). Biochemical prey recognition by planktonic protozoa. *Environmental Microbiology*, *9*(1), 216–222. https://doi.org/10.1111/j.1462-2920.2006.01130.x
38. Uribe-Querol, E., & Rosales, C. (2020). Phagocytosis: Our current understanding of a universal biological process. *Frontiers in Immunology*, *11*, 1066. https://doi.org/10.3389/fimmu.2020.01066
39. Boulais, J., Trost, M., Landry, C. R., Dieckmann, R., Levy, E. D., Soldati, T., Michnick, S. W., Thibault, P., & Desjardins, M. (2010). Molecular characterization of the evolution of phagosomes. *Molecular Systems Biology*, *6*(1), 423. https://doi.org/10.1038/msb.2010.80
40. Pittis, A. A., & Gabaldón, T. (2016). Late acquisition of mitochondria by a host with chimaeric prokaryotic ancestry. *Nature*, *531*(7592), 101–104. https://doi.org/10.1038/nature16941
41. Vosseberg, J., van Hooff, J. J. E., Marcet-Houben, M., van Vlimmeren, A., van Wijk, L. M., Gabaldón, T., & Snel, B. (2021). Timing the origin of eukaryotic cellular complexity with ancient duplications. *Nature Ecology & Evolution*, *5*(1), 92–100. https://doi.org/10.1038/s41559-020-01320-z
42. Tria, F. D. K., Brueckner, J., Skejo, J., Xavier, J. C., Kapust, N., Knopp, M., Wimmer, J. L. E., Nagies, F. S. P., Zimorski, V., Gould, S. B., Garg, S. G., & Martin, W. F. (2021). Gene duplications trace mitochondria to the onset of eukaryote complexity. *Genome Biology and Evolution*, *13*(5). https://doi.org/10.1093/gbe/evab055
43. Albers, S. V., & Meyer, B. H. (2011). The archaeal cell envelope. *Nature Reviews Microbiology*, *9*(6), 414–426. https://doi.org/10.1038/nrmicro2576
44. Arend, K. I., Schmidt, J. J., Bentler, T., Lüchtefeld, C., Eggerichs, D., Hexamer, H. M., & Kaimer, C. (2020). *Myxococcus xanthus* Predation of Gram-Positive or Gram-Negative Bacteria Is Mediated by Different Bacteriolytic Mechanisms. *Applied and Environmental Microbiology*, *87*(5), e02382–20. /aem/87/5/AEM.02382-20.atom. https://doi.org/10.1128/AEM.02382-20
45. Villanueva, L., von Meijenfeldt, F. A. B., Westbye, A. B., Hopmans, E. C., Dutilh, B. E., & Sinninghe Damsté, J. S. (2018). Bridging the divide: Bacteria synthesizing archaeal membrane lipids. *BioRxiv*, *448035*. https://doi.org/10.1101/448035
46. Matz, C., & Jürgens, K. (2005). High motility reduces grazing mortality of planktonic bacteria. *Applied and Environmental Microbiology*, *71*(2), 921–929. https://doi.org/10.1128/AEM.71.2.921-929.2005
47. Corno, G., & Jürgens, K. (2006). Direct and indirect effects of protist predation on population size structure of a bacterial strain with high phenotypic plasticity. *Applied and Environmental Microbiology*, *72*(1), 78–86. https://doi.org/10.1128/AEM.72.1.78-86.2006
48. Wildschutte, H., Wolfe, D. M., Tamewitz, A., & Lawrence, J. G. (2004). Protozoan predation, diversifying selection, and the evolution of antigenic diversity in Salmonella. *Proceedings of the National Academy of Sciences*, *101*(29), 10644–10649. https://doi.org/10.1073/pnas.0404028101
49. Matz, C., & Kjelleberg, S. (2005). Off the hook – how bacteria survive protozoan grazing. *Trends in Microbiology*, *13*(7), 302–307. https://doi.org/10.1016/j.tim.2005.05.009
