REVIEW/PERSPECTIVE August 2025

A Tubules-First Model for the Origin of Eukaryotic Membrane Traffic

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Abstract

The discovery of membrane trafficking proteins in Asgard archaea—the closest archaeal relatives of eukaryotes—reveals the deep evolutionary roots of the eukaryotic endomembrane system. This review synthesizes recent genomic, structural, and functional studies in archaea and eukaryotes to explore how these ancient proteins contributed to the emergence of intracellular membranes. While Asgard archaea appear to lack the canonical machinery for coated vesicle formation, they encode a full complement of proteins involved in membrane tubulation, scission, tethering, and fusion. This suggests that the archaeal ancestor of eukaryotes was capable of membrane remodeling, potentially via transient tubules. In early eukaryotes—following mitochondrial acquisition and ER internalization—tubules may have served as the principal carriers of membrane traffic. Spherical vesicles—which use coat proteins and sterol-rich membranes to stabilize high-curvature buds—may represent a later innovation, prior to the last eukaryotic common ancestor. Archaea-derived tubular trafficking pathways play essential roles in modern eukaryotic cells.

Keywords: evolutionary cell biology, eukaryogenesis, Asgard archaea, first eukaryotic common ancestor (FECA), membrane traffic, membrane tubules

Contents

1. INTRODUCTION

1.1. FECA, LECA, and eukaryogenesis

Over two billion years ago [119], in a habitat yet to be discovered, there lived a population of cells which would change the course of life on Earth: the earliest cells whose only living descendants are eukaryotes. Much later, perhaps by half a billion years or more, came the most recent cells whose descendants include all living eukaryotes. Somewhere along the path connecting these two points—the first eukaryotic common ancestor (FECA) and the last eukaryotic common ancestor (LECA)—a type of cell arose which harbored mitochondria (**Figure 1**). This watershed event is a conceptually convenient point to mark the origin of eukaryotes [21]. FECA, then, was a "prokaryote-grade" organism; and LECA was a "eukaryote-grade" organism recognizably similar to its present-day descendants, with mitochondria, a cytoskeleton, a nucleus, and a membrane traffic system [28][124]. Retracing the evolution of eukaryotes is challenging because the long path from FECA to LECA has (by definition) no extant branches to provide clues about intermediate cellular forms.

1.2. Reconstructing LECA

The most feasible approach is to start at the most recent end of the path: LECA. That is, we attempt to reconstruct the nature of the last eukaryotic common ancestor prior to the diversification of extant eukaryotic groups [83]. This could be based on molecular histories, or on trait histories. In the former case, we start with a toolkit of molecules known to drive various cellular processes, compute their phylogeny across the eukaryotic tree of life, and thereby infer LECA's molecular repertoire. In the latter case, for inference based on traits, we must understand where to root the eukaryotic tree of life. That is, we ask whether one group of extant eukaryotes is most closely related to LECA, and might therefore give us hints of LECA's biology. For example, a recent study suggests that the traits shared by the eukaryotic protist lineage known as excavates may be representative of LECA [124].

The current understanding of LECA's membrane trafficking system, and the ongoing evolution of trafficking pathways in extant eukaryotic lineages, has been summarized in several recent reviews [19][68][111]. It is clear from these studies that LECA already possessed sophisticated membrane traffic and other traits that are typical of present-day eukaryotes. To truly understand how these traits evolved, we are forced to look further back,

to the other end of the path: to FECA.

1.3. Mitochondria: early, intermediate or late?

We now know that eukaryotes are derived from a merger between archaeal host cells descended from FECA, and free-living bacterial cells which became endosymbiotic mitochondria [6][104][20] (**Figure 1**). Many questions about eukaryogenesis concern the timing of mitochondrial acquisition relative to the gain of other eukaryotic traits along the archaeal lineage [21][119][6][28]. In mitochondria-early scenarios, the host cell was a stereotypical archaeon [115]. In mitochondria-late scenarios, the host already possessed stereotypically eukaryotic traits, except for the endosymbiont. However, a growing body of data [120] supports a more nuanced mitochondria-intermediate scenario, in which the archaeal host possessed certain traits that are otherwise restricted to extant eukaryotes, including a cytoskeleton and a rudimentary membrane remodeling system. This review synthesizes new evidence in support of the mitochondria-intermediate scenario, and its implications for eukaryogenesis, specifically with respect to membrane traffic.

2. ARCHAEAL ANCESTRY OF EUKARYOTES

2.1. Two-domain classification of life

Carl Woese split all living organisms into three domains: bacteria, archaea, and eukaryotes [126]. Yet this could not be the entire story. Lynn Margulis's endosymbiont hypothesis argued for a bacterial origin of eukaryotic mitochondria [91][33], and it is now well established that mitochondria and present-day alpha-proteobacteria are sister lineages [21]. Endosymbiosis requires a host, but the nature of the host cell took longer to resolve. Early molecular data showed that eukaryotic ribosomes were more similar to archaeal rather than bacterial ribosomes [126]. Growing genomic evidence showed that eukaryotic DNA replication, transcription and translational machinery were archaeal in origin [85], strongly suggesting an archaeal host. These data are the basis for a two-domain classification of life, in which bacteria and archaea are the oldest lineages, and eukaryotes arise as a merger between them [119][21].

