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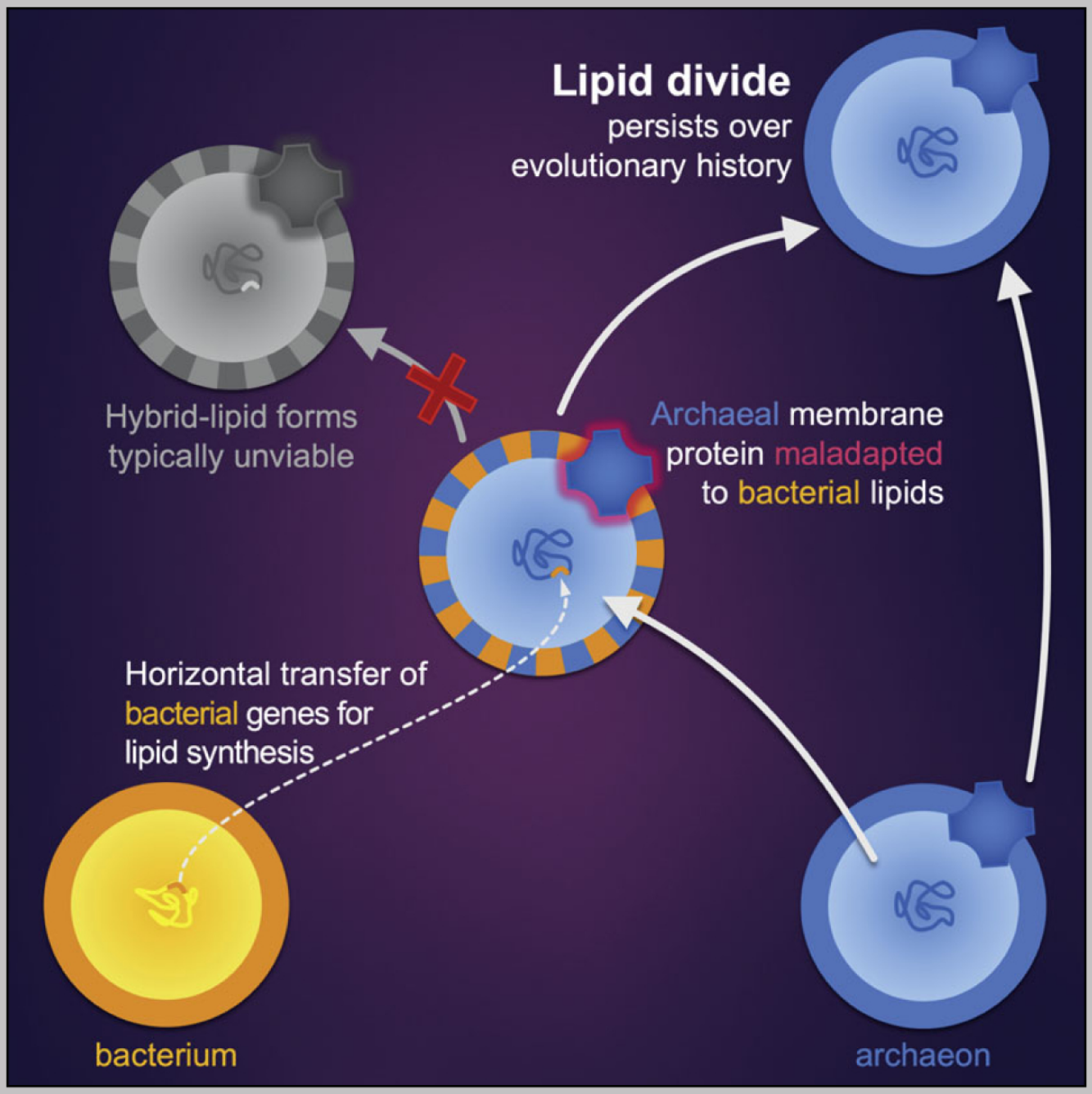

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Why the Lipid Divide? Membrane Proteins as Drivers of the Split between the Lipids of the Three Domains of Life

