The host-associated archaeome

Guillaume Borrel1, Jean-François Brugère2, Simonetta Gribaldo3, Ruth A. Schmitz4, 5 and Christine Moissl-Eichinger2, 4, 5, 9

Abstract | Host-associated microbial communities have an important role in shaping the health and fitness of plants and animals. Most studies have focused on the bacterial, fungal or viral communities, but often the archaeal component has been neglected. The archaeal community, the so-called archaeome, is now increasingly recognized as an important component of host-associated microbiomes. It is composed of various lineages, including mainly Methanobacteria and Methanomassiliicoccales (Euryarchaeota), as well as representatives of the Thaumarchaeota. Host–archaeome interactions have mostly been delineated from methanogenic archaea in the gastrointestinal tract, where they contribute to substantial methane production and are potentially also involved in disease-relevant processes. In this Review, we discuss the diversity and potential roles of the archaea associated with protists, plants and animals. We also present the current understanding of the archaeome in humans, the specific adaptations involved in interaction with the resident microbial community as well as with the host, and the roles of the archaeome in both health and disease.

Eukaryotes are inhabited by microorganisms, and there is increasing appreciation that this resident microbial community interacts with its host and influences host fitness and functionality. These communities likely evolved over millions of years of coexistence with their hosts, leading to the holobiont concept1, 2.

Most studies have focused on host-associated bacteria, but archaea have generally been neglected, despite the fact that they are also consistent members of the microbiomes associated with diverse hosts, including protists, plants, animals and humans. Specifically, we have known for almost half a century that methanogenic archaea thrive in the human gastrointestinal tract (GIT)3, and the first representative, Methanobrevibacter smithii, was isolated nearly 40 years ago4.

Archaea were originally discovered and isolated from extreme ecosystems, including volcanic environments, salt lakes and other biotopes that are characterized by extreme temperatures, pH values or ion concentrations. However, over the past decades, cultivation-independent studies have revealed that archaea are universally distributed and could be among the most abundant and active microorganisms in moderate environments such as the ocean water column5–11. The domain Archaea includes a vast diversity of lineages, some of which are mostly composed of uncultured representatives12, 13. These lineages are gathered into at least four large clades — namely the Euryarchaeota, the TACK (Thaumarchaeota, Aigarchaeota, Crenarchaeota, Korarchaeota) superphylum, the DPANN (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota) clade and the Asgard archaea (Fig. 1). The currently known host-associated archaea are phylogenetically diverse but are mostly composed of methanogens and, more recently discovered, Thaumarchaeota (Fig. 1). Today, research on the human archaeome (defined as the archaeal component of the human-associated microbial community) is still in its infancy. This is due to various reasons, including methodological issues resulting from the specific biology of archaea (BOX 1), that have also led to a general lack of knowledge about archaea in the microbiome research community. Recent studies have provided new insights into the human archaeome — including, for example, the discovery of novel host-associated lineages, such as the Methanomassiliicoccales, that might have a beneficial impact on human health14, 15; the discovery of archaea on the human skin and their possible link to age and skin physiology16, 17; and the finding that human archaea are recognized by the immune system and are involved in pro-inflammatory processes18, 19.

In addition, the development of specific archaea-targeting methods has updated the biogeography of the human archaeome and revealed previously undetected members20, 21. Furthermore, novel insights have also been gained into the role of archaea in plant physiology and the plant-specific profile of the archaeome22, as well as their association with animal skin23 and the GIT of primates24, which has fuelled debate on the archaeome’s host adaptation and co-evolution.

With novel methods in place, even if still imperfectly adapted to the archaeome (BOX 2), many fundamental questions about the contributions of archaea...
to the microbiome and to host physiology can now be addressed (see REFS 12–15).

In this Review, we aim to provide a comprehensive view of the current knowledge on the host-associated archaeome, in organisms including protists, plants, humans and other animals. In addition, we highlight the potential roles of archaea in human health and disease and explore the question of whether pathogenic archaea exist. Finally, we identify the knowledge gaps that remain to be addressed and advise on the next steps for research on the archaeome.

**Protist, plant and animal archaeomes**

The archaeome of anaerobic protists. Similarly to bacteria, archaea (namely members of the orders Methanobacteriales, Methanococcales, Methanosarcinales and possibly Methanomassiliicoccales) can live in the cytoplasm of anaerobic ciliates, amoebas and flagellates. These endosymbiotic methanogens interact closely with hydrogenosomes, which are the specific organelles of protists that generate H2 by oxidizing pyruvate and malate during carbohydrate degradation. Methanogens benefit from the H2 for methanogenesis, and in return they improve the energy degradation. Methanogens interact closely with hydrogenosomes, which are the specific organelles of protists that generate H2 by oxidizing pyruvate and malate during carbohydrate degradation. Methanogens benefit from the H2 for methanogenesis, and in return they improve the energy degradation. Methanogens live in the cytoplasm of anaerobic ciliates, amoebas and flagellates. These endosymbiotic methanogens interact closely with hydrogenosomes, which are the specific organelles of protists that generate H2 by oxidizing pyruvate and malate during carbohydrate degradation. Methanogens benefit from the H2 for methanogenesis, and in return they improve the energy degradation.
Reviews

Archaea also possess specific structural characteristics, such as unique membrane lipids (C\textsubscript{5} isoprenoid units ether-coupled to \(\alpha\)-glycerol at the (sn)-2,3 position\textsuperscript{160,161}). Their cell envelopes never contain bacterial-like peptidoglycan or lipopolysaccharides but can be composed of protein, pseudomurein or modified heteropolysaccharides. In some cases, even only single or double membranes without additional layers function as an outer shell\textsuperscript{162}. Consequently, archaea are not affected by antibiotics that target peptidoglycan, such as \(\beta\)-lactams, but are susceptible to antimicrobials that are also active against both bacteria and eukaryotes, such as metronidazole\textsuperscript{163}.

For motility, archaea use unique rotating flagella (‘archaella’) that are not homologous to those of bacteria and eukaryotes but instead are evolutionarily and structurally related to type IV pili\textsuperscript{173}. Archaeal surfaces can additionally bear pili or fimbriae, cannnulae, fibres or even grappling-hook-bearing hami\textsuperscript{174}. Many of these cell surface structures enable archaea to interact with surfaces, other (microbial) cells or viruses\textsuperscript{175}. Also, most archaea have a single membrane and lack the specific machineries that many Gram-negative bacteria use to deliver toxins into eukaryotic target cells, such as type III secretion systems\textsuperscript{176}.

Archaea can survive challenging conditions by switching to extreme slow growth or dormancy, but a capacity to form spores has not been observed to date. They are widespread in various ecosystems and include autotrophs, heterotrophs, phototrophs, chemotrophs, organotrophs and lithotrophs, as well as both aerobes and anaerobes. Archaea are considered to comply with chronic energy stress and are thus well adapted to nutrient-limiting ecological niches\textsuperscript{164}.

