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Opinion

Bacterial Vesicle Secretion and the Evolutionary Origin of the Eukaryotic Endomembrane System

Sven B. Gould,[1,*] Sriram G. Garg,[1] and William F. Martin[1,*]

Eukaryotes possess an elaborate endomembrane system with endoplasmic reticulum, nucleus, Golgi, lysosomes, peroxisomes, autophagosomes, and dynamic vesicle traffic. Theories addressing the evolutionary origin of eukaryotic endomembranes have overlooked the outer membrane vesicles (OMVs) that bacteria, archaea, and mitochondria secrete into their surroundings. We propose that the eukaryotic endomembrane system originated from bacterial OMVs released by the mitochondrial ancestor within the cytosol of its archaeal host at eukaryote origin. Confined within the host's cytosol, OMVs accumulated naturally, fusing either with each other or with the host's plasma membrane. This matched the host's archaeal secretory pathway for cotranslational protein insertion with outward bound mitochondrial-derived vesicles consisting of bacterial lipids, forging a primordial, secretory endoplasmic reticulum as the cornerstone of the eukaryotic endomembrane system.

Eukaryogenesis: A Matter of Compartmentalisation

Among the many traits that distinguish eukaryotic from prokaryotic cells, none is more conspicuous or significant than the eukaryotic **endomembrane system** (see Glossary). Like other eukaryotic-specific traits, such as mitosis and sex, its evolutionary origin remains obscure. The compartments of the endomembrane system are present throughout the major eukaryotic groups, as are the proteins that are specific to them [1]. Hence both were present in the eukaryote common ancestor [2], for which reason thoughts on the origin of the endomembrane system are linked to thoughts on the origin of eukaryotes themselves.

Despite many differences in their mechanistic details, theories for the origin of the endomembrane system traditionally derive it from inward invaginations of the plasma membrane, such that the endoplasmic reticulum (ER) lumen is topologically homologous to the environment [1,3–6]. This is true for theories that posit autogenous (nonsymbiotic) eukaryote origins [7] and for theories that posit eukaryotes to descend from symbiotic associations of prokaryotes [8]. Though most current theories now posit that mitochondria arose in an archaeal host through endosymbiosis (Box 1), the question of how the merger of two prokaryotic cells gave rise to a cell possessing a eukaryotic endomembrane system with elaborate vesicle trafficking (Figure 1) remains unanswered, as does the question of how **archaeal lipids** of the host's plasma membrane came to be replaced by bacterial lipids.

Though prokaryotes do not generate intracellular vesicle traffic of the kind found in eukaryotes, they do indeed generate OMVs, but these are secreted outwardly into the environment, not

Trends

Eukaryogenesis models struggle with explaining the origin of the endomembrane system and the transition from an archaeal plasma membrane based on isoprene ethers to a bacterial-type membrane based on fatty acid esters.

Bacteria and archaea secrete outer menbrane vesicles (OMVs) into their surroundings. If the endosymbiont that became the mitochondrion did so in the archaeal host, it physically generated the first vesicles of the endomembrane system.

Endosymbiont OMVs could only accumulate in the host's cytosol – fusion with each other could have generated compartments, fusion with the archaeal plasma membrane could have converted its chemical composition.

Starting endomembrane origin with outward flux of endosymbiont-derived OMVs integrates mitochondria, their lipids, and their energetics into current models of eukaryote origin, explaining why eukaryotes had a mitochondrion-bearing ancestor.

[1]Institute for Molecular Evolution, University of Düsseldorf, 40225 Düsseldorf, Germany

*Correspondence: gould@hhu.de (S.B. Gould) and bill@hhu.de (W.F. Martin).

Box 1. Endosymbiosis at Eukaryote Origin

The origin of eukaryotes hinges upon endosymbiosis, and eukaryotic cell complexity arose in the wake of mitochondrial origin, not as its prerequisite [57]. From the genomic standpoint a consensus is emerging that the origin of eukaryotes involved only two distinct partners: an archaeal host cell and an α-proteobacterial endosymbiont that became the mitochondrion [29,43,44,57,71,74–76]. This consensus does not touch upon whether the archaeal host bore a nucleus or not, but several issues require consideration concerning this discrepancy. It concerns, in particular, the purpose of a nucleus in an archaeal cell with cotranscriptional translation that remains unanswered in gradual models for eukaryogenesis that place the origin of the nucleus before that of the mitochondrion.