Victor Sojo

Recent results from engineered and natural samples show that the starkly different lipids of archaea and bacteria can form stable hybrid membranes. But if the two types can mix, why don't they? That is, why do most bacteria and all eukaryotes have only typically bacterial lipids, and archaea archaeal lipids? It is suggested here that the reason may lie on the other main component of cellular membranes: membrane proteins, and their close adaptation to the lipids. Archaeal lipids in modern bacteria could suggest that the last universal common ancestor (LUCA) had both lipid types. However, this would imply a rather elaborate evolutionary scenario, while negating simpler alternatives. In light of widespread horizontal gene transfer across the prokaryotic domains, hybrid membranes reveal that the lipid divide did not just occur once at the divergence of archaea and bacteria from LUCA. Instead, it continues to occur actively to this day.

1. Introduction

The three domains of life—archaea, bacteria, and eukaryotes—are markedly differentiated in the composition of their cellular membrane lipids. While the overall biological and chemical function is roughly the same in the three domains, the lipids of archaea are typically composed of isoprenoid chains ether-linked to an *sn*-glycerol-1-phosphate (G1P) headgroup, whereas bacterial and eukaryotic lipids are typically composed of fatty acids in ester linkage to an *sn*-glycerol-3-phosphate (G3P) headgroup (G3P being the mirror molecule, or enantiomer, of G1P).[1] This stark difference between the two lipid types is known as the "lipid divide."

The core topology of the tree of life remains contentious,[2–4] but evidence amassed over the last decade supports the view that eukaryotes as we presently know them arose from a merger of prokaryotic cells,[5–9] involving an archaeal host[10–12] (likely from within the recently described Asgard superphylum)[13,14] and an endosymbiotic[5] bacterium (likely from a lineage within or closely related to the modern Alphaproteobacteria).[15–17] If this was the case, eukaryotes likely inherited their membrane lipids from their prokaryotic forebears. This puts the question of the lipid divide, firstly, on the divergence of archaea and bacteria from the last universal common ancestor (LUCA)—hereby assuming that the root of the tree of life lies between the two prokaryotic domains.

Ideas abound, from a noncellular[1,18] or at least non-lipid-synthesizing LUCA,[18] to one that had only some but not all elements of modern lipids,[19,20] to a LUCA that had both sets of lipids in full.[21] Whatever the scenario, the divide occurred, and the two prokaryotic domains ended up with starkly different membrane lipids.

The exact amount and significance of large-scale horizontal-gene-transfer events between prokaryotes is a matter of debate,[22–25] but it can perhaps be safely assumed that the genes for making the other domain's lipids have likely been imported on a number of occasions. Indeed, whether transferred or ancestral, the ability to make so-called chimeric lipids (i.e., with components typically associated with the other domain) is well documented.[4,26–28] The full lipids, however, have persisted in their binary split.

The simplest explanation is that hybrid or "heterotypic" membranes (i.e., those containing both fully archaeal-type and fully bacterial-type lipids) must be structurally unstable in some way and would self-segregate into the two types if mixed, at least over evolutionary time.[21,29] However, results from chemically and genetically engineered hybrids show that archaeal- and bacterial-type lipids can indeed be successfully mixed under laboratory conditions, yielding stable membranes.

2. Mixed Archaeal–Bacterial Lipid Membranes Are Viable in the Laboratory

Liposomes made by mixing a 1:2 proportion of archaeal polar lipids (extracted from *Sulfolobus solfataricus*) with bacterial-like phosphatidyl choline (extracted from eggs) were more stable to leakage than vesicles made from either of the pure lipids (although notably, not under chemical stress).[30]

Similarly, heterotypic membranes in liposomes prepared by chemically mixing lipids from thermophilic archaea and bacteria (including *Aeropyrum pernix* and *Thermus thermophilus*) were more stable at high temperatures (up to 120 °C) than either purely archaeal or purely bacterial liposomes.[31]

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Further, and building on previous results showing that the archaeal lipid pathway can be successfully expressed in recombinant bacteria,[32] an *Escherichia coli* (*E. coli*) strain was recently engineered to contain the full set of archaeal lipid-making genes.[33] Not only did the chimeric bacteria successfully synthesize the archaeal lipids, but the heterotypic membranes actually proved advantageous under a number of environmental-stress conditions.

