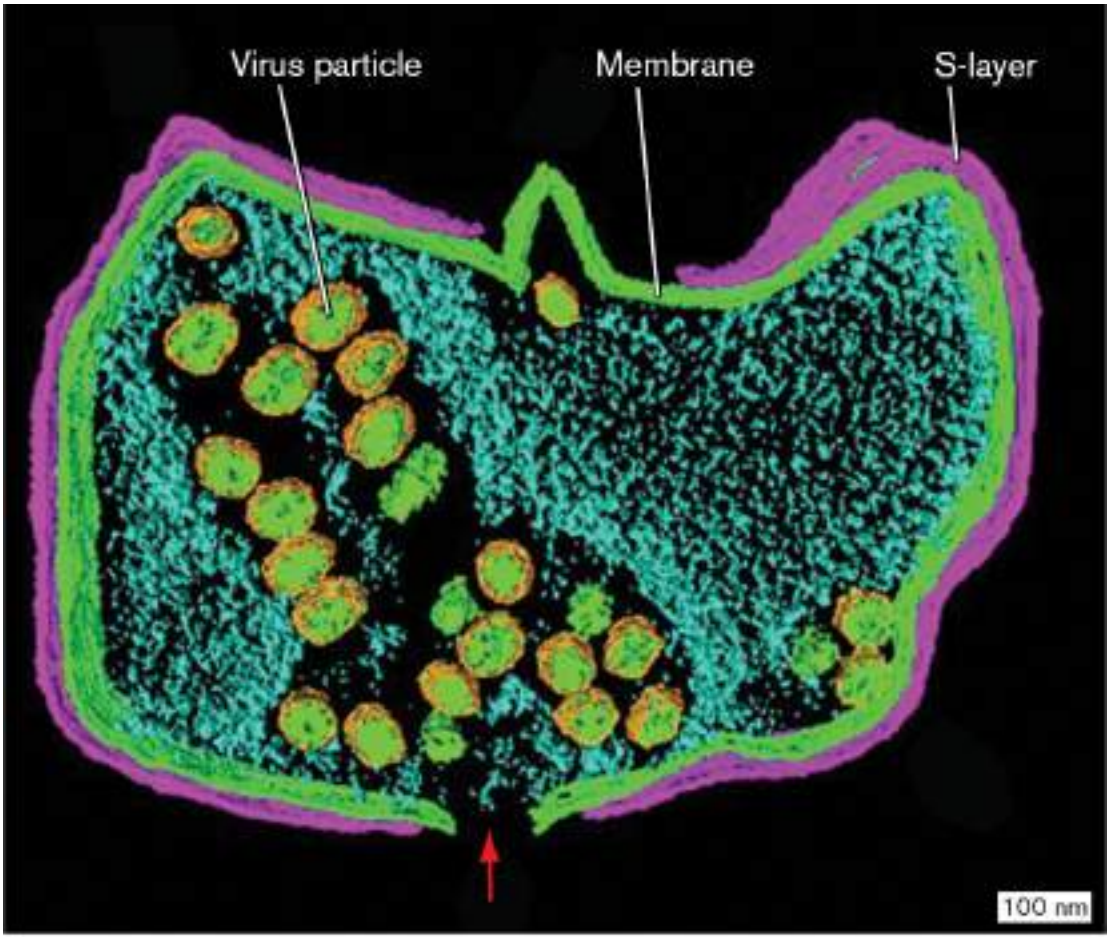
CHAPTER 19

Archaeal Diversity



A *Sulfolobus* archaeon from an acid hot spring in Costa Rica is infected by a virus. At temperatures near boiling, the virus programs its assembly within the archaeal cell. A viral enzyme breaks through the cell membrane and S-layer, allowing exit of progeny (red arrow). Cryo-electron

tomography, colorized purple (S-layer), green (cell membrane).

Source: Haina Wang et al. 2018. *J. Virol.* **92** :E01727-17.
H. WANG ET AL. 2018. *J VIROL* . **92** :E01727–17

Chapter Sections		Learning Objectives
19.1 Archaeal Diversity at a Glance	»	Explain how key traits of Archaea distinguish them from the Bacteria and Eukarya.
	»	Outline the phylogeny and traits of major groups of Archaea.
19.2 TACK Hyperthermophiles Eat Sulfur	»	Describe the adaptations and habitats of sulfur-oxidizing archaea of the TACK superphylum and the viruses that infect them.
19.3 Thaumarchaeota: Ammonia Oxidizers and Animal Symbionts	»	Explain how the Thaumarchaeota contribute to mesophilic ecosystems, including ammonia oxidation and symbiosis with animals.

Chapter Sections		Learning Objectives
Special Topic 19 Ammonia-Oxidizing Archaea Keep Your Fish Healthy		
19.4 Euryarchaeota: Methanogens from Gut to Globe	»	Outline the phylogeny and metabolism of diverse kinds of methanogenic Euryarchaeota.
	»	Explain how methanogens contribute to gut, soil, and sewage ecosystems and how methanogenesis accelerates global climate change.
19.5 Haloarchaea and Other Euryarchaeotes: Underground and Under Ocean	»	Describe the traits of thermophilic and halophilic archaea, including photoheterotrophy and archaeal motility.
19.6 DPANN Symbionts, Altiarchaeales, and Asgard:	»	Outline the phylogeny and traits of DPANN taxa, and explain the evidence for the Asgard relationship to Eukarya.

Chapter Sections		Learning Objectives
Branch to Eukaryotes		

eResearch Activity 19 Can an Archaeal Fuel Cell Make Electric Current in Boiling Water?

In 1977, Carl Woese revealed the existence of a third kind of life: the Archaea, a domain of life forms very different from the plants, animals, and bacteria that had long been known. Archaea are found in all soil and water habitats, in symbiosis with animals and plants, and in extreme environments that exclude bacteria and eukaryotes. Archaea include hyperthermophiles inhabiting Earth's hottest habitats, as well as Arctic and Antarctic psychrophiles. Other taxa grow in anaerobic soil and water, where they collaborate with bacteria in multispecies biofilms. Methanogens inhabit the digestive tracts of humans and other animals, where they interact with our immune system yet do not cause disease. Their methane production is a growing cause of global climate change.

In Chapter 19 we explore the diversity of archaea. We emphasize the unique structures and metabolic pathways of archaeal cells. While surveying their major taxonomic categories, we introduce research techniques used to study organisms in extreme environments and those with unique forms of metabolism. Extremophiles have interesting applications in industry, such as the production of high-temperature enzymes, and even a fuel cell that runs at temperatures near the boiling point of water.

19.1 Archaeal Diversity at a Glance

Archaea are known for including extremophiles such as *Sulfolobus* , which survives acid hot springs without even a cell wall—as do the viruses that infect it (see the chapter-opening image). Yet archaea also show surprising similarities to eukaryotes. *Ignicoccus hospitalis* is a spaghetti-tubed hyperthermophile whose form could be mistaken for a eukaryote (**Fig. 19.1**). This archaeon was isolated by the famed extremophile hunter Karl Stetter from a thermal vent off the coast of Iceland, 600 meters below sea level, where it grows best at 90°C. Microscopists Thomas Heimerl and Reinhard Rachel showed that *I. hospitalis* possesses an extensive endomembrane system within its cytoplasm. They used low-temperature processing and electron tomography on serial sections to map the extensive protrusions and tubules of the *Ignicoccus* endomembranes, which surprisingly resemble nuclear membranes with endoplasmic reticulum.

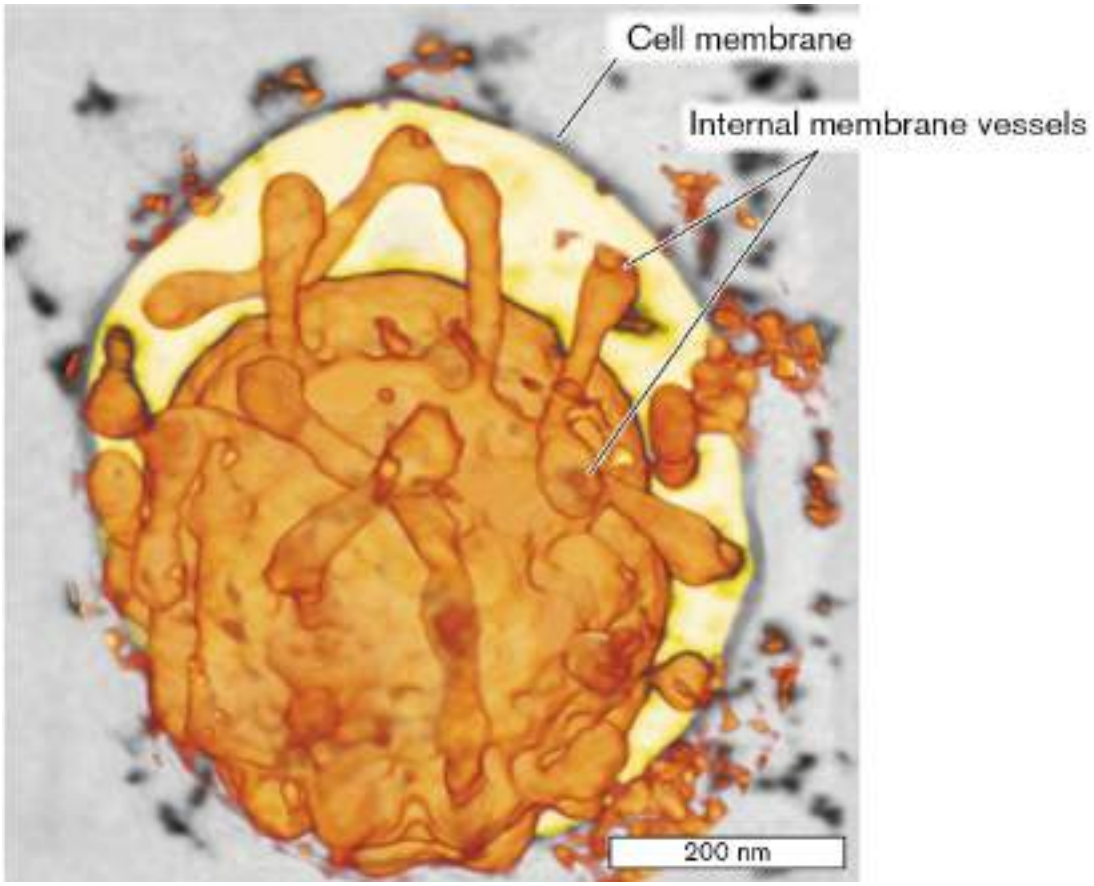


FIGURE 19.1 ■ *Ignicoccus hospitalis* , from a hydrothermal vent 600 meters below sea level, at Kolbeinsey Ridge volcano. *Ignicoccus hospitalis* has an extensive endomembrane system (orange) within an outer compartment contained by the cell membrane (yellow). Cryo-electron tomography, colorized.

COURTESY OF THOMAS HEIMERL

Other kinds of archaea proliferate throughout mesophilic soil and water and within the digestive tracts of humans and other animals. Even human skin microbiota include more than 4% archaea. Other archaea cooperatively colonize the surface of plant roots. Throughout these habitats, methanogens make a living off the H_2 and CO_2 from bacterial mixed-acid fermentation (see Chapter 13).

Do archaea more closely resemble bacteria or eukaryotes? Archaea share many metabolic traits with bacteria, such as multigene operons and the metabolic pathways of redox metabolism (discussed in Chapter 14). But archaea share with eukaryotes some core traits of DNA-RNA machinery and transcription factors. Key traits such as ether-linked membrane lipids are found mainly in archaea and in some bacteria via horizontal transfer from archaea (**Table 19.1**).

TABLE 19.1 Archaeal Traits Distinct from Bacteria and Eukaryotes

Trait	Archaea showing the trait	Alternative traits of bacteria and/or eukaryotes
Cell envelope		
Membrane lipids: isoprenoid *sn* -glycerol 1 -phosphate ethers or diethers	All archaea	Membrane lipids: *sn* -glycerol 3 -phosphate hydrocarbon diesters. Eukaryotic membranes have ~20%

Sources: O. Kandler and H. König. 1998. *Cell. Mol. Life Sci.* **54** :305–308; C. Bullock. 2000. *Biochem. Mol. Biol. Ed.* **28** :186–191.

		ether-linked lipids.
Membrane lipid chains stiffened by covalent cross-links or by pentacyclic rings	Most archaea	Chains stiffened by saturation
S-layer of glycoprotein	Crenarchaeota and Thaumarchaeota	Peptidoglycan (bacteria); cellulose and other polysaccharides (eukaryotes)
S-layer of protein, methanochondroitin, or sulfated polysaccharide	Methanogens and Haloarchaea	Peptidoglycan (bacteria); cellulose and other polysaccharides (eukaryotes)
Pseudopeptidoglycan sacculus contains talosaminuronic acid; peptide bridges contain only l-amino acids	Methanobacteriales, Methanopyrales	Peptidoglycan (bacteria); cellulose and other polysaccharides (eukaryotes)
Metabolism		

Sources: O. Kandler and H. König. 1998. *Cell. Mol. Life Sci.* **54** :305–308; C. Bullock. 2000. *Biochem. Mol. Biol. Ed.* **28** :186–191.

Nonphosphorylated intermediates of sugar catabolism and synthesis [Embden-Meyerhof-Parnas (EMP) glycolysis in some cases]	All archaea	EMP pathway of glycolysis, with phosphorylation mediated by NAD or NADP [or Entner-Doudoroff (ED) pathway]
Methanogenesis from H_2 and CO_2; or from CO, methanol, methyl sulfides, formate, or acetate	Methanogens	Anaerobic metabolism such as fermentation; no methane production
Archaeal coenzymes: cofactors F_{420} and F_{430}, and coenzyme M	Most methanogens	Flavin mononucleotide, coenzyme A, others
Retinal-associated light-driven membrane pumps for H^+ or Na^+	Haloarchaea	Chlorophyll-based photosynthesis (bacteria and eukaryotic chloroplasts)

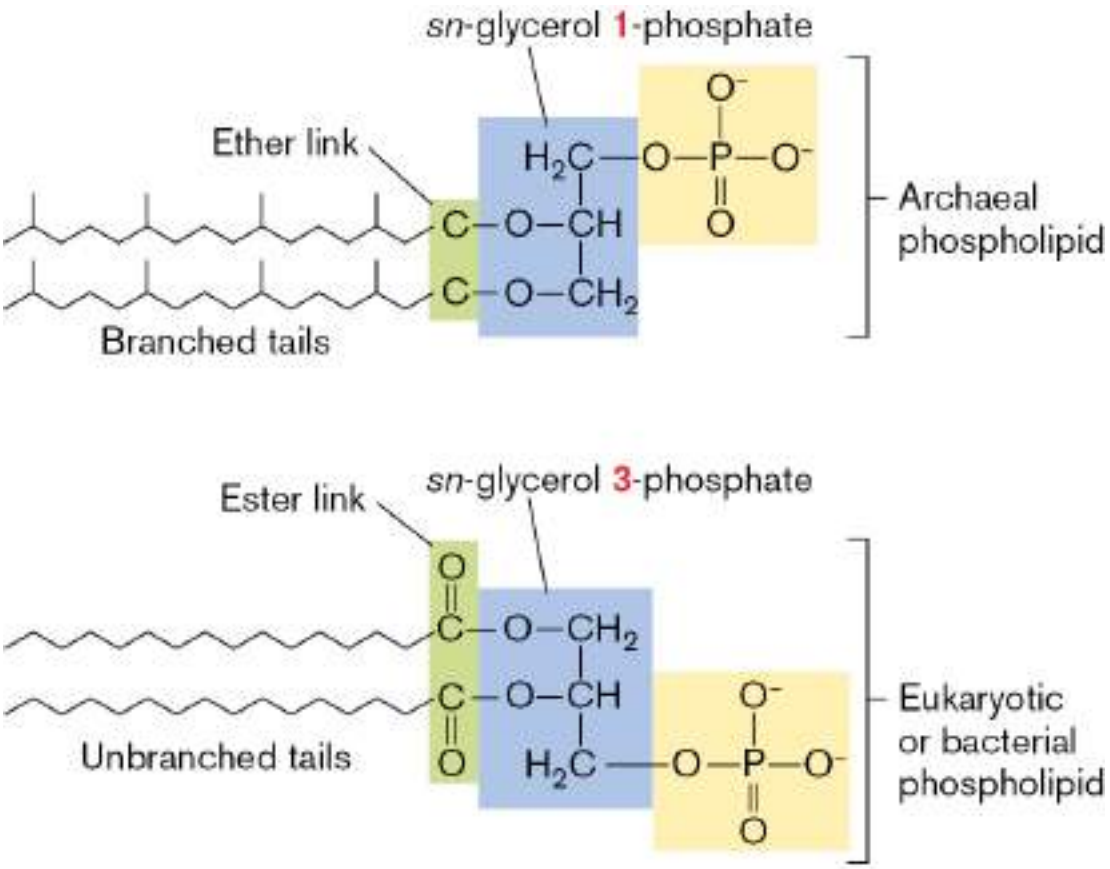
Sources: O. Kandler and H. König. 1998. *Cell. Mol. Life Sci.* **54** :305–308; C. Bullock. 2000. *Biochem. Mol. Biol. Ed.* **28** :186–191.

Nucleic acid structure and function		
Positive superturns generated by reverse gyrase, which protect DNA from extreme acid	Hyperthermophilic archaea	Negative superturns generated by gyrase
Unique base structures in tRNA, such as the guanine analog archaeosine	Most archaea	tRNA bases, such as queuosine, found only in bacteria and eukaryotes

Sources: O. Kandler and H. König. 1998. *Cell. Mol. Life Sci.* **54** :305–308; C. Bullock. 2000. *Biochem. Mol. Biol. Ed.* **28** :186–191.

Ether-Linked Isoprenoid Membranes

The most distinctive structure of archaea is their ether-linked phospholipids with methyl branches.



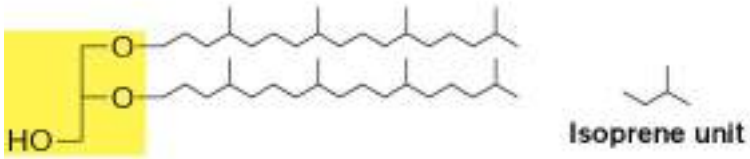
The ether linkage resists hydrolysis, compared to the mostly ester-linked lipids of bacteria and eukaryotes. In other words, it takes more energy to break the bonds in ether-linked membranes. Another major difference is that the ether linkages of archaea attach hydrocarbon chains to *sn* -glycerol **1** -phosphate, whereas bacterial and eukaryotic lipid chains are attached to *sn* -glycerol **3** -phosphate (the mirror image or enantiomer of *sn* -glycerol **1** -phosphate).

Thus, archaeal membrane lipids differ from most of those found in bacteria and eukaryotes, with the exception of a few thermophilic bacteria that obtained ether lipids horizontally from archaea in their shared high-temperature environment. Most features of archaeal lipids increase lipid stability in extreme environments such as high temperature or extreme acidity. Nevertheless, these ether lipids are also widespread in mesophilic archaea that grow at moderate temperatures.

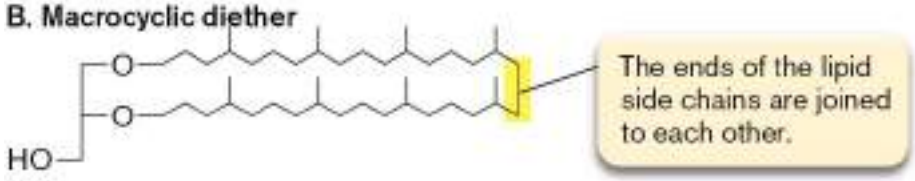
Overall, the distinctive features of archaeal ether lipids include:

- ***sn* -glycerol 1-phosphate.** Archaeal membrane lipids incorporate *sn* -glycerol **1** -phosphate (**Fig. 19.2A**), rather than the mirror-symmetrical form *sn* -glycerol **3** -phosphate, which is used by bacteria and eukaryotes. The two chiral forms show similar thermal stability, but their biochemistry requires different enzymes, and thus they represent a deep divergence in ancestry. In some archaea the glycerol is extended by six carbons, forming nonitol (nine OH groups).

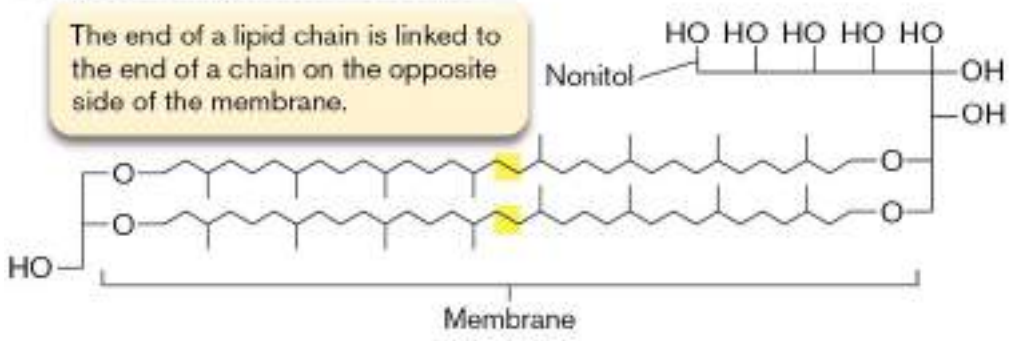
A. Diphytanylglycerol diether



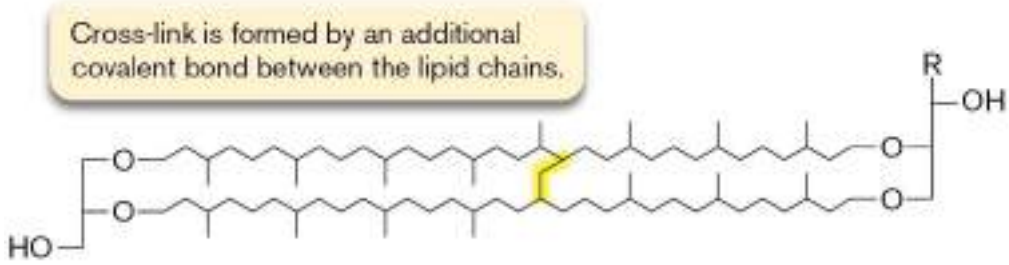
B. Macrocyclic diether



C. Glycerol dialkyl nonitol tetraether



D. Cross-linked tetraether



E. Tetraether with cyclopetane rings

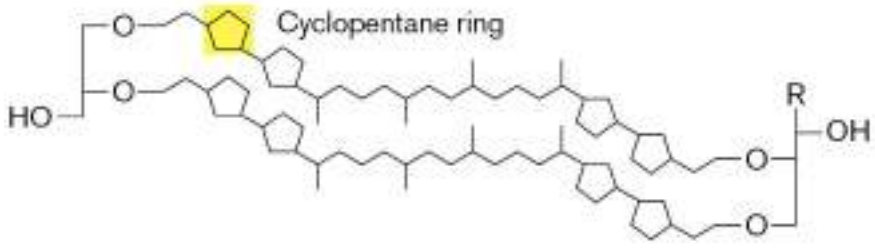


FIGURE 19.2 ■ Branched-chain ether lipids are characteristic of archaeal membranes.

Source: Modified from R. M. Daniel and D. A. Cowan. 2000. *Cell. Mol. Life Sci.* **57** :250–264.

- **Isoprenoid chains.** The side chains of archaeal lipids are branched at every fourth carbon. The methyl branches arise by condensation (C–C bond formation) of units of isoprene (**Fig. 19.2A**). Condensed isoprene chains are called isoprenoid or diphytanyl chains; thus, the overall lipid is diphytanylglycerol diether. Isoprenoid branched chains increase membrane stability by hooking each other in place.
- **Cross-linked lipids.** In some hyperthermophiles, the ends of side chains are linked covalently, either to each other (**Fig. 19.2B**) or to a lipid on the opposite side of the membrane (**Fig. 19.2C**). Two pairs of lipid chains cross-linked across the membrane form a tetraether , so called because the complex contains four ether links in all. In some cases, an additional covalent bond links the two linked pairs of side chains across the middle (**Fig. 19.2D**).
- **Cyclopentane rings.** In some archaea, the lipid's methyl branches cyclize, forming cyclopentane rings (**Fig. 19.2E**). Cyclopentane rings strengthen membranes at high temperature.

Most archaea possess a single cell membrane, without any outer membrane such as that of Gram-negative bacteria (shown in Chapter 3). Many possess no cell wall at all, only an S-layer of proteins plugged into the tetraether membrane (discussed in Section 19.2). Some archaea, such as methanogens and haloarchaea, do have a cell wall, but the structure differs fundamentally from the bacterial peptidoglycan (see Sections 19.4 and 19.5).

Similarly, many archaea possess filamentous protein structures for attachment and motility that are analogous to bacterial pili and flagella. Despite functional similarity, the archaeal protein structures are very different from those of bacteria. Archaeal flagella are called archaella (singular, **archaellum**). Archaella have rotary motors, but

they evolved independently from bacterial flagella, showing greater similarity to type IV pili.

Archaeal Gene Structure and Regulation

How do archaeal genomes compare with those of bacteria and eukaryotes? The genomes of archaea generally resemble those of bacteria in size and gene density, and genes of related function are arranged in operons, like those of bacteria. The genes encoding most archaeal proteins contain uninterrupted coding sequences, as in bacteria, but certain transfer RNA (tRNA) gene sequences are interrupted by introns (nontranslated sequences), similar to the tRNA introns found in eukaryotes.

The archaeal apparatus for DNA and RNA polymerases, transcription factors, and protein synthesis show remarkable similarity to those of eukaryotes. **Figure 19.3A** compares the components of an archaeal RNA polymerase (from *Thermococcus*) with those of a bacterium (*Escherichia coli*) and those of a eukaryote (*Saccharomyces cerevisiae*). Evidence for these models has come from many researchers, including Katie Shalvarjian and Dipti Nayak at UC Berkeley. The archaeal polymerase possesses two transcription factors (regulatory protein components) that are found in eukaryotes: TATA-binding protein (TBP, a subunit of transcription factor II D, TFIID) and transcription factor II B (TFIIB, which in archaea is designated TFB). By contrast, bacteria have no homologs of these factors. A consequence of the eukaryotic-like transcription and translation in archaea is that archaea are resistant to antibacterial antibiotics that target transcription and translation.

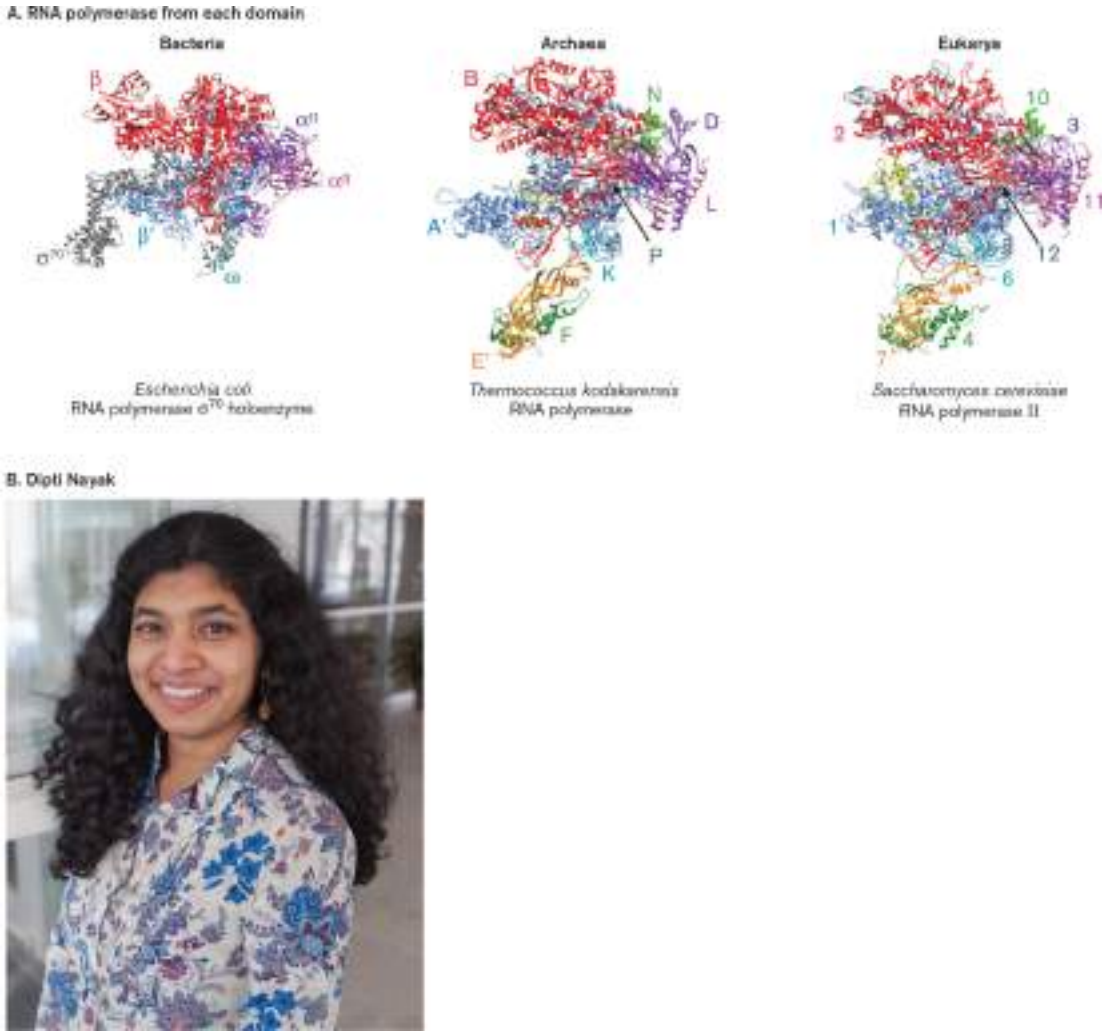


FIGURE 19.3 ■ Genetic enzyme complexes of Archaea resemble those of Eukarya. A. Structural overview of RNA polymerase (RNAP) derived from a bacterium (*Escherichia coli* , PDB ID: 4YG2); an archaeon (*Thermococcus kodakarensis* , PDB ID: 4QIW); and a eukaryote (polymerase II from *Saccharomyces cerevisiae* , PDB ID: 1WCM). Color indicates orthologous subunits of RNAP shared by the different organisms. **B.** Dipti Nayak leads research on the molecular biology of archaea at UC Berkeley.

K. E. SHALVARJIAN ET AL. 2021. *CURR OPIN MICROBIOL* . **60** :8–15
DIPTI NAYAK

Another eukaryotic structure for which archaeal homologs were discovered is that of histones , the fundamental packaging proteins

of DNA. The histone complex found in eukaryotic chromosomes contains a histone $(H3 + H4)_2$ tetramer flanked by two histone (H2A + H2B) dimers. The archaeal chromosome complex consists solely of $(H3 + H4)_2$ homologs. Histone homologs pack the DNA for many kinds of archaea, including *Sulfolobus* , *Thermococcus* , and methanogens.

A unique feature of archaeal DNA function is the reverse gyrase enzyme. Reverse gyrase adds superturns to overwind the DNA helix. By contrast, all bacteria and eukaryotes, as well as mesophilic archaea, have gyrase to form negative superturns that maintain their DNA in an "underwound" supercoiled state (presented in Chapter 7). But hyperthermophilic archaea with reverse gyrase maintain positive supercoiling to stabilize their DNA. **Figure 19.4** shows a model for the reverse gyrase mechanism and includes data from Taisaku Ogawa and colleagues at Waseda and Nagoya Universities, Japan. The enzyme catalyzes DNA strand breakage and then passes the complementary strand through the gap, adding a helical turn. To finalize the added turn, the broken strand is ligated.

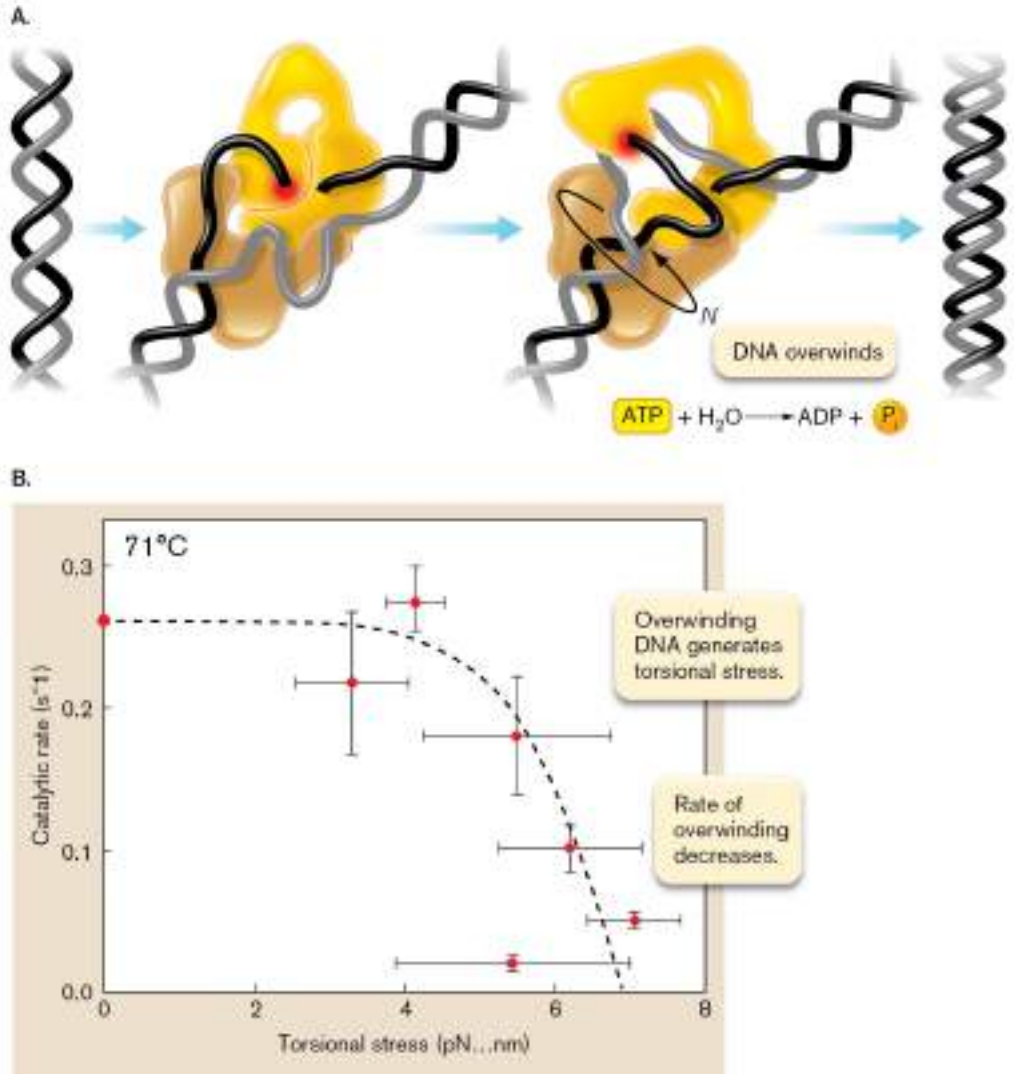


FIGURE 19.4 ■ Reverse gyrase overwinds DNA. A. The reverse gyrase catalyzes DNA strand breakage, then passes the complementary strand through the gap, adding a helical turn. The broken strand is ligated. Overall the reaction consumes one molecule of ATP. **B.** Catalysis of turn addition at 71°C. Adding helical turns increases torsional stress and slows the rate of catalysis. N = newtons.

