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Eukaryogenesis From FECA to LECA: Radical Steps Along the Way

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ABSTRACT

The characteristics of the last eukaryotic common ancestor (LECA) population and the root of the eukaryotic tree have been coming into focus lately. However, the trajectory taking the host, related to present-day Asgard archaea and the endosymbiont, related to present-day alphaproteobacteria, toward such fully integrated and complex organisms is still unclear. Here I marshal recent evidence supporting the *early* arrival of the "mitochondrion-to-be", setting up the evolutionary dynamic for a series of mutual adaptations leading to eukaryotes. Upon critical analysis of some presuppositions in phylogenomic reconstructions of eukaryogenesis, I again propose that pre-symbiosis, efficient ATP generation, internal reactive oxygen species (ROS) formation and enhanced retention of genes supplied by horizontal gene transfer (HGT) *interdependently* allowed this unique transformation to occur.

1 | Introduction and Background

Microbial eukaryotes (protists) represent the majority (~70%) of physiological and evolutionary eukaryotic diversity of our planet, and its extended sampling over the last decade has significantly increased both breadth and depth of the eukaryotic tree [1–5]. This means that the genetic and functional contours of the last eukaryotic common ancestor (LECA) population [6] and the root of the eukaryotic tree are finally coming into focus [7–12]. Thus, to quote a recent consensus overview: "Once assembled, a robust LECA gene set will be a useful tool for evaluating alternative hypotheses about the origin of eukaryotes…" [11]. Indeed, when it comes to thinking about such evolutionary trajectories toward a LECA population alternatives still abound [13]. This does not mean that major players, as well as processes involved, are not known, but there is intense discussion about how their interplay has given us this population of cells (such differences of opinion are nicely illustrated by the "mitochondria early/intermediate/late" discussion) [9, 13–17]. In the following section, I will describe the characteristics of the LECA population and the generally accepted contributors (see Figure 1) to its appearance. Next, I highlight some of the difficulties involved in reconstructing the pathway from FECA to LECA. I conclude by presenting a scenario to go from a merger of a "host-related" Asgard archaeon (belonging to the Heimdallarchaeota [18]) with an alphaproteobacterial organism to the LECA. I defend a model stressing the crucial role played by molecular oxygen (O_2) and its radical derivatives, reactive oxygen species (ROS), during this development. In doing so I try to enrich discussions about the evolution of eukaryotes, which seem to be dominated by phylogenomic analysis, with biochemical/cell biological considerations.

2 | LECA and Its Contributing Constituents

Over the last decade, a lot of progress has been made with regard to the characteristics of the LECA population, culminating in the following consensus picture. The population contained highly complex single-celled organisms [3, 7–9, 12] with, at minimum, the following canonical eukaryotic characteristics: a complex dynamic cytoskeleton, a nucleus, an endomembrane system, aer-