Altogether these results present substantial evidence that mixed-lipid membranes are, at least under laboratory conditions, viable.

But in spite of these successes with engineered liposomes and strains, wild-growing bacteria with archaeal lipids, or conversely, the presence of bacterial lipids in archaea living in the wild, have remained elusive until recently.

3. Bacteria with Archaeal Lipids Are Found in Nature

A recent study reports that members of the bacterial Fibrobacteres/Chlorobi/Bacteroidetes (FCB) superphylum contain an almost complete set of genes for synthesizing archaeal membrane lipids, in addition to the traditional bacterial lipids.[34] The initial results came from a metagenomic study of strains that are yet to be cultured in the laboratory, but were then shown to extend to multiple members of the FCB superphylum sequenced previously. Separate results have since confirmed the presence of the full archaeal lipid-biosynthesis machinery in multiple members of the FCB group, including homologs of the lipid headgroup-synthesizing enzyme *sn*-glycerol-1-phosphate dehydrogenase (G1PDH) in Fibrobacteres.[4] Moreover, expressing the relevant genes heterologously in *E. coli* produced archaeal-like lipids, even in the absence of any known G1PDH homologs.[34] The original cells themselves, however, are yet to be grown in the laboratory, so additional experimental tests are needed to determine exactly how archaeal-like the lipids of these bacteria are, and how much of them the membranes contain. Nevertheless, the results provide crucial support for an idea that has been growing over the last two decades: membranes with mixed archaeal and bacterial lipids are viable, and there do not seem to be any intrinsic genomic or structural reasons why the two types should not mix across domains.

Yet they largely do not. Recent observations notwithstanding, archaea typically have archaeal lipids and bacteria have bacterial lipids. So, if lipids themselves did not cause the lipid divide, what did?

I suggest here that it was likely the other main component of cellular membranes: membrane-associated proteins.

4. Membrane Proteins as Drivers of the Lipid Divide

Membrane proteins can compose half of the total volume of cellular membranes[35] and they mediate many of the cell's most crucial processes,[35–38] including bioenergetics, reproduction, motility, transport, nutrient acquisition, waste disposal, surface interactions, homeostasis, cell-to-cell communication, and more.

There is a strong association between the structure, and consequently function, of membrane proteins and the lipid environments in which they exert these functions.[39–41] For example, the bacterial DNA replication machinery is closely associated with the membrane,[42] and membrane shaping is mediated largely by proteins (most evidently in eukaryotes, in which cytokinesis and remodeling are driven by a host of proteins with highly specific interactions with the lipids).[43,44] These observations paint a picture of membrane proteins that have evolved, and continue to evolve, in close adaptation to their lipids.

Thus, while the word "membrane" is often used to refer specifically to the lipids, the sheer proportions of proteins within membranes, and their roles in providing shape, function, and structure to those membranes, suggest that the term should be used more inclusively.

The traditional fluid-mosaic view of membranes[37] has been correspondingly amended in the decades following its publication. Lipids are no longer seen as forming a regular monolithic slab into which proteins are sparsely embedded in a seamless match that does not perturb the lipids themselves. Instead, dense agglomerations of proteins and lipids interact with and shape each other, their local membrane environment, and ultimately the cell itself.[40,45,46] That is, like everything else in the cell, membrane proteins have evolved in close adaptation to their environment,[38] which to a great extent is the lipids with which they interact.[41]

I therefore suggest that the vast differences between archaeal and bacterial lipids may suffice to explain why they are largely inexistent across domains: not because of the lipids themselves but because of their interactions with membrane proteins (**Figure 1**).

This elicits the question of why members of the bacterial FCB superphylum would carry genes to synthesize the lipids typically associated with archaeal membranes. Whether these genes were acquired horizontally at the base of the group or were always there since LUCA and have since been lost in all other groups,[34] it remains an unusual example of heterotypic membranes that calls for an explanation.

