nature microbiology

PERSPECTIVE

https://doi.org/10.1038/s41564-020-0710-4

The Syntrophy hypothesis for the origin of eukaryotes revisited

Purificación López-García ✉ and David Moreira

The discovery of Asgard archaea, phylogenetically closer to eukaryotes than other archaea, together with improved knowledge of microbial ecology, impose new constraints on emerging models for the origin of the eukaryotic cell (eukaryogenesis). Long-held views are metamorphosing in favour of symbiogenetic models based on metabolic interactions between archaea and bacteria. These include the classical Searcy's and Hydrogen hypothesis, and the more recent Reverse Flow and Entangle–Engulf–Endogenize models. Two decades ago, we put forward the Syntrophy hypothesis for the origin of eukaryotes based on a tripartite metabolic symbiosis involving a methanogenic archaeon (future nucleus), a fermentative myxobacterial-like deltaproteobacterium (future eukaryotic cytoplasm) and a metabolically versatile methanotrophic alphaproteobacterium (future mitochondrion). A refined version later proposed the evolution of the endomembrane and nuclear membrane system by invagination of the deltaproteobacterial membrane. Here, we adapt the Syntrophy hypothesis to contemporary knowledge, shifting from the original hydrogen and methane-transfer-based symbiosis (HM Syntrophy) to a tripartite hydrogen and sulfur-transfer-based model (HS Syntrophy). We propose a sensible ecological scenario for eukaryogenesis in which eukaryotes originated in early Proterozoic microbial mats from the endosymbiosis of a hydrogen-producing Asgard archaeon within a complex sulfate-reducing deltaproteobacterium. Mitochondria evolved from versatile, facultatively aerobic, sulfide-oxidizing and, potentially, anoxygenic photosynthesizing alphaproteobacterial endosymbionts that recycled sulfur in the consortium. The HS Syntrophy hypothesis accounts for (endo)membrane, nucleus and metabolic evolution in a realistic ecological context. We compare and contrast the HS Syntrophy hypothesis to other models of eukaryogenesis, notably in terms of the mode and tempo of eukaryotic trait evolution, and discuss several model predictions and how these can be tested.

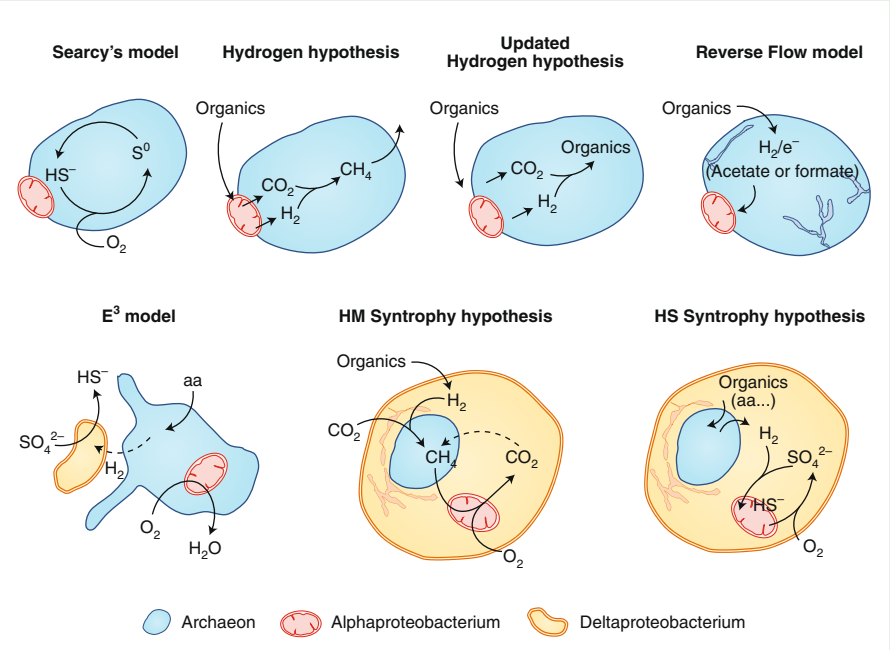
Eukaryogenesis was a unique major evolutionary transition resulting in a substantial increase in average cell complexity. This foundational event led to an impressive radiation of morphologically diverse phyla, most of them unicellular (protists) but many including multicellular taxa such as animals, fungi, kelp and land plants[1]. Elusive for a long time, reconstructing a mechanistically plausible and ecologically realistic model for the origin of eukaryotes appears now within reach thanks to recent advances in molecular phylogenomic tools, genome-binning from metagenomes and a better knowledge of microbial diversity and function in natural ecosystems. Until recently, notwithstanding the generally accepted endosymbiotic origin of mitochondria and chloroplasts, models proposing the symbiotic origin of eukaryotes directly from bacterial and archaeal ancestors were largely dismissed[2,3]. The prevailing view stated that an independent proto-eukaryotic lineage sister to archaea evolved most eukaryotic features (including a complex cytoskeleton, endomembranes, nucleus and phagocytosis) before it engulfed the alphaproteobacterial ancestor of mitochondria[4–6]. This view started to vacillate with the realization that truly primary amitochondriate eukaryotes were not known[7] and additionally deteriorated with phylogenomic trees where eukaryotes branched within archaea, albeit without clear sister groups[8]. The discovery of Asgard archaea, a phylogenetically deep-branching lineage sharing more and more similar genes with eukaryotes than other archaea[9,10], has further fostered this paradigm shift on eukaryogenesis. Eukaryotes are no longer on the same footing as archaea and bacteria as one of the original primary domains of life[11]; they are a third but secondary domain resulting from the evolutionary merging of specific archaeal and bacterial linages[2,6,12–14]. Moreover, current knowledge about Asgard archaea's general metabolic potential and preferred biotopes (mostly sediments and microbial mats, where intimate metabolic interactions are the rule[3,15]) realistically favour symbiogenetic models based on metabolic symbioses or syntrophies[16,17]. This is further supported by the syntrophic nature of the first cultured Asgard member, '*Candidatus* Prometheoarchaeum syntrophicum', an anaerobic organism able to grow in symbiosis with a sulfate-reducing deltaproteobacterium, a methanogenic archaeon or both[18]. Collectively, this strongly supports cooperative models for the origin of the eukaryotic cell[2,3,6,17] whereby higher complexity evolved from the physical integration of prokaryotic cells combined with extensive gene and genome shuffling[12,19–21].

The first symbiogenetic models date back to more than 20 years ago. Among them, the more detailed were the Serial Endosymbiosis theory[22–24], the Hydrogen hypothesis[25] and the Syntrophy hypothesis[26,27]. In the original Syntrophy hypothesis, we proposed that eukaryotes evolved from a tripartite metabolic symbiosis based on (i) interspecies hydrogen transfer from a fermenting deltaproteobacterial host to an endosymbiotic methanogenic archaeon and (ii) methane recycling by a versatile methanotrophic, facultative aerobic alphaproteobacterium[26] (hydrogen–methane (HM) Syntrophy). From an ecological perspective, these metabolic interactions were reasonable, being widespread in anoxic and redox transition settings[2]. However, previous knowledge about archaeal diversity and metabolism was much more fragmentary than it is today, and the metabolic potential of uncultured lineages remained inaccessible. The probable involvement of an Asgard archaeal relative in eukaryogenesis imposes new constraints, such that realistic models need to consider their metabolic potential and ecology. Accordingly, several symbiogenetic models are currently being put forward. They differ on the metabolic interactions proposed (Box 1) and, importantly,

Ecologie Systématique Evolution, CNRS, Université Paris-Saclay, AgroParisTech, Orsay, France. ✉e-mail: puri.lopez@u-psud.fr

Box 1 | Symbiogenetic models for the origin of eukaryotes based on metabolic exchange