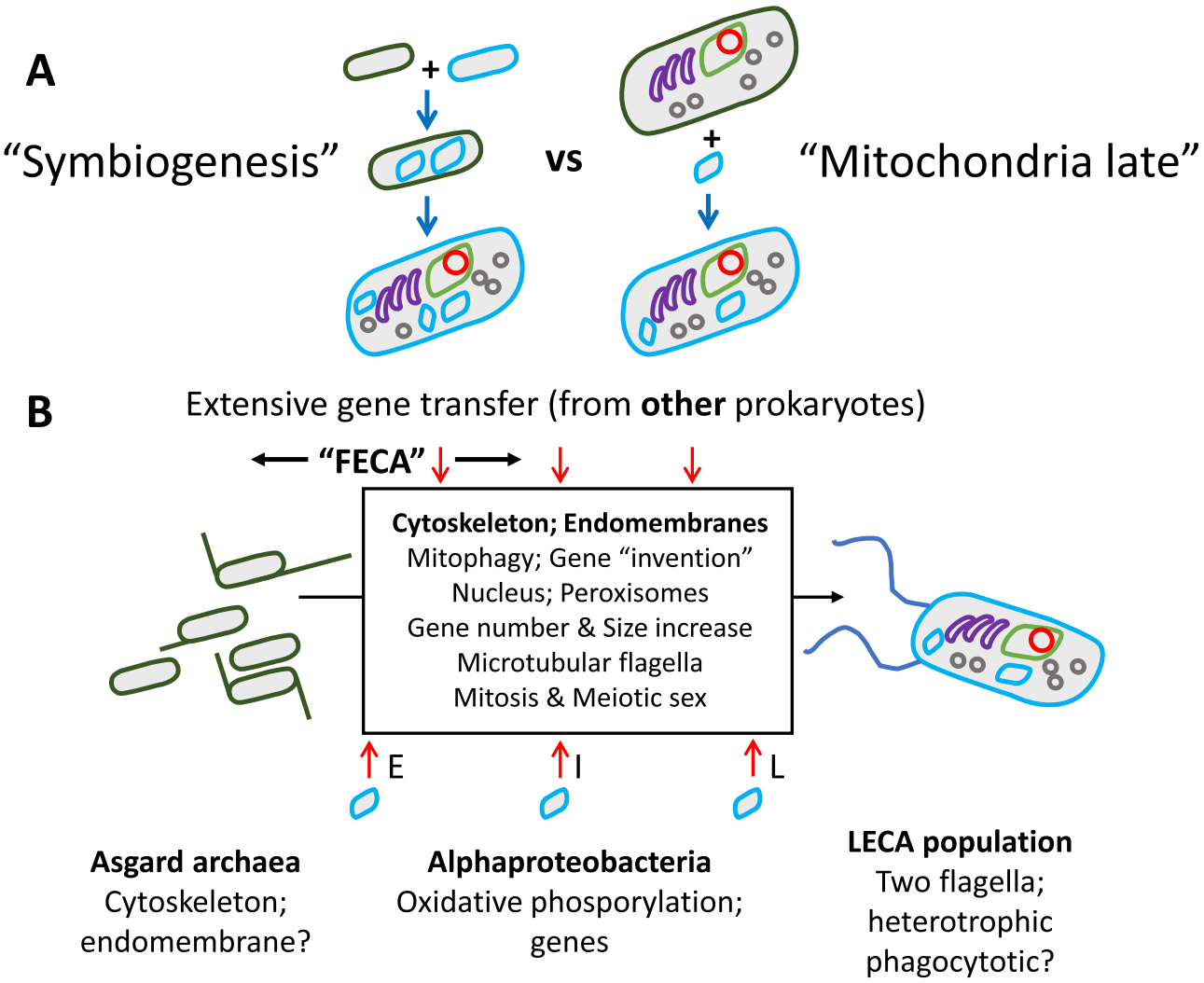

FIGURE 1 | (A) Opposite alternative scenarios for eukaryogenesis: "Symbiogenesis" [28, 131] versus "Mitochondria late" (Uptake upon pre-symbiosis by unknown mechanisms or by phagocytotic engulfment, respectively). (B) From FECA to LECA and the contributing factors: (i) Asgard archaea, (ii) alphaproteo (like) bacteria, and (iii) extensive HGT from other sources. FECA placement is arbitrary (see black arrows and main text). E – early; I – intermediate; L – late merger/acquisition. The "black box" with eukaryotic characteristics symbolizes our ignorance regarding the sequence of events. Color codes: Archaea/archaeal membranes—dark green; Bacteria/bacterial membranes/mitochondria – light blue; Nucleus/nuclear membrane – red; ER – light green; Golgi – purple; Peroxisomes – gray. Boxed changes: Cytoskeleton – The Asgard archaeon at minimum contained a precursor to the full complex eukaryotic three component cytoskeletal system of micro and intermediate filament and microtubules (cf. LECA flagella), allowing long extensions, either for effective metabolite exchange or a larger area of membrane potential [37, 58]. Gene invention – Both gene duplications and "new" genes (cf. increase). (Endo)membrane changes – large scale development of the endomembrane system and replacement of all archaeal membranes by bacterial ones. Could mitophagy [132] have been at the basis of all autophagy [133]? LECA probably was phagocytotic (see main text). All (other) major developments are discussed in the article. Adapted and extended from [55].