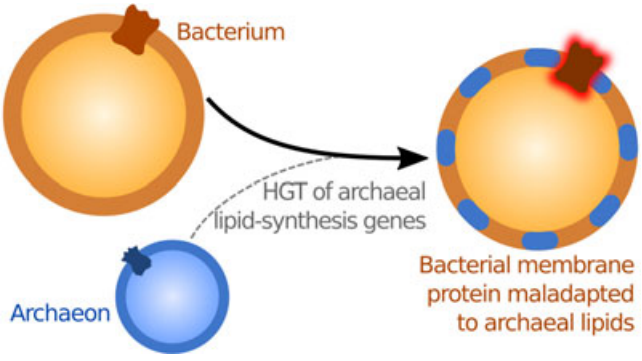

Figure 1. Maladaptive interactions of membrane proteins with foreign lipids could drive the lipid divide. Although the necessary genes have likely been imported a number of times, members of only one prokaryotic group are known to carry the genes to synthesize the other domain's lipids. Deleterious interactions (red shade, top right) of established membrane proteins with imported foreign lipids (blue dashes) may suffice as an explanation for the usual purity of cellular membranes across the tree of life. HGT: horizontal gene transfer.

Let us first consider horizontal transfer. Since the FCB bacteria with archaeal-lipid biosynthesis genes are not yet cultured, any conclusions regarding the functional importance of these putative membrane lipids will be limited to what we can infer from their genomes. However, this could perhaps be analogous to the ecological advantage granted by the import across domains of genes encoding foreign membrane proteins. A notable example is the clade-defining acquisition of the aptly named bacteriorhodopsin by the ancestor of Haloarchaea. The membrane-bound protein is central to the archaeon's bioenergetic budget and its acquisition from bacteria must have inevitably involved large adaptations to the archaeal membrane.[41,47]

If acquired horizontally at the base of the FCB superphylum, the archaeal lipids likely convey an ecological advantage to these bacteria (or at least did to their ancestors) in the same way that a bacterial membrane protein does to the Haloarchaea (discussed in further detail in Section 6).

Notably, the advantage could come from the lipids themselves (**Figure 2**). Lipids provide a number of functions to the cell but, at a minimalistic physicochemical level, they must 1) provide a highly impermeable barrier and 2) remain in a liquid-crystal state.[48] Membranes made of bacterial-like lipids only achieve both properties right at or slightly above the phase-transition temperature of the lipids, so bacteria tightly regulate the expression of a variety of lipids such that their membranes remain viable under different conditions. Conversely, membranes built with archaeal-like lipids remain impermeable and liquid-crystalline at the entire range of temperatures of life.[48] As results from the laboratory suggest,[33] such an advantage provided by archaeal lipids may suffice as an explanation for their presence in some bacteria (Figure 2A).

Alternatively, any imported archaeal-type lipids could be largely restricted to interactions with specific membrane proteins (Figure 2B). Notably, though, even in cases of crucial membrane proteins whose encoding genes were horizontally acquired by archaea from bacteria,[47,49,51] such as the aforementioned import of bacteriorhodopsin into Haloarchaea, the membranes have remained conservatively archaeal.

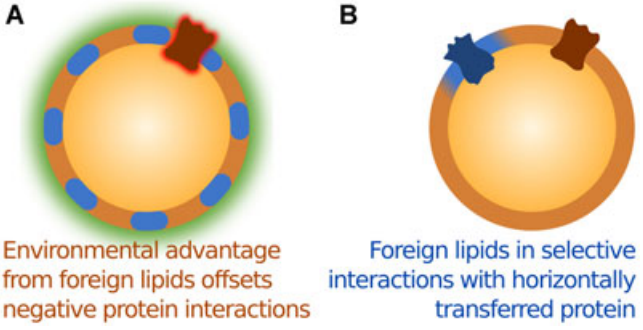

Figure 2. Two possible explanations for a successful import of lipids across domains. A) Early after a horizontal acquisition, the environmental advantage (green shade) provided by the imported lipids (blue dashes) outweighs any deleterious effects of these lipids on native membrane-associated proteins (red shade, as in Figure 1). Proteins would adapt to the novel lipids over time and vice versa. B) Foreign lipids may be constrained to interactions with similarly imported membrane proteins (both blue, top left), avoiding interactions with native proteins (top right).

The alternative scenario, if the genes for synthesizing archaeal lipids were not acquired horizontally, is that they have been in the FCB lineage ever since LUCA. I suggest that this would raise more questions than it answers.