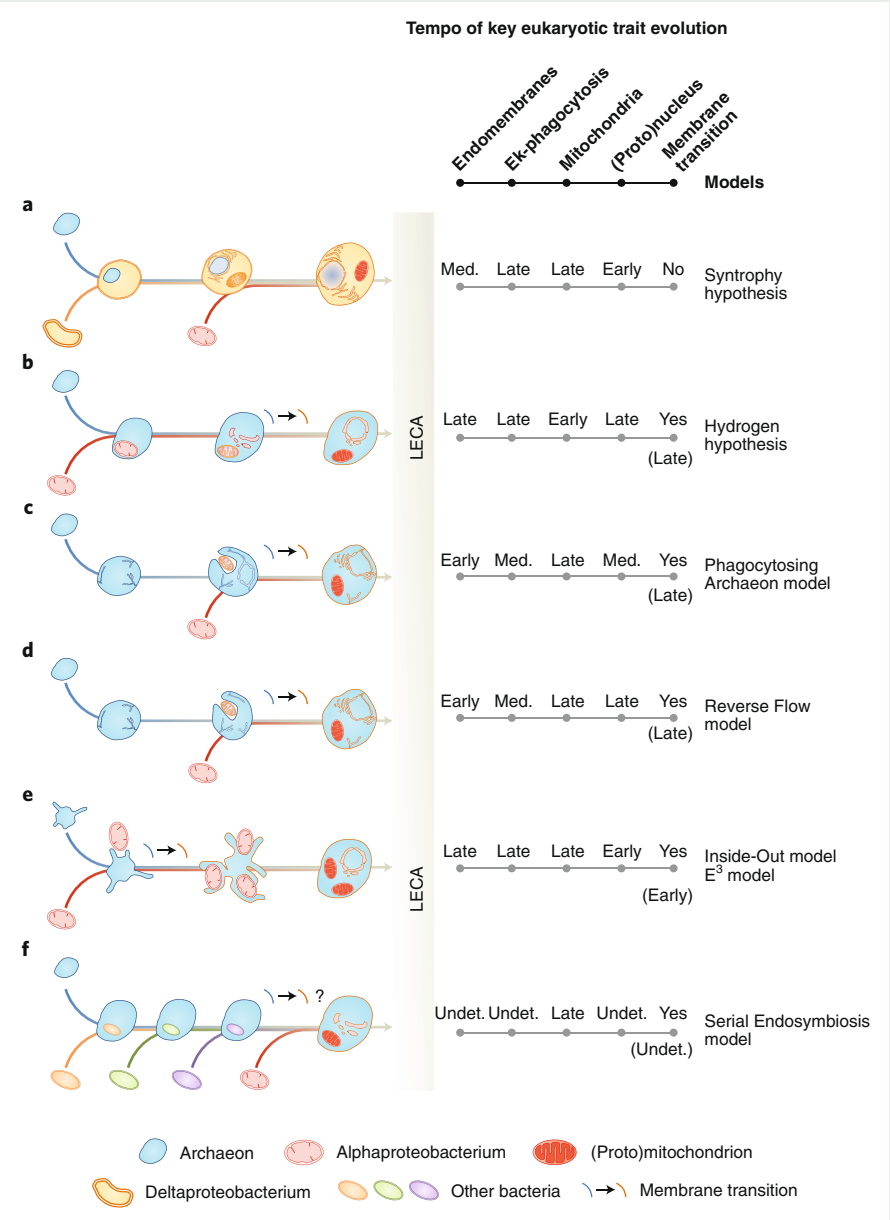
A variety of models propose that the eukaryotic cell evolved from a metabolic symbiosis (or syntrophy) established between archaeal and bacterial cells in anoxic or microoxic environments. Most of them involve only two partners, one archaeon and the alphaproteobacterial ancestor of mitochondria. However, some models invoke the participation of one additional bacterium, either transiently, as a facilitator of the eukaryogenetic symbiosis[18], or as an integral part of it[19,26]. One of the oldest proposals based on explicit syntrophy was that of D. Searcy, who stated that eukaryotes derived from a sulfur-mediated symbiosis between a wall-less, sulfur-respiring *Thermoplasma*-like archaeon and photo- or chemoautotrophic H_2S-utilizing bacterium[149,150]. The original Hydrogen hypothesis postulated a hydrogen-mediated symbiosis between a hydrogenoclastic methanogenic archaeon and a hydrogen-producing alphaproteobacterium, with hydrogen being used to reduce the CO_2 also released by the bacterium for methanogenesis[25]. In a more recent version of the Hydrogen hypothesis, the initial methanogenic host was abandoned in favour of an autotrophic, non-methanogenic archaeon that would use the Wood–Ljungdahl pathway to fix carbon using the hydrogen released by the mitochondrial ancestor[148]. Based on the inferred ancestral metabolism of Asgard archaea, which likely were organoheterotrophs with flexible potential for hydrogen consumption and production, Spang and co-workers put forward the Reverse Flow model. In this model, the eukaryogenetic syntrophy was based on hydrogen transfer (or electrons, that is, reducing equivalents, which might be also mediated by formate or acetate) from the anaerobic heterotrophic archaeon to the alphaproteobacterium[16]. The recent Entangle–Engulf–Endogenize (E^3) model[18] favours a dual symbiosis, in microoxic environments, of an Asgard archaeon that degraded amino acids to short-chain fatty acids and hydrogen with a sulfate-reducing bacterium (SRB) and an aerobic organotrophic alphaproteobacterium that scavenged toxic O_2. As the consortium progresses towards increasingly oxic zones, the interaction with the alphaproteobacterium becomes stronger until it is engulfed. The SRB symbiosis is transient and eventually lost[18]. Finally, the original version of the syntrophy hypothesis (HM Syntrophy) postulated a tripartite integrative symbiosis. First, a syntrophy based on interspecies H_2 transfer was established between a fermentative, ancestrally sulfate-reducing myxobacterium (Deltaproteobacteria) and a methanogenic archaeon using the fermentation-derived hydrogen for methanogenesis. Subsequently, a metabolically versatile alphaproteobacterium able to carry out facultative aerobic respiration but also able to oxidize methane (methanotroph) incorporated stably into the consortium[19,26]. In the revised variant of the Syntrophy model (HS Syntrophy), we hypothesize a symbiosis between a hydrogen-releasing Asgard archaeon able to degrade small organics and a complex, myxobacterial-like deltaproteobacterial host scavenging hydrogen (or reducing equivalents) for sulfate reduction. The alphaproteobacterial ancestor of mitochondria was a sulfide-oxidizing facultative aerobe, recycling sulfur in the consortium. It was also possibly a mixotrophic organism able to carry out anoxygenic photosynthesis using H_2S as electron donor.



the tempo and mode of evolution of key eukaryotic traits (Box 2). Here, we present an updated version of the Syntrophy hypothesis based on a tripartite metabolic symbiosis involving interspecific hydrogen and sulfur transfer (HS Syntrophy) occurring in redox transition ecosystems: a complex sulfate-reducing deltaproteobacterium (host), an endosymbiotic hydrogen-producing Asgard-like archaeon (future nucleus) and a metabolically versatile, facultatively aerobic, sulfide-oxidizing and potentially anoxygenic photosynthesizing alphaproteobacterium (future mitochondrion). We also briefly discuss the evolution of the endomembrane system, the nucleus and the genome[19] in the framework of the HS Syntrophy hypothesis. This model makes several predictions that differentiate it from alternative scenarios, including, notably, the two-step origin of the nucleus (first as a distinct metabolic compartment before its consecration as a major genetic reservoir and expression centre) and the bacterial origin of eukaryotic membranes and cytoplasm. We propose ways to specifically test some aspects of different eukaryogenesis models and offer suggestions for future avenues of research.