Source: Taisaku Ogawa et al. 2016. *FEBS J.* **283** :1372.

Adding a twist that overwinds DNA causes torsional stress and therefore requires energy provided by ATP hydrolysis. The positive

superturns decrease melting of DNA and thus prevent the helix from coming apart at high temperature. Reverse gyrase originated in Archaea, though it was transferred horizontally to some bacterial hyperthermophiles such as *Thermotoga* (discussed in Chapter 18).

Another unique feature of archaeal genetics is their modified bases in tRNA. In particular, the guanosine analog archaeosine (7-formamidino-7-deazaguanosine) occurs in tRNA of nearly all archaea, but not in tRNA of any bacteria or eukaryotes. Other unusual tRNA bases, such as queuosine, are found only in bacteria and eukaryotes, not in archaea.

Phylogeny of Archaea

What are the major kinds of Archaea? The answer to this question keeps changing, as DNA sequencing of uncultured organisms reveals a growing number of previously unknown phyla (**Fig. 19.5**). Two major advances in genomic analysis have enabled this remarkable pace of discovery, as described by Thijs Ettema (Uppsala University, Sweden) and colleagues.

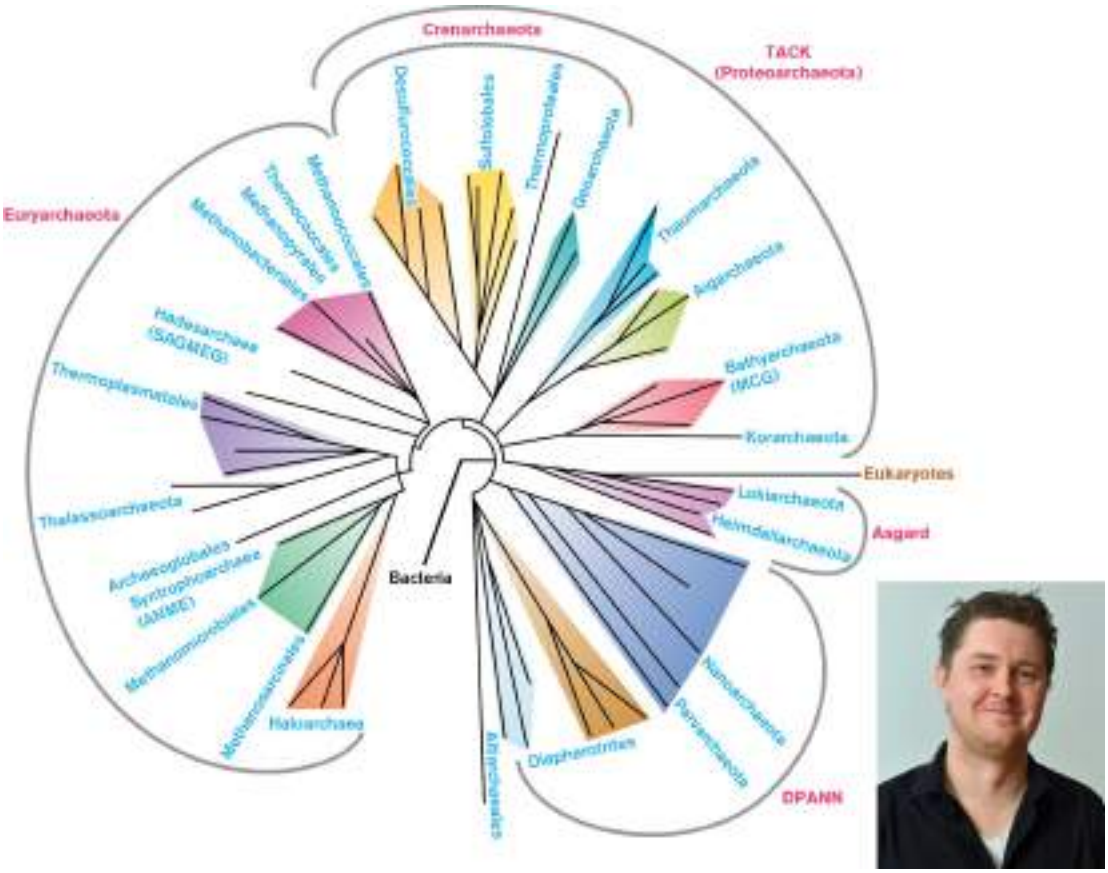


FIGURE 19.5 ■ Archaeal phylogeny. Major superphyla, phyla, and orders of Archaea. Divergence is based approximately on small-subunit ribosomal RNA (SSU rRNA) and genome sequences. (See Table 19.2 for abbreviations.) **Inset:** Thijs Ettema.

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The first major advance in phylogenetics was the assembly of metagenomes (the genome sequences of a microbial community) from mixed environmental samples (discussed in Chapter 21). Metagenomes enable construction of "genomic bins" that approximate the genomes of uncultured organisms. These genomic bins enable comparison of entire small-subunit ribosomal RNA (SSU rRNA) genes and ribosomal operons encoding ribosomal proteins and transfer RNAs, as pioneered by Jillian Banfield's lab at UC Berkeley. Ribosomal protein operons and other well-conserved

marker genes expand the volume of data available from SSU rRNA comparison, improving the resolution of taxonomic distances.

The second advance was the sequencing of single-cell genomes, most prominently developed at the Bigelow lab in Maine and by Tanja Woyke at the Joint Genome Institute of the U.S. Department of Energy. When a sequence is obtained from a single cell, it may be more fragmented than a genomic bin (Chapter 21), but we know that the entire sequence comes from one individual, not an artifactual hybrid. Thus, for example, we can see when an organism possesses multiple metabolic capabilities, such as the ability of some marine archaea to combine autotrophic and heterotrophic lifestyles.

Major archaeal taxa are outlined in **Table 19.2** . As of this writing, the clades labeled in blue in **Figure 19.5** are the most commonly studied.

TABLE 19.2	Archaeal Diversity [a]

TACK (Thaumarchaeota, Aigarchaeota, Crenarchaeota, Korarchaeota).

Aigarchaeota. Aerobic hyperthermophilic filaments in hot springs.

Bathyarchaeota (formerly Miscellaneous Crenarchaeote Group; MCG). Widely divergent autotroph-heterotrophs in diverse soil and water.

Korarchaeota. Anaerobic hyperthermophiles in Yellowstone hot springs and in deep-sea thermal vents.

Thaumarchaeota. Tetraether membranes include crenarchaeol. Marine and soil archaea that oxidize NH_3 with O_2. Important marine sources of nitrates for phytoplankton, and of methylphosphonate ($CH_3-PO_3^{2-}$), which bacteria convert to methane.

- **Cenarchaeales.** Sponge symbionts; grow at 10°C. *Cenarchaeum symbiosum* .
- **Nitrosopumilales.** Ammonia-oxidizing archaea (AOA), marine and soil. *Nitrosopumilus maritimus* , *Nitrososphaera gargensis* .
- **Psychrophilic marine thaumarchaeotes (uncharacterized).** Marine water, deep sea, and Antarctica. Anaerobic heterotrophs, sulfate reducers, nitrite-reducing methanotrophs.

Thermoproteota. Most have tetraether membranes surrounded by S-layer. Includes thermophiles in hot springs and marine vents; also marine mesophiles and

psychrophiles. **Crenarchaeota** is an alternative name encompassing Desulfurococcales, Sulfolobales, Thermoproteales, Geoarchaeota.

- **Caldisphaerales.** Thermoacidophilic heterotrophs that grow in hot springs. *Caldisphaera* spp.
- **Desulfurococcales.** Anaerobic sulfur reduction with organic electron donors. Irregularly shaped cells with glycoprotein S-layer; no cell wall (*Aeropyrum pernix* , *Desulfurococcus fermentans*). *Ignicoccus islandicus* . *Pyrodictium abyssi* , *Pyrodictium occultum* , *Pyrolobus fumarii* grow at marine thermal vents, up to 110°C.
- **Geoarchaeota.** Acidic thermal mats.
- **Sulfolobales.** Aerobic acidophiles; moderate thermophiles. Oxidize H_2S to H_2SO_4 . *Sulfolobus* , *Sulfurisphaera* , *Acidianus* .
- **Thermoproteales.** *Pyrobaculum* , *Thermoproteus* , *Vulcanisaeta* .

Euryarchaeota. Metabolism includes methanogenesis, halophilic photoheterotrophy, and sulfur and hydrogen oxidation; acidophiles and alkaliphiles. Methanogens and halophiles have rigid cell walls.

Anaerobic Methane-Oxidizing Euryarchaeota (ANME). Found in anoxic marine sediments. Oxidize methane from methanogens, in syntrophy with sulfate-reducing bacteria.

Archaeoglobales. Hyperthermophiles; sulfate oxidation of H_2 or organic hydrogen donors; reverse methanogenesis. *Archaeoglobus fulgidus* .

Hadesarchaea. Formerly South African Gold Mine Miscellaneous Euryarchaeotic Group (SAGMEG). Found in water from gold mines deep underground.

Haloarchaea. Halophiles; grow in brine (concentrated NaCl). Conduct photoheterotrophy by light-driven H^+ pump and Cl^- pump. *Haloarcula* , *Halobacterium* , *Haloferax* grow in salterns and salt lakes. *Haloquadra* (*Haloquadratum*) are square-shaped. *Halorubrum lacusprofundi* is an Antarctic psychrophile. *Natronococcus* spp. are alkaliphiles in soda lakes.

Methanogens (many classes). Generate methane from CO_2 and H_2 , formate, acetate, other small molecules; strict anaerobes. Pseudopeptidoglycan or sulfated chondroitin cell walls. Grow in anaerobic soil, water, or animal digestive tracts. Deep-sea psychrophiles generate methane hydrates.

- **Methanobacteriales.** Lack cytochromes; reduce CO_2 , formate, or methanol with H_2 by electron bifurcation. *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* inhabit human digestive tract.
- **Methanomicrobiales, Methanococcales, and Methanopyrales.** Lack cytochromes; reduce CO_2 , formate, or methanol with H_2 by electron bifurcation. *Methanocaldococcus jannaschii* , vent thermophile.
- **Methanosarcinales.** Includes acetoclastic methanogens.

Thermococcales. Hyperthermophiles (grow above 100°C) and barophiles (up to 200 atm pressure). Anaerobes; reduce sulfur. *Thermococcus* , *Pyrococcus abyssi* , *P. furiosus* .

Thermoplasmata. Extreme acidophiles; oxidize sulfur from pyrite (FeS_2), generating sulfuric acid. Mesophiles or moderate thermophiles.

- **Thermoplasmatales.** *Ferroplasma acidiphilum* and *F. acidarmanus* grow at 37°C–50°C. Oxidize sulfur from FeS

$_2$, generating ambient pH as low as pH 0. No cell wall. *Thermoplasma acidophilum* grows at 59°C and pH 2.
Asgard. Vent thermophiles named for Norse gods. Molecular features such as tubulin suggest shared ancestry with Eukarya.
Heimdallarchaeota. Formerly Ancient Archaeal Group (AAG).
Lokiarchaeota. Isolated from Loki's Castle hydrothermal vent, off coast of Norway. Formerly called Deep Sea Archaeal Group (DSAG) or Marine Benthic Group B (MBGB).
DPANN (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaeota). Diminished genomes and metabolic capabilities suggest obligate symbionts.
Nanoarchaeota. Vent hyperthermophiles; obligate symbionts attached to *Ignicoccus* . *Nanoarchaeum equitans* .
Altiarchaeales. Form grappling-hook biofilms in cold sulfidic water. *Altiarchaeum* . The position of Altiarchaeales within DPANN is uncertain.

For several reasons, the phylogeny of Archaea is a challenge to define. Most archaea are uncultured and are known solely through metagenomic bins. Many of their genomes are highly "recombinogenic." For example, two different samples of *Ferroplasma acidarmanus* show 99% identical SSU rRNA, yet their overall genomes differ by 22%, implying extensive horizontal gene exchange. And many archaeal genomes, particularly those of mesophiles, include large portions of DNA transferred from bacteria. Microbial ecologist Purificación López-García, at Paris-Sud University,

proposes that hyperthermophilic archaeal species evolved into mesophiles by acquiring genes from bacteria. According to her model, the bacterial genes provided key metabolic pathways and cell structures optimized for growth at moderate or cold temperatures.

TACK Superphylum

The TACK superphylum is named for four taxa: T haumarchaeota (or Nitrososphaerota), A igarchaeota, C renarchaeota (or Thermoproteota), and K orarchaeota (**Fig. 19.5** and **Table 19.2**). TACK includes many taxa that grow at temperatures above 90°C. Most TACK hyperthermophiles were discovered at deep-sea marine hydrothermal vents or at hot springs such as those of Yellowstone National Park. Major clades (designated orders) include Desulfurococcales , sulfur-reducing anaerobes at marine hydrothermal vents; Sulfolobales , sulfide-oxidizing acidophiles at hot springs; Thermoproteales , vent thermophiles; and Geoarchaeota , found in acid iron mats.

Many TACK thermophiles metabolize sulfur, either by anaerobic reduction (such as by H_2 to form H_2S) or by aerobic oxidation (by O_2 to form sulfuric acid). Anaerobic sulfur metabolizers include moderate thermophiles (growth range about 60°C–80°C) as well as hyperthermophiles (90°C–120°C). Many of the hyperthermophiles are also barophiles, growing under high pressure at hydrothermal vents on the ocean floor; an example is *Pyrodictium abyssi* .

Besides thermophiles, the TACK clades include a substantial proportion of mesophilic soil, marine, and benthic (marine sediment) microbial communities. Many are found in the microbiomes of plants and animals. Understanding these organisms is important for assessing their contribution to the global carbon cycle. The Bathyarchaeota (formerly Miscellaneous Crenarchaeote Group; MCG) include vast populations throughout the open ocean. The Thaumarchaeota are ammonia-oxidizing archaea (AOA), which oxidize ammonia to nitrite. These ammonia oxidizers play a major

role in the nitrogen cycle (discussed in Chapters 21 and 22). Other Thaumarchaeota include mesophilic heterotrophs and sulfur oxidizers in soil and water. Yet other TACK organisms are psychrophiles (growing at temperatures below 20°C) found in Antarctic lakes. Overall, the TACK superphylum spans the widest range of growth temperatures of any division of life.

Euryarchaeota: Methanogens, Halophiles, and Thermophiles

The superphylum Euryarchaeota also includes members throughout soil and water, and associated with plants and animals. The most highly divergent group of Euryarchaeota is methanogens, including several polyphyletic clades (clades not sharing a single common ancestor). Methanogens serve a key energetic role in ecosystems by offering an anaerobic mechanism for removing excess H_2 and other small-molecule reductants. But their metabolism releases methane —a potent greenhouse gas. The accelerating release of methane from Arctic tundra and from marine benthic sources has drastic consequences for our global climate (discussed in Section 19.4 and Chapter 22).

Another branch of euryarchaeotes is the Haloarchaea, extreme halophiles that are the only form of life to grow in concentrated brine (NaCl). Most Haloarchaea, such as *Halobacterium* species NRC-1, are photoheterotrophs that can supplement their metabolism with retinal-containing light-driven ion pumps, called bacteriorhodopsin . These ion pumps (for protons or for sodium ions) are the only known form of phototrophy in archaea (discussed in Chapter 14). Bacteriorhodopsins are found in Haloarchaea such as *Halobacterium halobium* ; the species was named before it was known to be an archaeon (discussed in Section 19.5). It turns out that Haloarchaea have transferred genes encoding light-absorbing proton pumps into many marine bacteria. By contrast, the chlorophyll-based phototrophy found in bacteria and plants is completely unknown in archaea.

Note: In **Table 19.2** , certain archaeal names contain a "bacteria" component; for example, the genus *Halobacterium* . Such organisms were known and named as bacteria before 1977, when the category "archaea" was first defined.

Euryarchaeotes include extreme acidophiles, as well as extreme alkaliphiles—from *Ferroplasma* (Thermococcales), growing at pH 0, to *Natronococcus* , growing at pH 10. With respect to pH, the euryarchaeotes show the widest range of any taxonomic group.

Unique metabolic pathways. Some archaea use distinctive metabolic pathways. TACK archaea catabolize glucose via several variants of the Entner-Doudoroff (ED) and Embden-Meyerhof-Parnas (EMP) pathways that rarely occur in bacteria (**Fig. 19.6**). For example, the sulfur thermophiles *Sulfolobus* and *Thermoplasma* convert glucose to gluconate without phosphorylation, ultimately generating pyruvate with no net production of ATP. (ATP is still produced by further breakdown of pyruvate.)

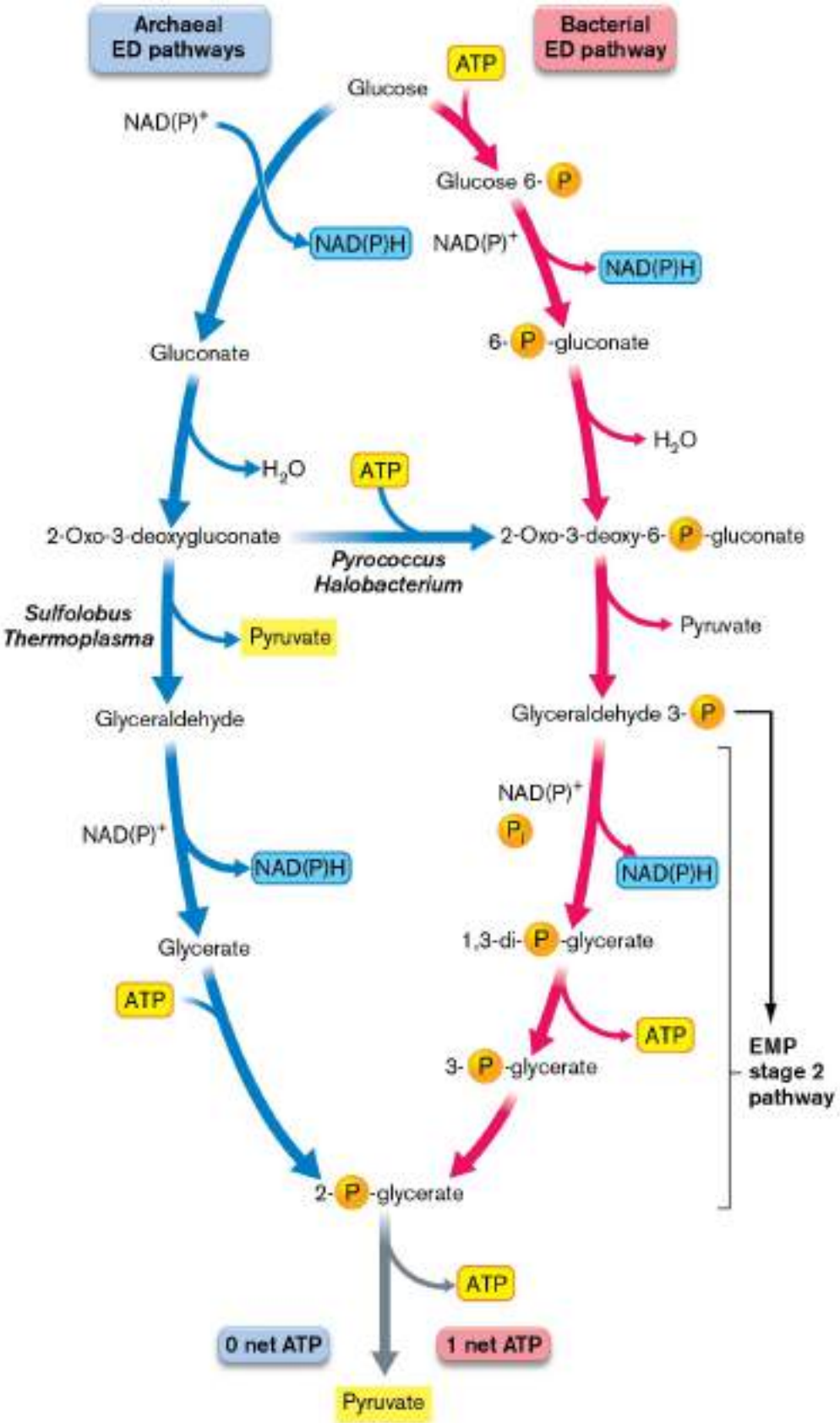

Archaeal ED pathways
Bacterial ED pathway
Glucose
ATP
NAD(P)+
Glucose 6-P
NAD(P)H
NAD(P)+
NAD(P)H
Gluconate
6-P-gluconate
H2O
H2O
ATP
2-Oxo-3-deoxygluconate
2-Oxo-3-deoxy-6-P-gluconate
Pyrococcus
Halobacterium
Sulfolobus
Thermoplasma
Pyruvate
Pyruvate
Glyceraldehyde
Glyceraldehyde 3-P
NAD(P)+
Pi
NAD(P)+
NAD(P)H
NAD(P)H
Glycerate
1,3-di-P-glycerate
ATP
ATP
3-P-glycerate
EMP
stage 2
pathway
2-P-glycerate
ATP
0 net ATP
1 net ATP
Pyruvate

FIGURE 19.6 ■ Glucose catabolism in archaea. Left: *Sulfolobus* and *Thermoplasma* species catabolize glucose to pyruvate via a modified ED pathway without phosphorylating glucose and produce no net ATP. **Right:** *Halobacterium* species phosphorylate 2-oxo-3-deoxygluconate and produce one net ATP via the EMP stage 2 pathway. *Pyrococcus furiosus* oxidizes glyceraldehyde 3-phosphate using ferredoxin instead of NAD^+ and avoids phosphorylation.

Among the Euryarchaeota, halophilic archaea such as *Halobacterium* phosphorylate the dehydrated product of gluconate (2-oxo-3-deoxygluconate), which enters the "standard" ED pathway. The ED pathway generates one molecule of pyruvate and one molecule of 3-phosphoglycerate, which produces one net ATP through the second stage of the EMP pathway. This variant may be compared with different variants of the EMP pathway seen outside the TACK group, in the euryarchaeotic vent thermophile *Pyrococcus furiosus* . *P. furiosus* oxidizes glyceraldehyde 3-phosphate using ferredoxin instead of NAD^+ and avoids phosphorylation. The reduced ferredoxin is then used to reduce $2H^+$ to H_2 in an energy-yielding reaction. Nevertheless, *P. furiosus* performs highly effective metabolism, and it can power a fuel cell that runs at near-boiling temperature (eResearch Activity 19).

Asgard, Eukaryotes, and DPANN

A fascinating clade of deep-sea archaea, the Lokiarchaeota , branch from a marine benthic microbial community. First identified at a thermal vent named Loki's Castle, the Lokiarchaeota genomes share surprising traits with eukaryotes. Several related phyla have been described and given similar Norse mythological names, such as the Heimdallarchaeota (formerly Ancient Archaeal Group; AAG). These clades are collectively known as the Asgard superphylum.

Eukaryotic-like genes found in Asgard archaea include those associated with an actin cytoskeleton and phagocytosis. These features, and phylogenetic trees, imply that the ancestor of all Eukarya—which includes protists, fungi, and plants and animals—branched relatively late from the Asgard line of Archaea. For further discussion, see Section 19.6 .

Another vast superphylum revealed by metagenomics is DPANN (named for the phyla D iapherotrites, P arvarchaeota, A enigmarchaeota, N anoarchaeota, and N anohaloarchaeota). The genomes of these organisms all show evolutionary loss of many genes, typical of obligate symbionts. The most studied example is the Nanoarchaeota species *Nanoarchaeum equitans* , a cellular parasite of *Ignicoccus hospitalis* (see Section 19.6).

Meanwhile, sequencing of metagenomes and single-cell genomes continues to reveal new deeply branching phyla with unexpected traits. For example, the Altiarchaeales clade includes *Altiarchaeum hamiconexum* , a soil archaeon that forms networked biofilms connected by harpoon-like appendages. As of this writing, the taxonomic position of Altiarchaeales (within DPANN or outside) remains in dispute.

Thought Question

19.1 Suppose that two deeply diverging clades each show a wide range of growth temperature. What does this suggest about the evolution of thermophily or psychrophily?

To Summarize

- **Archaeal membranes are composed of *sn* -glycerol 1-phosphate diether or tetraether lipids** having isoprenoid side chains that may include cross-links or pentacyclic rings. The membrane may be covered by a protein S-layer, but no cell wall. A few clades of Archaea possess a cell wall of pseudopeptidoglycan.
- **Glucose is catabolized by variants of the Entner-Doudoroff pathway.** Other metabolic pathways found in archaea include methanogenesis and retinal-associated light-driven ion pumps such as bacteriorhodopsin.
- **Central gene functions of archaea resemble those of eukaryotes.** These include the structure of DNA and RNA polymerases and of histone-like DNA-binding proteins.
- **Reverse gyrase overwinds DNA.** Reverse gyrase is unique to archaea and to a few bacteria that obtained it by horizontal gene transfer.
- **The TACK superphylum includes hyperthermophiles, sulfur reducers and oxidizers, and Thaumarchaeota ammonia oxidizers.** TACK organisms grow across the widest range of temperatures.
- **Euryarchaeota include methanogens and halophiles.** Methanogens reduce CO_2 and fermentation acids to methane, adding to climate change.
- **The deep-sea Asgard superphylum of hyperthermophiles may include the archaeal ancestor of all eukaryotes.**
- **Metagenomics reveals uncultured organisms.** We continue to discover deeply branching clades of archaea by sequencing metagenomes.

Glossary

isoprenoid

A condensed isoprene chain, found in archaeal membrane lipids.

tetraether
: A molecule containing four ether links. An example is found in archaeal membranes, when two lipid side chains form ether linkages with a pair of side chains from the other side of the bilayer.

archaellum *pl.* archaella
: A rotary complex for motility in archaea, analogous to the bacterial flagellum.

histone
: A protein that binds eukaryotic DNA and compacts chromosomes in nucleosomes.

reverse gyrase
: An enzyme that adds positive superturns to DNA in addition to the fundamental twist of the DNA helix. Found mainly in archaea, reverse gyrase differs from the gyrase of bacteria and eukaryotes, which adds negative superturns, against the twist.

Desulfurococcales
: A phylum of thermophilic archaea in the TACK superphylum that metabolize sulfur and organic compounds.

Sulfolobales
: A phylum of thermophilic archaea in the TACK superphylum that includes sulfur oxidizers.

Thermoproteales
: A clade of the phylum Thermoproteota, thermophilic archaea including hyperthermoacidophiles, in the TACK superphylum.

Geoarchaeota
: A clade of Thermoproteota, thermophilic archaea including mats at thermal vents, in the TACK superphylum.

Bathyarchaeota
: A phylum of thermophilic archaea in the TACK superphylum, widely distributed in anoxic soil, water, and marine sediments.

Thaumarchaeota
: A phylum of archaea in the TACK superphylum that includes ammonia oxidizers and symbionts of marine invertebrates

Euryarchaeota

A major division of Archaea, containing methanogens, halophiles, acidophiles, and thermophiles, as well as soil archaea that are not extremophiles.

bacteriorhodopsin
: A haloarchaeal membrane-embedded protein that contains retinal and acts as a light-driven proton pump; it is homologous to the bacterial proteorhodopsin.

Lokiarchaeota
: A phylum in the Asgard superphylum of Archaea that contains deep-sea thermophiles isolated from a thermal vent named Loki's Castle. Formerly known as the Deep-Sea Archaeal Group or Marine Benthic Group B (DSAG or MBGB).

Nanoarchaeota
: A phylum in the DPANN superphylum of Archaea that includes very small cells, which are obligate symbionts of vent hyperthermophiles.

Endnotes

1. Note a: Blue shade indicates superphylum. Return to reference a

19.2 TACK Hyperthermophiles Eat Sulfur

As noted earlier, the TACK acronym (**Table 19.2**) designates a superphylum including four clades identified as Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota. Added branches such as Geoarchaeota and Bathyarchaeota continue to be discovered. Many TACK organisms are hyperthermophiles found in hot springs or in undersea hydrothermal vents.

Hot Springs

Thermophilic archaea commonly grow in hot springs and geysers such as those of Yellowstone National Park (**Fig. 19.7**) or the Solfatara volcanic area near Naples, Italy. A hot spring occurs where water seeps underground above a magma chamber, which heats the water to near boiling. The heated water expands and is forced upward through fissures, coming out in a heated spring. In a geyser, the water is heated under pressure. As the water escapes upward, it turns into steam, which expands and jets upward, falling into a heated pool. These heated pools and their surrounding edges generate extreme ranges of temperature, mineral content, and acidity. They support a diverse range of microbial life, including thermophilic cyanobacteria and firmicutes, as well as archaea.



FIGURE 19.7 ■ Thermophiles colonize a hot spring. Morning Glory Pool, a hot spring in Yellowstone National Park, supports thermophilic archaea and bacteria.

HSUEH-YI CHEN/ALAMY STOCK PHOTO

Several features of hot springs and geysers are important for thermophiles:

- **Reduced minerals.** The heated water dissolves high concentrations of sulfides and other reduced minerals. When the water emerges and cools, the minerals precipitate. These reduced minerals serve as rich energy sources for autotrophs.
- **Low oxygen content.** At higher temperatures, the oxygen concentration of water declines. Therefore, hyperthermophiles tend to be anaerobic, although there are important exceptions, such as the aerobic sulfur oxidizer *Sulfolobus* .
- **Steep temperature gradients.** The temperature of the water falls dramatically within a short distance from the source, forming a steep gradient. Different species of thermophiles are adapted to different temperatures and grow in separate patches at the different temperatures, causing a variegated pattern.

- **Acidity.** Some hot-spring environments show extreme acidity. The acidity results from oxidation of sulfur or iron in reactions that generate strong inorganic acids, such as sulfuric acid (H_2SO_4).

A special subcategory of volcanic hot-spring habitats is that of submarine hydrothermal vents on the ocean floor. The vent thermophiles must evolve to grow at high pressure under several kilometers of ocean. Pressure increases by approximately 100 atm per kilometer of ocean depth. Organisms that grow only at high pressure are called barophiles (discussed in Chapter 5).

Desulfurococcales: Reducing Sulfur

In the TACK Crenarchaeota group, the Desulfurococcales archaea show distinctive cell structures and forms of metabolism (**Table 19.3**). All possess a membrane with a combination of diethers and tetraethers, surrounded by an elaborate S-layer. Many take advantage of the high temperatures that increase the thermodynamic favorability of sulfur redox reactions. An example is *Desulfurococcus fermentans* (**Fig. 19.8**), a motile coccoid cell with archaella, isolated from hot springs. *D. fermentans* grows optimally at 85°C. The species respires anaerobically by reducing elemental sulfur (S^0) to sulfide (HS^-). (Anaerobic respiration was discussed in Chapter 14 .) Sulfur reduction is coupled to oxidation of small organic molecules such as sugars.

TABLE 19.3 TACK Hyperthermophiles

Representative species	Growth temperature (°C)	Growth pH	Cell shape	Metabolism
Aeropyrum pernix	70–100°C	pH 5–9	Cocci	O_2 respiration
Desulfurococcus fermentans	78–87°C	pH 6	Motile cocci with archaella	Anaerobic S^0 respiration or fermentation

TABLE 19.3	TACK Hyperthermophiles			
Representative species	**Growth temperature (°C)**	**Growth pH**	**Cell shape**	**Metabolism**
Ignicoccus islandicus	70–98°C	pH 5–7	Cocci with periplasmic space	Anaerobic lithotrophy, S^0 oxidation of H_2
Pyrodictium abyssi	80–110°C	pH 5–7	Disks linked by cannulae	Anaerobic oxidation of H_2 by S^0 or $S_2O_3^{2-}$ or fermentation
Sulfolobus solfataricus	50–87°C	pH 2–4	Irregular cocci	O_2 respiration on S^0, producing H_2SO_4
Thermosphaera aggregans	65–90°C	pH 5–7	Motile cocci in aggregates	Anaerobic fermentation

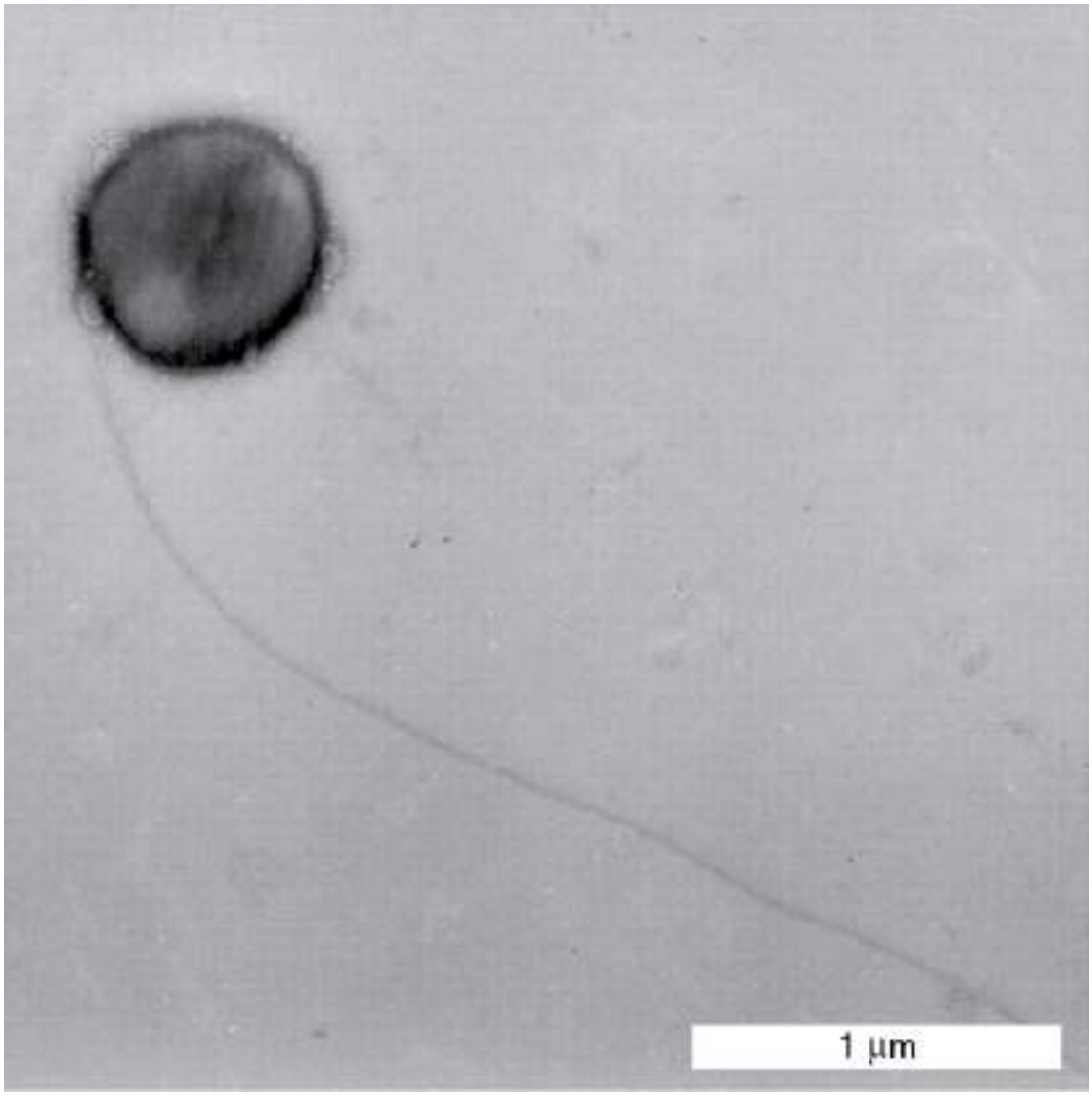


FIGURE 19.8 ■ TACK hyperthermophile. *Desulfurococcus fermentans* (stain shadow, TEM).

