Chapter 21

Archaea

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Just about half a century ago, all prokaryotes, i.e., cells without nucleus, were classified within one kingdom: Monera. However, in the late 1970s, scientists were starting to recognize that this classification system, based predominantly on morphological and metabolic traits, underestimated the vast diversity of prokaryotic life. Around the same time, the pioneering work of Carl Woese and George Fox led to the discovery that prokaryotes were, in fact, composed of two fundamentally different domains of life—the Bacteria and the Archaea (originally referred to as “Eubacteria” and “Archaebacteria,” respectively) [1]. Woese and coworkers used the RNA components of the ribosome to reconstruct the first phylogenetic tree of life based on molecular data [2], which divided cellular organisms into three separate domains of life.
Practical Handbook of Microbiology

(Figure 21.1A)—the Bacteria, Archaea, and Eukarya, the latter of which comprised all organisms with a true nucleus [2]. At that time, it was suggested that Archaea, in spite of their superficial similarity to Bacteria, may be more closely related to eukaryotes than Bacteria. In fact, they seemed to harbor simplified versions of eukaryotic informational processing machineries (replication, transcription, translation, and cell division), in addition to unique characteristics such as ether-bound isoprenoids rather than ester-bound fatty acid-based lipids (Table 21.1). Subsequent research on Archaea, accompanied by extensive methodological developments in environmental microbiology, sequencing technologies, physiology, cell biology, and phylogenetics, has further changed our view on the diversity of life, the tree topology, as well as the ecological and evolutionary importance of Archaea. In particular, the use of cultivation-independent techniques, such as metagenomics and single-cell genomics, which allow us to obtain genomes of uncultivated organisms directly from environmental samples [3, 4], have been a key element leading to our changed perception of archaeal diversity and distribution. While Archaea have originally been viewed as comprising predominantly “extremophilic” organisms inhabiting environments with high temperature, salinity, and high or low pH, they are now known to be ubiquitous in all environments on Earth, including marine waters and freshwater lakes, sediments, soils (including plant roots), aquifers, and the human microbiome to name a few [5–7]. With their widespread ecological distribution and important metabolic capabilities, Archaea are recognized as key players in a wide variety of biogeochemical processes, including the sulfur, nitrogen, and carbon cycles [8]. For instance, Archaea include the only known organisms able to conserve energy through the anaerobic production or consumption of methane in processes referred to as methanogenesis and anaerobic methane oxidation, respectively. Since methane is an extremely potent greenhouse gas, with a global-warming potential about 25 times greater than carbon dioxide, these Archaea have an essential role in the global carbon budget and consequently climate change [9]. Finally, the study of archaeal phylogenetic diversity and evolution has fundamentally changed our understanding of the eukaryotic cell (see below) [10].

Archaea and the Tree of Life

Since the discovery of the Archaea as a separate domain of life (Figure 21.1A), their relationship to Bacteria and eukaryotes has been a matter of debate and is regarded to be of fundamental importance for our understanding of the origin of eukaryotes. Eukaryotic cells are highly compartmentalized and it has long been recognized that eukaryotic compartments, such as mitochondria (the site of ATP generation via oxidative phosphorylation) and chloroplasts (the organelles in which photosynthesis occurs in plants), evolved as a result of endosymbiosis, i.e., mitochondria and chloroplasts seem to be derived from Alphaproteobacteria.

FIGURE 21.1  Schematic depictions of the relationship of Archaea with Bacteria and eukaryotes in the tree of life. A: Upon the discovery of Archaea as a separate domain, the tree of life was divided into three major domains. B: However, phylogenetic analyses of core informational proteins suggested later that eukaryotes may have evolved from within the Archaea, challenging the three-domain topology. C: Recent research, among others enabled by the discovery of the Asgard archaea, has shed further support on the branching of eukaryotes from within the Archaea (in terms of universal marker proteins). In turn, it has been suggested that the tree of life has two primary domains of life—the Archaea and Bacteria—and one secondary domain of life, which evolved from the former (see text for more details).
TABLE 21.1
Comparison of Selected Characteristics of the Major Domains of Life

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bacteria</th>
<th>Archaea</th>
<th>Eukarya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane-enclosed nucleus</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Chromosomal structure</td>
<td>Circular</td>
<td>Circular</td>
<td>Linear</td>
</tr>
<tr>
<td>Peptidoglycan in cell wall</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Membrane lipids</td>
<td>Ester-linked</td>
<td>Ether-linked</td>
<td>Ester-linked</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Glycerol-3-phosphate</td>
<td>Glycerol-1-phosphate</td>
<td>Glycerol-3-phosphate</td>
</tr>
<tr>
<td>Ribosomes (mass)</td>
<td>70S</td>
<td>70S</td>
<td>80S</td>
</tr>
<tr>
<td>Initiator tRNA</td>
<td>formylmethionine</td>
<td>methionine</td>
<td>methionine</td>
</tr>
<tr>
<td>Introns</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Operons</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>RNA polymerase</td>
<td>One (4 subunits)</td>
<td>One (8–12 subunits)</td>
<td>Three (12–14 subunits)</td>
</tr>
<tr>
<td>Transcription factors required</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>TATA box in promoter</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Archaeal transcription also shares several features in common with eukaryotes. While many archaeal genomes encode gene clusters reminiscent of bacterial operons, the archaeal transcription machinery represents a simplified version of their eukaryotic counterparts [29]. For instance, the archaeal DNA-dependent RNA polymerase (RNAP) consists of 12-13 subunits, which are homologous to the subunits of the three eukaryotic RNA polymerases (RNAP I-III) [29]. In contrast, RNAP of Bacteria consists of only five subunits, two of which are distantly related to archaeal RNAP subunits 1 and 2 (i.e., RpoA and RpoB). Transcription initiation, which is based on the same molecular mechanisms across the domains, also involves homologous transcription factors in Archaea and eukaryotes [29].

Similarities in the translational machinery between Archaea and eukaryotes are also evident. Archaeal ribosomes are of...
comparable size to bacterial ribosomes (70S), but share various ribosomal subunits uniquely with eukaryotes [30]. Additionally, translation in Archaea is initiated by an initiator tRNA carrying methionine and several translation initiation factors, as is seen in eukaryotic organisms but contrasts with the use of formylmethionine by bacteria. Further, a 22nd amino acid, pyrolysine, has been identified uniquely in certain members of the Archaea, in particular methanogens [31].

Notably, Archaea not only share homologous replication, transcription, and translation machineries with eukaryotes, but have also been found to encode various so-called eukaryotic signature proteins (ESPs) [32], i.e., proteins that are generally absent from bacterial genomes while being central to the integrity and functioning of eukaryotic cells. These proteins include, for instance, components of the eukaryotic cytoskeleton (such as actin and tubulins), cell division and vesicle trafficking machineries, endosomal sorting complexes required for transport (ESCRT), as well as the proteasome and ubiquitin system [10].

In particular, members of the TACK archaea (discussed later in this chapter) including among others the Cren-, Aig- and Thaumarchaeota have early on been found to encode certain ESPs that were absent from Euryarchaeota [15, 16, 33–35]. For instance, while Euryarchaeota use FtsZ as major cell division protein, many Cren- and Thaumarchaeota harbor a cell division system (also referred to as cdvABC system) that includes homologs of eukaryotic ESCRT-III and an ATPase related to vacuolar protein sorting-associated protein 4 (Vps4) [36–39]. Furthermore, archaeal actin homologs referred to as crenactin, which are distantly related to eukaryotic actins, have been discovered in the genomes of two species of Thaumarchaeota, “Candidatus Nitrosoarchaeum limnia” and “Candidatus Nitrosoarchaeum koreensis” [41], and an analysis of the “Candidatus Caldararchaeum subterraneum” composite genome revealed the presence of a presumably fully functional ubiquitin-like protein modifier system [42].