Box 2 | Symbiogenetic models for the origin of eukaryotes according to the timing and mode of evolution of key eukaryotic traits

Regardless of the metabolic basis of the symbiosis established between the Asgard archaeon and its bacterial partner(s) during eukaryogenesis, models differ in the proposed mechanisms that resulted in the physical integration of two or more cells in one (the future eukaryotic cell) and the evolution of typical eukaryotic traits as well as in the relative timing of the involved events (**a**–**e**). The Syntrophy hypothesis is the only model where a membrane transition is not needed, since the host (future cytoplasm) is a deltaproteobacterium naturally endowed with bacterial phospholipids (**a**). The future nucleus has an early origin in this model; it would derive from a distinct metabolic compartment (endosymbiotic archaeon) that is progressively confined by a host-derived secretory membrane. Mitochondria appear relatively late. In the Hydrogen hypothesis[25], the endosymbiosis of the alphaproteobacterial ancestor of mitochondria within an archaeon by means independent of classical, eukaryotic-like phagocytosis (Ek-phagocytosis) is the starting event triggering eukaryogenesis (**b**). The nucleus and the associated membrane system would form de novo from lipid vesicles produced by the alphaproteobacterium[126,151]. The archaeal membrane phospholipids would have been fully replaced by the bacterial phospholipids by the fusion of those bacterial vesicles with the outer plasma membrane and the progressive displacement of archaeal lipids[151]. Currently, in the most widely accepted type of eukaryogenetic models, which include the Phagocytosing Archaeon[81] hypothesis (**c**) and the related Reverse Flow model (**d**), the development of a complex cytoskeleton and endomembrane system predate the acquisition of the mitochondrial ancestor by classical phagocytosis[6,9,16]. Although these two models show similarities, in the Phagocytosing Archaeon model, the nucleus would appear before the mitochondrial acquisition in contrast with the Reverse Flow model. At any rate, in these models (**c**,**d**) the mitochondrial endosymbiosis would be the consequence of an already well-engaged eukaryogenetic process. From this perspective, these models represent the transposition of past scenarios based on the existence of a proto-eukaryotic lineage different from archaea and bacteria endowed with all of the typical eukaryotic traits but



Box 2 | Symbiogenetic models for the origin of eukaryotes according to the timing and mode of evolution of key eukaryotic traits (Continued)

mitochondria[4,5,79,152], to a nucleus-lacking proto-eukaryotic Asgard archaeon that is already seen as the FECA[6,153]. These two models and the Hydrogen hypothesis clearly differ in the timing of the mitochondrial acquisition and the endomembrane system but, in the three cases, the archaeal membrane phospholipids are replaced relatively late by bacterial-type phospholipids (**b**–**d**). In another set of models, including the Inside-Out[147] and the E^3 hypotheses, the mitochondrial ancestor is acquired relatively late by a slow process of engulfment. This involves archaeal membrane extrusions that progressively surround, entangle and eventually endogenize the future mitochondria (**e**). At least in the Inside-Out model, the archaeal-to-bacterial membrane phospholipid transition would occur relatively early, facilitated by close cell-to-cell contact and symbiotic gene transfer. According to this model, only bacterial membranes that are far more flexible than the archaeal ones would be able to form an endomembrane system and carry out phagocytosis[147]. Finally, in the Serial Endosymbiosis model, several sequential symbioses intervene[75,133]. Although the details remain undetermined, the mitochondrion would be acquired late (**f**). Med., medium; Undet., undetermined.

The ecological context of the eukaryogenetic symbiosis

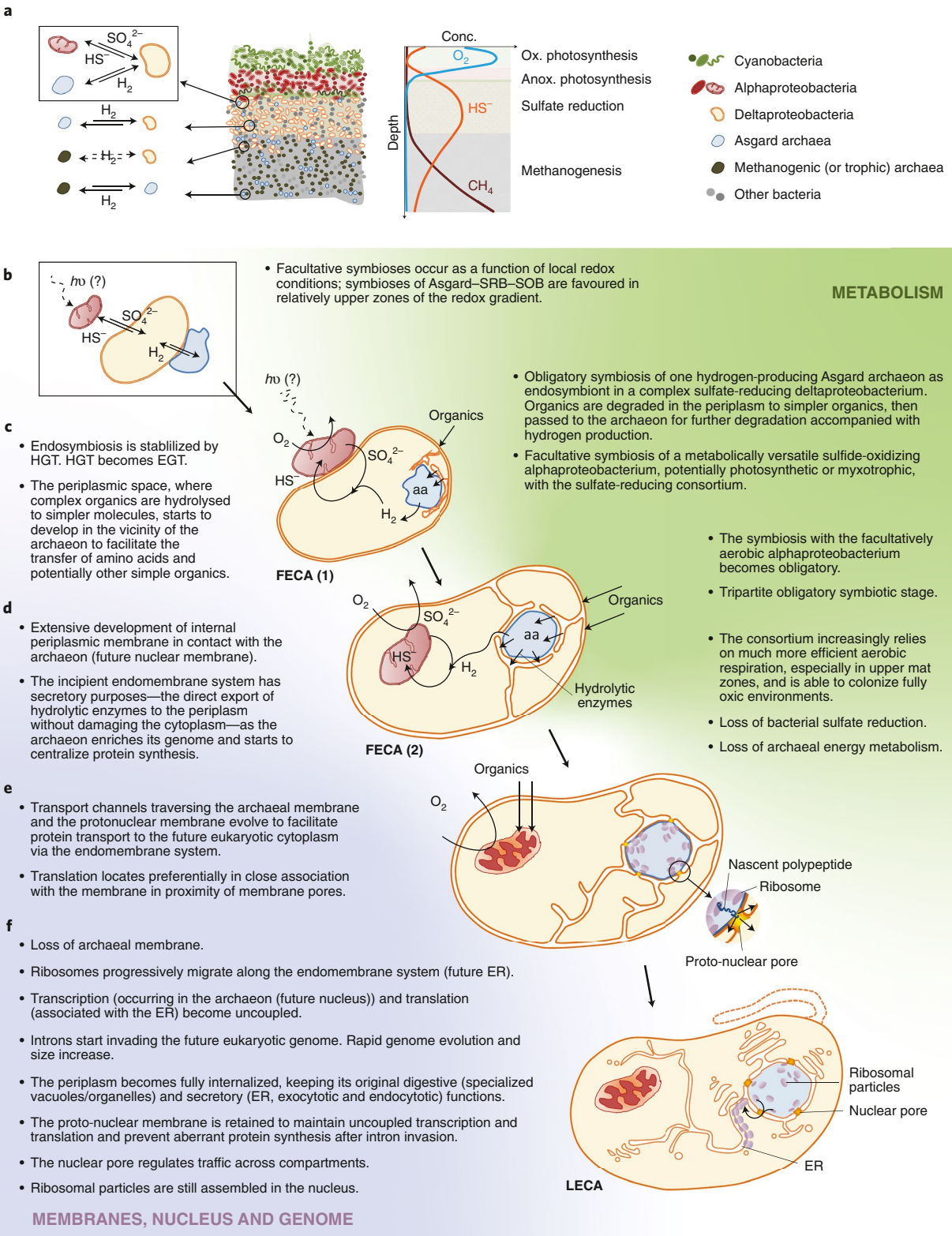
Despite the challenges associated to the interpretation of the earliest life traces, the oldest reliable eukaryotic fossils can be dated back to at least 1.65 billion years ago (Ga)[28]. This imposes a minimal age for the origin of eukaryotes that roughly agrees with the oldest boundaries of recent molecular dating estimates for the last eukaryotic common ancestor (LECA; 1.0–1.6 Ga)[29] and the eukaryotic radiation (<1.84 Ga)[30]. At the same time, LECA was complex, being endowed with mitochondria and resembling modern protists[20,21]. Although the alphaproteobacterial lineage that gave rise to the mitochondrion is yet to be precisely identified[31], it is clear that the mitochondrial ancestor was aerobic[32], but likely also possessed anaerobic respiratory capacities (as in many modern protists). This implies that aerobic respiration had already evolved in bacteria when the mitochondrial endosymbiosis occurred, and that oxic or microoxic conditions existed in the environment where the mitochondrial endosymbiosis took place or in its immediate vicinity. Aerobic respiration possibly evolved (almost) in parallel to cyanobacterial oxygenic photosynthesis, which led to the oxygenation of the atmosphere, the Great Oxidation Event (GOE), some 2.4 Ga ago at the beginning of the Proterozoic (2.5–0.5 Ga)[33,34]. Therefore, eukaryogenesis took place between the GOE and the minimum age of the oldest unambiguous eukaryotic fossils[28]. If some older, more difficult to affiliate, fossils[35] are indeed eukaryotic, eukaryogenesis might have occurred during the first three to five hundred million years after the GOE.