The selective pressures that brought forth the possession of the nuclear envelope (NE) as a permanent fixture of eukaryotic cells are, we suggest, distinct from the OMV-dependent ER origin of the NE itself. The presence of spliceosomes in the eukaryote common ancestor suggests that the initial selective advantage of possessing an NE was the spatiotemporal separation of spliceosomal splicing from translation, with spliceosomal introns stemming from group II introns acquired via endosymbiotic gene transfer from the mitochondrial symbiont [77]. Spliceosomal splicing requires a nucleus to exclude active ribosomes from intron-containing transcripts, because ribosomes operate much more rapidly than spliceosomes, such that cotranscriptional translation on nascent transcripts bearing spliceosomal introns would lead to defective polypeptides only. The physical exclusion of ribosomes from active chromatin via membranes would allow the slow process of splicing to go to completion before translation sets in. Similar to the intron hypothesis for the origin of the nucleus [77], our present suggestion for the origin of the endomembrane system requires a non-nucleated archaeal host with cotranscriptional translation at the origin of mitochondria.

inwardly into the cytosol. Decades ago, microbiologists observed that Gram-negative bacteria can secrete lipopolysaccharide (LPS) complexes [9] that presumably stem from the outer membrane [10] into the environment. As explained in the next section, quite a bit is now known about prokaryotic OMVs, but less about the proteins involved, which are, in some cases, homologous to those germane to vesicle scission into eukaryotic **multivesicular bodies (MVBs)** for example. Moreover, even mitochondria themselves are known to secrete **mitochondria-derived vesicles** (MDVs; Figure 1) into the cytosol [11–14]. No previous theory for the origin of the eukaryotic endomembrane system, however, incorporates the observations available for prokaryotic OMVs. Here we close that gap with an evolutionary inference that accounts for the origin of the eukaryotic endomembrane system in a novel and natural manner.

Prokaryotic Vesicle Secretion

As Deatherage and Cookson [15] write, it has long been known, but underappreciated, that bacteria and archaea generate OMVs. Both Gram-negative [16] and Gram-positive [17] bacteria secrete OMVs that stem from their outer membrane (Figure 2). In addition, some bacteria form nanowires, long tube-like protrusions of the outer membrane [18]. Bacterial OMV cargo ranges from outer membrane proteins to the content of the periplasmic space, which can be specifically apportioned for inclusion into OMVs [19]. OMVs are also clinically important as they can include key toxins associated with bacterial virulence and toxicity [20,21]. The rate of OMV secretion and the nature of their content can vary according to nutrient availability, stress, host–pathogen interactions, and exposure to antibiotics such as gentamicin [9,20]. The mechanistic details behind OMV release are still poorly understood, but in Gram-negative bacteria the release of OMVs is thought to result from the interplay of peptidoglycan, surface proteins, and the LPS complexes themselves [10,15,16,21,22].

Archaea also secrete OMVs [15,23], which contain proteins of the S-layer, components of the outer membrane [24], and in some cases also toxins [25]. The release of archaeal OMVs involves the Cdv (cell division) proteins A, B, and C [24,26], which are homologous to members of the eukaryotic **ESCRT** III protein family involved in membrane vesicle scission [27]. In addition to their role in OMV secretion, archaeal Cdv proteins are involved in cell division (Figure 2). While bacteria require FtsZ for cell division, many archaea lack FtsZ, with the formation of the division ring and the final scission of the daughter cells being mediated by Cdv proteins [26]. Similar to their role in cell division [26,27], Cdv proteins could aid in the tethering and scission of the membraneous neck that leads to the release of the nascent OMV from the archaeal plasma

Glossary

Archaeal lipids: membrane lipids composed of isoprenoid hydrocarbon side chains linked via an ether bond to glycerol-1-phosphate.
Autophagosomes: double-membrane-bound compartments involved in the degradation of intracellular proteins and organelles through autophagy. Outer membrane fuses with the lysosome to form the autolysosome.
Bacterial lipids: membrane lipids composed of a glycerol-3-phosphate linked to fatty acid side chains via an ester linkage.
Coatomer: class of proteins involved in vesicle coat formation. Many share a similar domain architecture uniting a β-propeller and an α-solenoid domain.
Endomembrane system: elaborate membrane system unique to eukaryotes; it includes the nucleus, the endoplasmic reticulum, the Golgi apparatus, the lysosome, the peroxisome, autophagosomes, and the myriad vesicle-trafficking processes that interconnect them with each other and the plasma membrane.
Endosomal sorting complex required for transport (ESCRT): multicomponent machinery subdivided into ESCRT-0, I, II, III; it facilitates membrane vesicle budding 'away' from the cytoplasm.
Flagellar pore complex (FPC): also known as the ciliary pore complex, a structure composed of many proteins that share a high degree of homology with the nuclear pore complex (NPC) and regulates transport into the flagellum.
Glyoxysome: specialized type of peroxisome found in plants and some fungi.
Golgi apparatus: highly dynamic structure of ordered stacks that act as a sorting station for vesicular trafficking from ER to the plasma membrane and other compartments.
Lokiarchaea: recently discovered archaeal phylum that monophyletically branches with eukaryotes.
Lysosome: acidified compartment and final destination for the degradation of proteins and particles coming from multivesicular bodies (MVBs).
Mitochondria-derived vesicles (MDVs): vesicles that originate from the mitochondria and fuse with

membrane into the environment [24]. Importantly, prokaryotic OMV flux, whether bacterial or archaeal, is not inward but outward (Figure 2) in cases reported to date.