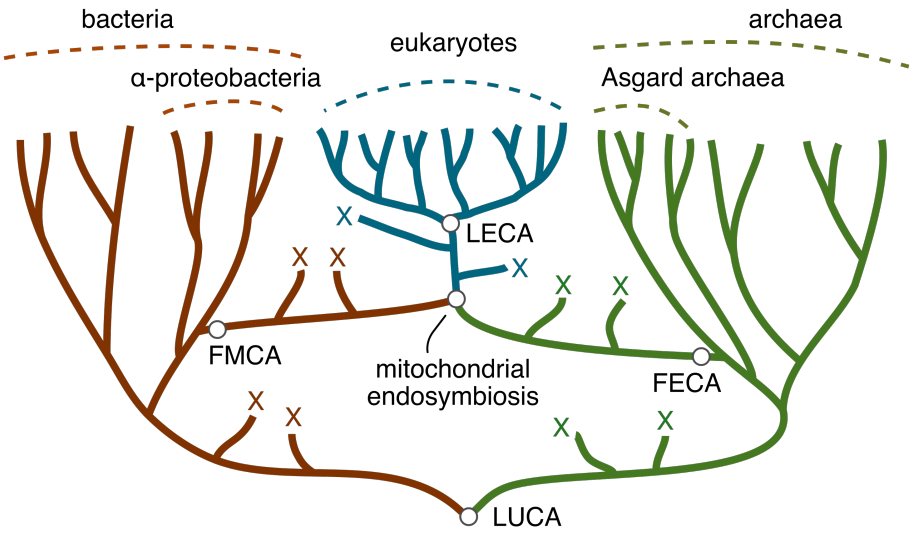

Figure 1: Emergence of eukaryotes as a merger between archaeal and bacterial cells. Time increases in the upward direction. X represents extinct lineages. LUCA: Last universal common ancestor. FMCA: First mitochondrial common ancestor. FECA: First eukaryotic common ancestor. LECA: Last eukaryotic common ancestor.

2.2. Discovery of the Asgard phylum

A key question is to understand which present-day archaea are most closely related to the ancestral archaeal host [35]. The study of eukaryogenesis was reinvigorated with the discovery, one decade ago, of the archaeal group Lokiarchaeota [105], first based on purely phylogenomic evidence from deep-sea samples. Strikingly, the genomes of these cells encoded many eukaryotic signature proteins (ESPs)—that is, proteins that previously were thought to be restricted to eukaryotes [105]. This was quickly followed by the discovery of several archaeal lineages related to Lokiarchaeota, such as Heimdallarchaeota, Thorarchaeota, and Odinarchaeota, which collectively were evocatively named the Asgard superphylum [131] (now reclassified phylum Asgardarchaeota, containing classes Lokiarchaeia, Heimdallarchaeia, etc. [84]). Phylogenomic analyses based on concatenated marker proteins have expanded and classified Asgard diversity, and found that eukaryotes branch robustly from within this phylum [23] as a sister lineage to the Heimdallarchaeia [132]. In summary, the Asgard archaea represent the closest living archaeal relatives of eukaryotes [131]. FECA likely branched off from the ancestors of the Asgard archaea prior to the Great Oxidation Event, over 2.4 billion years ago [132]. LECA is much more recent, dating to between 1.8 and 1.6 billion years ago [9][11] (**Figure 1**).

2.3. Unique cell biology of Asgard archaea

The ESPs encoded by Asgard genomes include a large number of genes encoding proteins involved in cytoskeletal and membrane traffic machinery [131][56][23]. This suggests that members of the Asgard phylum are atypical archaea [115]. Consider the example of cytoskeletal proteins. Bacterial structural homologs of actin are widespread and play roles in cell division and chromosome segregation [121], but they are highly diverged from eukaryotic actin. In contrast, many archaea encode eukaryote-like actins; and Asgard archaea encode both actins and several homologs of eukaryotic actin regulators [106][16]. The tubulin homolog FtsZ is found across bacteria and most archaeal lineages [121] where it drives cytokinesis [43] (in certain archaeal lineages cytokinesis is driven by the ESCRTIII complex [41]; we will return to ESCRTIII in a later section). However, non-FtsZ-like tubulin homologs with unknown function have been identified in multiple Asgard lineages [1][127], with sequences and structures close to that of eukaryotic tubulins [1].

Genomic evidence alone is not sufficient to establish the existence of novel cell-biological mechanisms [20]. Much stronger support for the uniqueness of Asgard archaea comes from landmark studies reporting the successful cultivation and subsequent cell-biological investigation of two members of this clade: *Prometheoarchaeum syntrophicum* [42] and *Lokiarchaeum ossiferum* [87][127]. Both these representatives of the Asgard phylum are small coccoid cells about 500 nm in diameter. They have several linear or branched membrane protrusions, about 50 nm to 100 nm in diameter, and sometimes several microns in length [42][87]. In *L. ossiferum* these protrusions are associated with a network of cytoskeletal filaments composed of an Asgard actin homolog [87]. Its genome also encodes a tubulin homolog that assembles into microtubles in vitro, and appears to form large filamentous structures in vivo [127]. In cryo-tomograms of *L. ossiferum* cells, intracytoplasmic vesicles are sometimes seen [87].

3. EMERGENCE OF THE ENDOMEMBRANE LUMEN

We next move from present-day Asgard archaea to the ancestral FECA, and trace its presumed path through the course of eukaryogenesis. The creation of the nucleus, within which chromatin is located, is a key step in the establishment of the modern eukaryotic cell plan. Indeed, the combination of nucleus and mitochondria was originally considered as diagnostic of eukaryotes [93], though these features likely arose at different times. Since

we are interested in the origin of eukaryotic membrane traffic, we will not remark on the timing of mitochondrial acquisition. Instead, we will focus on the series of steps through which the endomembrane system would have developed.

3.1. Homology of the archaeal plasma membrane with the eukaryotic ER

In extant eukaryotes the largest endomembrane compartment is the endoplasmic reticulum (ER): a tubular-planar network that accounts for about half the total membrane content of a cell [65]. The bulk of the phospholipid content of eukaryotic cells is made at the cytoplasmic and nucleoplasmic leaflets of the ER [44][5]. This includes most lipids destined for mitochondria, with only a few specialized lipids synthesized at the mitochondrial inner membrane [44][3]. The ER is also the site for the synthesis of trans-membrane and secreted proteins, accounting for about a third of the cellular proteome [4][109]. Proteins are synthesized by ribosomes on the cytoplasmic face of the ER. Trans-membrane and secreted proteins have special signal peptides that enable them to translocate across the ER membrane during synthesis, facilitated by a protein complex known as the translocon [4][109]. These proteins are exported from the ER in membrane-bounded carriers, and reach the plasma membrane via the secretory pathway [22][62].