50. Lancaster, C. E., Ho, C. Y., Hipolito, V. E. B., Botelho, R. J., & Terebiznik, M. R. (2019). Phagocytosis: What's on the menu? *Biochemistry and Cell Biology*, *97*(1), 21–29. https://doi.org/10.1139/bcb-2018-0008
51. Greub, G., & Raoult, D. (2004). Microorganisms resistant to free-living amoebae. *Clinical Microbiology Reviews*, *17*(2), 413–433. https://doi.org/10.1128/CMR.17.2.413-433.2004
52. Gong, J., Qing, Y., Zou, S., Fu, R., Su, L., Zhang, X., & Zhang, Q. (2016). Protist-bacteria associations: Gammaproteobacteria and alphaproteobacteria are prevalent as digestion-resistant bacteria in ciliated protozoa. *Frontiers in Microbiology*, *7*, 498. https://doi.org/10.3389/fmicb.2016.00498
53. Davis, K. M., & Weiser, J. N. (2011). Modifications to the peptidoglycan backbone help bacteria to establish infection. *Infection and Immunity*, *79*(2), 562–570. https://doi.org/10.1128/IAI.00651-10
54. Chow, J., Lee, S. M., Shen, Y., Khosravi, A., & Mazmanian, S. K. (2010). Host–bacterial symbiosis in health and disease. *Advances in Immunology*, 107, 243–274. Elsevier. https://doi.org/10.1016/B978-0-12-381300-8.00008-3
55. McFall-Ngai, M. J. (2015). Giving microbes their due—Animal life in a microbially dominant world. *Journal of Experimental Biology*, *218*(12), 1968–1973. https://doi.org/10.1242/jeb.115121
56. Horn, M., & Wagner, M. (2004). Bacterial endosymbionts of free-living amoebae1. *The Journal of Eukaryotic Microbiology*, *51*(5), 509–514. https://doi.org/10.1111/j.1550-7408.2004.tb00278.x
57. Tekle, Y. I., Lyttle, J. M., Blasingame, M. G., & Wang, F. (2021). Comprehensive comparative genomics reveals over 50 phyla of free-living and pathogenic bacteria are associated with diverse members of the amoebozoa. *Scientific Reports*, *11*(1), 8043. https://doi.org/10.1038/s41598-021-87192-0
58. Casadevall, A., & Pirofski, L. (2000). Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. *Infection and Immunity*, *68*(12), 6511–6518. https://doi.org/10.1128/IAI.68.12.6511-6518.2000
59. Batut, J., Andersson, S. G. E., & O'Callaghan, D. (2004). The evolution of chronic infection strategies in the α-proteobacteria. *Nature Reviews Microbiology*, *2*(12), 933–945. https://doi.org/10.1038/nrmicro1044
60. Matilla, M. A., & Krell, T. (2018). The effect of bacterial chemotaxis on host infection and pathogenicity. *FEMS Microbiology Reviews*, *42*(1), 40–67. https://doi.org/10.1093/femsre/fux052
61. Flannagan, R. S., Cosío, G., & Grinstein, S. (2009). Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. *Nature Reviews Microbiology*, *7*(5), 355–366. https://doi.org/10.1038/nrmicro2128
62. Simons, A., Alhanout, K., & Duval, R. E. (2020). Bacteriocins, antimicrobial peptides from bacterial origin: Overview of their biology and their impact against multidrug-resistant bacteria. *Microorganisms*, *8*(5), 639. https://doi.org/10.3390/microorganisms8050639
63. Morton, J. T., Freed, S. D., Lee, S. W., & Friedberg, I. (2015). A large scale prediction of bacteriocin gene blocks suggests a wide functional spectrum for bacteriocins. *BMC Bioinformatics*, *16*(1), 381. https://doi.org/10.1186/s12859-015-0792-9
64. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature*, *415*(6870), 389–395. https://doi.org/10.1038/415389a