The discovery of the Asgard archaea [17, 18], the currently most closely related archaeal sister lineage of eukaryotes, has recently revealed a variety of additional ESPs in Archaea. For instance, Asgard archaea not only encode additional homologs of eukaryotic informational processing machineries but also harbor simplified versions of the eukaryotic oligosaccharyl-transferase-complex and ubiquitin modifier system. Furthermore, they encode an extended set of small GTPases [17, 18], which are key regulators in eukaryotic cells with a central role in vesicle trafficking machineries [43]. Additional central components homologous to eukaryotic vesicle transport and tethering were identified in the genomes of the Thorarchaeota [18]. Further, Asgard archaea harbor protein domain homologous to the key domains of the three major eukaryotic ESCRT machinery complexes (ESCRT I-III) and a diversity of cytoskeleton-related proteins that are much more similar to their eukaryotic counterparts than those previously identified in Archaea. These include the lokiacitins found across the Asgard representatives, as well as bona fide tubulins in Odinarchaeota [10, 17, 18]. Notably, Asgard archaea also encode actin-regulating proteins, such as the profilins [18], which were recently shown to be functionally equivalent to those of eukaryotes [44].

Altogether, archaeal information processing machineries as well as an extended set of ESPs in members of the TACK and in particular the Asgard archaea, further testify to the archaeal origin of the eukaryotic cell. Importantly, the study of these complexes in Archaea can help to provide a better understanding of eukaryotic cell biology and provide insight into the relative timing of the evolution of cellular complexity.

Archaeal Cell Membranes and Cells Walls

The composition of archaeal cell membranes differs fundamentally from those of Bacteria and eukaryotes [45]. For instance, the glyceral used to make archaeal phospholipids is a stereoisomer of the glyceral used to build bacterial and eukaryotic membranes, i.e., while Archaea use glyceral-1-phosphate, eukaryotes and bacteria have glyceral-3-phosphate. Furthermore, Archaea harbor isoprenoid side chains instead of the fatty acid side chains found in Bacteria and eukaryotes. These isoprenoids are bound to the glyceral backbone by ether linkages contrasting with the ester linkages formed between the bacterial and eukaryotic glyceral and fatty acid moieties. Archaeal isoprenoid side chains in the two monolayers of the lipid bilayer can be linked, thereby giving rise to transmembrane phospholipids. The isoprenes can also form five-carbon ring structures, which may function in the stabilization of the membranes of archaeal species that live in high temperature environments. More than 100 different ether-type polar lipids, such as phospholipids and glycolipids, have been identified in Archaea [46].

Different archaeal representatives differ with regard to their cell walls. In contrast to Bacteria, Archaea lack peptidoglycan and are thus naturally resistant to antibiotics that impair the synthesis of peptidoglycan, such as penicillins. Some species of methanogenic Archaea contain cell walls of pseudopептидоглэн (pseudomurein) that superficially resemble bacterial peptidoglycan but contain different components (e.g., N-acetylglucosaminuronic acid instead of N-acetylglucosamine) and have β-1,3 instead of β-1,4-glycosidic bonds. Yet, most archaeal species lack pseudomurein and instead harbor cell walls made of proteins, glycoproteins, or polysaccharides [47]. For instance, a common cell wall structure found in Archaea is composed of a paracrystalline surface layer, termed S-layer, consisting of protein or glycoprotein moieties arranged in hexagonal patterns. Finally, some Archaea, such as certain members of the order Thermoplasmatales, lack cell walls altogether.

Taxonomic Diversity of Archaea

The Archaea were originally divided into two major phyla, termed Crenarchaeota and Euryarchaeota [2]. However, recent advances in culture-independent, high-throughput sequencing techniques have uncovered a large diversity of novel archaeal lineages, most of which remain uncultivated [5]. Many of these newly discovered archaeal lineages are only distantly related to established lineages within the Cren- and Euryarchaeota, which has led to the proposal of many additional archaeal phyla and superphyla during the past years [7]. Figure 21.2 summarizes the
FIGURE 21.2 Depiction of the phylogenetic diversity of the Archaea and the presence/absence of key features. The unrooted phylogenetic tree was inferred with maximum likelihood using the IQ-tree software (with the C20+LG+F+R mixture model) and was based on an alignment of 11399 positions from a representative set of 569 archaeal taxa. Highly supported clusters (assessed by ultrafast bootstraps [220] and SH-like approximate-likelihood ratio test [221]) are indicated with gray or black dots based on their branch support values (see figure inlet). Taxa were predominantly named according to Adam et al. [7] (bold face), but alternative names suggested by GTDB (https://gtdb.ecigenomic.org/) are indicated in parenthesis when available. Numbers in brackets indicate the number of genomes/taxa per cluster. The presence and absence of certain features are shown with black and gray circles for each major taxonomic lineage (see figure inlet). Please note that the last column only reports those archaeal taxa that have been confirmed to be part of the human archaeome.
current understanding of the archaeal phylogeny, including established and proposed phyla, classes, and orders, as well as the general physiological grouping and certain features discussed below. However, please note that there is currently no consensus on how to best classify archaeal lineages. Therefore, a widely accepted taxonomy of the Archaea remains to be established [5]. In particular, there are currently two main classification schemes used: the classification suggested by Adam and coworkers that is implemented in NCBI [7] and the system introduced by the developers of the Genome Taxonomy Database (GTDB) (https://gtdb.ecogenomic.org/). The latter of these was suggested to provide a standardized and rank-normalized genome-based classification system, which was recently used to revise the bacterial taxonomy [48].

Euryarchaeota

The Euryarchaeota (Figure 21.2) comprise various cultivated and well-characterized archaeal species including the globally important methanogens (i.e., methane producers) as well as anaerobic methane-oxidizing Euryarchaeota (ANME) [49, 50]. Methanogens and ANME play a key role in the carbon cycle by anaerobically producing or consuming the potent climate gas, methane [8, 9, 50–52]. However, research during the past years has shown that the Euryarchaeota are a phylogenetically and physiologically much more diverse radiation than originally thought [5, 7]. Indeed, it remains to be elucidated whether Euryarchaeota comprise a monophyletic group or phylogenetically distinct divisions, some of which may be more closely related to the TACK and Asgard archaea [7, 53, 54]. In the following, we provide an overview of the major lineages comprising canonical and recently discovered lineages affiliating with the Euryarchaeota.

Methanotecta

The Methanotecta (Figure 21.2), a recently proposed superclass [7], comprise the so-called class II methanogens (Methanosarcinales, Methanomicrobiales, Methanocellales), several phylogenetically distinct ANME archaeal lineages, the Haloarachaeota, Archaeoglobales, as well as the more recently described archaeal orders referred to as Methanomatronarchaeota, Syntrophoarchaeales, Methanoliparales, and Methanophagales. We present major features of these different lineages below.

Methanomicrobiales

The order Methanomicrobiales comprises several families, such as the Methanocalculusaceae, Methanoregulaceae, Methanospirillaceae, Methanomicrobiaceae, and Methanocorpusculaceae (e.g., reviewed in [55]), and can be found in a variety of anoxic habitats, including wetlands, soil, oceans and freshwater, landfills, rice paddies, as well as associated with animals [50]. Members of the Methanomicrobiales have diverse cell shapes, ranging from rods to cocci to plates, including motile and nonmotile species, and grow between 0°C and 60°C [55]. Cells are often surrounded by glycoprotein-containing S-layers. Many Methanomicrobiales use hydrogen and carbon dioxide to form methane and all species are obligate anaerobes. They can use formate and alcohol but not acetate and methylated C1-compounds as substrates for methanogenesis, distinguishing them from the Methanosarcinales [9, 55].