The Sec61/SecY translocon (known as Sec61 in archaea and eukaryotes and SecY in bacteria) is conserved across all domains of life. In bacteria and archaea it is located on the cytoplasmic face of the plasma membrane. Eukaryotic Sec61 is likely derived from an archaeal ancestor [109]. In eukaryotes, Sec61 works with the TRAP and OST complexes on the cytoplasmic face of the ER [109][47][123]. TRAP and OST subunits are among the ESPs found in Asgard archaeal genomes [23], and are inferred to have high structural homology with their eukaryotic counterparts [15]. When expressed in human cell lines, *P. syntrophicum* TRAP and OST subunits (both trans-membrane and soluble subunits) localize to the ER [15]. These observations of Sec61/TRAP/OST properties strongly support the homology of protein machinery at the Asgard plasma membrane and the eukaryotic ER.

3.2. Inside-out and outside-in models of eukaryogenesis

The nucleus is not in fact a membrane-bounded compartment [6]: the nucleoplasm is a specialized sub-domain of the eukaryotic cytoplasm, connected to it by nuclear pores; and the nuclear envelope is a specialized sub-domain of the ER, with its own lumenal space.

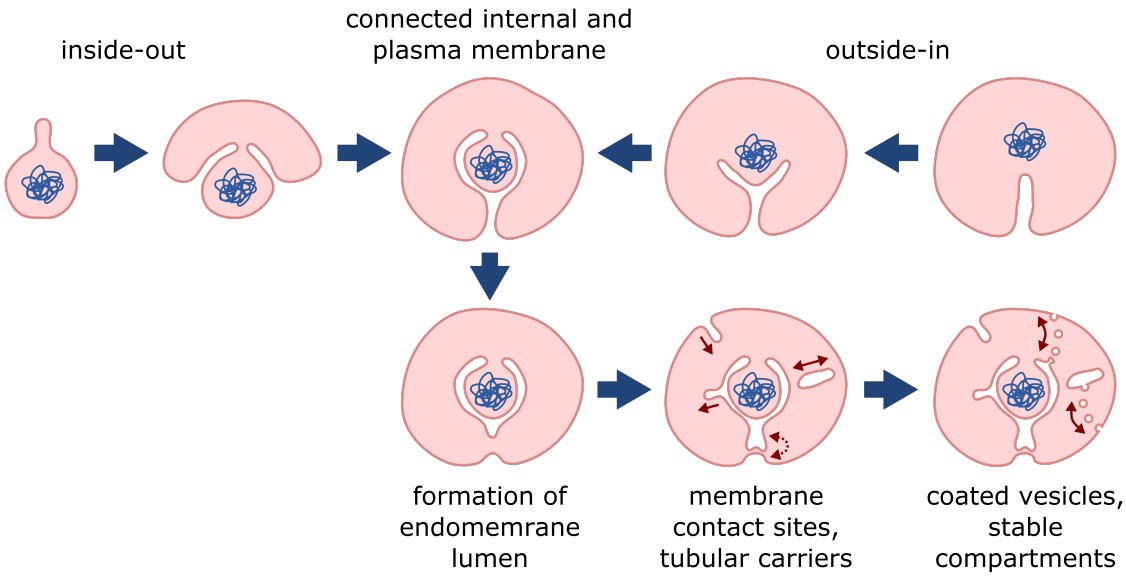

Figure 2: Formation of a separate lumen, nucleoplasm and cytoplasm. These schematic figures represent minimal endomembrane configurations (the 3D configuration is the solid of revolution about the vertical axis, except for last two diagrams). The inside-out and outside-in models make different predictions about the nature of the ancestral archaeal host. Both scenarios reach a common intermediate point in which internal and plasma membranes are connected by tubules or more complex structures. The formation of the proto-ER and the endomembrane lumen marks a critical step. Chromatin (blue squiggle) is eventually contained in the nucleoplasm and surrounded by the nuclear envelope/proto-ER; the nucleoplasm is connected to the cytoplasm by the nuclear pore. Transport of lipids and proteins from the proto-ER to the plasma membrane first occurs via membrane contact sites or tubular carriers. Coated vesicles and non-ER endomembrane compartments (potentially stabilized versions of tubules) come later. Tubular carriers may have existed in the archaeal host prior to the formation of the lumen.

The development of an endomembrane lumen is a critical step in eukaryote evolution, and should be considered as distinct from the emergence of the nucleus.

There are three major classes of models for the emergence of a separate lumen, nucleoplasm and cytoplasm during eukaryogenesis [6][63] (**Figure 2**). In mitochondrial-derived membrane models, eukaryote endomembranes are not of archaeal origin, but instead arise from outer-membrane vesicles (OMVs) shed by mitochondria [63]. Though OMVs may have played a role in eukaryogenesis, an exclusively mitochondrial origin of the eukaryotic endomembrane is disfavored by the emerging support for pre-mitochondrial membrane remodeling capacity in Asgard archaea [19]. In outside-in models, invaginations of the archaeal plasma membrane go on to form the nuclear envelope, with the creation of the

lumen and the nucleoplasm possible in either order. This implies that archaeal and eukaryotic plasma membranes are homologous, and the archaeal and eukaryotic cytoplasms are homologous, while the nucleoplasm is a novel territory. In inside-out models [7] tubules protruding outward from the archaeal plasma membrane eventually expand and engulf the original cell; the nucleus arises before the lumen, and the base of the tubules corresponds to the nuclear pore. This implies that archaeal plasma membrane and the ER are homologous, and the archaeal cytoplasm and the nucleoplasm are homologous, while the eukaryotic cytoplasm is a novel territory.