A Bacterial Vesicle Model for the Origin of the ER

The essence of our proposal is that the α-proteobacterial ancestor of mitochondria was also able to produce OMVs, that it did so as it became an endosymbiont in its archaeal host, and that those OMVs provided the initial seed of the eukaryotic endomembrane system. This suggestion is compatible with the widespread production of OMVs among modern prokaryotes and with the more recent observation that mitochondria themselves generate MDVs within eukaryotic cells today [11–14]. Upon endosymbiosis, the archaeal secretion system (**SecY/Sec61p**) and its associated **N-glycosylation** machinery integrated readily into the endosymbiont's OMVs, giving rise to a primordial ER that provided the founding stock from which all other endomembrane compartments, including the nucleus, arose (Figure 3, Key Figure).

In terms of the number and nature of evolutionary innovations required to evolve a basic endomembrane system with selectable ER function, our minimal premises can hardly be underbid. We require that the eukaryote common ancestor possessed a mitochondrial symbiont [28], which is now consensus among evolutionary cell biologists [2,29]. We also require that the host was a normal archaeon [28], lacking both a nucleus and its own pre-existing endomembrane system at mitochondrial acquisition, its archaeal chromosomes located in the cytosol and subject to cotranscriptional translation [30]. Thus, our model requires a mechanism of entry for the endosymbiont that is independent of **phagocytosis** and thus demands that one prokaryote can become an endosymbiont within another prokaryote. Clear examples for such symbioses do indeed abound [31,32]. Phagocytosis is not a prerequisite to endosymbiosis and in light of archaeal physiology is problematic for reasons discussed below (and in Box 2).

If the OMV-producing ancestor of mitochondria continues with its natural activity of producing OMVs consisting of bacterial lipids in an archaeal host with cytosolic chromosomes, what happens? Quite a lot happens, and a quite sudden transition appears possible, too, without even requiring evolutionary inventions, merely spatial reorientation of pre-existing host (archaeal) and symbiont (bacterial) components by virtue of endosymbiosis (Figure 3). Notably, prior to endosymbiosis, the symbiont's OMVs diffuse into the environment. In the closed quarters of an archaeal cytosol, the membrane vesicles have no place to go. They can fuse, either with themselves to generate larger vesicular compartments, or with the plasma membrane to export their contents to the cell exterior. The former generates a basic ER topology. The latter constitutes, we propose, the ancestral outward state of eukaryotic membrane flux, and furthermore converts the chemical composition of the host's plasma membrane from isoprene ethers to bacterial fatty acid esters. Importantly, these three salient eukaryotic traits – cytosolic vesicles, outward membrane flux, and the accumulation of bacterial lipids in the archaeal plasma membrane – arise without need for any evolutionary invention (Figure 3). They arise as the result of an OMV-producing bacterium living as an endosymbiont within an archaeon.

The presence of bacterial OMVs in the archaeal cytosol provide a fundamentally new and continuously arising membrane system in the host's cytosol. The consequence is that proteins once destined to the host's plasma membrane via the secretory pathways now have an additional, alternative target: cytosolic OMVs. Accordingly, the host's SecY/Sec61p system, which, prior to symbiosis, facilitated the cotranslational insertion of membrane proteins into the only membrane facing the cytosol – the plasma membrane – now has a new target: OMVs of endosymbiotic origin. This formed a primordial ER membrane architecture, which matches exactly the topology of the modern eukaryotic SEC complex at the rough ER (Figures1 and 3). Initially, this primitive vesicle flux to the plasma membrane required not a single invention, only endosymbiosis, and it provided ground for natural selection (e.g., of **coatomer** proteins and

various other compartments such as peroxisome and MVBs.

Multivesicular bodies (MVBs): membrane-bound compartments containing cytoplasm-derived vesicles destined for degradation at the lysosome.

N-glycosylation: adds oligosaccharide side chains to certain asparagines in proteins. Typically occurs in the ER lumen of eukaryotes.

Nuclear pore complex (NPC): multiprotein complex that spans the nuclear envelope and regulates transport. Many NPC proteins share similarities with proteins of the flagellar pore complex (FPC) and vesicle coat.

Peroxisome: compartment involved in the catabolism of fatty acids, polyamines, and hydrogen peroxide.

Phagocytosis: uptake of large particles such as entire bacterial cells by macrophages or amoebae. Food-particle-containing phagosomes fuse with MVBs and ultimately the lysosome for degradation.

Ribophorin I: protein of the rough ER that binds to the SEC complex, promoting N-glycosylation by serving as a substrate-specific chaperone.

Sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA): a P-type ATPase found in the ER that regulates Ca^{2+} storage in the ER lumen.

SecY/Sec61p: main translocon of the SEC complex involved in translocation of nascent polypeptides from ribosomes into the ER lumen.

V-ATPase: a type of proton pump that acidifies compartments, commonly found in vacuoles and lysosomes. The eukaryotic V-ATPase shares significant homology with the archaeal plasma membrane ATPase (or the A-ATPase).