What did the Earth look like at that time? Before the GOE, the atmosphere and oceans were essentially anoxic, which constrained existing biogeochemical cycles. The atmosphere rapidly oxygenated from 2.33 Ga, but sulfate levels in oceans increased more slowly[33], limiting the biological S cycle[36,37]. This means that oceans were oxygen-poor during the early Proterozic, when eukaryotes evolved; the deep ocean remained anoxic until the beginning of the Phanerozoic (500 million years ago (Ma))[38–40]. If an aerobic mitochondrial ancestor suggests oxygen availability at or near the environment where eukaryotes finally evolved, current knowledge on Asgard archaea ecology and metabolism strongly suggests that the archaeon involved in eukaryogenesis, and hence the first eukaryogenetic steps, were strictly anaerobic. Asgard archaea are mostly found in deep-sea sediments[9,10,18,41] and microbial mats[10], including thermophilic ones[10,42]. Thus, with the exception of some derived planktonic Heimdallarchaeota, which more recently acquired the capacity to oxidize organics using nitrate or oxygen as terminal electron acceptors[16,43], the vast majority of Asgard archaea thrive in anoxic environments, as their ancestors did, degrading organics and producing or consuming hydrogen[16]. These observations argue in favour of redox transition environments—where anoxic and oxic or microoxic zones are in close proximity—as preferred ecosystems for eukaryogenesis. Furthermore, since the deep ocean during the early Proterozoic was anoxic, it is more likely that eukaryotes evolved in shallow sediments or microbial mats, where redox gradients established, like today, from the oxygen-enriched surface where cyanobacterial oxygenic photosynthesis took place to the increasingly anoxic layers below.

Phototrophic microbial mats are particularly interesting potential eukaryogenesis cradles. They were the Proterozoic 'forests', dominating shallow aquatic and terrestrial habitats, as abundant fossil stromatolites (lithified microbial mats) show[34,44,45]. These light- and redox-stratified microbial communities are phylogenetically and metabolically diverse[42,46,47]. Although microorganisms in modern mats are different from their Proterozoic counterparts, core metabolic functions have been mostly preserved across phyla[48] and at the ecosystem level, suggesting that functional shifts observed in mats across redox gradients today reflect early metabolic transitions[49]. Most primary production occurs in upper layers, where light can penetrate, via photosynthetic carbon fixation. The upper cyanobacterial oxygenic photosynthesis layer is typically followed by a reddish layer dominated by oxygen-tolerant anoxygenic photosynthesizers (Alpha- and Gammaproteobacteria) and, often, an underlying green layer of photosynthetic Chloroflexi and/or Chlorobi. Organic matter fixed in the upper mat layers is progressively degraded in deeper, anoxic layers by extremely diverse microbial communities[42,50,51]. Two broad zones can be distinguished in vertical anoxic profiles where sulfate reduction and methanogenesis dominate, respectively[46] (Fig. 1a).

Fig. 1 | Environmental context, metabolic interactions and (endo)membrane evolution during eukaryogenesis according to the HS Syntrophy hypothesis. **a**, Eukaryogenesis took place in phototrophic microbial mats where steep redox gradients occur. Syntrophic interactions based on interspecies hydrogen and/or sulfur transfer are widespread depending on local physicochemistry; they notably involve methanogens, Asgard archaea, SRB and SOB. **b**–**f**, Different eukaryogenesis steps. **b**, Initial facultative symbiosis stage involving a hydrogen-producing Asgard archaeon, a sulfate-reducing deltaproteobacterium and a sulfide-oxidizing alphaproteobacterium possibly able to carry out anoxygenic photosynthesis. **c**, First integration of the Asgard archaeon as an endosymbiont (the future nucleus). **d**, Second integration step involving the endosymbiosis of the alphaproteobacterium. Stages c and d may have been coetaneous (FECA stage) or, more likely, decoupled in time (FECA 1 and 2). **e**, Advanced integration stage involving important changes in metabolism (the consortium relies on aerobic respiration with all other previous metabolic interactions between partners being lost) and endomembrane evolution. **f**, LECA stage. The position of H_2 or any other substrate by an arrow (over or under) implies transfer in the sense of the arrow; when it is on two arrows of opposed directionality, transfer may occur either way. aa, amino acids; Conc., concentration; Ox., oxygenic; Anox., anoxygenic; *hυ*, photon-derived energy.

Here, like in anoxic sediments, the degradation of organic matter involves syntrophy[52], mostly implicating interspecies hydrogen (or, directly, electron[53]) transfer. In anoxic environments, pairs of electron donors and acceptors display low redox potential differences such that many energy-generating metabolic reactions can only proceed in the presence of syntrophic sinks[54]. Methanogenic archaea and SRB belonging to the Deltaproteobacteria are frequently engaged in syntrophy. Deltaproteobacteria are metabolically diverse and can use or produce hydrogen or, directly, electrons[55], and are frequently involved in interspecies hydrogen or electron transfer[53]. Many of them oxidize organic compounds with sulfate, but they can also be autotrophic[56] (including in syntrophy[57]), can use other electron donors and acceptors (including metals such as arsenic[58]) and can ferment or switch between metabolisms depending on the



environmental conditions[59]. Deltaproteobacteria establish widespread syntrophies with archaea, such as with methanogens when acting as hydrogen producers and with methanotrophic archaea when acting as hydrogen-consuming sulfate-reducers[60]. Mutualistic interactions between methanogens and deltaproteobacteria can be rapidly selected, leading to specialized syntrophy[61,62]. Deltaproteobacterial SRB also establish symbioses with sulfide-oxidizing or other bacteria and eukaryotes[2,59,63]. In addition to methanogens and SRB, a wide variety of uncultured lineages occur in anoxic sediment and microbial mat layers, where archaea thrive. Many of these archaea seem to be involved in cycling organics, particularly alkanes, and are likely being engaged in syntrophies with hydrogen scavengers[49,52,64]. Indeed, the first cultured Asgard archaeon can grow by degrading amino acids in syntrophy with either a sulfate-reducing deltaproteobacterium and/or a methanogen[18].

Eukaryogenetic syntrophies

Considering this historical and ecological context, we favour the idea that eukaryogenesis occurred in microbial mats (or similarly stratified shallow sediments) with marked redox gradients (Fig. 1a) that were potentially mildly warm. Oxygenic photosynthesis—and, in close proximity, aerobic respiration—might have first evolved in warm environments. Indeed, most deep-branching cyanobacteria are thermophilic[65,66]. Although universal molecular mechanisms to cope with reactive oxygen species exist[67,68] and might have been co-opted from antioxidant-prone compounds very early[69,70], oxygen toxicity would have been advantageously relieved in thermophilic mats by its rapid release into the atmosphere (oxygen is poorly soluble at high temperature). The original (HM) Syntrophy hypothesis postulated, on solid microbial ecology grounds, the evolution of eukaryotes from well-known widespread symbioses between fermenting (hydrogen-producing), ancestrally SRB, deltaproteobacteria and methanogenic archaea. SRB and methanogens, which compete for hydrogen, can readily evolve stable syntrophy in co-culture[61,62]. We additionally favour a myxobacterial-like deltaproteobacterium due to the similarities shared by these complex social bacteria and eukaryotes[19,26]. This symbiosis would have established at the sulfate–methane transition zone but evolved upwards in the redox gradient, where an additional symbiosis formed with a versatile methanotrophic alphaproteobacterium that scavenged the methane released by the primary consortium. We cannot completely reject such tripartite metabolic symbiosis at the origin of eukaryotes since methanogenesis, originally thought to be exclusive to Euryarchaeota, occurs across archaeal phyla and might have been ancestral to the archaeal domain[71–74]. However, although methanogenesis might be eventually discovered in Asgard archaea (some Asgard archaea do have methyl-coenzyme M reductases, probably involved in the reverse anaerobic alkane oxidation reaction[41]), current genomic comparisons seem to exclude it from their ancestral metabolic capacities[16].