Some of the archaea-specific pathways correspond to functions that are also present in bacteria (sugar metabolism, CO\textsubscript{2} fixation and biosynthetic pathways) but that involve archaea-specific enzymes\textsuperscript{176,177}. This is exemplified by ammonia oxidation\textsuperscript{179}, a capacity with high ecological impact in marine and soil environments, whose pathway in archaea is distinct from the bacterial counterpart, owing to a lack of haem-based enzymes\textsuperscript{180,181}. Methane metabolisms (methanogenesis and methanotrophy) are widely distributed among archaea\textsuperscript{182,183} and involve a specific enzymatic complex, the methyl-coenzyme M reductase. Methanogenesis is a unique metabolic trait of archaea that results in methane production via four main pathways: two of them commonly use hydrogen as an electron donor (hydrogenotrophic pathways) — that is, CO\textsubscript{2}-reducing and methyl-reducing methanogenesis — whereas the other two do not require an external hydrogen source and include methylothrophic and acetoclastic methanogenesis\textsuperscript{184,185}. Methanogens are strict anaerobes and are widely distributed in various environments, such as freshwater and marine sediments, (wetland) soils and the digestive tracts of animals. Methanogenesis relies on a limited number of simple compounds (for example, H\textsubscript{2}, CO\textsubscript{2}, formate, methyl compounds and acetate) that are metabolic by-products of organic matter degradation in anoxic environments. By keeping H\textsubscript{2} concentrations low, methanogens enable secondary fermentation (for example, the utilization of volatile fatty acids, lactate and alcohol) to remain thermodynamically favourable, and they increase the energy yield of primary fermenters using complex molecules such as carbohydrates\textsuperscript{186}. Thus, by contributing to the overall efficiency of energy retrieval during the digestion of organic matter, methanogens are considered to represent keystone microorganisms in anoxic ecosystems. The contribution of methanogenesis to climate change is being observed with concern, as most biologically produced atmospheric methane originates from archaeal metabolism (~69%; REF\textsuperscript{187}). Methanotrophic archaea play an important role in the mitigation of methane emissions to the atmosphere, particularly from marine sediments. In contrast to bacterial methanotrophy, the archaean pathway relies on the reverse use of enzymes involved in methanogenesis. Moreover, some archaea couple methane oxidation with the direct reduction of electron acceptors not used by bacteria — such as iron and manganese oxides, nitrate or humic acids\textsuperscript{188–191} — or with indirect electron transfer to sulfur compounds via sulfate-reducing bacteria\textsuperscript{192}.

For entries denoted as ‘Yes’, the specific trait is present in either all or some members.

| Feature                     | Bacteria                        | Archaea                     | Eukaryotes            |
|-----------------------------|--------------------------------|-----------------------------|-----------------------|
| Nucleus                     | No                             | No                          | Yes                   |
| Organelles                  | No                             | No                          | Yes                   |
| Spliceosomal introns        | No                             | No                          | Yes                   |
| Chromosome shape            | Circular and linear            | Circular                    | Linear                |
| Operons                     | Yes                            | Yes                         | Rare                  |
| RNA polymerase              | Bacteria-like                  | Eukaryote-like              | Eukaryote-like        |
| DNA polymerase              | Bacteria-like                  | Eukaryote-like              | Eukaryote-like        |
| Ribosome type               | 70S                            | 70S                         | 80S                   |
| Translation start (amino acid) | Formylmethionine                | Methionine                  | Methionine            |
| Histones                    | No                             | Yes                         | Yes                   |
| Peptidoglycan (PG)          | Yes                            | No; pseudo-PG in some       | No                    |
| Motility                    | Bacteria-type flagellum        | Archaea-type flagellum      | Eukarya-type flagellum|
| Lipopolysaccharide          | Yes                            | No                          | No                    |
| Membrane lipids             | Ester links (glycerol-1-phosphate backbone) | Ether links (glycerol-3-phosphate backbone) | Ester links (glycerol-1-phosphate backbone) |
| Methanogenesis              | No                             | Yes                         | No                    |
| Oxidogenic photosynthesis   | Yes                            | No                          | Yes                   |
| Spores                      | Yes                            | No                          | Yes                   |
| Human pathogenicity         | Yes                            | No                          | Yes                   |
**Pseudogenization**
Conversion of a gene into a nonfunctional gene-like sequence in a symbiotic relationship.

**Rhizosphere**
Soil area around a plant root, influenced by root exudates and inhabited by a specific population of microorganisms.

**Endosphere**
Internal regions of plant tissues, which are inhabited by endophytic microorganisms.

**Phyllosphere**
All above-ground parts of plants, serving as habitat for free-living microorganisms.

different methanogens. Moreover, recent genomic analyses of endosymbiotic archaea have revealed only a few differences from their free-living counterparts, but the nature of these variations seems partly similar between evolutionarily distant endosymbiotic archaea, which suggests convergent adaptations to this lifestyle. In addition, the high level of genes undergoing pseudogenization in these genomes probably indicates a recent and still ongoing process of adaptation. Together, these studies suggest that endosymbiotic archaea form stable associations with their host, probably at the strain level, but are periodically replaced by a novel archaeal symbiont. However, most of these observations derive from associations between archaea and free-living protists, and the question still remains open for host-associated protists.

**The plant archaeome.** Microbial communities in plants have an essential role, as they can affect plant growth, productivity, adaptation, diversification and health. Overall, archaea are distributed differently in the rhizosphere, endosphere and phyllosphere. Microniche differentiation is supported by their competition with bacteria and fungi, as well as by abiotic factors, including nutrient availability and exposure in the phyllosphere, the presence of root exudates in the rhizosphere and the more stable conditions in the soil. For the widespread leafy green plant arugula (garden rocket), the diversity of archaea was found to be lowest in the phyllosphere, which indicates unfavourable habitat conditions, whereas the diversity of archaea in the soil and rhizosphere was much richer, so this may be the preferred habitat.

However, knowledge on the interaction of archaea and plants is very restricted and is based on a few specific types of plant–archaeome interactions. The most prominent example of plant-associated archaea is the methanogens residing in the anaerobic rhizosphere of rice in oxygen-depleted wetlands, mostly represented by Methanocellales, Methanosetaeaceae and Methanoregulaceae. A large part of the methanogen produced in the rice rhizosphere is derived from the breakdown of organic compounds produced by the plant, and the methane primarily escapes to the atmosphere via the plant gas vascular system, thus bypassing bacterial aerobic methanotrophs. Although methane emission in rice fields is a subset of the overall plant-mediated methane emission in wetlands, it is responsible for 10% of the global budget of atmospheric methane.

Signatures of ammonia-oxidizing Thaumarchaeota were also found to be abundant in the leaves of...
Mediterranean olive trees, which reveals cultivar-specific abundance patterns. Similar observations were made in tomato plants, where the abundance of the archaeal community (Thaumarchaeota (60%), Methanosarcina (12.6%), Methanoculleus (3.4%); Fig. 3) was found to be dependent on plant genotype and habitat. Notably, the archaeal abundance and diversity were comparably low in seeds, and no indication of plant-mediated vertical transmission of archaeal microbiome components was detected.

Plants in alpine bogs harbour a substantial archaeal community that is composed of 60 different genera. Notably, metagenomic analyses revealed potential archaeal functions in, for example, the promotion of plant growth through auxin biosynthesis, nutrient supply, and protection against oxidative and osmotic stress. Additional genetic capacities for CO₂ and N₂ fixation were also observed. Similar functions were reported for arugula, with mainly Thaumarchaeota and Euryarchaeota being detectable and visible in both the rhizosphere and phyllosphere. In particular, ‘Candidatus Nitrosocosmicus’ (Thaumarchaeota) seems to be involved in positive interaction with plants, as Nitrosocosmicus oleophilus MY3 was found to colonize the root surfaces of Arabidopsis thaliana plants and to trigger systemic resistance against the plant pathogens Pectobacterium carotovorum subsp. carotovorum SCC1 and Pseudomonas syringae pv. tomato DC3000 (Ref. 70).