Methanosarcinales

The Methanosarcinales are closely related to the Methanomicrobiales and include families such as the Methanosarcinaeae, Methanotrichaceae (formerly Methanosaetaceae), and Methanomiccaceae (Table 21.1), as well as the Methanoperedenaceae (ANME-2d). While this order comprises diverse methanogenic organisms, it also includes representatives of the anaerobic methane-oxidizing Euryarchaeota ANME-2 and -3 lineages [50, 52, 56]. Similar to the Methanomicrobiales, representatives of the Methanosarcinales are found in a range of anoxic habitats [50]. Yet, in contrast to other methanogenic orders, the Methanosarcinales are known for their much wider substrate range for methanogenesis, i.e., members of this group not only use hydrogen and formate as substrates but also a variety of methylated compounds and acetate [8, 9]. Considering that methanogenesis based on acetate may contribute up to two-thirds of methane released to the atmosphere, members of this group have important roles in the global carbon cycle [8, 51]. Representatives of the ANME-2 and -3 lineages use the reverse methanogenesis pathway to anaerobically oxidize methane [52]. While some ANME-2 members can grow independently using nitrate, nitrite, or Fe(III) as electron acceptors [57–59], other ANME-2 grow in syntrophic consortia with bacterial partners (especially sulfate reducers) that serve as external electron sinks [52, 60]. Members of these groups are particularly abundant in the sulfate-methane transition zone in marine sediments and play an important role in the global carbon cycle by reoxidizing a large fraction of the methane produced in marine sediments before it can enter the atmosphere [52, 61].

Methanophagales (ANME-1)

The Methanophagales comprise another lineage of anaerobic methane-oxidizing archaea, also known as the ANME-1 lineage. While originally thought to affiliate with the Methanosarcinales, they were recently shown to represent a sister lineage of the Syntrophoarchaeales [7] (Figure 21.2 and later in this chapter). Similar to the ANME-2 and -3 lineages that belong to the Methanosarcinales, members of this group occur in diverse marine, terrestrial, and freshwater environments [62], are particularly abundant in the sulfur-methane transition zone [61], and use the reverse methanogenesis pathway for the anaerobic oxidation of methane (AOM) [63]. While ANME-1 has not been cultivated thus far, various lines of research have suggested that members are able to oxidize methane in syntrophy with sulfate-reducing bacteria (SRB) through direct electron transfer [52, 60, 64].

Methanocellales

Methanocellales represents a more recently described order of hydrogenotrophic methanogens that were originally referred to as Rice Cluster I (RC-I) [65] due to their initial discovery in rice paddy fields, where they are important producers of methane [66]. The first representative of this order, Methanocella paludicola, was isolated from an aerobic,
propionate-containing enrichment culture [65] and represents a nonmotile anaerobe with rod-shaped cells thriving at temperatures between 25°C and 40°C [65]. While the isolate performs methanogenesis using hydrogen, carbon dioxide, and formate, it uses acetate as a carbon source. Hydrogen is provided by its syntrophic partner, the bacterium *Syntrophobacter fumaroxidans* [67]. Similar metabolic features were found in other representatives of this order, including *M. arvoryzae* [68] and *M. conradii* [69].

**Syntrophoarchaeae**

*Syntrophoarchaeae* (sometimes assigned to the *Methanocarcinales*; Table 21.1) represent a recently discovered group of anaerobic, alkane-oxidizing archaea usually found in hydrocarbon-rich sediments [70, 71]. For example, the first two representatives of this lineage, *Syntrophoarchaeum butanivorans* and *Syntrophoarchaeum caldarius*, were originally isolated from hydrothermal- and hydrocarbon-rich marine sediments of the Guaymas Basin [71, 72]. Notably, *Syntrophoarchaeae* grow by the anaerobic oxidation of butane as well as propane, which are thought to be metabolized using the reverse methanogenesis pathway also operating in ANME archaea [73]. In particular, they encode subunits homologous to the Methyl-Coenzyme M Reductase (MCR) complex, which represents the key enzyme of methanogens catalyzing the demethylation of CH₃-S-CoM to methane [51]. In *Syntrophoarchaeae*, MCR is thought to be used in reverse and to mediate the first step of the breakdown of short-chain alkanes eventually yielding carbon dioxide as an end product [71]. As indicated by the names of members of this group, studied representatives grow syntrophically with the sulfate-reducing bacterium *Candidatus Desulfoturvus auxilli.*

**Archaeoglobales**

The *Archaeoglobales* comprises species belonging to the genera *Archaeoglobus*, *Ferroglobus*, and *Geoglobus* [74]. The *Archaeoglobus* sp. is believed to be predominantly composed of strictly anaerobic and hyperthermophilic members, growing optimally at 80°C and neutral pH. The best studied representatives are autotrophs and/or organotrophs and can reduce sulfite or sulfite during respiration [75]. Species of *Ferroglobus* grow by oxidation of Fe(II)S²⁻ and H₂ [75], whereas *Geoglobus* grows anaerobically in the presence of acetate and ferric iron [74]. Recently, genomes of so far uncultivated members of the *Archaeoglobi* were reconstructed from environmental samples and shown to encode MCR-like protein complexes similar to those of methanogens and ANME archaea [76, 77]. Based on genomic inferences, it was suggested that the respective organisms may be able to grow by the oxidation of methane or alternative short-chain alkanes.

**Methanoliparales**

*Methanoliparales* is an uncultivated lineage within the *Methanotecta* that phylogenetically places between *Archaeoglobales* and a cluster comprising *Syntrophoarchaeae* and *Methanophagales*. *Methanoliparales* were first discovered in two metagenomes from a petroleum-enrichment culture and an oil seep and are represented by two metagenome-assembled genomes: *Candidatus Methanoliparum thermophilum* NM1a and *Candidatus Methanoliviera hydrocarbonicum* NM1b [78]. Genomic analyses suggest that *Methanolipirales* are methanogens that encode the Wood-Ljungdahl carbon fixation pathway and are capable of beta-oxidation. Interestingly, both genomes code for two distinct MCR complexes, which may be involved in methanogenesis and the oxidation of alkanes, respectively.

**Haloarchaeota**

*Halobacteria*, herein referred to as *Haloarchaeota*, are a diverse group of *Archaea*, mostly of which are adapted to high salinity. Salt requirements of these species range from 1.5 to 5.2 M NaCl, although most strains grow best between 3.5 and 4.5 M NaCl, at or near the saturation point of salt (36% w/v salts). In order to maintain osmolarity of their cells in high-salt environments, haloarchaeal members accumulate up to 5 M intracellular levels of KCl to counterbalance high extracellular salt concentration. As a result, the entire intracellular machinery, including enzymes and structural proteins, must be adapted to high salt levels. The proteins of all haloarchaeal species have a very low isoelectric point and the genomes contain high GC contents that are well above 60% [79]. Some species of *Haloarchaeota* are motile by means of tufts of flagella, although many species are nonmotile [75]. *Haloarchaeota* comprise various aerobic or facultative anaerobes and show diverse morphologies and shapes, including rods, cocci, and a multitude of pleomorphic forms [75, 80]. The lack of turgor pressure within haloarchaeal cells enables the cells to tolerate the formation of corners, and as such, some species are even triangular or square-shaped [75, 80]. Cell envelopes of coccoid *Haloarchaeota* are stable in the absence of salt, while noncoccoid species maintain their integrity only in the presence of high concentrations of NaCl or KCl [75]. Non-coccoid species have a proteaceous cell envelope with glycoprotein subunits forming a hexagonal pattern [75]. Species of *Haloarchaeota* are abundant in salt lakes, inland seas, and evaporating ponds of seawater, such as the Dead Sea and solar salterns. *Haloarchaeota* often tint the water column and sediments in bright colors due to the presence of retinal-based pigments. Some of these pigments are capable of the light-mediated translocation of ions across cell membranes. The best known halobacterial pigment is bacteriorhodopsin, which is an outwardly directed proton pump. Bacteriorhodopsin is involved in energy conservation and is the only nonchlorophyll-mediated light energy transducing system known to date [79]. Other retinal-based pigments found in *Haloarchaeota* include halorhodopsin, which is an inward chloride pump involved in osmotic homeostasis, as well as sensory rhodopsin I and II (SRI and SRII, respectively). SRI and SRII can mediate positive and/or negative phototaxis [79].

