OPINION

The early evolution of lipid membranes and the three domains of life

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Abstract | All cell membranes are composed of glycerol phosphate phospholipids, and this commonality argues for the presence of such phospholipids in the last common ancestor, or cenancestor. However, phospholipid biosynthesis is very different between bacteria and archaea, leading to the suggestion that the cenancestor was devoid of phospholipid membranes. Recent phylogenomic studies challenge this view, suggesting that the cenancestor did possess complex phospholipid membranes. Here, we discuss the implications of these recent findings for membrane evolution in archaea and bacteria, and for the origin of the eukaryotic cell.

Omnis cellula e cellula ('Every cell originates from a cell')[1].

With this eloquent epigram more than 150 years ago, Virchow established the basis of contemporary cell theory, which states that cells are the basic units of life and invariably originate from other cells by division[1]. This was in perfect agreement with Darwin's 'common descent' hypothesis[2]. That cells come from cells, and organisms from organisms, implies an unavoidable physical continuity, justifying the Darwinian idea that all species are inter-related within a tree of life (that is, a tree of cells or organisms), with its deepest node occupied by the last universal common ancestor, or cenancestor (according to Fitch)[3].

All cells are bound by lipid membranes that ensure the individuality and integrity of cells and mediate their interactions with the surrounding environment[4]. Despite the crucial role of membranes in allowing the genetic and metabolic systems to interact and evolve together, most studies on the origin and early evolution of life have focused on the emergence of either the genetic system or energy and carbon metabolism[5,6]. This long-standing dichotomous debate — replication first versus metabolism first — left little room for membranes and, consequently, the origin and evolution of membranes has received much less attention than the origin of the genetic material or of energy and carbon metabolism. This is particularly surprising in the context of metabolism-first views, as the establishment of an electrochemical gradient across membranes to yield free energy that can be chemically stored[7] is a universal feature that links membranes to energy metabolism.

Historically, the origin of membranes has been mostly approached from a bottom-up perspective, focusing on how amphiphilic molecules form vesicles under prebiotic conditions and serve as primordial boundaries for protocells (BOX 1). By contrast, a top-down approach, allowing the characteristics of the cenancestor's boundaries to be inferred by comparing present-day organisms, came much later, after the discovery of archaea and their distinct membranes. This led to a paradox. According to the cell theory, as cells come from cells and modern cells are bounded by lipid membranes composed of similar molecules (phospholipids), a cenancestor with phospholipid-based membranes is the most parsimonious inference. However, two different, albeit structurally similar, kinds of phospholipids exist in nature (FIG. 1). Bacteria and eukaryotes have the same membrane biochemistry, with ester-linked fatty acid phospholipids that are based on glycerol-3-phosphate (G3P). These G3P phospholipids were thought to be universal, but the surprise came when pioneering studies of archaeal biochemistry showed that archaeal phospholipids are made of glycerol-1-phosphate (G1P) that is ether linked to isoprenoid chains[8–10]. This chemical disparity mirrors the use of different phospholipid biosynthesis pathways in archaea and bacteria, and in particular the use of a distinctive glycerol phosphate dehydrogenase to synthesize G1P[11]. When they were discovered, these archaeal pathways were considered to be unique and non-homologous to those of bacteria and eukaryotes[11].

How can these findings be reconciled with the logical inference that phospholipids are ancestral membrane components? Did their biosynthesis evolve independently in archaea and bacteria–eukaryotes? Does this imply that the cenancestor lacked lipid membranes? If so, what was the nature of the cenancestral membranes? These questions have led to controversy and raised additional, rarely explicit, issues on the evolution of eukaryotes. In this Opinion article, we explore the different hypotheses that have been proposed to answer these questions and discuss them in the light of recent phylogenomic data.

Phospholipid biosynthesis: evolved twice?

Various hypotheses have addressed the fundamental differences between archaeal and bacterial–eukaryotic phospholipids and, more specifically, the apparently unrelated nature of the pathways that synthesize the two opposed glycerol phosphate stereoisomers (FIG. 1). Koga *et al.*[11] openly deserted the cell theory by proposing that the cenancestor was acellular (that is, it had no membrane) (FIG. 2a). According to this radical view, phospholipid biosynthesis emerged late (relative to other views) and independently in the ancestral lineages that led to contemporary archaea and bacteria. Although this hypothesis accounts directly

for the differences between archaeal and bacterial membrane phospholipids, it is at odds with the increasing evidence pointing to a complex cenancestor that contained several hundred genes[12]. Such complexity would have required Darwinian evolution (based on natural selection and other evolutionary forces) to operate on individualized entities, a situation that would have been achieved by membrane compartmentation[13]. To reconcile this requirement with the apparent lack of homology between the archaeal and bacterial lipid biosynthesis pathways and, hence, the absence of lipids in the cenancestor, Martin and Russell[14] envisaged that the cenancestor had mineral, instead of lipid, membranes. In this model, the first cells would correspond to three-dimensional iron monosulphide compartments in a submarine chimney in which the redox, pH and temperature gradients were established by hydrothermal venting. Geochemistry would have been replaced progressively by biogeochemistry, leading to a complex cenancestor possessing ribosomes and other universally conserved features enclosed by mineral membranes. Phospholipid biosynthesis would have evolved independently during the evolution of the archaeal and bacterial lineages, allowing their respective release from the maternal chimney (FIG. 2b). However, hydrothermal systems are largely transient in nature, with timescales ranging between 1 and 10,000 years for complete hydrothermal fields and typically of less than 100 years for the individual chimneys[15]. As mineral-bounded cells would not have had the capacity to move between different chimneys, the whole evolutionary pathway between the origin of life and the emergence of complex archaeal and bacterial cells would have to have occurred in the same unique hydrothermal chimney at a surprising speed. More importantly, this proposal fails to postulate a mechanism to couple the formation of these mineral compartments with the replication of the inner biological components, and therefore compromises the link between the different constituents that is necessary for Darwinian evolution to act on individuals[16].