A. A. PEREVALOVA ET AL. 2005. *INT. J. SYST. EVOL. MICROBIOL.* **55** :995–999

Another coccoid cell, *Ignicoccus hospitalis* , introduced earlier (Fig. 19.1), has an outer membrane surrounding its cytoplasmic membrane, with a large aqueous compartment between them. This outer compartment suggests a model for an intermediate stage of evolution of the eukaryotic nucleus. *I. hospitalis* , unlike *Desulfurococcus* , is a marine organism, growing at temperatures as high as 90°C. It is a lithotroph, oxidizing hydrogen with sulfur:

$$H_2 + S^0 \longrightarrow H_2S$$

Most of the cultured species of Desulfurococcales are obligate anaerobes. An exception is *Aeropyrum pernix* , one of the first archaea to have its genome sequenced. *A. pernix* is an aerobic heterotroph, respiring with O_2 on complex compounds during growth at 70°C–100°C.

While *Desulfurococcus* species are motile, other TACK hyperthermophiles form dense biofilms. *Thermosphaera aggregans* forms colonies so tightly bound that they cannot be dissociated by protease treatment or sonication.

Thought Question

19.2 What might be the advantages of archaellar motility for a hyperthermophile living in a thermal spring or in a black smoker vent? What would be the advantages of growth in a biofilm?

Sulfolobales: High Temperature and Extreme Acid

Some archaea grow in extreme acid, as well as high temperature. Organisms that grow under multiple conditions considered "extreme" are called "polyextremophiles." The phylum Sulfolobales includes species that respire by oxidizing sulfur (instead of reducing it as *Desulfurococcus* does). These organisms, such as *Sulfolobus* species, grow at 80°C–90°C within hot springs and solfataras (volcanic vents that emit only gases). Ken Stedman and colleagues at Portland State University study *Sulfolobus solfataricus* , a species that grows at 80°C and pH 2 (**Fig. 19.9**). Unlike most archaea, *S. solfataricus* is readily cultured in the laboratory, and thus many interesting traits have been documented.

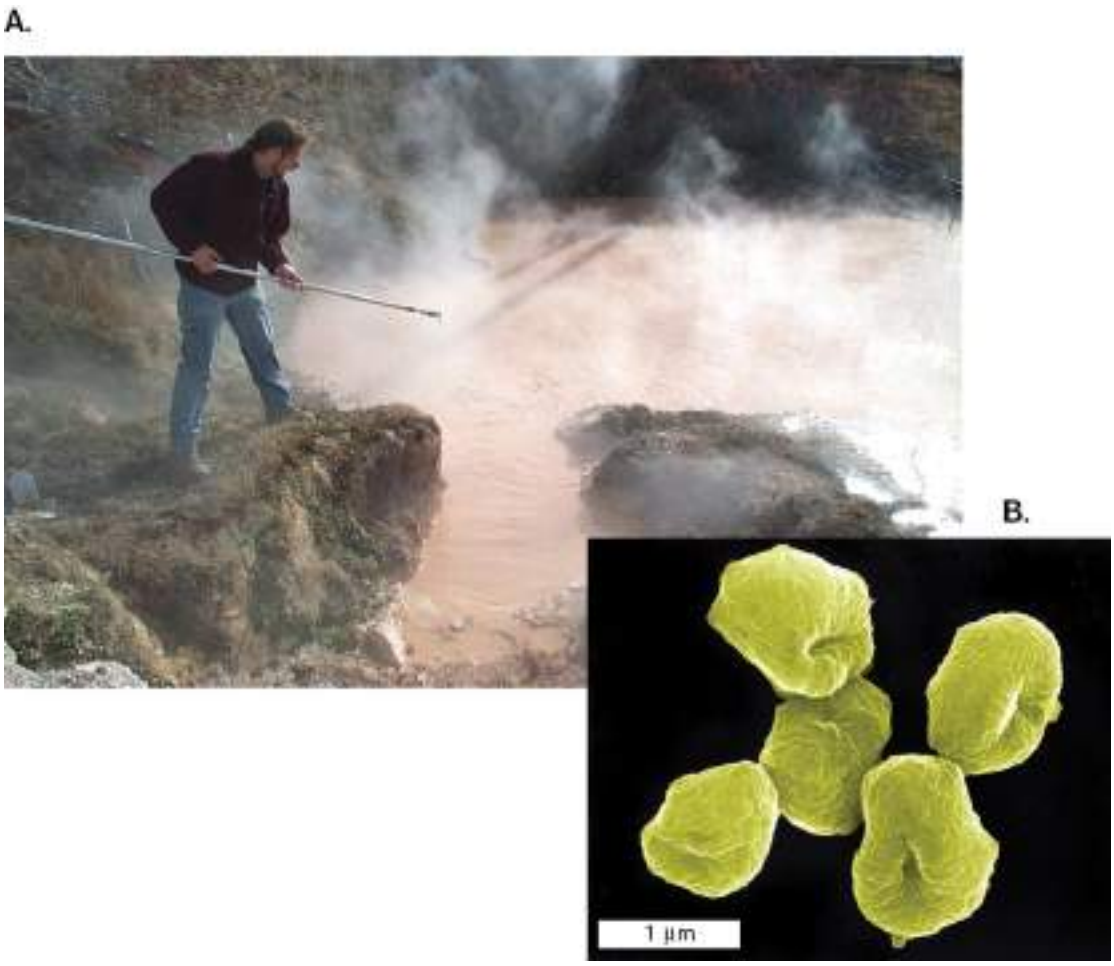


FIGURE 19.9 ■ Isolating *Sulfolobus* at Yellowstone. A. A researcher holds a collecting tube at length to obtain samples from a steaming spring, Rabbit Creek, at Yellowstone National Park. **B.** *Sulfolobus* species grow at 80°C at pH 2–3.

COURTESY OF KEN STEDMAN
EYE OF SCIENCE/SCIENCE SOURCE

Cell membrane and S-layer. *Sulfolobus* cells have a membrane composed mainly of tetraethers that join the two membrane leaflets into one; the membranes are further stiffened by forming cyclic pentanes (Fig. 19.2E). Tetraether membranes are common in acidophilic thermophiles, probably because they are exceptionally impermeable to protons. The number of cyclic pentanes can vary with temperature, pH, and energy stress; in this way, the cell adjusts its membrane fluidity.

Like most hyperthermophiles, *Sulfolobus* lacks any cell wall but has an S-layer of glycoprotein (**Fig. 19.10** ; also see Chapter 3). The S-layer proteins

of *Sulfolobus* species lock together in a geometric array, forming a sturdy protective coating. While keeping the cell intact, the S-layer protein array nonetheless allows a flexible cell, in contrast to the more rigid structures of most bacteria.

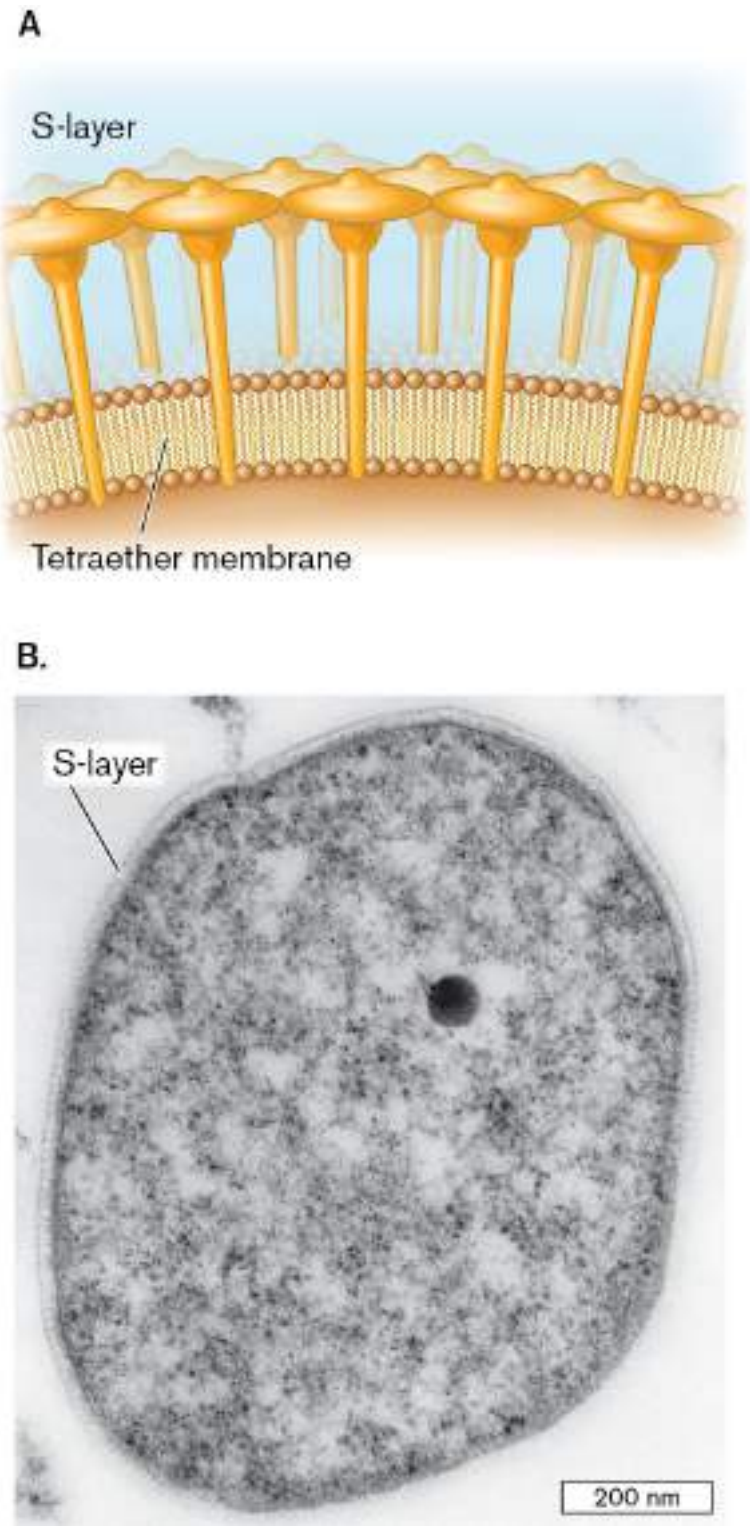


FIGURE 19.10 ■ *Sulfolobus* . A. S-layer proteins plugged into the tetraether membrane of *Sulfolobus* . **B.** *Sulfolobus* cell with tough, flexible S-layer.

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Sulfolobus species swim using archaella (**Fig. 19.11**). Like bacterial flagella, *Sulfolobus* archaella are helical filaments driven by a motor embedded in the plasma membrane (**Fig. 19.11B**). The motor structure is embedded between S-layer proteins. Unlike the bacterial flagellum, which is driven by proton motive force, the archaellum is driven by ATP hydrolysis catalyzed by FlaI motor subunits (orange in **Fig. 19.11B**).

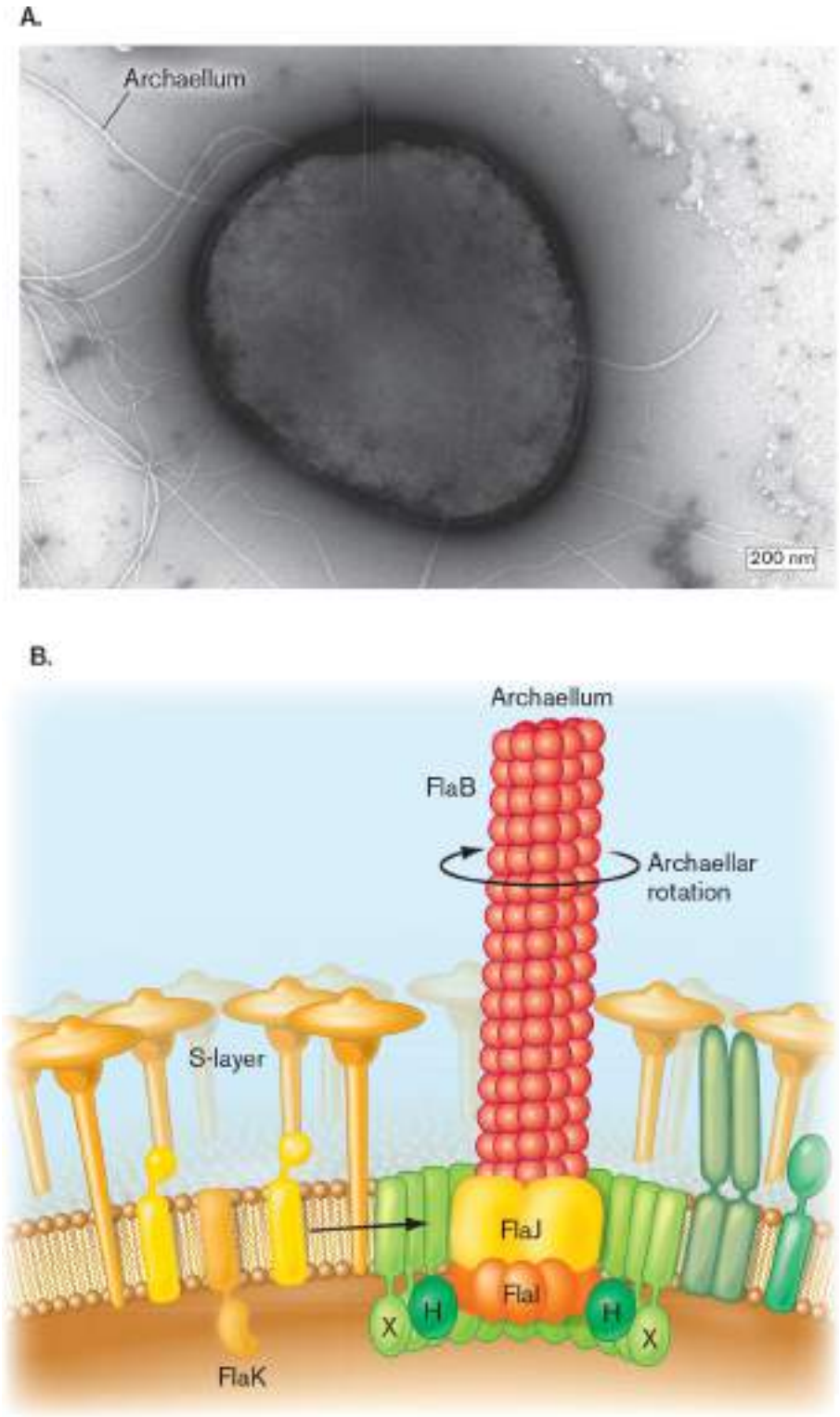


FIGURE 19.11 ■ *Sulfolobus* sp. A20 possesses archaella. A. Motile *Sulfolobus* cell with archaella (TEM). **B.** Archaellum structure is embedded between S-layer subunits.

Source: Part B modified from Sonja-Verena Albers and Ken F. Jarrell. 2015. *Front. Microbiol.* **6** :23, fig. 1B.

COURTESY OF LI HUANG

Sulfide oxidation. *Sulfolobus* species oxidize organic compounds with oxygen or they oxidize S^0 or H_2S to sulfuric acid:

$$2S^0 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 \longrightarrow 4H^+ + 2SO_4^{2-}$$

As a result of sulfuric acid production, the pH of the organism's surroundings falls to pH 2–3, effectively excluding all but acidophiles. While other archaea grow at higher temperatures (up to 120°C) or lower pH (below pH 0), *Sulfolobus* is of interest as a "double extremophile," requiring both high temperature and extreme acidity simultaneously.

Sulfolobus species can grow heterotrophically on sugars or amino acids. In fact, many species are easily cultured in tryptone broth at 80°C, pH 3. Their internal pH is typically pH 6.5; thus, they maintain more than three units of pH difference across their membrane. The full metabolic potential of this organism is revealed by annotation of its genome, which contains homologs of sugar and amino acid transporters, as well as the non-ATP-forming Entner-Doudoroff pathway of glucose catabolism. Genes are present for enzymes to oxidize HS^- and thiosulfate ($S_2O_3^{2-}$), as well as S^0. The main redox carrier for respiration appears to be ferredoxin (instead of NADH, which is relatively unstable at high temperature).

Thought Question

19.3 What problem with cell biochemistry is faced by acidophiles that conduct heterotrophic metabolism?

Viruses of *Sulfolobus*. A fascinating discovery in *Sulfolobus* was that of archaeal viruses. *Sulfolobus* species are attacked by a number of viruses (see the chapter-opening image). The sequential process of a viral infection has been observed in *S. solfataricus* (**Fig. 19.12**). Cells were infected with *Sulfolobus* turreted icosahedral virus (STIV), a virus isolated from a boiling-acid hot spring in Yellowstone National Park. Each icosahedral particle of STIV

has 12 turret-like projections, as shown in the cryo-electron microscopy (cryo-EM) image reconstruction in **Figure 19.12A** . Mature virions contain a double-stranded DNA genome coated with lipid within the capsid. The thin section of an infected cell (**Fig. 19.12B**) shows progeny virions packed into a hexagonal array, portions of which poke through the host cell's S-layer in pyramidal bulges. After lysis (**Fig. 19.12C**), the S-layer complex is all that remains of the empty cell. The overall S-layer appears surprisingly intact, indicating the strength of its subunit interactions.

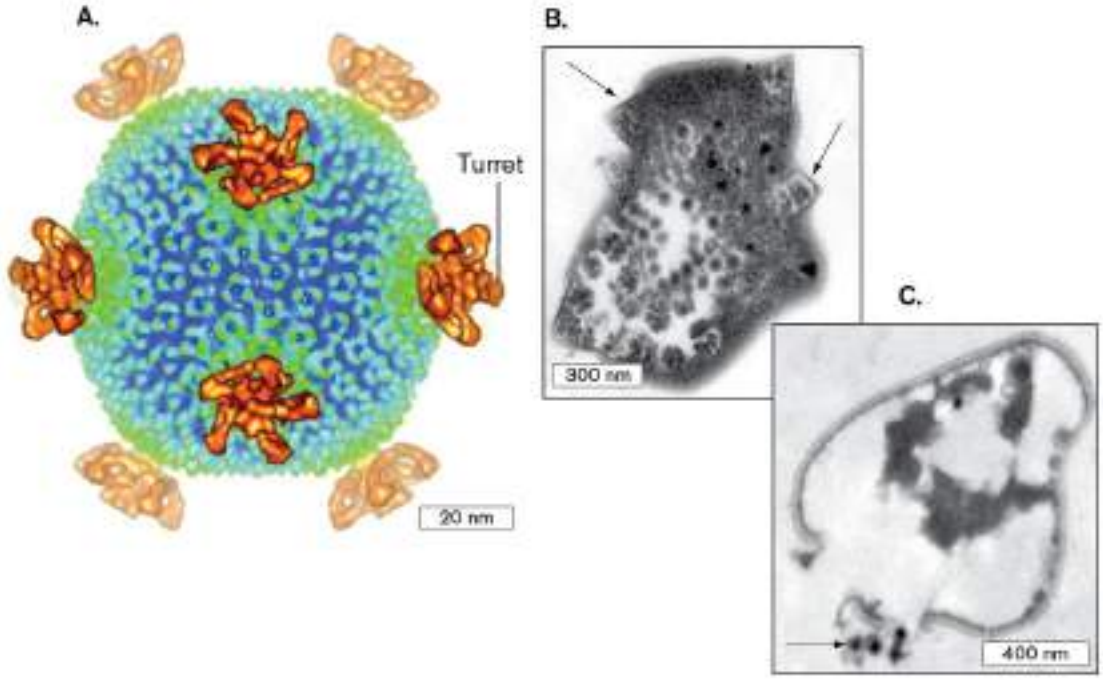


FIGURE 19.12 ■ *Sulfolobus* turreted icosahedral virus (STIV) infects *Sulfolobus solfataricus* . A. STIV capsid with "turrets" (cryo-EM). Capsid diameter 60 nm. **B.** A cell of *S. solfataricus* packed with a hexagonal array of STIV particles. Arrows point to pyramidal bulges where virus arrays poke through a breach in the S-layer. **C.** Empty cell membrane and S-layer following lysis and viral release. Arrow points to released virus particles.

GEORGE RICE ET AL. 2004. *PNAS* **101** :7716–7720, FIG. 2A
SUSAN K. BRUMFIELD ET AL. 2009. *J. VIROL.* **83** :5964–5970. © 2009, AMERICAN SOCIETY FOR MICROBIOLOGY. ALL RIGHTS RESERVED
SUSAN K. BRUMFIELD ET AL. 2009. *J. VIROL.* **83** :5964–5970. © 2009, AMERICAN SOCIETY FOR MICROBIOLOGY. ALL RIGHTS RESERVED

The STIV lytic cycle resembles lytic and fast-release cycles of bacterial and eukaryotic viruses, although the capsid "turrets" of the capsid and the

pyramidal bulges of the lysing cell are unique to archaea. The structure of a capsid protein component shows surprising homology to both bacterial and eukaryotic viral proteins.

Another unusual virus infecting *Sulfolobus* species is the fusellovirus (**Fig. 19.13**). The spindle shape of fusellovirus is found only in viruses of archaea. The DNA genome of this virus—which must retain its structure at high temperatures—is positively supercoiled. The structure and genetics of fusellovirus have been studied, including its ability to integrate in the host genome at a tRNA-encoding gene.

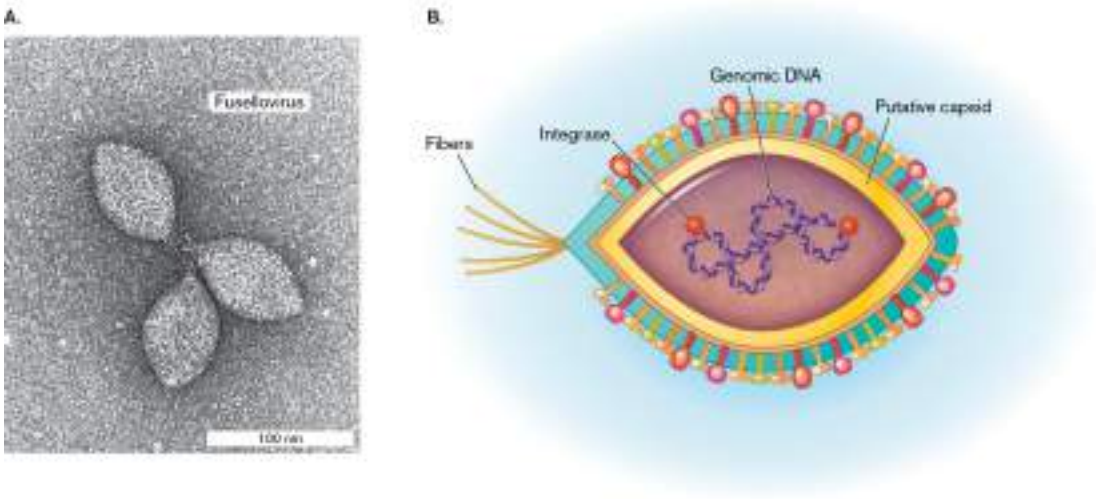


FIGURE 19.13 ■ Fusellovirus of *Sulfolobus* species. A. Spindle-shaped fusellovirus particles (TEM, with heavy-atom stain shadowing). **B.** Virion structure, including positively supercoiled DNA genome with integrase and capsid and envelope proteins.

REPRINTED BY PERMISSION FROM SPRINGER NATURE: D. PRANGISHVILI, D. BAMFORD, P. FORTERRE, ET AL. 2017. *NAT. REV. MICROBIOL.* **15** :724–39

Numerous other archaeal viruses have been discovered in high-temperature environments, including icosahedral, tailed, and filamentous forms. Nearly all known viruses of archaea have genomes consisting of double-stranded DNA, suggesting that only double-stranded DNA is stable enough for virus particles to persist at high temperature. Nevertheless, in 2012, Eugene Koonin's group at the National Center for Biotechnology Information identified gene sequences of an RNA virus from a Yellowstone hot spring, including an archaeal RNA-dependent RNA transcriptase. This finding suggests there may exist RNA viruses that infect hyperthermophilic archaea.

Thought Question

19.4 What hypotheses might we propose about archaeal evolution if viruses of mesophilic archaea are found to have RNA genomes? What if, instead, all archaeal viruses have DNA genomes only?

Besides Sulfolobales, the group Thermoproteota includes other clades of thermoacidophiles. Caldisphaerales is a clade of thermoacidophiles first isolated from a Philippine hot spring at Mount Makiling. Members of Caldisphaerales, such as *Caldisphaera* , typically grow at pH 3 up to 80°C. Unlike *Sulfolobus* species, *Caldisphaera* species are anaerobes or microaerophiles, tolerating only low concentrations of oxygen. They grow by fermentation or anaerobic respiration. Another clade, Thermoproteales, includes hyperthermoacidophiles isolated from marine vents; thus, they survive extreme pressure, as well as heat and acid! *Thermoproteus* species grow at temperatures up to 97°C and at pH values lower than pH 3. Their rod-shaped cells are less than 0.3 µm in length—one of the smallest cell types known. They have autotrophic metabolism, gaining energy by reducing sulfur with H_2 to H_2S.

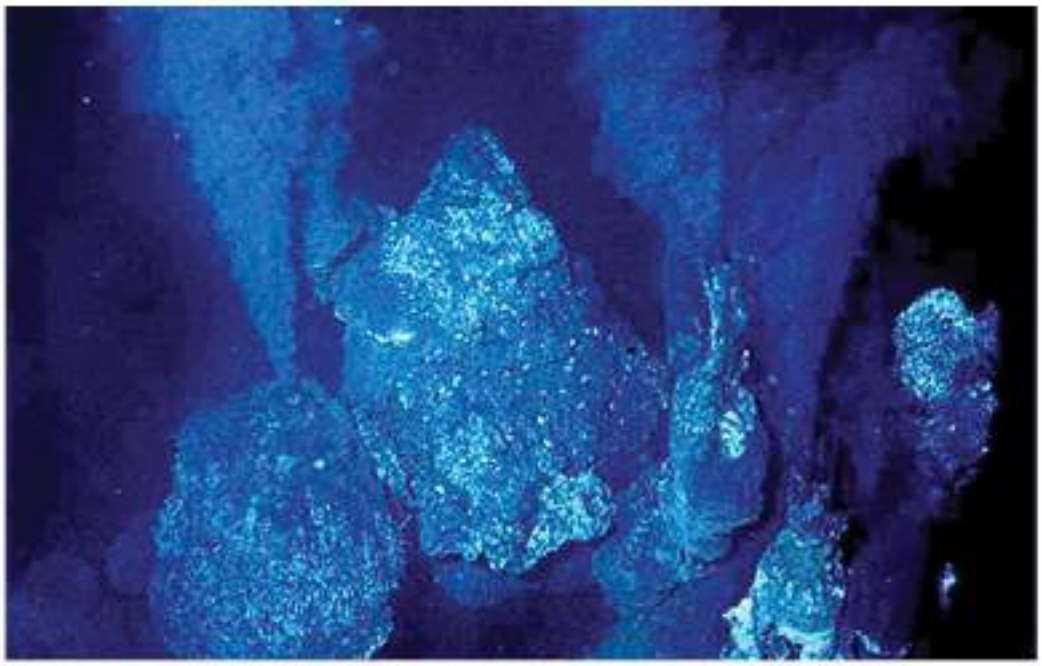
Barophilic Vent Hyperthermophiles

The most extreme hyperthermophiles are barophiles adapted to grow near hydrothermal vents at the ocean floor. The high pressure beneath several kilometers of ocean allows water to remain liquid at temperatures above 100°C; the highest known temperature for growth of an organism is 125°C (for *Pyrolobus fumarii*).

A common feature of thermal vents is the black smoker (**Fig. 19.14**). A black smoker is a chimney-like structure resulting from the upwelling of seawater superheated by an undersea magma chamber. As in a geyser aboveground, the heated water is forced upward through a small opening. Because the thermal vent is under steam pressure, the water can reach temperatures of over 400°C, enabling it to dissolve high concentrations of minerals such as iron II sulfide (FeS). When the rising water escapes, however, it immediately cools, depositing iron sulfide around the edge of the vent chimney and precipitating iron sulfide particles that cloud the water—hence the term "black smoker." While no organism can grow at 400°C, various species of archaea are adapted to grow in the range of 100°C–120°C,

where the vent stream meets the seawater and minerals precipitate (**Fig. 19.14B**).

A.



B.

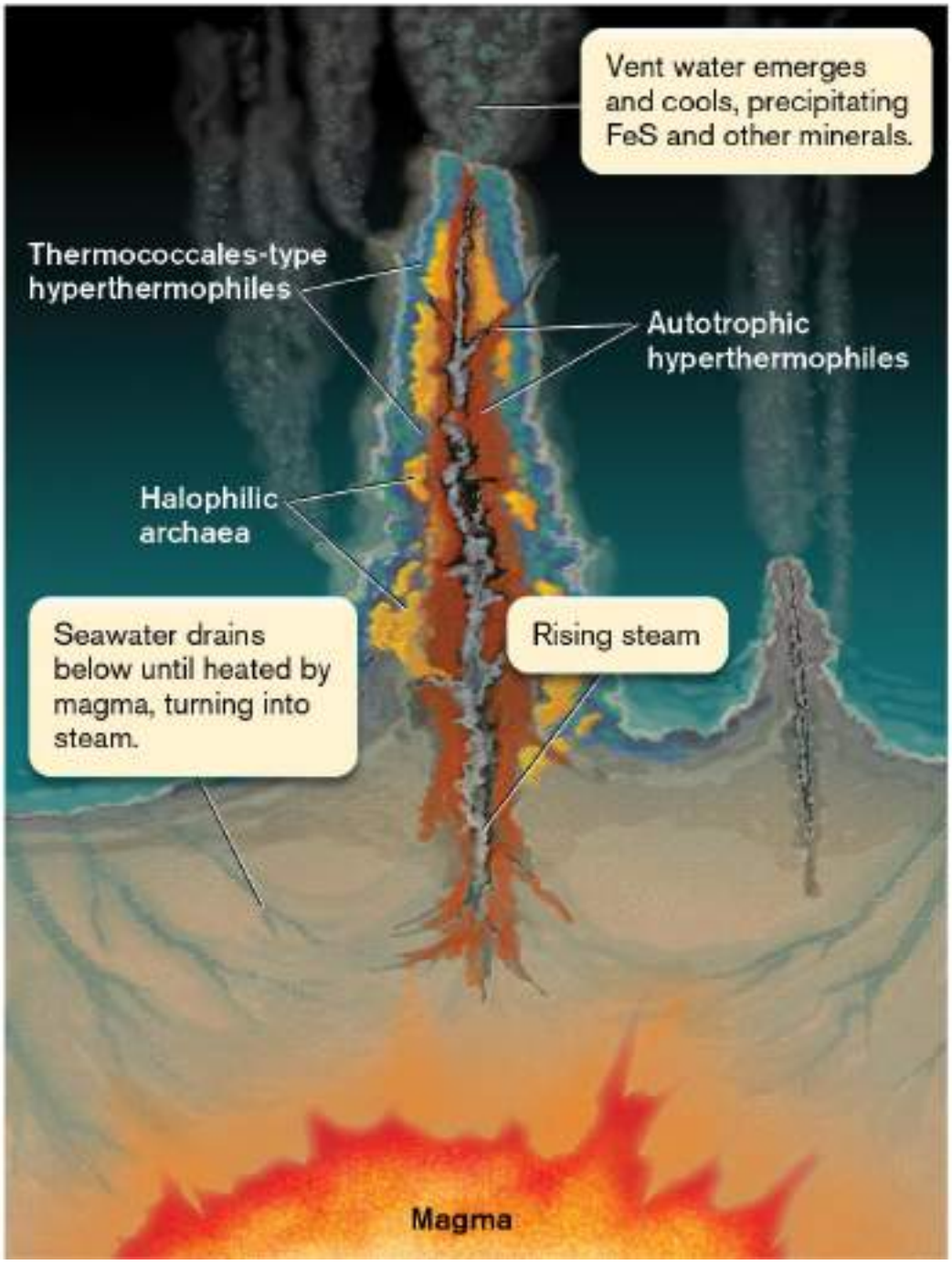


FIGURE 19.14 ■ Extreme temperature and pressure: black smoker vents. A. Black smoker vent with fluid escaping from "chimneys" of sulfide minerals that crystallize as the 350°C fluid hits the cold, 2°C ocean seawater. The photo was taken by the submersible *Alvin* at a depth of 2,250 m, at the Juan de Fuca Ridge, off the coast of Oregon. **B.**

Different parts of the smoker vent system support different classes of archaea.

DR. MICHAEL PERFIT, UNIVERSITY OF FLORIDA, AND NOAA VENTS PROGRAM

How do we study organisms under such extreme conditions? To study hyperthermophiles from black smoker vents requires specialized equipment. The isolation of such organisms is a challenge because their habitats endanger our own survival. Undersea vent systems must be approached by a special submersible device with a robotic arm. An example is the Environmental Sample Processor from the Monterey Bay Aquarium Research Institute (**Fig. 19.15A**). The robotic system samples temperature and other properties of fluid emerging from a black smoker hydrothermal vent. It can then sample organisms for study. An advanced version of this device can actually process the organism's DNA. Thus, the DNA can be obtained from vent-adapted microbes that could not survive transfer to a laboratory at sea level. The robotic sample processor is supported by NASA as a model for a future space probe to explore one of Jupiter's moons, Europa, considered a possible source of extraterrestrial life.

Organisms that do survive transport to sea level must nonetheless be maintained at high pressure and temperature to ensure viability. In the laboratory, all devices for microscopy and cultivation must be kept under pressure and at high temperature (**Fig. 19.15B**). The culture must be provided with reduced minerals and gases needed for the growth of vent microbes.

A.



B.