The animal archaeome. Animals with known symbiotic associations with archaea include sponges, insects and vertebrates. The first representative genome of Thaumarchaeota, namely ‘Candidatus Cenarchaeum symbiosum’, was retrieved from a marine sponge, which lives in close symbiosis with its archaeal inhabitant. In some cases, Thaumarchaeota even dominate the microbial communities associated with sponges. It has been suggested that these archaea might remove the host’s nitrogenous waste products and, in turn, provide carbon to the host.

Methanogenic archaea, and in particular Methanobrevibacter species, are extraordinarily well-adapted to interact with animal hosts and non-archaeal components of their microbiomes. Through their oxidation of pyruvate (Pyr), the methane is emitted mainly by the eructation of cattle and by flatulence. The figure shows a comparison of the global methane emission rates of cattle, termites, sheep, goats, pigs (considering one billion pigs worldwide, according to the UN Food and Agriculture Organization (FAO)), humans and chickens (considering 23 billion chickens worldwide, according to the FAO), in billions of tons per year (btpa). The methane levels released into the atmosphere from cattle are the highest among all livestock and represent one of the largest sources of anthropogenic methane emissions. MeOH, methanol; TMA, trimethylamine.
consumption of various small fermentation end products, *Methanobrevibacter* species are flexible supporters of syntrophic interactions. *Methanobrevibacter* species are the predominant archaea in the GITs of various ruminants and non-ruminants, including cattle, yaks, sheep, reindeer, goats, buffalo, deer, pigs, wallabies, rhinoceroses, chickens, iguanas, termites and many other species (see REF. 23) (FIGS 2,3). Other methanogenic archaea (*Methanosphaeroides*, *Methanosarcina*, *Methanomassiliicoccus* and *Methanomicroccocus* species) have also been identified in various animals (for example, in cattle, sheep, goats, deer, horses, pigs, kangaroos, rhinoceroses, hoatzins, iguanas and termites), but they are usually less abundant (FIG. 4). Owing to the resulting massive methane production and impact on global warming (FIG. 2), methanogenic archaea–ruminant and archaea–termite symbiosis has been a subject of active research. It has been estimated that a single cow emits up to 7001 of methane per day24 (on average, 150.7 g per day; see REF. 25), and these studies have also aimed to reduce methane emissions26. In addition, the activity of methanogenic archaea negatively affects the weight gain and efficiency of feeding, so that a reduction of methanogenic archaea negatively affects the weight of animals and non-archaea (particularly *Halococcus*; FIG. 4) in animals and plants27, raises many questions about the origin and types of interaction of these archaea28, particularly as it has been proposed that in humans, halophilic archaea could be contaminants from salted food29.

This is also true for the unclassified ‘*Candidatus Woesearchaeota*’ (DPANN), which has also been identified in human samples, mainly from the respiratory tract30,31. The function of Woesearchaeota in the environment remains elusive, but owing to metabolic deficiencies, a probable dependency on syntrophic microorganisms has been discussed32; to date, details about their potential role in the human body are completely unknown.

**The human archaeome**

The human microbiome carries numerous archaea, in particular on the skin, in the respiratory tract and in the GIT. Whereas the specific role of non-methanogenic archaea in the human body remains to be explored, methanogens maintain numerous syntrophic relationships with resident bacteria. Owing to their dependence on bacterial metabolic activity for the availability of their own substrate, methanogens could be indicators of the microbiome status per se33,34,35. Despite our limited knowledge of host interactions, both archaeal genomes and experiments with cultured methanogen representatives have revealed a profound adaptation strategy to the human GIT.

**Presence, abundance and activity of archaea in the human microbiome.** A substantial component of the human microbiome consists of archaea from a wide diversity of lineages, including Methanobacteriales, Methanomassiliicoccales, Methanomicrobiales, Methanosarcinales, Halobacteriales, Thaumarchaeota (Nitrososphaerina) and members of the DPANN clade36,37,38 (FIGS 1,3). In the GIT, the most prevalent and abundant archaea are representatives of the Methanobacteriales and the Methanomassiliicoccales39. Methanobacteriales are mainly represented by two species — namely *Methanobrevibacter smithii* and *Methanosphaeroides stadtmanae*, with prevalences of up to 97.5% and 23%, respectively40. Human gut-associated Methanomassiliicoccales consist of at least nine species, the most common of which are ‘*Candidatus Methanomassiliicoccus intestinalis*’, ‘*Candidatus Methanomethylphilus alvus*’, Mx-02, Mx-03 and Mx-06,
archaea in several human populations\textsuperscript{41,62}. Whether this increase is due to host physiological changes, such as prolonged gastrointestinal transit time with age, or to multiple acquisitions during the human lifetime remains unclear. Another factor is host genetics, as the abundance of \textit{M. smithii} has been found to be more similar between monozygotic twins than between dizygotic twins\textsuperscript{47,48}, and to correlate positively with a single-nucleotide polymorphism in a long non-coding RNA of the human genome\textsuperscript{46}. However, the presence and activity of methanogens is also associated with non-archaeal members of the host microbiome. For instance, the prevalence of Methanomassiliicoccales, which use H\textsubscript{2} to reduce trimethylamine (TMA) for methanogenesis, was found to correlate positively with the number of different TMA-producing pathways present in the bacterial microbiome\textsuperscript{45}. Similarly, a correlation of \textit{M. smithii} with certain bacterial taxa from the Firmicutes was noted. In particular, the abundance of \textit{Christensenellaceae}, representing a highly heritable clade\textsuperscript{89,102}, was found to be positively correlated with the abundance of \textit{Methanobrevibacter Christensenella} representatives support \textit{M. smithii} through efficient H\textsubscript{2} transfer via close physical interactions, as was shown in co-culture experiments with \textit{Christensenella minuta}\textsuperscript{103}. In these experiments, the hydrogen consumption by \textit{M. smithii} shifted the \textit{C. minuta} metabolism towards acetate production rather than butyrate production, an effect that was less pronounced with \textit{Bacteroides thetaiotaomicron}, a taxon not correlated with \textit{M. smithii} abundance in the human gut. Interestingly, non-bacterial microbiome members such as \textit{Candida} fungi were also found to co-occur with \textit{M. smithii}, which suggests additional syntrophic relationships\textsuperscript{40,41}.

Besides methanogens, Halobacteria (for example, \textit{Halofex massiliense}) have been detected and isolated from human stool samples, including from patients suffering from inflammatory bowel disease\textsuperscript{63,67,79,102,103}. However, the impact of halophilic archaea on both the human microbiome and the host remains unclear, and their presence has been discussed as possibly being transient and associated with the consumption of salt-containing food products\textsuperscript{104}.

