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THINK AGAIN

Insights & Perspectives

Zombie ideas about early endosymbiosis: Which entry mechanisms gave us the "endo" in different endosymbionts?

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Abstract

Recently, a review regarding the mechanics and evolution of mitochondrial fission appeared in Nature. Surprisingly, it stated authoritatively that the mitochondrial outer membrane, in contrast with the inner membrane of bacterial descent, was acquired from the host, presumably during uptake. However, it has been known for quite some time that this membrane was also derived from the Gram-negative, alphaproteobacterium related precursor of present-day mitochondria. The zombie idea of the host membrane still surrounding the endosymbiont is not only wrong, but more importantly, might hamper the proper conception of possible scenarios of eukaryogenesis. Why? Because it steers the imagination not only with regard to possible uptake mechanisms, but also regarding what went on before. Here I critically discuss both the evidence for the continuity of the bacterial outer membrane, the reasons for the persistence of the erroneous host membrane hypothesis and the wider implications of these misconceptions for the ideas regarding events occurring during the first steps towards the evolution of the eukaryotes and later major eukaryotic differentiations. I will also highlight some of the latest insights regarding different instances of endosymbiont evolution.

KEYWORDS
endosymbiont, eukaryogenesis, membrane replacement, serial endosymbiosis, symbiogenesis

INTRODUCTION

In their highly informative and exhaustive review of the mitochondrial "divisome," responsible for mitochondrial fission, Kraus and coworkers make an unsubstantiated, and alas, erroneous, claim regarding the evolutionary origin of the mitochondrial outer membrane (OM).[1] They claim an "…inner membrane of prokaryotic origin that is bordered by an outer membrane derived from the host-cell plasma membrane;" for the ancestry of the mitochondrial membranes. However, not only is the inner membrane (IM) of prokaryotic origin, but most researchers in the field agree that the OM is derived from the original OM of the Gram-negative bacterial endosymbiont as well.[2–6] Interestingly, this also holds true for the OM of modern-day chloroplasts.[7] That this misconception represents a real zombie idea is nicely illustrated by a critical June 2010 "Sandwalk" blogpost, which can be found at https://sandwalk.blogspot.com/2010/06/on-origin-of-double-membrane-in.html, and which I reread after the article by Kraus et al[1] appeared. The Sandwalk ("Strolling with a skeptical biochemist") is a regular, high quality blog by the biochemist Laurence Moran. In this specific post he discusses another instance of the persistent error in the context of the "3 Quarks Daily 2010 prize for best blog posting about science," where one of the prizes went to a contribution describing the evolution of Chloroplasts, that, as Moran

Abbreviations: ATOM, atypical translocase of the outer membrane; IM, inner membrane; OM, outer membrane; SELMA, symbiont-specific ERAD-like machinery

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puts it "repeats the common myth". Because of its crispy clarity I quote his reaction in full: "This is very wrong. The original bacteria had a double membrane and that double membrane was an integral part of the energy producing pathway that became so important for the eukaryotic cell. It's simply not true that the double membranes of bacteria and chloroplasts were the result of endocytosis." The provenance of the OMs of mitochondria and chloroplasts, thus, tells us nothing about how they ended up in the host. But just repeating here that this idea is wrong will get me nowhere. Why do we (think) we know better? If readers want to have an excellent, highly concise, overview of the scientific background for the following considerations, I would refer them to the aptly named primer "Membranes and evolution" by Sven B. Gould.[8]

For the sake of balance, I also want to stress here that though I explore the possibility that the Gram-negative bacteria which gave rise mitochondria (and possibly even those that gave rise to chloroplasts, but see below) were never surrounded by a host membrane, because they did not enter using a phagocytic mechanism, others, following the lead of the recently deceased Tom Cavalier-Smith, favour a phagotrophic origin for both[9], most of all because it represents a known mechanism. Of note, Cavalier-Smith also, already in 1982, speculated that the OM of the primary endosymbionts indeed represents the original Gram-negative bacterial OM, in his case because he inferred the loss of original surrounding phagosomal membranes; see for example.[10]

