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Bacterial gene import and mesophilic adaptation in archaea

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Abstract

It is widely believed that the archaeal ancestor was hyperthermophilic, but during archaeal evolution, several lineages — including haloarchaea and their sister methanogens, the Thaumarchaeota, and the uncultured Marine Group II and Marine Group III Euryarchaeota (MGII/III) — independently adapted to lower temperatures. Recent phylogenomic studies suggest that the ancestors of these lineages were recipients of massive horizontal gene transfer from bacteria. Many of the acquired genes, which are often involved in metabolism and cell envelope biogenesis, were convergently acquired by distant mesophilic archaea. In this Opinion article, we explore the intriguing hypothesis that the import of these bacterial genes was crucial for the adaptation of archaea to mesophilic lifestyles.

Although the temperature at which the cenancestor (the last common ancestor of all extant organisms) lived continues to be a matter of controversy^{1–7}, the hyperthermophilic nature of the last common ancestor of archaea has seldom been challenged. Owing to their ability to grow in hot, acidic, anoxic and/or salty environments, archaea were originally thought to be extremophiles by nature; all of the deepest branches of the two archaeal groups that were known at the time archaea were discovered (the kingdoms Crenarchaeota and Euryarchaeota) corresponded to organisms that could thrive optimally at 80°C or above^{1,8,9}. Consequently, a hyperthermophilic archaeal ancestor seemed to be the most parsimonious explanation. With the advent of molecular tools to explore archaeal diversity in natural environments, mesophilic lineages were discovered in oceans and soils, notably Group I (recently renamed as the phylum Thaumarchaeota¹⁰), Marine Group II and Marine Group III (MGII/III; both of which belong to the phylum Euryarchaeota^{9,11–13}), and more recently, several less well-characterized mesophilic environmental lineages, such as the miscellaneous Crenarchaeota group (MCG) and marine benthic group D (MBG-D)^{14,15}. However, all of these new lineages have thermophilic ancestors or are nested in larger clades, the deepest branches of which are occupied by hyperthermophiles^{16–19}. Collectively,

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Supplementary information

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these findings suggest that all of the mesophilic archaea that are known have adapted secondarily and, in many cases, independently to mesophily from hyperthermophilic ancestors. These transitions to life at lower temperatures must have been paralleled by the acquisition of an array of adaptive molecular traits to enable nucleic acids, proteins and membrane lipids to function properly^{3,20–22} (BOX 1).

In the past few decades, it has become clear that horizontal gene transfer (HGT) is a major ongoing force in prokaryotic evolution²³ and that it has also shaped the origin and evolution of eukaryotes, mostly via endosymbiotic gene transfer²⁴. Pervasive HGT might, in principle, be considered an obstacle to retrieving meaningful vertical phylogenetic relationships among taxa^{23,25,26}; however, despite the phylogenetic ‘noise’ introduced by HGT, recent work shows that shared ancestral HGT events can be used to support the monophyly of specific organismal lineages^{23,27–29}. HGT contributes to innovation and adaptation to changing or new environments through the expansion of gene families and the import of genes encoding novel metabolic functions^{30,31}. Although novel functions acquired by HGT may be adaptive for environmental change, there is a trade-off owing to the inherent cost that accompanies the expression of a foreign gene. Organisms from closely related taxa are thought to exchange genes more easily because they have similar expression systems, are more likely to be compatible metabolically and often use similar mechanisms to adapt to their environment^{31,32}. By contrast, the risk of faulty expression is higher for foreign genes from distant lineages, and the gene itself may have a deleterious effect if, for example, it encodes a protein that needs to function within an autochthonous multiprotein complex²⁵. In such cases, only those genes that provide crucial selective advantages tend to be retained after ‘long-distance’ transfers²⁵.

One emblematic example of such a long-distance massive transfer that led to adaptation to a new lifestyle concerns the only two bacterial taxa that contain truly hyperthermophilic species: the orders Aquificales and Thermotogales. Initially, these taxa were thought to be the earliest branching bacterial lineages, and thus a hyperthermophilic ancestor for the bacterial domain seemed plausible. This hypothesis is consistent with the idea of a hyperthermophilic cenancestor that gave rise to the respective hyperthermophilic ancestors of bacteria and archaea¹, and also with more recent studies based on ancestral gene reconstruction that, despite inferring a mesophilic cenancestor, also infer thermophilic bacterial and archaeal ancestors⁵. However, the early branching phylogenetic position of these two bacterial lineages was later contested by other phylogenetic analyses that used more phylogenetic markers and better taxonomic sampling than the initial analyses; these independent results suggested that the bacterial ancestor and possibly also the cenancestor were mesophilic or moderately thermophilic⁴. The presence of a large number of genes that seemed to have been transferred from hyperthermophilic archaea to *Aquifex aeolicus*³³ and *Thermotoga maritima*^{34,35} subsequently led to the suggestion that the acquisition of these genes facilitated the secondary adaptation of these bacteria to hot environments³¹. One of the key genes that seems to have promoted such a transition is that encoding reverse gyrase, a type I topoisomerase with possible protective functions that introduces positive supercoils into DNA and is often considered a hallmark of hyperthermophily³⁶ (BOX 1). Thus, reverse

gyrase was most probably acquired by these two hyperthermophilic bacterial groups from archaea^{36,37}.