The overwhelming majority of studies on human-associated archaea have been conducted on stool samples, and knowledge about specific body sites is still sparse. It seems likely that the GIT contains a larger diversity of archaea than has been identified from stool samples, as signatures of Methanomicrobioales, Methanobacterium and DPANN have been reported in biopsy samples but not in the stool\textsuperscript{20,92}. Moreover, similar to bacteria, human-associated archaeal communities group on the basis of body location, with Thaumarchaeota signatures predominating on the skin, methanogens in the GIT, a mixed Thaumarchaeota or methanogen landscape for the upper respiratory tract, and DPANN (Woesearchaeota) in the lung\textsuperscript{50}. On the skin, two studies have revealed that archaea generally represent up to 1% of the microbiota\textsuperscript{41,117}. Interestingly, the positive correlation between age and the abundance of archaea observed for the gut also holds true for the skin\textsuperscript{7}.

\textbf{Fig. 3} | \textbf{Archaeal taxa detected in human, other animal and plant samples.} To create evolutionary trees, 165 rRNA gene sequences from isolated strains, publicly available clone sequences (for example, \textit{Saccharolectus}) and amplicon-based studies of humans\textsuperscript{15}, other animals\textsuperscript{11,12} and plants\textsuperscript{13,14,15} were quality-filtered (no singletons, length > 100 bp, alignment score > 10, alignment identity > 40%), grouped at 57% similarity and processed through SILVA SINA classification\textsuperscript{169}. Trees were calculated via RAxML, on the backbone of three neighbour sequences per query that were used to stabilize the tree (‘add to neighbours tree’ option; neighbour representatives are shown in the tree with an unlabelled node) (for a detailed overview, please see the supplementary figures). For the human archaeome tree (top panel), lineages found in only one publication are not shown; this filtering was not applied for the animal archaeome tree (bottom left panel) or the plant archaeome tree (bottom right panel), owing to the small number of available studies. The output was completed with meta-information (sample origin and isolate) using iTOL\textsuperscript{169}. Thaumarchaeota (corresponding to \textit{Nitrososphaeria}, in shades of orange), Woesearchaeota (in very soft red), Halobacteria (in shades of grey), Methanomicrobiales (in shades of red), Methanococcales (in shades of dark blue), Methanosarcinales (in shades of blue), Methanomassiliicoccales (in shades of purple) and Methanobacterales (in shades of green) were found in all groups in different sample types — that is, oral cavity, respiratory tract, gastrointestinal tract (GIT) (including faeces, gut biopsies and rumen samples) and skin samples, as well as green plant and/or seed samples — as indicated by the circles outside each tree, which are linked to the individual archaeal representatives.
Adaptations to the human host and interaction with the immune system. As we discussed above, the archaea in the human gut are generally dominated by a few specific taxa (Fig. 3). These taxa are rarely reported from environments outside of the animal GIT, which suggests a high degree of specialized adaptation. Such adaptations are mirrored by a number of specific traits that differentiate archaea residing in the GIT from free-living ones. These traits include, for example, modifications of the cell surface (for improved adhesion and biofilm formation) and the possession of bile salt hydrolases to defeat the host’s defences (Fig. 5).

M. smithii, M. stadtmanae and host-associated Methanomassiliicoccales encode a large number of membrane-bound adhesin-like proteins (ALPs) that have been suggested to be involved in binding to different host sites and syntrophic commensal bacteria. Because the expression levels of M. smithii ALPs are influenced by environmental conditions (for example, the presence of bacteria or substrate availability), it has been suggested that M. smithii has a high ability to colonize different microniches in the gut. It could be hypothesized that the specific physical interaction of M. smithii with C. minuta is promoted by some of these ALPs. Methanobacterales and Methanomassiliicoccales ALPs comprise different protein domains, possibly indicating different niche adaptations and evolutionary origins. Phylogenetic approaches have revealed that several of the M. smithii ALPs were probably acquired through horizontal gene transfer (HGT). Methanomassiliicoccales probably also acquired their ALPs via HGT, as the type they possess has not been found in other archaea but is present in high numbers in several gut-associated bacteria.

Besides ALPs, diverse glycosyltransferases seem to have been acquired via HGT in Methanobrevibacter and Methanosphaera species. These glycosyltransferases could account for a modification of polysaccharides present at the cell surface, which could improve adherence to both abiotic and biotic surfaces. Supporting this observation, M. stadtmanae cells strongly adhere to human immune and epithelial cells and easily aggregate into biofilm structures, most probably due to the secretion of extracellular polysaccharides. Incidentally, oral biofilms were reported to contain Methanobrevibacter oralis (in at least every second patient suffering from periodontal disease) and members of the Methanomassiliicoccus genus.

Bile acids are important regulators of the human microbiome and exert a strong selective pressure on the microbial population. Microorganisms have developed strategies to counteract bile toxicity via bile salt hydrolases (BSHs), which are also important for secondary bile acid synthesis. Similar to various bacteria, several gut methanogens — M. smithii, M. stadtmanae and ‘Ca. M. alvus’ — could detoxify this molecule using BSH. Methanobacterales and Methanomassiliicoccales BSHs are distantly related and could have been acquired via independent HGT events, probably from Firmicutes, in the case of Methanobacterales. M. oralis (present mainly in the mouth) and Methanomassiliicoccales from an environmental clade lack BSH, an observation that supports the acquisition of this enzyme due to a very specific adaptation to the intestine.

The innate immune system is the very first line of host defence against microorganisms, through methods including the production and release of anti-bacterial compounds such as antimicrobial peptides (AMP), along with cytokines. These are excreted by epithelial cells right after the recognition of microorganism-associated molecular patterns of bacteria, such as flagellins, peptidoglycan and lipopolysaccharides. Although they are probably not directly the target, human archaea are exposed to the various AMPs secreted by the host to control the bacterial microbiota. Notably, susceptibilities against AMPs were found to be substantially
different among mucosa-associated methanogenic strains\textsuperscript{19,114}, with pseudomurein-containing archaea, such as \textit{M. stadtmanae}, being more resistant against the lytic effects of AMPs than, for example, members of the Methanosarcinales or Methanomassiliicoccales.

In recent years it has clearly been shown that archaea interact with and activate the human immune system. The activation of human immune cells, as well as pro-inflammatory cytokine responses by peripheral blood mononuclear cells and by monocyte-derived dendritic cells, initiated by phagocytosis and endosomal lysis, has been demonstrated\textsuperscript{14}. A strong response — that is, high release of pro-inflammatory cytokines, including interleukins as well as interferons — was exclusively observed when stimulating the immune system with \textit{M. stadtmanae} cells\textsuperscript{18,113}. Interestingly, the other two GIT archaeal isolates tested, \textit{M. smithii} and \textit{Methanomassiliicococcusc luminyiensis}, showed only mild responses, if any at all\textsuperscript{14,17}. As \textit{M. smithii} has the capacity to produce glycans that mimic those found in the human gut\textsuperscript{18}, it is attractive to hypothesize that those host-like glycans enable \textit{M. smithii} to escape from the host immune system. Although these studies demonstrated not only an innate but also an adaptive immune response by human immune cells in response to \textit{M. stadtmanae}, initially no specific receptor involved was identified. Only recently was it demonstrated that RNA from \textit{M. stadtmanae} is a potent immune stimulator, and Toll-like receptor (TLR) 7 and TLR8 were identified as the involved pattern recognition receptors\textsuperscript{16}. Moreover, this molecular interaction led to TLR8-dependent triggering of the NLRP3 inflammasome, in a new and alternative path of inflammasome activation. To the best of our knowledge, archaea do not trigger any other innate immune receptor and thus may be unique among microbial stimulators in triggering RNA-dependent signalling only. Hence, this TLR8-dependent alternative inflammasome activation may be archaea specific.