obic mitochondria with the complete oxidative phosphorylation (OXPHOS) system, a centriole, and peroxisomes. These cells were also capable of mitosis [19–21] and the related process of full meiotic sex [22–27]. Somewhat in contrast, the first eukaryotic common ancestor (FECA) concept remains rather more elusive and arbitrary. Where does one "stop going back"? Most researchers opt for an Asgard (like) archaeon that would engulf the precursor of the mitochondrion [13], but, taking the merger of an archaeon and a bacterium as the crucial event, the FECA could also be situated here [28, 29]. Both fit with seeing FECA as the organism splitting from its closest archaeal kin, before evolving the defining traits of eukaryotes. One point of concern: this nomenclature implicitly downgrades the contribution of the endosymbiont (we could consider eukaryogenesis as a "merger instead of an acquisition" [28]). Apart from these two cellular contributions there is a stunning and crucial third source (see Figure 1): a large array of other prokaryotes [30]. This source has sometimes been seen as reflecting remnants of serial endosymbiosis [31–33], but this has been questioned (see e.g., [34]), and it is now generally considered the be the result of extensive horizontal gene transfer (HGT). The question then becomes: what allowed this extraordinary number of HGT instances? I will argue that *mitochondria allowed the massive retention of HGT* derived genes (Figure 1) [35]. This early consequence of internal OXPHOS activity also brings into focus another major contributor to eukaryogenesis: ROS formation *inside* the newly formed entity.

3 | How to Get From FECA to LECA: The Difficulties of Phylogenomics

As illustrated in Figure 1, an enlightening framework to structure our discussion is a focus on the question when the precursor of the mitochondrion arrived on the scene. In "A" two extremes are depicted (leaving space for all kinds of *intermediate* scenarios). The timing of arrival would then be correlated with the number of eukaryotic characteristics possibly evolving in response to endosymbiont entry (the earlier, the more pleiotropic its effects). For the sake of clarity, I should stress that even a "late" arrival of the endosymbiont does not preclude some post-merger adaptations (e.g., a model in which the eukaryotic cell develops meiotic repair upon endogenous ROS formation by the newly acquired endosymbiont). Thus, the two extremes do not reflect an absolute dichotomy in this regard.

One scenario has mitochondria arriving (e.g., by way of "modern" eukaryotic phagocytosis; see Box 1) late in already highly complex pre-eukaryotic cells [14, 36]. This scenario stresses the well-documented genomic complexity of the Asgard archaeal clade, as compared to other prokaryotes [37], and it might try to explain the large number of genes coming in as the result of the close association of Asgardian archaea with other prokaryotes in complex symbiotic networks and/or the early development of phagocytosis. The other, with the endosymbiont arriving early, illustrates so-called symbiogenic models. Of note, in the context of eukaryogenesis, this term refers to models where necessary mutual adaptations of the merging organisms explain many, if not all, of the emerging eukaryotic characteristics. Thus, the *early* arrival of the bacterium, either upon phagocytosis or via another mechanism [38], is seen as a *crucial prerequisite for* eukaryogenesis [28, 39, 40]. For instance, a recent addition to symbiogenic hypotheses speculates that genome conflict drove innovations such as the nucleus, a complex cytoskeleton, and the synaptonemal complex [41]. So, how does one try to distinguish between these models, for instance using reconstructions of the LECA's proteome? One popular approach is by comparing the phylogenetic distances (i.e., the number of substitutions compared to their prokaryotic source, as reflected in the time span, T, for a gene/protein branch) of LECA protein families of alphaproteobacterial ancestry and mitochondrial localization with those of other prokaryotic origins or cellular localization. Then, "the smaller the relative change of the mitochondrial proteins, the more recent the acquisition" the reasoning goes. Using such kinds of reasoning in different phylogenetic contexts, research groups find the arrival to be late or intermediate [14, 17, 42]. In an excellent contribution, Roger and co-workers discuss the pitfalls of these approaches [13]. Most of the assumptions made stand up to scrutiny, they conclude, but a few problematic ones remain. The most important one: the assumption that proteins of different origins, on average, all evolve at the same rate (there should be no *systematic biases* in evolutionary rates). I quote: "However, eukaryogenesis must have involved significant shifts in evolutionary constraints on proteins as they adopted new functions." [13] Interestingly, this observation seems to invalidate the method as a means of timing endosymbiont (mitochondrion) entry, because there might indeed be differences between the evolutionary pathways of "host" and "organelle", both with regard to proteins and the genes encoding them. Let's list some here.

i. Often forgotten, the endosymbiont was a fully aerobic bacterium containing the complete respiratory chain as part of OXPHOS. It is well documented that amino acid composition and sequence determine proteome vulnerability to oxidation-induced damage [43]. Thus, the endosymbiont genome/proteome would not have to adapt further to normal levels of ROS formation, while the, originally anaerobic, host would.