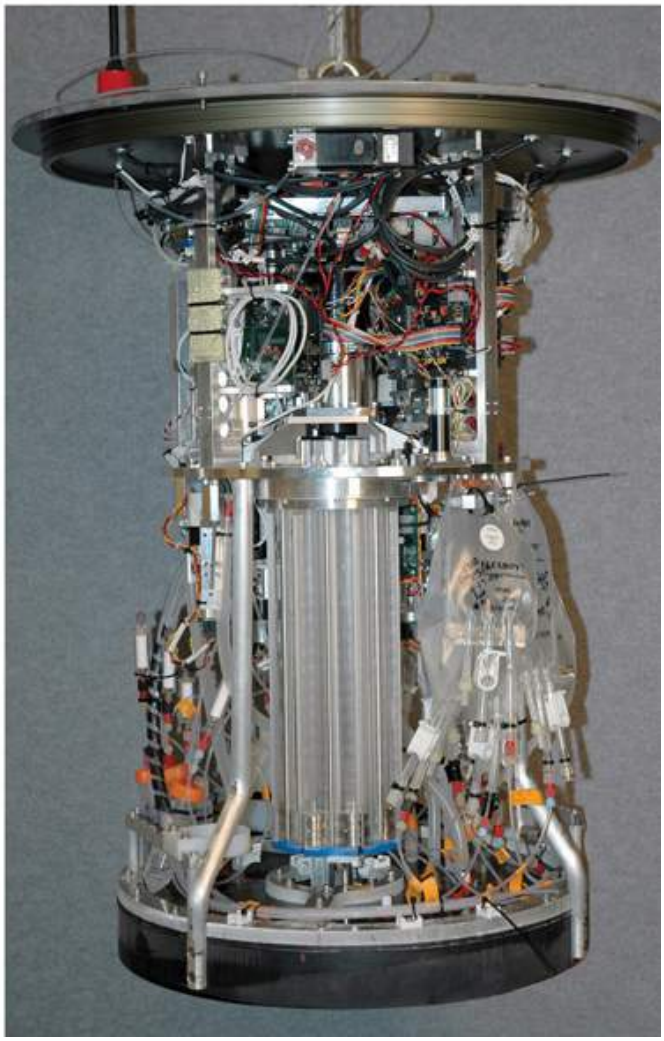


FIGURE 19.15 ■ Robotic sampling from a black smoker vent. A. Engineer Gene Massion, from the Monterey Bay Aquarium Research Institute, deploys the submersible Environmental Sample Processor with robotic collection arm at an ocean site off the coast of Maine. **B.** Pressurized device for sampling and cultivation of vent organisms.

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TODD WALSH © 2006 MBARI

Vent-adapted members of Desulfurococcales include *Pyrodictium abyssi* (**Fig. 19.16**), *P. occultum* , and *P. brockii* ; the latter is named for Thomas Brock (1926–2021) of the University of Wisconsin–Madison, a pioneering researcher of hyperthermophiles. For energy, *Pyrodictium* species reduce

sulfur to H_2S, either with molecular hydrogen or with organic compounds. A membrane-embedded sulfur-reducing complex and a proton-translocating ATP synthase have been isolated from *P. abyssi* . The complexes are extremely heat stable, exhibiting a temperature optimum of 100°C.

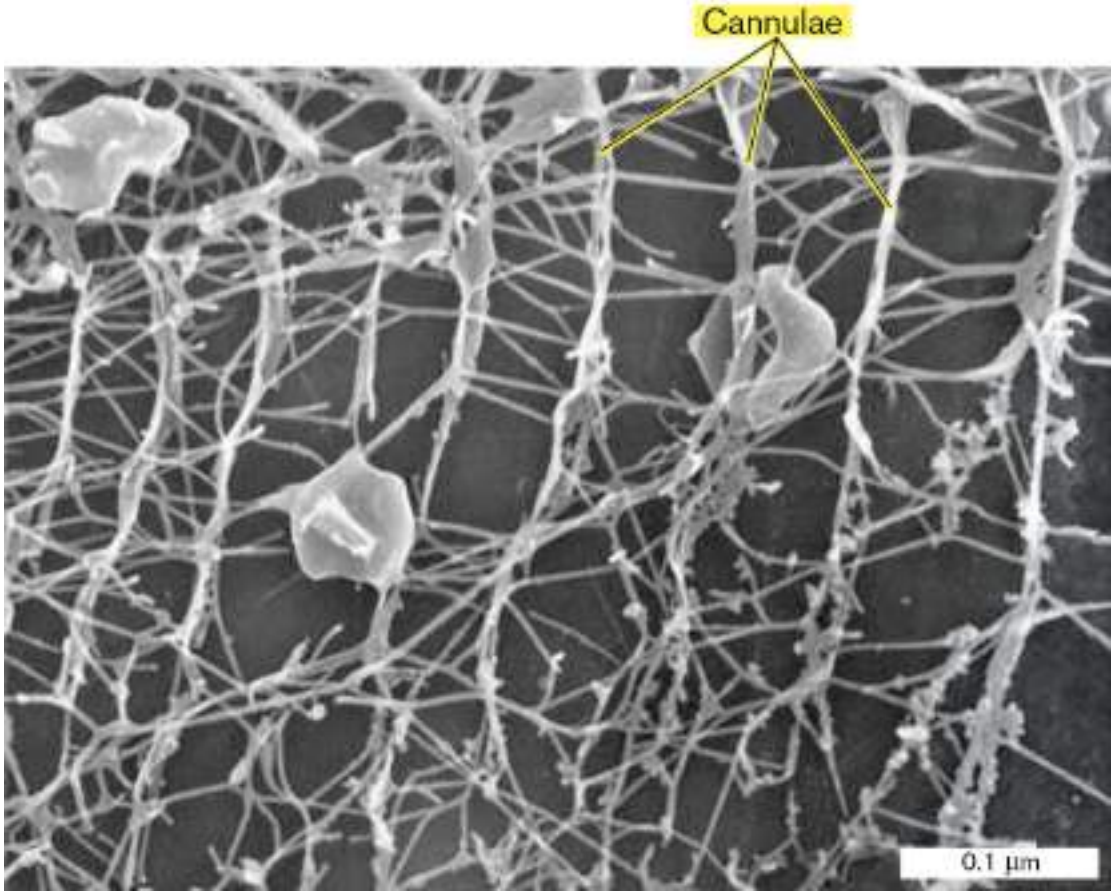


FIGURE 19.16 ■ *Pyrodictium abyssi* , growing as networks of cells linked by cannulae. SEM.

S. Y. M. NG ET AL. 2008. *J. BACTERIOL.* **190** :6039. REPRODUCED WITH PERMISSION FROM AMERICAN SOCIETY FOR MICROBIOLOGY

Pyrodictium species grow as flat, disk-shaped cells that can be as thin as 0.1 µm. The cells contain a periplasm and outer membrane with an S-layer that is coated with zinc sulfide, a mineral that precipitates from the vent. The cell disks are interconnected by glycoprotein tubules called cannulae (singular, cannula). The cannulae can extend to more than 0.1 mm, forming complex networks of connections (**Fig. 19.16**). In liquid culture, the networks grow into white balls up to 10 mm in diameter. Cryo-electron tomography of a *Pyrodictium* cell shows that the cannulae bridge the

periplasm between cells, but not the cytoplasm. The cannulae may enable *Pyrodictium* cells to share nutrients and maintain a biofilm, while keeping their cellular identity distinct with separated cytoplasm.

What happens when a *Pyrodictium* cell divides? Cells of *P. abyssi* generate new cannulae as they undergo fission (**Fig. 19.17**). Some of the new cannulae form as loops connecting the two daughter cells while simultaneously pushing the two cells apart. In this fashion, the cell division process expands the cell network.



FIGURE 19.17 ■ *Pyrodictium abyssi* , undergoing cell division. Cells of *Pyrodictium abyssi* (arrows) generate new interconnecting cannulae as they divide.

CHRISTIAN HORN ET AL. 1999. *J. BACTERIOL.* **181** :5114–18

Note: Two genera of vent thermophiles have similar names but only distant genetic relatedness: *Pyrodictium abyssi* , from TACK Desulfurococcales; and *Pyrococcus abyssi* , from Euryarchaeota.

Korarchaeota and Aigarchaeota: Cryptic Filaments of the Deep

Metagenomic analysis continues to reveal previously unknown branches of life. An example of a deeply branching clade is the Korarchaeota (the "K" in TACK). Korarchaeota are hydrothermal vent hyperthermophiles isolated by Susan Barns and Norman Pace. The organisms have not been isolated in pure culture, but in 2008 the genome of one korarchaeote was sequenced from an enriched mixed culture at 80°C–90°C at Obsidian Pool, Yellowstone National Park (**Fig. 19.18**). The organism, provisionally named " *Candidatus* Korarchaeum cryptofilum," grows in long, thin filaments less than 200 nm wide. Its genes suggest that it gains energy mainly from anaerobic peptide

fermentation, yet we still do not know enough to culture this organism in the laboratory.

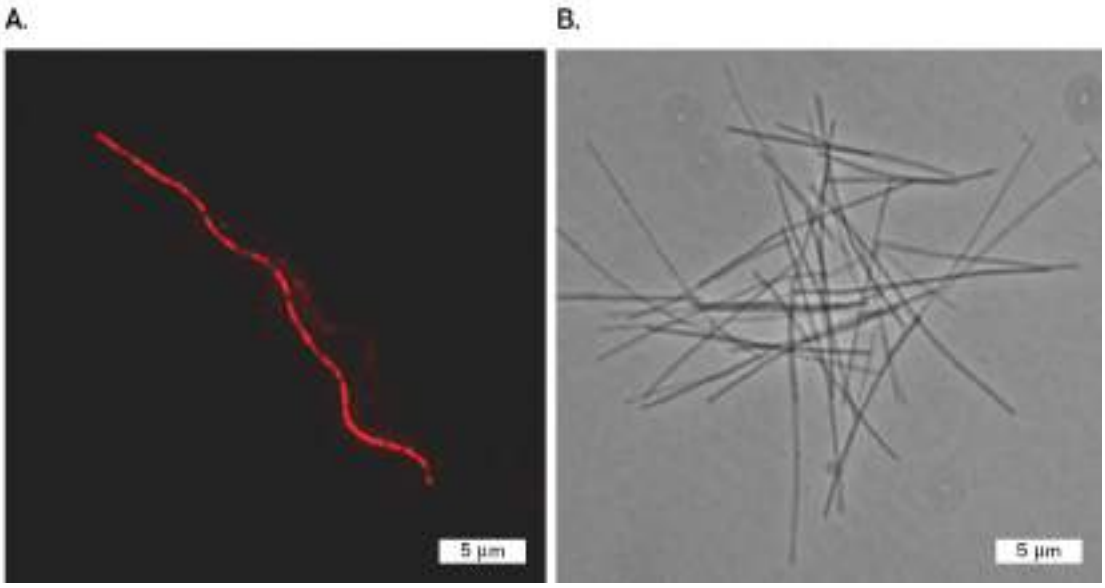


FIGURE 19.18 ■ Korarchaeota: a filamentous vent hyperthermophile. A. Filamentous chain of a candidate species: *Korarchaeum cryptofilum* (fluorescence microscopy, FISH with fluorescent DNA probe). **B.** *K. cryptofilum* under phase contrast.

J. G. ELKINS ET AL. 2008. *PNAS* **105** :8102, FIG. 1A
J. G. ELKINS ET AL. 2008. *PNAS* **105** :8102, FIG. 1B

Another interesting discovery, from a terrestrial subsurface hydrothermal ecosystem, is the phylum Aigarchaeota . The Aigarchaeota include filamentous heterotrophs that are aerobic, catabolizing many organic molecules using oxygen as their electron acceptor. Aigarchaeota is the "A" in the TACK superphylum name.

To Summarize

- **Habitats for hyperthermophiles** include hot springs and submarine hydrothermal vents. Vent organisms are barophiles as well as thermophiles. Anaerobic hyperthermophilic acidophiles include Caldisphaerales and Thermoproteales.

- **Desulfurococcales includes diverse thermophiles.** Most are anaerobes that use sulfur to oxidize hydrogen or organic molecules.
- ***Sulfolobus*** species oxidize sulfur or H_2S to sulfuric acid, and they catabolize organic compounds.
- **Archaella are driven by rotary motors.** Rotation is powered by ATP hydrolysis. The archaellum may share ancestry with type IV pili.
- **Viruses infect *Sulfolobus* .** Archaeal viruses show unique shapes and positively supercoiled DNA.
- **Marine hydrothermal vents support archaea that are barophiles as well as hyperthermophiles.** *Pyrodictium* species are disk-shaped cells interconnected by cytoplasmic bridges called cannulae.
- **Korarchaeota and Aigarchaeota are filamentous hyperthermophiles.**

Glossary

Desulfurococcales
: A phylum of thermophilic archaea in the TACK superphylum that metabolize sulfur and organic compounds.

Sulfolobales
: A phylum of thermophilic archaea in the TACK superphylum that includes sulfur oxidizers.

black smoker
: An oceanic thermal vent containing high concentrations of dark minerals such as iron sulfide.

cannula *pl.* cannulae
: A narrow tubule. For *Pyrodictium* species, glycoprotein cannulae interconnect cells at their periplasm.

Korarchaeota
: A phylum of archaea in the TACK superphylum that contains anaerobic hyperthermophiles in hot springs and deep-sea thermal vents.

Aigarchaeota
: A phylum of archaea in the TACK superphylum that contains filamentous aerobic hyperthermophiles in hot springs.

barophile
: Also called *piezophile* . An organism that requires high pressure to grow.

Fig. 19.1

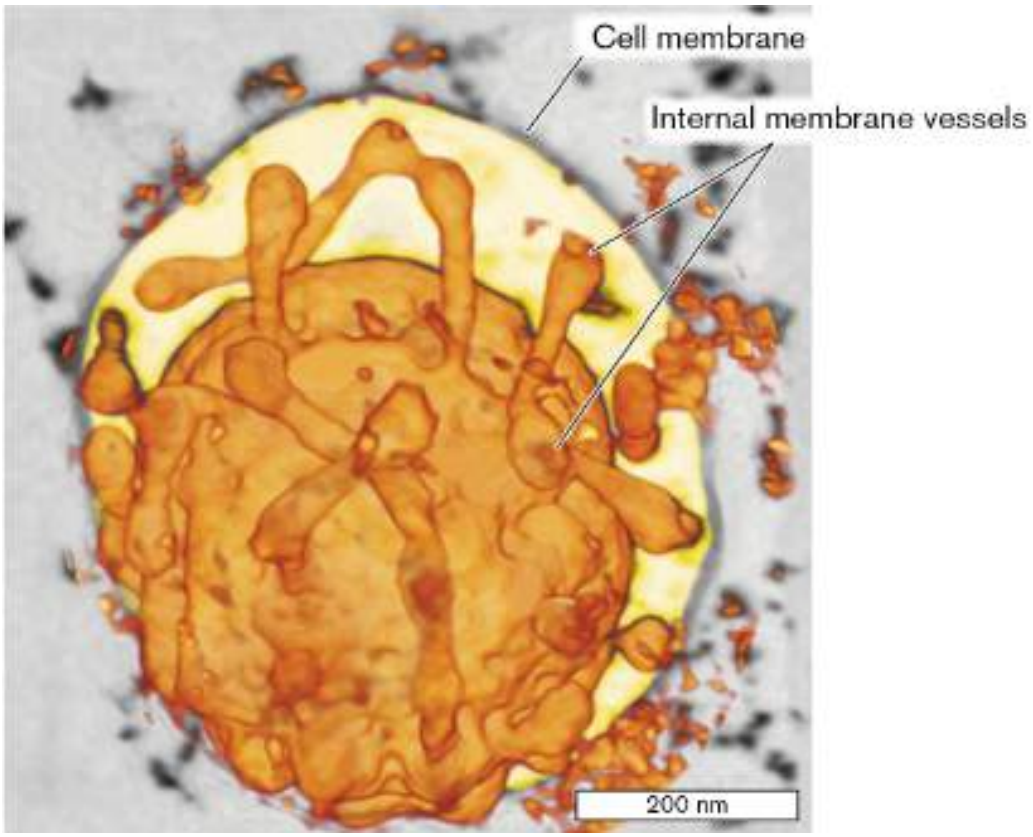


FIGURE 19.1 ■ *Ignicoccus hospitalis*, from a hydrothermal vent 600 meters below sea level, at Kolbeinsey Ridge volcano. *Ignicoccus hospitalis* has an extensive endomembrane system (orange) within an outer compartment contained by the cell membrane (yellow). Cryo-electron tomography, colorized.
COURTESY OF THOMAS HEIMERL

Fig. 19.2E

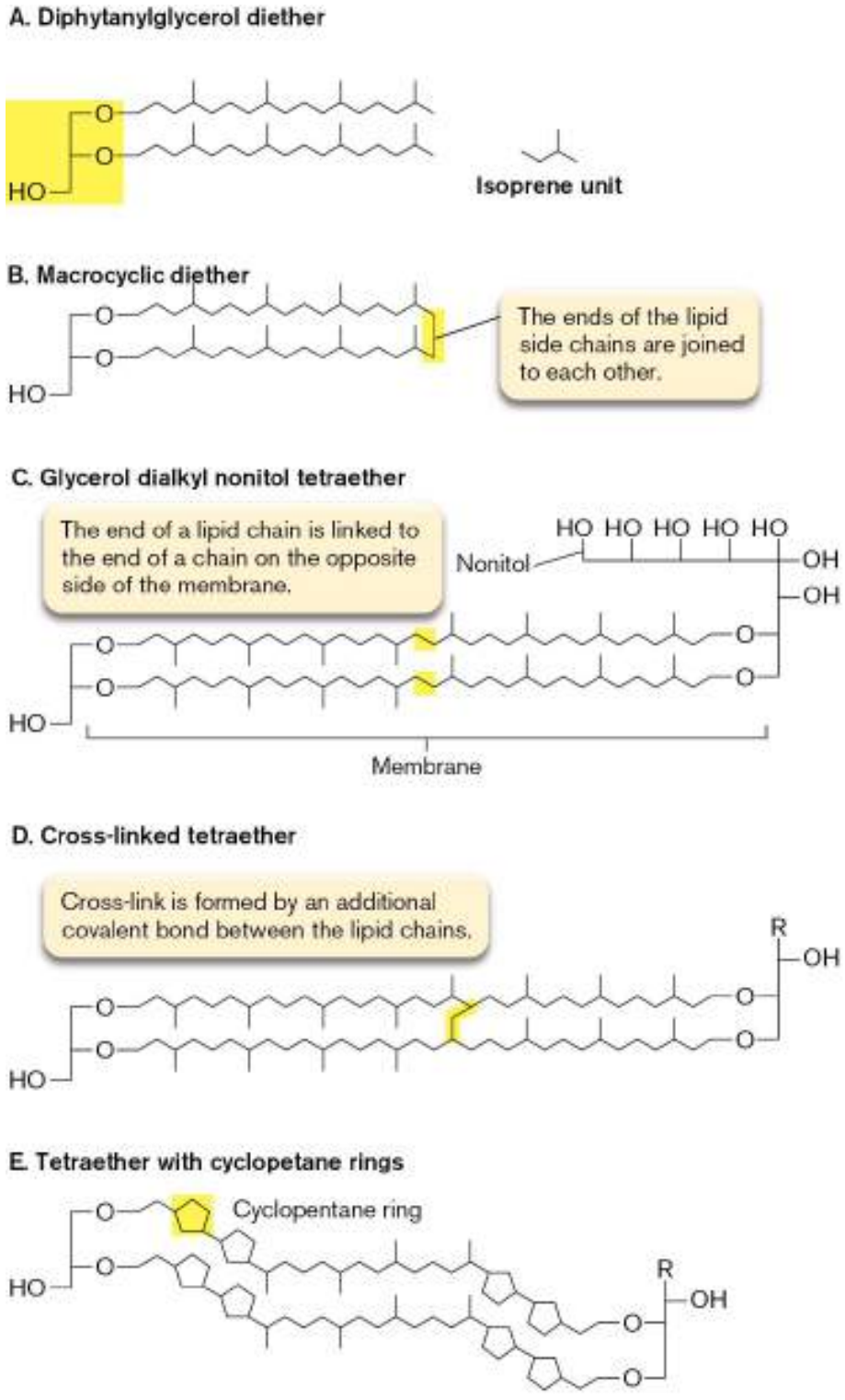


FIGURE 19.2 ■ Branched-chain ether lipids are characteristic of archaeal membranes.

Source: Modified from R. M. Daniel and D. A. Cowan. 2000. *Cell. Mol. Life Sci.* **57** :250–264.

19.3 Thaumarchaeota: Ammonia Oxidizers and Animal Symbionts

Because archaea were first isolated from extreme habitats, it came as a surprise when SSU rRNA probes revealed mesophilic TACK archaea (then identified as Crenarchaeota) in moderate habitats throughout the biosphere. The first marine mesophilic archaea were found in 1992 by Jed Fuhrman, University of Southern California (**Fig. 19.19A**). Edward DeLong (**Fig. 19.19B**), Massachusetts Institute of Technology, further sampled the Pacific Ocean at the Hawaii Ocean Time-series station, where he found high numbers of mesophilic archaea, then called Crenarchaeota (now the "C" in TACK; **Fig. 19.19C**). The abundance of archaea, predominantly Thaumarchaeota, varied according to season and increased with depth, typically comprising 40% of the total microbial population at depths of 1,000 meters, where temperatures are cold.

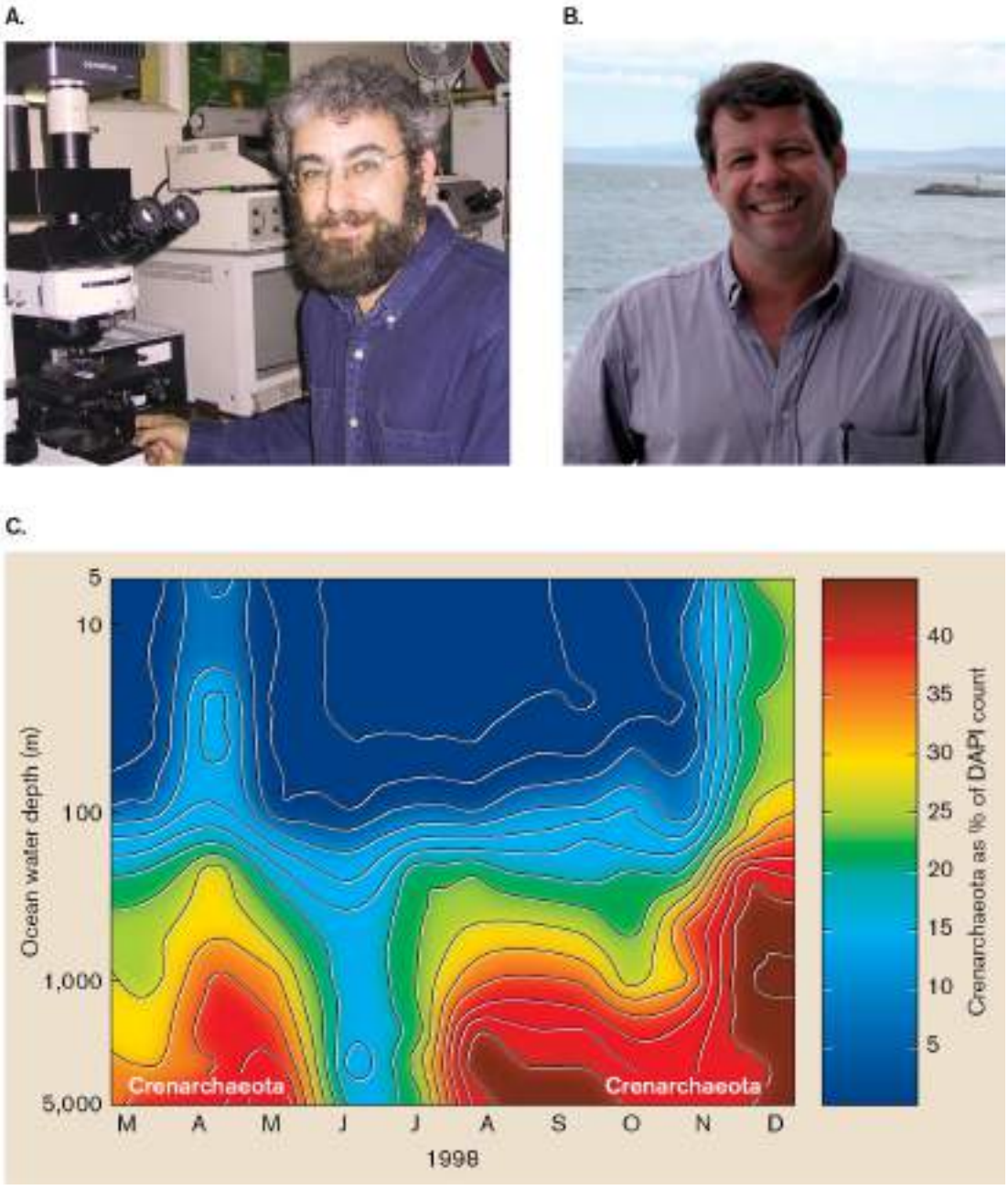


FIGURE 19.19 ■ Crenarchaeota in the Pacific Ocean. A. Jed Fuhrman samples mesophilic archaea in the Pacific Ocean. **B.** Ed DeLong mapped the abundance of marine mesophilic archaea. **C.** The proportion of TACK mesophilic archaea (color profile), measured as a function of depth and season, in the Hawaii Ocean Time-series station. Archaea were identified by fluorescence in situ hybridization (FISH). A fluorescein-labeled DNA probe specific to TACK archaea was hybridized to a cellular rRNA sequence; DNA

was detected using the DNA-binding fluorophore 4′,6-diamidino-2-phenylindole (DAPI).

Source: Part C modified from Markus Karner et al. 2001. *Nature* **409** :507.

COURTESY OF JED FUHRMAN
COURTESY OF ED DELONG

Mesophilic archaea may have evolved from hyperthermophiles by gene acquisition from mesophilic bacteria. Many—perhaps the majority of archaea overall—grow in water or soil, often in association with plants and animals.

Ammonia-Oxidizing Thaumarchaeotes Cycle Global Nitrogen

From a global standpoint, the most significant thaumarchaeotes discovered are the ammonia-oxidizing archaea (AOA), such as those of the order Nitrosopumilales. The best-studied ammonia-oxidizing thaumarchaeote, *Nitrosopumilus maritimus* , was isolated from a marine water tank at the Seattle Aquarium by David Stahl and colleagues at the University of Washington (**Fig. 19.20**).

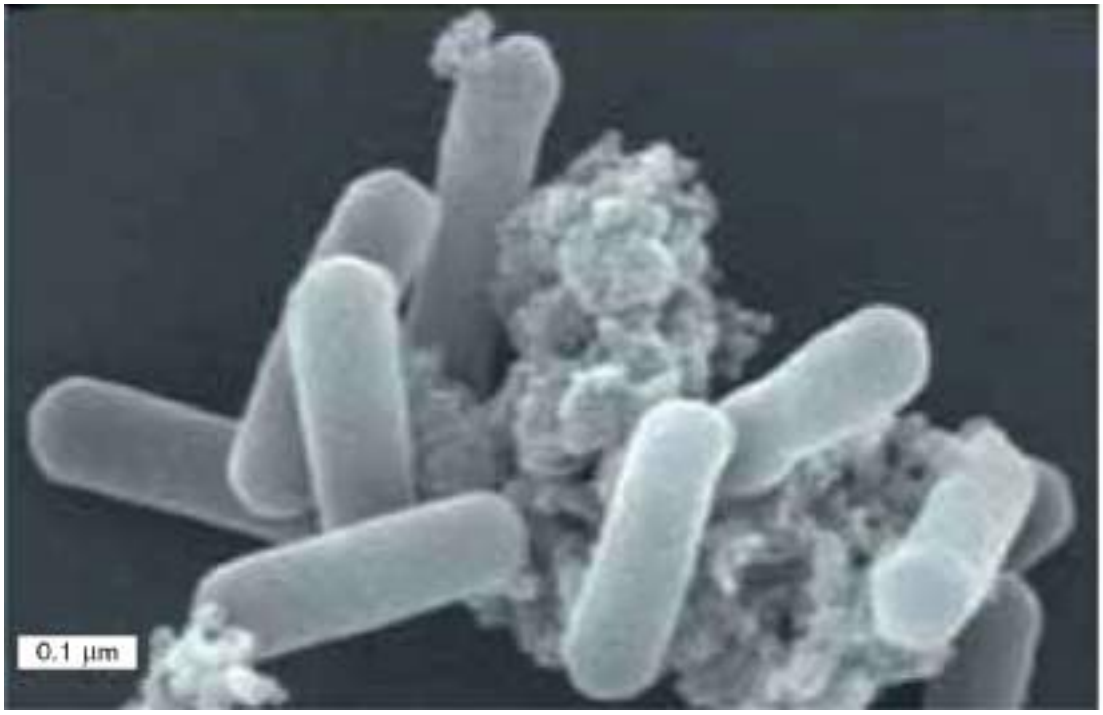


FIGURE 19.20 ■ *Nitrosopumilus*, an ammonia-oxidizing archaeon. *Nitrosopumilus maritimus* cells clustered on sediment (SEM).

M. KÖNNEKE ET AL. 2005. *NATURE* **437** :543–546, FIG. 2D

Ammonia-oxidizing archaea gain energy by aerobically oxidizing ammonia to nitrite:

$$2NH_3 + 3O_2 \longrightarrow 2NO_2^- + 2H_2O + 2H^+$$

This lithotrophic reaction yields redox energy, enabling the microbe to fix CO_2 for biomass (discussed in Chapter 14). In marine environments, excreted ammonia can build up to high levels, until it is oxidized by archaea and bacteria. In some habitats, the Thaumarchaeota perform most of the recycling of ammonia, such as the ammonia excreted by fish in your aquarium. The thaumarchaeotes thus help balance the ecology of intertidal pools and beaches. The reaction plays a key role in the global nitrogen cycle, as the first step of returning organic nitrogen to atmospheric N

$_2$ (discussed in Chapters 21 and 22). It also provides a major source of nitrite for marine phytoplankton.

Ammonia-oxidizing archaea are ubiquitous in marine environments. Tatsunori Nakagawa and colleagues at Nihon University, Japan, used enrichment culture (described in Chapter 4) to hunt for new kinds of AOA (**Fig. 19.21**). Samples of sand from the seafloor at Tanoura Bay, Japan, were serially diluted in medium containing ammonium sulfate and adjusted to pH 8, a pH high enough for significant deprotonation of ammonium ion (NH_4^+) to ammonia (NH_3). During the enrichment culture, ammonia was progressively consumed and converted to nitrite (NO_2^-). In the culture, the gene encoding ammonia oxidase (*amoA*) was identified by polymerase chain reaction (PCR) amplification. The AOA were identified by fluorescence in situ hybridization (FISH) using a fluorophore attached to DNA that hybridizes to rRNA of *Nitrosopumilus* species.

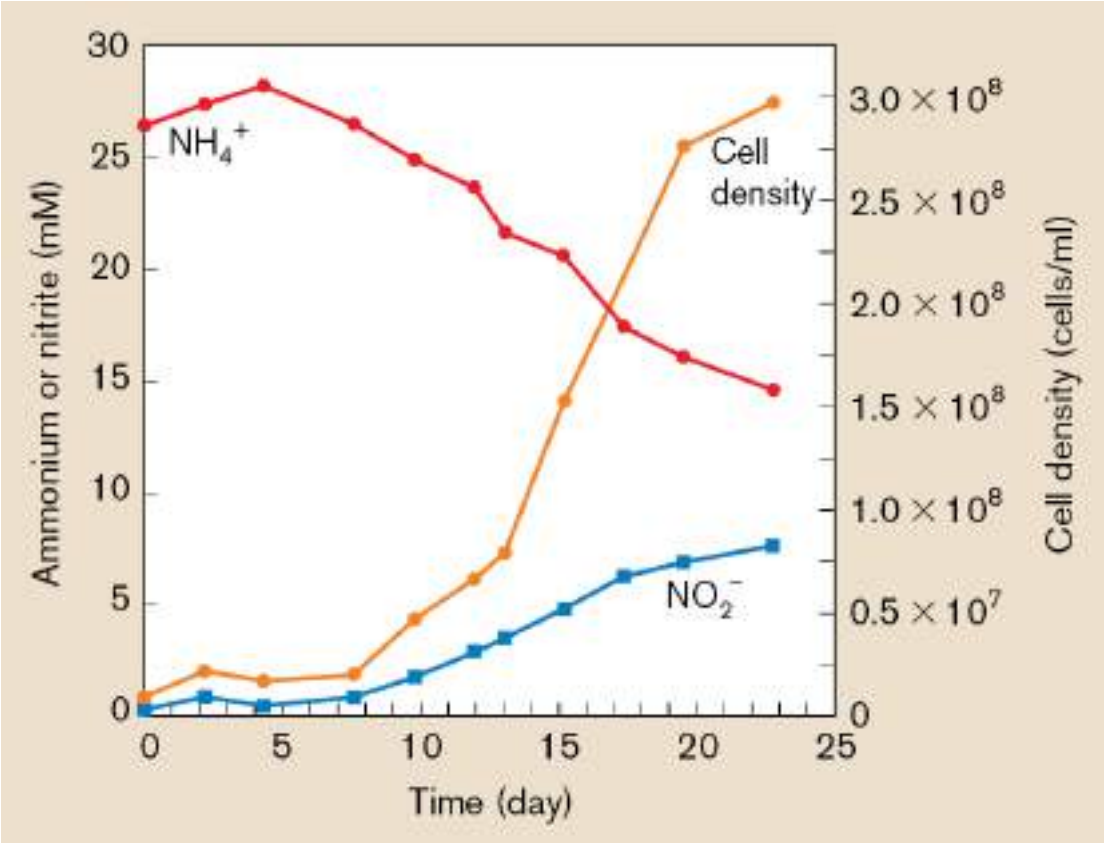
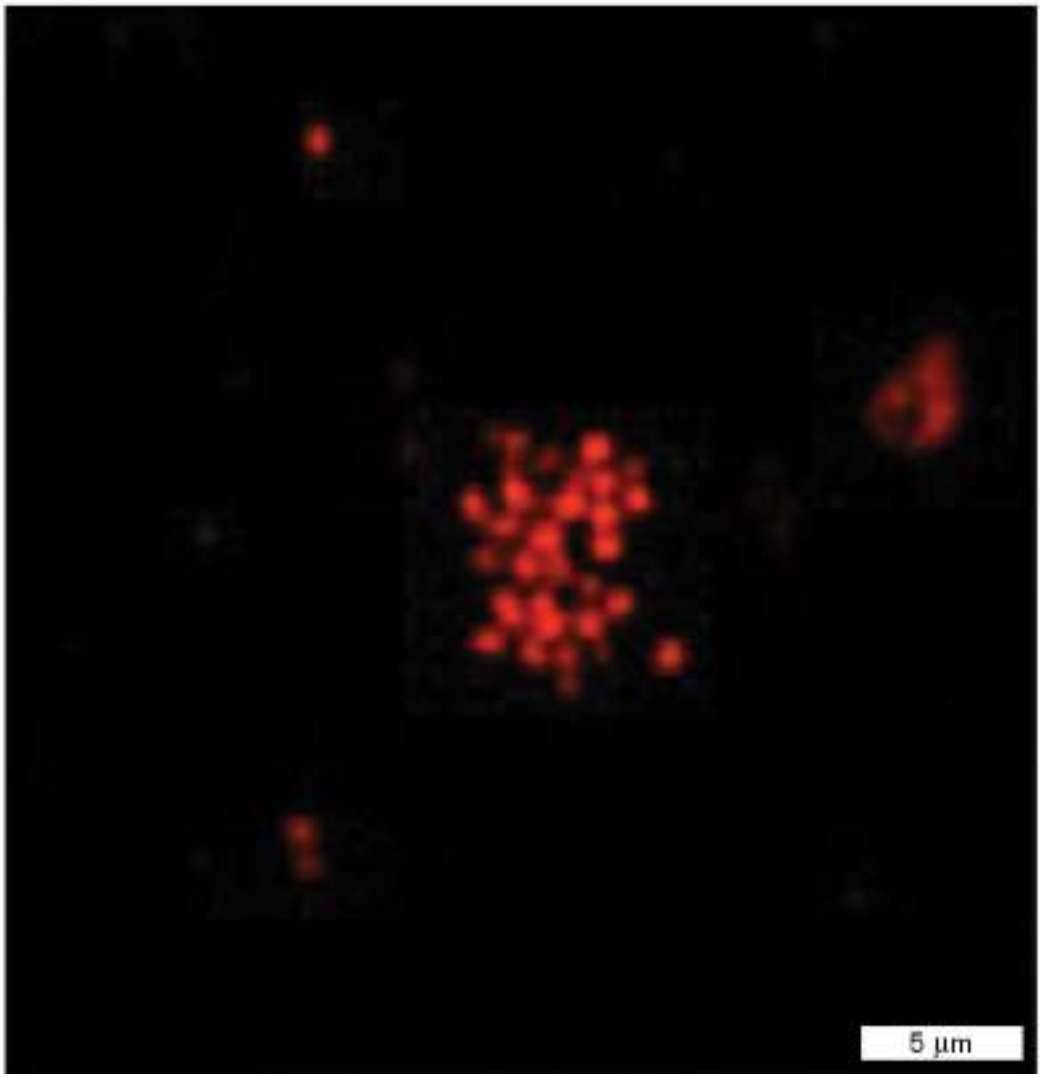


FIGURE 19.21 ■ Enrichment culture for ammonia oxidizers. Ammonia-oxidizing microbes from eelgrass seafloor samples convert ammonia to nitrite.