\textbf{Archaea in human health and disease.} By using the indirect detection of methanogenic activity via methane breath tests, possible correlations between the occurrence of methanogens in the human GIT and various diseases have been analysed since the late 1970s\textsuperscript{19}. Since then, the relationship of methanogen abundance (found through cultivation-based, quantitative PCR (qPCR)-based and next-generation sequencing (NGS)-based analyses) or breath methane content with disease has been assessed in various (gastrointestinal) diseases and physiological states of the host. These include colon cancer, diverticulosis, diabetes, obesity and anorexia, inflammatory bowel diseases and many other conditions (summarized in Refs\textsuperscript{26,35,118}). However, due to the above-mentioned methodological issues related to bacteria-centric methods or other pitfalls (BOX 2), the available information is contradictory, and the involvement of archaea in human health and disease often remains blurry\textsuperscript{31,35,118}.

When subject to dysbiosis or infection, several sites of the human body are known to present a higher prevalence and abundance of methanogenic archaea. For example, \textit{M. smithii} was reported in the vagina only in patients suffering from vaginosis and was absent in healthy individuals\textsuperscript{19,122}. Moreover, \textit{M. smithii} was found in individuals with muscle abscesses\textsuperscript{104}, pneumonia\textsuperscript{112} and urinary tract infections\textsuperscript{121}, and together with \textit{M. oralis} in patients with refractory sinusitis\textsuperscript{123}. \textit{M. oralis} was also reported in brain abscesses\textsuperscript{15,124} and has been associated with periodontitis\textsuperscript{119,120} or peri-implantitis\textsuperscript{105}. Interestingly, \textit{M. oralis} is more prevalent and abundant in severe periodontitis, but it was not detected in healthy sites adjacent to periodontal pockets and was no longer present after healing, which highlights the specific association of \textit{M. oralis} with the inflamed site\textsuperscript{120}. All these dysbioses and infections consist in a strong increase or de novo colonization by anaerobic fermentative bacteria. This shift to anaerobic fermentative bacteria is accompanied by an increase of archaea, which can reach a high proportion of the whole microbial community.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
\textbf{Enzymes that catalyse the transfer of glycosyl (sugar) residues to an acceptor molecule} & \\
\hline
\hline
\textbf{Microorganism-associated molecular patterns} & The conserved molecules characteristic of microbes, which are recognized by the immune system. \\
\hline
\end{tabular}
\caption{Microbial ligands.}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5.png}
\caption{Interaction of the gastrointestinal archaeome with the host and the bacterial microbial community. The host provides a stable biotope, including nutrition, to the archaeal community and regulates the composition of the microbial community as a whole through antimicrobial peptides (AMPs) and bile acids. In addition, the host has been shown to release pro-inflammatory cytokines in response to some archaeal components. Archaea in the human gut may exhibit a high degree of specialized adaptation, which is mirrored by a number of specific traits, such as reduced and/or adapted physiological capacity (not shown), as well as defence and attachment mechanisms (adhesin-like proteins (ALPs), glycans, bile salt hydrolases and biofilm formation). Methanogenic archaea produce methane, a potential neuromodulator and immunomodulator (see the main text), which is excreted by the host, contributing to global methane emissions (see FIG. 2) and affecting human physiology, such as gut motility. The host-associated bacteriome provides substrates for the archaeome (including formate, trimethylamine (TMA), methanol, H\textsubscript{2} and CO\textsubscript{2}). Moreover, the bacteriome may be a source of genetic material for archaeal members of the microbiome via horizontal gene transfer, allowing the acquisition of such mechanisms as ALPs, glycosyltransferases and bile salt hydrolases. Archaeal methanogenesis benefits the bacteriome by maintaining a low H\textsubscript{2} concentration and so improving energy gain. The interactions of the bacteriome with the host are not shown in this simplified schematic.}
\end{figure}
— namely, up to ~25% in brain abscesses and 18% in severe periodontitis. Although methanogens are never the only microorganisms present at these infected sites, they are likely to promote the outgrowth of fermentative bacteria involved in inflammation by lowering H₂ concentrations. In fact, other hydrogen-consuming microorganisms, such as sulfate-reducing bacteria, show an increased abundance in severe periodontitis and could fulfill the same role as methanogens. Thus, methanogenic archaea might participate in such polymicrobial diseases through syntrophic interactions, representing one component of a “unit of pathogenicity” in addition to bacterial partners. Interactions of methanogenic archaea with pathogenic bacteria could actually occur in various diseases and not be limited to syntrophic partnerships. Indeed, a recent genome survey revealed that more than 200 pathogens have genes involved in hydrogen consumption or production. This suggests a potential dual role for methanogens, as syntrophic partners of fermentative pathogens and as potential competitors with hydrogenotrophic pathogens.

Beyond this indirect role, it is unknown whether archaea can be directly involved in inflammation at these infected sites. Some indications exist for M. smithii, as so far it has only been detected in inflamed areas. In this respect, it has also been shown that the pro-inflammatory potential of archaea varies among species — for example, M. stadtmanae triggers a stronger immune response than either M. smithii or M. luminyensis using monocyte-derived dendritic cells. Moreover, increased abundance of M. stadtmanae was found to correlate frequently with disease and inflammation, in particular in inflammatory bowel disease. In combination with its observed high pro-inflammatory potential, the activation of the inflammasome and the strong B cell and T cell responses within draining lymph nodes due to M. stadtmanae entering the bloodstream suggest its potential involvement in the development and/or manifestation of disease. By contrast, a recent publication showed an association between M. stadtmanae presence and a lower risk of asthma in young children, which is indicative of a beneficial role for M. stadtmanae.

Besides the direct interaction of archaeal cells and the immune system, the gaseous product of methanogenic archaea, methane, could in itself have a physiological effect on the host, as indicated by a growing number of recent studies. For example, a direct influence of methane on gut motility was shown on dogs, by slowing intestinal transit by up to 59%. This slowdown is possibly caused by a direct action of methane on the cholinergic pathway of the enteric nervous system, possibly also explaining the association of methanogens with constipation. Constipation, or slower intestinal transit, also eases the colonization of microorganisms with longer generation times, such as the preferred syntrophic partners of methanogens (for example, Clostridiales cluster XIV) and the methanogens themselves. Treatment with statins, which specifically inhibit the archaeal fatty acid synthesis pathway, is considered a possible way to improve constipation and associated disorders. Other recent works, performed with methane-rich saline in rodent models, have indicated that methane might be involved in other important processes, such as enhanced exercise capacity, increased secretion of glucagon-like peptide-1 (GLP-1, which has a role in insulin secretion and appetite suppression), or anti-inflammatory and neuroprotective effects. Whether the methane formed by methanogens directly in the GIT also has these effects remains to be analysed.

In addition to these potential positive effects of methane (which need to be re-evaluated in the human setting with biogenic methane supplied by methanogens), several other positive roles for archaea have been proposed. For example, Thaumarchaeota found on the skin could contribute to the oxidation of ammonia compounds delivered by sweat and thus could lower the skin pH. Their presence has in fact been linked with drier skin, but details on beneficial, commensal or opportunistic pathogenic activities are still missing. Another important positive role of members of the Methanomassiliicoccales in human health could be through their utilization of TMA as a substrate for methaneogenesis. TMA is generated during dietary compound degradation by intestinal bacteria and is then oxidized into trimethylamine-oxide (TMAO) in the liver. TMAO is involved in the development of cardiovascular and chronic kidney diseases, and TMA itself is associated with a genetic metabolic disorder, trimethylaminuria.