The idea that hyperthermophilic bacteria adapted to high temperature by acquiring genes from hyperthermophilic archaea raises the possibility that the opposite may also have occurred, such that archaea secondarily and independently adapted to mesophilic lifestyles by importing genes from bacteria. In this Opinion article, we explore recent phylogenomic data that suggest that this intriguing possibility did indeed occur, and we consider future studies that are needed to shed more light on this hypothesis.

Diversity and ecology of Archaea

At the onset of the 1990s, Carl R. Woese and colleagues formally segregated the Archaea as a third phylogenetic domain of life⁸ consisting of two kingdoms (now most often referred to as phyla): Crenarchaeota and Euryarchaeota. The kingdom Crenarchaeota comprised hyperthermophilic organisms only, which thrived in hot springs and deep-sea hydrothermal vents^{38,39}. Hyperthermophiles, which are defined as those organisms that grow optimally at 80 °C, had been discovered by Thomas D. Brock after his pioneering work on thermophilic bacteria that live at 50–75 °C (REF. 39). At the beginning of the 1970s, he also isolated several sulfur-oxidizing strains that grew at even higher temperatures (80–85 °C), which he placed in the new genus *Sulfolobus*⁴⁰. Initially thought to be bacteria, these *Sulfolobus* strains were soon affiliated with the newly defined Archaea domain, becoming the first members of the Crenarchaeota ever cultured in the laboratory. The Crenarchaeota now encompasses three orders of hyperthermophilic organisms (Sulfolobales, Thermoproteales and Desulfurococcales; FIG. 1a), several of which thrive optimally at >100 °C. Most of these organisms are strict anaerobic chemolithoautotrophs, although some are microaerophilic and heterotrophic, such as *Sulfolobus* spp.^{38,39}. Woese believed that the Crenarchaeota resembled the most ancestral phenotype of archaea, and he thus suggested a hyperthermophilic anaerobic chemolithoautotrophic ancestor for the Archaea domain⁸.

By contrast, the kingdom Euryarchaeota was much more diverse phenotypically and comprised not only hyperthermophilic lineages but also moderately thermophilic and mesophilic species^{8,38,39}. Most of the first described euryarchaeotal orders contained methanogenic organisms, which derive energy from the reduction of CO₂ to CH₄ using hydrogen under strict anaerobic conditions. Methanogenesis probably evolved only once, and with the potential exception of the Thermococcales (an early branching order of hyperthermophilic anaerobic heterotrophs that are abundant in deep-sea vents^{38,39}), all other known non-methanogenic members of the Euryarchaeota are likely to have evolved from methanogenic ancestors^{19,41}. Members of the Euryarchaeota that are non-methanogenic include three orders: the hyperthermophilic Archaeoglobales, some members of which produce methane in trace amounts; the thermoacidophilic Thermoplasmatales, which includes some genera that lack a cell wall (such as *Thermoplasma* and *Ferroplasma*) and are abundant in volcanic areas and acid mine drainage sites; and the halophilic Halobacteriales (referred to hereafter as haloarchaea)^{38,39}. Haloarchaea, which were among the first archaea to be isolated and characterized, are mesophilic organisms that live in environments where the salt concentration exceeds 150–200 g L⁻¹; they partially compensate for the high salt

levels with high intracellular K⁺ concentrations (up to 4 M). The remaining euryarchaeotal orders with cultured representatives are all methanogenic⁴¹, are adapted to a wide array of temperature and salt conditions, and are particularly widespread in anoxic sediments and in the gut of many metazoan species^{38,39}. Basal methanogenic orders (such as Methanobacteriales and Methanococcales) contain hyperthermophilic, thermophilic and mesophilic species, but late branching methanogenic orders (Methanosarcinales, Methanomicrobiales and Methanocellales) comprise mesophilic species only, some of which are halophilic. Recently, a new clade of methanogens has been proposed to constitute a seventh methanogenic order, Methanomassiliicoccales, which is related to the order Thermoplasmatales⁴².