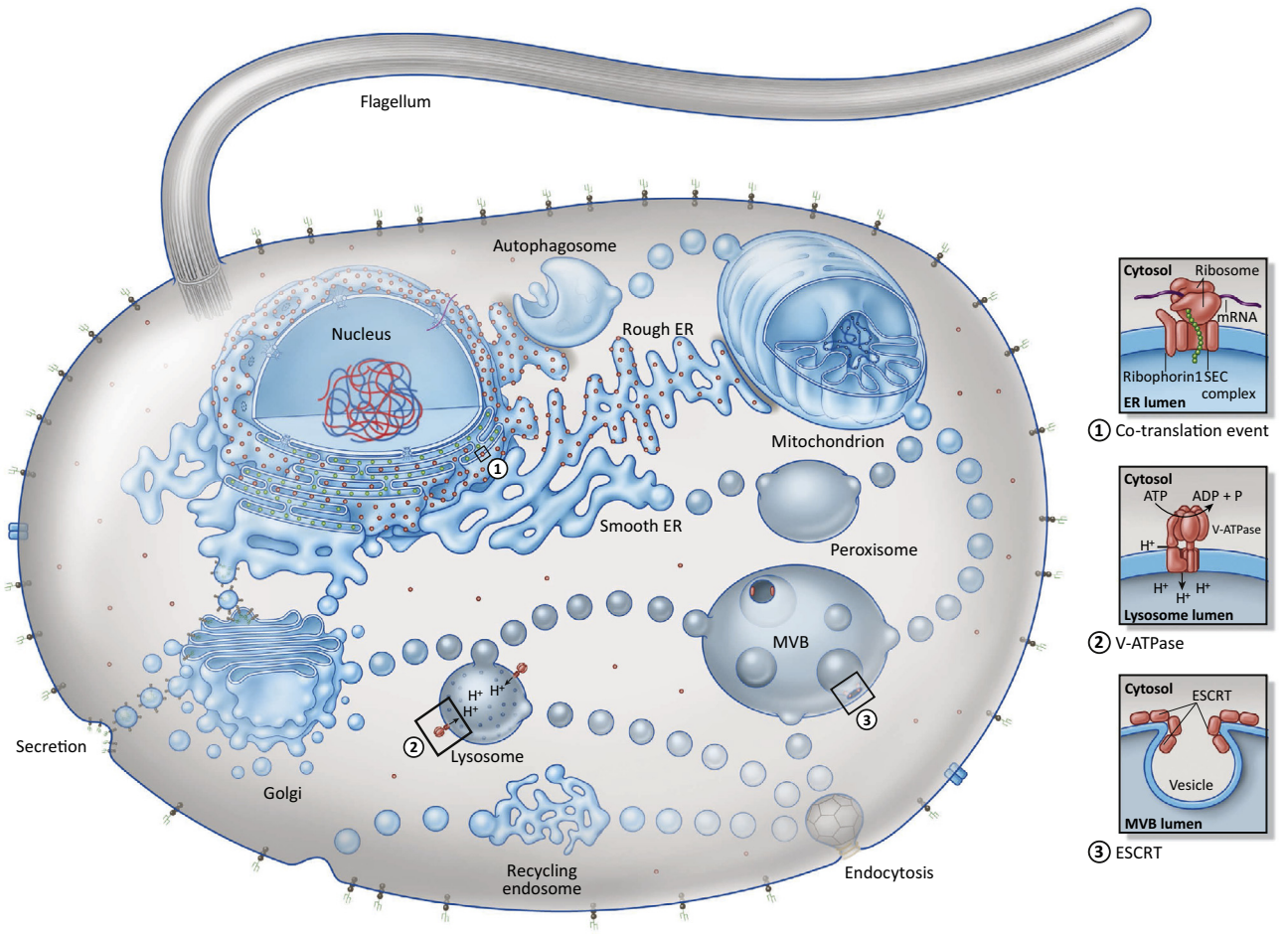

Figure 1. The Eukaryotic Cell. The one decisive trait of the eukaryotic cell is its elaborate endomembrane system. At its centre stands the smooth and rough endoplasmic reticulum (ER) [78], the latter being studded with ribosomes that cotranslationally transport proteins across the SEC complex [41]. For N-glycosylation, **ribophorin I** associates with the Sec61 translocon and serves as a substrate-specific chaperone [79]. Vesicles that bud from the ER can transverse the Golgi – for further modification of cargo and lipids and subsequent sorting – or generate, and continuously supply, other compartments, such as the peroxisome and phagosome [50]. Mitochondria-derived vesicles (MDVs) help to form autophagosomes that originate at the ER–mitochondria contact sites [40] and peroxisomes [12]. Multivesicular bodies (MVBs) represent specialised endosome-associated compartments that contain internal vesicles [80]. They also receive MDVs for subsequent degradation at the lysosome [13]. ESCRT proteins mediate the scission of membranes to release vesicles into the MVBs [80]. The endomembrane system of the eukaryotic cell is a merger of host (red) and endosymbiont (blue) components.

targeted flux) to work on. Because our proposal posits the endosymbiont's OMVs to physically generate the primordial ER, it directly accounts for the observation that only eukaryotes, the cells descended from a mitochondrion-bearing ancestor, possess a *bona fide* endomembrane system. In the following, we briefly consider the properties and components of the eukaryotic endomembrane system, and their homologies.