Methanomassiliicoccales species with the genetic potential to use TMA were found to be associated with a lower concentration of faecal TMA than in subjects without Methanomassiliicoccales or with Methanomassiliicoccales that were not capable of TMA consumption. The removal of TMA by Methanomassiliicoccales before it enters the bloodstream could therefore help prevent the development of diseases and metabolic disorders associated with this dietary nutrient.

The anaerobic TMA-degradation pathway, which requires the 22nd proteinogenic amino acid pyrrolysine, is shared by only a few bacteria and some archaea, and in the human GIT it currently seems to be unique to the Methanomassiliicoccales. Therefore, the prevention of these diseases could rely on the supplementation of TMA-consuming Methanomassiliicoccales (so-called “archaeobiotics”). As a proof of concept, a single inoculation of M. luminyensis B10 (the so-far-unique isolate of Methanomassiliicoccales) significantly lowered the concentration of plasma TMAO in standard C57BL/6 laboratory mice through a 30-day experiment, despite a very poor colonization. M. smithii and two TMA-utilizing methanogenic archaea (nonhuman and environmental) also showed a protective effect on a mouse model prone to atherosclerosis.

Do archaeal pathogens exist? Despite the above-described possible involvement of methanogenic archaea in several polymicrobial diseases, to date, archaeal pathogens according to Koch’s postulates and their per se pathogenicity have not yet been identified.

Nonetheless, in theory, archaea have all the preconditions to develop into pathogens: they are genetically and metabolically diverse, widespread in the environment and capable of engaging in warfare with their close relatives by various anti-archaeal compounds (such as sulfolobicin); have been interacting with different...
hosts for millions of years; and are recognized by the host immune system\textsuperscript{46-50}. Thus, it has been proposed that the current lack of identified archaeal pathogens might simply reflect a lack of knowledge due to our current inability to correctly detect them in disease patterns\textsuperscript{50}.

By contrast, it has also been proposed that no archaeal pathogens exist, which could be due to various reasons. One hypothesis is that archaea use different cofactors than eukaryotes do and as such have no inherent advantage in becoming pathogens to acquire resources from their host\textsuperscript{134}. However, archaea could take advantage by acquiring metabolites other than vitamins\textsuperscript{135}.

Another hypothesis for the absence of archaeal pathogens is that they have unique viruses and thus cannot acquire virulence factors from bacterial and eukaryotic viruses; in addition, the abundance of archaeal lytic viruses may prevent the maintenance of virulence factors in the mobile genetic pool\textsuperscript{136}. However, lysogenic or integrated viruses can also carry virulence factors, and our current knowledge of the diversity and genetic pool of archaeal viruses is highly incomplete, covering only a few non-host-associated lineages\textsuperscript{136}. Even less information is available on the virome of host-associated archaea and its interplay with that of host-associated bacteria and eukaryotes.

Another prospect is that the emergence of pathogenesis is a rare event that occurred in only a few bacteria and eukaryotes, but never in archaea\textsuperscript{137}. Among the very few bacterial pathogens (estimated to comprise less than 1% of all bacterial species\textsuperscript{137}), pathogenic Gram-negative proteobacteria deliver virulence factors into their target cells by using specialized molecular needles such as type III secretion systems, which are absent from archaea. However, many Gram-positive pathogens exist, and they deliver their virulence factors by other machineries that can also be found in the archaea, so there is no de facto reason why an archaean could not have acquired the capability to use one of these systems to deliver virulence factors to a eukaryotic cell.

As we discussed in previous sections, only a few lineages of the Archaea have engaged in associations with eukaryotic hosts (FIG. 1). This small number of transitions from a free-living to a host-associated lifestyle in Archaea may have narrowed the chances to develop virulence in a certain time frame, in particular when considering that a minimum of 42 independent events of adaptations to a host-associated lifestyle have occurred in Bacteria\textsuperscript{138}.

This opens another question: why would archaea be inherently less capable than bacteria of adapting to a host-associated lifestyle? The answer may be linked to a previously proposed common ecological trait of archaea\textsuperscript{139}; their adaptation to chronic energetic stress. This trait relies primarily on the specific membranes in archaea that enable them to dominate in extreme environments by minimizing their maintenance energy and to outcompete or be as competitive as bacteria in niches with low available energy. According to this hypothesis, the inherent tendency of archaea to thrive under chronic energy stress would therefore be incompatible with the rapid adaptability that is a distinguished feature of many pathogens\textsuperscript{139}. Moreover, this ancestral trait might have restricted the environmental distribution of many archaea to specific niches, such as extreme environments and/or deep anoxic sediments, where they are unlikely to encounter a potential eukaryotic host.

In conclusion, it is striking that nearly 40 years after the first description of human-associated archaea, we still have no evidence for one of their members being the primary cause of a disease. The reasons for this are still unclear and probably lie in a combination of all the above-mentioned hypotheses, including the fact that the archaea that are most strongly associated with animal hosts, notably methanogens, depend on the availability of metabolites (H\textsubscript{2}, methyl compounds) produced by other resident bacteria and as such, may be unable to act as independent pathogens.

**Conclusions and outlook**

To summarize the current knowledge, it is evident that archaea are abundant, diverse and active components of numerous microbiomes in plants, animals and humans. The current literature indicates that their presence has a substantial influence on their hosts and on other members of the microbiome. However, it is still unclear whether these interactions rely on archaea-specific traits or on properties shared with and/or acquired from other microbiome components. Either way, such adaptations are likely to be the result of a long-term co-evolution. From a host perspective, and beyond the enigma of the absence of pathogenic archaea, the extent to which their interactions are beneficial, neutral or deleterious is still unknown. Moreover, no data are available on the intraspecies and interspecies levels of communication between archaea and both their syntrophic partners and hosts. Open questions also include how and when archaea are acquired during the lifetime of their hosts and why their diversity and abundance seem to increase with a host’s age. These questions are central but still unanswered, because of methodological limitations and the complex confounding factors that affect archaeal distribution (for example, host age or ethnicity). More efforts will therefore be required in order to characterize and capture the diversity of host-associated archaea by using targeted approaches on well-characterized populations. There is also an urgent need for growing, isolating and characterizing a higher number of host-associated archaeal strains, which will provide key knowledge about their physiology beyond what can be inferred from genome or metagenome sequences alone. This would also reveal the determinants that have enabled certain archaea to successfully colonize their host. Finally, it will be important to study archaea–host associations through the development of plant and animal models with defined microbiomes. In addition to the above-mentioned perspectives, future research on human health will need to include more clinical studies and take into consideration the inflammation potential of archaea and their interaction with the immune system, especially of archaea that are strongly associated with inflamed body sites. These studies should also address the role of archaeal metabolisms and their products, such as the influence of methane on the human body.

Published online 20 July 2020
First proposal of providing archaea as live therapeutic products in order to prevent some human diseases. Bennett, G. et al. Genomics and metagenomics of trimethylamine- utilizing Archaea in the human gut microbiome. ISME J. 11, 2059–2074 (2017).