Source: Modified from Naoki Matsutani et al. 2011. *Microbes Environ.* **26** :23–29.

Nitrosopumilus and related AOA oxidize ammonia at extremely low concentrations. Thus, AOA quickly remove a substance toxic to fish. AOA have since been found throughout marine and freshwater communities, as well as in soil, where they perform the important function of ammonia removal. They appear in industrial waste sludge, revealed by fluorescent DNA probes (**Fig. 19.22**). While ammonia is also oxidized by bacteria, in many habitats the AOA are the dominant oxidizers. Ammonia-oxidizing thaumarchaeotes continue to be discovered in other habitats, including Antarctic lakes down to the temperature of −20°C—as well as habitats closer to home, such as the sponge filter of your own freshwater aquarium (Special Topic 19).

A.



B.

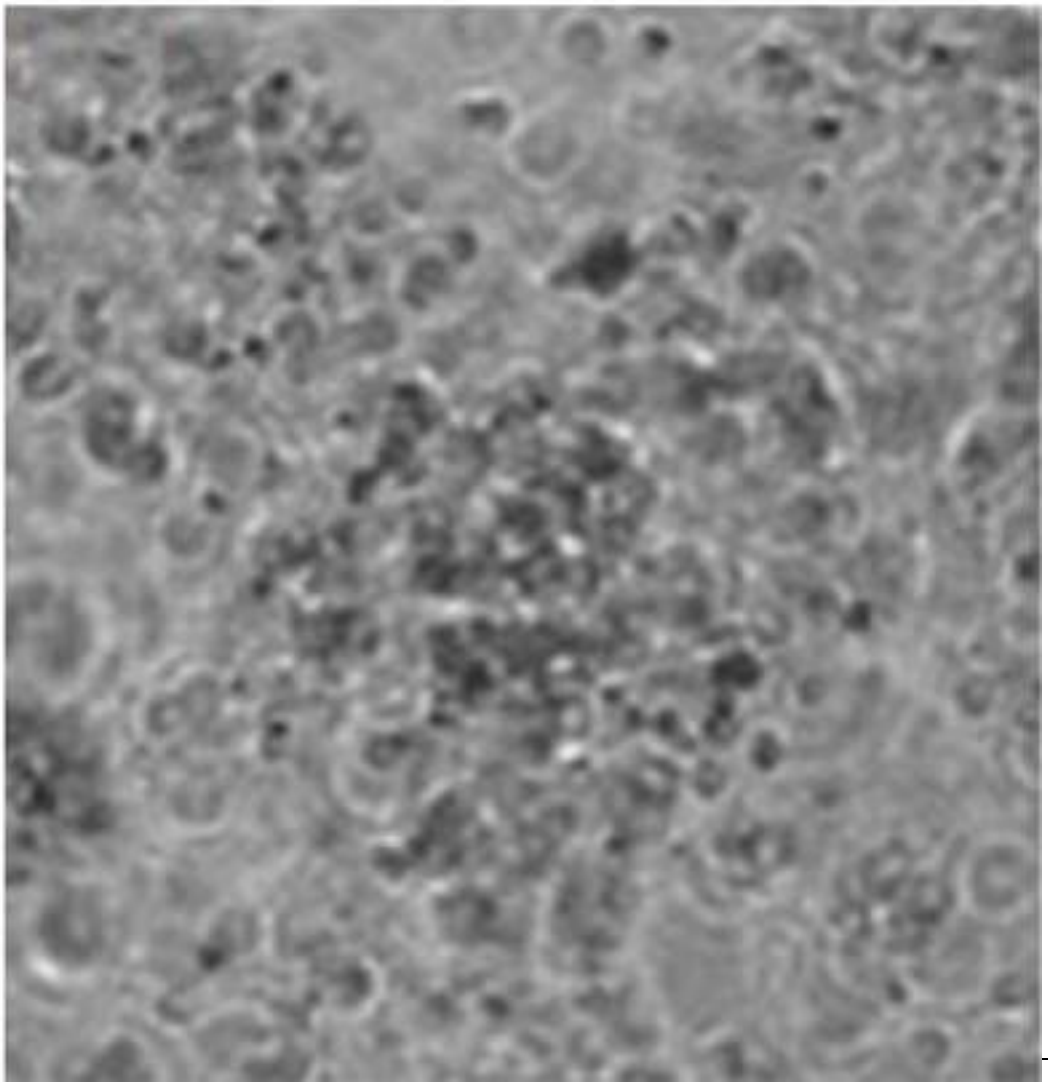


FIGURE 19.22 ■ Ammonia-oxidizing Thaumarchaeota in nitrogen-rich industrial sludge. A. FISH probe hybridizes with rRNA sequence specific to thaumarchaeotes. **B.** Ammonia-oxidizing archaea within a particle of sewage sludge (LM).

MARK MUSSMANN ET AL. 2011. *PNAS* **108** :16771

MARK MUSSMANN ET AL. 2011. *PNAS* **108** :16771

SPECIAL TOPIC 19 Ammonia-Oxidizing Archaea Keep Your Fish Healthy

We often think of archaea as living in exotic environments far from a human home. But many kinds of archaea live in our homes, on our skin, and even in the water of a typical home aquarium (**Fig. ST 19.1**). How do they interact with the fish? Laura Sauder and colleagues at the University of Waterloo, Canada, hypothesized that unidentified Thaumarchaeota would be found oxidizing ammonia that the fish excrete. Some bacteria are known to oxidize ammonia, and commercial cultures can be purchased to improve the aquarium water and extend the life of the fish. But what if in natural systems archaea are more prevalent, and perhaps do a better job than bacteria?

Sauder sought to find ammonia-oxidizing thaumarchaeotes in an aquarium's sponge filter. A sponge filter traps particulate matter as a motor pumps water through, thus providing cleaner water for the fish. But microbes from the trapped particles can proliferate as a biofilm on the sponge, where they continually receive nutrients from the pumped water. Sauder investigated whether any of these sponge biofilms contain archaea. She cultured the sponge biofilm in a mineral salts medium supplemented with ammonium chloride (NH_4Cl), providing the ammonium ion that Thaumarchaeota can oxidize. This approach to favoring the culture of a metabolic type is known as enrichment culture (discussed in Chapter 4). The medium also contained antibiotics that target bacteria but not archaea (see Section 19.1). Samples of the culture were then observed by fluorescence microscopy with fluorescence in situ hybridization (FISH; described in Chapter 2). The FISH fluorophore-labeled DNA probe hybridizes to SSU rRNA at a sequence specific to archaea (**Fig. ST 19.1** , inset). In the micrograph, spots showing green fluorescence indicate archaea, whereas blue fluorescence arises from a different fluorophore that generally

stains all DNA. A majority of the spots are green, showing the presence of archaea.

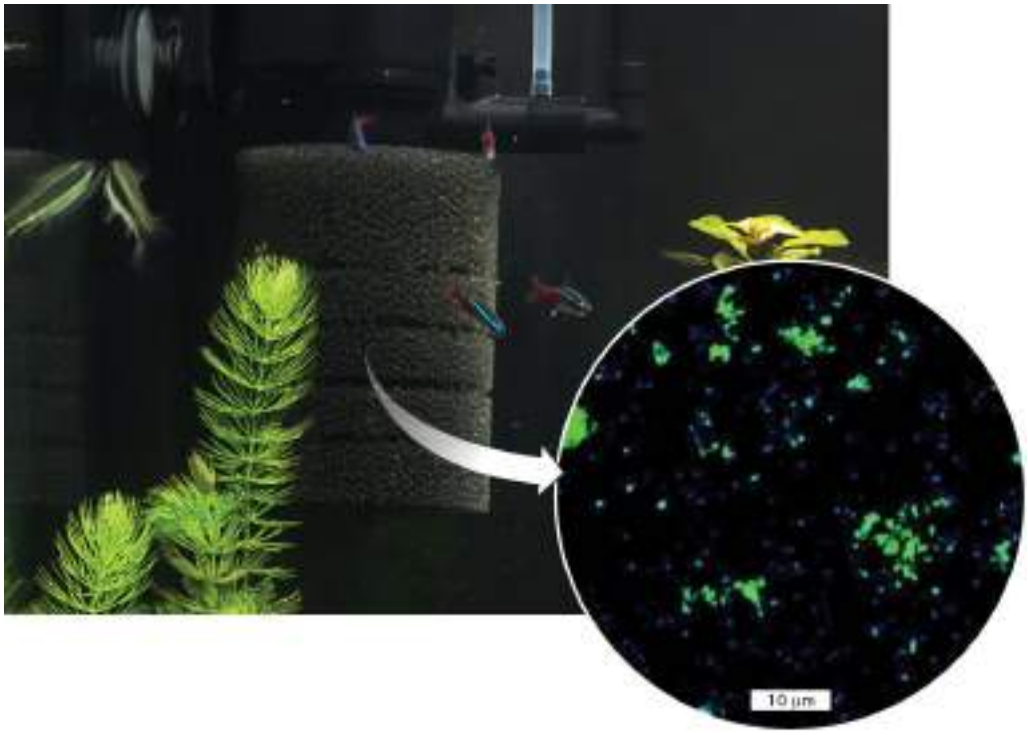


FIGURE ST 19.1 ■ " *Candidatus* Nitrosotenuis aquarius" cultured from a sponge filter. In a freshwater aquarium, fish benefit from ammonia-oxidizing archaea on a sponge filter. **Inset:** " *Candidatus* Nitrosotenuis aquarius" (green fluorophore, FISH) and other microbes (blue fluorophore, FISH) were cultured from a sponge filter.

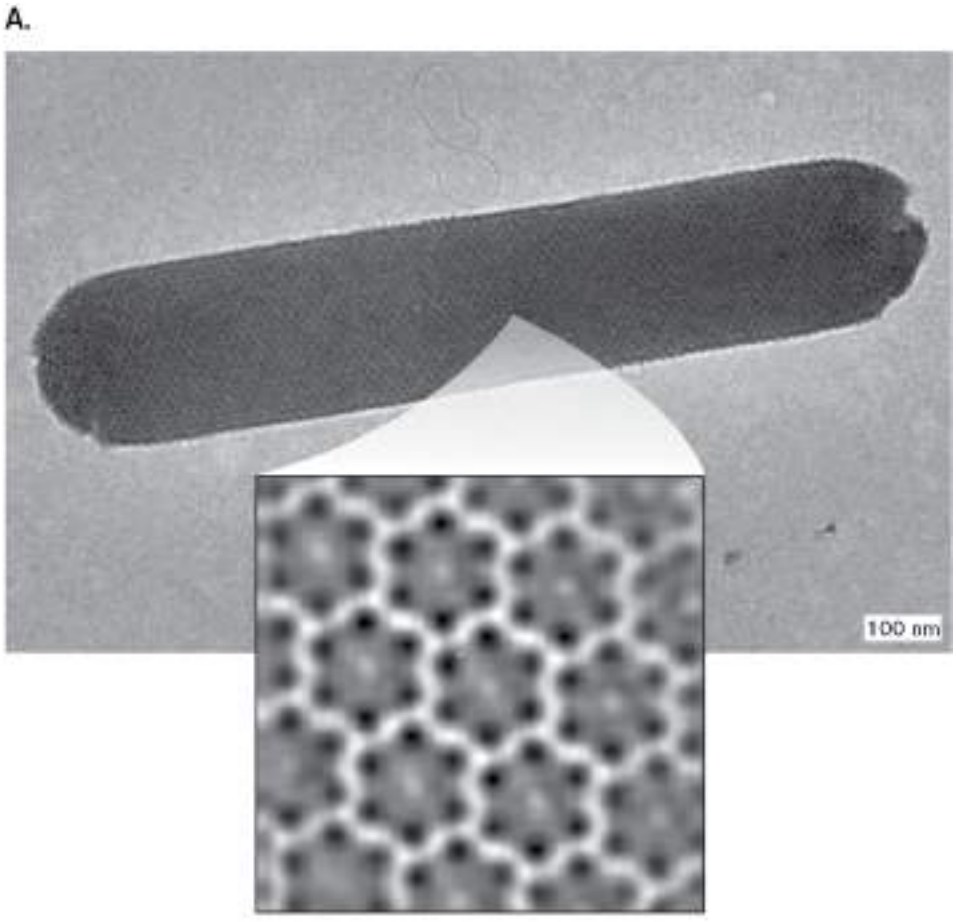
Source : Laura Sauder et al. 2018. *Appl. Environ. Microbiol.* **84** :e01430.

MPRODUCTION/DEPOSITPHOTOS
L. A. SAUDER ET AL. 2018. *APPL ENVIRON MICROBIOL* . **84** :E01430–18

The archaea obtained were then isolated as clones, and their genomes were sequenced by Illumina sequencing. The genomes resembled those of several ammonia-oxidizing marine archaea within the genus *Nitrosotenuis* . For Sauder's aquarium isolate, the researchers proposed the name " *Candidatus* Nitrosotenuis aquarius." (For a candidate species, *"Candidatus"* is italicized but

the proposed genus and species name is not.) The genome of this isolate shows the genes *amoABC* encoding the classic enzyme ammonia monooxygenase as well as ammonium transporters.

Sauder's team also characterized her isolate by electron microscopy (**Fig. ST 19.2A**). The archaeal cell is an elongated lozenge with the hexagonal packing of S-layer subunits. The pattern resembles that of the *Nitrosopumilus* sp. S-layer imaged by cryo-electron tomography.



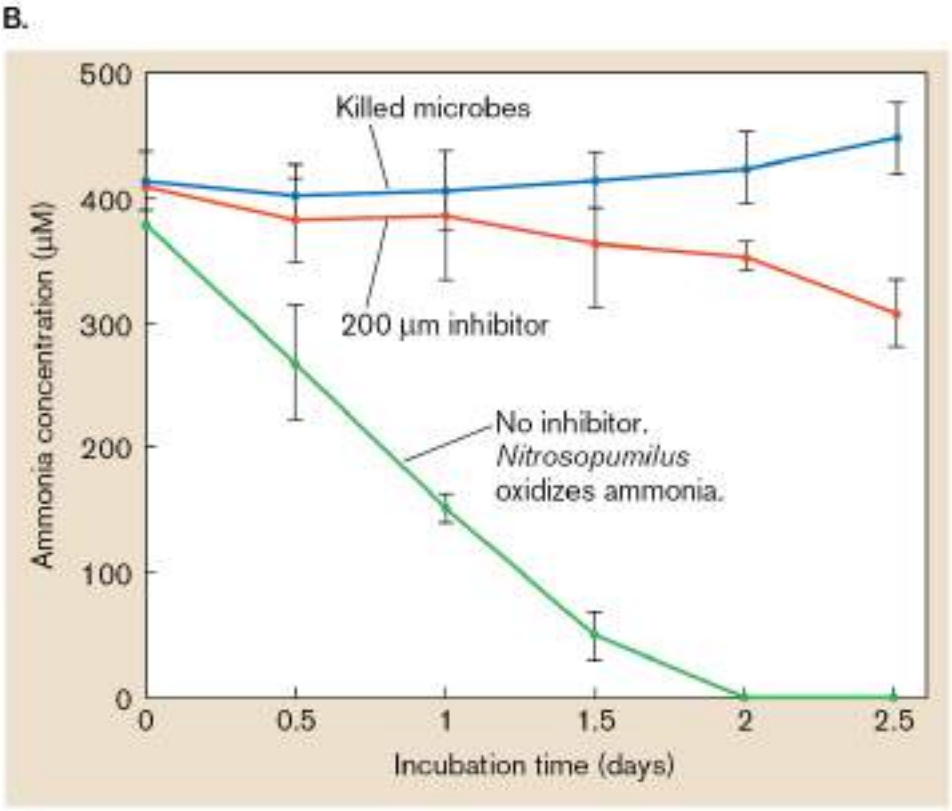


FIGURE ST 19.2 ■ " *Candidatus* Nitrosotenuis aquarius" S-layer and ammonia filtration. A. Cell stained with uranyl acetate shows hexagonal pattern of S-layer. **Inset:** S-layer of related species, *Nitrosopumilus* SCM1 (cryo-electron tomography). **B.** Ammonia removed by the sponge filter biomass. *Source:* Laura Sauder et al. 2018. *Appl. Environ. Microbiol.* 84:e01430. Inset source: Wei Qin et al. 2017. *Int. J. Syst. Evol. Microbiol.* **67** :5067.

L. A. SAUDER ET AL. 2018. *APPL ENVIRON MICROBIOL* . **84** :E01430–18
W. QIN ET AL. 2017. *INT J SYST EVOL MICROBIOL* . **67** :5067–5079

The effectiveness of the sponge filter biomass was confirmed by ammonia assay (**Fig. ST 19.2B**). The assay was conducted with controls including an inhibitor of the ammonia monooxygenase, and a control with the microbes inactivated. Overall, these newly described archaea turn out to be hard-working partners in a microbial ecosystem close to home. Laura

Sauder has now joined a biotech company, Indigo Ag, that develops sustainable approaches to agriculture including microbial partners.

RESEARCH QUESTION

How do archaea regulate their ammonia oxidation? What environmental factors enhance the microbial ammonia removal in your fish tank?

Sauder, Laura A., Katja Engel, Chien-Chi Lo, Patrick Chain, and Josh D. Neufeld. 2018. " *Candidatus* Nitrosotenuis aquarius," an ammonia-oxidizing archaeon from a freshwater aquarium biofilter. *Applied and Environmental Microbiology* **84** :e01430.

Symbiotic ammonia-oxidizing archaea. Human sweat glands release nitrogenous materials that bacteria break down to ammonia. Are ammonia-oxidizing archaea found on humans? Christine Moissl-Eichinger, at the Medical University of Graz, Austria, discovered AOA on human skin, where they are the major archaeal component of the skin microbiome. The human body continually emits ammonia, so AOA may help metabolize this toxic substance while controlling skin pH for the microbial community. The finding of ammonia-oxidizing thaumarchaeotes on the skin, as well as methanogens in the gut, may help explain another discovery made by Moissl-Eichinger; namely, the DNA signatures of thaumarchaeotes and methanogens in hospital intensive care units and industrial clean-room facilities.

In the deep ocean, there are thaumarchaeote psychrophiles (cold-adapted microorganisms). Some psychrophilic thaumarchaeotes live as endosymbionts of marine animals such as sponges. The thaumarchaeote *Cenarchaeum symbiosum* inhabits the sponge *Axinella mexicana* (**Fig. 19.23A**). *C. symbiosum* has yet to be grown in culture, but the presence of the microbes is shown by the fluorescence of a DNA probe that hybridizes

to sequences specific to *C. symbiosum* (**Fig. 19.23B**). How the microbe benefits its sponge host is unknown, but the sponge and its endosymbionts can be cocultured in an aquarium for many years. A hypothesis investigated by researchers is that *C. symbiosum* produces antimicrobial agents that protect the sponge from pathogens or predators (see Chapter 15). Products of *C. symbiosum* are now being tested for their pharmacological properties.

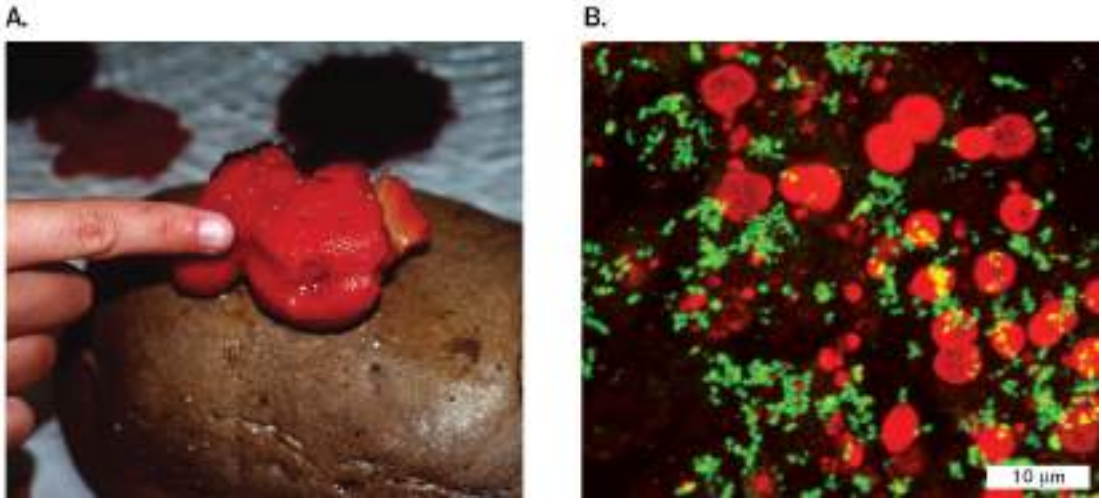


FIGURE 19.23 ■ Symbiosis between a thaumarchaeote and a sponge. A. *Cenarchaeum symbiosum* inhabits the sponge *Axinella mexicana* . **B.** Differential fluorescent staining of *C. symbiosum* (green fluorescence) present in sponge tissue visualized by FISH (fluorescent probes that hybridize rRNA; discussed in Chapter 2). Host cell nuclei fluoresce red (propidium iodide stain).

EDWARD F. DELONG. 2003. *ASM NEWS* **69** :503–511, FIG. 3A
CHRISTINA PRESTON, MONTEREY BAY AQUARIUM RESEARCH INSTITUTE

To Summarize

- **Oceans, soil, plant roots, and animals** provide habitats for mesophilic and psychrophilic TACK archaea.

- ***Nitrosopumilus* and other ammonia-oxidizing archaea (AOA) gain energy by aerobically oxidizing ammonia to nitrite.**
- **Ammonia-oxidizing archaea** make essential contributions to local and global nitrogen cycling in water and soil.
- **Ammonia-oxidizing archaea comprise a significant part of the human skin microbiome.**
- **Thaumarchaeotes include symbionts of marine sponges.** Emerging thaumarchaeotes include mesophiles, psychrophiles, and thermophiles in many environments.

19.4 Euryarchaeota: Methanogens from Gut to Globe

A third major branch of known archaea is Euryarchaeota, the "broad-ranging archaea" (**Table 19.2**). The euryarchaeotes include multiple branches of methanogens , species that derive energy through reactions producing methane. Methanogenesis is a form of energy-yielding metabolism unique to archaea—another profound distinction of the archaeal domain.

Most methanogens are strict anaerobes; while their metabolism is chemolithotrophic, they depend on bacterial communities releasing CO_2 , H_2 , and other simple carbon compounds. Methanogens release methane from soil and animal digestive habitats found nearly everywhere on Earth. For the methanogen, methane is just a by-product of its energy-yielding metabolism, but this by-product is a greenhouse gas with profound consequences for our biosphere (discussed in Chapter 22).

Figure 19.5 shows the approximate branch points of five major clades of methanogens. The branches are polyphyletic—that is, they lack a distinctive shared ancestor—and they are interspersed by nonmethanogens.

- Methanosarcinales (**Fig. 19.24A**) are found in ocean and soil, breaking down acetate, alcohol, and amines to methane.

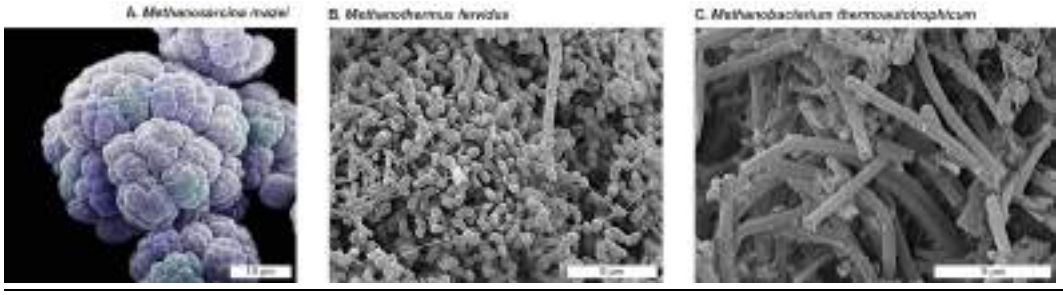


FIGURE 19.24 ■ Methanogens show a wide range of shapes. A. *Methanosarcina mazei* , a lobed coccus form lacking flagella (SEM). **B.** *Methanothermus fervidus* , a short bacillus (SEM). **C.** *Methanobacterium thermoautotrophicum* , an elongated bacillus (SEM).

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- Methanobacteriales (**Fig. 19.24B and C**) inhabit soil and animal digestive tracts. They include main human gut methanogens *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* .
- Methanopyrales include hyperthermophilic methanogens growing at up to 122°C.
- Methanococcales include mesophilic and hyperthermophilic marine organisms.

- Methanomicrobiales are marine methanogens.

The methanogens branch deeply, as diverse as all the euryarchaeotes together. A possible explanation of this phylogeny would be that the ancestral euryarchaeote was a methanogen, but many descendant lineages lost the metabolism. Alternatively, divergent euryarchaeotes could have acquired the genes for methanogenesis later, independently, by horizontal gene transfer. As yet, we lack sufficient genomic data to determine which model is correct. Furthermore, genes encoding enzymes of methanogenesis were found in one deep-branching clade outside the Euryarchaeota, the TACK phylum Bathyarchaeota.

Biogenic Paths to Methane

Biogenic methane formation has actually been observed since 1776, when the Italian priest Carlo Campi and physicist Alessandro Volta (1745–1827) investigated bubbles of "combustible air" from a wetland lake. Volta wrote:

> Being in a little boat on Lake Maggiore, and passing close to an area covered with reeds, I started to poke and stir the bottom with my cane. So much air emerged that I decided to collect a quantity in a large glass container. . . . This air burns with a beautiful blue flame.

Volta noted that methane arose from wetlands containing water-saturated decaying plant material. In 1882, the German medical researcher Hermann von Tappeiner (1847–1927) combined plant materials with ruminant stomach contents (a source of methanogens) and showed that both components were essential to produce methane. During the late nineteenth century, in England, methane was collected from manure and sewage and used as a fuel for street lamps.

Different species generate methane from different substrates, such as CO_2 and H_2 or small organic compounds, generally fermentation products of bacteria. Each pathway of methanogenesis generates a small free energy change ($\Delta G°'$) that is just enough to drive processes of carbon fixation and biosynthesis. All types of methanogenesis are poisoned by molecular oxygen and therefore require extreme anaerobiosis. Major substrates and reactions include:

Carbon dioxide: $CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$
Formic acid: $4CHOOH \longrightarrow CH_4 + 3CO_2 + 2H_2O$
Acetic acid: $CH_3COOH \longrightarrow CH_4 + CO_2$
Methanol: $4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$
Methylamine: $4CH_3NH_2 + 2H_2O \longrightarrow 3CH_4 + CO_2 + 4NH_3$
Dimethyl sulfide: $2(CH_3)_2S + 2H_2O \longrightarrow 3CH_4 + CO_2 + 2H_2S$

In CO_2 reduction, the most common form of methanogenesis, carbon dioxide plus molecular hydrogen combine to form water and methane. CO_2 -reducing methanogens

are autotrophs, growing solely on CO_2 and H_2 with a source of nitrogen and other minerals. Methanogenesis from other carbon sources, such as formate, acetate, or methanol, is heterotrophic and generates CO_2 as a product in addition to methane. The nitrogen-containing substrate methylamine generates ammonia in addition to methane and CO_2, whereas the sulfur-containing substrate dimethyl sulfide generates hydrogen sulfide.

Note that only a narrow range of substrates supports methanogenesis. For unknown reasons, most methanogens lack the vast array of energy-yielding pathways found in soil bacteria such as *Rhodopseudomonas* or *Streptomyces*. Thus, methanogens generally require close association with bacterial partners to provide their substrates—a relationship called syntrophy (discussed in Chapter 13). Syntrophic methanogens usually grow in habitats with minimal resource flux, where hydrogen and carbon dioxide gases can be trapped for their use. Removal of these gases then enhances the metabolism of partner bacteria.

Thought Question

19.5 Anoxic soil contains bacteria and methanogens. What will happen to the microbial populations when the soil is tilled and aerated?

Many kinds of methanogens can be cultured in the laboratory. Their culture poses special challenges because the reactions of methanogenesis are halted by oxygen. Thus, most methanogens are strict anaerobes, although a few that tolerate oxygen have been found. To exclude oxygen, microbes are cultured and manipulated within an anaerobic chamber. Furthermore, methanogens that use CO_2 and H_2 as substrates must receive a steady supply of these gases. Even more challenging is the culture of hyperthermophilic methanogens, such as *Methanopyrus*, a deep-ocean vent hyperthermophile that grows at scorching temperatures up to 122°C. For these organisms, we must maintain both high temperature and high pressure.

During growth, how do methanogens form biomass? Many euryarchaeotes fix CO_2 into biomass by the Wood-Ljungdahl pathway of acetogenesis (acetate formation), also known as the reductive acetyl-CoA pathway (presented in Chapter 15). Biochemical and genomic comparisons suggest that two-carbon assimilation by acetogenesis may be the oldest means of carbon fixation, used by the ancient ancestral archaea.

A striking feature of methanogens is the wide range of growth temperatures among closely related species. Thermophiles and even hyperthermophiles branch from closely related mesophiles; for example, the order Methanobacteriales includes *Methanobrevibacter ruminantium* (37°C–39°C), *Methanobacterium thermoautotrophicum* (50°C–75°C), and *Methanothermus fervidus* (60°C–97°C). **Table 19.4** summarizes five major orders of methanogens.

TABLE 19.4	Methanogens			
Representative species	**Growth temperature (°C)**	**Growth pH**	**Cell shape**	**Substrates for methanogenesis**
Methanobacteriales				
Methanobrevibacter ruminantium	37–39°C	pH 6–9	Chains of short rods	H_2 and CO_2
Methanobacterium thermoautotrophicum	50–75°C	pH 7–8	Filaments of long rods	H_2 and CO_2, formate
Methanothermus fervidus	60–97°C	pH 6–7	Rods	H_2 and CO_2
Methanococcales				
Methanococcus vannielii	20–40°C	pH 7–9	Cocci with archaella	H_2 and CO_2
Methanocaldococcus jannaschii	48–94°C	pH 6–7	Cocci with archaella	H_2 and CO_2
Methanomicrobiales				
Methanoculleus olentangii	30–50°C	pH 6–8	Cocci	Acetate, complex nutrients
Methanospirillum hungatei	20–45°C	pH 6–7	Spirilla	Acetate
Methanopyrales				
Methanopyrus kandleri	84–122°C	pH 6–8	Long rods (2–14 μm)	H_2 and CO_2
Methanosarcinales				
Methanosarcina barkeri	20–50°C	pH 5–7	Aggregates of cocci	H_2 and CO_2, methanol, methylamine, acetate
Methanosaeta concilii	10–45°C	pH 6–8	Filaments of rods	Acetate

Methanohalophilus zhilinae	45°C	pH 8–10	Cocci	Methanol, methylamine, dimethyl sulfide
Source: Harald Huber and Karl O. Stetter. 2002. *The Prokaryotes* . Springer.				

Methanogens Show Diverse Cell Forms

Despite their metabolic similarity, methanogens display an astonishing diversity of form, perhaps as diverse as the entire domain of bacteria (**Fig. 19.24**). For example, *Methanocaldococcus jannaschii* cells grow as cocci with numerous archaella attached to one side, while *Methanosarcina mazei* forms peach-shaped cocci lacking archaella. *Methanothermus fervidus* cells are short, fat rods without archaella, and *Methanobacterium thermoautotrophicum* grows as elongated rods reminiscent of the bacterial genus *Bacillus* . Still others, such as *Methanospirillum hungatei* , form wide spirals.

The morphological diversity of methanogens may be explained in part by their rigid cell walls, which can maintain a distinctive shape. The composition of methanogen cell walls is much more diverse than that of bacteria. *Methanobacterium* species have a cell wall composed of pseudopeptidoglycan , or **pseudomurein** , a structure in which chains of alternating amino sugars are linked by peptide cross-bridges analogous to those of peptidoglycan (**Fig. 19.25**). The bacterial *N* -acetylmuramic acid, however, is replaced by a related sugar, *N* -acetyltalosaminuronic acid, and the sugar linkage is β(1,3) instead of β(1,4) as in bacteria. As a result, these archaea are resistant to lysozyme, which degrades bacterial cell walls at the β(1,4) sugar linkage. The peptide cross-bridges of pseudopeptidoglycan differ as well, causing resistance to penicillin (discussed in Chapter 3).