65. Andrä, J., Herbst, R., & Leippe, M. (2003). Amoebapores, archaic effector peptides of protozoan origin, are discharged into phagosomes and kill bacteria by permeabilizing their membranes. *Developmental & Comparative Immunology*, *27*(4), 291–304. https://doi.org/10.1016/S0145-305X(02)00106-4
66. Peters, B. M., Shirtliff, M. E., & Jabra-Rizk, M. A. (2010). Antimicrobial peptides: Primeval molecules or future drugs? *PLoS Pathogens*, *6*(10), e1001067. https://doi.org/10.1371/journal.ppat.1001067
67. Jana, B., & Salomon, D. (2019). Type VI secretion system: A modular toolkit for bacterial dominance. *Future Microbiology*, *14*(16), 1451–1463. https://doi.org/10.2217/fmb-2019-0194
68. Hernandez, R. E., Gallegos-Monterrosa, R., & Coulthurst, S. J. (2020). Type VI secretion system effector proteins: Effective weapons for bacterial competitiveness. *Cellular Microbiology*, *22*(9). https://doi.org/10.1111/cmi.13241
69. Husnik, F., & Keeling, P. J. (2019). The fate of obligate endosymbionts: Reduction, integration, or extinction. *Current Opinion in Genetics & Development*, *58–59*, 1–8. https://doi.org/10.1016/j.gde.2019.07.014
70. Sousa, F. L., Neukirchen, S., Allen, J. F., Lane, N., & Martin, W. F. (2016). Lokiarchaeon is hydrogen dependent. *Nature Microbiology*, *1*(5), 16034. https://doi.org/10.1038/nmicrobiol.2016.34
71. Papa, S., Capitanio, G., & Papa, F. (2018). The mechanism of coupling between oxido-reduction and proton translocation in respiratory

chain enzymes: Protonmotive respiratory chains. *Biological Reviews*, *93*(1), 322–349. https://doi.org/10.1111/brv.12347
72. Guo, H., Suzuki, T., & Rubinstein, J. L. (2019). Structure of a bacterial ATP synthase. *ELife*, *8*, e43128. https://doi.org/10.7554/eLife.43128
73. Brand, M. (2003). Approximate yield of ATP from glucose, designed by donald nicholson: Commentary. *Biochemistry and Molecular Biology Education*, *31*(1), 2–4. https://doi.org/10.1002/bmb.2003.494031010178
74. Krulwich, T. A., Sachs, G., & Padan, E. (2011). Molecular aspects of bacterial pH sensing and homeostasis. *Nature Reviews Microbiology*, *9*(5), 330–343. https://doi.org/10.1038/nrmicro2549
75. Mencía, M. (2020). The archaeal-bacterial lipid divide, could a distinct lateral proton route hold the answer? *Biology Direct*, *15*(1), 7. https://doi.org/10.1186/s13062-020-00262-7
76. Pinegin, B., Vorobjeva, N., Pashenkov, M., & Chernyak, B. (2018). The role of mitochondrial ROS in antibacterial immunity. *Journal of Cellular Physiology*, *233*(5), 3745–3754. https://doi.org/10.1002/jcp.26117
77. West, A. P., Brodsky, I. E., Rahner, C., Woo, D. K., Erdjument-Bromage, H., Tempst, P., Walsh, M. C., Choi, Y., Shadel, G. S., & Ghosh, S. (2011). TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature*, *472*(7344), 476–480. https://doi.org/10.1038/nature09973
78. Abuaita, B. H., Schultz, T. L., & O'Riordan, M. X. (2018). Mitochondria-derived vesicles deliver antimicrobial reactive oxygen species to control phagosome-localized *Staphylococcus aureus*. *Cell Host & Microbe*, *24*(5), 625–636.e5. https://doi.org/10.1016/j.chom.2018.10.005
79. Kawai, Y., Mercier, R., Wu, L. J., Dominguez-Cuevas, P., Oshima, T., & Errington, J. (2015). Cell growth of wall-free L-form bacteria is limited by oxidative damage. *Current Biology*, *25*(12), 1613–1618. https://doi.org/10.1016/j.cub.2015.04.031