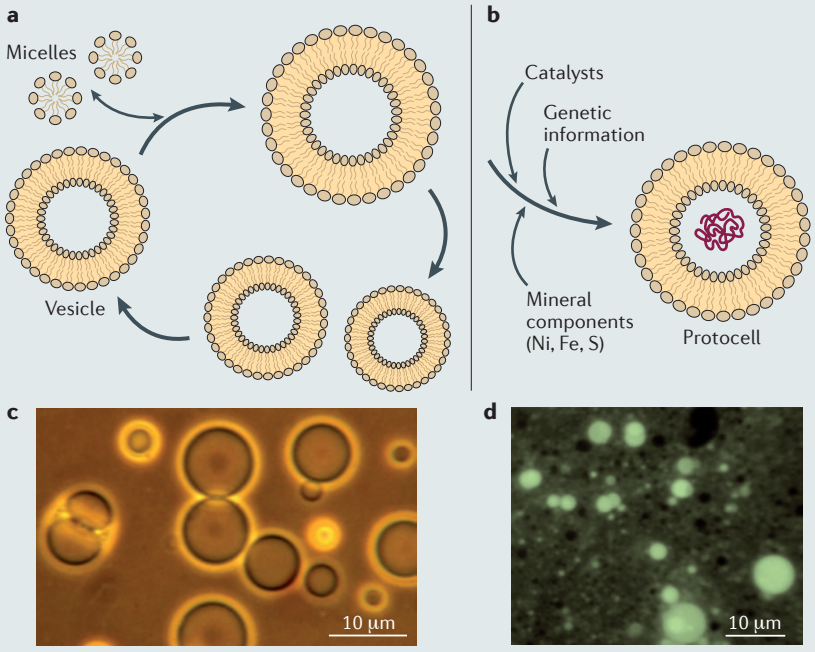
In contrast to these 'late membrane origin' hypotheses, other models invoke a much earlier origin of phospholipids. In an extension to his 'iron–sulphur world' hypothesis, Wächtershäuser[17] speculated that early cellularization occurred via membranes composed of simple lipids that were synthesized non-enzymatically by either inorganic transition metals or primitive non-stereospecific enzymes, leading to a community of cenancestral pre-cells (FIG. 2c). Pre-cell heterochiral membranes would have been replaced by more stable homochiral membranes when stereospecific enzymes appeared, triggering the divergence of archaea and bacteria. Therefore, despite acknowledging the presence of lipid membranes in pre-cells, Wächtershäuser

Box 1 | **Prebiotic origin of membranes**

Although modern cell membranes are bilayers of glycerol phospholipids, the first cell membranes probably self-assembled from simple, single-chain amphiphilic molecules, such as monocarboxylic acids or alcohols[73]. In contrast with monolayered micelles, vesicles expose hydrophilic groups to both the exterior and interior of their bilayer boundary, being able to encapsulate a certain volume of solution[74] and, depending on the bilayer permeability, creating gradients of particular molecules and ions[75]. Furthermore, vesicles can grow and divide spontaneously[76] (see the figure). From an origin-of-life perspective, these are interesting properties that made vesicles of amphiphilic compounds replace Oparin's 'coacervates' (proteinaceous aggregates)[77] in models of primeval-cell formation[78,79]. The possibility of incorporating the building blocks of replicating genetic polymers inside such vesicles has converted these vesicles into an attractive and tractable model for synthetic-biology experiments and protocell formation *in vitro*[13,67]. This avenue of research is progressing quickly, to the point that the traditional focus on self-maintenance (metabolism) as a major property of life, together with self-replication (a genetic system), is shifting towards a focus on self-assembly (membranes) in contemporary origin-of-life thinking[67,80,81].

What kinds of amphiphilic compounds were available on the early Earth and could serve for the self-assembly of protomembranes? Two sources of such compounds are known. The first source is extraterrestrial and consists of the organic matter delivered by carbonaceous chondrites. These primitive meteorites are enriched in organic compounds, including amino acids and a variety of amphiphilic molecules; such amphiphilic molecules can assemble into vesicles spontaneously, as Deamer[78] showed in 1985. The second source is terrestrial and corresponds to the abiotic formation of hydrocarbons by Fisher–Tropsch synthesis, involving the reaction of carbon monoxide and hydrogen to form hydrocarbons in the presence of iron catalysts under hydrothermal conditions. These serpentinization reactions may have been very active in the early Archean ocean[45]. Hydrocarbons can easily oxidize into mixtures of long-chain carboxylic acids and alcohols that, in the presence of glycerol, can form phospholipids[79]. Membranes with an increasing presence of phospholipids may have triggered new selective pressures for the evolution of metabolism and transport[45].

The figure shows the vesicle growth and division cycle (part **a**) and the formation of protocells (part **b**). The micrographs show self-assembled vesicles formed from the amphiphilic C_3–C_{11} carboxylic acids and polycyclic hydrocarbon derivatives found in the Murchison meteorite (part **c**) and from decanoic acid (part **d**). The vesicles formed from decanoic acid have incorporated short, fluorescently labelled DNA fragments during wet–dry–wet cycles. Micrographs courtesy of D. Deamer (University of California, Santa Cruz, USA).