In the early 1990s, the exploration of microbial diversity in the environment, using 16S rRNA gene amplicon sequencing, led to the discovery of seemingly mesophilic archaeal lineages (initially termed Group I–Group III) in marine plankton^{11–13}. Group I archaea branched with the classical Crenarchaeota and were found to be diverse and widespread not only in the oceans, where they are particularly abundant at high depth^{43–45}, but also in freshwater and soils^{46,47}. The isolation of the first culturable member of this group, the aerobic ammonia-oxidizing chemolithoautotroph *Nitrosopumilus maritimus*, from fish-tank sediment led to the discovery that Group I archaea have a major ecological role as nitrifiers in the global nitrogen cycle^{48,49}. Moreover, their distinct position in phylogenetic trees of ribosomal proteins suggested that they constitute an independent phylum; thus, members of the Group I Crenarchaeota are now referred to as the phylum Thaumarchaeota¹⁰. The occurrence of a separate phylum of mesophilic archaea initially raised the possibility that the archaeal ancestor was not a thermophile¹⁰; however, the discovery of early branching thaumarchaeotal lineages in hot springs and aquifers^{50–52}, as well as the monophyly of Thaumarchaeota with the deeper branching hyperthermophilic Aigarchaeota⁵³ and Korarchaeota⁵⁴ (all three of which, together with Crenarchaeota, form the ‘TACK’ superphylum^{16–18}, otherwise known as Proteoarchaeota¹⁹ (FIG. 1a)), strongly argues for a hyperthermophilic ancestry of the entire clade. Compared with Thaumarchaeota, the environmental MGII and MGIII archaea, which belong to the phylum Euryarchaeota and are found exclusively in the ocean, remain enigmatic. MGII occurs throughout the water column, and its abundance peaks in the photic zone^{43,44,55}, whereas MGIII is typically found in bathypelagic and abyssal zones^{13,45}. Although members of MGII and MGIII have not yet been cultured, metagenomic studies suggest that MGII archaea from the upper water column are proteorhodopsin-based photoheterotrophs^{56–58}, whereas those found in the deep sea seem to lack proteorhodopsin²⁸. MGII and MGIII archaea form a monophyletic clade (which we refer to hereafter as MGII/III) that also seems to have adapted secondarily to a mesophilic lifestyle; their closest (albeit highly divergent) cultured relatives are *Aciduliprofundum boonei* and the Thermoplasmatales, which are thermophiles with optimal growth temperatures of ~60 °C (REF. 38) (FIG. 1a).

Several other environmental archaeal lineages have since been discovered in ocean sediment and probably include mesophilic members. Among them, the more widespread and diverse are the MCG and MBG-D members^{14,15}, which branch within the TACK superphylum and the Euryarchaeota, respectively. In addition, a recent study including partial genomes from

single cells identified an apparently monophyletic clade of deeply branching archaea, the 'DPANN', which includes the phyla Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota and Nanohaloarchaeota. The DPANN archaea are hyperthermophilic (with the exception of Parvarchaeota and the halophilic Nanohaloarchaeota) and are very small cells that are probably parasitic or symbiotic⁵⁹. Although the deepest branches within the proposed DPANN lineage seem to be hyperthermophilic (which would argue in favour of a hyperthermophilic archaeal ancestor), the presence of fairly deeply branching mesophilic lineages might cast some doubt on the thermophilic nature of the DPANN ancestor and, hence, of the archaeal ancestor. However, the existence of the DPANN group as a natural clade has been questioned. The genomes of these archaea seem to be highly reduced and contain rapidly evolving sequences, which are prone to well-known artefacts that may affect phylogenetic reconstruction, especially the long-branch attraction artefact⁶⁰. The use of complex models of sequence evolution and more accurate methods of phylogenetic reconstruction show that the DPANN group is actually composed of various independent rapidly evolving lineages nested within the Euryarchaeota^{19,61,62}. Therefore, these lineages would not be informative about whether the archaeal ancestor was thermophilic or not.

The currently known diversity of archaea, the topology of the archaeal tree and the distribution of optimal growth temperature across lineages (FIG. 1a) together suggest not only that the last common archaeal ancestor was probably hyperthermophilic, but also that at least three major independent and ecologically successful adaptive events occurred to lead to the transition from a hyperthermophilic to a mesophilic lifestyle. These events involved the Thaumarchaeota, the MGII/III archaea, and the clade encompassing haloarchaea and the related mesophilic methanogens of the orders Methanosarcinales, Methanocellales and Methanomicrobiales. Independent adaptation to life at lower temperatures may have also occurred in two other methanogenic orders, both encompassing deeply branching hyperthermophilic species and several (Methanobacteriales) or a few (Methanococcales) mesophilic species.