**Methanonatronarchaeota**

Another lineage of halophilic archaea are the *Methanonatronarchaeota*, which were first recovered from hypersaline anoxic lake sediments [81] and are currently represented by isolates from two distinct subgroups: the soda lake isolate *Methanonatronarchaeum thermophilum* AMET and the salt lake isolate *Candidatus Methanohalarchaeum thermophilum*
other metals [91]. which tolerate high concentrations of iron, copper, zinc, and
of the most extreme acidophilic organisms known, members of
Together with members of Picrophilum
obligate acidophiles that are able to grow at pH values around 0.
Representatives of this family are cell wall-lacking extreme and
man-made), as well as in areas with geothermal activity [90, 91].
ore deposits, mines, and acid mine drainage systems (natural or
commonly using ferrous iron as energy and inorganic carbon as a
Ferroplasma
lineage of the
Methanomassiliicoccales
as electron acceptors, such as methanol or trimethylamine, and formate or hydrogen as electron donors [81]. The
16S rRNA gene analyses indicate that Methanomassiliicoccales
Picrophilus
are the first cultured representatives of the SA1 group, which is
are mostly found in hypersaline environments [81, 83]. Yet, the
exact placement of Methanomassiliicoccales in the archaeal tree
is life is still debated. While initial phylogenetic analyses placed
this lineage sister to Haloarchaea [81], recent analyses have sug-
gested that the Methanomassiliicoccales form an early diverging
lineage of the Methanotecta [84].

Diaforarchaeae
The Diaforarchaeae comprise a recently suggested superclass [7]
that includes the Thermoplasmata and related lineages, such as the
diverse and abundant Marine Group II and III archaea [85, 86],
also known as the Poseidioniales and Pontarchaeales, respec-
tively, as well as a recently discovered new order of methanogens,
the Methanomassiliicoccales [87].

Thermoplasmatales
The Thermoplasmatales comprise the genera Acidiplasma,
Thermoplasma, Picrophilus, Cuniculiplasma, and Ferroplasma.
Cuniculiplasma, Thermoplasma, and Ferroplasma are the only
cultivated archaeal representatives that lack cell walls [75, 88].
Species of Thermoplasma are facultative anaerobes and obligate
heterotrophs, using elemental sulfur for respiration. Most mem-
bers of this group are thermoacidophiles and grow optimally at
60°C and pH 2 [75]. For instance, representatives may be found
in self-heating coal refuse piles and in acidic solfatara fields [75].

Members of the Picrophilus are the most acidophilic organisms
known so far [89]. They form irregular cocci that are 1–1.5 μm
in diameter and contain S-layer cell walls [75]. Picrophilus are
thermophilic and hyperacidophilic and grow at temperatures
between 47°C and 60°C and pH ranges of 0–3.5 [75]. Their ability
to grow at pH values near 0 and at high temperatures has
shifted the physicochemical boundaries at which life was con-
considered to exist.

In contrast to other members of the Thermoplasmatales,
Ferroplasma are not thermophilic and can grow autotrophi-
cally using ferrous iron as energy and inorganic carbon as a
carbon source. Representatives can be found in a variety of
acidic environments with stable chemical conditions, such as
ore deposits, mines, and acid mine drainage systems (natural or
man-made), as well as in areas with geoehydrological activity [90, 91].
Representatives of this family are cell wall-lacking extreme and
obligate acidophiles that are able to grow at pH values around 0.
Together with members of Picrophilum, they comprise a group
of the most extreme acidophilic organisms known, members of
which tolerate high concentrations of iron, copper, zinc, and
other metals [91].

Aciduliprofundales
Aciduliprofundales, formerly named the “deep-sea hydrothermal
vent euryarchaeota 2” (DHVE2) lineage, is currently represented
by the cultivated Aciduliprofundum boonei [92, 93]. As the ori-
ignal name suggests, Aciduliprofundales are predominantly found
across hydrothermal vents, where they can represent up to 15% of
the archaeal community [92–94]. A. boonei is an anaerobic het-
erotroph that ferments peptides and is able to reduce elemental
sulfur or ferric iron at a pH between 3.3 and 5.8 (optimum pH 4.6)
and an optimal growth temperature of 70°C [92]. This organism is
motile with a single flagellum and has pleomorphic cells of about
0.6–1 μm in diameter that are surrounded by a single S-layer.

Methanomassiliicoccales
The order Methanomassiliicoccales represents the first lin-
eage of the Thermoplasma known to comprise methano-
genic members [87], several of which have been isolated, such as
Methanomassiliicoccus luminyensis [95, 96], Candidatus Methanomethylophilus albus [97], and Candidatus Methano-
plasma termiteum [98]. Methanomassiliicoccales are widely
distributed in wetlands and sediments as well as the gastroin-
testinal tracts of animals including those of humans and cows
[87, 99, 100]. Members of this group comprise H2-dependent
methylo trophic methanogens, which are able to use methyl-
ated amines [100] including mono-, di-, and trimethylamines
for methanogenesis. Considering that the latter compounds have
been implicated in human disease, gut-associated members of the
Methanomassiliicoccales may play an important role in
human health [100].

Poseidioniales
The Poseidioniales [101], formerly Marine Group II (MG II), lack
any cultured representatives and are mainly known from 16S
rRNA gene diversity assays and genomic analyses. Poseidioniales
are often found in the photic zone of marine waters and can pres-
et up to 15% of archaeal cells in the Atlantic ocean [102–104].
They are further divided into Candidatus Poseidonacea (MGIIa)
and Candidatus Thalassarchaeaceae (MGIIb), whose
abundances seasonally fluctuate, i.e., members of MGIIa and
MGIIb are more abundant in the summer and winter, respectively
[105]. Members of this group comprise aerobic heterotrophs with
the potential to utilize a range of substrates such as proteins, pep-
tides, amino acids, fatty acids, carbohydrates, xenobiotics, and
agar [101, 106–110]. In addition, some representatives of the class
Ca. Poseidonia ii, found in the photic zone, encode proteorhodop-
sin indicative of a photoheterotrophic lifestyle [101, 107, 110].

Pontarchaeales
The order Pontarchaeales, or Marine Group III, are often found
in the deep ocean, while being less abundant in the photic zone
[102, 111]. Based on genomic data, it was inferred that deep-
sea Pontarchaeales likely represent motile heterotrophs that
might degrade proteins, carbohydrates, and lipids [112]. In con-
trast, surface dwelling members of the Pontarchaeales seem
to encode photolyase and rhodopsin genes and in turn may be
photoheterotrophs [111]. Notably, both the Pontarchaeales
and the Poseidioniales lack the key archaeal lipid biosynthesis
gene encoding glycerol-1 phosphate dehydrogenase, such that
it is currently unclear whether members of these orders encode
canonical archaeal lipids [45]. In particular, the presence of genes for glycerol-3-phosphate dehydrogenase, which is essential in the synthesis of bacterial lipids, has led to the suggestion that these Archaea may have mixed membranes [45, 101].

Other Euryarchaeota

The following section provides an overview of additional lineages affiliating with the Euryarchaeota, including methanogenic lineages that have been extensively studied in the past. However, some analyses indicate that at least some of these orders may be more closely related to the TACK and Asgard archaea [7, 53, 54].

Methanococcales

As the name implies, the Methanococcales include representatives with coccoid shapes and proteinous cell walls [75]. All members of this lineage are thought to be strict anaerobes that obtain energy by the reduction of CO₂ to methane [9] and comprise mesophilic (e.g., Methanococcus) to thermophilic (e.g., Methanothermococcus) to hyperthermophilic (e.g., Methanocaldovococcus) taxa [75].