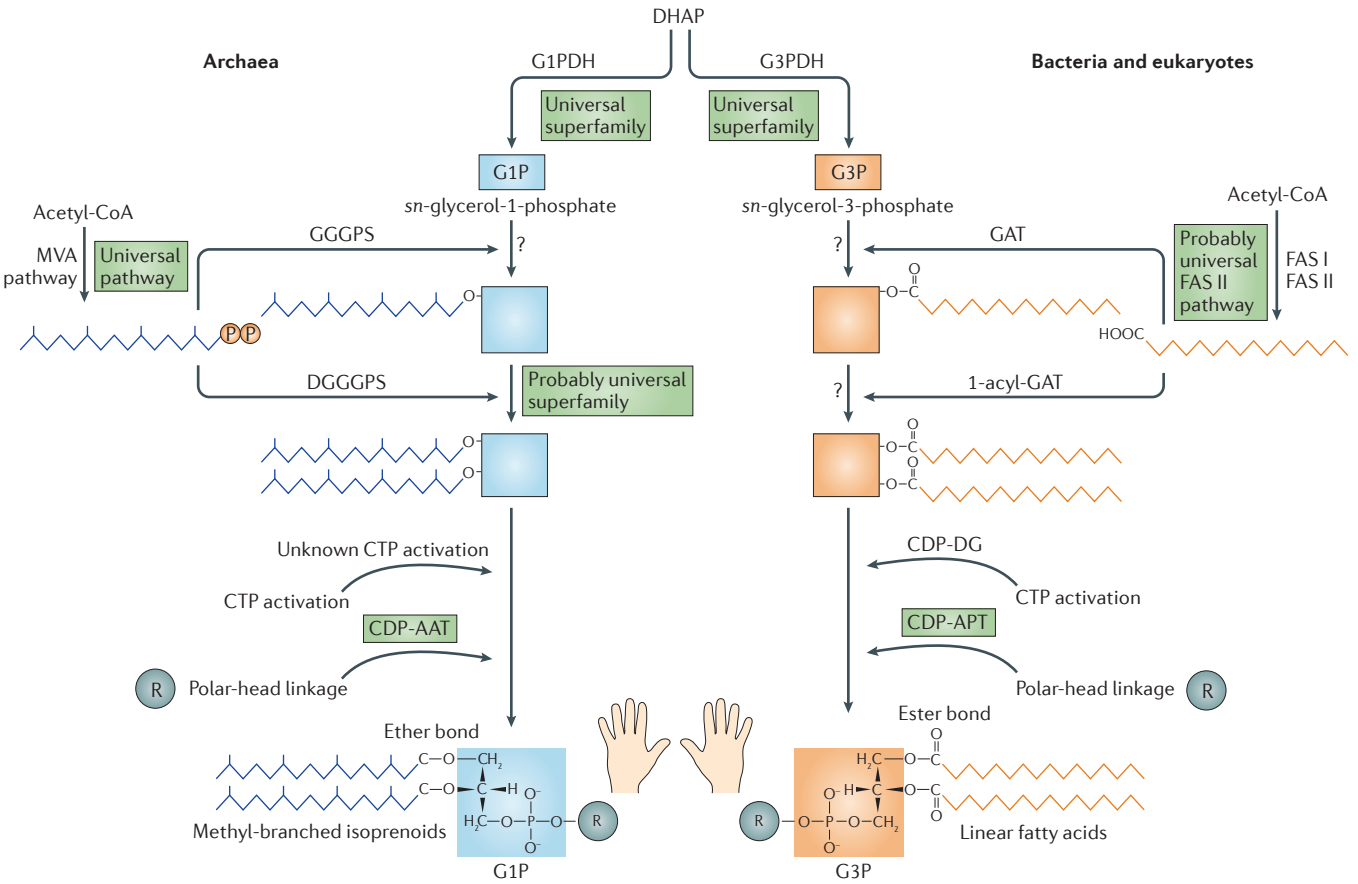


Figure 1 | **Phospholipid biosynthesis pathways in archaea, bacteria and eukaryotes.** Phospholipid components and the enzymes that synthesize them are different in modern archaea versus modern bacteria and eukaryotes. For some steps in the pathways, there is phylogenomic evidence either supporting the hypothesis that homologous enzymes carried out a particular step in the cenancestor (universal proteins or pathways) or indicating that the presence of the relevant enzymes in the cenancestor cannot be excluded (probably universal proteins or pathways). Cytidine diphosphate-alcohol archaetidyltransferase (CDP-AAT) and CDP-alcohol phosphatidyltransferase (CDP-APT) are homologous in the two pathways. Polar head radicals can be serine, ethanolamine or glycerol, among others. A question mark indicates that information is unknown. CDP-DG, CDP-diacylglycerol synthase; CTP, cytidine triphosphate; DGGGPS, digeranylgeranylglyceryl phosphate synthase; DHAP, dihydroxyacetone phosphate; G1P, *sn*-glycerol-1-phosphate; G1PDH, G1P dehydrogenase; G3P, *sn*-glycerol-3-phosphate; G3PDH, G3P dehydrogenase; GAT, G1P acyltransferase; GGGPS, geranylgeranylglyceryl phosphate synthase; FAS, fatty acid synthesis; MVA, mevalonate.

thought that specific lipid biosynthesis pathways evolved independently in archaea and bacteria.