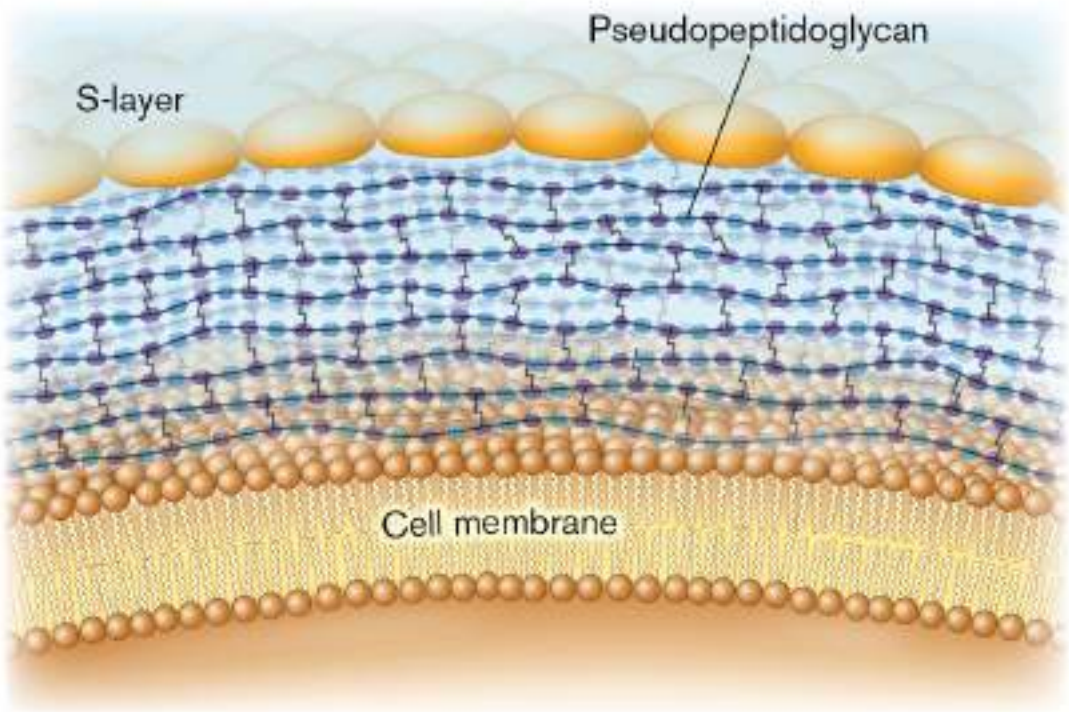


FIGURE 19.25 ■ Methanobacteriales and other methanogenic species have a cell wall of pseudopeptidoglycan between their membrane and S-layer.

By contrast, *Methanosarcina* species have a cell wall composed of sulfated polysaccharides. The genera *Methanomicrobium* and *Methanococcus* have protein-derived cell walls.

Filamentous methanogens form chains of large cells similar to those of filamentous cyanobacteria. Filaments of *Methanosaeta* perform a key function in the treatment of sewage waste (**Fig. 19.26** , presented in Section 22.2). In waste treatment, the raw sewage first undergoes aerobic respiration by bacteria, in which the organic materials are converted into small molecules such as CO_2 and acetate, and then the remainder is digested anaerobically. The bacteria performing anaerobic decomposition become trapped in filaments of *Methanosaeta* species, which convert bacterial fermentation products such as acetate into methane and CO_2 . The methanogenic filaments serve a key function by trapping bacteria into granules that settle out from the liquid.

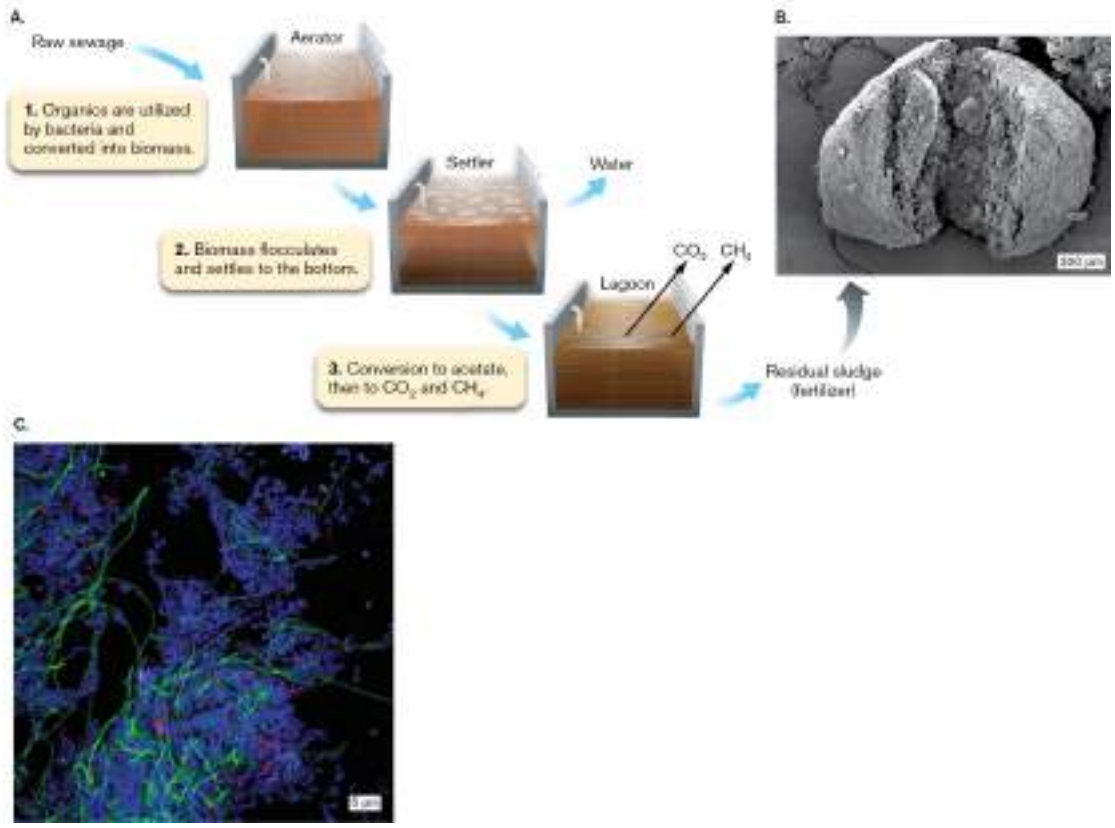


FIGURE 19.26 ■ Filamentous methanogens bind bacterial communities in waste treatment. A. Raw sewage is aerated and decomposed by bacteria, followed by anaerobic incubation. Under anaerobiosis, the bacterial waste products are converted to methane and CO_2. The bacteria are packed together by filamentous methanogens to form sludge. **B.** Sludge (wastewater sediment) forms granules packed with bacteria and methanogens (discussed in Chapter 22). **C.** Filamentous methanogens entangle bacteria, forming "flocs" that settle in the waste treatment tank. Methanogens fluoresce green with a DNA probe, while other wastewater microbes fluoresce blue (DAPI stain).

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Methanogenesis in soil and landfills. A major methanogenic environment is the anaerobic soil of wetlands. The wetlands that generate the most methane are typically disturbed or artificial wetlands, particularly rice paddies, which contain high levels of added fertilizer that bacteria convert to the substrates used by methanogens. From the standpoint of the organism, methane is an incidental by-product, but this product has great significance for our biosphere as a greenhouse gas (discussed in Chapter 22).

Chinese farmers have found that one way to decrease methane production is to drain the soils used for rice production. Changsheng Li and colleagues at the University

of New Hampshire showed in experimental plots that drained soil produces less methane. The drained soil receives more oxygen, which blocks methanogenesis. Draining soil also stimulates rice root development and accelerates decomposition of organic matter in the soil to release nitrogen. Sufficiently dry rice paddy soil can actually become a sink for atmospheric methane, thanks to methane-oxidizing bacteria.

Another major source of methanogens is landfills. Landfills such as those outside New York City are among the largest human-made structures on Earth. They are rich in organic wastes, which bacteria ferment to CO_2, H_2, and short-chain organic molecules that methanogens convert to methane. As a result, large amounts of gas can build up and spontaneously combust, causing explosions. To avoid explosions, the methane needs to be piped out. In some cases, the gas can be collected and used to generate electricity.



In Arctic tundra, from Alaska and Canada to Siberia, many psychrotrophic methanogens lie buried. As the planet's temperature increases, formerly frozen regions thaw. Buried communities of bacteria activate their metabolism, releasing fermentation products that include CO_2 and H_2. These molecules then serve as substrates for methanogens. So much methane is released that, trapped beneath ice, it can burst into flame (**Fig. 19.27**). Rising temperatures accelerate methanogenesis, and the methane bubbles up so fast that it melts holes in the ice sheets covering Arctic lakes. In 2012, Andrew McDougall and colleagues at the University of Victoria, British Columbia, modeled the permafrost carbon release and its feedback effect on global warming. They project that permafrost could release more than a quarter of its carbon stores by the year 2100, and that this amount could add another 1.5°C to the global temperature by the year 2300.



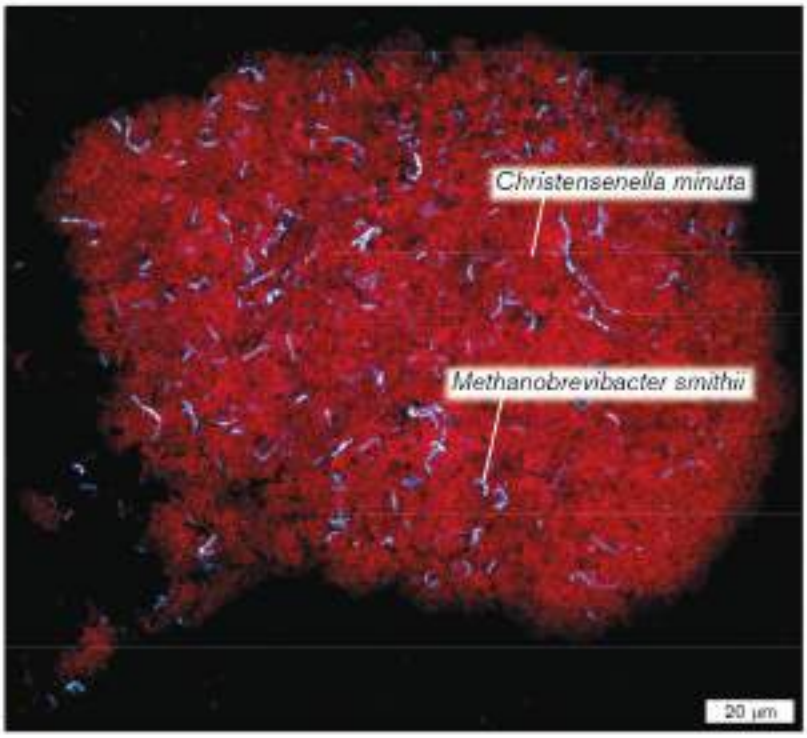
FIGURE 19.27 ■ Polar methane release. Dr. Katey Walter Anthony determines if an Alaskan lake contains methane by igniting the gas flux.
UNIVERSITY OF ALASKA FAIRBANKS/NICHOLAS HASSON

Methane hydrates and Syntrophoarchaeota. Psychrophilic marine methanogens grow at or beneath the seafloor. These seabed methanogens generate large volumes of methane that seep up slowly from the sediment. Under the great pressure of the deep ocean, the methane becomes trapped as methane gas hydrates , which are crystalline materials in which methane molecules are surrounded by a cage of water molecules. The methane hydrates accumulate in vast quantities. Methane hydrates are of interest as a potential source of natural gas. But if a large part of this methane were to be released, it could greatly accelerate global warming (discussed in Chapter 22).

Fortunately, much of the methane produced by seafloor methanogens is oxidized to CO_2 by anaerobic methanotrophs. These methanotrophs are methane oxidizers, classified as Anaerobic Methane-Oxidizing Euryarchaeota (ANME) , also known as Syntrophoarchaeota. The ANME euryarchaeotes oxidize methane in syntrophy with sulfate-reducing bacteria such as *Desulfobacterales* (discussed in Chapter 18). The bacteria reduce sulfate (abundant in seawater) to sulfide. Coupled together, the reactions of methane oxidation and sulfate reduction have the negative value of ΔG needed to drive metabolism for both kinds of organisms.

Digestive methanogenic symbionts. Numerous methanogens also grow within the digestive fermentation chambers of animals such as termites and cattle (**Fig. 19.28** ; discussed in Chapter 21). Termites have to aerate their mounds continually in order to remove methane; when rainfall temporarily clogs the mound, the mound can be ignited by lightning and explode. Cattle support methanogenesis within their rumen and reticulum (a common veterinary trick is to insert a tube into the rumen and ignite the escaping methane gas). Bovine methanogenesis diverts carbon from meat production, and it makes a significant contribution to global methane.

A.



B.



FIGURE 19.28 ■ Methanogens in the human gut microbiome. A. Coculture of *Methanobrevibacter smithii* (blue fluorophore, FISH) and bacteria *Christensenella minuta* (red fluorophore, FISH) from human gut microbiome. **B.** Ruth Ley's laboratory group at Max Planck Institute, Tübingen, Germany.

A. RUAUD ET AL. 2020. *MBIO* **11** :E03235–19
MAX PLANCK INSTITUTE FOR BIOLOGY TÜBINGEN

Methanogens also contribute to human digestion. Species such as *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* may constitute about 10% of gut anaerobes. *Methanobrevibacter smithii* increases the efficiency of digestion by consuming excess reduced products (formate and H_2) from bacterial mixed-acid fermentation (discussed in Chapter 13). High levels of H_2 inhibit bacterial NADH dehydrogenases and thus decrease the proton potential for ATP production. **Figure 19.28** shows a coculture of *Methanobrevibacter smithii* with a gut bacterium, *Christensenella minuta*, obtained by the lab group of Ruth Ley at the Max Planck Institute, Tübingen, Germany. Ley's group showed that *M. smithii* metabolism is intimately connected with that of the H_2-producing bacteria, for a net energy yield favorable to both—another example of syntrophy.



If methanogens influence the fermentation efficiency of gut bacteria, do they affect the caloric content we obtain from food? Studies have found that mice colonized with both *Bacteroides thetaiotaomicron* and *M. smithii* store more fat after consuming the same quantity of food than do mice colonized solely with *B. thetaiotaomicron*. Human studies suggest that methanogenesis may increase the efficiency of gut bacterial catabolism and human fat storage. How the composition of intestinal microbiota may influence obesity is discussed in Section 23.2.

Biochemistry of Methanogenesis

Methanogenesis is extremely important for our environment because the process releases a potent greenhouse gas. Knowledge of methanogenic biochemistry may help us develop ways to minimize methane output from cattle. For example, feeding red seaweed to cattle decreases their methane release by 80%. How does this happen?



Cofactors for methanogenesis. The process of methanogenesis uses a series of specific cofactors to carry each carbon from CO_2 (or other substrates) as it becomes progressively reduced by hydrogen. The hydrogen atoms also require redox carriers. Most of the cofactors are unique to methanogens, although the general structural types resemble those of redox cofactors that we saw in Chapter 14 (**Fig. 19.29**). For example, cofactor F_{420} is a heteroaromatic molecule (containing nitrogens in the aromatic rings) that undergoes a redox transition by acquiring or releasing two hydrogens (**Fig. 19.29C**)—a transition similar to that of the nicotinamide ring of NADH.

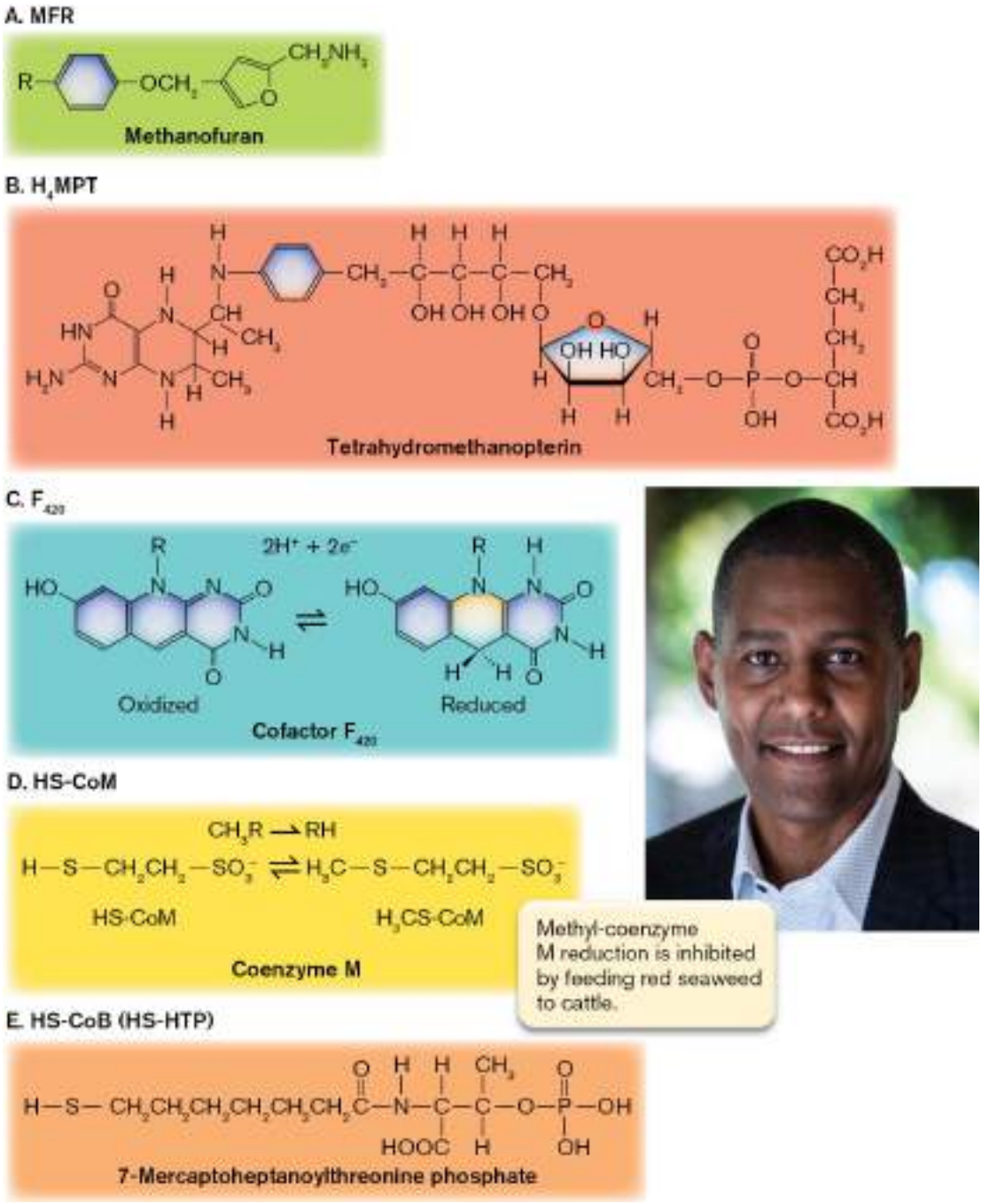


FIGURE 19.29 ■ Cofactors for methanogenesis. Cofactors specific for methanogenesis transfer the hydrogens and the increasingly reduced carbon to each enzyme in the pathway. **Inset:** Ermias Kebreab, UC Davis, showed that feeding seaweed to cattle inhibits methanogenesis by blocking the enzyme that reduces methyl-coenzyme M.

ERMIAS KEBREAB

One key reaction in methanogenesis is the reduction of methyl-coenzyme M to form methane. This reduction step can be inhibited by a halogenated defense molecule produced by the red seaweed *Asparagopsis* . Ermias Kebreab, at UC Davis, showed that feeding seaweed to cattle inhibits methanogenesis. Thus, by understanding the biochemistry of methanogenesis, we could potentially decrease the global methane emissions from cattle.

Methanogenesis from CO_2 . The formation of methane from CO_2 and H_2 (**Fig. 19.30**) is technically a form of anaerobic respiration in which H_2 is the electron donor and CO_2 is the terminal electron acceptor (discussed in Chapter 14). The process fixes CO_2 onto the cofactor methanofuran (MFR) and then passes the carbon stepwise from one cofactor to the next, each time losing an oxygen to form water or gaining a hydrogen carried by another cofactor.

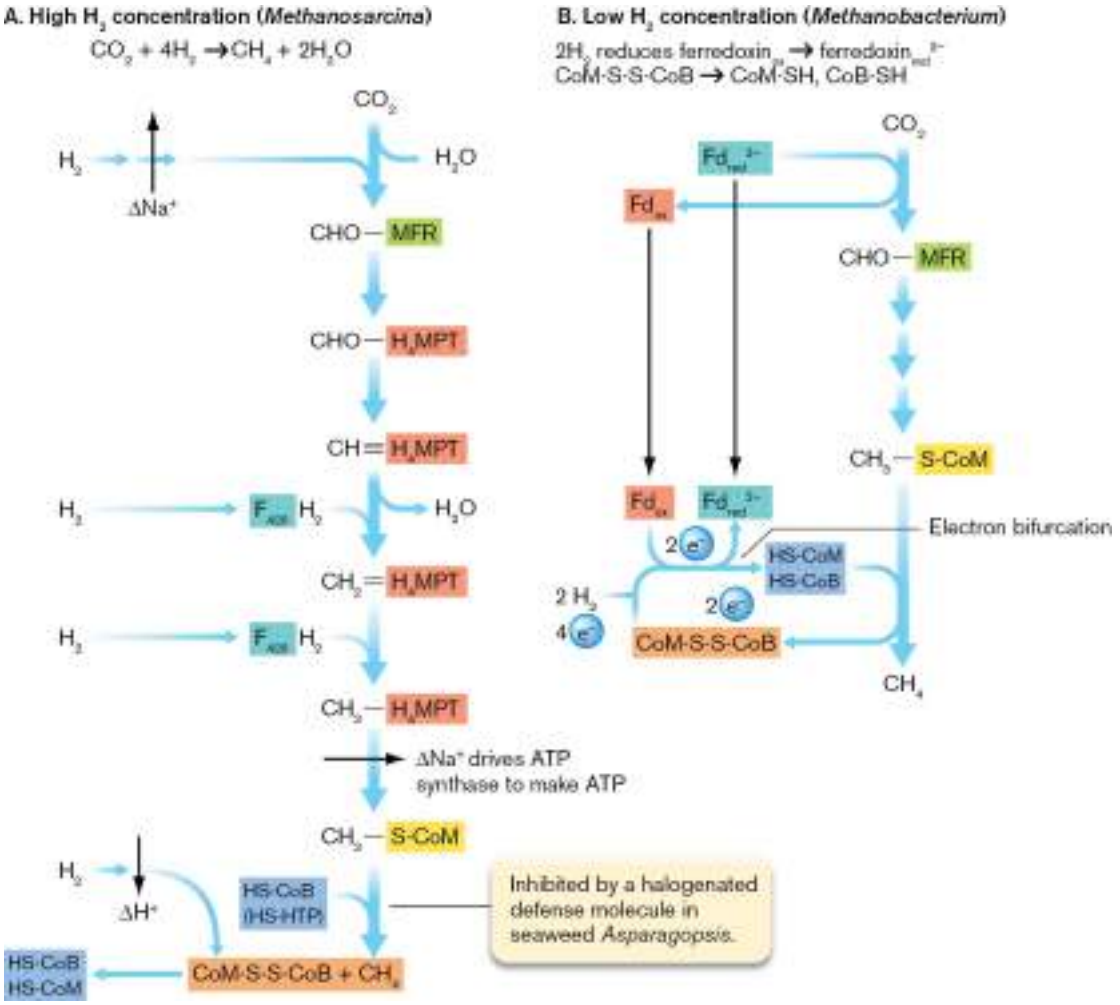


FIGURE 19.30 ■ Methanogenesis from CO_2 and H_2 . All steps require specific enzymes (not shown). **A.** At high [H_2], the initial incorporation of H_2 requires a coupled sodium potential (ΔNa^+). The step from CH_3 –H_4 MPT to CH_3

–S-CoM generates a transmembrane sodium potential (ΔNa^+), which drives ATP synthesis. **B.** At low [H_2], electron donation to ferredoxin (Fd) requires energy input from coupled electron donation to CoM-S-S-CoB. This coupled process involves electron bifurcation.

The first step in the conversion of CO_2 and H_2 to methane is fixing the carbon onto methanofuran (for its chemical formula, see **Fig. 19.29A**). This fixation step requires reaction of protons with oxygen to form water. The mechanism of methanogenesis depends on the available concentration of H_2 . High-H_2 environments, such as oil wells and sewage sludge, favor genera such as *Methanosarcina* (**Fig. 19.30B**). The high H_2 concentration allows electron donation to CO_2 from an electron transport system (ETS). The ETS may provide energy from a sodium potential (ΔNa^+). Most methanogens require Na^+ for growth, unlike bacteria, many of which can grow without sodium. Methanogenesis ultimately generates a Na^+ potential that drives ATP synthesis. The sodium requirement of methanogens is something they share with another division of Euryarchaeota, the halophiles (discussed in Section 19.5).

Other methanogens, such as *Methanobacterium* and *Methanococcus* , use much lower concentrations of H_2 (<1/10,000 atm), common in soil or water. At lower concentrations of substrate, the free energy change (ΔG) of reaction becomes less favorable (discussed in Chapters 13 and 14). So the methanogen needs to couple CO_2 reduction to an energy-spending reaction, the reduction of CoM-S-S-CoB (**Fig. 19.30B**). This coupling of an energy-spending electron transfer (CO_2 reduction via ferredoxin) to an energy-yielding electron transfer (CoM-S-S-CoB reduction) is called electron bifurcation . The cost of electron bifurcation (compared to the ETS used with high H_2) is that fewer ATPs can be made, but it allows many more types of methanogens to grow in a much wider range of natural habitats. Electron bifurcation is shown in **Figure 19.30B** .

Later steps in methanogenesis reduce the carbon with H_2 to methane. In the final step, CoM-S-S-CoB serves as an anaerobic terminal electron acceptor for an ETS accepting electrons from H_2 . Overall, H_2 reduces CoM-S-S-CoB back to the two cofactors HS-CoM and HS-CoB through an ETS, generating a proton motive force. The proton motive force drives ATP synthase.

Another important feature of methanogenesis is that the enzymes catalyzing each step require several transition metals. For example, the hydrogenase that reduces F_{420} requires both nickel and iron. The enzyme catalyzing the reaction

$$CO_2 + \text{methanofuran} + 3H \longrightarrow$$

$$\text{CHO-methanofuran} + H_2O$$

requires either molybdenum or tungsten, depending on the species; some species have two alternative enzymes, depending on which metal is available. Another metal, cobalt,

is required for a B_{12} -related cofactor that participates in methane production from methanol and methylamines.

Thought Question

19.6 What do the multiple metal requirements suggest about how and where the early methanogens evolved?

Methanogenesis from acetate. Methane production from acetate is particularly important for wastewater treatment, where most of the substrate consists of short-chain bacterial fermentation products. The acetate methanogenesis pathway is not fully understood, but the initial incorporation of H_2 probably requires a coupled gradient of sodium ion, as it does for CO_2 methanogenesis. The two carbons from acetate enter different pathways, which eventually converge:

$$CH_3\text{–S-CoM} + \text{HS-CoB} \longrightarrow CH_4 + \text{CoM-S-S-CoB}$$

To Summarize

- **Methanogens gain energy through redox reactions that generate methane** by using H_2 to reduce CO_2 , formate, acetate, and other small molecules. Methanogens require association with bacteria whose fermentation generates the needed substrates.
- **Methanogens have rigid cell walls of diverse composition in different species** , including pseudopeptidoglycan, protein, and sulfated polysaccharides.
- **Species of methanogens show a wide range of different shapes** , including rods (single or filamentous), cocci (single or clumped), and spirals.
- **Methanogens inhabit anaerobic environments** such as wetland soil, marine benthic sediment, and animal digestive organs.
- ***Methanobrevibacter smithii* and *Methanosphaera stadtmanae*** are the main methanogens of the human colon. They show possible involvement with caloric efficiency of digestion.
- **Biochemical pathways of methanogenesis** involve transfer of the increasingly reduced carbon to cofactors that are unique to methanogens.

Glossary

Anaerobic Methane-Oxidizing Euryarchaeota (ANME) *or* anaerobic methane oxidizers
: A group of archaea in the superphylum Euryarchaeota that oxidize methane. Some, such as the *Methanoperedenaceae* , oxidize methane independently whereas others oxidize methane syntrophically with sulfate-reducing bacteria (SRB).

methanogen
: An archaeon that uses hydrogen to reduce CO_2 and other single-carbon compounds or acetate to methane, yielding energy. Clades of methanogens branch within the superphylum Euryarchaeota.

methanogenesis
: An energy-yielding metabolic process that releases methane, commonly from hydrogen gas and oxidized one- or two-carbon compounds. It is unique to archaea.

syntrophy
: Metabolic cooperation between two different species; usually one member releases a product whose removal by the second species enables the pair to metabolize with a negative value of ΔG .

pseudopeptidoglycan *or* pseudomurein
: A peptidoglycan-like molecule composed of sugars and peptides that is found in some archaeal cell walls.

methane gas hydrate
: A crystalline material in which methane molecules are surrounded by a cage of water molecules. This molecular configuration is found in the deep ocean.

electron bifurcation
: A biochemical reaction in which an electron transfer that yields energy is coupled to an electron transfer that consumes energy.

Figure 19.5

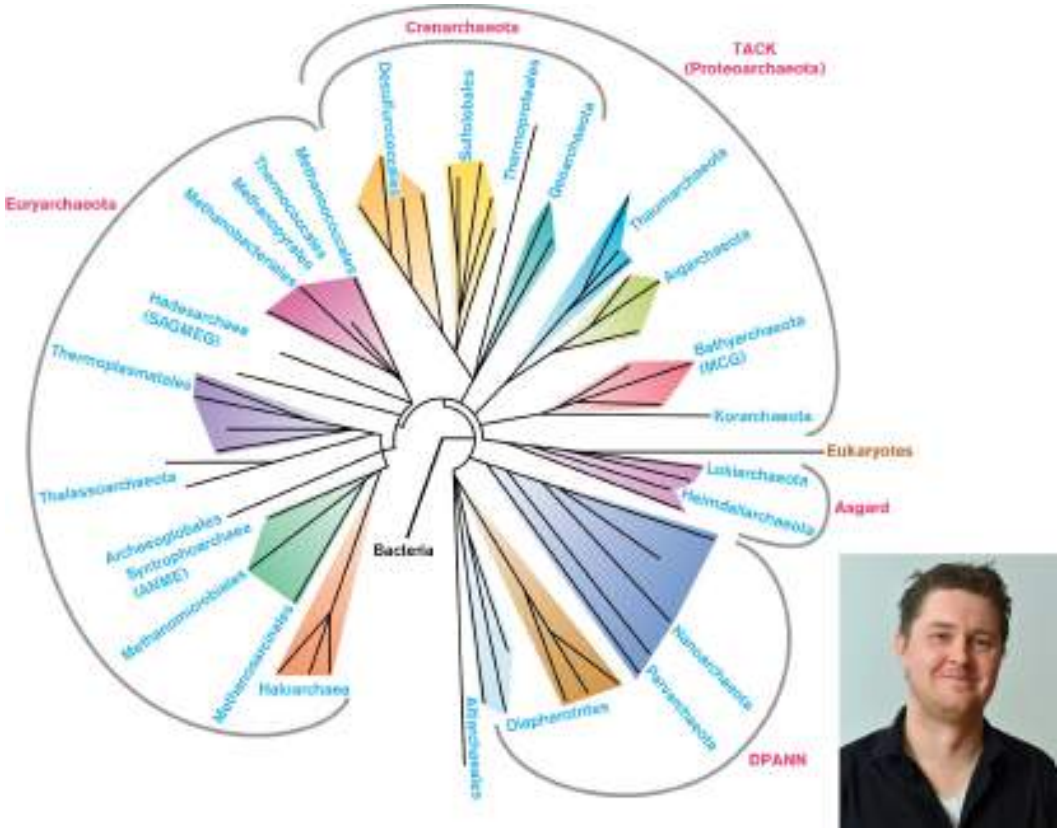


FIGURE 19.5 ■ Archaeal phylogeny. Major superphyla, phyla, and orders of Archaea. Divergence is based approximately on small-subunit ribosomal RNA (SSU rRNA) and genome sequences. (See Table 19.2 for abbreviations.) **Inset:** Thijs Ettema.

COURTESY OF THIJS ETTEMA

19.5 Haloarchaea and Other Euryarchaeotes: Underground and Under Ocean

In the saturated brine of Utah's Great Salt Lake or Israel's Dead Sea, few living things can grow except for halophilic euryarchaeotes called Haloarchaea . Elsewhere, still other euryarchaeotes include sulfur hyperthermophiles. Some are also acidophiles—hyperthermoacidophiles that grow under the most extreme acid as well as high temperatures.

Haloarchaea: Life in Salt

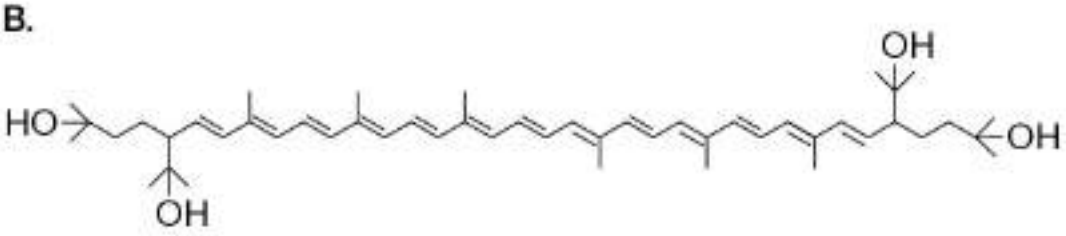
The halophilic archaea require at least 1.5-M NaCl or equivalent ionic strength, and most grow optimally at near saturation (about 4.3 M, seven times the concentration of seawater). Salted foods such as meat and fish and salt-cured hides can be spoiled by haloarchaea.

Most haloarchaea belong to a monophyletic clade that was formerly named Halobacteria, before the archaea were classified as distinct from bacteria. Haloarchaea show relatedness to methanogens (Fig. 19.5), but they do not conduct methanogenesis. Most haloarchaea grow as photoheterotrophs, using light energy to drive a retinal-based ion pump to establish a proton potential. Their photopigments color salterns, brine pools that are evaporated to mine salt (**Fig. 19.31A**). The red pigment bacterioruberin protects cells from damage by light (**Fig. 19.31B**).

A.



B.



Bacterioruberin

FIGURE 19.31 ■ A saltern for salt production. A. Aerial view of a solar saltern facility in Grantsville, Utah. **B.** The red pigment bacterioruberin protects Haloarchaea from damage by light.

COURTESY OF DON GREEN PHOTOGRAPHY

Note: The terms "Halobacteria" and "Haloarchaea" are synonymous; all members of this clade are indeed archaea. Outside the Haloarchaea, true bacteria that grow in elevated NaCl are called halophilic bacteria or bacterial halophiles.