Thermococcales

Members of the Thermococcales represent anaerobic heterotrophs that utilize a wide range of organic compounds, including amino acids, a variety of sugars, and organic acids such as pyruvate. When available, they can use elemental sulfur as the terminal electron acceptor. Extensive research has been carried out on the metabolism of cultivated representatives and led to the discovery of unique enzymes and pathways [113]. Certain members of the Thermococcales represent important model organisms. For example, the hyperthermophilic Pyrococcus furiosus, which grows anaerobically at temperatures near 100°C using carbohydrates and peptides as carbon and energy sources [75], has been extensively used to study thermostable enzymes and adaptations to high-temperature environments [114].

Methanobacteriales

The Methanobacteriales comprise another lineage of methanogenic archaea that reduce CO₂ or methyl compounds with H₂, formate, or secondary alcohols as electron donors. They include rod-shaped, lancet-shaped, or coccoid members, which contain cell walls made of pseudopeptidoglycan. Methanobacteriales are widely distributed in nature and are found in anaerobic habitats such as aquatic sediments, soil, anaerobic sewage digesters, and the gastrointestinal tracts of animals to name a few [50, 75].

Methanopyrales

The Methanopyrales consists of a single genus, Methanopyrus, comprising rod-shaped members with cell walls made of pseudopeptidoglycan [75]. Known Methanopyrus are hyperthermophilic, and grow between 84°C and 110°C, with optimal growth at 98°C. Similar to other methanogenic lineages, members of this group have a chemolithoautotrophic lifestyle converting CO₂ and H₂ to methane [9, 75]. While it has proven difficult to resolve the exact phylogenetic placement of the Methanopyrales relative to other archaea, it has recently been suggested that this lineage forms a monophyletic clade together with the Methanobacteriales and the Methanococcales referred to as Methanomada [7]. However, it remains to be determined whether these so-called group I methanogens [9] are indeed closely related phylogenetically (Figure 21.2).

Methanofastidiosales

Methanofastidiosales represent a recently discovered and thus far uncultivated archaeal lineage (also known as WSA2 or Arc I), whose members are present in diverse environments including sediments, groundwater, and bioreactors [115–117]. Metagenomic approaches have enabled the reconstruction of genomes of representatives of the Methanofastidiosales from wastewater-treatment bioreactors [117]. While members of this group encode key genes for methanogenesis, they lack genes related to carbon-fixation pathways and were suggested to solely use methylated thiols as substrates for methanogenesis [117].

Theionarchaeota

Theionarchaeota (formerly Z7ME43) represents another clade of uncultivated archaea, which forms a sister lineage of the Hadesarchaeota (see next) and was originally discovered in water-filled limestone sinkholes in northeastern Mexico [118]. This clade is currently represented by two genomes that were recovered from the White Oak River Estuary in North Carolina [119]. Genomic analyses indicated that Theionarchaeota might conserve energy by peptide fermentation.

Hadesarchaeota

The Hadesarchaeota, which were originally referred to as the South-African Gold Mine Miscellaneous Euryarchaeal Group (SAGMEG), are distributed in a variety of anoxic environments, including the terrestrial subsurface as well as marine sediments, which cover a wide span of temperatures [120–123]. The first genomes of members of this clade were reconstructed from the water column of the White Oak River estuary [123] as well as Yellowstone National Park (YNP) hot spring sediments and indicated the capability of anaerobic CO oxidation potentially coupled to nitrite or H₂O reduction [123]. Notably, another genome of a member of the Hadesarchaeota was recently obtained from a hot spring metagenome and shown to encode a mcr-like operon. Based on phylogenetic analyses of MCR subunits as well as genomic analyses, it was suggested that these Hadesarchaeota may represent alkane-oxidizing archaea similar to members of the Syntrophoarchaeales [124] and perhaps some representatives of the Bathyarchaeota [50].

Persephonarchaeae

The Mediterranean Sea Brine Lakes 1 (MSBL1) clade, now referred to as the Persephonarchaeae [7], is another lineage of uncultivated archaea that is closely related to the Hadesarchaeota. The Persephonarchaeae are commonly found in marine hypersaline environments [125, 126] and comprise potential anaerobic mixotrophs that may conserve energy through sugar fermentation but may also be able to fix inorganic carbon [127]. Genomic inferences suggest that MSBL1 archaea synthesize trehalose as putative osmolyte to encounter the high salt conditions in their environment [127].

Hydrothermarchaeota

The Hydrothermarchaeota [7], also known as the Marine Benthic Group-E (MBG-E), were originally discovered in marine deep-sea
putative anaerobic chemolithoautotrophs that use carbon monoxide and/or hydrogen as electron donors as well as a variety of electron acceptors including nitrate and sulfate [132, 133].

**The TACK Superphylum**

The TACK superphylum was originally introduced to describe the *Crenarchaeota* and the related phyla referred to as the *Thaumarchaeota, Aigarchaeota, and Korarchaeota* [16]. During the past years, many additional lineages affiliating with the TACK archaea have been discovered through metagenomics and single cell genomics approaches and the TACK lineage has therefore been suggested to be referred to as the *Proteoarchaeota* [134]. However, a consensus has yet to be reached regarding both the naming as well as the validity of using a superphylum as a taxonomic level. In the following sections, we introduce canonical and recently discovered clades belonging to the TACK archaea.

**Crenarchaeota**

The *Crenarchaeota* includes a diversity of (hyper-) thermophilic archaeal species, many of which have been discovered through cultivation-based approaches before the onset of the genomics era in microbiology and now represent important model organisms. This taxon is composed of a single class, the *Thermoprotei*, which is subdivided into three to five subclades, the *Thermoproteales, Sulfolobales, Desulfurococcales* as well as the *Fervidococcales and Acidilobales*. However, the latter two may in fact belong to the *Desulfurococcales* (Figure 21.2). Cultured crenarchaeal species are morphologically diverse, and include rods, cocci, filamentous, and disk-shaped cells. Almost all cultured species are obligate (hyper-) thermophile, with optimal growth temperatures ranging from 70°C to 113°C and many members are also acidophiles and capable of metabolizing sulfur. Representatives of the *Crenarchaeota* thrive in environments such as hot solfataras, volcanic areas, as well as hydrothermal vents at the bottom of the ocean. A variety of metabolic capabilities have been described in the different members of the *Crenarchaeota*. For instance, some *Thermoproteales* are chemolithoautotrophs, using carbon dioxide as a carbon source and conserving energy by the conversion of hydrogen and elemental sulfur to hydrogen sulfide. Others respire various organic substrates using oxygen, sulfur, nitrate, or nitrite as electron acceptors [75]. Many members of the *Desulfurococcales* are strict anaerobes and neutrophiles to weak acidophiles, growing optimally at pH 5.5–7.5 [135]. Representatives of the *Sulfolobales* are acido-philic hyperthermophiles, which can grow lithoautotrophically by oxidizing sulfur or chemoheterotrophically on simple reduced carbon compounds using sulfur derivatives as electron acceptors. Notably, the *Crenarchaeota* include several members that have been shown to be hosts of the small-celled *Nanoarchaeota* [136–140] (see later in this chapter). In particular, the biocenosis between *Ignicoccus hospitalis*, a member of the *Desulfurococcales*, and its nanoarchaeal ecosymbiont, *Nanoarchaeum equitans*, has been extensively studied and provides important insights into archaeal cell biology and cell-cell communication [141]. For instance, investigation of *I. hospitalis* revealed remarkable cellular features including the presence of two outer membranes surrounding a large periplasmic space as well as an endomembrane system reminiscent of eukaryotic cells [142].