EVOLUTIONARY EVIDENCE FOR THE CONTINUITY OF A BACTERIAL OM IN MITOCHONDRIA

I first have to dismantle a spurious argument often used to support the idea of a bacterial origin of the OM. Though modern theories and current data have it that the "host" cell taking up the ancestors of mitochondria was an archaeon, the fact that the OM contains "bacterial" lipids (fatty-acid based; ester-linked hydrophilic head group) instead of archaeal lipids (isoprenoid based; ether-linked hydrophilic head group) in itself cannot be used to argue that the OM is not host derived. This is simply due to the fact that all archaeal membranes have been replaced in eukaryotes, and though the building blocks of the eukaryotic cell membrane have been replaced by bacterial ones, no one would argue that it does not represent in some sense the original barrier of the archaeon (as e.g., illustrated by the kind of proteins we find inside; see below). I will discuss some of the possible explanations for this complete lipid replacement later on.

The real evolutionary evidence for the bacterial origin of the OM in mitochondria can be found in its protein composition, which is clearly of alphaproteobacterial origin. The OM of mitochondria and plastids contain many specific β-barrel proteins (such as porins and Tom40 in mitochondria, and Omp85 in chloroplasts) which are only found in the OMs of Gram-negative bacteria.[3,7] These β-barrel proteins are inserted and assembled into complexes in the bacterial OM by an in-situ molecular machine. It's central component, known as Omp85 or BamA, is a highly conserved bacterial protein involved in the recognition of the C-termini of OM proteins. A homologue (often referred to as SAM50) is found in mitochondria.[2] This homologue is also essential for assembly of β-barrel proteins in the mitochondrial OM.[4] In the highly divergent eukaryotic parasite *Trypanosoma brucei*, a conventional Tom40 translocase is missing from the OM, while an atypical translocase of the OM (ATOM) is found instead. Electrophysiological single channel properties of ATOM show it to resemble bacterial-type translocases rather than "normal" eukaryotic Tom40. Thus, ATOM further strengthens evolutionary links between bacterial translocases and the ones found in mitochondria of other eukaryotes.[5] Functional studies also indicate that the mitochondrial OM is the evolutionary descendant of the bacterial OM. A prime example can be found in research of a pathogenic bacterial protein, PorB, which is normally targeted from pathogenic Neisseria to host cell mitochondria. It could be shown that PorB uses an evolutionary conserved 'bacterial like' mechanism, allowing it to enter the mitochondrial OM.[11] The observation that bacterial proteins with so-called carboxyl-terminal tail anchors appear to be capable of spontaneous insertion into the mitochondrial OM, might also be relevant here.[12]

WHAT DOES MEMBRANE PROTEIN COMPOSITION REALLY PROVE?

Now, one might counter that these multi-enzyme complexes have just been retargeted to membranes of host origin, but such a proposal goes against all observations so far. Individual proteins, complete metabolic pathways, as well as (mostly peripheral) factors of molecular machines can indeed be, and often are, repurposed and retargeted, but this has not been observed for complete membrane bound multicomponent molecular machines, such as the mitochondrial protein import machinery.[13] Indeed, the specific evolutionary background of a large membrane complex is often used to pinpoint the origins of a specific membrane in the context of complex primary and secondary endosymbiosis.

A major event in the increase of cellular complexity was the uptake of a red alga by a eukaryote (an instance of secondary endosymbiosis; see below). This gave rise to multiple membranes surrounding the original red plastid, in all later, incredibly diverse, descendants. The fact that the (now even more complicated) import of proteins into the new organelle is partially taken care of by the so-called SELMA complex is used as evidentiary material to postulate that the second outermost membrane in this instance of secondary endosymbiosis is derived from an ER membrane. The proof for this assertion? The SELMA complex located in this particular membrane is considered to be a derivate of an ancient ER-associated molecular machine (ERAD; Endoplasmic-reticulum-associated protein degradation) involved in targeting and exporting proteins for subsequent degradation. In this context a few important points should be stressed:

(i) The fact that the SELMA multi-protein machinery seems to be universal also indicates that the transformation of a red alga into a complex red plastid succeeded only once.

(ii) The outermost membrane of red complex plastids seems to be derived from an ER as well. Indeed, in cryptomonads (as well as in haptophytes and heterokonts) the outermost membrane was abundantly demonstrated to be contiguous with the host ER.[14]

(iii) Importantly, because in secondary endosymbiosis we are considering uptake of a complete eukaryote by another eukaryote, the ER-related membranes could, in principle, have come from either organism and might have allowed for dual protein targeting. Possible membrane fusions can play a role as well.