Connections of the ER with Mitochondria and Other Compartments

Ostensibly, relics of the ER's origin from endosymbiotic OMVs are still visible today. Prokaryotes synthesize their lipids directly at the plasma membrane, but not so in eukaryotes. Eukaryotic lipid synthesis – which is similar to bacterial membrane lipid synthesis and not to the archaeal lipid synthesis pathway – occurs mainly at the ER and involves considerable exchange with the mitochondria [33,34]. In traditional invagination models for the origin of the endomembrane

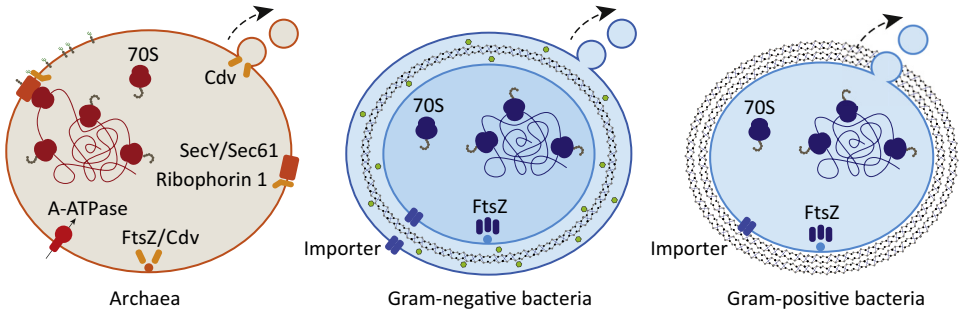

Figure 2. Prokaryotic Membrane Vesicle Secretion. Both bacteria and archaea release outer membrane vesicles (OMVs) into the environment that bud from the outer membrane. In archaea the Cdv proteins, which are involved in cell division and are homologous to proteins of the eukaryotic ESCRT machinery, mediate vesicle budding. All prokaryotes use 70S ribosomes for protein translation, and all bacteria, but only some archaea, make use of FtsZ for cell division. The illustration focuses on components discussed in the context of the proposal for the origin of the eukaryotic endomembrane system, such as the storage of Ca^{2+} (green hexagons) in the periplasmic space of Gram-negative bacteria or ribophorin 1, a protein involved in N-glycosylation.

system, the ER lumen is homologous to the environment. In our model, the ER lumen is homologous to the periplasm of the mitochondrial endosymbiont (Figure 3), the mitochondrial intermembrane space. The main sites of Ca^{2+} storage (mediated by **SERCA**) and signalling in eukaryotes are the ER and mitochondria [35], and indeed *Escherichia coli* concentrates Ca^{2+} between its inner and outer membrane under certain conditions [36]. The ER furthermore temporarily connects to other compartments and mitochondria through dedicated 10–30 nm long contact sites [37,38], from which autophagosomes arise and expand through MDV secretion [39]. Vesicle transport between mitochondria and peroxisomes, mediated by Vps35, has been recently observed [12], as well to MVBs [13]. Such contact sites are crucial for lipid biosynthesis, ion exchange and storage, signaling, and a range of membrane dynamics [34].

In addition, eukaryotic protein secretion commences at the rough ER through the binding of the 80S ribosome to the SEC complex that receives the nascent polypeptide chain to initiate cotranslational targeting into the ER lumen [40]. The entire eukaryotic SEC machinery is of archaeal origin [41,42], as is the ribosome [43,44]. The same is true for Rpn1 that stems from archaea [44]. By inference, the entire archaeal SecY/Sec61p/Rpn1 system for cotranslational N-glycosylation [45] was seamlessly integrated early into mitochondrial OMVs, which subsequently fused with the host's plasma membrane (Figure 3B). One might interject that the archaeal SecY/Sec61p system would be unlikely to integrate into a bacterial lipid bilayer, but the crystal structure of the archaeal Sec61 complex was determined from proteins expressed in *E. coli* [46].

N-glycosylation itself is not as unique to eukaryotes as once believed, being widespread among prokaryotes [47,48]. A continuous flow of bacterial lipid OMVs to the archaeal plasma membrane, also for the release of N-glycosylated proteins, would have naturally transformed the lipid composition of the archaeal plasma membrane from ether-linked isoprenes to ester-linked fatty acids (Figure 3B). Considering the diameter of bacterial OMVs (10–300 nm; [15]) and the diameter of an average archaeon (1 μm; [49]), less than 10 000 OMVs can generate enough membrane material to transform a surface area equal to that of an average archaeon. A clear implication of our proposal is that the lipid transition in eukaryotes could have occurred very rapidly in evolutionary terms, without precisely defining rapid in specific years or generations.