In this context, we now favour a similar eukaryogenetic process based on alternative, albeit equally ecologically relevant, metabolic symbioses (Fig. 1). In our HS Syntrophy hypothesis, we propose that eukaryotes evolved from the syntrophic interaction of a sulfate-reducing (hydrogen- or electron-requiring) deltaproteobacterium, possibly sharing some complex traits with myxobacteria, and a hydrogen-producing Asgard-like archaeon. This deltaproteobacterium may have been metabolically versatile or mixotrophic, but in symbiosis with the archaeon, it respired sulfate. This initial facultative symbiosis was stabilized by the incorporation of the archaeon as endosymbiont (Fig. 1b,c). This consortium likely established first in deeper anoxic layers and subsequently migrated upwards in the redox gradient, where it established a second (initially facultative) symbiosis with a sulfide-oxidizing alphaproteobacterium that acted as both a sulfide sink and a sulfate donor for the Asgard–deltaproteobacterium consortium (Fig. 1b,c). Alternatively, the two facultative symbioses might have co-existed, although, in this case, we favour a later obligatory endosymbiosis of the alphaproteobacterial ancestor[19]. This would be in line with genomic evidence suggesting a late mitochondrial symbiosis[75]. Given the dominance of H_2S-dependent anoxygenic photosynthesizing bacteria in microbial mats and their interaction with SRB for sulfur cycling in upper layers[76,77], the versatile, facultatively aerobic mitochondrial ancestor was likely also photosynthetic (or mixotrophic). Interestingly, the possibility that mitochondrial cristae evolved from intracytoplasmic membranes typical of photosynthetic Alphaproteobacteria has been highlighted[78]. This tripartite symbiotic consortium became definitely stabilized when the alphaproteobacterium became an endosymbiont within the deltaproteobacterium (Fig. 1d). In our view, the first eukaryotic common ancestor (FECA) is neither an archaeon nor a bacterium, but the first obligatory symbiogenetic consortium. Strictly speaking, this would correspond to the integrated symbiosis of the three partners that contributed to the final formation of the eukaryotic cell and genome. But the FECA stage could also be decoupled in time in two subsequent stages corresponding to the integration of the Asgard archaeon within the deltaproteobacterium (FECA 1) and the acquisition of the mitochondrial endosymbiont (FECA 2).

In our model, up to the FECA stage, the eukaryogenetic syntrophies were based on the same metabolic exchange that occurred in the corresponding facultative symbioses (hydrogen between the archaeon and the SRB, and sulfide or sulfate between the SRB and sulfide-oxidizing bacteria (SOB)). However, the incorporation of the mitochondrial ancestor as an obligatory endosymbiont implied a radical change in the metabolism of the whole consortium, constraining the outcome of the eukaryogenetic process. Because the mitochondrial ancestor was also aerobic and could obtain a much higher energy yield by directly oxidizing organics, the consortium started to rely solely on aerobic respiration (Fig. 1e). This resulted in the loss of the less efficient anaerobic archaeal metabolism and bacterial sulfate reduction. At the same time, the proto-eukaryote migrated to the fully oxic layers of the mats, spreading on oxic surfaces and, following the development of motility mechanisms, colonizing the planktonic realm. Cellular changes, including the development of an extensive endomembrane system, led to the LECA stage (Fig. 1f).

The HS Syntrophy model implies that three prokaryotic partners became integral parts of the future eukaryotic cell. However, other pre-eukaryogenetic symbioses might have occurred at the facultative syntrophy stage, eventually leaving historical traces in the form of transferred genes to the eukaryogenetic symbiotic partners. One traditional criticism to symbiogenetic models proposing the endosymbiosis of one prokaryote within another prokaryote is the absence of phagocytosis in prokaryotes[2,4,6,79,80]. Mainstream models now accept a symbiogenetic origin of eukaryotes but only under the premise that an endomembrane system, a developed cytoskeleton and phagocytosis evolved in the archaeal ancestor prior to the engulfment of the mitochondrial ancestor[6,81]. However, prokaryotes harbouring endosymbiotic prokaryotes are known and may be more frequent than currently thought. In addition to the well-known cases of gammaproteobacterial symbionts within betaproteobacterial endosymbionts in mealybugs[82] and Rickettsiales in tick mitochondria[83], old electron microscopy studies[84–86] and more recent observations[87] suggest the potential occurrence of prokaryotic endosymbionts in bacteria. Interestingly, a recent report of prey engulfment by Planctomycetes[88] suggests that bona fide bacterial phagocytosis exists, albeit based on different molecular grounds than eukaryotic phagocytosis[88]. In the case of archaea, although Nanoarchaeota can be associated with the inter-membrane space in the archaeon *Ignicoccus hospitalis*[89], true endosymbionts remain to be observed. Consequently, regardless of the mechanism, these collective observations suggest that prokaryotic endosymbioses, at least within bacteria, are feasible.

Membranes and endomembranes

The eukaryotic plasma membrane and endomembrane system, including the endoplasmic reticulum (ER), the nuclear membrane, the Golgi apparatus and other vesicular components, including vacuoles and lysosomes, are interconnected (either continuously or by fusing and merging). They share a similar composition, with typical bacterial-like phospholipids[2,90]. The phospholipid bilayer in eukaryotes is particularly flexible and can undergo deformation, bending, fusion and fission. This is achieved thanks to a highly developed cytoskeleton[91], coating components (including clathrin adaptor protein complexes AP1–5, the coatomer protein complexes COPI and COPII, the trafficking complex TSET and the retromer and endosomal sorting complexes required for transport; ESCRT), ADP-ribosylation factor (ARF)/ARF-like GTPases and their regulators, and fusion machinery (involving the SNAP receptor protein complex (SNARE), multisubunit tethering complexes, Rab GTPases and regulatory factors)[92–94]. Both the cytoskeleton and membrane remodelling and fusion complexes were already present in LECA, which was capable of phagocytosis, secretion and trafficking, and have a chimeric origin[20,93,95–97]. In addition to innovations[21,95], some cytoskeletal and membrane-remodelling proteins are archaeal-like (for example, actin, profilin, ESCRT proteins[6,98] and, perhaps, some GTPases[99], although some of these may be bacterial[100,101]), but a significant number of endomembrane system-related proteins could also be of bacterial, though not alphaproteobacterial, origin[75]. The biosynthesis of sterols is notably of bacterial origin[102].

Most eukaryogenetic models propose a two-partner symbiosis in which the archaeal host incorporated the alphaproteobacterial ancestor of mitochondria. This implies a shift of the host membrane from the more rigid archaeal, glycerol-1-phosphate (G1P)-based ether-linked isoprenoid phospholipids to the more flexible and permeable bacterial G3P-based, usually ester-linked, fatty acid phospholipids[2,90] (Box 2). However, such a transition, with a G1P-to-G3P-based phospholipid shift in particular, has never been observed in nature (some thermophilic bacteria use ether links, long-known exceptions[90]). Recently, an engineered *Escherichia coli* strain was forced to express archaeal phospholipids, making up to 30% of the total membrane phospholipids[103]. The engineered heterochiral membrane strain was viable and, interestingly, the expressed archaeal lipids recruited G1P, suggesting that stereospecificity is somehow linked to the phospholipid composition, in a peculiar form of membrane heredity. However, if more than 30% archaeal lipids were incorporated into the membrane, severe growth impairment was observed and the shape of cells became aberrant; they produced numerous vesicles and underwent asymmetric cell division[103]. One could therefore ask how the expression of archaeal phospholipids affects *E. coli* fitness and whether such engineered strains would be able to survive competition with normal bacteria in natural environments. Archaeal and bacterial phospholipids impose very different local physicochemical conditions that constrain integral membrane proteins[104]. As a consequence, a membrane lipid composition shift implies an extensive adaptation of the entire membrane-associated proteome[105]. In this context, neither the stability of heterochiral liposomes[106] nor the (partial) expression of archaeal phospholipids in engineered *E. coli*[103] can be taken as evidence for an archaeal-to-bacterial membrane transition. While the bacterial nature of eukaryotic phospholipids represents a serious difficulty for models invoking an archaeal host, it is naturally explained by the bacterial nature of the host in the syntrophy hypothesis (Box 2; Fig. 1).