ii. More speculatively, the host evolves into a more complicated center of control, with "…innovation in informational, endomembrane, signalling and cytoskeletal systems." [13], while the endosymbiont loses "autonomic" functions and "just" has to maintain metabolic contributions (though possibly at higher levels), which could be reflected in more changes in host genes, overall.

iii. Related to this, I should point out that we are looking at a *very skewed sample* of the endosymbiont genes/proteins. At least 40% (probably a significant underestimation) of the original alphaproteobacterial genes were lost [44]. Of those that were retained, practically all migrated to the nucleus. NB, a significant fraction of these preserved genes encodes proteins which function in the cytoplasm or other organelles [45, 46], and not in mitochondria.

Thus, finding the genes associated with alphaproteobacterial ancestry and mitochondrial localization changing less does not have to be due to "late endosymbiont entry" at all. At the very least, these considerations warn against basing the timing of endosymbiont entry exclusively on phylogenomic analyses.

4 | How to Get From FECA to LECA: Explaining Specific Features

Symbiogenic (syntrophic) models got a boost from a recent Nature Ecology & Evolution paper by Santana-Molina et al. [45, 47], in which they show that modern eukaryotes still bear the hallmarks of an ancestral syntrophy between "an asgardarchaeal host cell and an alphaproteobacterial endosymbiont" in the eukaryotic genes for central carbon metabolism [45, 48]. Symbiogenesis would predict mutual adaptations in each partner of this primordial merger, and there are many changes in the host that could be candidates for such adaptations (see below). However, are there *mitochondrion-specific* changes that have occurred during eukaryogenesis which could also make sense in this light?

Strikingly, some features of the endosymbiont were intensely altered during this instance of primary endosymbiosis only: the ribosomes [49] and electron transfer chain (ETC) complexes (especially complex I, NADH dehydrogenase [50, 51]) have become conspicuously bigger, with many more components as compared to their bacterial ancestors. A thorough analysis by van der Sluis et al. showed this to be eukaryogenesis-specific, as such extensive changes are not observed at all for chloroplasts, when compared to their cyanobacterial ancestors [52]. One could speculate that this is due to a period of increased ROS production by the endosymbiont upon metabolic rewiring and rapid switching between metabolic substrates, as can still be observed

occurring in versatile eukaryote model organisms today [53]. This would also help to account for the constructive neutral like character of complexity increase observed here [52, 54], because the changes seem to just laboriously repair damage [52], while small population sizes (see Box 2) would allow them to become fixed. Internal ROS increase might also help to explain the massive loss and relocation of alphaproteobacterial genes in the early stages of symbiogenesis [55].

Looking at host changes from a symbiogenic standpoint we first have to point out a difficulty. We actually do not have a good idea what the host looked like. Based on genomic analysis of its present-day closest relatives there are quite a few gene homologues that could have encoded a more complex archaeon/pre-eukaryote [56, 57], *if they functioned as they do now* in eukaryotes. However, so far, apart from the long extensions [58] (see legend Figure 1), the Asgard archaea have not been found to contain complex internal membrane systems. Recently, Asgard archaea ("Hodarchaeales") more closely related to the host, also turned out to be internally simple syntrophic cells [59]. Thus, the level of "host complexity" remains a matter of speculation. Many symbiogenic models have been proposed for the evolution of general eukaryotic characteristics. Just a few examples must suffice here: (i) Nucleus—the nuclear envelope would allow mRNA splicing (introns coming from genes transferred by the endosymbiont) to end before translation, restricting it to mature reading frames [60] or it would allow countering adverse effects of genome dynamics, not only symbiogenic ones [41]. (ii) Endomembrane system—resulting from co-evolution of Asgard archaea regulatory proteins (e.g., Arf GTPases [57]) and the release of outer membrane vesicles from the endosymbiont, also explaining the replacement of archaeal membranes by bacterial ones in the eukaryotes [61]. Another recent symbiogenic analysis also posits the mitochondrial endosymbiosis as being crucial for the emergence of compartmentalisation and organellar networks [62]. (iii) Peroxisomes—reducing endosymbiont ROS production by partially moving beta-oxidation [63, 64]. (iv) Meiotic sex – hypothesised to allow merging of endosymbiont and host genomes in early eukaryote evolution [65] or evolved as a repair mechanism upon the challenge of increased internal ROS formation by the endosymbiont [27]. These innovations all illustrate the general eukaryotic increase in size and complexity, which brings us to a vexing bioenergetic question: how can you afford to build all that?