5. A LUCA with Both Sets of Lipids Presents a Challenging Evolutionary Scenario

If the archaeal lipid-synthesis genes in the FCB superphylum are indeed ancestral and can be traced back to LUCA, this would require an explanation for why (so far) only this group kept the ability to synthesize the lipids that we now associate with the other domain and, conversely, why all other lineages lost it. Redundancy might be enough, but this in turn prompts a deeper question: why would LUCA need two sets to begin with?

Let us assume that LUCA did indeed have the genes for synthesizing both sets of lipids. Since the enzymes for the biosynthesis of the two types are entirely unrelated,[1,52] it is difficult to imagine that LUCA's ancestors evolved both types in full at exactly the same time. Instead, one type was likely completed first, followed by the other. It is not easy to envision what kinds of selective pressures could have made LUCA's ancestors benefit from developing a second full set of lipids after already having a perfectly functional first one,[53] considering that almost no organism known at present seems to experience such pressures. And this only for all archaea to selectively lose one type while almost all bacteria lost the other, despite the mix being stable and in some cases even advantageous.[33]

As discussed above, lipids with characteristics typically associated with the other domain are certainly known, but the presence of both types in full appears to be extremely rare and narrowly distributed. Perhaps archaea and bacteria emerged from a complex LUCA population with a variety of lipid elements or, even less parsimoniously, one of the prokaryotic domains arose from within the other and for some reason needed to re-invent membrane lipids. Developing a second kind of lipids could have provided an ecological advantage (as per Figure 2A), but these scenarios put rather demanding evolutionary and chronological constraints not only on the divergence of archaea and bacteria from LUCA, but also on the diversification of both domains. A horizontal transfer at the base of the FCB superphylum would seem less taxing.

Further parsing of FCB and other genomes should allow to ascertain whether horizontal gene transfer played a role at the foundation of the group, or if the genes do indeed seem ancestral.

6. Bacterial Membrane Proteins in Archaea: Exceptions Could Prove the Rule

I have argued for the importance of the adaptation between membrane proteins and their native lipids. The presence of bacterial membrane proteins in archaeal lipids, such as bacteriorhodopsin in Haloarchaea, would thus seem to present a challenge.

Bacteriorhodopsin is crucial to the Haloarchaea: it is centrally involved in adenosine triphosphate (ATP) production and amounts to around 75% by mass and over 50% by surface of the "purple membranes" that it composes.[54] The protein granted the emerging

Haloarchaea the ability to exploit sunlight, which enabled them to colonize a previously unavailable niche in drastic differentiation from their methanogenic ancestors.[47]

The lipids may have remained archaeal simply because the advantage of having this and other imported proteins sufficed on its own, in spite of their predictably poor adaptation to the archaeal lipids at first. Indeed, membrane proteins are known to evolve faster than cytosolic proteins,[38,55] which in cases of horizontal transfers could reflect adaptation to the lipids in addition to the external environment.[38]

However, it has been implied that the genes for bacterial-lipid biosynthesis may have been concomitantly imported with those for bacteriorhodopsin.[47] If that was the case, a further reason Haloarchaea may have kept their archaeal lipids, despite the importance of bacteriorhodopsin, is that the predictably better efficiency of bacteriorhodopsin in its native lipids would have been outweighed by the cost of enforcing the bacterial lipids on the resident archaeal proteins (the opposite outcome to that in Figure 2A). Allowing the imported bacterial proteins to adapt to the archaeal lipids over time was preferred.

This in turn brings forth the question of the early evolution of eukaryotes, in which both archaeal and bacterial membrane lipids and proteins may have been present early on, but whose lipids are now typically bacterial.

7. Why Do Eukaryotes Have Bacterial Membrane Lipids?

If eukaryotes came to be from an association between bacterial endosymbionts[5] and an archaeal host,[6] this immediately raises the question of why they presently have bacterial-type lipids, not only in both mitochondrial membranes but also in the nucleus and all other organelles, as well as, notably, in the plasma membrane.