In the HS Syntrophy model, the endomembrane system results from the invagination of the deltaproteobacterium inner membrane and the internalization of the periplasm. The outer bacterial plasma membrane would be retained as the eukaryotic plasma membrane. Many bacteria harbour endomembrane compartments linked to specialized biochemical functions[107]. These include the well-known cyanobacterial thylakoids, but also compartments in magnetotactic bacteria[108], anammox bacteria[109] and Poribacteria[110]. Some Planctomycetes develop a thoroughly studied nuclear-like compartment[111], and a similar structure has been recently described in the candidate phylum Atribacteria[112]. This implies that the internalization of membranes is relatively common across bacterial phyla. Although Deltaproteobacteria with endomembranes have not been described, their diversity is far from fully explored and they have membrane-remodelling potential. For instance, developed cytoskeletons (a prerequisite for extensive membrane remodelling) exist in the predatory *Bdellovibrio*[113] but also in myxobacteria, which are able to generate protruding membrane tubes that interconnect cells[114]. In the HS Syntrophy model, similarly to the former HM Syntrophy[19], the initial driving force for endomembrane evolution is the establishment of a secretory system connecting the endosymbiotic archaeon with the periplasm (Fig. 1c,d). As in contemporary heterotrophic deltaproteobacteria, the periplasm was the digestive space of the host deltaproteobacterium in which complex organics taken up from the environment were hydrolysed to simpler organics. Some of these simpler organics (such as amino acids and short hydrocarbons) were used by the archaeon for its organoheterotrophic metabolism, which yielded hydrogen used in turn by the SRB host. By being an endosymbiont, the archaeon maximized its uptake surface for small organics from the bacterial cytoplasm. In turn, the deltaproteobacterial host maintained an optimal uptake surface for complex organics from the environment while having a ready internal source of hydrogen (or electrons) for sulfate reduction. As the symbiosis evolved, many genes were transferred from the deltaproteobacterium to the archaeon, which progressively centralized genes and gene expression for the whole consortium. Notably, this included many hydrolytic enzymes required for the periplasmic degradation of complex organics. These enzymes were transported from the archaeal compartment towards the original bacterial periplasm via an incipient endomembrane system that, eventually, fully surrounded the archaeon and constituted the future nuclear membrane. This implied the evolution of a transport system only through the archaeal membrane since, on the bacterial side, transporters for the export of newly synthesized hydrolytic enzymes to the periplasm and the environment already existed. Initially, bacterial transporters may also have been inserted into the archaeal membranes (following gene transfer to the archaeal genome), but later replaced by channels communicating with bacteria-derived pore-like structures and allowing for the export of increasingly bigger and varied substrates (see next section). The transfer of hydrolytic enzymes to the digestive periplasmic space via the endomembrane system was essential to prevent the hydrolysis of cytoplasmic components (Fig. 1d). At the same time, in this way, the digestive space largely increased. Hence, the initial digestive and trafficking-related endomembrane system was the precursor not only of the nuclear membrane and the ER, but also of the different eukaryotic vesicles related to digestive processes (lysosomes, peroxisomes and digestive vacuoles). Upon the endosymbiosis of the mitochondrial ancestor and the loss of the archaeal and SRB metabolism, the organic compounds were directly oxidized via aerobic respiration by the alphaproteobacterium and the endomembrane system was retained for the trafficking of proteins synthesized in the proto-nucleus (Fig. 1e) and, with time, in association with the ER itself (Fig. 1f). At the same time, the ancient periplasm was completely internalized and the former digestive periphery transferred to independent vesicular compartments (Fig. 1f). The secretory Golgi apparatus and other endocytotic and exocytotic systems developed in parallel. The association of archaeal membrane-bending systems (notably ESCRT) with the host bacterial membranes facilitated the process of endomembrane formation.

The origin of the nucleus

Most eukaryogenetic models fail to advance convincing selective forces to explain why the nucleus evolved[2]. We propose, like in the

HM Syntrophy[19], a two-step process entailing two sequential selective forces. First, a proto-nucleus evolved as a different metabolic compartment. This chimeric compartment was composed of the endosymbiotic archaeon and the surrounding proto-nuclear membrane of deltaproteobacterial origin (Fig. 1c,d). The first selective force for the evolution of the nuclear membrane was the need to export bacterial enzymes already synthesized by the archaeon—after their genes were transferred to the archaeal genome—to the periplasmic space. Its first role was therefore secretory (exporting towards the trafficking endomembrane system). Other proteins of archaeal origin also began to be exported, contributing to the evolution of several chimeric eukaryotic systems. Once the archaeal genome started to host essential genes from its symbiotic partners, and these genes were lost from the donor genomes, the archaeon started to centralize protein synthesis for the whole consortium. This entailed the development of a transport mechanism from the archaeal cytoplasm to the bacterial endomembrane system, which was at the origin of the nuclear pore. This implied the formation of coordinated apertures through the archaeal membrane and the proto-nuclear membrane, although these apertures might have formed only on the bacterial membrane (the future nuclear pores) with archaeal membrane transporters facilitating export prior to archaeal membrane loss. The potential to establish communicating pores exists in both archaea and deltaproteobacteria. Archaea are able to establish intercellular cytoplasmic bridges and fuse[115]. Myxobacterial deltaproteobacteria are also able to fuse their membranes[116,117] and develop contact-dependent abilities, including coordinated gliding via junctional pore complexes[118–121]. Progressively, ribosomes concentrated around these incipient communicating pores, eventually migrating to the host's cytoplasm along the endomembrane system (the future ER), where protein synthesis started to take place. This led to a progressive decoupling of translation, which became associated to the ER, and transcription, which took place in the archaeal cytoplasm (future nucleoplasm).

As the consortium evolved between FECA and LECA, after the mitochondrial ancestor was fixed in the consortium and the archaeal and SRB metabolisms were lost in favour of the more efficient mitochondrial respiration, the archaeal membrane became useless and was completely lost. Membrane loss is not infrequent in the framework of endosymbiosis[122]. However, during this evolutionary process, extensive genome evolution took place[19]. This involved (endo)symbiotic gene transfer (EGT) to the archaeal genome, likely accompanied by other horizontal gene transfer (HGT) events, which was largely facilitated by active processes fostering genome evolution such as gene and genome fragment duplication and reshuffling. As transcription and translation decoupled, introns invaded the future eukaryotic genome. They likely derived from the original self-splicing introns of the alphaproteobacterial endosymbiont[123] and were possibly complemented by other mechanisms[124,125]. Once introns invaded the genome, the proto-nuclear membrane was selectively retained (exapted) to maintain the transcription–translation uncoupling. Therefore, preventing the deleterious massive synthesis of aberrant proteins was the second selective force acting during nuclear evolution[19]. Intron invasion has been proposed as the exclusive selective force for the origin of the nucleus[126]. However, in our view, transcription–translation uncoupling—and therefore a nuclear membrane—must pre-exist in order for introns to spread and not the opposite[2]. Not only would the insertion of one or a few introns in essential genes be immediately deleterious, but the evolution of a continuous nuclear membrane requires intermediate steps, during which transcription and translation are still coupled, that intron invasion as a selective force cannot explain.

The initial chimeric proto-nuclear pore evolved into the modern nuclear pore as a traffic check-point and hub of gene regulation[127]. We view the nucleolus and the ribosomal particle assembly process as remnants of the archaeal origin of the nuclear compartment[19]. The assembly of eukaryotic ribosomes is a complex and energy-costly process that takes place in the nucleolus. Ribosomal proteins are synthesized in the cytoplasm and transported to the nucleus. After assembly with ribosomal RNA in the nucleolus, ribosomal particles are transported back to the cytoplasm, where they associate to the ER for function[128]. The set of proteins involved (processome) is essentially of archaeal origin[129].