**Thaumarchaeota**

Environmental 16S rRNA-based surveys in the early 1990s have led to the discovery of uncultivated archaeal lineages distantly related to the *Crenarchaeota* in moderate marine and terrestrial ecosystems. The subsequent cultivation of the first representatives of these so-called mesophilic *Crenarchaeota* (also MGI) from marine and terrestrial environments [143] and the study of the first genomes of members of this group [144, 145], revealed that they form a separate phylum within the *Archaea* referred to as the *Thaumarchaeota* that distantly affiliates with the *Crenarchaeota*. Most cultivated *Thaumarchaeota* are chemolithoautotrophic ammonia-oxidizing archaea (AOA), which play an important role in the nitrogen and carbon cycles in both aquatic and terrestrial environments [146]. However, the reconstruction of genomes of deep-branching *Thaumarchaeota* has recently led to the suggestion that not all members of this group are AOA but instead represent chemoorganotrophs that may reduce oxygen, nitrate, or sulfur [147]. This notion was recently confirmed with the isolation of the thermoacidophilic, sulfur- and iron-reducing organoheterotrophic *Conexivisphaera calidus*, a potentially early diverging member of the *Thaumarchaeota* [148].

**Aigarchaeota**

The *Aigarchaeota* represent another proposed candidate phylum that comprises species of the Hot Water Crenarchaeotic Group I (HWCGI), members of which have not been cultivated so far. Genomic analyses of the first representatives of this group have suggested that the *Aigarchaeota* comprise both facultative and obligate anaerobes, which may respire a variety of organic substrates and perhaps also hydrogen and carbon monoxide using oxygen or oxidized sulfur or nitrogen compounds as electron acceptors [42, 149–152]. Furthermore, several representatives seem to have the ability to fix inorganic carbon. *Aigarchaeota* seem to predominantly inhabit thermally heated terrestrial and marine ecosystems, including hot springs, subsurface aquifers, and mine fracture waters [150, 152].

**Korarchaeota**

The *Korarchaeota* comprises a group of uncultivated *Archaea* that had already been discovered in the late 1990s in terrestrial and marine thermal environments [153]. The first member of this clade, referred to as *Ca. Korarchaeum cryptoophilum,* was shown to comprise ultra-thin, needle-shaped cells measuring up to 100 μm in length. Genomic analyses indicated that this organism represents a peptide fermenter with a unique set of informational processing genes, which early on indicated that it comprises the first member of a distinct archaeal phylum [154]. Recently, genomes of additional members of the *Korarchaeota* have been recovered from sediments [128] and represent an uncultivated archaeal lineage widely distributed in deep subseafloor environments. Genomes from members of this group have been reconstructed from metagenomes of the Juan de Fuca Ridge flank, Guaymas Basin hydrothermal sediments, and the Mid-Atlantic Ridge of the South Atlantic Ocean [129–131]. Genomic analyses have indicated that *Hydrothermarchaeae* are metabolically versatile [131] and include putative anaerobic chemolithoautotrophs that use carbon monoxide and/or hydrogen as electron donors as well as a variety of electron acceptors including nitrate and sulfate [132, 133].
deep-sea hydrothermal vent sediments [130] and hot spring environments [18, 124, 155] providing novel insights into the metabolic features of this clade. Notably, genomic analyses revealed that certain members of the *Korarchaeota* harbor the key genes for methanogenesis, [155] which may for instance enable methanogenesis from methanol and hydrogen or the coupling of the anaerobic oxidation of methane with sulfite reduction [155].

**Bathyarchaeota**

*Bathyarchaeota* were originally discovered through 16S rRNA gene surveys in hot springs [153] and were referred to as Miscellaneous Crenarchaeota Group (MCG) [156] due to their distant affiliation with cultivated *Crenarchaeota*. This extremely diverse phylum is now subdivided into at least 25 subgroups, which are defined at family and order level [157]. Notably, members of this putative phylum-level lineage can be found in a diversity of anoxic marine, terrestrial, and hydrothermal environments including marine sediments and often represent the most abundant archaeal community members [157–159]. Based on genomic analyses, it is inferred that many *Bathyarchaeota* are heterotrophs with a wide substrate range including acetate, proteins, and aromatic compounds such as lignin [157, 160]. However, the *Bathyarchaeota* also includes putative acetogenic species [161] as well as organisms with *mcr* genes [162], which are closely related to those of *Syntrophoarchaeota* [71]. In turn, it has been suggested that some members of the *Bathyarchaeota* may be able to mediate the anaerobic oxidation of short-chain alkanes [50].

**Geoarchaeota**

*Geoarchaeota*, also Novel Archaeal Group 1 (NAG1), are often found in hypoxic to oxic, hot, acidic, iron-rich springs [163–165] and represent a lineage of thus far uncultivated archaea which seem to be closely related to or part of the Crenarchaeota [164, 166]. Based on genomic inferences, it has been suggested that the *Geoarchaeota* are likely motile and might conserve energy through the oxidation of carbon monoxide, peptides, and/or carbohydrates using oxygen as a terminal electron acceptor [149, 164].

**Verstraetearchaeota**

*Verstraetearchaeota* were originally discovered in deep South-African Gold mine microbial communities through 16S rRNA gene surveys and were referred to as Terrestrial Miscellaneous Crenarchaeota Group (TMCG) [120]. Members of this group seem to be widely distributed and are also found in hydrocarbon-rich environments, sediments, soil, and wetlands [167]. First insights into the metabolic features of members of this group were derived from genomes assembled from anoxic digesters, named *Methanomethylicus* sp. and *Methanosuratus* sp. [167]. Subsequently, additional representatives were discovered and referred to as *Methanohydrogenales* and *Methanomediales* [168]. Notably, the *Verstraetearchaeota* comprise members with *mcr*-gene operons most similar to those found in methanogenic *Euryarchaeota*. In turn, based on genomic inferences, it was suggested that the *Verstraetearchaeota* likely include anaerobic methylo trophic as well as hydrogenotrophic methanogens [167–169].

**Nezhaarchaeota**

*Nezhaarchaeota* are a recent addition to the TACK superphylum represented solely by uncultivated members, whose genomes were assembled from hot spring metagenomes and hydrothermal sediments [77]. Notably, the *Nezhaarchaeota* encode a MCR protein cluster and are potential hydrogenotrophic methanogens [77].

**Marsarchaeota**

The *Marsarchaeota*, or “Novel Archaeal Group 2” (NAG2), are typically found in geothermal, iron oxide-rich mats [163]. The first genomes of members of this lineage were recently recovered from thermal (50–80°C) and acidic (pH 2.5–2.5) microbial mats from Yellowstone National Park [170] and led to the suggestions that the *Marsarchaeota* are aerobic chemoorganotrophs that degrade lipids, peptides, and carbohydrates and may be able to reduce ferric oxide.

**Geothermarchaeota**

The *Geothermarchaeota* represents one of the most recent additions to the Archaea and is thus far only represented by uncultivated members, whose genomes have been reconstructed from metagenomes from the Juan de Fuca Ridge subseafloor [129] and hydrothermal vent sediments in the Guaymas Basin [130]. Little is yet known about the lifestyle and ecological roles of *Geothermarchaeota*, and in-depth genomic analyses will be necessary to infer their metabolic potential.

**The Asgard Superphylum**

The Asgard superphylum is a recently described archaeal radiation, which comprises several different archaeal clades of high taxonomic rank (likely phylum-level) [17, 18]. Notably, phylogenetic and comparative genomic analyses have indicated that this archaeal clade includes the closest archaeal sister lineage of eukaryotes (discussed previously in this chapter). Members of this superphylum have originally been discovered in sediments all around the world, in which they can comprise a significant fraction of the microbial diversity. In the following, we briefly introduce the major metabolic features of the currently known members of the Asgard archaea, i.e., the *Loki-, Thor-, Odin-, Hel-, and Heimdallarchaeota*.