More complex scenarios which build on these basic steps are possible. For example, archaeal homologs of eukaryotic fusexins—proteins which mediate cell-cell fusion during meiosis—have recently been discovered in haloarchaea [67]. Cells of this archaeal clade can form tissue-like multicellular structures [78]. This raises the possibility of a multi-cell origin of eukaryotic cell architecture and polyploidy [88]. Fusexins have not, however, been confirmed in Asgard archeaeal genomes [67], so models of this type are speculative for the moment.

3.3. An epoch of tubules connecting internal and plasma membranes

In outside-in models the translocon, originally on the archaeal plasma membrane, would need to be re-localized to the internal membrane which eventually becomes the nuclear envelope. In inside-out models the translocon would remain at its original location on the archaeal plasma membrane, precursor of the nuclear envelope, rather than move outward to the new eukaryotic plasma membrane [7]. In either class of models there is an extended epoch during which an internal membrane remains connected to the plasma membrane by tubules or more complex structures, and its interior remains continuous with the extracellular milieu (**Figure 2**). The eventual scission of these connecting structures to create intracellular membranes with a true lumen is a required step in eukaryogenesis. The possibility of tubule-associated machinery in Asgard archaea, reviewed in detail in later sections, is consistent with this picture.

What were the selective pressures that drove the development of membrane traffic?

The costs associated with membrane traffic include: energetic costs associated with membrane remodeling [14], and the genomic burden of maintaining the complex protein machinery required for regulating membrane traffic [111]. The potential benefits of intracellular compartments include: efficient digestion of nutrients [110][95]; increased surface and volume for biosynthesis [95]; and optimization of metabolic and regulatory processes by splitting across compartments [46] or localizing to 2D membranes [52]. Even in the absence of compartments, the ability to control membrane curvature and create chemically distinct membrane sub-domains may itself aid the regulation of cellular processes [14]. Considering these costs and benefits, we can ask if the archaeal host was capable of phagocytosis or pinocytosis (the uptake of solid and soluble nutrients, respectively, via membrane carriers) prior to mitochondrial acquisition. Quantitative bio-energetic estimates show that phagocytosis would be costly in a prokaryotic cell, but would be beneficial once ATP synthesis was localized to mitochondria [63]. Similarly, flux-based models [95] suggest that the costs of pinocytosis generally outweigh the benefits in terms of nutrient uptake. In contrast, the use of internal membranes for biosynthesis is beneficial, providing a high surface area for these processes while simultaneously freeing up the plasma membrane for nutrient uptake [95]. These calculations are consistent with the development of an endomembrane system specialized for biosynthesis in early in eukaryogenesis.

3.4. Lipid transfer from the proto-ER to the plasma membrane

During the epoch when internal membranes are connected to the plasma membrane, protein and lipid synthesis could in principle occur at both locations. However, following the separation of the ER from the plasma membrane and the emergence of a lumen, there are three possible outcomes. Biosynthesis could occur on all membranes, or be localized either to the plasma membrane or to the proto-ER. Quantitative flux models suggest it is more efficient to restrict biosynthesis to high-surface-area internal membranes, leaving more plasma membrane area free for nutrient uptake [95] (discussed further in the Box). The localization of lipid synthesis to the ER, rather than across all membranes, may have also paved the way for the development of compartment-specific lipid identity markers. Such markers are crucial in preventing the ER from fusing to plasma membrane, thereby

protecting the integrity of the lumen.

Once secreted and trans-membrane proteins and lipids are synthesized at the ER, there must be a way for these molecules to be transported to the plasma membrane (and also to mitochondria). In present-day eukaryotes, lipid transport is often carried out by soluble lipid transfer proteins (LTPs) at membrane contact sites (MCSs) [97] (**Figure 2**): regions where membranes of distinct organelles brought into close apposition by tethering machinery. The ER makes such contact sites with most intracellular membranes, including the plasma membrane [92] and the mitochondrial outer membrane [3]. Certain classes of eukaryotic LTPs appear to have a bacterial origin [53]. Interestingly, a recent study confirmed the existence of LTPs in Asgard archaea, which are capable of carrying out lipid transfer in vitro and when expressed in yeast [55]. The function of these proteins in archaeal cells is not yet known.

Even if MCSs existed in early eukaryotes, this still does not solve the problem of transporting transmembrane and secreted proteins from the proto-ER to the plasma membrane. The only way to achieve this is by using membrane-bounded carriers. Assuming that tubule-like tunnels existed during the epoch of a connected internal and plasma membrane, we could hypothesize that internalized versions of these structures may have served as the primordial membrane-bounded carriers (**Figure 2**).

4. MEMBRANE TRAFFICKING PROTEINS IN ASGARD ARCHAEA

The discovery of Asgard archaea prompted a renewed search for membrane trafficking machinery that might predate mitochondrial endosymbiosis. The earliest phylogenomic analyses of Lokiarchaeia and other Asgard members already identified several small GTPases, ESCRT complex components and the COPII components Sec23/24 [105][131]. Later targeted searches for membrane trafficking proteins revealed a larger repertoire of interesting candidates [49][20].