Phylogenomics of lipid biosynthesis

Although textbooks often emphasize the differences between archaeal and bacterial phospholipids, the distinction is actually not so sharp. Ether links are found in bacterial (and eukaryotic) phospholipids[18,19], fatty acids have been detected in archaea[20–22], and isoprenoids are universally distributed membrane components[23,24] (TABLE 1). The asymmetry of the glycerol phosphate stereoisomers — G1P in archaea and G3P in bacteria and eukaryotes —that are synthesized by non-homologous glycerol phosphate dehydrogenases[11] is the only inviolate difference. However, phylogenomic approaches, based on molecular phylogenetic analyses of genes from complete genome sequences, have questioned the strength of this distinction, as they have uncovered the fact that both G1P dehydrogenases and G3P dehydrogenases belong to large multi-enzymatic superfamilies that are widespread in the three domains of life, and that at least one member of each superfamily probably evolved before the separation of archaea and bacteria[25]. Therefore, the cenancestor might have used those ancestral enzymes to synthesize a mix of both G1P and G3P (FIG. 2d). Subsequent duplication of the ancestral enzymes and the recruitment of different copies in archaea and bacteria would have led to the evolution of the specific G1P dehydrogenases and G3P dehydrogenases as the two domains diverged.

Recent phylogenomic analyses also revealed that the mevalonate pathway of isoprenoid biosynthesis, which is highly conserved in all archaea and eukaryotes and in several bacterial phyla, was probably present in the cenancestor and was lost secondarily in most bacteria, in which it was replaced by the non-homologous methylerythritol phosphate pathway[26]. Similarly, archaeal genomes have homologues of bacterial fatty acid biosynthesis genes[25], and although these genes generally belong to large multigene families with complex evolutionary histories, an ancestral origin cannot be excluded[27]. For example, a biotin-dependent carboxylase, which catalyses the incorporation of a CO_2 moiety into biotin-bearing substrates and is required for fatty acid biosynthesis, was probably present in the cenancestor[28]. Finally, in addition to the enzymes that synthesize these phospholipid building blocks, those enzymes that link glycerol

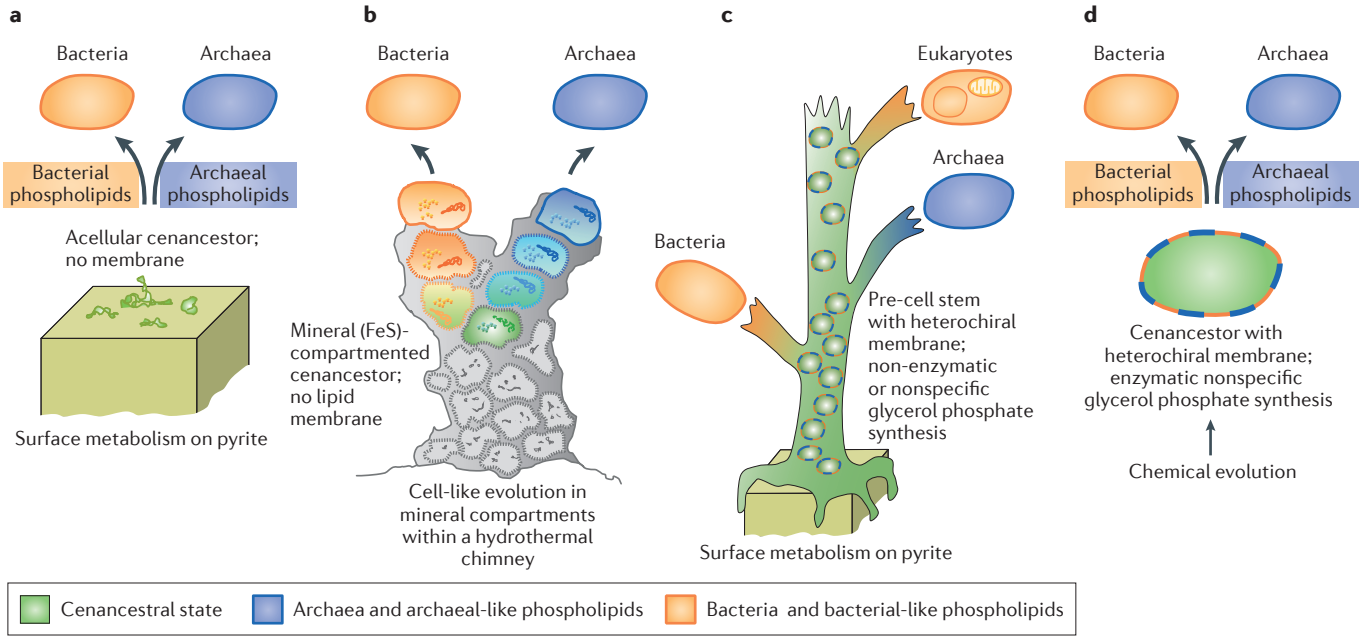


Figure 2 | **Models explaining the early evolution of archaeal and bacterial phospholipid biosynthesis.** **a** | Independent evolution of phospholipid biosynthesis from an acellular cenancestor[11]. **b** | Independent evolution of phospholipid biosynthesis from a mineral-bounded cenancestral compartment[14]. **c** | Evolution of archaeon-specific and bacterium-specific phospholipid biosynthesis pathways from a stem of pre-cells with heterochiral membranes[17]. **d** | Evolution of archaeon-specific and bacterium-specific phospholipid biosynthesis pathways from a cellular cenancestor with heterochiral membranes that are synthesized via universal but substrate-nonspecific enzymes[25].

phosphate to hydrocarbon chains and polar head groups also belong to universal gene families and are probably also ancestral[29–31] (TABLE 1).