The make-up of a composite genome

During eukaryogenesis, various mechanisms shaped the evolving eukaryotic genome. These included HGT, EGT, gene duplication, gene loss and new gene creation, accompanied by the invasion of introns and mobile selfish elements. The directionality of gene transfer to the archaeal genome and its establishment as the future nuclear genome may have been simply dictated by chance, the consequence of an essential gene transfer from one genome to the other followed by a gene loss in the donor[19]. The retention of the archaeal endosymbiont genome as the future nuclear genome is often criticized on the grounds that in extant cases of endosymbioses, endosymbionts tend to reduce their genomes in favour of the host's. However, known extant endosymbioses occur within eukaryotes, which are already composite cells harbouring mitochondria and eventually chloroplasts, in which many essential organellar genes already reside in the nuclear genome. Therefore, the eukaryotic nuclear genome is essential and must centralize genes coming from any new incoming endosymbiont. The situation was radically different at the origin of eukaryotes when organelle reliance on the nuclear genome was not yet established and symbiotic partners were mutually dependent. In the Syntrophy hypothesis, the archaeal genome became the future nuclear genome. Bacterial components were thus included in an archaeal genomic background, leading to the well-recognized mixed heritage of eukaryotic genomes, with 'informational' genes (related to DNA replication, transcription and translation) being archaeal-like, and 'operational' genes (involved in energy and carbon metabolism) being bacterial-like[130]. While true in general terms, a closer look at the bacterial-like genes in eukaryotes poses some questions.

Most symbiogenetic models invoke only two partners: an archaeal host and the alphaproteobacterial ancestor of mitochondria (Box 1). Consequently, two predictions follow: (i) host (archaeal-like) genes must dominate over the endosymbiont (alphaproteobacterial-like) genes and (ii) most bacterial-like eukaryotic genes must be of alphaproteobacterial origin. However, neither of these predictions hold. Bacterial-like genes are more abundant than archaeal-like genes in eukaryotic genomes[131], and genes with alphaproteobacterial ancestry only represent a minority of bacterial-like genes in modern eukaryotes[75,132] and LECA[133]. To explain this 'silent' non-alphaproteobacterial bacterial majority in eukaryotic genomes, the progressive erosion of ancient phylogenetic signal—which makes it difficult to pinpoint the precise origin of those genes—and massive HGT from diverse bacterial donors to the archaeal and/or the alphaproteobacterial symbiotic partners, have been invoked[131,134]. High bacteria-to-archaea HGT levels have been observed in several phyla[135], including the Asgard archaea[9,10]. However, the patterns observed in eukaryotic genomes could be only explained if genes transferred to the archaeal and/or alphaproteobacterial ancestors of eukaryotes had been subsequently lost in all their sister lineages, which is unlikely[133]. In addition, eukaryotic alphaproteobacterial-like genes have significantly shorter branches than other bacterial-like genes in phylogenetic trees including prokaryotic homologues[75]. This suggests a late mitochondrial arrival in a host with an already chimeric genome[75]. Moreover, if alphaproteobacterial-like genes mostly relate to mitochondrial functions, bacterial genes of non-alphaproteobacterial ancestry seem to be involved in other essential eukaryotic traits such as the endomembrane system, reinforcing the idea that they evolved prior

Table 1 | Key open questions and possible ways of progress to discriminate or refine current symbiogenetic models of eukaryogenesis

	Means of obtaining answers	Models favoured or disfavoured
Predictions of the HS Syntrophy hypothesis		
Existence of a versatile SRB deltaproteobacterial lineage closer to eukaryotes, possibly sharing complex traits in common with myxobacteria.	Explore microbial ecosystems relevant for eukaryogenesis (sediments and microbial mats) in search for novel deltaproteobacterial lineages followed by phylogenomic analyses.	The detection of deltaproteobacterial lineages sharing a common and stronger phylogenetic signal with eukaryotes, as compared to other bacteria, would be consistent with the HS Syntrophy model.
Existence of an alphaproteobacterial lineage of versatile S-oxidizers, perhaps photosynthetic, closer to mitochondria.	Explore microbial ecosystems relevant for eukaryogenesis in search for novel alphaproteobacterial lineages followed by phylogenomic analyses.	The detection of sulfide-oxidizing, potentially photosynthetic, alphaproteobacterial lineages sharing a common phylogenetic signal with eukaryotes to the exclusion of other bacteria would be consistent with the HS Syntrophy model.
A large fraction of bacterial genes in eukaryotes predates the mitochondrial endosymbiosis and derives from Deltaproteobacteria.	Improve phylogenetic analyses of bacterial genes across eukaryotes, particularly those present in LECA.	The presence, function and relative amount of deltaproteobacterial-like genes in eukaryotes as compared to other bacterial-like genes might support the involvement of a deltaproteobacterial symbiont in eukaryogenesis.
Deltaproteobacterial genes mostly relate to membrane, cell signalling and cytoplasm functions.	Improve phylogenetic analyses and functional annotation of deltaproteobacterial-like genes in eukaryotes.	The involvement of deltaproteobacterial genes in membrane, cell signalling and cytoplasmic functions would be supportive of the HS Syntrophy model.
Bacterial genes widespread in eukaryotes (present in LECA) largely derive from EGT, not HGT.	Improve phylogenetic analyses of bacterial genes present in LECA and look for potential homologues in Asgard archaea and in the closest alphaproteobacterial ancestors of mitochondria.	If those bacterial-like genes in eukaryotes are missing in Asgard archaea or in Alphaproteobacteria, or the potential homologues are more distantly related than genes from other prokaryotic lineages, models invoking bacterial symbioses prior to the mitochondrial symbiosis would be favoured.
More general questions and/or problems		
Prokaryotic endosymbiosis and the origin of the nucleus		
Do endosymbiotic prokaryotes exist in free-living prokaryotes? Do endosymbiotic archaea exist within bacteria?	Search for potential prokaryotic endosymbionts in anoxic- or redox-transition ecosystems, such as sediments or microbial mats.	If prokaryotic endosymbionts occur within prokaryotes, models proposing early prokaryotic endosymbionts would be equally favoured as compared to models for which eukaryotic-like phagocytosis is a prerequisite. Finding archaeal endosymbionts within bacteria would relieve constraints for models proposing the endosymbiosis of one archaeon within a bacterium during eukaryogenesis.
Mitochondria: original metabolism and timing		
What was the metabolism of the alphaproteobacterial mitochondrial ancestor like? Did it have genes from other bacteria?	Explore microbial ecosystems in search of novel alphaproteobacterial lineages closely related to the mitochondrial lineage.	If the closest alphaproteobacteria to the mitochondrion are identified, they might provide clues about the metabolic properties of the mitochondrial ancestor, potentially favouring specific eukaryogenesis models. If non-alphaproteobacterial genes in eukaryotes can be mapped back to these alphaproteobacteria, the origin of those genes would be more easily explained by HGT to the mitochondrial ancestor from other bacteria.
Will the inclusion of more bacterial and archaeal genomes, potentially more closely related to eukaryotes, lead to the discovery of genes displaying similarly long branches in phylogenetic trees as compared to alphaproteobacterial genes?	Enrich the taxonomic sampling of Asgard archaea and bacteria that have close homologues in eukaryotic genomes.	The discovery of an Asgard and/or bacterial lineage closer to eukaryotes and displaying branches of equivalent length to that of alphaproteobacterial-like genes in eukaryotes in phylogenetic trees may imply a simultaneous or temporally close symbiotic interaction of archaea and/or other bacteria during eukaryogenesis.
Origin and nature of eukaryotic membranes		
Can bacteria expressing archaeal phospholipids be stably maintained?	Carry out experiments progressively expressing more archaeal phospholipids in bacteria until the complete replacement of bacterial phospholipids (eventually knocking out bacterial phospholipid synthesis genes).	If bacteria bearing membranes where bacterial phospholipids have been fully replaced by archaeal phospholipids can be experimentally produced, a bacterial-to-archaeal membrane transition would have been historically feasible.
If so, what is the fitness cost?	Study fitness of bacteria with archaeal phospholipids in their membrane in long-term experiments with and without competition with the wild type and other strains, and as a function of environmental conditions.	If fitness decreases and if bacteria cannot compete under any tested environmental conditions with the wild type and other strains and/or other bacteria, a membrane transition would have been historically unlikely.

continued

Table 1 | Key open questions and possible ways of progress to discriminate or refine current symbiogenetic models of eukaryogenesis (Continued)