Import of bacterial genes

Extensive bacteria-to-archaea gene transfer

Studies in the early 2000s^{63,64} suggested that interdomain gene transfer has occurred in haloarchaea, and this finding was recently confirmed on a genome-wide scale⁶⁵. This recent study revealed that ~1,000 bacterial genes were acquired by the methanogenic ancestor of haloarchaea, so the authors proposed that this massive gene transfer enabled a metabolic shift from anaerobic chemolithoautotrophic methanogenesis to aerobic heterotrophy (or bacteriorhodopsin-based photoheterotrophy). Furthermore, many bacterial genes were also identified in mesophilic methanogens of the orders Methanosarcinales, Methanocellales and Methanomicrobiales⁶⁵. Extensive HGT from bacteria to archaea has also been reported in some gut methanogens that belong to the order Methanobacteriales^{66,67}. These massive gene transfers from bacteria to archaea were recently confirmed by a larger analysis that included more than 267,000 protein-coding genes belonging to 134 sequenced archaeal genomes, and their homologues from 1,847 reference bacterial genomes²⁹. This latter study reports high levels of transfer of bacterial metabolic genes at the base of each of the 12 studied archaeal

orders (Thermoproteales, Desulfurococcales, Sulfolobales, Thermococcales, Methanobacteriales, Methanococcales, Thermoplasmatales, Archaeoglobales, Methanomicrobiales, Methanocellales, Methanosarcinales and Halobacteriales (haloarchaea)), suggesting that the acquisition of these metabolic functions were key innovations at the origin of these archaeal groups²⁹.

Similarly, phylogenetic analyses of two other archaeal groups (the Thaumarchaeota and the MGII/III euryarchaeotes) also suggest high levels of HGT from bacteria^{68,69}. This finding is consistent with BLAST-based comparisons of the first composite MGII euryarchaeote genome that was assembled using marine metagenomic sequences⁵⁷. The observed high levels of interdomain HGT in the Thaumarchaeota and the MGII/III euryarchaeotes were recently confirmed using pangenomes that were reconstructed from available reference genome sequences and marine metagenomic fosmid sequence data (corresponding to ~16 thaumarchaeotal and ~9 euryarchaeotal genomes)²⁸. The analysis suggests that ~25% and ~30% of the genes in the Thaumarchaeota and the MGII/III euryarchaeotes, respectively, were acquired from distant donors, mostly bacteria (90% of HGT cases; the rest of the genes was acquired from distant archaeal lineages or, in very few cases, eukaryotes), and about half of these genes (9–12%) are ancestral to the respective taxa.

Interdomain HGT seems to be not only extensive but also highly asymmetrical; apart from the transfer of archaeal genes to the bacterial orders Aquificales and Thermotogales^{33–35}, most interdomain HGT seems to have involved transfers from bacteria to archaea⁷⁰; indeed, transfers in this direction are estimated to have been at least five times more frequent than archaea-to-bacteria transfers²⁹. The reason for this asymmetry is unclear, although several potential causes have been suggested, including statistical bias (owing to the natural dominance of bacteria in most environments), mechanistic bias (such that foreign genes are more readily incorporated by archaea than by bacteria), selective bias (a potential lower fitness cost for archaea than for bacteria following gene transfer) or adaptive bias (in which the genes acquired by archaea are, for example, associated with a key metabolic advantage that enables the colonization of a new niche, whereas those acquired by bacteria are less likely to be, owing to the more widespread ecological distribution of bacteria)²⁸.

More ancestrally imported genes in mesophilic archaea

The idea that the import of bacterial genes might have paralleled the adaptation of archaea to mesophilic lifestyles was suggested a decade ago, after the analysis of a metagenomic fosmid clone from a marine thaumarchaeote revealed a high level of interdomain HGT⁶⁸. However, the generality of this observation was unclear because at that time the availability of genome sequences from diverse mesophilic archaea was limited. However, recent phylogenetic analyses of genes in genome sequences from distantly related archaeal groups^{28,29,65} enable this hypothesis to be tested. Indeed, the proportion of bacterial genes that are found in the ancestors of different archaeal taxa is considerable (FIG. 1b) and is negatively correlated with average optimal growth temperature (FIG. 2a). This observation is based on data from three studies^{28,29,65} that differ slightly in the methodology used: in the first study²⁸, the phylogenetic trees of the Thaumarchaeota and the MGII/III euryarchaeotes were inspected manually for classification into ancient (ancestral) and recent HGT events²⁸,

whereas in the second and third studies^{29,65}, a more automated procedure was used, in which any HGT that was shared by two different genomes in the same archaeal clade was considered to be ancestral. The latter approach may overestimate the true number of ancestral HGT events, as it includes in this category recent gene acquisitions that may, for example, occur in two closely related species in the same genus but be absent in the rest of the clade. To accommodate this possibility, we also consider the estimates proposed by this study²⁹ to be a reflection of all HGT events, both ancestral and recent, that occurred in the different archaeal lineages. Thus, the total number of all bacterial genes acquired by archaea (not limited to those genes that are considered ancestral) is also negatively correlated with average optimal growth temperature (FIG. 2a). Together, these findings suggest that mesophilic archaeal lineages have imported more genes from bacteria than hyperthermophilic archaea. Extensive import of foreign genes entails an increase in genome size; consistent with this, the number of genes acquired from bacteria seems to be positively correlated with archaeal genome size (FIG. 2b), such that the genomes of mesophilic taxa are, on average, larger than those of hyperthermophilic archaea. In fact, the observed high levels of bacterial gene acquisition in several archaeal groups supports predictions based on ancestral gene content inference, which suggest that gene gain in archaeal genomes is largely due to HGT¹⁸.