One possibility is that the archaeal host had purely bacterial-like lipids to begin with, instead of the traditional archaeal lipids. However, this would make the host unlike any archaeon known at present. No such case is known in bacteria either: the members of the FCB superphylum that carry genes to synthesize archaeal-like lipids retain those for the traditional bacterial lipids as well.[34]

A second possibility, perhaps more plausible in light of the recent findings in FCB bacteria, is that the host archaeon was able to synthesize the lipids of both types. Genes for making multiple elements of the bacterial lipid biosynthesis machinery have been detected in several of the Asgard-archaea genomes.[4,28] However, these do not branch together in phylogenetic trees with the homologous genes in eukaryotes.[4] Moreover, none of the known members of the (undoubtedly still undersampled) Asgard superphylum seem to have the ability to synthesize fully bacterial membrane lipids such as the ones that eukaryotes carry.

The origin of the distinctly bacterial eukaryotic lipids thus remains unresolved, but these recent phylogenetic analyses point to an early mitochondriate eukaryotic common ancestor that carried genes for both archaeal and bacterial lipid biosynthesis.

Lipids are known to mix across the multiple membranes of the eukaryotic cell,[56,57] so if the adaptation of membrane proteins to their lipids is indeed crucial, it is reasonable to predict that the inevitably heterotypic lipid membranes would have been disfavored after the acquisition of mitochondria. This was not necessarily because of instability of the mixed membranes themselves, but because of maladaptation of the membrane proteins to the mutually foreign lipids. There would have been an advantage to sticking to only one kind. But which? It is not immediately obvious why eukaryotes chose the bacterial lipids over the archaeal ones, whose genes were already in the (proto)nucleus and have now been replaced there with the bacterial analogs.

I suggest that the choice was due to the increasing reliance of eukaryotes on (proto)mitochondrial ATP production (among multiple other functions),[16] which is driven largely by a host of ancestrally bacterial membrane proteins closely associated with the bacterial-like membrane.

The discussion here notably leaves out the possibility of the so-called "three-domains tree," in which eukaryotes are sister to the archaea and all three domains are monophyletic in their origin.[58] Such a scenario would allow for a eukaryotic ancestor that, from the start, brought with it traits that we now associate with the archaea and the bacteria, including an archaeal-like information machinery but bacterial-like membrane lipids. This view is not without supporters,[3] but even allowing for a later mitochondrial endosymbiosis it would seem to be at odds with much of the evidence amassed in recent years.[7,8,11]

8. Conclusions and Outlook

Bacteria with archaeal lipids, whether natural or engineered, show that the two remarkably distinct types of lipids can coexist. This suggests that lipids themselves did not cause the divide that has seen all known archaea share one type of lipid, while almost all bacteria (and all eukaryotes) share a starkly different, if largely analogous, type. Something else must have driven the split.

Since membrane proteins play such crucial roles in the cell, from reproduction to bioenergetics to homeostasis, and amount to approximately one-third of protein-coding genes[59] and half of the volume of the membranes themselves,[35,54] I suggest that the driver was, and still is, their adaptation to and close interactions with the lipids.

A quick scan of the Protein Data Bank (PDB)[60] shows that only under 4% of structures in the database (many of which are determined using heterologous expression) are presently annotated as membrane proteins. There are multiple challenges associated with membrane-protein structure determination,[61] but the hypothesis here predicts that forcing membrane proteins into unfamiliar lipids should hinder their heterologous expression prior to structure determination. This seems to be the case even within the same domain,[62] suggesting that the principle put forward here extends beyond the divide between domains. This may in turn bear some relevance to why archaea have never picked up photosynthesis (a heavily membrane-associated process) or bacteria methanogenesis from CO_2 (a key enzyme of which is the ion gradient-genarating membrane-bound methyl transferase, Mtr).

Similarly, engineering archaea with bacterial proteins, or vice versa, should be easier with water-soluble than with

membrane-bound proteins, as should the transfer of protein-coding genes between the two domains (although this may be outweighed by the advantage of picking up genes in a new environment).[38]

In light of pervasive horizontal gene transfer across the prokaryotic domains,[63] I suggest that the lipid divide did not occur just once at the divergence of archaea and bacteria from LUCA. Instead, it has continued to occur actively over 4 billion years of evolution to the present. If lipids themselves are not causing the lipid divide, perhaps the proteins embedded in them are.

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archaea, bacteria, divergence, eukaryotes, evolution, horizontal gene transfer, last universal common ancestor (LUCA)

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