5 | Internal Mitochondrial Membranes Dedicated to OXPHOS Were Crucial for Eukaryogenesis: ATP and ROS

Was the entry of the "pre-mitochondrion" necessary for the development of the much more complex eukaryotes (now able to retain and express genes obtained by HGT) with smaller effective population sizes (N_e)? Whether we look at it using the theoretical frameworks of population genetics or bioenergetics, the increase in cell size and mitochondrial inner membrane surface seems to have been necessary for this "jump in complexity". One basic reason: such increased complexity is expensive to maintain and, in the end, cannot be covered by the proton motive force (PMF) of further plasma membrane expansion (following a squared function), because energetic needs will increase according to a cubic function. Thus, the highly efficient internal mitochondrial dynamo with extended membrane invaginations using O_2, freely crossing the plasma membrane [35], as the final acceptor (in OXPHOS) looks to be crucial in making the leap from prokaryotes to eukaryotes [48]. This is illustrated by the fact that most anaerobic eukaryotes, though not all [66], are simplified

BOX 1 Was phagocytosis involved in the uptake of the "pre-mitochondrion" or not?

How did the endosymbiont end up in the archaeal host? Many recent examples of (temporary) endosymbiont uptake use phagocytotic mechanisms, so suggesting this well-established mechanism for the mitochondrion-to-be as well is obviously appealing [91]. Though the LECA was envisaged to be a "Predatory protist" [92], Bremer et al. used bioinformatic analysis to even dispute that the LECA itself was phagotrophic [93], and an extensive review of phagocytosis also tends toward several independent later developments [94]. However, recent research regarding the eukaryotic tree [9, 10, 12] pictures a typical phagocytotic heterotrophic (bi)flagellate with an excavate-like feeding groove at the root [95], the architecture of which traces back to the LECA population, as already stated in Hampl et al. [66]. Concerning its architecture: it was probably relatively small (25 μm or less) and flagellated to feed on bacterial prey in suspension [12]. However, even if we accept that LECA was phagocytotic, this tells us nothing about the primordial uptake mechanism of the alphaproteobacterial ancestor. The fact that there are relatively small phagotrophic eukaryotes and even ones that have lost their mitochondria is of course not informative about this first uptake at all [66]. Of note, not long ago, a planctomycetal bacterium with phagocytosis-like cell engulfment of prey was discovered [96]. This observation further falsified notions that prokaryotes are unable to engulf other cells, that only big cells are capable of phagocytosis, or that phagocytosis itself leads to major increases in cell complexity [66]. This instance concerns a bacterium, not an archaeon, and the mechanism, though unknown, must be different from the eukaryotic one. Regarding eukaryotic phagocytosis, there are several reasons to suppose it was not involved in pre-mitochondrial uptake [66], and I'll just mention two here. Though we still know very little about the ecology of Asgard archaea, they form part of symbiotic networks [37, 58, 59], in which contacts are not along the lines of predator prey relations [97], but likely concentrated around exchange of partially reduced metabolites, as we still find today in the eukaryotic cell [48], or maybe even heat exchange [98, 99]. Apart from that, in the most likely scenario, the incorporation of the endosymbiont released the archaeal membrane from PMF duties (see Box 2), thus allowing eukaryote-like phagocytosis only later on. But how could an Asgard archaeon have taken up an alpha-proteobacterial cell then? One exciting possibility is by outer membrane-fusion derived acquisition of a gram-negative bacterium in ecologically crowded (e.g., symbiotic) environments, as described in [38]. Such a mechanism could be involved in the uptake of gram-negative bacteria by non-phagocytotic, parasitic kinetoplastid protists, of which quite a few examples have been described [100, 101].

BOX 2 Debating bioenergetic efficiency: were mitochondria needed to start eukaryogenesis?