(iv) Originally, most researchers preferred another interpretation, with the four membranes, characterizing such a plastid, corresponding to host endomembrane, plasma membrane of the engulfed alga followed by the two membranes of the primary plastid on the inside (see below).[15] However, this would have implied complete SELMA retargeting.

(v) The molecular steps at the origin of the red complex plastids are still heavily debated, as there are no simple models explaining all our observations. An interesting attempted reconstruction, heavily based on the conservation of the SELMA complex, can be found in.[16]

(vi) The fact that membranes surrounding secondary plastids contain complexes with an evolutionary link to protein degradation might seem to hint at phagosomal forms of uptake, but tells us nothing regarding the mechanism of uptake during primary endosymbiosis.

Thus, the evolutionary reconstruction based on the location of the SELMA complex does not have to start with phagocytic entry (though it clearly does not exclude it either, because the outermost membrane is indeed of host origin). It could just imply that the red alga might have been surrounded by stacking vesicles at a certain stage of acquisition, which helps explain the continuity of both outer membranes with the ER. This resembles theories for the formation of the double layered nuclear membrane during eukaryogenesis (see asterisk in Figure 1) and could have made use of mechanisms still on display in eukaryotes during the process of autophagy/mitophagy in which the ER membranes plays a major role.[17,18] However, the phagocytic nature of secondary endosymbiont uptakes (including that of the original red alga) is widely accepted and looking even more likely because of the following recent finding by Keeling and co-workers. They describe two species of protists preying on eukaryotes which seem closely related to red algae (phylum Rhodelphidia).[19] However, they represent flagellate predators with surprisingly gene-rich genomes, unlike the reduced genomes normally found in this group. They also have a relic (genome-lacking) primary plastid possibly participating in, amongst others, haem synthesis and nuclear mRNA splicing. This makes it likely that certain ancient Archaeplastida (descendants of the eukaryote that took up the cyanobacterium at the basis of the chloroplast) displayed mixotrophic feeding. Such a combination of predation and phototrophy helps explain the origins of red algae. Going further, one might speculate that this mixotrophic organism itself could have resulted from a phagocytic uptake of the ancestral cyanobacterium by an early predatory eukaryote.

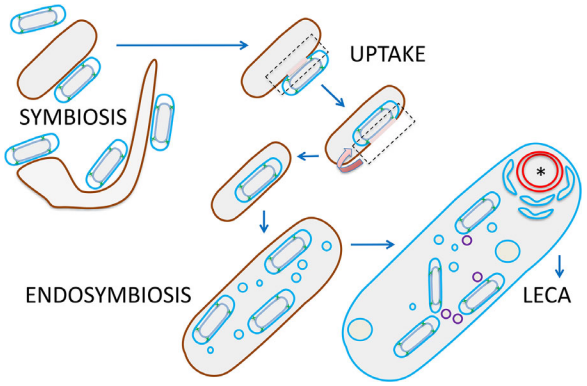

FIGURE 1 From prokaryotic symbiosis to eukaryotes? Starting out with the symbiosis of Asgard archaea (brown) and bacteria related to the Alpha-proteobacteria (OM – blue; IM – gray) necessitating close membrane to membrane contact (due to metabolite exchange), a fleeting membrane fusion (dashed box) could have allowed uptake without major direct damage to either participant (NB: Both prokaryotes retain membrane integrity with respect to the outside environment). The replacement of archaeal membranes by bacterial OM ones, as well as the development of an endomembrane system (functionally new structures, indicated in purple; the stacked double nuclear membrane indicated in red), possibly derived from OM vesicle formation inside the merged system[27] is also depicted. The asterisk indicates the host (nuclear) DNA protected by the nuclear membrane. This double membrane structure could have evolved from stacking vesicles, which might explain the continuity with the ER. For further explanations see the main text.