Convergent import of bacterial genes

We conducted a manual examination of the phylogenetic trees of imported bacterial genes in the three distant archaeal lineages that independently adapted to mesophily (the Thaumarchaeota, the MGII/III euryarchaeotes and haloarchaea; haloarchaea were chosen as a representative of the clade that they form with their sister methanogens, as they contain the most bacterial genes^{28,29,65}), and interestingly, this analysis reveals recurrent cases of convergent gene acquisition (FIG. 3). Most of these events preceded the diversification of the three groups, which suggests that the transfers are ancestral. For example, imported bacterial genes in haloarchaea represent up to 31% and 23% of ancestral genes imported by the Thaumarchaeota and the MGII/III euryarchaeotes, respectively²⁸. These percentages are based on ancient transfers (core genes) only, and the data for the number of core genes shared by these groups are provided in Supplementary information S1,S2 (figures). This overlap in imported genes might correspond either to the independent acquisition of the same gene from different bacterial donors by each of the three archaeal groups (FIG. 4a) or to transfer to one or two of these lineages followed by subsequent interarchaeal transfer (FIG. 4b; see Supplementary information S3 (figure)). Most of those genes found in all three groups are involved in energy conversion, amino acid transport and metabolism, and lipid or membrane biogenesis (see Supplementary information S1,S2 (figures) for specific details regarding the numbers of shared genes and the COG and KEGG functional classifications).

Convergent acquisition of the same bacterial genes by these three distantly related archaeal groups — either directly (from bacterial donors) or indirectly (by means of interarchaeal transfer) — highlights the important adaptive nature of such transfers at the origin of the three archaeal lineages the Thaumarchaeota, the MGII/III euryarchaeotes and haloarchaea. So what could be the common selective advantage underlying these convergent

acquisitions? The nature of the functions encoded by the transferred genes provides interesting clues.

Adapting to mesophilic lifestyles

In the case of haloarchaea, it has been proposed that the import of a large number of bacterial genes resulted in a metabolic advantage that enabled the transformation of strictly anaerobic chemolithoautotrophic methanogenic ancestors into oxygen-respiring heterotrophic haloarchaea⁶⁵. The hypothesis that the acquired genes reflect an adaptation to oxic conditions is consistent with the observation that many of the acquired genes shared by haloarchaea, the Thaumarchaeota and the MGII/III euryarchaeotes are related to oxygen respiration (including several electron transport chain and cytochrome *bc* complex genes)^{28,65}. However, the fact that late branching mesophilic methanogens independently acquired hundreds of bacterial genes^{29,65} but have remained strict anaerobes (so lack the genes involved in oxygen respiration) and are autotrophic (many thaumarchaeotes are also autotrophic⁴⁹) questions whether adaptation to an oxic environment is the only or the primary explanation. Is there a common characteristic among all of the archaeal lineages that have undergone massive import of bacterial genes?

The most common feature that is shared among these groups, some of which are anaerobic, autotrophic or both, is the ability to grow at lower temperatures than their hyperthermophilic ancestors. At lower temperatures, oxygen dissolves more efficiently, which makes it easily accessible, such that heterotrophy based on oxygen respiration is not only possible but is also a dominant mesophilic lifestyle. Therefore, the acquisition of genes for aerobic respiration certainly seems crucial for the ability to colonize many low-temperature environments. However, living at lower temperatures requires a variety of other molecular adaptations that favour the flexibility of nucleic acids, proteins and membranes (BOX 1). A closer look at specific HGT events reveals the transfer of some genes that are potentially involved in such adaptive mechanisms, such as the independent acquisition of DNA gyrase by the MGII euryarchaeotes and the ancestor of haloarchaea, including their sister groups⁷¹. This bacterial type II topoisomerase introduces negative supercoils to relax the positive supercoils generated during replication and transcription⁷²; its unwinding activity is one potential strategy for the adaptation of DNA-dependent processes to lower temperatures, and thereby has the opposite effect of reverse gyrase in hyperthermophiles³. Although thaumarchaeotes lack DNA gyrase, they do possess histones, and so they might rely solely on the strategy used by eukaryotes (which also lack DNA gyrase) to generate negative supercoiling, which involves the wrapping of DNA around nucleosomes (composed of histones) followed by relaxing of the DNA by topoisomerases⁷³. Many euryarchaeotes also possess histones, and the acquisition of DNA gyrase may have had a synergistic effect on DNA-dependent processes in these organisms, with accompanying changes in transcriptional patterns⁷¹, which may have further contributed to their bacteria-like adaptation to mesophily.