4.1. Remote homologs and the search for Asgard membrane traffic machinery

A recent study using an expanded set of Asgard species identified new members of previously-known membrane trafficking ESPs, as well as new classes of proteins and

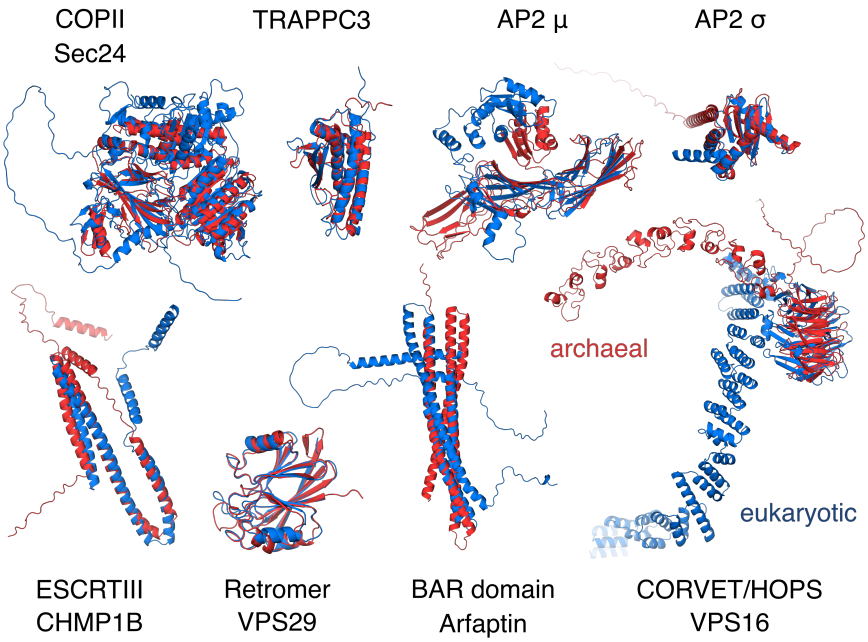

Figure 3: Structural homology between putative Lokiarchaeia membrane trafficking proteins and their eukaryotic counterparts. We show bidirectional strong Foldseek [114] hits for structural homology as predicted by Alphafold [116]. The only exception is for CORVET/HOPS VPS16, where no bidirectional strong hits can be found. Though the figure shows Alphafold structures to enable like-to-like comparison, each eukaryotic protein has an experimentally-determined PDB structure (see Table 1 for details).

complexes [23], based on sequence homology. These include the OST/TRAP translocon (discussed earlier), the adaptor protein (AP) complex, the retromer complex, and the CORVET/HOPS complex. We will discuss these proteins in detail in a later section. The detection of these types of remote protein homologs is challenging, because extant eukaryotes and Asgard archaea are separated by over two billion years of evolution. Many of the protein complexes involved in membrane traffic involve paralogs, that is, proteins which duplicated and diverged from a common ancestor [111][19]. Examples include subunits of the adaptor protein complex [38] or CORVET/HOPS tethers [99]. Therefore, even if the inference of homology is correct, the assignment of which specific subunits of a complex are in correspondence may not be: all of them will appear related at the sequence level.

The development of Alphafold has boosted our ability to detect remote homologs even when the underlying sequences are highly diverged [116][40][114]. A recent structure-based search identified a large number of new Asgard ESPs, highly diverged at the sequence level. The reuse of the same domains and folds across proteins will mean that structural

searches will often identify proteins of unrelated function. It is therefore important to enforce a bidirectional-strong-hit criterion for both the Asgard and the eukaryotic structural homologs. Even by this stringent criterion, many Asgard ESPs appear to be true homologs in a structure-based search (**Figure 3**, Table 1). There are exceptions, however. While sequence search [23] has identified Asgard homologs for subunits of the endolysosomal CORVET/HOPS tether and the CATCHR-type GARP tether [107], they are not bidirectional strong structural hits with eukaryotic proteins (**Figure 3**). Such homologies can be validated by experimentally demonstrating similar structures (e.g. [1][15][112]) or functions (e.g. [87][127][15][55][117][133]) as their eukaryotic counterparts.

4.2. Asgard archaea appear to lack canonical vesicle coats

Coats such as clathrin, COPI and COPII scaffold membranes to generate spherical vesicles [50]. The scaffolding subunits of these coats (the clathrin heavy chain, the COPI α and β' subunits, and the COPII subunits Sec13/Sec31) have a conserved architecture: one or more β-propellers linked to an α-solenoid, the latter forming the struts of the scaffold [89]. The same architecture is shared by subunits of the CORVET/HOPS tethering complexes [89]. All these proteins are thought to derive from an ancestral "protocoatomer" [89][19][26]. Several Asgard proteins are predicted to have either α-solenoid or β-propeller structures [49], but there are none with the combined β-α coatomer architecture [25]. Remote homologs of the CORVET/HOPS tether detected in archaeal genomes [23] show poor structural similarity with their eukaryotic counterparts, in particular they do not have a clear β-α architecture (**Figure 3**). It is possible that this architecture arose as a fusion between contiguous β-propeller and α-solenoid archaeal genes at some stage on the path leading from FECA to LECA [19].

Clathrin, COPI and COPII vesicles also require adaptor proteins which mediate interactions with vesicle cargo: the heterotetrameric AP complexes for clathrin and F-COPI for COPI, all of which share a common ancestry [38]; and the unrelated Sec23/24 for COPII [130]. Sequence homologs of all subunits of the AP complexes and Sec23/Sec24 have been found in Asgard genomes [23] and do appear to be good structural homologs as well (**Figure 2**, Table 1). Since Asgard archaea lack the scaffolding coatomers [25] and sterol-rich membranes [11] required for coated vesicle formation in present-day eukaryotes, it is interesting to ask what role the adaptor proteins, and the rest of the putative membrane trafficking apparatus, are playing. One possibility is that all these proteins have roles un-

related to membrane traffic. Another is that they do mediate membrane traffic, but using carriers other than spherical coated vesicles. What if the primordial carriers were tubules? If this were true, we would predict that the Asgard ancestors of eukaryotes expressed protein machinery required to generate and regulate such tubular carriers.

5. BIOPHYSICS AND CELL BIOLOGY OF TUBULATION

It is useful consider tubulation from a biophysical perspective, so we can understand the repertoire of molecules that may be required to generate and regulate tubules in cells.