Origin and nature of eukaryotic membranes		
Does the whole membrane proteome evolve?	Study how the proteome changes in experimental evolution as a function of archaeal phospholipid content in bacterial membranes.	If important changes in the proteome are observed, this imposes constraints for models invoking a membrane-transition.
Can we answer in the same way to the three previous questions in this table section for the opposite case of archaea expressing bacterial phospholipids?	Engineer archaeal cells with bacterial phospholipids and carry out similar experiments as described in the three previous table section lines for bacteria expressing archaeal phospholipids.	If archaea bearing membranes with only bacterial phospholipids can be produced, an archaeal-to-bacterial type membrane transition could have been historically feasible. If not, the fitness cost may be too high for those engineered archaea to compete with wild-type archaea and/or in natural environments and/or the proteome significantly affected, meaning an archaeal-to-bacterial type membrane transition would have been historically unlikely.

to the mitochondrion[75]. Non-alphaproteobacterial genes appear to derive from various bacterial phyla (with Deltaproteobacteria and Actinobacteria among the most frequent donors), suggesting successive ancient waves of HGT from these phyla and/or the implication of several bacterial symbionts during eukaryogenesis (Box 2e). Symbiogenetic models involving more than two partners are often dismissed by a simplistic parsimony argument. However, parsimony is not evolutionary evidence per se and, in most complex ecosystems, multiple symbioses are widespread[3]. The first cultured Asgard archaeon can indeed grow in symbiosis with one sulfate-reducing deltaproteobacterium and one methanogenic archaeon[18]. If we transposed a similar symbiosis at the onset of eukaryogenesis, a significant number of deltaproteobacterial-like genes in eukaryotes might be explained by HGT during the long-term coexistence with a symbiont that later disappeared without integrating the consortium[18]. Additional bacterial ecto- or endo-symbionts might have also transferred genes, leading to the mosaic origin of eukaryotic bacterial-like genes as proposed in the 'pre-mitochondrial symbiosis' model[133] and in line with the 'shopping-bag model' proposed for the evolution of plastid genomes[136].

However, the presence of many non-alphaproteobacterial bacterial-like genes in eukaryotes is compatible with the HS Syntrophy model. Accordingly, deltaproteobacterial genes would have been acquired by EGT. Interestingly, deltaproteobacterial-like genes seem to be the most abundant non-alphaproteobacterial category, and are also among the oldest[75]. Inferring the precise phylogenetic origin of genes of different ages in eukaryotic genomes is far from trivial due to mutational saturation and the erosion of phylogenetic signal in increasingly older genes[137]. Furthermore, each potential additional symbiont could contribute a number of genes acquired by HGT from different donors in such a way that the apparent number of eukaryogenetic symbiotic partners would appear inflated. Nonetheless, a strong phylogenetic signal supports the deltaproteobacterial origin of many eukaryotic genes involved in diverse functions and structures. In addition to early identified deltaproteobacterial-like eukaryotic genes[26], the list also includes antimicrobial defensins[138], serine/threonine/tyrosine protein kinases[139], phosphoprotein phosphatases[140], high mobility group A proteins[141], isoprenoid biosynthesis enzymes[142], cyclitol synthases[143] and some kinetochore proteins[97]. Mitochondria also recruited some deltaproteobacterial proteins, potentially reflecting an alpha–delta–proteobacterial symbiosis, such as thiolases[144], fatty acid beta-oxidation enzymes[145] and, possibly, some proteins involved in anaerobic metabolism. Stemming from the versatility of many alphaproteobacteria, we view the mitochondrial ancestor as a facultative aerobe able to carry out not only anaerobic respiration with various electron acceptors but also substrate-level phosphorylation[27]. Several genes involved in these reactions seem ancestral in eukaryotes, branching close to deltaproteobacteria and other anaerobic bacteria[146]. Although they are usually interpreted as independent HGT acquisitions from various donors[146], these observations can alternatively support a deltaproteobacterial anaerobic respiration toolkit in ancestral eukaryotic mitochondria that was subsequently lost to different degrees in aerobic lineages.

Future prospects

Any model on eukaryogenesis must account for the evolution of key eukaryotic traits (including, notably, genome complexity, nature and origin of eukaryotic membranes or endomembranes and the nucleus), in a way that is mechanistically plausible and explains the observed patterns and causes (selective forces) for the evolution of those traits in a realistic ecological context. In this framework, our HS Syntrophy model considers constraints imposed by the discovery of Asgard archaea and their ancestral metabolic potential to put forward one of the most comprehensive eukaryogenetic models. This model presents some difficulties, notably in the centralization of the genome and protein synthesis by the archaeon with subsequent export to the deltaproteobacterial host. However, it is ecologically relevant, fits well with the observed chimerism of eukaryotic genomes and has the advantage, over archaeal host-based models (Box 1), of readily explaining the bacterial-like nature of eukaryotic membranes[2,90]. The HS Syntrophy hypothesis makes several predictions that differentiate it from other hypotheses (Table 1). Some of these are shared with the HM Syntrophy model and include the presence of EGT-derived deltaproteobacterial genes in eukaryotes that should be mostly involved in membrane, cell-signalling and cytoplasmic functions. Others, such as the involvement of a potentially photosynthetic S-oxidizing alphaproteobacterium, specifically characterize the HS Syntrophy. These predictions suggest that such specific alpha- and deltaproteobacterial lineages, which are phylogenetically closer to eukaryotes than other bacterial lineages, might exist. The nature of the alphaproteobacterial ancestor of mitochondria is indeed still cryptic[31]. If such lineages were discovered through environmental studies in microbial mats or sediments, the HS Syntrophy would gain support. These syntrophy models are realistically based on well-known metabolic interactions in microbial mats or sediments, but metabolic variants involving a similar tripartite symbiosis and eukaryogenetic process might be also envisaged within a more general Syntrophy model; its distinctive features being the nuclear origin from an archaeal endosymbiont in a bacterial cytoplasm and the independent acquisition of the mitochondrial ancestor.

Beyond the syntrophy hypothesis, progress in several areas is needed to answer open questions and differentiate major model types (Table 1). In archaeal host-based symbiotic models[16,18,147,148], three major issues need attention. First, the archaeal-to-bacterial membrane transition remains a major drawback. Cases of mem-

brane transitions implying complete phospholipid-type replacement and concomitant membrane proteome adaptation are not observed in nature. If bacteria and, more particularly, archaea, can be engineered to (i) accomplish the full replacement of membrane phospholipids for the opposite type and (ii) be competitive in real environmental conditions (have permissive fitness cost), the historical feasibility of such transition would be supported. So far, the evidence is lacking. In some models, the emission of cell protrusions and a progressive engulfment of an alphaproteobacterium is preferred over immediate phagocytosis[18,147]. This long process would favour phospholipid exchange and replacement of the rigid archaeal phospholipids for more flexible bacterial ones[147]. However, it is unclear how such a 'slow phagocytosis' process would occur across prokaryotic generations, which are needed for evolution to take place (including phospholipid replacement and concomitant mebrane proteome adaptation) prior to true engulfment. Second, two-partner models need to propose convincing detailed evolutionary mechanisms and selective forces for the origin of the eukaryotic nucleus, which are so far lacking. Finally, eukaryogenetic models need to explain the 'silent bacterial majority' in eukaryotic genomes. In two-partner models, HGT from different bacteria to any of the partners might be the most logical explanation, but this does not necessarily explain why groups of functionally-related genes seem to come from a few bacterial groups[75,133], even if some HGT and phylogenetic reconstruction noise are likely involved in these observations. In-depth phylogenomic analyses including a broad taxon sampling and, if identified, the closest bacterial and archaeal relatives of eukaryotes will be needed. Collectively, the information gathered from environmental, experimental cell biology and phylogenomic studies should help to discriminate existing models, refine them or envisage new ones. More than ever, solving the eukaryogenesis riddle seems at hand.

Received: 1 August 2019; Accepted: 13 March 2020;
Published online: 27 April 2020

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Acknowledgements

The authors acknowledge funding from the European Research Council (ERC) grants ProtistWorld (to P.L.-G.; agreement no. 322669) and Plast-Evol (to D.M.; agreement no. 787904), and the French Agence Nationale de la Recherche (to P.L.-G.; grant no. ANR-18-CE02-0013-1).

Author contributions

P.L.-G. and D.M. conceived and discussed the ideas presented in the manuscript. P.L.-G. wrote the manuscript with critical input from D.M.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence should be addressed to P.L.-G.