Among the abundance of evidence for subsequent serial phagocytic uptake (in this case of a complete red alga, that is, representing tertiary endosymbiosis), the presence of a so-called nucleomorph (a tiny, vestigial, eukaryotic nucleus located between the inner and outer membrane pairs) in the cryptomonads takes pride of place.[20] Characterization of the SELMA machinery took place in cryptomonads first, and its ER connections are still seen by some as proof that such machineries can relocate to other membranes. Supposing this indeed impossible, however, brings us the question: how did the plasma membrane disappear in that case? Membranes can indeed be lost and I will return to that question in the section "Further observations on endosymbiont uptakes" below. It should be stressed here that all these differences of opinion do not extend to the OMs of primary plastids or mitochondria: these are clearly derived from the OM of Gram-negative bacteria. We are, thus, left with questions: how did the complete Gram-negative precursors of mitochondria and chloroplasts end up in the cytoplasm without any further membranes surrounding them? Were both, or the cyanobacterium only, taken up by a form of phagocytosis, after which the uptake membrane disappeared, or were entirely different mechanisms involved?

MEMBRANES: MIXED MESSAGES?

As such, it is striking that the old way of thinking regarding the evolutionary origins of the OM persists, but this state of affairs also

has quite pernicious side effects. Automatically interpreting the OM as host-derived, limits the nature of models worthy of consideration in several ways. First of all, it gives rise to an implicit acceptance of the idea that uptake of the primary endosymbiont resulted from phagocytic behaviour. Secondarily, it reinforces the concept of a complex "pre-eukaryote" with phagocytic capabilities engulfing an alphaproteobacteria-like organism during the later stages of eukaryogenesis. Thus, it also runs counter to so-called symbiogenic theories that try to explain most, if not all, fundamental eukaryotic traits as a result of the archaeon bacterium merger. However, finding a "complete" Gram-negative bacterium (albeit with a highly reduced genome) inside modern-day eukaryotes is compatible with many symbiogenic scenarios, such as a more accidental uptake following extensive membrane contacts allowing symbiotic metabolite exchange between an (Asgard) archaeon and a Gram-negative bacterium. The Asgard lineage seems most closely related to the original "host" at eukaryotic origins.[21] Such an archaeon has recently been cultivated in symbiosis with other prokaryotes, including bacteria.[22] Interestingly, this archaeon, christened *Prometheoarchaeum syntrophicum*, displays extreme membrane protrusions, probably to optimize metabolite exchange with its symbiotic partners. These observations led the authors to propose an alternative conjecture for some of the early steps in eukaryotic evolution: the entangle–engulf–endogenize (or E^3) model.[22] Metabolite exchange might even already have had some aspects reminiscent of modern cytoplasm/mitochondrion exchange in response to changes in cellular oxygen/metabolite concentrations.[23]

The E^3 model is rather vague with regard to the actual mechanism allowing uptake after entanglement. However, one can speculate. If we postulate that (primitive) phagocytic mechanisms either did not exist yet, or did not play a role in this instance, then some form of temporary membrane fusion between bacterial OM and archaeal plasma membrane must have been involved. Such membrane fusion indeed occurs much more easily under conditions of close membrane proximity and high membrane irregularities in adjacent bilayers (e.g., due to the presence of protein structures). Membrane fusion can be non-leaky, but for this to happen, it has to be really rapid.[24] With regard to possible barriers towards the mixing of the two types of membranes (ether-bound isoprenoid lipids and ester-bound fatty acid lipids): when archaeal lipids were recombinantly expressed in *E. coli*, stably mixed archaeal/bacterial membranes could be detected[25], so this does not seem to be an intrinsic difficulty of this scenario. In the instance of a hypothetical membrane fusion between our host and the precursor of the endosymbiont it not only should occur rapidly, it should be fleeting, because the bacterial OM has to be released from the cell membrane and reclose in the cytoplasm of the archaeon, restoring a functional intermembrane space.

This might seem to be a rather tall order, unless one considers the following. The fact that both precursors to the primary eukaryotic endosymbionts (mitochondrion and "chloroplast") were Gram-negative bacteria with an OM, *might not be a coincidence*. Could it be that only this bacterial architecture allows for the entry mechanism just described? In such cases the "cytoplasmic/matrix" part of the bacterium is not disturbed by the entry process and the OM can be dragged along for re-closure by existing contact sites between OM and IM. Such contacts sites in Gram-negative bacteria can for instance take the form of inter-membrane bridges formed by ordering of IM proteins dictated by OM proteins which have the intrinsic property to cluster into islands with restricted lateral mobility.[26] After the uptake, prolonged OM vesicle secretion inside the merged cell system might have laid the groundwork for the complete replacement of archaeal by bacterial membranes, as well as the development of the full eukaryotic endomembrane system, including the nuclear double membrane structure as proposed by Garg and colleagues.[27] The complete hypothetical scenario for the progenitor of the mitochondrion is depicted in Figure 1. The cyanobacterium uptake (initiating the Archaeplastida) could either have been the result of phagocytosis by a eukaryote or also have followed the route depicted.