Study of the (meta)genomic and metabolic diversity of methanogenic archaeons in a human elderly cohort, with evidence of lower faecal TMA concentration being associated with some specific markers. Muller, M. et al. Humforming archaea as live biotherapeutic products in order to prevent some human diseases. Bennett, G. et al. Genomics and metagenomics of trimethylamine- utilizing Archaea in the human gut microbiome. ISME J. 11, 2059–2074 (2017).

First demonstration of the immunogenic activity of human-associated methanogens (severe pro-inflammatory response of peripheral blood mononuclear cells associated with) with disease. Muller, M. et al. Human age and skin physiology shape diversity and abundance of Archaea on skin. Sci. Rep. 7, 40357 (2017).

Bang, C., Weidenbach, K., Gutsmann, T., Heine, H. & Schmitz, R. A. The Archaeal archaeon Methanoplasma stadtmanae and Methanobrevibacter smithii stimulate human dermal cells. PLoS One 9, e99411 (2014).

First demonstration of the immunogenic activity of human-associated methanogens (severe pro-inflammatory response of peripheral blood mononuclear cells associated with) with disease. Muller, M. et al. Human age and skin physiology shape diversity and abundance of Archaea on skin. Sci. Rep. 7, 40357 (2017).

Bang, C., Weidenbach, K., Gutsmann, T., Heine, H. & Schmitz, R. A. The Archaeal archaeon Methanoplasma stadtmanae and Methanobrevibacter smithii stimulate human dermal cells. PLoS One 9, e99411 (2014).

First demonstration of the immunogenic activity of human-associated methanogens (severe pro-inflammatory response of peripheral blood mononuclear cells associated with) with disease. Muller, M. et al. Human age and skin physiology shape diversity and abundance of Archaea on skin. Sci. Rep. 7, 40357 (2017).

Bang, C., Weidenbach, K., Gutsmann, T., Heine, H. & Schmitz, R. A. The Archaeal archaeon Methanoplasma stadtmanae and Methanobrevibacter smithii stimulate human dermal cells. PLoS One 9, e99411 (2014).

First demonstration of the immunogenic activity of human-associated methanogens (severe pro-inflammatory response of peripheral blood mononuclear cells associated with) with disease. Muller, M. et al. Human age and skin physiology shape diversity and abundance of Archaea on skin. Sci. Rep. 7, 40357 (2017).

Bang, C., Weidenbach, K., Gutsmann, T., Heine, H. & Schmitz, R. A. The Archaeal archaeon Methanoplasma stadtmanae and Methanobrevibacter smithii stimulate human dermal cells. PLoS One 9, e99411 (2014).
74. Kinsman, R., Sauer, F. D., Jackson, H. A. & Wolnyetz, M. S. Methane and carbon dioxide emissions from dairy cows in full lactation monitored over a six-month period. J. Dairy Sci. 78, 2760–2766 (1995).

75. Dui, E. C. et al. Mode of action uncovered for the specific reduction of methane emissions from rumen-protected 

Nopolon. Proc. Natl Acad. Sci. USA 113, 6172–6177 (2016).

76. Shabat, S. K. et al. Specific microbiome- dependent biochemical pathways underlie the energy-harvesting ef- 

78. Liu, X. et al. Insights into the ecology, evolution, and metabolism of the widespread Archaeoarchaeota lineages. Microbiome 6, 102 (2018).

79. Saunois, M. et al. The global methane budget calculated over time. Annu. Rev. Earth. Planetary. Sci. 47, 1, 8–18 (2019).

80. Kinsman, R., Sauer, F. D., Jackson, H. A. & Polag, D. & Keppler, F. Global methane emissions from domesticated animals. Proc. Natl Acad. Sci. USA 115, 13306–13311 (2018).

81. Saunois, M. et al. Halophilic archaea in the human intestinal mucosa. Environ. Microbiol. 12, 2353–2410 (2010).

82. Liu, X. et al. Insights into the ecology, evolution, and metabolism of the widespread Woesearchaeota lineages. Microbiome 6, 102 (2018).

83. Anumagum, M. et al. Enterotypes of the human gut microbiome. Nature 473, 174–180 (2010).

84. Haji-shaghali, G., Darveau, R. P. & Curtis, M. A. The Keystone-pathogen hypothesis. Nat. Rev. Microbiol. 10, 677–687 (2012).

85. Dridi, B., Henry, M., El Khechine, A., Raoult, D. & Drancourt, M. High prevalence of Methanobrevibacter smithii and Methanomassiliicoccales detected in the human gut using an improved DNA detection protocol. PLoS One 4, e7063 (2009).

86. Polag, D. et al. Global methane emissions from the human body: past, present and future. Atmos. Environ. 214, 116823 (2020).

87. Suyama, M. & O’Keefe, S. J. D. Why do African Americans get more colon cancer than Native Africans? Am. J. Gastroenterol. 102, 698–704 (2007).

88. Drancourt, M. High prevalence of methanogenic archaea in refractory sinusitis, an emerging clinical entity. Front. Public Health 7, 12 (2019).

89. Dancronc, M. et al. Environmental data from methane archaea in refractory sinusitis, an emerging clinical entity. Front. Public Health 7, 12 (2019).

90. Boros, M. & Keppler, F. Methane production and global warming. Front. Public Health 7, e00006 (2018).

91. Wiggins, H. Breath-methane in patients with cancer of the rectum: a randomised controlled trial. J. Clin. Microbiol. 133, 1495–1500 (2018).

92. Tottey, W. et al. Colonic transit time is a driven force on the human body: health implications and future perspectives. J. Neurogastroenterol. Motil. 26, 1029–1036 (2017).

93. Jones, B. V., Begley, M., Hill, C., Cahan, C. G. M. & Marchesi, J. R. Functional and comparative metagenomic analysis of bile salt hydroxylase activity in the human gut microbiome. Proc. Natl Acad. Sci. USA 105, 13550–13555 (2008).

94. Bang, C. et al. Effects of antimicrobial peptides on methanogenic archaea. Antimicrob. Agents Chemother. 56, 4125–4130 (2012).

95. Blais, K. et al. Metabolic properties of archaeal species found in bioaerosols. PLoS One 6, e23526 (2011).

96. Davenport, E. R., Clark, A. G. & Ley, R. E. The relationship between the human genome and microbiome comes into view. Annu. Rev. Genet. 51, 415–433 (2017).

97. Bondon, M. J. et al. Identification of host genetics on the gut microbiome. Nat. Genet. 48, 1407–1412 (2016).

98. Goodrich, J. J. K. et al. Human genetics shape the gut microbiome. Nature 510, 415–418 (2014).

99. Raulud, A. et al. Synthrophy via interspecies H2 transfer between Christensennella and Methanobrevibacter underlies global occurrence in the human gut. mBio 11, e02355-19 (2020).

100. Hoffmann, C. et al. Archaea and fungi of the human gut. Proc. Natl Acad. Sci. USA 104, 10645–10650 (2007).

101. Xu, J. et al. Insights into the ecology, evolution, and metabolism of the widespread archaea lineages. Microbiome 6, 102 (2018).

102. Anumagum, M. et al. Enterotypes of the human gut microbiome. Nature 473, 174–180 (2010).
153. Mackay, R. J., McEntyre, C. J., Henderson, C., Lever, M. & George, P. Trimethylaminuria: causes and diagnosis of a socially distressing condition. Clin. Biochem. Rev. 32, 33 (2011).