The secretion of vesicles to the plasma membrane is hardly the sole function of the ER. Vesicles that originate from the ER fuse with, and form, all other endomembrane-bounded cell

Key Figure

A Model for the Evolutionary Origin of the Eukaryotic Endomembrane System

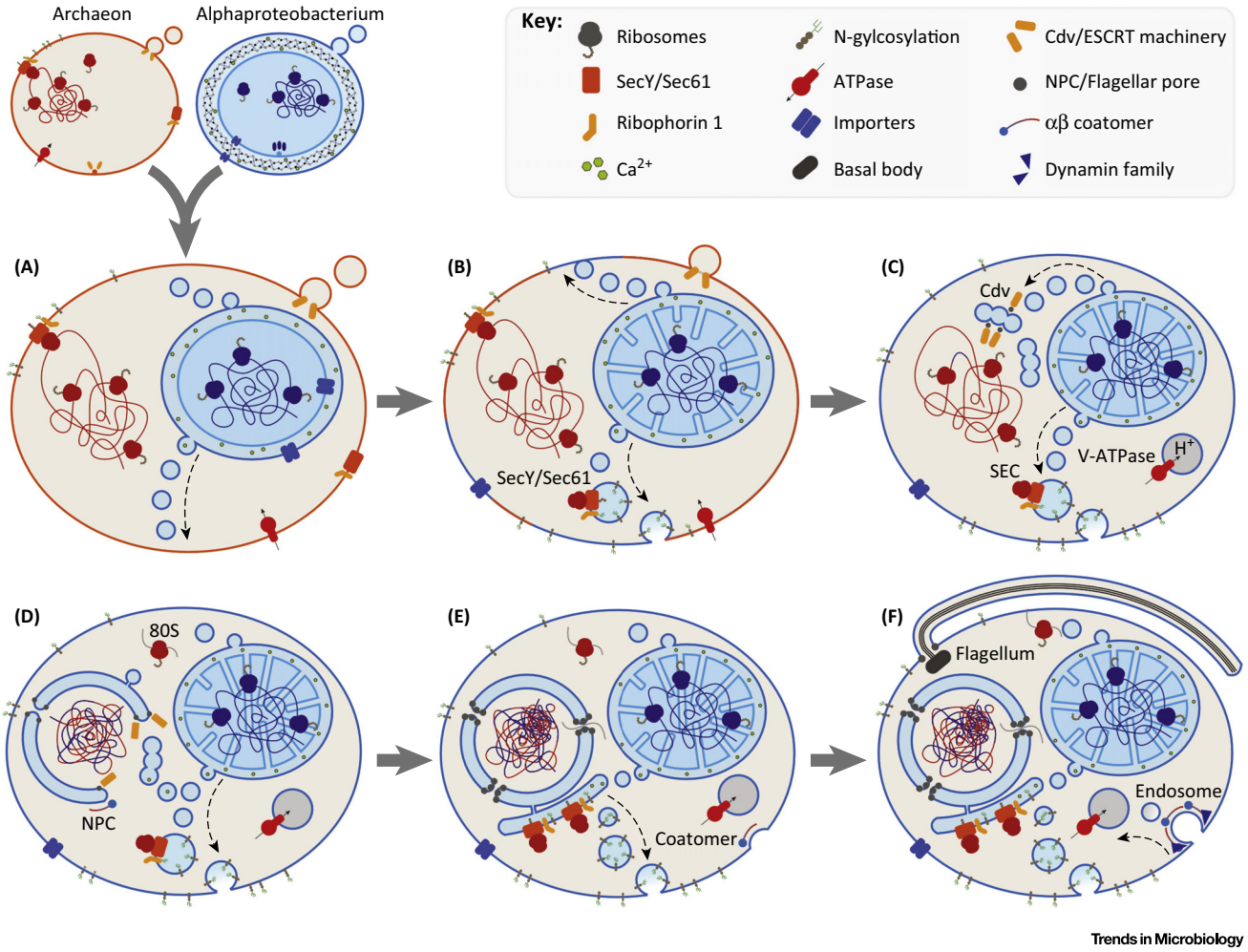

Figure 3. (A) After endosymbiosis, the endosymbiont continues with OMV secretion, thereby generating the first vesicular flux inside the archaeal cytosol. (B) Cytoplasmic OMVs provided an alternative target for the archaeal SecY/Sec61 and N-glycosylation machinery, generating a primitive endoplasmic reticulum (ER) and stabilizing OMV flux towards the plasma membrane (PM), where N-glycosylated proteins are released. This simultaneously initiated the conversion of the PM from archaeal ether-linked isoprenes (orange) to bacterial ester-linked fatty acids (blue). (C) The invasion of group II introns through endosymbiotic gene transfer drove the formation of the nucleus, may be with the aid of Cdv proteins. The presence of a primitive ER allowed the evolution of additional compartments, including a vacuole acidified by the archaeal A-ATPase (today the V-ATPase). (D) Together with the nucleus, the nuclear pore complex (NPC) was formed using proteins that may be originated from the fusion of genes encoding β-propeller folds and α-solenoid domains, ribosomes were now excluded from the nucleus. (E) A fully functional endomembrane system, in which the nuclear envelope (NE) is continuous with the ER, was in place, and coatomer proteins initiated the emergence of the first inbound vesicle budding (endocytosis). (F) Simultaneously, the flagellum evolves from proteins that originate from the NPC and/or the endosome. A virtue of this model is that the ancestral direction of membrane flux is a natural consequence of a fully formed pre-existing property of the mitochondrial endosymbiont that continues until today (Figure 1).

compartments, and these include the **Golgi apparatus**, the lysosome, the peroxisome, the **glyoxysome**, and the autophagosome [50]. Analyses of peroxisomal function and proteins, such as those for fatty acid β-oxidation, indicate that mitochondria predated peroxisomes in evolution [51,52], which is consistent with our model. In our proposal, the ER is the central hub upon which the biogenesis of all other nonendosymbiotic compartments depends, including the nucleus.