FURTHER SPECULATIONS AND CLARIFICATIONS REGARDING MEMBRANE MERGERS

A few further considerations seem in order. First of all, if this form of fusion, under the conditions of close proximity as required by symbiotic metabolite exchange, was not such an extremely rare occurrence, even the kind of cellular syncytia, sometimes proposed as intermediates during eukaryogenesis[28], could have appeared. Such syncytia could replace the dominant model of "two cells that become one," and invoke a larger membrane enclosed unit coming from many symbiotic prokaryotic cells in highly diversified environments. It is speculated that such entities would allow more "wriggle room" for the many eukaryotic evolutionary inventions because of genomic redundancies. This could have been an evolutionary intermediate stage before the release of the free-living eukaryotes. What this hypothetical scenario would mean for the characteristics of the last eukaryotic common ancestor is unclear. Secondly, when we talk about the fusion of "different" membranes, we should distinguish the mixing of archaeal and bacterial membranes from the mixing of eukaryotic membranes. Though these are clearly bacterial in origin, they also display profound, functionally important, differences. As an example, the outer and inner leaflets of the plasma membrane of eukaryotes differ, with the outside containing mostly sphingomyelin, phosphatidylcholine and glycolipids, while the inner leaflet is dominated by fatty acids linked via phosphates to several different hydrophilic head groups, such as phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine. This diversity is also seen when, for instance comparing ER, Golgi and plasma membrane, the amount of phosphatidylcholine steadily declines towards the outside, while cholesterol and sphingomyelin concomitantly increase in concentration.[29] All mitochondrial IM's are characterized by the almost complete absence of cholesterol and an abundance (~20% of all lipids of the IM) of cardiolipin: an atypical, four (!) fatty acid tails containing molecule of bacterial origin. Like other such lipids (compare the galactolipids of cyanobacterial origin always present in photosynthetic plastids) of endosymbiont origin, cardiolipin seems to be essential for both the stability of bio-energetic membranes (i.e., those maintaining a $\Delta\Psi$) and the embedded respiratory

complexes of the electron transport chain responsible for generating the potential in question. In mitochondrial "ghosts" (hydrogenosomes and mitosomes) the loss of the ability to respire and generate a $\Delta\Psi$, correlates with the absence of cardiolipin. The universal presence of the lipid, thus, also points to the major role molecular oxygen played during eukaryogenesis.[23] These outspoken functional differences in membrane composition have to be maintained, which means that the eukaryotic cell is also characterized by a large protein machinery for highly specific vesicle transport.[30] However, all of this does not mean that we can dismiss beforehand that fusions between different eukaryotic membranes have never happened, for example, during, or after, the uptake of an endosymbiont-to-be.

It seems obvious that instinctively sticking with the "natural" idea of a predatory phagocytic organism (and to be clear, a prokaryote able to take up other prokaryotes, in the form of a planctomycete bacterium, though completely unrelated to our archaeal host and using an unknown mechanism, has indeed recently been found[31]), restricts our ability to come up with models such as the one depicted in Figure 1. Of note, this proposal might be tested under laboratory conditions, by looking at whether a Gram-negative bacterium, such as *E. coli*, could pass through a membrane of isoprenoid lipids, while retaining viability. Even more enlightening would be the answer to the question under which circumstances this would be most likely to occur. Maybe membranes are not so limited in what they can accomplish after all.

PHAGOCYTOSIS: EARLY OR LATE?