Phospholipid membranes in the cenancestor

The phylogenomic evidence discussed above suggests that the cenancestor possessed a complete toolkit for making both isoprenoid- and fatty acid-based phospholipids, possibly using a mixture of G1P and G3P stereoisomers[25–27] (TABLE 1). The early origin of phospholipids is further supported by the universal conservation of several contemporary membrane-embedded proteins[12,32]. These include proteins involved in membrane bioenergetics — notably an ATPase that specifically exploits transmembrane ion gradients[29,33], and integral membrane proteins (for example, hydrogenases and dioxygen reductases) that are involved in respiratory chains[34–37] — and also proteins of the secretion and membrane-targeting machineries, such as the signal recognition particle and the Sec, YidC and Tat (twin-arginine translocation) protein export and membrane insertion pathways[38–41]. In an attempt to reconcile the compelling evidence that these membrane proteins were cenancestral with the proposal for an iron monosulphide-bounded cenancestor, Koonin and Martin[42] proposed that lipid patches, but not a continuous lipid membrane, accumulated on mineral walls to host these proteins. However, the co-occurrence of a respiratory chain and ATPases in the cenancestor strongly suggests that ATPases had already evolved to exploit a transmembrane proton (or sodium, according to recent suggestions[43]) gradient, which requires a continuous membrane[29].

In summary, in our opinion, the cenancestor probably had lipid membranes and the enzymatic machinery to synthesize modern phospholipid components, including G1P, G3P, isoprenoids and fatty acids. Contrary to previous assumptions[17], heterochiral membranes formed by G1P- and G3P-based phospholipids do not appear to be intrinsically less stable than homochiral ones[44]. Therefore, on the basis of phylogenomic and physicochemical considerations, we propose that the cenancestor possessed a heterochiral, complex, modern-like phospholipid membrane (FIG. 3). The large number of enzymes required to synthesize it is compatible with a complex cenancestor having a large genome[12]. Differences between archaeal and bacterial membranes would have evolved as these two domains diverged from the cenancestor. From an ecological standpoint, a single ancient origin for lipid membranes seems realistic. In fact, it is difficult to imagine that phospholipid membranes, and thus true cellularization, originated twice because when a given ancestral organism acquired a lipid membrane it would gain a strong selective advantage and supersede less efficient competitors[45]. This probably did occur but would have been earlier in evolution, when cellularization first appeared, at a moment that might be considered the true origin of life.

The archaea–bacteria 'lipid divide'

Most early evolution scenarios[11,13,15,23] consider Archaea and Bacteria as the primary domains (that is, the two domains that diverged directly from the cenancestor), and the domain Eukarya as having originated secondarily, containing chimeric organisms that were derived from the symbiosis of an archaeon (or a member of a proto-eukaryotic sister lineage to archaea) and at least one bacterium, the ancestor of mitochondria.

If the cenancestor had complex heterochiral membranes, what was the driving force triggering the 'lipid divide' — the differentiation of archaeal and bacterial membranes? The first possible explanation to be invoked was the instability of mixed membranes[17,46], but experiments with liposomes containing archaeal and bacterial phospholipids showed that the stability of homochiral

Table 1 | **Phylogenomic arguments in favour of a cenancestral phospholipid membrane**

Observations	Implications for the cenancestor
Phospholipid components	
• Ancestral presence of at least one member of the two dehydrogenase superfamilies to which the contemporary dehydrogenases synthesizing G1P (archaea) and G3P (bacteria and eukaryotes)[25] belong.	• Possible enzymatic synthesis of G1P and G3P
• Presence of isoprenoids in the three domains of life • Conservation of the mevalonate pathway of isoprenoid synthesis in archaea, bacteria and eukaryotes[26]	• Biosynthesis of isoprenoids
• Presence of fatty acids in the three domains of life • Conservation of key enzymes for fatty acid synthesis and degradation in archaea, bacteria and eukaryotes[25,27]	• Probable biosynthesis and degradation of fatty acids
• Broad distribution of ether-linked phospholipids and of homologues of the archaeal enzyme superfamily responsible for ether link formation in the three domains of life[30]	• Possible hydrocarbon chain attachment to glycerol phosphate (at least) via ether links
• Presence of one representative of the CDP-APT family involved in polar head group attachment in archaea and bacteria[31]	• Phospholipid head group attachment in the cenancestor
Lipid membrane-associated proteins	
• Universally conserved H^+ (or Na^+) ATPase[32,33,83]	• Synthesis of ATP exploiting a transmembrane ion gradient • Need for a continuous lipid membrane
• Universally conserved components of respiratory chains (cytochrome *b*, Rieske protein, hydrogenases and dioxygen reductases)[34–37]	• Likely presence of a respiratory chain in the membrane that could generate a proton gradient across the membrane
• Universally conserved SRP system (SRP domain and its receptor)[38]	• Targeting of proteins to the membrane
• Universally conserved Sec, YidC and Tat pathways[39–41]	• Protein export and insertion into the membrane

CDP-APT, cytidine diphosphate-alcohol phosphatidyltransferase; G1P, *sn*-glycerol-1-phosphate; G3P, *sn*-glycerol-3-phosphate; SRP, signal recognition particle; Tat, twin-arginine translocation.