In terms of protein structure, genes encoding chaperones (which are involved in facilitating the correct folding of proteins) are abundant among the bacterial genes that are shared by the Thaumarchaeota, the MGII/III euryarchaeotes and haloarchaea^{28,65} (see Supplementary

information S1 (figure)). Transfer of the gene encoding the well-known chaperone heat shock protein 70 (Hsp70) from bacteria to a few archaeal groups was observed 15 years ago⁷⁴. Hsp70 is found in mesophilic archaea, including the Thaumarchaeota and haloarchaea, but thus far seems to be absent from hyperthermophilic archaea⁷⁵. A potential role in the adaptation to mesophily was recently proposed for the chaperone DnaK–Hsp70 system, its co-chaperone DnaJ and the nucleotide exchange factor GrpE⁷⁶, which are involved in the heat shock and general stress responses. Although the phylogenetic history of the encoding genes is complex, it has been proposed that the genes were acquired by archaea following multiple independent HGT events from bacteria (with some input from eukaryotes), followed by interarchaeal transfer⁷⁶. Homologues of the gene encoding cold shock protein A (CspA) represent another example of a gene that has been transferred on multiple occasions and has a potentially adaptive role in mesophily. This gene was transferred to the ancestor of haloarchaea and to either the Thaumarchaeota or the MGII/III euryarchaeotes, followed by intra-archaeal gene transfer between the two latter groups (see Supplementary information S3 (figure)). CspA seems to bind mRNA and regulate translation, the rate of mRNA degradation and the termination of transcription in bacteria⁷⁷.

A third type of adaptation to mesophily probably involved the acquisition of genes with roles in regulating membrane fluidity and transport across membranes, as most of the imported bacterial genes belong to these two functional categories^{28,29,65} (see Supplementary information S1,S2 (figures)). Membranes need to maintain a high permeability barrier and a liquid crystalline phase. Ether-linked isoprenoid phospholipids that are found in archaeal membranes support these requirements and are naturally more resistant to proton leakage than bacterial membranes²². Although monolayer tetraether lipids and their degree of cyclization were initially thought to be more stable adaptations to high temperature, as compared with diether lipids, tetraether lipids are also found in mesophilic archaea⁷⁸. By contrast, bacterial fatty acid ester lipids are stable only in mesophilic conditions, such that bacteria must adjust the composition of lipids in their membranes under thermophilic conditions, often mimicking archaea (by incorporating ether links, and monolayer tetraester and tetraether lipids, and by adjusting the fatty acid composition of the membrane)²². Some archaea include a proportion of ether fatty acid phospholipids in their membranes and, although the full biosynthetic pathway remains to be ascertained, several of these genes seem to have been acquired from bacteria by some archaeal groups^{79,80}. Consistent with this, bacterial proteins involved in fatty acid biosynthesis (such as enoyl-CoA hydratase, acyl-CoA dehydrogenase and 3-hydroxyacyl-CoA dehydrogenase) have been acquired by the Thaumarchaeota, the MGII/III euryarchaeotes (in agreement with previous suggestions⁵⁷) and haloarchaea^{28,29,65}. The import of genes encoding proteins that are part of complexes involved in energy generation at the membrane (such as the cytochrome *bc* complex) has been proposed to be dependent on the acquisition of genes involved in fatty acid biosynthesis⁸⁰. Whether the incorporation of fatty acids into archaeal membranes is required only for the function of cytochrome complexes or whether these fatty acids contribute to higher membrane flexibility is an intriguing question that remains to be explored. Changes in membrane lipid composition not only alter passive permeability, such as the passage of protons or water molecules, but also modify the local physicochemical environment of integral membrane proteins. This includes protein systems that are

responsible for the transport of a wide variety of metabolites across membranes, which must adapt to retain their specificity and efficiency (BOX 1). Thus, in addition to fatty acid synthesis, many transporter genes were convergently acquired by mesophilic archaea from bacteria, consistent with the idea that they need to adapt to their novel membrane environment and adjust solute specificity accordingly⁸¹.

Concluding remarks

Temperature is among the most important drivers of the composition of microbial communities⁸² and seems to be the major determinant of molecular evolutionary rates in archaea⁷⁵. The findings described in this Opinion article suggest that the hyperthermophilic archaeal ancestors adapted multiple times to mesophily and that most mesophilic archaeal taxa acquired many genes from bacteria^{28,29,65}. Interdomain gene transfer seems to have occurred preferentially from bacteria to archaea^{29,70}, except for the intriguing case of hyperthermophilic bacteria, which seem to have imported archaeal genes that probably contributed to their secondary adaptation to high temperature.