5.1. Protein machinery to regulate tubular carriers

The compressive stresses required for tubule formation can arise from a variety of mechanisms, including crowding, phase separation, or osmotic shock [59]. Tubulation and other shape instabilities can occur spontaneously due to active lipid fluxes, even in the absence of specialized protein machinery [96][80]. From a cellular viewpoint, it may be more relevant to consider how the generation of tubules due to intrinsic physical mechanisms may be regulated in space and time. The full cycle of membrane transport via tubular carriers requires the following successive steps (**Figure 4**): initial curvature generation and tubule formation from a source membrane; loading of cargo within the tubule; scission of the tubule to generate a mobile carrier; and finally tethering and fusion to the target membrane. We will later encounter specific proteins involved in the regulation of each of these steps, in various eukaryotic tubulation pathways [77][31]. For the present, we focus on the critical initial step: curvature generation.

5.2. Mechanisms to generate membrane curvature

There are different scales of curvature associated with the generation of membrane carriers such as vesicles and tubules [50], ranging from tens to hundreds of nanometers. The lipid composition of a membrane influences its preferred curvature, stiffness, and other biophysical properties. For example, sterols can reduce energy barriers during the formation of high-curvature vesicles and tubules [2], due to a combination of effects involving insertion, inter-leaflet flipping, and phase separation [2][76]. Sterols are universal and essential constituents of extant eukaryotic membranes, but appear to have arisen relatively late on the path from FECA to LECA [11].

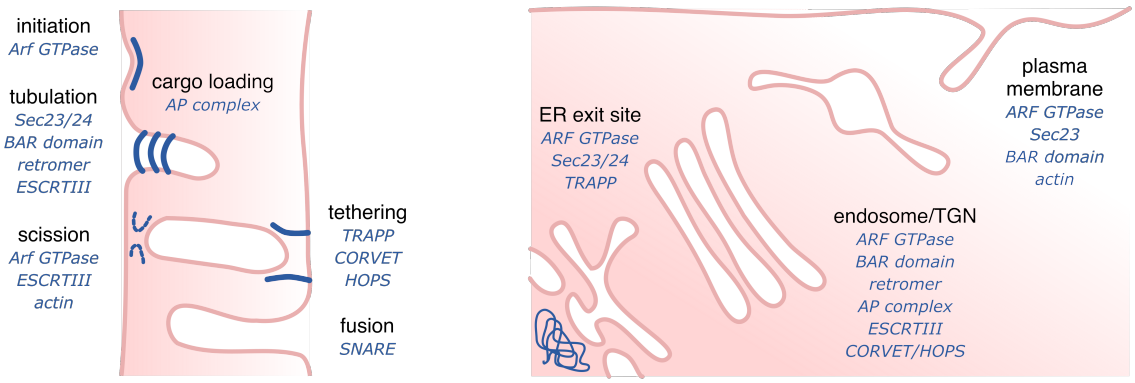

Figure 4: Asgard-derived eukaryotic signature proteins involved in tubular trafficking in eukaryotes. Proteins are mapped to each step in the generation of tubular carriers (left) and to sites of tubulation in eukaryotic cells (right).

The generation of high-curvature structures in the range of less than 100 nm often requires specific protein machinery. There are three basic mechanisms by which proteins can generate membrane curvature [100][50]: insertion, which creates asymmetry in the lipid bilayer; crowding, which creates an entropic force to curve the membrane; and scaffolding, in which large-scale polymeric protein structures constrain the shape of the underlying membrane. The diverse class of BAR domain proteins play key roles in membrane curvature generation and sensing in a variety of cellular contexts [100]. These proteins assemble on and stabilize the highly-curved necks that form at intermediate stages of vesicle or tubule budding. BAR domains have a preference for tubular membranes [101], while vesicle coat proteins such as clathrin are typically associated with spherical membranes [50]. In vitro experiments demonstrate that tubular structures generated by BAR domain scaffolds can undergo friction-driven scission [102].

6. ARCHAEA-DERIVED TUBULAR TRAFFICKING PATHWAYS IN EXTANT EUKARYOTES

In the absence of cell-biological studies on Asgard archaeal specimens, how can we empirically infer the roles Asgard ESPs are playing? One way is to study the function of their eukaryotic homologs. A second is to study the behaviour of the archaeal proteins in vitro. And a third is to express the archaeal proteins in eukaryotic models and observe what they do. Many recent studies of this type, when taken together, paint a compelling picture that Asgard membrane trafficking proteins are specialized for tubulation.

6.1. Functional conservation between eukaryotic and Asgard Arf GTPases

Small GTPases are important regulators of modern eukaryote membrane traffic: Rab GTPases play roles as membrane identity markers [51], while Arf GTPases regulate the initiation of vesicle formation and cargo sorting [108]. Phylogenetically, these proteins can be classified into Ras-like (including Rabs), Arf-like and Rag-like GTPases [49][117], and representatives of all three families can be found in Asgard genomes. These archaeal GPTases are similar to their eukaryotic counterparts, both structurally [112][117] and in the location of sequence motifs [133]. Phylogenetic studies show that eukaryotic Arf-like and Rab-like GTPases are derived from archaeal ancestors [49][117]. GEFs and GAPs act as positive and negative regulators of Rab and Arf activity, by switching them toward GTP-bound or GDP-bound states [51][108]. These GTPase regulators often multimerize via roadblock/longin domains [54]. Asgard genomes also encode several roadblock/longin domain proteins [49], with structures similar to their eukaryotic counterparts [112].

When Asgard Arf GTPases are expressed in yeast, they localize to membranes [117][133]. While the Asgard proteins lack the myristoylation site which assists with membrane interaction in eukaryotes [117][133], they do have the N-terminal amphipathic helix, characteristic of eukaryotic Arfs, required for membrane insertion [117]. Some Asgard Arfs show membrane association that depends on their GTP-bound state, and GTP-to-GDP switching can be regulated by eukaryotic GEFs and GAPs [133]. They also bind to eukaryotic Sec23/Sec24 (components of the COPII coat, discussed below) [133]. These results demonstrate a remarkable degree of functional conservation between Asgard and eukaryotic GTPases.