I have dealt with this question in the context of eukaryogenesis before.[32] It is worthwhile to reiterate that a pre-symbiotic stage based upon metabolite exchange cannot easily be reconciled with a phagocytic uptake. However, such previous exchange makes the unlikely wholesale integration necessary for a successful merger of two prokaryotes clearly easier to accept. Also, many authors have pointed out the incompatibility of prokaryotic bio-energetic membranes, that have to maintain a $\Delta\Psi$, with recurring classic phagocytosis. Further analysis of microfossil traces and the great variety of phagosome associated proteins within extant eukaryotes by Daniel Mills seem to hint at several independent, "late," origins within this domain.[33] This brings him to also speculate about alternative mechanisms of uptake at the basis of eukaryogenesis, though repeated later independent evolution, as such, does not exclude an older "primitive" form. As a last consideration, though sometimes criticized because the use of O_2 as the final electron acceptor in respiratory processes is not without complications due to its extreme electronegativity and reactivity,[34] I would invoke bioenergetics.[35] It seems clear that the extended internal membranes of mitochondria, studded with the complexes involved in oxidative phosphorylation generate ATP in a highly efficient manner. This might have been a prerequisite before all the expensive eukaryotic capabilities (including phagocytosis) could ever evolve. Current analysis makes the involvement of an efficient fully aerobic bacterium related to the alpha-proteobacteria at eukaryotic origins perfectly feasible, because a protein family analysis demonstrated a burst of emerging oxygenases and other oxidoreductases, indicative of sufficient early biosphere oxygenation, to have occurred more than 3 billion years ago, well before the emergence of the eukaryotes (~2 billion years ago).[36]

Taken together, this makes a more accidental entry as depicted in Figure 1, instead of phagocytic uptake, seem to be more likely. However, the recent descriptions of some internal membrane structures in an archaeon (*Ignicoccus hospitalis*) have prompted speculation that the eukaryotic endomembrane system (and possibly phagocytosis) might have originated within the Archaea.[37] As mentioned, the bacterial (planctomycete) example of cellular uptake already expanded our horizons where prokaryotic capabilities are concerned. Though these findings might give rise to a healthy debate, the phylogenomic data and physiological considerations still favour a non-phagocytic uptake of the bacterium which would end up as our mitochondrion, I think. The major advantage of such a symbiogenic stance: the gulf in complexity between prokaryotes and eukaryotes can be explained in the light of the mutual adaptations necessary for a successful merger of the prokaryotes involved. Many of these make a lot of sense in the light of internal ROS formation by the new endosymbiont.[38]

FURTHER OBSERVATIONS ON ENDOSYMBIONT UPTAKES

Before further discussing the instances of remnants of host membranes still surrounding organelles/endosymbionts (I shall use both terms interchangeably, as the distinction is somewhat arbitrary[15]) in eukaryotes, it should be pointed out that later endosymbionts are surprisingly abundant. They also give indications that many (all?) later uptakes in eukaryotic lineages were phagocytic. As mentioned, instances of eukaryotic lineages taking up complete red/green algae in so-called secondary endosymbiotic events are for instance found in cryptomonads, haptophytes and heterokonts (uptake of a red alga) and euglenids (uptake of a green alga). It is commonly accepted that the uptake in these cases involved phago/endocytosis. Going through the excellent Figure 2 of Patrick Keeling's 2010 review[15], we encounter further examples, such as the secondary uptake of a green alga in the chlorarachniophytes (retaining a nucleomorph[39]), an instance of primary endosymbiosis in the case of the freshwater amoeba *Paulinella* taking up a cyanobacterium[40], and illustrations of tertiary endosymbiosis in several dinoflagellate species.[41] The incredible diversity and plasticity gives rise to highly dynamic evolving partnerships, such as serial algal replacement. Here I want to highlight a very recent discovery illustrating the evolutionary plasticity. Ciliates are a group of eukaryotic heterotrophs, mostly feeding on smaller organisms (bacteria and algae). However, they might have evolved from a photosynthetic (red alga containing) ancestor, which lost its endosymbiont.[42] "Normal" ciliate heterotrophes use their mitochondria and oxygen for ATP generation, but several species of ciliates are able to cope with anaerobic environments using mitochondrial remnants in the form of hydrogenosomes.[43] It is important to note that under these circumstances protons serve as the electron acceptors, generating H_2, while ATP generation occurs via substrate level phosphorylation during

fermentation. But, we now find that ciliate plasticity and endosymbiosis might go even further: an anaerobic ciliate has picked up a new endosymbiont ("*Candidatus* Azoamicus ciliaticola") capable of using nitrate as its terminal electron acceptor. The respiratory denitrification pathway possibly allows the transfer of ATP to the host. This ciliate, with mitochondrial remnants only, seems to have secondarily acquired an alternative energy-providing endosymbiont.[44]