**Lokiarchaeota**

The *Lokiarchaeota* represents an archaeal lineage originally referred to as the Deep Sea Archaeal Group (DSAG) or Marine Benthic Group B (MBGB) archaea, which are abundant in diverse marine sediments [94, 171]. For example, the *Lokiarchaeota* comprise up to 10% of the microbial community in cold sediments near Loki’s Castle hydrothermal vent field from which the first metagenomes were obtained [17, 18]. Members of the *Lokiarchaeota* might be autotrophs using the Wood-Ljungdahl pathway for carbon fixation [172]. However, genomic analyses also revealed the potential for the use of a variety of organic carbon substrates, suggesting that representatives of the *Lokiarchaeota* may predominantly rely on fermentative growth [20]. In fact, the successful cultivation of the first *Lokiarchaeote, Candidatus*...
Prometheoarchaeum syntrophicum, revealed that this organism ferments amino acids enabled through a syntrophic interaction with hydrogen- or formate-consuming partner organisms [22].

Thorarchaeota

The Thorarchaeota share many metabolic features with the Lokiarchaeota [20, 173, 174]. Currently known representatives harbor a variety of genes likely encoding proteins involved in the usage of organic substrates. Furthermore, they encode the Wood-Ljungdahl pathway, which could be used for carbon fixation or serve as an electron sink during growth on organics. In contrast to currently known Lokiarchaeota, members of this group also harbor a putative NADH dehydrogenase that may enable respiratory growth in addition to fermentation [20]. Based on current environmental survey data, the Thorarchaeota seem less abundant than the Lokiarchaeota but occur in a wide variety of anoxic environments [18].

Heimdallarchaeota

Thus far known representatives of the Heimdallarchaeota are metabolically diverse and differ from other Asgard lineages [20]. While genomic analyses indicate that they are able to utilize a large variety of organic substrates similar to other members of the Asgards, they do not seem to be fermentative organisms and current members lack the Wood-Ljungdahl pathway. Instead, they encode a membrane-bound electron chain, which allows growth using oxygen and nitrite as electron acceptors [20, 21]. Heimdallarchaeota are currently thought to comprise the archaeal lineage most closely related to the archaeal ancestor of eukaryotes. However, though found in a variety of environmental samples including anoxic sediments and oxygenated waters, they are generally less abundant than the Loki- and Thorarchaeota.

Odinarchaeota

Odinarchaeota are currently represented by a single genome, which was obtained from a hot spring metagenome [18]. Similar to other members of the Asgard superphylum, Odinarchaeum encodes the ability to use organic compounds as growth substrates [20]. Yet, it lacks the key enzyme of the Wood-Ljungdahl pathway and instead encodes membrane-bound hydrogenases, which suggests that the thermophilic Odinarchaeum may conserve energy through fermentation of organic substrates to hydrogen, acetate, and carbon dioxide. Members of the Odinarchaeota are thought to predominantly inhabit thermal environments such as hot spring sediments and hydrothermal vents [18].

Helarchaeota

The Helarchaeota represent the most recently discovered clade within the Asgard archaea [175]. While they harbor similar gene sets as the Loki- and Thorarchaeota, currently known representatives of this lineage also contain mcr-gene clusters. Phylogenetic analyses of the encoded proteins revealed their close relationship with proteins of Syntrophoarchaeum opening the possibility that certain members of the Asgard archaea have the potential to anaerobically oxidize short-chain alkanes, perhaps in syntrophy with microbial partners [175]. However, the environmental distribution of the Helarchaeota and the functional importance of this potential alkane metabolism in Asgard archaea remain to be determined.

The DPANN Superphylum

The DPANN superphylum is the fourth major radiation in the Archaea, besides the Euryarchaeota, TACK, and Asgard archaea [149, 176, 177]. Currently, this radiation is assumed to comprise a large diversity of distinct archaeal clades most of which seem to predominantly include members with extremely small genomes and cell sizes that are thought to depend on partner organisms for growth and survival [177]. While first defined in reference to the Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, and Nanohaloarchaeota (DPANN) [149], additional lineages such as the Micarchaeota, Pacarchaeota, Woesarchaeota, and Huberarchaeota are now also considered members of this group [177, 178]. Furthermore, the Altarchaeota [179], representatives of which do not have reduced genomes, are sometimes considered to belong to the DPANN [177, 180]. However, the boundaries between certain clades of DPANN and other archaea (in particular the Euryarchaeota) are not well defined and it remains to be established which lineages indeed belong to a monophyletic (i.e., sharing a common ancestor) DPANN clade [180].

Nanoarchaeota

The first representative lineage of the DPANN archaea was already discovered in 2002, i.e., long before the DPANN radiation was known. In particular, Huber and coworkers discovered a small-celled organism in cultures of the crenarchaeum, I. hospitalis, which they referred to as N. equitans [136]. Initially, it was suggested that this organism is the first representative of a novel phylum called Nanoarchaeota or may represent a highly derived member of the Euryarchaeota [136, 181]. However, upon the genomics-based discovery of additional archaeal lineages represented by organisms with small genomes, which affiliated with Nanoarchaeota, it was proposed that the Nanoarchaeota belong to the DPANN radiation [149]. Notably, the nanosized hyperthermophilic N. equitans is obligately host-dependent and grows as an ectoparasite on the surface of I. hospitalis [182, 183]. It lacks genes for many major metabolic pathways and in turn depends on its host for the acquisition of diverse metabolites likely including lipids, amino acids, and ATP. In line with this, the genome of N. equitans represents one of the smallest known genomes of any extracellular organism (480 kb) [184]. However, compared to the genomes of many bacterial endosymbionts, the genome of N. equitans does not show evidence of pseudogenes and contains a full complement of tightly packed genes encoding informational processing machineries [184]. Similar trends have recently been seen in other representatives of the DPANN radiation [177]. Members of the Nanoarchaeota have been found in a variety of thermal environments ranging from hydrothermal vents to hot springs and are now assumed to infect a variety of different crenarchaeal hosts. For instance, additional Nanoarchaeota, such as Candidatus Nanobisiansus stetteri, Candidatus Nanopusillus acidilobi, and Candidatus Nanoolepta minutus have recently been successfully co-cultivated with their crenarchaeal hosts referred to as
Altiarchaeota and its Symbiont—A Member of the Huberarchaeota

The Altiarchaeota represent a lineage variably affiliating either with the DPANN archaea or Euryarchaeota [6, 135, 177, 179, 180, 191] in phylogenetic analyses depending on the type of analysis (e.g., with regard to model choice) and data used. Altiarchaeota (formerly also referred to as SMI Euryarchaeota) were originally discovered in a cold (−10°C), sulfurous Moor in Germany [135] but can also be found in sulfidic springs [192, 193], marine sediments, hot springs, and aquifers [191]. Notably, some members of the Altiarchaeota are found in microbial consortia that display a unique morphology described as a “string-of-pearls,” which is several millimeters in length and consists of tiny white pearls (0.5–3 mm diameter) connected by thin threads [135]. The outer part of the pearl is composed of bacteria, such as the Gammaproteobacterium Thiotoxrix unzi [194] or the Epsilonproteobacterium IMB1 [195], while the inside is dominated by Altiarchaeota [135]. The large size of the consortium allows for the effective enrichment of Altiarchaeota on polyethylene nets that can consist of ~98% of archaeal cells and ~2% bacteria [196]. Other representatives of the Altiarchaeota occur in almost single-member biofilms (~5% bacteria, ~95% Altiarchaeota) in sulfidic springs [192, 193].

Notably, Altiarchaeota have not only been found in symbiosis with bacteria but represent the hosts of members of the Huberarchaeota, a recent addition to the DPANN superphylum [178, 197]. Similar to other DPANN archaea, known members of the Huberarchaeota have reduced genomes and lack proteins related to energy metabolism, regeneration of redox equivalents and nucleotide biosynthesis indicating that they depend on a variety of compounds from their hosts.