Box 2. The Energetic Price of Phagocytosis

If the archaeal host that acquired the mitochondrion were phagocytotic, it must have had all the parts required for phagocytosis, namely, a fully developed cytoskeleton, food vacuoles, and an endomembrane system. In other words, assuming a phagocytotic origin of mitochondria [3–6,65] means assuming that the host had evolved eukaryotic complexity without the participation of mitochondria. That rekindles the archezoa theory, which was rejected over a decade ago [81] because its predictions failed. Mitochondria are tied to eukaryote origin, hence to the origin of complexity. Why? The origin of eukaryote complexity required energy, mitochondrial energy. Eukaryotic cell complexity emerges from massive amounts of proteins that constitute and modulate the cytoskeleton and membrane flux in the eukaryotic cytosol. Protein synthesis is 75% of a cell's energy budget; mitochondria afforded eukaryotes internalized bioenergetic membranes that scale freely with increasing cell volume [57], and that covered the costs of that protein synthesis.

Prokaryotes synthesize ATP at the plasma membrane. Eukaryote origin witnessed the loss of all ATP synthesis at the plasma membrane and the transition to compartmentalized energy metabolism with glycolytic ATP synthesis in the cytosol and chemiosmotic ATP synthesis in mitochondria [28,57,70]. The bioenergetic transition at eukaryote origin was evolutionarily rapid. How so? The archaeal host's plasma membrane ATPase did not become a pseudogene. It remained under functional constraint, was targeted via the Sec pathway to a novel endomembrane, and reversed function to acidify food vacuoles at cytosolic ATP expense.

Phagocytosis first theories fail to account for the source of cytosolic ATP required to acidify food vacuoles. One might counter that fermentation was the source, but archaeal fermenters are chemiosmotic, using their ATPase at the plasma membrane [82]. Provided that carbon was supplied to the mitochondrion through importers integrated into the archaeal plasma membrane, only one key innovation was required to transform the mitochondrial endosymbiont into an ATP-exporting organelle – the ADP/ATP carrier in the mitochondrial inner membrane [83]. This and other independent lines of evidence [84,85] all suggest rapid eukaryote origin.

Phagocytosis demands a fully functional endomembrane system, in turn requiring proteins that facilitate vesicle flux and the membrane vesicles themselves. Key proteins of the endomembrane system have homologous domains in prokaryotes, they underwent duplication, diversification and functional specialization at eukaryote origin [1,2]. Their diversification required the intracellular vesicles that afforded these proteins their selectable functions. Mitochondria not only supplied the energy needed to evolve eukaryotic endomembrane proteins, they physically provided the vesicles and source for their natural selection. Similar to energetics, bacterial outer membrane vesicles (OMVs) place mitochondria before phagocytosis in the sequence of events at eukaryote origin.

On the Origin of the Nucleus

The ER is contiguous with the nuclear envelope (NE), which, similar to the ER, is a single folded membrane with two leaves (Figure 1). In eukaryotes with open mitosis, the NE arises from ER vesicles, which store proteins of the NE when the nucleus disintegrates during cell division [53,54]. In eukaryotes with closed mitosis, the NE increases in size during cell growth via lipids supplied via the ER [55]. In other words, the nuclear envelope can be viewed as a functionally distinct extension of the ER. As with the emergence of the eukaryotic SEC system, the archaeal host added crucial components to cytosolic membranes of endosymbiont origin (Figure 3). ESCRT III and the p97 AAA-ATPase control annular fusion of the newly forming NE [56], a process topologically resembling membrane fusion at the end of cytokinesis. Archaeal cell division and OMV secretion depends on CdvB and CdvC that are homologous to eukaryotic ESCRT III proteins and Vps4, respectively [24,26,27]. The formation of a primordial ER, and later an NE, could have involved archaeal precursors of ESCRT proteins and small GTPases of the archaeal host, such as those identified in **Lokiarchaea** [44]. Consistent with their function during cell division, we suggest that, during evolution, the NE emerged from the ER (Figure 3C), while the ER emerged from the mitochondrion. This order is independent of, but fully compatible with, bioenergetic considerations that identify the origin of the mitochondrial endosymbiont in an archaeal host (Box 1) as the rate-limiting event at eukaryote origin from which all other processes of eukaryogenesis unfold [57].