We have argued that the import of bacterial genes is likely to have contributed to the adaptation of divergent archaeal lineages to life at lower temperatures. But did this interdomain gene transfer actually trigger the adaptation to mesophilic lifestyles, or was it just an accompanying process resulting from the overabundance of mesophilic bacteria compared with thermophilic bacteria and the consequential increased probability for the transfer of genes associated with mesophily? Although it is difficult to differentiate between these two possibilities, the fact that many of the transferred genes encode proteins that regulate DNA topology (for example, DNA gyrase), protein folding (for example, Hsp70 and CspA) and membrane permeability (many transporters and, potentially, some fatty acid biosynthesis proteins), and also that these genes were independently acquired by the ancestors of different mesophilic archaeal lineages, does indeed suggest that these HGT events did promote adaptation to mesophily. It is possible that adaptive and non-adaptive HGT events became intertwined in a positive feedback loop, such that some archaea might have originally imported key adaptive genes that facilitated their colonization of cooler environments, and these archaea were subsequently exposed to a wide diversity and abundance of mesophilic bacteria, resulting in further HGT events that were not directly related to mesophilic adaptation. These more recent HGT events may have involved genes for energy metabolism (such as those involved in oxygen respiration) that might have provided selective advantages in lower-temperature environments, such as the use of novel resources (dissolved gases and organic compounds) and the colonization of new ecological niches. In other words, we suggest that hyperthermophilic archaea initially acquired a few bacterial genes that triggered adaptation to progressively lower temperatures, thus shifting their ecological preference to milder environments. Following this, archaeal mesophilic lifestyles expanded, as these lineages could import many more genes from the mesophilic bacteria that were present in the new environment. These additional imports could be considered 'opportunistic' (REF. 83) and did not necessarily have a role in adaptation to low temperature per se but resulted in further archaeal diversification and adaptation to a variety of mesophilic lifestyles.

Our hypothesis is based on current knowledge of archaeal diversity and the topology of the archaeal tree, which imply that the deepest archaeal branches correspond to hyperthermophilic organisms. This view is open to change should bona fide ancestral mesophilic lineages predating the divergence of hyperthermophilic clades on the archaeal tree be discovered, should the root of the archaeal tree be replaced among mesophilic lineages or should the genomes of newly discovered free-living mesophilic archaeal groups be shown to lack bacterial genes. Thus, our hypothesis that archaea adapted secondarily to mesophily by acquiring adaptive bacterial genes has some testable predictions. For instance, it would predict that newly discovered lineages of mesophilic archaea, such as the MCG or MBG archaea, would have higher levels of bacterial genes in their genomes than hyperthermophilic archaea and that a fraction of these genes would correspond to driver genes that are directly related to mechanical adaptations of the cellular components to lower temperatures. Future genomic and phylogenetic analyses of these novel archaeal clades will enable such hypotheses to be tested.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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BOX 1**Adaptation to high and low temperatures**

Life at both high and low temperatures requires specific molecular adaptations^{3,20–22}. As temperature increases, molecular motion intensifies; although this is beneficial for enzyme catalysis, hot conditions may lead to protein and nucleic acid denaturation and thermodegradation. Similarly, high temperatures may disrupt the integrity of the cell membrane and cause proton leakage, which impairs the proton motive force for energy transduction and alters the transport of solutes^{22,84}. Molecular adaptations to thermophily and hyperthermophily tend to counteract these deleterious effects by increasing molecular compaction and stability. Thus, hyperthermophiles typically possess DNA-binding proteins, specific topoisomerases and repair mechanisms that protect against DNA damage^{3,36,37,85}, and their structural RNAs are GC-rich⁵. They also possess more rigid membranes that contain membrane-spanning ether-linked phospholipids^{22,86}, less permeable Na⁺ (instead of H⁺) pumps⁸⁴ and high-affinity transporters⁸¹, as well as proteins that are smaller and have more intermolecular and intramolecular interactions via hydrophobic networks of ion pairs or hydrogen bonds^{85,87–89}. By contrast, low temperatures reduce molecular movement, which diminishes enzyme activity and dynamic cellular functions. Accordingly, several adaptations favour increased molecular motion to maintain the flexibility and fluidity that is compatible with biological activity^{20–22}. Thus, mesophiles and psychrophiles possess molecular mechanisms to unwind the double-stranded DNA helix (DNA gyrase^{3,72} or histones and relaxing type II topoisomerases⁷³), more-fluid membranes with more unsaturated and often branched lipids^{21,22}, and more-flexible proteins that have reduced hydrophobic cores, less charged surfaces, more lateral chains and, in the case of enzymes, higher substrate affinity (and they thus require less activation energy)^{21,87–90}. Specific chaperones and cold or heat shock proteins are present in both hyperthermophiles and mesophiles to facilitate protein folding and to prevent denaturation or inactivation^{68,75}.

An important adaptation of proteins to enable them to function at different temperatures involves adjusting the amino acid composition to include more hydrophobic residues and residues that have altered charge or polarity. These changes occur in transmembrane proteins, such as transporters, that need to adapt to different membrane environments⁹⁰. Changes in overall amino acid composition imply corresponding changes in codon bias; thus, temperature adaptation determines not only the proteome but also the composition of the genome⁸⁹. This is evidenced by the increase in amino acid and nucleotide substitution rates that paralleled the secondary adaptation of archaea to mesophilic lifestyles⁷⁵.