and heterochiral mixed membranes is similar, challenging this idea[44]. The past evolutionary constraints faced by the two domains might constitute another explanation. It is widely accepted that the last common ancestor of archaea was hyperthermophilic[47], so the composition of archaeal membranes may result from ancestral adaptation to extremely hot environments. Extreme physicochemical conditions induce chronic energy stress, which has to be managed by the tight regulation of membrane permeability. Thus, archaeal membranes have evolved to prevent proton leakage at high temperature and to control transmembrane electrochemical gradients at extreme pH and high salinity[48]. In the bacterial lineage, the evolution of the acyl-carrier protein allowed efficient fatty acid synthesis, and this pathway was recruited for phospholipid synthesis, relegating isoprenoid biosynthesis to other cellular functions[27]. Finally, the idea of a 'frozen accident' cannot be discarded. Components of bacterial phospholipids are also present in archaea and vice versa, but these components function in alternative cellular processes in the other domain: G1P is used in bacterial envelopes[49,50], fatty acids are used in archaeal metabolism[51] and the universally distributed isoprenoids are involved in a range of functions[23,24]. This suggests again that these components are ancestral but were recruited for different uses in archaea and bacteria. Their recruitment may have occurred accidentally or by drift. It could be that enzymes from different ancestral dehydrogenase families specialized in the synthesis of opposed glycerol phosphate stereoisomers.

The origin of eukaryotic membranes

The origin of eukaryotic membranes is a problem that is rarely addressed by the different hypotheses that have been proposed to explain the emergence of eukaryotes (FIG. 4). Eukaryotic membranes have typical bacterial-like phospholipids. By contrast, the apparent conservation of the isoprenoid biosynthesis mevalonate pathway in archaea and eukaryotes, and its loss in most bacteria, could support a relationship between archaea and eukaryotes. However, recent phylogenomic analyses show that there are major differences between the archaeal and eukaryotic mevalonate pathways; archaea have the most divergent pathway, whereas eukaryotes and several bacteria appear to have retained the ancestral version[26]. This suggests that eukaryotes inherited their membranes directly from bacteria or from a common ancestor of bacteria and eukaryotes to the exclusion of archaea. This is at odds with the classical Woesian three-domain phylogeny rooted on the bacterial branch[52]. With regard to the eukaryotes, this phylogeny implies that the last common ancestor of archaea and eukaryotes would have had either an archaeal-like membrane that was subsequently replaced by bacterial-like phospholipids in eukaryotes (FIG. 4a), or an ancestral mixed membrane with both G1P and G3P phospholipids that evolved towards a modern archaeal-like membrane in archaea and towards a bacterial-like membrane in eukaryotes after the divergence of both lineages (the pre-cell-like model) (FIG. 4b).

Both options are problematic. Unless considering massive horizontal transfer of all the necessary genes, the mixed-membrane model implies the less parsimonious assumption that bacterial-like membranes evolved twice from the ancestral mixed membrane, in bacteria and eukaryotes independently. The fact that no archaeal-to-bacterial membrane transition has been identified so far also undermines the hypothesis that an archaeal-like membrane was secondarily replaced in eukaryotes. This also affects the view of Cavalier-Smith[53] that archaea and eukaryotes evolved from a Gram-positive bacterial ancestor and that archaea replaced the

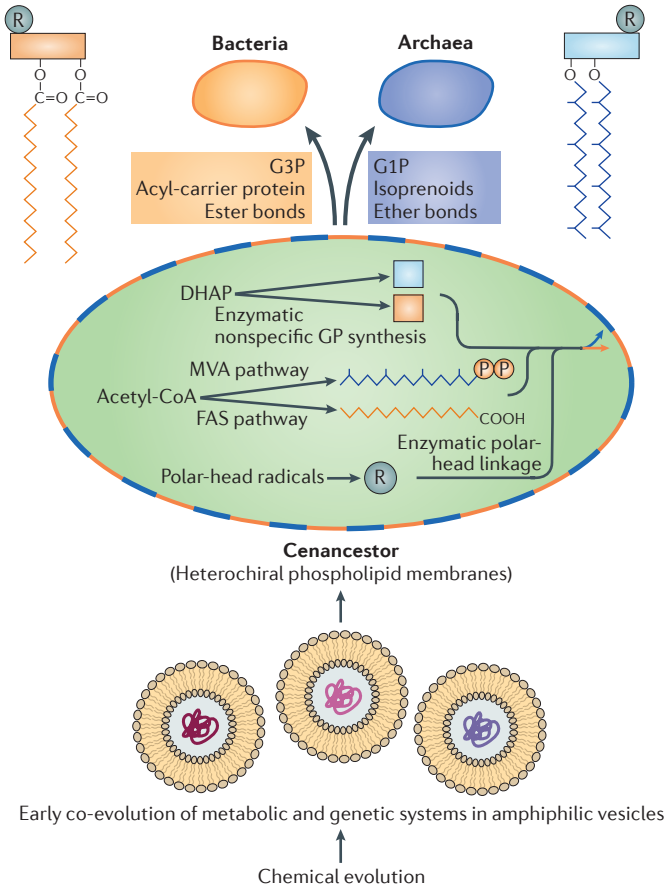


Figure 3 | **Complement of enzymes involved in the biosynthesis of phospholipid components in the cenancestor, and their evolution during the archaea–bacteria split.** This complement of enzymes is inferred by phylogenomic analysis of complete genome sequences of contemporary species. The cenancestor would have been able to synthesize heterochiral phospholipid membranes with a mix of *sn*-glycerol-1-phosphate (G1P) (blue) and *sn*-glycerol-3-phosphate (G3P) (orange) produced from dihydroxyacetone phosphate (DHAP), bound to isoprenoid and fatty acid lateral chains and to polar head radicals. We propose that the first cells were surrounded by amphiphilic vesicles that were synthesized abiotically and that the cenancestor already possessed a sophisticated enzymatic machinery for lipid biosynthesis. The divergence of bacteria and archaea from the cenancestor was paralleled by the specialization of their membranes. Bacteria use G3P that is bound via an ester link to fatty acids which are synthesized in an efficient way owing to the acyl-carrier protein. By contrast, archaea use G1P that is bound via an ether link to isoprenoids. GP, glycerol phosphate; MVA, mevalonate; FAS, fatty acid synthesis.