154. Srinivasan, G., James, C. M. & Krzycki, J. A. Pathogenic archaebacteria: do they not exist? J. Bacteriol. 182, 2988–2988 (2000).

155. Martin, W. Pathogenic archaeabacteria: do they not exist because archaeabacteria use different vitamins? BioEssays 26, 592–595 (2004).

156. Gill, E. E. & Brinkman, F. S. L. The proportional lack of archaeal pathogens: do viruses/phages hold the key? BioEssays 33, 248–254 (2011).

157. Prangshilvi, D. et al. Sulfoflexins, specific protease toxins produced by strains of the extremely thermophilic archael genus Sulfolobus. J. Bacteriol. 182, 2985–2988 (2000).

158. Cavicchioli, R. & Curti, P. Response to William Martin’s letter. BioEssays 26, 595 (2004).

159. Gill, E. E. & Brinkman, F. S. L. The proportional lack of archaeal pathogens: do viruses/phages hold the key? BioEssays 33, 248–254 (2011).

160. Prangshilvi, D. et al. The enigmatic archaean virosphere. Nat. Rev. Microbiol. 15, 724–739 (2017).

161. No authors listed. Microbiology by numbers. Nat. Rev. Microbiol. 9, 628 (2011).

162. Sachs, J. L., Skophammer, R. G. & Regus, J. U. Evolutionary transitions in bacterial symbiosis. Proc. Natl Acad. Sci. USA 108, 10800–10807 (2011).

163. Valentine, D. L. Adaptations to energy stress dictate the ecology and evolution of the Archaea. Nat. Rev. Microbiol. 15, 115–115 (2007).

164. Castelle, C. J. & Banfield, J. F. Major new microbial groups expand diversity and alter our understanding of the tree of life. Cell 172, 1181–1197 (2018).

165. Borrel, C. et al. Wide diversity of methane and short-chain alkanes metabolisms in uncultured archaea. Nat. Microbiol. 4, 603–615 (2019).

166. Food and Agriculture Organization. Statistical Yearbook of the Food and Agriculture Organization of the United Nations (FAO, 2016).

167. Montero, A. L. G. et al. The role of small luminants on global climate change. Acta Sci. Anim. Sci. 40, 45124–45124 (2018).

168. Sanderson, M. G. Biomass of termites and their emissions of methane and carbon dioxide: a global database. Glob. Biogeochem. Cycles 10, 543–557 (1996).

169. Dridi, B., Fardeau, M.-L., Ollivier, B., Raoult, D. & Quast, C. et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596 (2013).

170. Albers, S.-V. & Meyer, B. The archaeal cell envelope. Nat. Rev. Microbiol. 9, 414 (2011).

171. Becker, K. W. et al. Unusual butane- and pentanetriol-based tetaen-rich lipids in Methanomassiliicoccus luminyensis, a representative of the seventh order of methanogens. Appl. Environ. Microbiol. 82, 4505–4516 (2016).

172. Klingl, A. S-layer and cytoplasmic membrane—exceptions from the typical archaeal cell wall with a focus on double membranes. Front. Microbiol. 5, 1–6 (2014).

173. Shahapure, R., Driessen, R. P. C., Haurat, M. F., Albers, S. & Danne, R. The archaebium: a rotating type IV plus. Mol. Microbiol. 91, 716–726 (2014).

174. Chaudhury, P., Quax, T. E. F. & Albers, S. Versatile cell surface structures of archaea. Mol. Microbiol. 107, 358–358 (2018).

175. Guenther, E. R. J. et al. First insights into the entry process of hyperthermophilic archaean viruses. J. Virol. 87, 13578–13585 (2013).

176. Sato, T. & Tomi, H. Novel metabolic pathways in archaea. Curr. Opin. Microbiol. 14, 507–514 (2011).

177. Bransen, C., Esser, D., Rauch, B. & Siebers, B. Carbohydrate metabolism in archaea: current insights into unusual enzymes and pathways and their regulation. Microbiol. Mol. Biol. Rev. 78, 89–175 (2014).

178. Leininger, S. et al. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809 (2006).

179. Stein, L. Y. Insights into the physiology of ammonia-oxidizing microorganisms. Curr. Opin. Chem. Biol. 49, 9–15 (2019).

180. Mand, T. D. & Metcalfe, W. W. Energy conservation and hydrogenase function in methanogenic archaea, in particular the genus methanosarcina. Microbiol. Mol. Biol. Rev. 83, e00200-19 (2019).

181. Borrel, C., Adam, P. S. & Grigoladou, S. Methanogenesis and the Wood–Ljungdahl pathway: an ancient, versatile, and fragile association. Genome Biol. Evol. 8, 1706–1711 (2016).

182. Stams, A. J. M. & Pluge, C. M. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat. Rev. Microbiol. 7, 568–577 (2009).

183. Ettwig, K. F. et al. Archaea catalyze iron-dependent anaerobic oxidation of methane. Proc. Natl Acad. Sci. USA 113, 12792–12796 (2016).

184. Cai, C. et al. A methanotrophic archaean couples anaerobic oxidation of methane to Fe(II) reduction. ISME J. 12, 1929–1939 (2018).

185. Scheller, S., Yu, H., Chadwick, G. L., McClynn, S. E. & Orphan, V. J. Artificial electron acceptors decouple archael methane oxidation from sulfate reduction. Science 351, 703–707 (2016).

186. Wegener, C., Krukenberg, V., Redel, D., Tegetmeyer, H. E. & Boeius, A. Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. Nature 526, 587–590 (2015).

187. Koning, A., Konig, H., Winter, J. & Leininger, T. Purification and use of Methanobacterium wolfei pseudomurein endopeptidase for lysis of Methanobacterium thermosautotrophicum. J. Bacteriol. 169, 1010–1016 (1987).

188. Lee, Z., Bussem, C. & Schmidt, T. mtdB: documenting the number of RNA and tRNA genes in bacteria and archaea. Nucleic Acids Res. 37, D489–D493 (2009).

189. Sun, Y., Liu, Y., Pan, J., Wang, F. & Li, M. Perspectives on cultivation strategies of archaea. Microb. Ecol. 79, 770–784 (2020).

Acknowledgements

The authors gratefully acknowledge A. Mahnert for support in the preparation of Figs. 5, and M. Blohs for input on methane production in humans. Funding given by the Austrian Science Fund (FWF) to C.-M. E. (Project ID P 50796 and P 52697) is highly appreciated, as is the funding from the German Science Foundation (DFG) given to R.A.S. (CHI1052/11-1/2). The French National Agency for Research is gratefully acknowledged for funding to G.B. and S.G. (Grants ArchEvol ANR-16-CE02-0005-01 and Metheval ANR-19-CE02-0005-01), and we acknowledge a grant to J.-F.B. from Hub Innovernige (Investissements d’Avenir 16-IDEX-0001 CAP 29–25).

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Microbiology thanks U. Cophina, I. Mizrahi, D. Reisman and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Publisher’s note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information

Supplementary information is available for this paper at https://doi.org/10.1038/s41579-020-0407-y.

RELATED LINKS

The Food and Agriculture Organization: www.FAO.org

© Springer Nature Limited 2020