6.2. Tubules at ER exit sites involving COPII, COPI and TRAPP

Secretory cargo exits the ER in carriers of various morphologies, including coated vesicles and tubules [22][79]. A tubular morphology is essential for the secretion of large cargo such as collagen in mammalian cells [62]. ER-to-Golgi transport in the model plant Arabidopsis is mediated by a tubular network [27]. The inner (Sec23/24) and outer (Sec13/31) COPII coats can together assemble onto tubes or vesicles, a process that requires the Sar1 Arf-like GTPase [130][58]. Sec23/24 can form liquid condensates, [61] and their assembly into arrays may influence the final carrier morphology [130]. The final scission of the carrier is driven by a structural transition of Sar1 by GTP hydrolysis [74][75][79]. These results show that eukaryotic Sec23/Sec24 and an Arf-like GTPase with an aphipathic helix, all of

which have homologs in Asgard archaea, can remodel membranes into tubules.

Super-resolution imaging in mammalian cells shows that ER-to-Golgi transport occurs via a complex tubular-vesicular network involving COPII components at the ER exit site—at the base of the tubules [98]—and COPI components at the tubule tips [128][122]. (COPI can also mediate the generation of tubules during intra-Golgi transport [129].) ER-derived carriers are tethered to the Golgi by the TRAPP complex, via an interaction with Sec23 [13][125]. The TRAPP tether is a multi-subunit Rab GEF, with several subunits containing longin domains [30][29]. Homologs of the TRAPPC3/Bet3 subunit are found in Asgard archaea [131][49] and are structurally similar to eukaryotic TRAPPC3/Bet3 [112].

6.3. Endosomal tubules involving retromer, adaptor protein complexes, and CORVET/HOPS tethers

Retromer and other sorting nexins generate tubular carriers at endosomes [32]. The three "core" retromer proteins (VPS26/29/35) form a tubular scaffold, while oligomeric BAR domain proteins (e.g. VPS5/17 in yeast, SNX1/2 in mammals) form an inner coat which interacts with cargo [17]. Similarly, while the adaptor protein (AP) complexes are usually found in endosome-derived vesicles when clathrin is present, they associate with endosome-derived tubules when clathrin is absent [39][31]. Though AP1 typically forms the inner layer of clathrin-coated vesicles, it can load cargo into tubular structures at the trans-Golgi network and on endosomes [86], a process which is enhanced by the knockdown of clathrin [39]. AP1 and Arf1 (also found in Asgard [117]) can together form a tubular membrane coat in vitro [39]. The adaptor protein AP3 can form either vesicles or tubules at endosomes, depending on whether it is associated with clathrin or with the tubular coat BLOC1 [10]. These results show that retromer and adaptor proteins can facilitate tubular membrane traffic in the absence of canonical coatomers. All retromer subunits and AP complex subunits have homologs in Asgard genomes [23]. Endosome-derived tubular carriers are tethered to their target compartments by CORVET/HOPS proteins [113][69]. Asgard archaea also encode homologs of CORVET/HOPS tethers [23], though as discussed earlier, these may not have the typical eukaryotic β-α architecture.

6.4. Membrane tubulation by eukaryotic and Asgard ESCRTIII proteins

ESCRT complexes (ESCRT-I, ESCRT-II and ESCRT-III in eukaryotes) are a diverse class of membrane remodeling machines [118]. In the "reverse" or "negative" topology, they generate tubes which protrude away from the cytoplasm, assembling internal to the tube. In the "normal" or "positive" topology, they generate tubes which pull into the cytoplasm, assembling external to the tube. The eukaryotic ESCRT-III complex consists of a set of duplicated proteins which break into two classes: CHMP1-3/IST1, and CHMP4-7. Eukaryotic CHMP4B filaments bind to flat membranes, but can tubulate membranes in when combined to CHMP2A [8]. Eukaryotic CHMP1B and IST1 can bind to and constrict tubules in the positive topology, as observed both in vitro [73][64] and within cells [64][18][39][31]. CHMP1B and IST1 contribute to the formation and friction-driven-scission of endosome-derived tubules [18][12].

The ESCRT system is likely ancestral to archaea [60]. In TACK-superphylum archaea, ESCRT-III proteins are specialized to drive abscission during cell division, working in the negative topology [41]. Asgard archaea encode members of all three ESCRT complexes, and these complexes link to the ubiquitin system, just as they do in eukaryotes [36]. Eukaryotic ESCRT machinery appears to be descended from Asgard ESCRTs [57]. Asgard archaea encode two types of ESCRT-III proteins: type III-A is related to CHMP1-3/IST1 and type III-B is related to CHMP4-7 [70][103]. Like their archaeal homologs, Asgard type III-B proteins assemble into filaments on flat membranes, while type III-A proteins assemble onto tubules. In combination, the archaeal ESCRTIII-B and ESCRTIII-A proteins can drive tubulation in vitro [66][103]. The role of these proteins in archaea is not clear.

6.5. Endocytic tubules at the plasma membrane involving Arf GTPases and BAR-domain proteins

Multiple routes of clathrin-independent endocytosis involving tubular carriers operate at the plasma membrane [24][81]. These structures are generated by BAR domain proteins such as endophilin in Shiga toxin uptake [82] or IRSp53 and PICK1 in the CG fluid uptake pathway [94]. Extended tubules can undergo friction-driven scission due to actin polymerization [82][94][102]. Endocytosis via tubular carriers can be initiated by GTPases such as Arf1 [94][81]. The CG pathway also appears to be upregulated by Sec23 [34], in apparent

symmetry with the initiation of carriers at ER exit sites [22]. Asgard archaea encode homologs of all the machinery required for tubular endocytosis: BAR domain proteins, Arf1, Sec23, and actin [23].