80. Kawai, Y., Mercier, R., Mickiewicz, K., Serafini, A., Sorio de Carvalho, L. P., & Errington, J. (2019). Crucial role for central carbon metabolism in the bacterial L-form switch and killing by beta-lactam antibiotics. *Nature Microbiology*, *4*, 1716–1726. https://doi.org/10.1038/s41564-019-0497-3
81. Claessen, D., & Errington, J. (2019). Cell wall deficiency as a coping strategy for stress. *Trends in Microbiology*, *27*(12), 1025–1033. https://doi.org/10.1016/j.tim.2019.07.008
82. Gould, S. B., Garg, S. G., & Martin, W. F. (2016). Bacterial vesicle secretion and the evolutionary origin of the eukaryotic endomembrane system. *Trends in Microbiology*, *24*(7), 525–534. https://doi.org/10.1016/j.tim.2016.03.005
83. Uribe-Querol, E., & Rosales, C. (2017). Control of phagocytosis by microbial pathogens. *Frontiers in Immunology*, *8*(1638), 23. https://doi.org/10.3389/fimmu.2017.01368
84. Nardone, G., & Compare, D. (2015). The human gastric microbiota: Is it time to rethink the pathogenesis of stomach diseases? *United European Gastroenterology Journal*, *3*(3), 255–260. https://doi.org/10.1177/2050640614566846
85. Gogarten, J. P., Kibak, H., Dittrich, P., Taiz, L., Bowman, E. J., Bowman, B. J., Manolson, M. F., Poole, R. J., Date, T., Oshima, T., Konishi, J., Denda, K., & Yoshida, M. (1989). Evolution of the vacuolar H+-ATPase: Implications for the origin of eukaryotes. *Proceedings of the National Academy of Sciences*, *86*(17), 6661–6665. https://doi.org/10.1073/pnas.86.17.6661
86. Mindell, J. A. (2012). Lysosomal acidification mechanisms. *Annual Review of Physiology*, *74*(1), 69–86. https://doi.org/10.1146/annurev-physiol-012110-142317
87. Nauseef, W. M. (2019). The phagocyte NOX2 NADPH oxidase in microbial killing and cell signaling. *Current Opinion in Immunology*, *60*, 130–140. https://doi.org/10.1016/j.coi.2019.05.006
88. Hajjar, C., Cherrier, M. V., Dias Mirandela, G., Petit-Hartlein, I., Stasia, M. J., Fontecilla-Camps, J. C., Fieschi, F., & Dupuy, J. (2017). The NOX family of proteins is also present in bacteria. *MBio*, *8*(6), e01487–17. https://doi.org/10.1128/mBio.01487-17
89. Speijer, D. (2021). Zombie ideas about early endosymbiosis: Which entry mechanisms gave us the "endo" in different endosymbionts? *BioEssays*, *43*(7), 2100069. https://doi.org/10.1002/bies.202100069
90. Giorgi, C., Marchi, S., & Pinton, P. (2018). The machineries, regulation and cellular functions of mitochondrial calcium. *Nature Reviews Molecular Cell Biology*, *19*(11), 713–730. https://doi.org/10.1038/s41580-018-0052-8
91. Mayer, B., & Oberbauer, R. (2003). Mitochondrial regulation of apoptosis. *Physiology*, *18*(3), 89–94. https://doi.org/10.1152/nips.01433.2002
92. Rout, M. P., & Field, M. C. (2017). The evolution of organellar coat complexes and organization of the eukaryotic cell. *Annual Review of Biochemistry*, *86*(1), 637–657. https://doi.org/10.1146/annurev-biochem-061516-044643
93. Jékely, G. (Ed.). (2007). *Eukaryotic membranes and cytoskeleton: Origins and evolution*. Springer Science+Business Media.

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