bacterial membrane secondarily (FIG. 4c). Cavalier-Smith postulates that the cenancestor was the last common bacterial ancestor and was bounded by two membranes (that is, was Gram negative), and that there was a single transition of double-membrane cells to single-membrane cells during evolution. Consequently, in his view, archaea, eukaryotes and Gram-positive bacteria, being bounded by single membranes, are monophyletic. However, in addition to the problem of a bacterial-to-archaeal membrane transition, the recent discovery of an archaeon with a double membrane, *Ignicoccus hospitalis*[54], invalidates the idea that the number of cell membranes is an extremely conserved characteristic that was altered only once during the history of the three domains. Thus, this characteristic cannot be used to support the monophyly of archaea, eukaryotes and Gram-positive bacteria.

Today, there is little doubt that the eukaryotic cell is a chimera that is derived from the endosymbiosis of the alphaproteobacterial ancestor of mitochondria within a host cell[55]. However, the nature of that host cell is still highly debated. The classical Woesian models propose that the host was a member of an independent proto-eukaryotic sister lineage to the archaea, with a nucleus and phagocytic capacity[56–58]. However, direct evidence for the existence of this amitochondriate proto-eukaryotic lineage is lacking, so several models (often called chimeric or symbiogenetic models) propose that eukaryotes derive directly from a symbiosis between archaea and bacteria[56,59]. This would account for the mosaic distribution of characteristics that is observed in eukaryotes, with the genes involved in replication, transcription and translation being of archaeal origin and most genes involved in metabolism and other cellular functions having bacterial homologues[60,61].

How do these models explain the bacterial-like nature of eukaryotic membranes? The models can be divided into two types, which are best illustrated by, respectively, the hydrogen hypothesis and the syntrophy hypothesis for the origin of eukaryotes. The hydrogen hypothesis proposes that eukaryotes originated from the endosymbiosis of an alphaproteobacterium within an archaeon[62], implying, similarly to some classical models, that there was a transformation of the original archaeal cell membrane into a bacterial-like membrane[63]. In this model, the nuclear membrane would form *de novo* from nucleus-encoded bacterial phospholipids that would form vesicles encapsulating the archaeon-derived nuclear genome (FIG. 4d). The second type of chimeric models, such as the syntrophy hypothesis[64,65], postulate that eukaryotes derive from the endosymbiosis of an archaeon within a bacterium, whereas mitochondria derive from a second endosymbiotic event[64–66]. In this case, the outer membranes of the consortium would be bacterial, eliminating the need for the aforementioned transition (FIG. 4e). This model additionally proposes that the external bacterial-like membrane developed an extensive endomembrane system with secretory functions, leading to the formation of the future nuclear membrane in an analogous manner to that postulated by classical autogenous models for the origin of the nucleus[65]. The membrane of the ancestral archaeon would then simply be lost.

In summary, most classical and chimeric models for the origin of eukaryotes require an archaeal-to-bacterial membrane transition. However, traces of this type of transition have never been found in contemporary bacteria or archaea, indicating that either this transition was extremely rare or it never occurred. In that case, the distribution of phospholipids in contemporary membranes would actually argue in favour of a bacterium at the origin of the cytoplasmic membrane of the proto-eukaryotic host that acquired mitochondria.