In this coming and going of endosymbionts, many parts of their physical make-up are either "stolen" (e.g., transfer of their genes to the nucleus) or lost completely (e.g., in the case of eukaryotic endosymbionts, their nuclei, with nucleomorphs reflecting intermediate stages on their way out). How about (plasma) membranes? Though most membrane configurations are even numbered (two or four), instances of three sequential membranes can be found, implying specific losses. However, reconstructing the events giving rise to this state of things, is perilous.[45] In conclusion: many aspects of the mechanistic events leading to the uptake of future endosymbionts are still not completely understood, but there is an excellent chance that the first uptakes (especially that of the mitochondrion-to-be) followed a different path than later uptakes by fully evolved eukaryotes. There are a few reasons why a phagocytic uptake of the primal cyanobacterium (if this indeed was the method of entry) is more easily accepted. Uptake was performed by an organism related to LECA (a highly complex organism with mitochondria, protein targeting mechanism, etc.). It is also worth stressing that stable incorporation of an auxotrophic organism is more easily envisaged.

Still, many critics are puzzled by the fact that, as phagocytosis is a well understood mechanism among eukaryotes for acquiring and hosting symbionts, it is not automatically accepted for the entry of the pre-mitochondrion as well. But symbiogenesis stresses that eukaryotic phagocytosis seems to presuppose the presence of mitochondria as well as a highly complicated preceding merger, possibly only succeeding because of prior "normal" symbiosis between the cells involved. Then, the merger itself was crucial in developing the salient eukaryotic characteristics, with phagocytic capabilities possibly among them.[32,46] However, as I mentioned, others have speculated that the archaeal host already had some primitive phagocytic capacity. This is a point of contention as well. [47,48]

THE "HOPEFUL MONSTERS" OF CELLULAR MERGERS AND ACQUISITIONS

The interesting, controversial, biologist Richard Goldschmidt, once coined the phrase "hopeful monsters" to describe homeotic mutants in the fruit fly Drosophila, because they revealed the evolutionary potential of genetic alterations in developmental processes, such as mutations in (the expression of) Hox genes.[49] In so doing he probably made too crude a distinction between micro and macro evolution, which is often seized upon by those opposing evolutionary frameworks. However, I do think it constitutes an apt description of the symbiogenic merger of prokaryotes at the basis of the eukaryotes[50,51] and many of the later acquisitions by eukaryotes themselves, we have been dealing with here. It clearly is a tall order to get organisms to "become one" in a stable configuration. That mitochondria and the primary plastid originated by endosymbiosis is abundantly clear, but how this integration took place, we still have to extensively speculate about. Other, later endosymbiotic systems are clearly more common (though it is wise to remember that all the different groups containing (remnants of) a red alga, came from one (!) old secondary endosymbiotic event, and seem to involve phagocytosis.[15] Not only is it extremely difficult to evolve into an integrated partnership, it also does not seem easy to maintain. Over longer time periods especially, obligate endosymbionts seem to be "reduced out of existence".[52] However, the "hopeful" aspect is abundantly borne out by the overwhelmingly successful eukaryotic diversity we find everywhere around us.

CONCLUSIONS

It is a pity that Kraus et al.[1] so easily subscribe to an outdated idea regarding OM origins in an otherwise outstanding review regarding mitochondrial fission and the roles played by the diverse divisome incarnations. This is especially the case because the field of models regarding eukaryotic origins is already beset by multiple misconceptions and anachronistic reasoning.[32,46] It is also true in light of the fact that describing the OM as the remnant of the host phagocytic membrane is still often, however spuriously, invoked as an extra proof for the endosymbiotic origins of both mitochondria and chloroplasts.

Whether we will ever be able to fully elucidate the processes that gave rise to the eukaryotes and the (in my opinion, crucial) role that the uptake of the future mitochondrion played during eukaryogenesis is uncertain. One of the reasons for this is the intrinsic nature of all biological evolution, produced by the interplay of chance and selection. The relative contributions of both are intensely debated in individual biological processes. If chance (sometimes referred to as "neutral" evolution) played a major role in this instance, we will be at a disadvantage. However, let us not make things even more difficult by misinterpreting the clues that mitochondria present us with: thus reviving zombie ideas regarding the evolutionary origins of their OM.

CONFLICT OF INTEREST

The author declares no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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