Figure 1. Growth temperature and horizontal gene transfer from bacteria to the archaeal lineages

a | Schematic representation of a Bayesian phylogenetic tree of the Archaea domain that is rooted by bacterial sequences, based on the concatenation of 32 ribosomal proteins and 38 conserved non-ribosomal proteins (including a total of 10,963 amino acid sites)¹⁹. The numbers shown in the branches are posterior probabilities and maximum likelihood bootstrap values¹⁹: all values at the branch points are 1/100, unless otherwise indicated. 1/- refers to bootstrap values that are lower than 50, which indicates that the node is fully

supported by posterior probability but unsupported by bootstrap analysis. The scale bar indicates the number of substitutions per alignment site. Median optimal growth temperatures for each of the different lineages are colour coded. For details of growth temperature values, see Supplementary information S4 (table). **b** | The number of bona fide archaeal genes and genes imported from bacteria, for different archaeal lineages^{28,29,65}. MGII/III, Marine Group II and Marine Group III euryarchaeotes; TACK, Thaumarchaeota–Aigarchaeota–Crenarchaeota–Korarchaeota.

Figure 2. Proportion of bacterial genes transferred to the ancestors of archaeal taxa as a function of average optimum growth temperature and genome size

Optimal average growth temperatures³⁸ and genome sizes for the different archaeal groups are given in Supplementary information S4 (table). The numbers of genes ancestrally acquired from bacteria by various archaeal groups are those published in previous work^{28,29,65}. The blue data points correspond to the proportion of horizontal gene transfer (HGT) events that are considered to be ancestral to the major archaeal taxa in REFS 29,65 and those that are ancestral in the Thaumarchaeota and ancestral to a lineage containing both Marine Group II and Marine Group III (MGII/III) euryarchaeotes in REF. 28. The orange

data points include all of the HGT events that occurred in the major archaeal taxa as described in REFS 29,65 but are interpreted here to include both ancestral and recent transfers (as the approach used in these studies^{29,65} may interpret some recent transfers as ancestral), together with the total number of ancestral and recent HGT events identified in the Thaumarchaeota and MGII/III euryarchaeotes in REF. 28. **a** | The acquisition of bacterial genes is negatively correlated with average optimal growth temperature in archaea. For the blue line, $R^2 = 0.618$ and $p = 6 \times 10^{-6}$ (in which R^2 is the coefficient of determination and p is the p value). For the orange line, $R^2 = 0.552$ and $p = 6 \times 10^{-6}$. **b** | The acquisition of bacterial genes is positively correlated with average archaeal genome size. For the blue curve, $R^2 = 0.463$ and $p = 0.38$. For the orange curve, $R^2 = 0.376$ and $p = 0.39$.

Figure 3. Imported bacterial genes shared by distant lineages of mesophilic archaea

The Venn diagram shows the number of genes that have been convergently acquired by the Thaumarchaeota, a lineage containing both Marine Group II and Marine Group III (MGII/III) euryarchaeotes, and haloarchaea. The diagram is based on previously published data and includes ancient and recent gene transfers^{28,29,65}; the number of genes belonging to the core (referring to ancient transfers) and shell (referring to more recent transfers) gene categories are shown in Supplementary information S1,S2 (figures).

Figure 4. Maximum likelihood phylogenetic trees showing cases of convergent bacterial gene acquisition by mesophilic archaea

a | A phylogenetic tree of riboflavin synthase subunit- α ; the encoding gene (*ribC*) was acquired independently by the ancestors of the Thaumarchaeota, a lineage containing both Marine Group II and Marine Group III (MGII/III) euryarchaeotes, and haloarchaea. The tree was reconstructed using 112 conserved amino acid positions. *ribC* has also been exchanged between thermophilic bacteria (*Thermus* spp. and *Thermosipho* spp.) and species in the order Thermococcales (*Thermococcus* spp. and *Pyrococcus* spp.), which shows that horizontal gene transfer (HGT) between distantly related organisms that coexist in the same habitat is possible. The two triangles for Gammaproteobacteria correspond to different

species of Gammaproteobacteria that do not cluster together when using this gene as phylogenetic marker. **b** | Phylogenetic tree of haem/copper-type cytochrome/quinol oxidase subunit 1; the encoding gene (*qoxB*) was acquired independently by the ancestor of MGII/III euryarchaeotes, and either the ancestor of the Thaumarchaeota or that of haloarchaea, followed by an internal archaeal transfer between the ancestors of the two latter groups. The tree was reconstructed using 439 conserved amino acid positions. Details about the phylogenetic reconstruction of each tree are provided in REF. 28 and in Supplementary information S5 (box). CFB, *Cytophaga–Flavobacterium–Bacteroides* (phylum Bacteroidetes).