Haloarchaeal form and physiology. Halophilic microbes need a way to maintain turgor pressure; that is, to avoid cell shrinkage as cytoplasmic water runs down the osmotic gradient toward external high salt. Most bacterial halophiles compensate for high external salt by uptake or synthesis of other kinds of osmolytes, such as small organic molecules. Haloarchaea, however, adapt to high external NaCl by maintaining a high intracellular concentration of potassium chloride (about 4-M KCl). Potassium ion concentrations are moderately high in most microbial cells (commonly 200 mM), but the exceptionally high KCl concentration within haloarchaea requires major physiological adaptations:

- **High GC content of DNA.** High salt concentration decreases the fidelity in base pairing of DNA. Because the triple hydrogen bonds of GC pairs hold more strongly than the double hydrogen bonds of AT pairs, the exceptionally high GC content of haloarchaea (above 60% for most species) may protect their DNA from denaturation in high salt.
- **Acidic proteins.** Most haloarchaeal proteins are highly acidic, with an exceptional density of negative charges at their surface. The high negative charge maintains a layer of water in the form of hydrated potassium ions (K^+). This unusual hydration layer keeps the acidic proteins soluble in the high salt within the cytoplasm.

The high salt content of haloarchaea provides a convenient way to lyse cell contents for analysis: Simply transfer cells into low-salt buffer, and they fall apart owing to osmotic shock. This technique provides a quick way for beginning students to isolate DNA. This and other techniques have made the organism *Halobacterium* sp. NRC-1 a model system for molecular biology education.

The properties of diverse halophiles are presented in **Table 19.5** . The haloarchaea are generally uniform with respect to temperature range (mesophilic). With respect to pH, some halophiles grow at neutral pH, whereas others are alkaliphiles, growing in soda lakes above pH 9, such as Lonar Lake, Maharashtra State, India. The cell envelopes of most haloarchaea contain rigid cell walls of glycoprotein, as do some of the methanogens. Most species possess archaella for phototaxis and gas vesicles for maintaining their buoyancy in the upper layer of the water column. Unlike phospholipid vesicles, gas vesicles are made entirely of protein, and they are filled with air.

TABLE 19.5 Halophilic Archaea

Representative species	NaCl range (M)	Growth temperature (°C)	Growth pH	Cell shape	Location of isolate
Haloarcula quadrata	2.7–4.3	53°C	pH 7	Square flat; pleomorphic	Sabkha, Sinai, Egypt
Haloarcula valismortis	3.5–4.3	40°C	pH 7.5	Pleomorphic rods	Salt pools, Death Valley, CA
Halobacterium salinarum	3.0–5.2	35–50°C	pH 7	Rods	Salted cowhide
Halococcus morrhuae	2.5–5.2	30–45°C	pH 7	Cocci	Dead Sea, Israel
Haloferax volcanii	1.5–5.2	40°C	pH 7	Pleomorphic; dish-shaped	Dead Sea, Israel
Halorubrum lacusprofundi	1.5–5.2	1–44°C	pH 7	Long rods (12 μm)	Deep Lake, Antarctica
Natronococcus occultus	1.4–5.2	30–45°C	pH 9.5	Cocci	Lake Magadi, Kenya
Natronomonas pharaonis	2.0–5.2	45°C	pH 9–10	Rods	Wadi El Natrun, Egypt

Sources: Aharon Oren. 2006. The order Halobacteriales. In M. Dworkin et al. (eds.), *The Prokaryotes* , Vol. 3. Springer. The *Halorubrum lacusprofundi* growth temperature range comes from Ricardo Cavicchioli. 2006. *Nat. Rev. Microbiol.* **4** :331.

Shiladitya DasSarma at the University of Maryland School of Medicine proposed to use recombinant gas vesicles of *Halobacterium* NRC-1 as a delivery vehicle for recombinant vaccines against typhoid bacteria. The recombinant haloarchaeal vaccine can be embedded in salt crystals (**Fig. 19.32**)—a cheap and convenient delivery method for developing countries where typhoid is widespread.



FIGURE 19.32 ■ Pink-pigmented salt crystals deliver haloarchaeal vaccine. *Halobacterium* NRC-1 embedded in salt crystals contain gas vesicles formed of a recombinant protein. The protein is harvested as a vaccine. Crystals are held here by Shiladitya DasSarma.

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With respect to cell shape, Haloarchaea display considerable diversity. Some species form symmetrical rods, as in *Halobacterium* NRC-1. Other species form pleomorphic cells, flattened like pancakes, and still others form regular cocci (*Haloferax mediterranei* ; **Fig. 19.33A**). A few species grow as flattened squares—the only microbial cells known to be square (*Haloquadratum walsbyi* ; **Fig. 19.33B and C**). The mechanism that maintains the various shapes is unknown. In oligotrophic (low-nutrient) environments, it is likely that the greater surface-to-volume ratio gives flattened cells a competitive edge in obtaining nutrients.

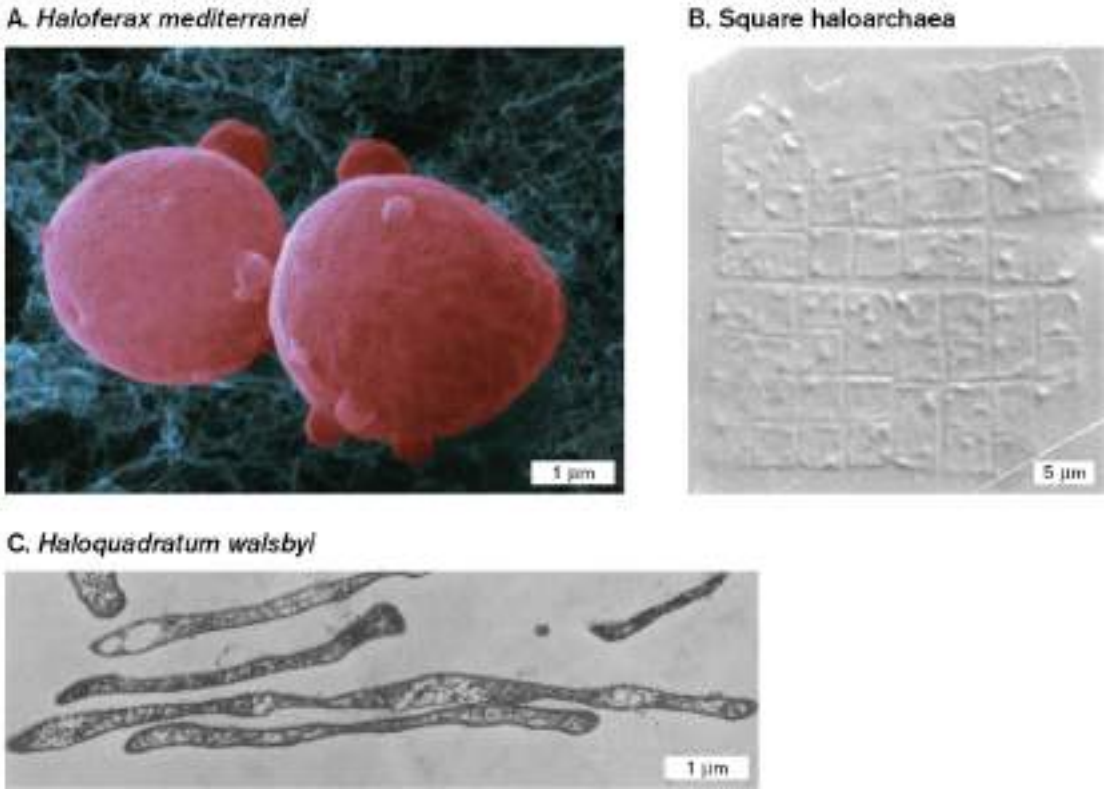


FIGURE 19.33 ■ Diverse haloarchaea. A. *Haloferax mediterranei* (SEM). **B.** Square haloarchaea (*Haloquadratum walsbyi*) during their sixth round of cell division, which alternates in vertical and horizontal directions (photomicrograph, Nomarski optics). **C.** Cross-sectioned cells of square haloarchaea show their thinness (TEM).

EYE OF SCIENCE/SCIENCE SOURCE

W. STOECKENIUS. 1981. *J. BACTERIOL* . **148** :352–360

M. KESSEL AND Y. COHEN. 1982. *J. BACTERIOL* . **150** :851–860

Hypersaline habitats. Hypersaline (high-salt) habitats differ with respect to pH, temperature, and the presence of other minerals, such as magnesium ion. Different kinds of hypersaline habitats support different species of haloarchaea (**Table 19.5**), as well as halophilic bacteria. Major types of habitats include:

- **Thalassic lakes.** Thalassic lakes (from the Greek *thalassa* , meaning "ocean"), such as the Great Salt Lake in Utah, contain saturated salts with ionic proportions similar to those of the ocean: Na^{+} and Cl^{-} (NaCl) predominate, followed by Mg^{2+} , K^{+} , and SO_4^{2-} . Thalassic lakes (also called brine lakes) support genera such as *Halobacterium* . Antarctic brine lakes support growth of cold-adapted halophiles such as *Halorubrum lacusprofundi* .

- **Athalassic lakes.** Athalassic lakes, such as the Dead Sea in Israel, contain higher proportions of magnesium ions. These habitats favor genera such as *Haloarcula* species, which require 100 mM Mg^{2+} for growth and grow best at magnesium concentrations above 1 M.
- **Solar salterns.** These are artificial pools of brine, or saturated NaCl, that evaporate in sunlight, precipitating halite (salt crystals) for commercial production. Commercial evaporation pools for salt actually benefit from the red microbes, whose light absorption accelerates heating and evaporation.
- **Brine pools beneath the ocean.** Undersea brine pools collect near geothermal vents, as in the Gulf of Mexico, Mediterranean Sea, and Red Sea. These hypersaline regions contain salts brought up by vent water. They support hyperthermophilic halophiles.
- **Alkaline soda lakes** , such as Lake Magadi in Kenya, where carbonate salts drive the pH above pH 9. These lakes support alkaliphilic haloarchaea such as *Halobacterium* .
- **Underground salt deposits** contain micropockets of salt-saturated water where halophiles may survive for thousands of years.

Retinal-based photoheterotrophy. Most haloarchaea are photoheterotrophs, in which light-directed energy acquisition supplements respiration on complex carbon sources.

Haloarchaea respire with oxygen or anaerobically with nitrate. A typical habitat for haloarchaea starts out as a pool with moderate salt content, containing a range of bacterial and archaeal species with varied tolerance to salt. As the pool evaporates, the salt concentrates, and the various bacteria die and lyse, releasing cell components that the haloarchaea can metabolize. The halophiles supplement their utilization of organic substrate energy by using light-driven ion pumps.

Respiration is supplemented by light-driven proton pumps containing retinal. Light in the green-yellow range is captured by retinal-containing proton pumps called bacteriorhodopsin and the chloride pump halorhodopsin (**Fig. 19.34**). (The role of light-driven ion pumps in phototrophy is discussed in Chapter 14 .) The proton pump bacteriorhodopsin was named to indicate that it is a prokaryotic version of the better-known eukaryotic retinal rhodopsins; the name was chosen before the domain Archaea was recognized. The chloride pump halorhodopsin was discovered and named in reference to the chloride ion (a halide). The two proteins are homologs with similar structure and mechanism. In the cell membrane, they form complexes that aggregate in patches called purple membrane.

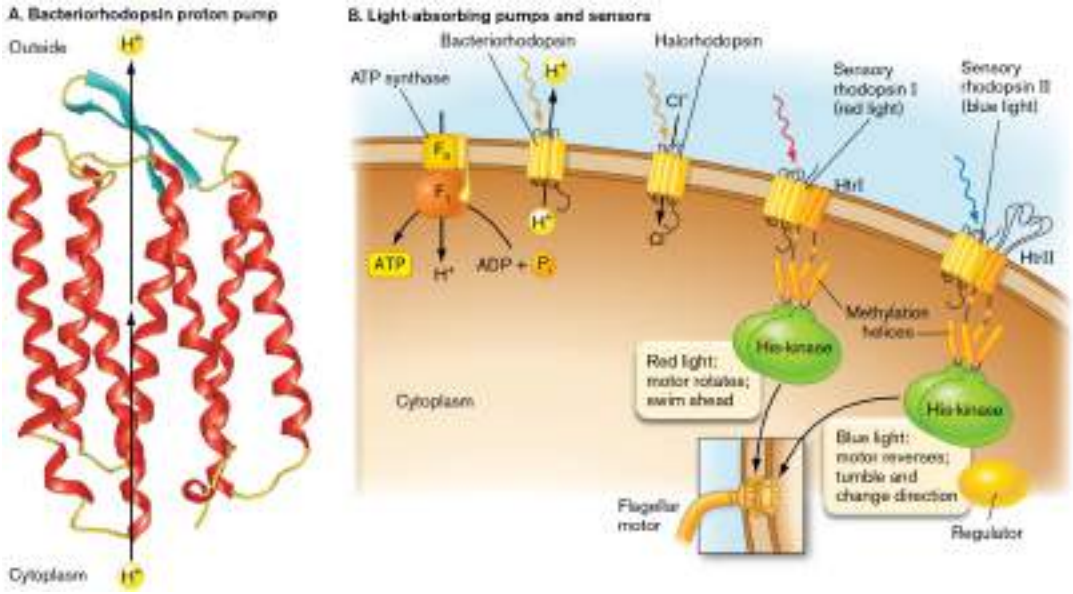


FIGURE 19.34 ■ Light-driven ion pumps and sensors. A. Bacteriorhodopsin absorbs light and pumps H^+ out of the cell (PDB code: 1QKO), whereas light-activated halorhodopsin pumps chloride into the cell (not shown). **B.** Rhodopsin family molecules in the membrane of *Halobacterium* : bacteriorhodopsin, light-driven proton extrusion; halorhodopsin, light-driven chloride intake; sensory rhodopsins I and II , with their signal transduction proteins Htr I and Htr II . The sensory transduction proteins phosphorylate and dephosphorylate a protein that regulates the direction of rotation of the flagellar motor.

Each bacteriorhodopsin proton pump contains seven alpha helices that traverse the membrane (**Fig. 19.34A**), surrounding a buried molecule of retinal, the same light-absorbing molecule found in photoreceptors of the human retina. Light absorption triggers a conformational change that enables a proton from the cytoplasmic face to be picked up by an aspartate residue. The proton is then transferred stepwise through several other amino acid residues in the protein, leading to release of the proton outside the cell. The net result of proton transfer by bacteriorhodopsin is generation of a proton motive force that can run a proton-driven ATP synthase, storing energy as ATP (**Fig. 19.34B**).

Halorhodopsin has a similar structure, in which chloride (instead of H^+) is pumped into the cell instead of outward. Because chloride is negatively charged, this chloride transport contributes to the proton motive force. Light-driven chloride pumps are unique to haloarchaea.

Haloarchaea also possess homologs of bacteriorhodopsin that serve as sensory devices: the sensory rhodopsins I and II. These proteins, too, each have seven alpha helices containing retinal. When the sensory rhodopsins absorb light, they signal the cell to swim using its archaella. The activated sensory rhodopsin I

directs the cell to swim toward red light. Sensory rhodopsin II is activated to reverse the archaellar motor and make the cell swim away from blue and ultraviolet light, which causes photooxidative damage to DNA. The rhodopsins signal through the chemotaxis machinery to the archaellar motor, using histidine kinase enzymes that phosphorylate regulatory proteins (see **Fig. 19.34B**). This response to light, or phototaxis, is an important model for archaeal molecular regulation.

Anaerobic haloarchaea. Until recently, all haloarchaea were thought to be aerobic or facultative respirers—essentially, heterotrophs with an added boost from light-driven ion pumps. In 2016, Dmitri Sorokin's group at the Russian Academy of Sciences, Moscow, discovered a strictly anaerobic haloarchaeon (*Hal anaero archaeum*) from a Siberian chloride-sulfate lake. This anaerobe requires 3- to 5-M NaCl and oxidizes acetate with sulfate, a form of sulfate respiration (discussed in Chapter 14). A related genus from anaerobic sediment of a brine lake was shown to oxidize H_2 or formate with sulfur. Thus, the known metabolic capacity of Haloarchaea has been expanded to include anaerobes with sulfur reduction.

Hot and Hotter: Thermococcales and Archaeoglobales

A major group of hyperthermophilic euryarchaeotes is the order Thermococcales, including genera such as *Thermococcus* and *Pyrococcus* (**Fig. 19.35**). These genera are most commonly isolated from thermal vents at the ocean floor and from submarine solfataras, volcanic vents that emit only gases. Besides high temperature, most of these microbes experience high pressure (they are barophiles). Most Thermococcales are anaerobes that ferment complex carbon sources such as peptides or carbohydrates. Despite deep genetic divergence, their traits superficially resemble those of TACK (crenarchaeote) sulfur hyperthermophiles. Unfortunately, their names also are similar.

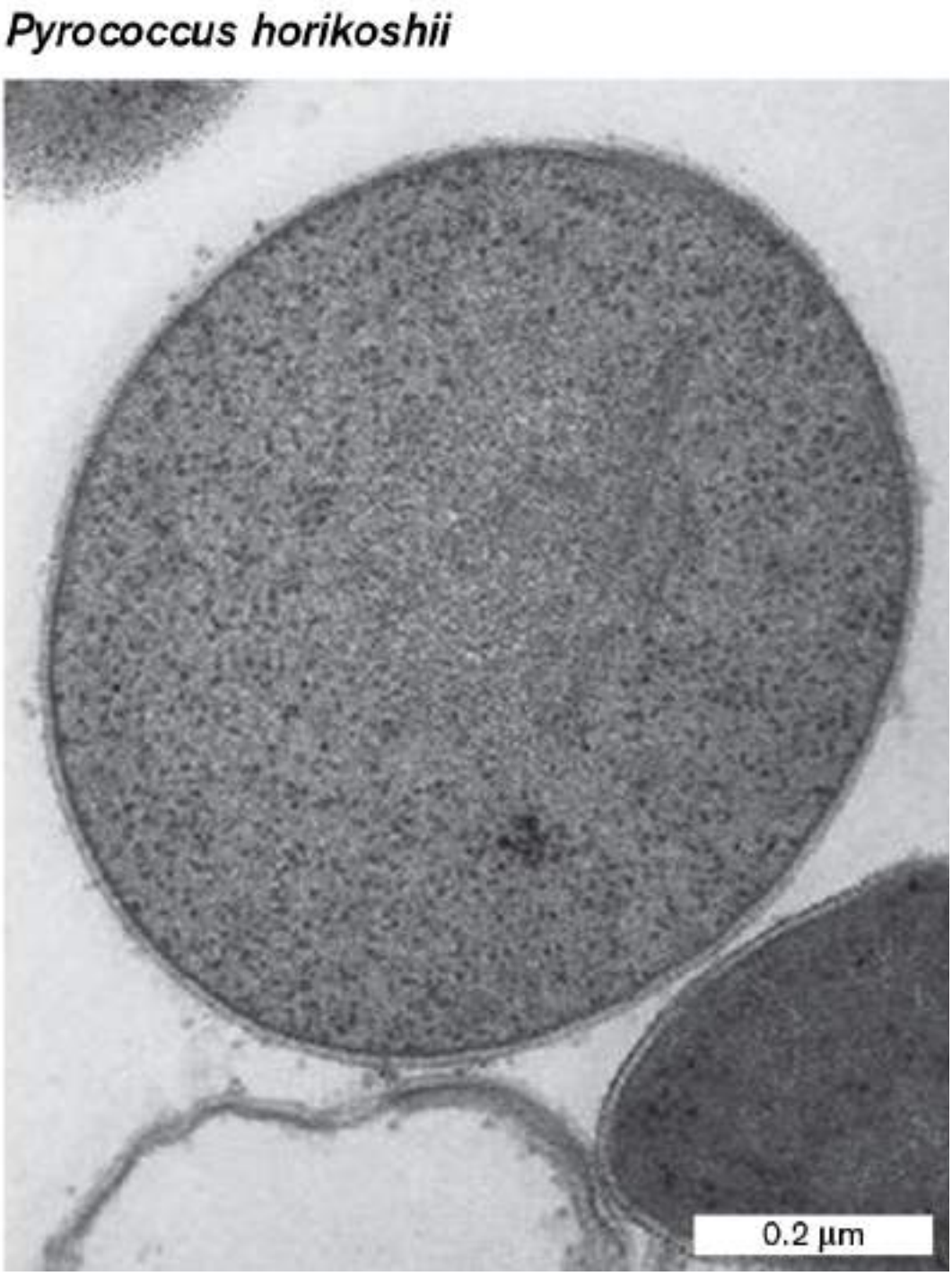


FIGURE 19.35 ■ Hyperthermophilic Euryarchaeota. *Pyrococcus horikoshii* , isolated from a hydrothermal vent at the Okinawa Trough (TEM).
J. GONZALES ET AL. 1998. *EXTREMOPHILES* **2** :123–130

Note: Distinguish the euryarchaeotes *Thermococcus* and *Pyrococcus* from the TACK genera *Thermoproteus* and *Pyrodictium* , respectively.

Sulfur reducers and oxidizers. Most *Thermococcus* and *Pyrococcus* species grow at temperatures well above 90°C; *Pyrococcus woesei* , for example, grows at temperatures as high as 105°C. Their growth is accelerated by the use of elemental sulfur as a terminal electron acceptor for anaerobic respiration:

$$2H^+ + 2\,e^- + S^0 \longrightarrow H_2S$$

Alternatively, these species can oxidize molecular hydrogen with sulfur:

$$H_2 + S^0 \longrightarrow H_2S$$

Species of Thermococcales are the source of "vent polymerases" for PCR amplification. They now replace the enzyme Taq polymerase obtained from the Yellowstone hot-spring bacterium *Thermus aquaticus* , which grows only at temperatures up to 90°C. The Taq enzyme has only limited stability at or above 95°C, but DNA polymerases with greater stability at higher temperatures and increased accuracy in replication have been produced from vent-dwelling archaea such as *Pyrococcus furiosus* and *Thermococcus litoralis* . These vent polymerases now allow higher-temperature denaturation and synthesis of GC-rich sequences that were difficult to amplify with the original thermostable enzyme.

The first member of Thermococcales whose genome was sequenced was *Pyrococcus abyssi* . (Remember to distinguish *Pyro coccus abyssi* from the TACK species *Pyro dictium abyssi* .) The genome of *Pyrococcus abyssi* reveals an especially high number of eukaryotic homologs for DNA replication, transcription, and protein translation. Examples of homologs include the eukaryotic-like primase, helicase, and endonuclease for generation of Okazaki fragments in the lagging strand of DNA synthesis. At the same time, the *Pyrococcus* genome also shows bacterial homologs for cell division and DNA repair. Thus, *Pyrococcus abyssi* offers a striking view of mixed heritage from the common ancestor of the three domains.

Pyrococcus species possess enzymes conducting most of the classic conversions of the EMP pathway of glycolysis, but several steps use enzymes unrelated to those in bacteria. Examples include two ADP-dependent enzymes—glucokinase and phosphofructokinase—as well as the phosphate-independent enzyme glyceraldehyde 3-phosphate ferredoxin oxidoreductase. The glyceraldehyde 3-phosphate conversion is unique in that it sidesteps the use of inorganic phosphate, which is required at this step by bacterial glycolysis (shown in Fig. 19.6).

Pyrococcus and other members of Thermococcales have enzymes requiring tungsten, a metal rarely required outside the archaea. (Tungsten is present in elevated concentrations at hydrothermal vents.) Another unusual feature of

Pyrococcus is that both proton motive force generation and the ATP synthase appear to involve a Na^+/H^+ antiport system. This dependence on sodium is a trait shared with the methanogens and the halophiles.

Thought Question

19.7 Compare and contrast the metabolic options available for *Pyrococcus* and for the TACK organism *Sulfolobus* .

Archaeoglobales reduce sulfate and reverse methanogenesis. While many benthic archaea reduce sulfur (S^0), only one clade, Archaeoglobales, is known to reduce sulfate ion (SO_4^{2-}) without a bacterial partner. *Archaeoglobus fulgidus* reduces sulfate by way of an acetyl-CoA degradation pathway that reverses part of methanogenesis. This process may be important for eliminating methane from methane hydrates before they release gas to the atmosphere.

Life in Hot Acid: Thermoplasmatales

A clade whose habitat is extreme even by archaeal standards is Thermoplasmatales . This clade includes thermophilic acidophiles with no cell wall and no S-layer, but only a plasma membrane. How they maintain their cells against the most extreme conditions known on Earth is poorly understood.

An example is a thermophile isolated from self-heating coal refuse piles, *Thermoplasma acidophilum* , growing at 59°C and pH 2. *Thermoplasma* cells are motile, but it is unclear how their archaella maintain a torque against the membrane without a rigid envelope or S-layer for support. The metabolism of *T. acidophilum* , like that of *Pyrococcus* , is sulfur (S^0) respiration of organic molecules.

The genome sequence of *T. acidophilum* contains just 1.5 million base pairs—one of the smallest known for a free-living organism. It shows substantial evidence of horizontal transfer from *Sulfolobus* , a TACK hyperthermophile that shares the same range of habitats. Horizontal transfer between distant relatives turns out to be common among the hyperthermophiles. For example, *T. acidophilum* has genes encoding an entire protein degradation pathway that was transferred horizontally from an ancestor of *Sulfolobus* .

Another acidophilic genus of the order Thermoplasmatales is *Ferroplasma* (**Fig. 19.36**). *Ferroplasma* species are mesophiles or thermophiles, found in mines containing iron pyrite ore (FeS_2). Using dissolved Fe^{3+} as an oxidizing agent in

the presence of H_2O, they oxidize the sulfur to sulfuric acid. The chemical equation is:

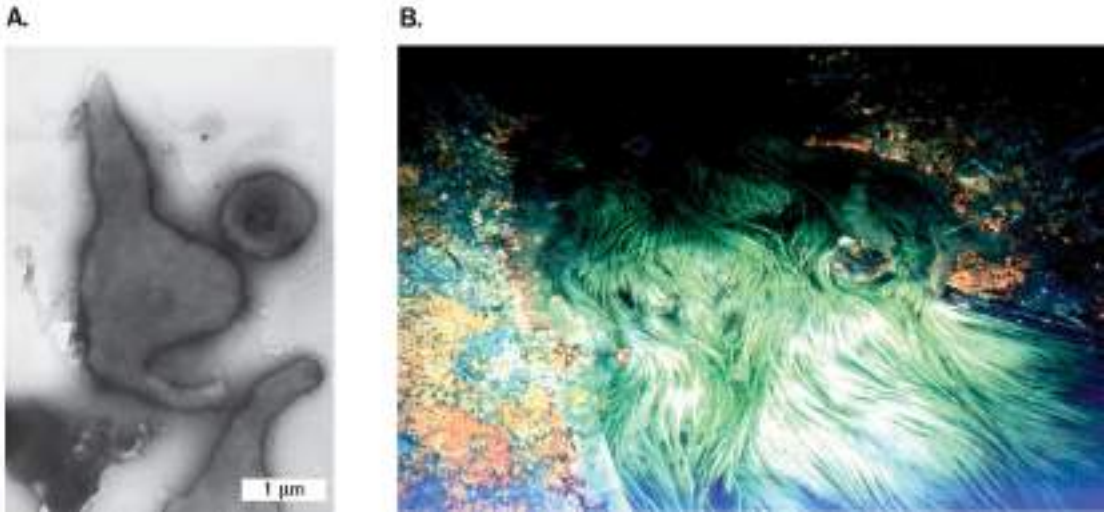


FIGURE 19.36 ■ The extreme acidophile *Ferroplasma* . A. *Ferroplasma acidiphilum* grows at pH 0 (TEM). **B.** Streamers of *F. acidarmanus* anchored to deposits of pyrite within the Iron Mountain mine in California. The stream is about a meter across, its water about pH 0. This level of acidity will dissolve a metal shovel in a day.

T. A. PIVOVAROVA ET AL. 2002. *MICROBIOLOGY* **71** :698–706
K. J. EDWARDS ET AL. 2000. *SCIENCE* **287** :1796

$$FeS_2 + 14Fe^{3+} + 8H_2O \longrightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$$

The reaction generates pH values below pH 0 (1-M H^+). This degree of acidity can dissolve a metal shovel within a day.

The amorphous cells of *Ferroplasma acidarmanus* grow in biofilms that form long streamers into water draining from the mine (**Fig. 19.36B**). The oxidative disintegration of iron-bearing ores can be useful for leaching of minerals, but it also causes acid mine drainage into aquatic systems (discussed in Chapter 22).

Thought Question

19.8 Compare and contrast sulfur metabolism in *Pyrococcus* and in *Ferroplasma* .

To Summarize

- **Haloarchaea are extreme halophiles** , growing in NaCl at a concentration of at least 1.5 M. They are isolated from salt lakes, solar salterns, underground salt micropockets, and salted foods.
- **Haloarchaea show diverse cell shapes** , including slender rods (*Halobacterium*), cocci (*Halococcus*), and flat squares (*Haloquadratum*). The cell envelopes of most haloarchaea contain rigid cell walls of glycoprotein. Gas vesicles enable haloarchaea to remain near the top of the water column.
- **Molecular adaptations to high salt** include DNA of high GC content and acidic proteins (proteins with a high number of negatively charged residues).
- **Retinal-based photoheterotrophy** involves the proton pump bacteriorhodopsin or the chloride pump halorhodopsin. Both pumps contain retinal for light absorption.
- **Thermococcales are hyperthermophiles.** Most species use sulfur to oxidize complex organic substrates.
- ***Pyrococcus* and *Thermococcus* are the source of vent polymerases** for PCR. Thermococcal enzymes notably use tungsten at their active site.
- ***Archaeoglobus* hyperthermophiles reduce sulfate and oxidize benthic methane hydrates.** The methyl group of acetate is oxidized to CO_2 by partly reversing methanogenesis.
- **Thermoplasmatales includes extreme acidophiles.** *Ferroplasma* oxidizes iron pyrite ore (FeS_2) in a process that generates concentrated sulfuric acid, causing acid mine drainage.

Glossary

Haloarchaea
: A monophyletic clade of Euryarchaeota that contains extremely halophilic archaea, inhabiting high-salt environments. Formerly known as Halobacteria.

gas vesicle
: An organelle that traps gases to increase the buoyancy of aquatic microbes.

bacteriorhodopsin
: A haloarchaeal membrane-embedded protein that contains retinal and acts as a light-driven proton pump; it is homologous to the bacterial proteorhodopsin.

halorhodopsin
: A haloarchaeal membrane-embedded protein that contains retinal and acts as a light-driven chloride pump; it is homologous to bacteriorhodopsin.

Thermoplasmatales
An order of extreme thermoacidophiles in the Euryarchaeota superphylum.

Fig. 19.5

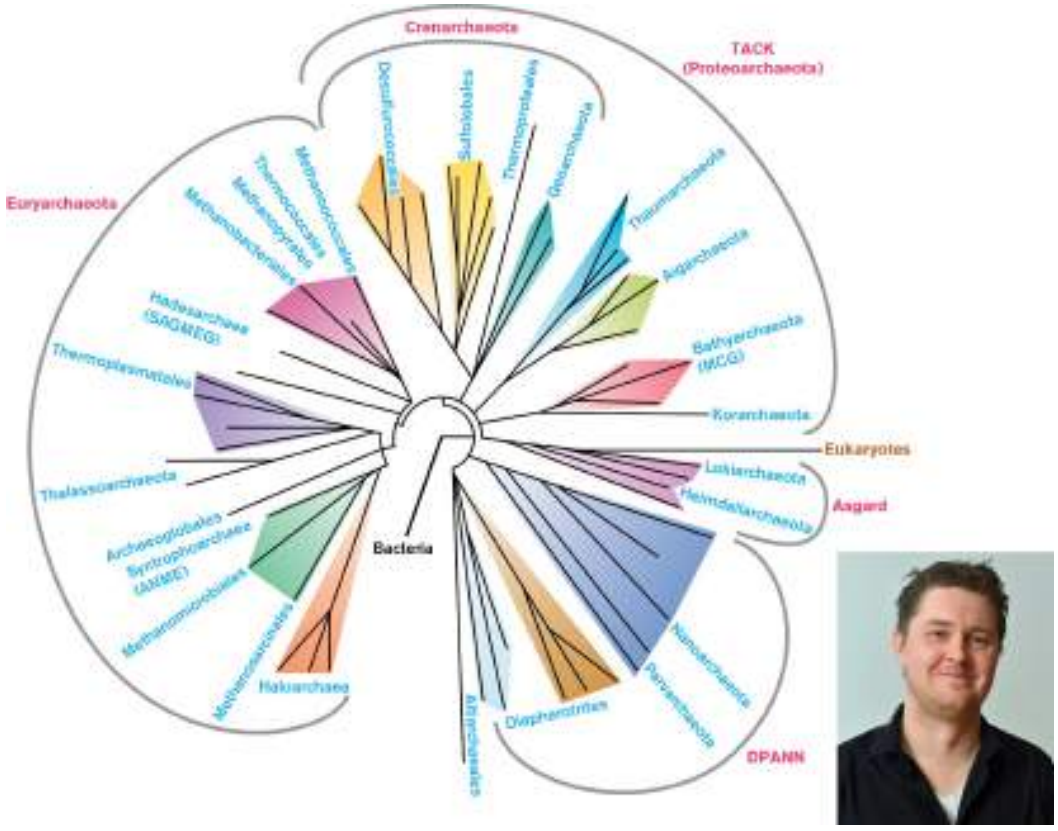


FIGURE 19.5 ■ Archaeal phylogeny. Major superphyla, phyla, and orders of Archaea. Divergence is based approximately on small-subunit ribosomal RNA (SSU rRNA) and genome sequences. (See Table 19.2 for abbreviations.) **Inset:** Thijs Ettema.