Did mitochondria open up evolutionary possibilities by (much) more efficient ATP generation? This has been almost acrimoniously discussed. I think some of this could be due to (i) discussions over concepts obscuring more basic agreements (see below); (ii) instances of erroneous anachronistic reasoning, as explained in [102]; (iii) scientific biases, for instance against rapid, revolutionary, change; and (iv) recently, one of the participants in many of these discussions claimed that systematic overestimations of the ATP requirements for growth obscure the crucial bioenergetic impact of the mitochondrial symbiosis; for details see [103].

Let's begin with some present-day eukaryotic examples, illustrating the energy efficiency of aerobic mitochondria. Resting (!) human adults use their bodyweight in ATP per day [104, 105], and tissue differences [106] spell out direct correlations between mitochondrial morphology, cristae, membrane surface area dedicated to proton motive force generation, and its use for massive ATP production [35, 107, 108]. Just like metazoans, unicellular eukaryotes demonstrate such overall correlations between cellular metabolism, mitochondrial use and cristae morphologies [35, 109]. It was claimed that, due to *internal* mitochondrial membranes, eukaryotic cells accrue 5×10^3 more metabolic power (W) per cell than prokaryotes. This would allow metabolically expensive maintenance and expression of many more genes in more complicated genomes [68, 110]. However, all calculations were performed using O_2 as the final acceptor, while at the same time these studies downplayed its importance, explicitly stating when discussing the salient advantage mitochondria provide: "It is not aerobic respiration—many mitochondria are anaerobic,…" [68] while referring to anaerobic eukaryotes [111]. But this is of course a prime example of anachronistic reasoning, such as also found in [66], where the existence of anaerobic as well as amitochondriate phagotrophic eukaryotes is invoked to throw doubt upon "…the assumption that evolution of complex features requires extra ATP,…" or the need "…to postulate that a mitochondrion must have preceded phagocytosis in eukaryogenesis." (see also Box 1). However, all of these examples are always "*reduced*" descendants of an *aerobic* LECA and that biological trajectory could make all the difference. There are (at least) two crucial differences between the ancient prokaryotic and the eukaryotic world which should give us pause: (i) Random genetic drift can "overrule" natural selection to allow change much more easily in eukaryotes relative to prokaryotes, due to smaller effective population sizes (N_e); [112] and (ii) The present-day biosphere is much richer, with many more diversified ecological niches. I will mention just one fact to boost this claim. Our best guess with regard to the biomass distribution (in Gigatons Carbon) at present for eukaryotes versus. prokaryotes is 468–77 (85%–15%) [113]. Assuming a stable prokaryote biomass, this would represent a huge increase in complex ecological niches for eukaryotes to adapt to, illustrated by many of the niches modern eukaryotic anaerobes have stepped into. Specific modern eukaryotes, especially anaerobic ones, can thus be very misleading as models illustrating steps from FECA to LECA.

That the increase in eukaryotic complexity was linked to superior mitochondrial energetics, along the lines formulated by Lane and Martin [68], was criticized by Lynch and Marinov [114]. Interestingly, they maintain that lifetime ATP requirements to sustain DNA, RNA, and protein levels just decline with cell volume, whether prokaryote or eukaryote. Consequently, only large eukaryotic cells, which have small effective population sizes (N_e), could integrate more and more genes. Together with the crucial observation that genetic drift allows genomic changes, even slightly disadvantageous ones, to become easily fixed in tiny populations [112, 115, 116], this would account for the prokaryote-eukaryote divide. This view is compatible with another interpretation, proteome allocation theory, stating that while early high relative energetic costs of new genes are suppressing growth rates, they can end up allowing higher survival capacity [117].

Here are some of the (counter)arguments during the prolonged discussions [68, 114, 118–121]. (i) Only a tiny part of the mitochondrial membrane contains ATP synthase dimers at the cristae edge [35, 122]. Counter: the PMF, generated by the number of respiratory ETC, which correlates with surface area, seems the actual limiting factor for ATP synthesis. (ii) Prokaryotic biomass forms the vast majority of the total [119]. Counter: recent estimates (85% is eukaryotic), quoted above [113], notwithstanding the fact that the prokaryotes outnumber eukaryotes a thousand-fold [123]. (iii) The plasma membrane has to relinquish bioenergetic contributions (Asgard archaea seem to use it thus [124]) abolishing any gain in capacity. Counter: this dismisses surface/volume considerations, but even worse places the loss of plasma membrane PMF in a negative light. But being replaced by a PMF generated by an extended, *internal* membrane almost completely dedicated to OXPHOS, opens up possibilities for the plasma membrane to be involved in new behaviors (such as phagocytosis). (iv) Lifetime ATP requirements maintaining DNA, RNA, and protein levels correlate *smoothly* with cell volume from prokaryotes to eukaryotes. Counter: we still have two clearly separate cellular distributions with extremes (large prokaryotes and small eukaryotes) having some overlap, but averages far apart. The overlap is partly filled with "reduced" eukaryotes which ought not to be interpreted anachronistically.

unicellular organisms, except for one very basic metazoan [67]. More details of the arguments involved are given in Boxes 2 and 3.