The first insights into the metabolism of the Altiarchaeota came from the metagenome-assembled genome (MAG) of Candidatus Altiarchaeum hamiconexum, which was reconstructed from a cold, sulfidic spring in Germany [179]. Genomic analyses suggested this representative is an anaerobic autotroph, potentially capable of growth on carbon dioxide and possibly acetate, formate, and carbon monoxide [179]. Ca. A. hamiconexum is a biofilm-forming, nonmotile organism with coccolid cells (0.4–0.7 μM in diameter) and a double membrane [179]. Cells can be surrounded by up to 100 hair-like proteinaceous appendages of 2–3 μM length, so-called hami, which mediate adhesion to various surfaces [198]. However, representatives of the Altiarchaeota from sediments lack genes encoding proteins involved in ham formation and show specific adaptations to their respective environments [191].

Archaia as Part of the Human Microbiome

For a long time, it was thought that Archaea played minor roles in the microbiomes of humans and other mammals and true archaeal pathogens remain to be discovered. The first archaean associated with humans was described in 1982, the methanogenic Methanobrevibacter smithii, which was isolated from human feces [199] suggesting that the methane exhaled by a certain proportion of humans may be produced by methanogens residing in the gastrointestinal tract [200]. Since then, several archaeal species have been identified to be associated with the intestinal, oral, gut, nasal, vaginal, lung, and skin microbiota of both humans and other animals [201–203]. However, their roles in human health and disease remain poorly understood [201–205]. In the following, we summarize current knowledge regarding the diversity and function of human-associated archaea.

Oral Archaeome

Methanogenic archaea are part of the oral archaeome with Methanobrevibacter oralis being the most frequently detected species [205, 206]. Notably, M. oralis seems to be correlated with periodontitis severity, supporting a potential pathogenic role of methanogenic archaea [206–208]. While no direct experimental evidence has demonstrated the virulence pattern of M. oralis and other oral archaeal species, the unique metabolism of methanogenic archaea provides insight into possible drivers of oral disease. For instance, methanogens in periodontal pockets may serve as an H2 sink, which would favor the proliferation of syntrophic pathogenic microbes [206–209]. Recent investigation into microbial communities in the oral cavity has shown significant positive correlations between the abundance of methanogens with that of Prevotella intermedia, a known bacterial pathogen involved in periodontal infections such as periodontitis, gingivitis, and necrotizing ulcerative gingivitis [208].
The relationship between these two groups in periodontal pockets is still unknown, but indirect and direct associations between the methanogens and the local environment may be driving the proliferation of *P. intermedia* through a series of possible syntrophic interactions [208]. A current key research interest is to further determine the immediate role of archaea in the pathogenesis of periodontal infections [206, 210]

**Gut Archaeome**

To date, three species of methanogenic archaea have been cultivated and isolated from gut-derived samples, i.e., from human stool: *M. smithii*, *Methanosphaera stadtmanae*, and *Methanomassiliicoccus luminyensis* [95, 199, 211]. With the help of molecular tools, two candidate-species, *Candidatus Methanomassiliicoccus intestinalis* and *Candidatus Methanomethylovorus alvinus*, in addition to several unknown members of the orders *Methanosarcinales*, *Methanobacterales*, *Methanococcales*, *Methanobacterales*, and *Methanopyrales*, have been shown to inhabit the human gastrointestinal tract [202]. Further, the presence of methanogens in biopsy samples suggests that they may be associated with the mucosal lining in addition to their presumed presence in the lumen [202]. *M. smithii* is the major archaeal component in the human gut, while *M. stadtmanae* and *M. luminyensis* are less frequently detected species [201, 202] and appear to play an important role as *H*2-consumers in the complex microbial ecosystem of the gut [201, 205, 209]. Fermentative microorganisms produce short-chain fatty acids and *H*2, the former being consumed by the host and the latter being scavenged and consumed by the archaea. This removal of *H*2 from the host by methanogens makes the fermentative processes kinetically more favorable and continuously drives this cyclical syntrophy [201, 202, 205]. Furthermore, there is evidence that methanogens may be involved in inflammatory bowel disease, irritable bowel syndrome, colorectal cancer, diverticulosis, and obesity [201, 205]. However, it is unclear whether methanogens directly or indirectly contribute to the development of gastrointestinal disorders and without doubt, more research is needed to unravel the role of archaea in intestinal disorders [204, 212]. For instance, it has also been suggested that some human-associated archaea may be mutualistic, providing health benefits and influencing host metabolism [202, 213].

Not all gut-associated archaea are methanogens however [202]. For instance, a halophilic archaeon belonging to the halobacteria, *Haloforax massiliensis*, was recently isolated from a human stool sample, molecular tools the debate over whether halophiles may colonize the gut [214, 215]. Other studies have revealed a diversity of halobacteria-related sequences in fecal samples collected from healthy Korean people, with analyzed sequences representing *Halorubrum alimentarium* and *Halorubrum koreense*. Interestingly, both *H. alimentarium* and *H. koreense* had previously been isolated from salt-fermented sea foods suggesting native cuisine and eating habits may contribute to the propensity of these organisms in the gut environment [201, 216].

**Global Human Archaeome**

Technological advancements in high-throughput sequencing have further improved insights into the human microbiome and revealed unexpected diversity of representatives from archaeal phyla that had not previously been detected in human habitats, including members of the DPANN archaea. In particular, members of the *Woesearchaeota* appear to be present in the human lung, and while it is speculated that they may exhibit parasitic/symbiotic lifestyles, their environmental role remains unclear [202]. Analytical exploration into the distribution of archaeal signatures in the human body has revealed site-specific patterns that shape a preliminary biogeographical view of the human archaeome: (1) *Thaumarchaeota* on the skin, (2) methanogenic *Euryarchaeota* in the gut, (3) mixed skin-gut nasal archaeal communities, and (4) *Woesearchaeota* inhabiting the lungs [202].

While *M. smithii*, *M. oralis*, *M. stadtmanae*, *M. luminyensis*, and *H. massiliensis* are the only archaeal species that have been successfully isolated and cultivated from human habitats, efforts are underway to culture more archaeal species associated with humans in order to better understand their roles as potential pathogens or commensal members with potentially positive physiological impacts. For instance, a major step toward a better understanding of the function and dynamics of human-associated archaea may be gained through the Human Microbiome Project [209, 217].

Concurrent with efforts to culture archaeal species infecting humans and elucidate their potential roles in human pathogenesis, there are several initiatives aiming to identify antimicrobial agents that are effective against *Archaea*. Research shows that archaea are resistant to antibiotics used to treat bacterial infections, in part due to morphological and physiological features that impede the action of many bacterial-targeting antimicrobial agents. *In vivo* and *in vitro* experiments have indicated susceptibility of several archaeal groups to certain protein synthesis inhibitors, including fusidic acid and imidazole derivatives [218]. Nonetheless, antibiotic-resistant archaea may become indirectly susceptible to antimicrobial treatments when relying on chemically susceptible bacterial partners within their complex communities. To date, there are a limited number of antimicrobials that target archaea directly. Greater exploration into archaea as causative agents of human disease would also require further investigation into antiaarchaeal compounds and treatments [210, 218].

**Summary**

Thought to be of limited ecological relevance originally, *Archaea* are now known to inhabit a wide range of ecosystems and to play a key role in major biogeochemical cycles [8]. Furthermore, *Archaea* have proven to be of fundamental importance for our understanding of the evolution of complex eukaryotic cells [10] and have emerged as important model systems. Notably, representatives of the *Archaea* are now known to form a stable part of the human microbiome and may even be involved in disease. Unique metabolic and cellular features of *Archaea* are being utilized in a variety of biotechnological applications as well as the development of novel adjuvants in the use of vaccines utilizing the unique membrane lipids of archaeal membranes [219]. Considering that a large fraction of *Archaea* of high-taxonomic rank likely still awaits discovery [5], the coming years will certainly witness further insights into the role of *Archaea* in ecological food webs, the evolution of life and human biology.
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