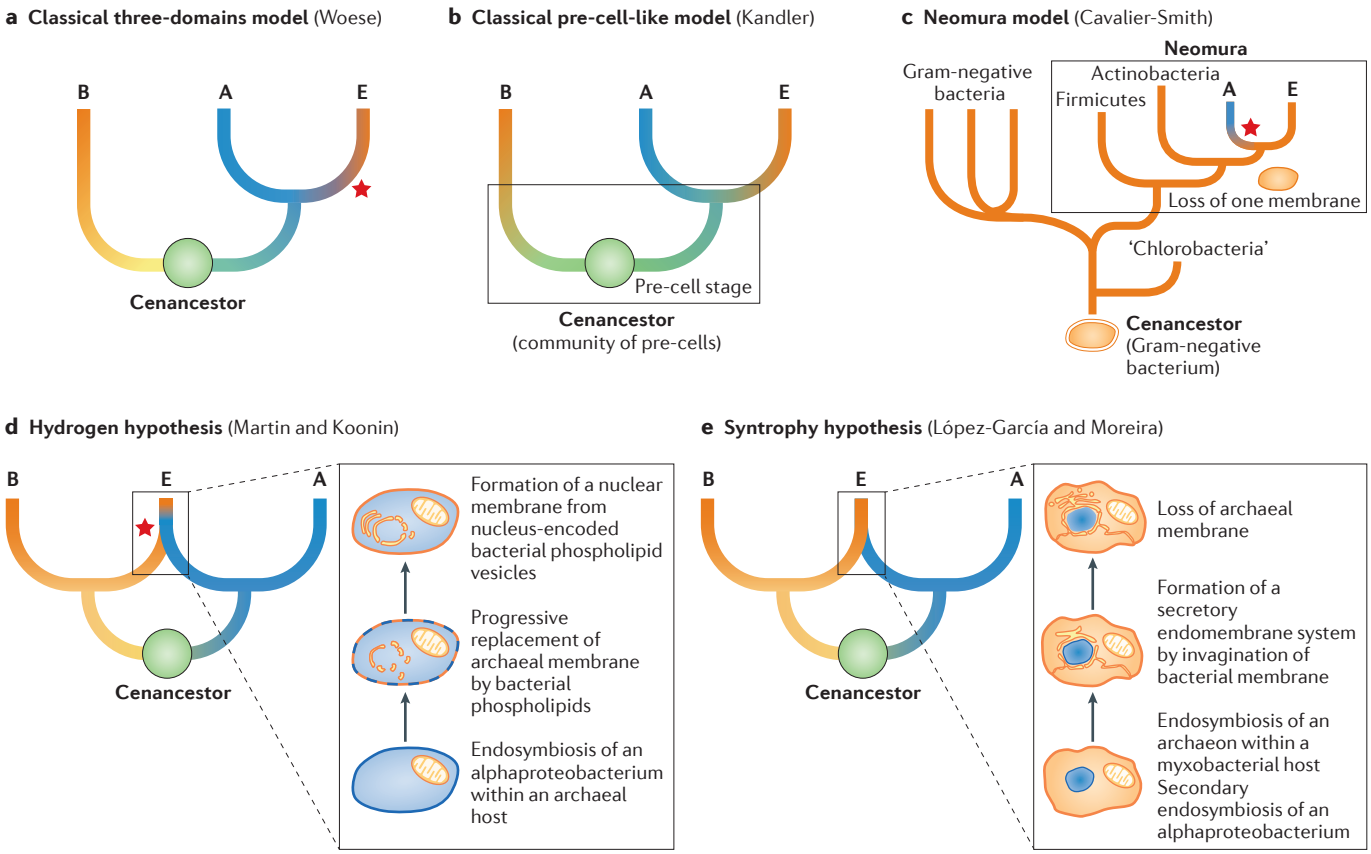


Figure 4 | **Models explaining the bacterial-like nature of phospholipid membranes in eukaryotes.** Different views of the evolutionary relationships among the three domains of life are depicted as simplified phylogenetic trees. The cenancestor (green) is placed at the root of the trees. Orange branches correspond to organisms with bacterial-like phospholipids, and blue branches correspond to organisms with archaeal-like phospholipids. Red stars indicate transitions from one type of phospholipid (archaeal or bacterial) to the other. Insets in the hydrogen and syntrophy hypotheses provide details about lipid evolution after the chimeric origin of eukaryotes by a symbiosis between archaea and bacteria. **a** | The classical three-domain model from Woese[52]. **b** | The classical pre-cell-like model from Kandler[82]. **c** | The Neomura model from Cavalier-Smith[53]. **d** | The hydrogen hypothesis as detailed by Martin and Koonin[63]. **e** | The syntrophy hypothesis as detailed by López-García and Moreira[65]. A, Archaea; B, Bacteria; E, Eukarya.

Conclusion and perspectives

Recent phylogenomic results suggest that pathways for the biosynthesis of the different phospholipid moieties and, hence, of phospholipid membranes, are ancient (TABLE 1). They date back at least to the cenancestor, which in our opinion probably possessed phospholipid membranes that were predated by simpler membranes in earlier evolutionary periods. As a corollary, and in contrast with hypotheses proposing a late origin of lipid membranes, this opens the possibility that lipid membranes have existed all along the evolution of life, from prebiotic times, when the first protocells were formed by vesicles of amphiphilic molecules of abiotic origin (BOX 1), to fully modern-like cells endowed with an enzyme set for phospholipid synthesis. This would extend the cell theory back to a protocellular era. Therefore, bottom-up approaches based on prebiotic chemistry studies can be enriched by top-down phylogenomic analyses that shed light on the characteristics of early cell membranes. Such synergy is necessary to clarify two of the most challenging and interlinked issues in origin-of-life research, namely the origin of the first cells, and the coupling of metabolism and genetic information[5]. The field of synthetic biology may contribute to filling this gap in our knowledge by fostering the study of vesicles that are built with prebiotically plausible lipids containing simple replicative polymers, and using these vesicles as primordial life models[13,67].

In addition to the information that can be inferred about the cenancestor and even earlier life forms, membranes are crucial to understanding the evolution of the three domains of life. Bacteria and archaea have different phospholipids, but also different DNA replication mechanisms[68]. Could the driving forces behind the lipid and DNA replication machineries be the same? Considering that DNA replication, chromosome segregation and cell division are intimately coupled in bacteria and archaea[69,70], this possibility is worth exploring. Also, an accurate biochemical description of modern membranes, considering not only the major but also the rare lipids, is still missing. This information could overcome the supposedly insurmountable difficulties in explaining the differences between archaeal and bacterial lipids. For example, the presence of fatty acids in archaea, as described several decades ago[20,21,71,72], is often neglected, as is the presence of archaeal-like ether lipids in some bacteria[18,19]. Phylogenomic analyses, which so far have mostly focused on the evolution of glycerol phosphate dehydrogenases, isoprenoids and fatty acids, must also address the origin and evolution of the enzymes that catalyse the formation of links among these elements and the lipid modifications such as glycosylation. Advances in these areas will improve our understanding not only of cell

membrane evolution but also of the origin of life and the diversification of the three domains.

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