Apart from efficient ATP synthesis, what are the consequences of having highly charged membranes, relying on O_2, *inside* the cell? Lane & Martin note that deviations from an optimal mitochondrial membrane potential are detrimental, risking "a rise in free-radical leak" [68]. These ROS are already abundantly formed when mitochondria function normally [69], for instance because breakdown of different metabolites results in differential use of electron entry points in the respiratory chain [53, 70, 71]. Using the mitochondrial PMF constitutes a complicated balancing act, and both sudden drops and elevations of the PMF are associated with ROS formation [72, 73], helping to explain the evolution of so-called uncoupling proteins [74, 75]. A host of other eukaryotic characteristics might be understood as evolutionary adaptations to using O_2 and its consequences in the form of internal ROS formation too [76]. As mentioned above, peroxisomes could have evolved in the context of lessening

BOX 3 An endosymbiotic ratchet model of endosymbiosis

The deliberations here have been mostly theoretical, so far. Are there no data available for what occurs in the first stages of primary endosymbiosis which have not been obliterated by vast time (> billion years) lapses? Luckily, we have a more recent example in the photosynthetic amoeba *Paulinella chromatophora*, where primary cyanobacterial endosymbiosis (giving rise to a "chromatophore") occurred "only" ~120 million years ago [125, 126]. The pertinent analyses come from Bhattacharya and co-workers. Recently, they argued for "…a central role for effective population size (N_e) in accelerating divergence post-endosymbiosis due to limits to dispersal and reproductive isolation that reduce N_e, leading to local adaptation" and stated that populations "…assort themselves in non-overlapping niches to minimize competition during the early, rapid evolutionary phase of organelle integration." These quotes come from the abstract of their aptly entitled article "Endosymbiotic ratchet accelerates divergence after organelle origin" [126]. The *Paulinella* data seem to fit a picture in which a difficult population bottleneck is survived within a specific niche (120 Mya ago), only later (60 Mya ago) allowing successful diversification and spread of these photosynthetic amoebae. Some of the obvious difficulties encountered during integration of a prokaryotic organism they discuss in "Why is primary endosymbiosis so rare?", introducing a simplified 'chassis and engine' model, which describes how host cells (chassis) and engulfed bacteria (engines) have to interact during primary endosymbiosis. For further details see [127]. Recently, another example of "new" (~1 Mya ago) primary endosymbiosis was discovered: the uptake of an N_2-fixing cyanobacterium functioning as a "nitroplast" of marine unicellular algae [128, 129]. This system might also be probed for general characteristics of ongoing primary endosymbiosis in eukaryotes [130].

The findings seem to dovetail with symbiogenic models of eukaryogenesis itself. Let me end the discussion of endosymbiotic ratchets with the following observations. All the primary cyanobacterial uptakes (leading to the Archaeplastida, the chromatophore in *Paulinella*, and the nitroplast) occurred in fully formed eukaryotes, but the uptake of the pre-mitochondrion did *not*. If we explain the relative scarcity of primary (as compared to secondary and tertiary) endosymbiosis by the availability of genes enabling metabolic integration (e.g., those involved in organellar protein import) in only one or in both partners [127], we have a further indication for the even greater challenge posed by eukaryogenesis itself. The absence of extant intermediates from the FECA to LECA transition seems to reflect a difficult population bottleneck, the effects of which might have been exacerbated by the relative rarity of aerobic niches on a still largely anaerobic planet. As mentioned in a recent report about the cultivation of a possible FECA-like host: "…FECA was a simple-celled anaerobic syntrophic peptide-degrading archaeon with …potential adaptations toward an oxygenated planet—the archaea-eukaryote transition was steep in both cell structure and aerobiosis." [59] However, while lacking intermediates might point to a bottleneck, this does not guarantee they will never be found.