6.6. Asgard homologs of the SNAREs which drive eukaryotic membrane fusion

SNARE proteins are conserved across eukaryotes, and play essential roles in the fusion of both vesicular and tubular [10] carriers to their target compartments [72]. Fusion is promoted by the energy released when a SNARE singlet on one membrane zips together with a SNARE triplet on another membrane to form a tight four-helix bundle [45]. In eukaryotes the four members of the helix, designated Qa/Qb/Qc/R, belong to four phylogenetically ancient families. SNARE homologs have been identified in multiple Asgard species [23], and appear to represent an ancestral state prior to the divergence of the Qa/Qb/Qc/R families [72]. (The only other prokaryotes in which SNAREs have been found are the γ-proteobacterial Legionellales; SNAREs in these eukaryotic pathogens are likely the result of horizontal gene transfer [72].) In biochemical assays, a Heimdallarchaeial SNARE is observed to interact with eukaryotic SNAREs [72]. The archaeal SNARE is promiscuous, able to substitute for eukaryotic R-, Qb-, or Qc-SNARES in complex. SNARE-like proteins from two different Heimdallarchaeial species can also interact to form a complex.

7. DISCUSSION

7.1. Do Asgard archaea have internal membranes?

We have seen that ESPs in Asgard archaeal genomes include proteins that facilitate each step in the generation of tubular carriers (**Figure 4**): Asgard Arf GTPases associate with membranes when expressed in eukaryotic cells [133][117]; Asgard ESCRTIII proteins drive membrane tubulation in vitro [103][66]; and Asgard SNAREs form complexes with their eukaryotic counterparts [72]. Yet, apart from fragmentary hints of intracytoplasmic vesicles in *L. ossiferum* [87], there is no current evidence that Asgard archaea have internal membranes. There are three possible explanations for this. First: it is possible that these ESPs have unknown roles in Asgard archaea, and were exapted for membrane trafficking only after mitochondrial acquisition. However, the strong structural and functional similarities between Asgard and eukaryotic ESPs weighs against this. Second: It is possible that intra-

cellular tubules are transient structures which only arise under specific conditions. Internal membranes may yet be discovered as more Asgard species are cultivated and subjected to cell-biological investigation. Third: the scattered pattern of ESPs across different Asgard lineages [23] may indicate that the known Asgard species represent streamlined versions of a more complex ancestor, which have lost their ancestral ability to generate internal compartments. In this case, ESPs may carry out rudimentary membrane remodeling in Asgard archaea, but fall short of sustaining a bona fide membrane traffic system.

7.2. Beyond tubular traffic: could coated vesicles have enabled stable endomembrane compartments?

There are two major innovations that represent the steps after the hypothetical tubule-based membrane trafficking system explored here (**Figure 2**). One is the origin of coated vesicles [19], which enable more precise and regulated transport of cargo. Another is the origin of endomembrane compartments other than the ER [111]. An intriguing possibility is that these two innovations are related. Suppose non-ER endomembrane compartments were stabilized versions of ancestral tubular carriers. Such structures are typically unstable (either shrinking away, growing without bound, or fluctuating randomly in size) [90]. One way to prevent instability is to supply and remove matter from such compartments on carriers that are much smaller in scale. The emergence of coated vesicles may thereby have created conditions which favored the maintenance of stable compartments. This could explain the existence of endomembrane structures at three distinct length-scales: the extensive ER, moderately-sized secretory and endocytic compartments, and small transport vesicles to supply and remove material from compartments.

7.3. Outlook

It seemed, until recently, that it might be impossible to distinguish between several, equally plausible, accounts for the origin of eukaryotic cells. The sheer timescales involved, and the lack of experimental data to guide the way, created a situation in which evolutionary approaches were seen as tangential to cell biology. This has now changed, because of the discovery of new archaeal and eukaryotic species, and the development of cell-biological models representative of the true breadth of the tree of life. By embracing the diversity of these organisms, both their unique characteristics and their universal traits, we will not only understand how eukaryotic cells came to be, but how they function today.

Table 1: Bidirectional strong structural homologs as predicted by Alphafold, between eukaryotic and Lokiarchaeia membrane trafficking proteins.

Protein	Euk. Uniprot	Arch. hit E-value	Arch. Alphafold	Euk. hit E-value	Euk. PDB (Ref.)
COPII Sec24	P40482	5.54e-25	A0A842VVG2	2.37e-24	4bzi [130]
TRAPPC3	Q9VSY8	2.89e-5	A0A524DX44	8.33e-6	7b6d [30]
AP2 μ	P84092	4.61e-10	A0A842VVK1	4.43e-10	4uqi [48]
AP2 σ	P62743	4.16e-5	A0A7K4CMM7	6.52e-6	4uqi [48]
ESCRTIII CHMP1B	Q7LBR1	1.19e-3	A0A497QK14	1.26e-3	6tz9 [73]
Retromer VPS29	Q9UBQ0	3.27e-18	A0A842QEB7	4.70e-19	2r17 [37]
BAR domain Arfaptin	P53365	2.06e-4	A0A524ERU1	3.49e-5	4dcn [71]
CORVET/HOPS VPS16	Q03308	N/A	A0A842VTQ5	N/A	8qx8 [99]

Starting with an Alphafold-predicted structure for the eukaryotic bait (Euk. Uniprot), we find a strong Alphafold-predicted archaeal hit (E-value). We then start with the Alphafold-predicted structure of that archaeal protein as bait (Arch. Alphafold), and find a strong experimentally-determined structural hit (Euk. PDB) for the original eukaryotic protein. For VPS16 no bidirectional hit can be found. We show a representative example of an archaeal protein with a visually similar predicted structure (**Figure 2**). These analyses were done using the EMBL-EBI AlphaFold Protein Structure Database (March 2025 update) [116].

DISCLOSURE STATEMENT

The author declares no competing interests.

ACKNOWLEDGMENTS

I thank Madan Rao, Jitu Mayor, Vivek Malhotra and Ishier Raote for discussions about the biophysics and cell biology of tubules; and Shachi Gosavi and Arkadeep Banerjee for help in visualizing protein structural alignments. This work was supported by the Simons Foundation (287975 to MT).

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