Some proteins of the **nuclear pore complex (NPC)**, the **flagellar pore complex (FPC)**, intraflagellar transport, and some coatomer proteins that form vesicle coats share similar structural properties (or even entire proteins) and have hence been suggested to be evolutionarily

linked [1,58–60]. Many of these proteins are characterised by a WD-domain containing β-propellers followed by an α-solenoid domain. This architecture allows for membrane interaction and bending, two important requirements for re-shaping membranes. Although WD-domains and solenoid-like proteins are widespread among prokaryotes [61,62], their domains are rarely organized as in eukaryotic cells [63], although the eukaryotic organization could result from simple gene fusion events [64]. Phylogenetic analysis suggests that these eukaryotic WD-domain proteins arose at roughly the same time [1]. In light of the present considerations, the nuclear envelope and pore came first, later followed by the emergence of the flagella and endocytosis, and not *vice versa* as according to recent suggestions [3,6,65].

Phagocytosis and Energetics

Many traditional models for the origin of the endomembrane system posit that phagocytosis arose prior to mitochondrial origin [3–5,65]. Phagocytosis requires a multiprotein machinery that forms the phagosomal cavity, which might have evolved multiple times independently during eukaryote evolution [66]. Scission of endosomes and phagosomes from the plasma membrane involve dynamins, a family of large GTPases [67]. Phylogenomic analysis of the dynamin segments suggests that the ancient version responsible for mitochondrial division was also the one mediating scission of early endomembrane vesicles [68]. Dynamin-related proteins such as DynA are common in bacteria, and the only three dynamin-like protein encoding genes found in archaea are of bacterial origin [69]. It appears that eukaryotic dynamins evolved from endosymbiotic dynamin-like proteins, speaking in favor of mitochondria proceeding phagocytosis.

Phagocytosis also requires a process that acidifies the food vacuole. Food vacuoles are useless if their contents cannot be broken down by proteases, which in eukaryotes are acid-activated [70]. Eukaryotic vacuole acidification requires, in turn, an important archaeal component, the **V-ATPase**, which consumes ATP to acidify vacuoles rather than synthesizing ATP from redox-generated ion gradients [71]. During eukaryote origin, the host's plasma membrane rotor–stator archaeal A-type ATPase (the eukaryotic version is called the vacuolar or V-type ATPase [72]) was re-targeted to a new compartment of OMV origin (now the lysosome), concomitant with a functional reversal of direction from ATP synthesis in archaea to ATP consumption in eukaryotes. This raises an interesting question seldom, if ever, asked in the context of eukaryote endomembrane origin: if phagocytosis preceded the mitochondrion, where was the cytosolic ATP coming from that allowed the V-ATPase to run backwards? And given that all the enzymes of chemiosmotic energy harnessing in eukaryotes stem from bacteria [69,73], why did the host lose all components of membrane bioenergetics other than the A-type ATPase? We suggest that the answer to both questions is mitochondria, for two reasons (Box 2).

Concluding Remarks

The present proposal for the origin of the ER and derived membranes differs in premises and substance from previous suggestions in the literature, in that it is based on outward vesicle flux, not inward. Our proposal requires almost no innovations, exceptional or unique evolutionary processes in either the mitochondrial ancestor or the archaeal host in order to bring forth a basic ER function with outward vesicle flux. Our proposal raises new questions (see Outstanding Questions), while directly accounting (i) for the archaeal ancestry, localisation and orientation of the secretory machinery that performs cotranslational insertion of proteins into eukaryotic membranes, (ii) for the circumstance that eukaryotes store Ca^{2+} in the ER lumen, which is, in this model, homologous to the ancestral mitochondrial periplasmic space, (iii) for the ancestral ground state and bacterial lipid composition of eukaryotic endomembranes, (iv) for the archaeal nature of eukaryotic ribosomes and N-glycosylation at the ER, (v) for the finding that eukaryotic lipid synthesis occurs predominantly at the ER and mitochondria, not at the plasma membrane, (vi) for the transitional mechanism that converted the composition of eukaryotic membranes from archaeal to bacterial lipids, (vii) for the formation of the nucleus from the ER during cell

Outstanding Questions

As new archaeal lineages become characterized that are, by the measure of ribosomal phylogeny, more closely related to the host that acquired the mitochondrion, will we find large, complex, eukaryote-like cells that never harboured mitochondria (like the archezoa theory once predicted), or will they be morphologically normal archaeal cells?

To what extent do mitochondria-derived vesicles exist among eukaryotic supergroups, and what functions do they perform?

Given technologies to insert OMV-producing bacteria into archaeal cells or suitable synthetic analogues, will it be possible to generate analogues of primitive endomembrane systems?

Could the fusion of prokaryotic β-propeller and α-solenoid domains generate protocoatomer proteins that facilitate positive membrane curvature?

Do mitochondria-derived vesicles contribute to the rebuilding of the ER and nucleus after mitosis in some eukaryotic lineages?

development, not vice versa, and (viii) for the archaeal ancestry, localisation, and orientation of the eukaryotic V-ATPase in food vacuoles. From our proposal, a natural evolutionary order in the origin of several key characters of eukaryotic cells unfolds in that, during eukaryogenesis, the ER represented the first autogenous (nonendosymbiotic) cell compartment, formed from OMVs secreted by the mitochondrion, subsequently giving rise to both the nuclear envelope and an ancestrally outward endomembrane flux.

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