ROS formation associated with fatty acid breakdown [63] and meiotic sex in response to an increase in free-radical mediated mutation rates [26, 27, 77]. Apoptotic processes, associated with impaired mitochondrial function and enhanced ROS formation probably go far back also [78]. Other essential characteristics possibly linked to endogenous ROS formation can be found in table 1 in [55]; even the nuclear membrane itself might be seen as a protective device against internal ROS formation [28]. One other contribution almost certainly derived from the endosymbiont, the replacement of all archaeal by bacterial membranes (possibly even contributing to the development of the eukaryotic endomembrane system by secreting outer membrane vesicles into the cytoplasm [61]), has already been mentioned as well. However, nothing is known about the relative ROS sensitivity of bacterial and archaeal membranes. In conclusion, ROS, an intrinsic, unavoidable by-product of highly efficient mitochondrial ATP production, seems to have been crucial during eukaryogenesis.

6 | Some Further Things to Ponder

One of the difficulties in understanding biological processes, let alone major transitions, is the sheer complexity of the interactions we are contemplating. This implies that identifying *crucial* factors will always remain challenging. However, we can try to illustrate the likelihood of a dominant contribution of the internal OXPHOS system provided by the endosymbiont by listing the following observations:

i. The eukaryotes only appeared after the great oxygenation event (~2.3 Ga -billion years-ago) approximately 2.0–1.8 Ga ago, [79, 80] while prokaryotic life had been around for ~4.2 Ga, according to the most recent insights [81]. A recent article concluded: "…the rise in complexity was temporally consistent with … the rise in oxygen. This suggests a causal relationship stemming from the increased energy needs of complex life fulfilled by oxygen" [80].

ii. There is a general, wide, gap between prokaryotic and eukaryotic complexity, not only in regard to obvious aspects, such as gene content, chromosomal organization or size, but also, as recently observed, with regard to potential for regulatory RNAs [82].

iii. Eukaryotes are characterized by extensive adaptations to oxidative processes including the associated damage and use intensive ROS signaling, mostly relayed by the redox states of critical cysteine residues and interconnecting, amongst others, epigenetics, mitochondria, gene regulation, proteostasis, and circadian rhythms [83–86].

iv. Many eukaryotic inventions can be (partly) understood in the light of *internal* ROS formation; see for example, [55].

v. There is a general correlation between levels of OXPHOS potential and eukaryotic complexity.

vi. Considering the possibility of such a dominant contribution of the mitochondrion (arriving at the start, delivering metabolic power, allowing extensive retention of genes acquired by HGT, and supplying membranes) the cur-

rent use of the FECA concept exposes the dominance of phylogenomic analysis.

In the future, phylogenomic analyses performed to illuminate the fascinating, but still enigmatic, FECA to LECA transition should be enhanced by some of the cell biological and biochemical considerations that were discussed. I want to end a bit more provocatively, briefly addressing why there is still broad resistance to symbiogenic models of eukaryogenesis, by highlighting the following aspects. (i) Most of the evolutionary reconstructions are of necessity phylogenomic, and one might wonder if ancient periods of rapid change could ever be accurately detected by this kind of analysis. (ii) The implicit rejection of revolutionary occurrences as illustrated by interpreting the pre-mitochondrial endosymbiosis as just one of many; earlier ones being seen as lost in time or leaving traces in the form of peroxisomes [87] or hydrogenosomes [88], later ones being so numerous as to illustrate the relative ease of their occurrence [89], downplaying the momentous differences involved in this single event [28, 52, 55]. (iii) Even deeper, as scientists we have the tendency to balk at "mere chance" disrupting predictable, lawlike behavior. However, in biology we have to accept its influence at every level: evolution is a fundamentally historic process. (iv) There is a partial connection to a common prejudice that evolution has an intrinsic tendency toward ever greater complexity (recently again dismantled in [90]) which does not sit well with a scenario of accidental uptake by a "tiny prokaryotic" archaeon, followed by an unlikely bottleneck of mutual adaptations, sculpted by chance, ever increasing ATP generating efficiency, and the challenges of internal ROS formation. But such a symbiogenic model for eukaryogenesis might be our best bet in light of the recent evidence.

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Conflicts of Interest

The author declare no conflict of interest.

Data Availability Statement

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

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