COURTESY OF THIJS ETTEMA

Fig. 19.6

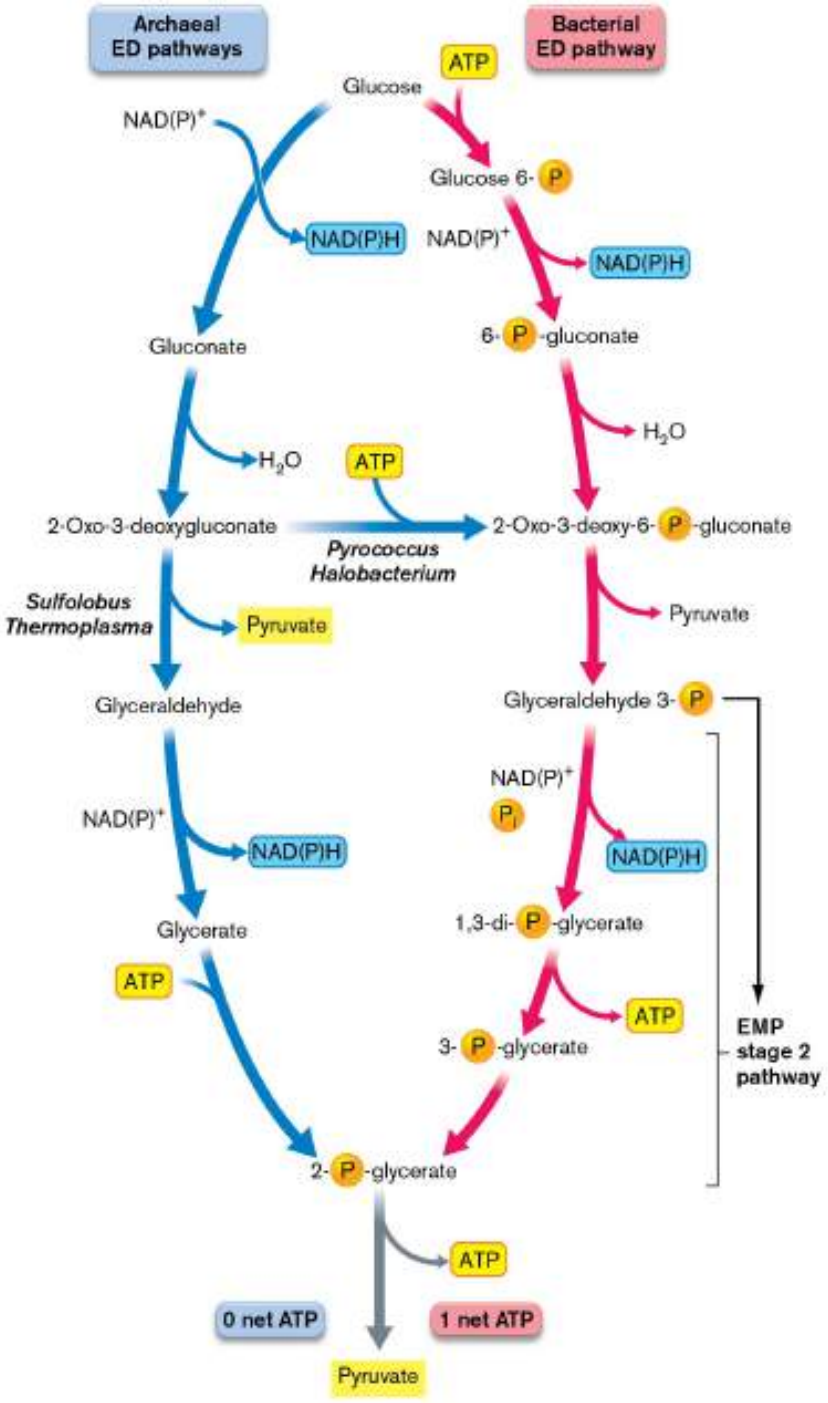


FIGURE 19.6 ■ Glucose catabolism in archaea. Left: *Sulfolobus* and *Thermoplasma* species catabolize glucose to pyruvate via a modified ED pathway without phosphorylating glucose and produce no net ATP. **Right:** *Halobacterium* species phosphorylate 2-oxo-3-deoxygluconate and produce one net ATP via the EMP stage 2 pathway. *Pyrococcus furiosus*

oxidizes glyceraldehyde 3-phosphate using ferredoxin instead of NAD^+ and avoids phosphorylation.

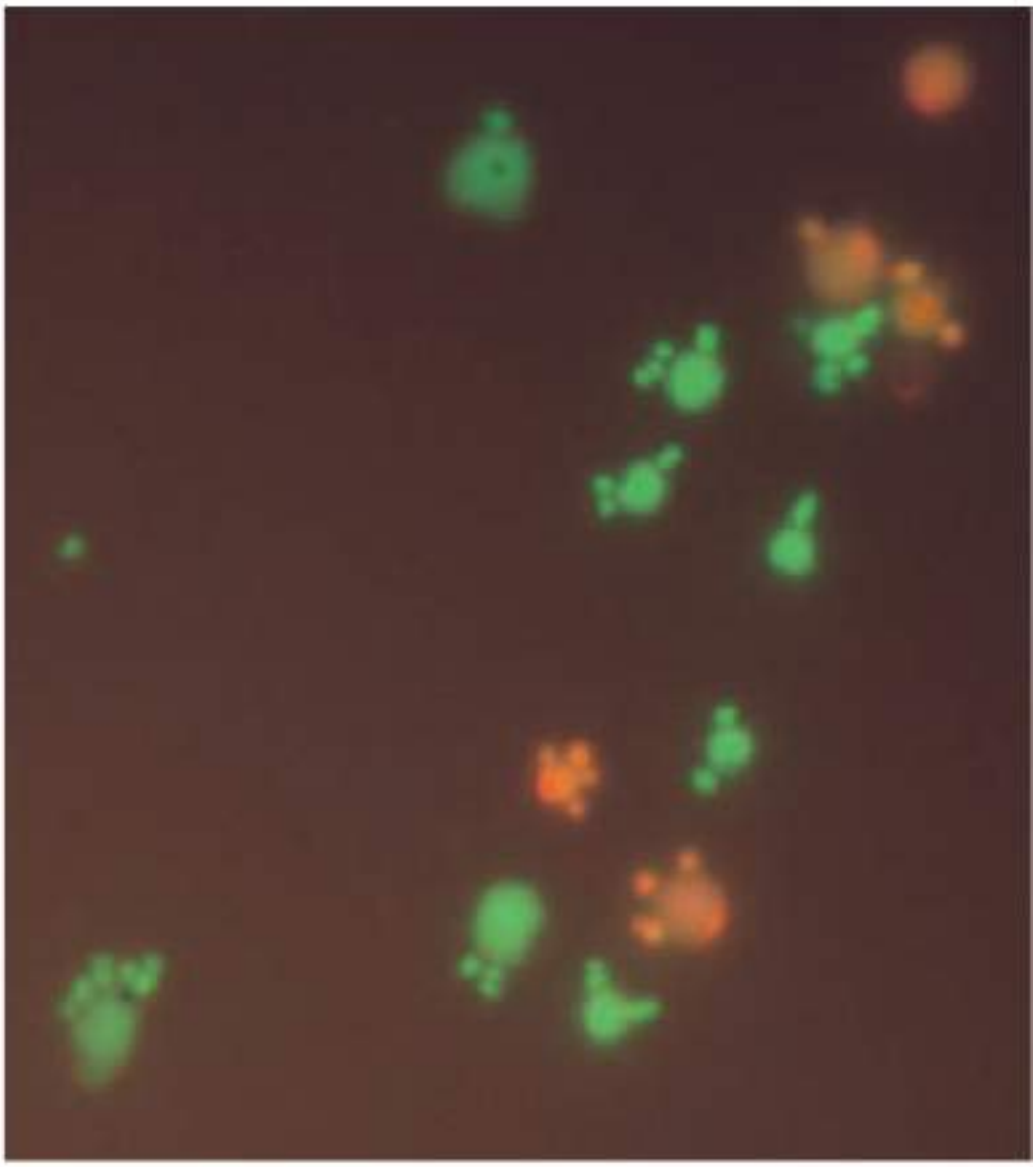
19.6 DPANN Symbionts, Altiarchaeales, and Asgard: Branch to Eukaryotes

The DPANN superphylum includes several deeply branching clades of ultrasmall cells that have shrunken genomes, have lost major metabolic pathways, and appear to require a symbiotic partner. They are most commonly found in freshwater and marine environments. A related group, Altiarchaeales, is free-living and shows unusual harpoon-like appendages called "hami." Finally, the deeply branching Asgard group includes marine thermophiles that may be the most closely related to the domain Eukarya.

Nanoarchaeota: Parasites on a Hyperthermophile

The best-studied clade of the DPANN superphylum is the Nanoarchaeota. The cultured species *Nanoarchaeum equitans* consists of exceptionally small cells that are obligate symbionts of the TACK hyperthermophile *Ignicoccus* presented earlier (Fig. 19.1). The *Ignicoccus-Nanoarchaeum* isolates were obtained from Kolbeinsey Ridge hydrothermal vents. The host cell may harbor up to four of the smaller cells (**Fig. 19.37A**). The *N. equitans* cell in **Figure 19.37B** is attached to *I. hospitalis* by a membrane bridge, which has been shown to connect the intracellular membrane tubules of *Ignicoccus* . How the *Nanoarchaeum* symbiont maintains this intimate connection is unknown.

A.



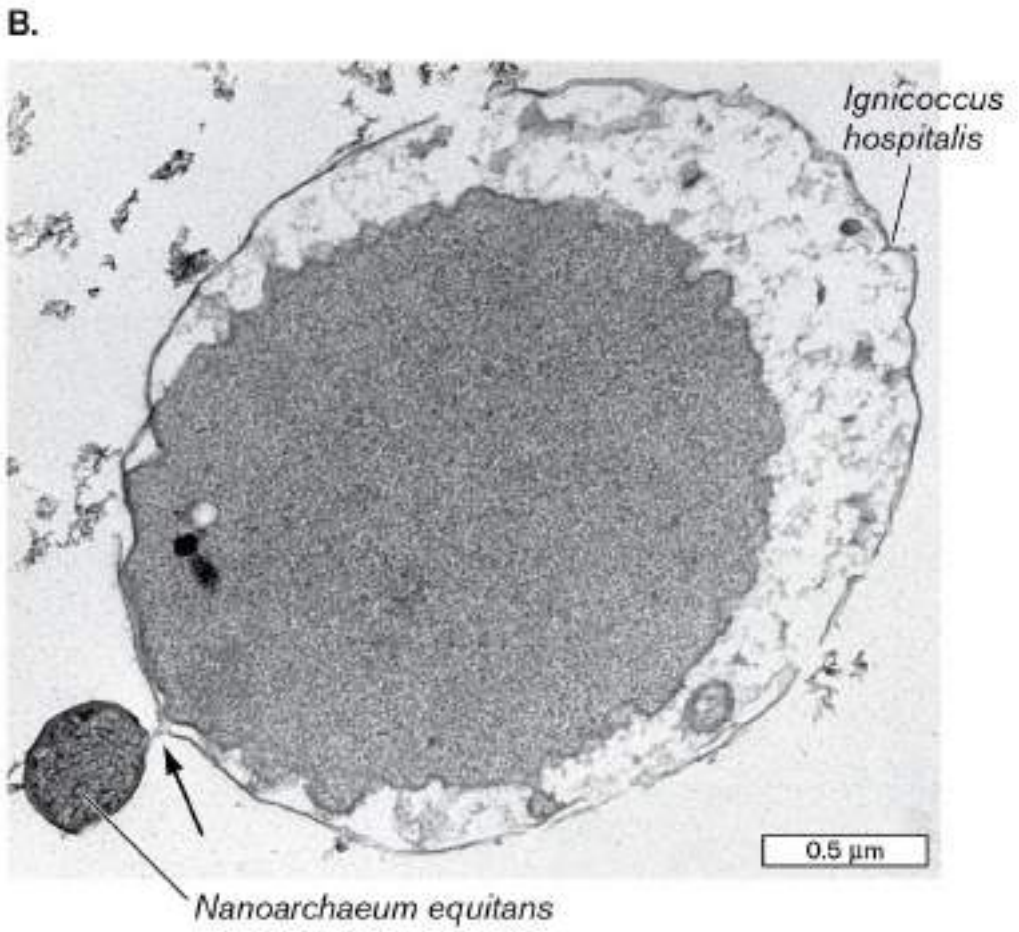


FIGURE 19.37 ■ *Nanoarchaeum equitans* , a small obligate symbiont attached to *Ignicoccus hospitalis* . A. Each *Ignicoccus hospitalis* cell here has one to four attached cells of *Nanoarchaeum equitans* . Fluorescence microscopy. Green (live cells); orange (dead cells). **B.** *Ignicoccus* , showing intracellular compartment and one cell of *N. equitans* attached by a membrane bridge (arrow; TEM).

U. JAHN ET AL. 2008. *J. BACTERIOL.* **190** :1743, FIG. 2B

U. JAHN ET AL. 2008. *J. BACTERIOL.* **190** :1743. REPRODUCED WITH PERMISSION FROM AMERICAN SOCIETY FOR MICROBIOLOGY

Both host and symbiont genomes have been sequenced, revealing extensive coevolution of the two. The *N. equitans* genome is exceptionally small (less than 500 kb) and shows evidence of rapid degenerative evolution. The diminished genome is typical of a dependent organism that has lost numerous genes for functions now

provided by a host. Growth rate comparisons show that the host *I. hospitalis* grows faster in the absence of *N. equitans* ; thus, *N. equitans* is inferred to be a parasite. It remains unclear, however, whether *N. equitans* makes any metabolic contribution to its host.

Members of other DPANN phyla, such as Diapherotrites, are identified from marine and underground sources. They show evidence of evolutionary gene loss as symbionts, but also the gain of some bacterial genes that may offer free-living alternatives. Their genes encoding SSU rRNA show multiple differences from the sequences generally used as primers for PCR amplification. Thus, it is hard to identify such organisms, much less study them.

Altiarchaeales: Archaea with Grappling Hooks

An interesting archaeon, *Altiarchaeum hamiconexum* , was found in sulfide-rich marsh water from the Sippenauer Moor in Germany. First known as the SM1 euryarchaeon, the microbe was discovered in 2004 by Rudolph Huber and his students at the University of Regensburg, Germany. These archaea fix CO_2 by an unusual pathway related to methanogenesis.

A surprising trait of *Altiarchaeum* was discovered by Christine Moissl-Eichinger. Her students showed how this organism forms netlike biofilms by use of pilus-like grappling-hook appendages called hami (singular, hamus ; **Fig. 19.38**). Each grappling hook contains paired barbs along an extended protein filament many times the length of the cell.

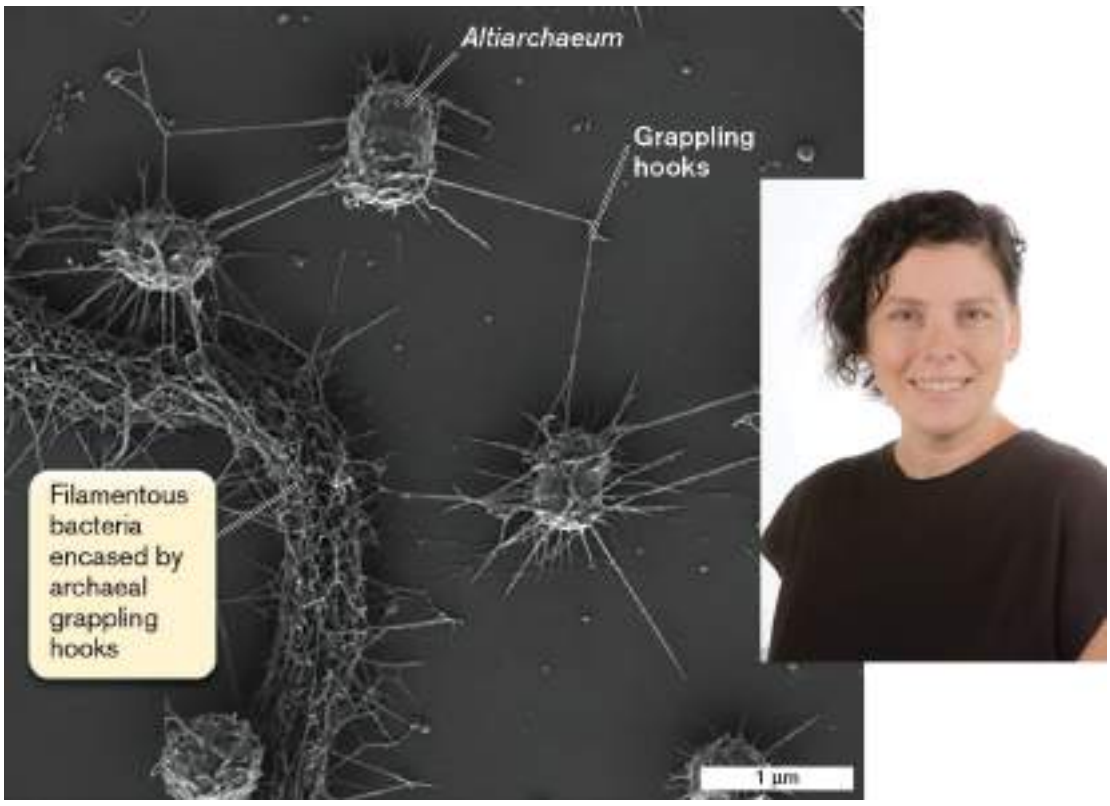


FIGURE 19.38 ■ *Altiarchaeum* forms chains with grappling hooks (hami). Inset: Christine Moissl-Eichinger.
ALEXANDRA K. PERRAS ET AL. 2014. *FRONT. MICROBIOL.* **5** :397
MARTIN WIESNER

The filament ends with a triple fishhook that enables filaments to clasp neighboring cells in a biofilm matrix (**Fig. 19.39**). No other kind of cell is known to make this type of appendage.

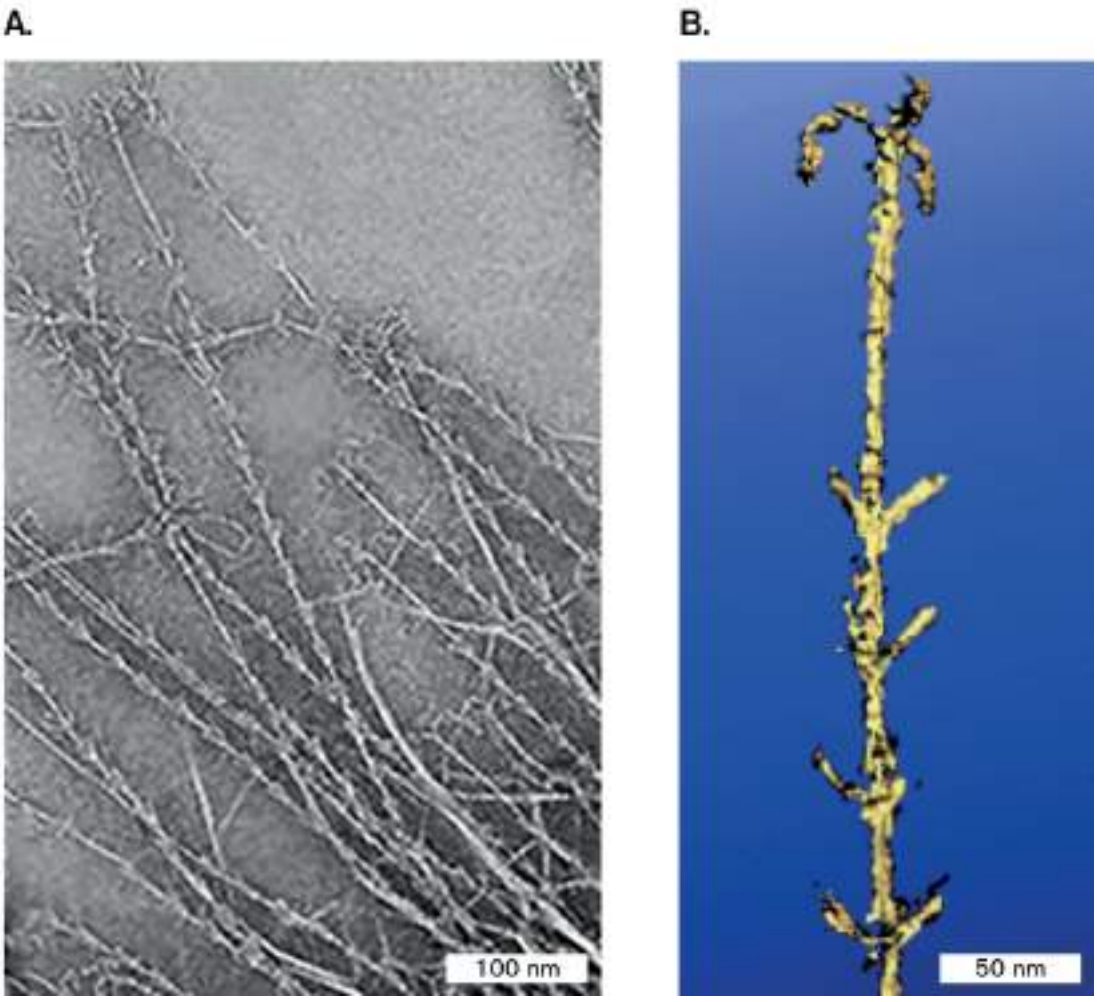


FIGURE 19.39 ■ Grappling hooks (hami) of *Altiarchaeum* species. A. Hooked appendages extending from a cell (TEM). **B.** Cryo-electron tomography model of a grappling hook (hamus).

ALEXANDER K. PERRAS ET AL. 2014. *FRONT. MICROBIOL.* **5** :397
CHRISTINE MOISSL ET AL. 2005. *MOL. MICROBIOL.* **56** :361, FIG. 4C

The matrix of grappling hooks also coats filaments of sulfide-oxidizing bacteria that may cooperate with *Altiarchaeum* . Thus, this archaeon represents yet another type of intimate community involving archaea.

Asgard, the Norse Gods Superphylum

The superphylum Asgard (meaning "world of the gods") was named for its inclusion of the Lokiarchaeota, originally identified from a hydrothermal ridge known as Loki's Castle. Related deeply branching phyla were fancifully named for other Norse gods, such as Heimdallarchaeota. Asgard genomes have been sequenced from various marine and freshwater sediments. All show such deeply branching divergence that standard SSU rRNA primers may fail to amplify their DNA.

Asgard genome sequences show genes with unusual similarity to signature genes of the domain Eukarya, such as those encoding components of cytoskeleton, signal transduction, and nucleocytoplasmic transport. Some Asgard archaea possess chromosomal histone proteins with N-terminal "tails" like those that regulate gene expression in eukaryotes. The archaeal phylogenetic tree, as computed by Thijs Ettema and co-workers (Fig. 19.5), shows the domain Eukarya branching from the Lokiarchaeota lineage. These sequence data are evidence that the original eukaryotic ancestor evolved from an Asgard ancestor that later acquired Alphaproteobacterial symbionts such as mitochondria.

The placement of many deep-branching clades, including the Eukarya branch, remains controversial. The sequences diverge so deeply that small adjustments in tree computation may yield different branch points for distant clades.

One artifactual effect that may occur is called long branch attraction (**Fig. 19.40**), a phenomenon in which two fast-evolving lineages show such a large number of sequence mismatches that their sequences actually appear more similar to each other than to more closely related lineages that evolve more slowly. Long branch attraction occurs because each sequence position has only four possible base pairs, so as the rate of DNA mutation increases, the chance increases that two distant lineages might independently change to the same base at a given position. Thus, two distantly evolved lineages may branch together because they share dissimilarity to more slowly evolving organisms. Further study of emerging genomes, and the culturing of live isolates, may help resolve the fascinating question of the origin of eukaryotes.

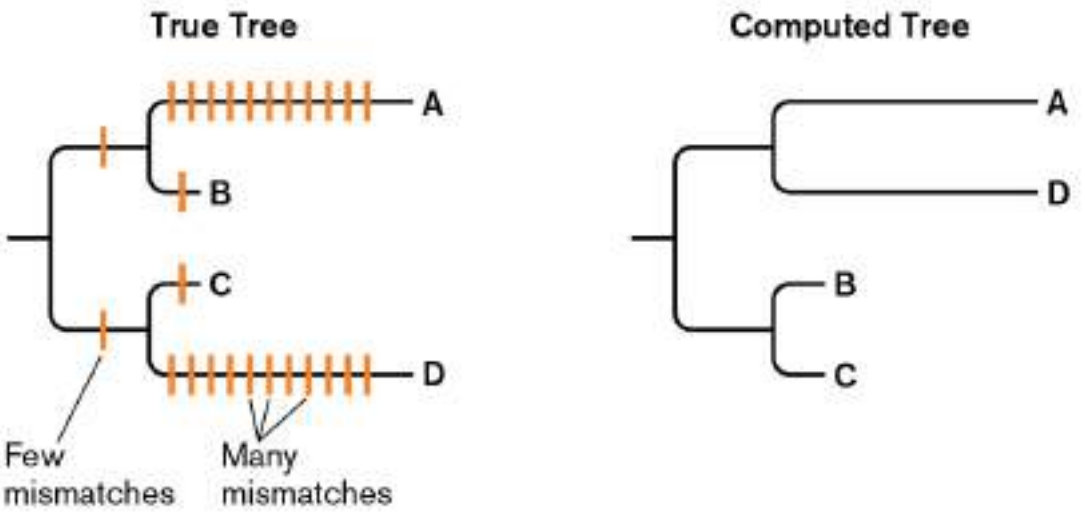


FIGURE 19.40 ■ Long branch attraction. Two fast-evolving lineages may show such a large number of sequence mismatches that their sequences actually appear more similar to each other than to more closely related lineages that evolve more slowly.

Overall, species of archaea inhabit a wider range of environments than either bacteria or eukaryotes do—from extreme heat to extreme cold, from high pH to extreme acid, as well as temperate environments such as soil and animal digestive tracts. Archaea show unique cell structures, such as grappling-hook appendages, and unique forms of metabolism, such as methanogenesis. Yet we probably know less about the actual scope of Archaea than we do about the other two domains because so many members remain uncultured. Much of what we call Archaea remains to be explored.

To Summarize

- **The DPANN phylum Nanoarchaeota includes *Nanoarchaeum equitans*, a tiny obligate symbiont of the marine hyperthermophile *Ignicoccus hospitalis*.** *N. equitans* is a parasite, affecting the growth of its host.

- **Altiarchaeales includes *Altiarchaeum hamiconexum* , an archaeon with unique grappling-hook appendages.** *A. hamiconexum* forms netlike biofilms that may include a relationship with sulfide-oxidizing bacteria.
- **The Asgard superphylum includes archaea that branch with the domain Eukarya.** Asgard genomes show a number of signature proteins found in eukaryotes.
- **Long branch attraction is a computational artifact that can lead distantly branching lineages to appear more similar than, in fact, they are.**

Glossary

hamus *pl.* hami

: An archaeal cell appendage with grappling hooks that enable cells to connect with each other, adhere to a surface, and form biofilms.

Fig. 19.1

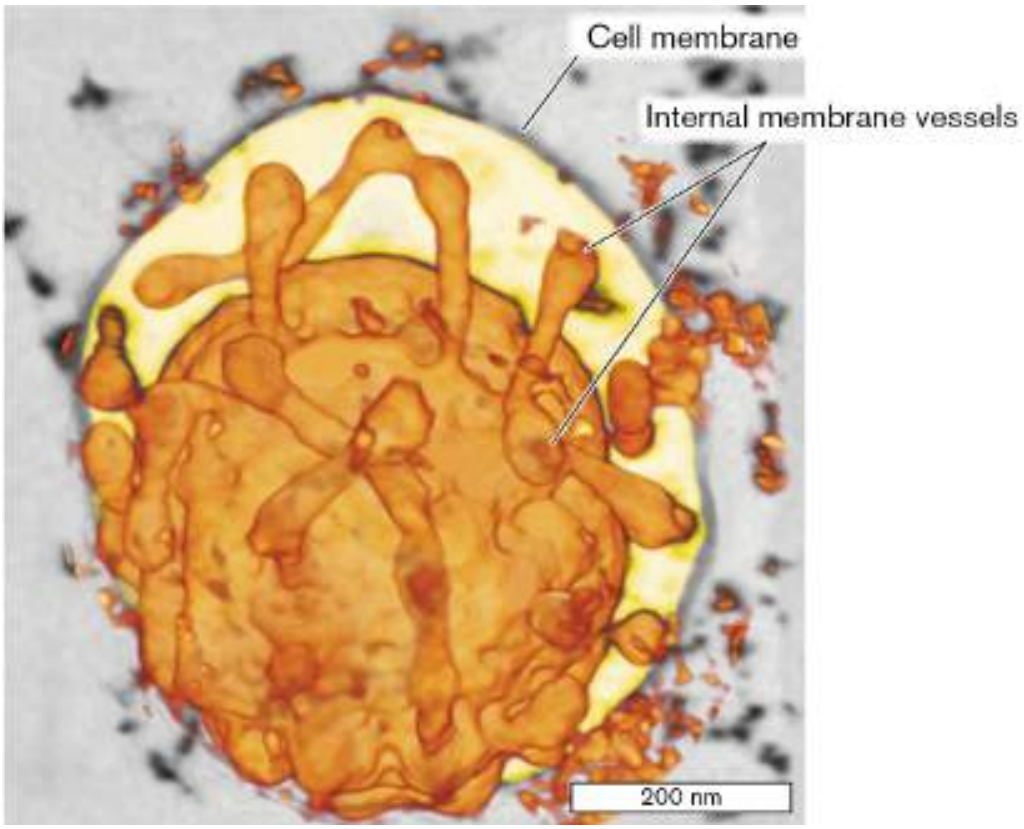


FIGURE 19.1 ■ *Ignicoccus hospitalis* , from a hydrothermal vent 600 meters below sea level, at Kolbeinsey Ridge volcano. *Ignicoccus hospitalis* has an extensive endomembrane system (orange) within an outer compartment contained by the cell membrane (yellow). Cryo-electron tomography, colorized.

COURTESY OF THOMAS HEIMERL

Fig. 19.5

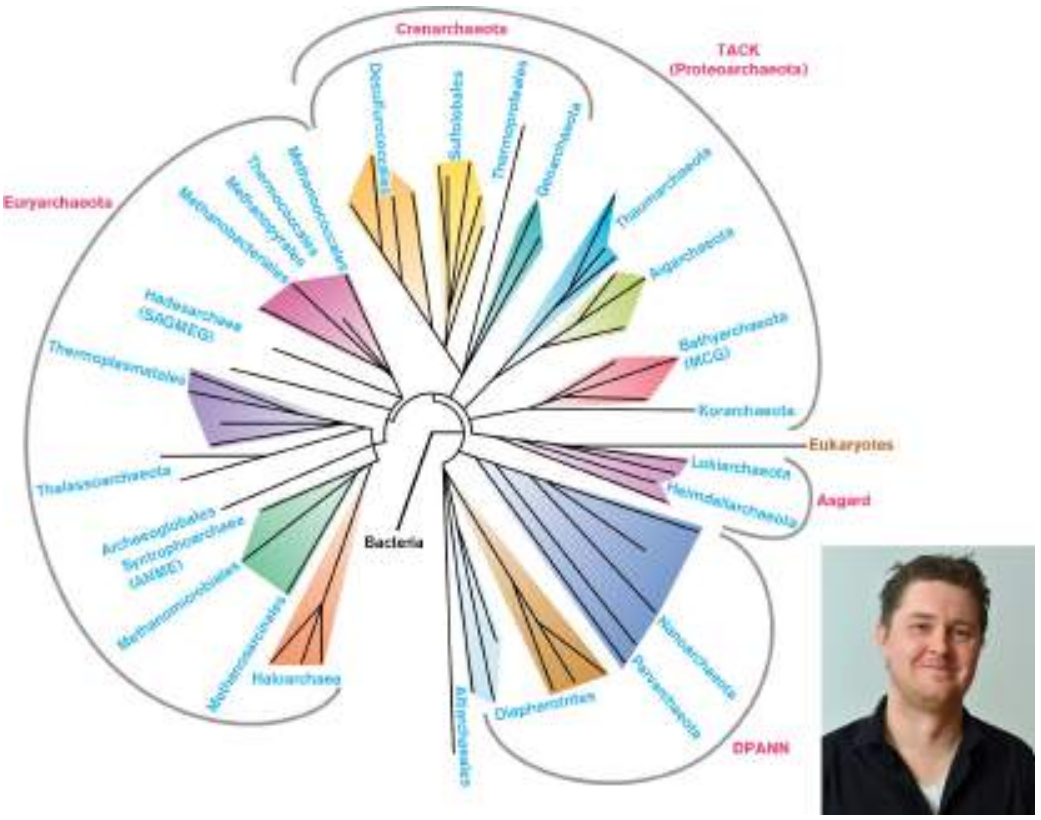


FIGURE 19.5 ■ Archaeal phylogeny. Major superphyla, phyla, and orders of Archaea. Divergence is based approximately on small-subunit ribosomal RNA (SSU rRNA) and genome sequences. (See Table 19.2 for abbreviations.) **Inset:** Thijs Ettema.

COURTESY OF THIJS ETTEMA

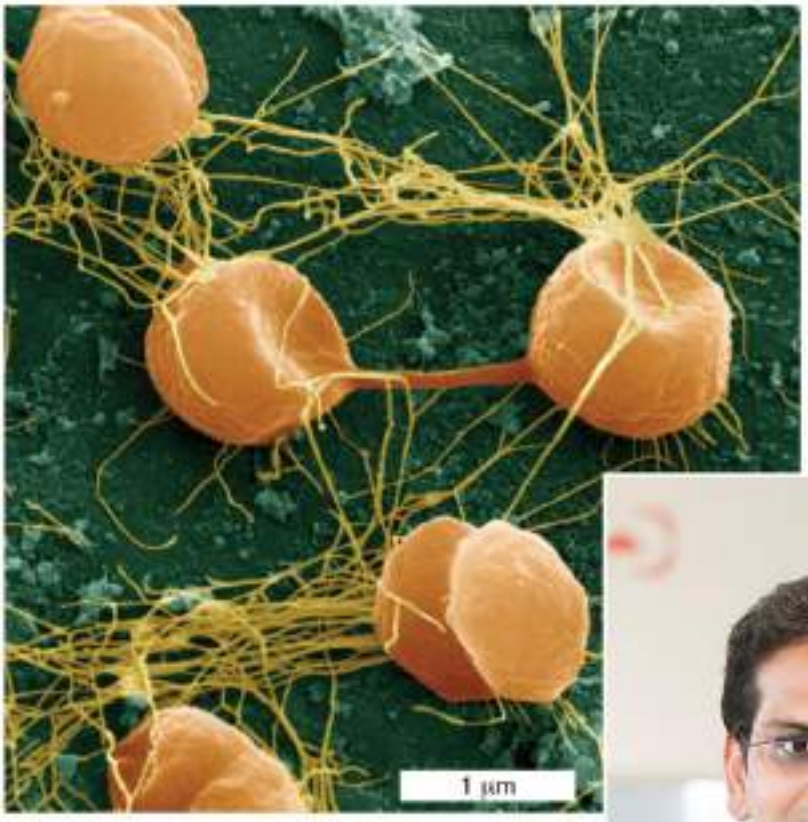
eResearch Activity 19

Can an Archaeal Fuel Cell Make Electric Current in Boiling Water?

In Chapter 14 we learned how bacteria can colonize an electrode and donate electrons, generating an electric current. Such bacterial electricity can be used to make fuel cells for human technology. Could hyperthermophilic archaea do something similar? What if archaea could make a fuel cell at temperatures near boiling? Such a fuel cell could be useful for manufacturing processes that release heat and generate high temperatures.

Narendran Sekar, in the lab of Ramaraja Ramasamy at the University of Georgia, set out to make an archaeal fuel cell at the highest possible temperature. He started with *Pyrococcus furiosus* , a vent hyperthermophile discovered by Karl Stetter and given a name that means "fireball" (**Fig. ERA 19.1**). The optimal temperature for growth of *P. furiosus* is 100°C, and Sekar managed to achieve electric current above 90°C. **Figure ERA 19.1B** shows cells of *P. furiosus* colonizing the surface of carbon fibers of an anode (an electrode that attracts particles of negative charge).

A.





B.

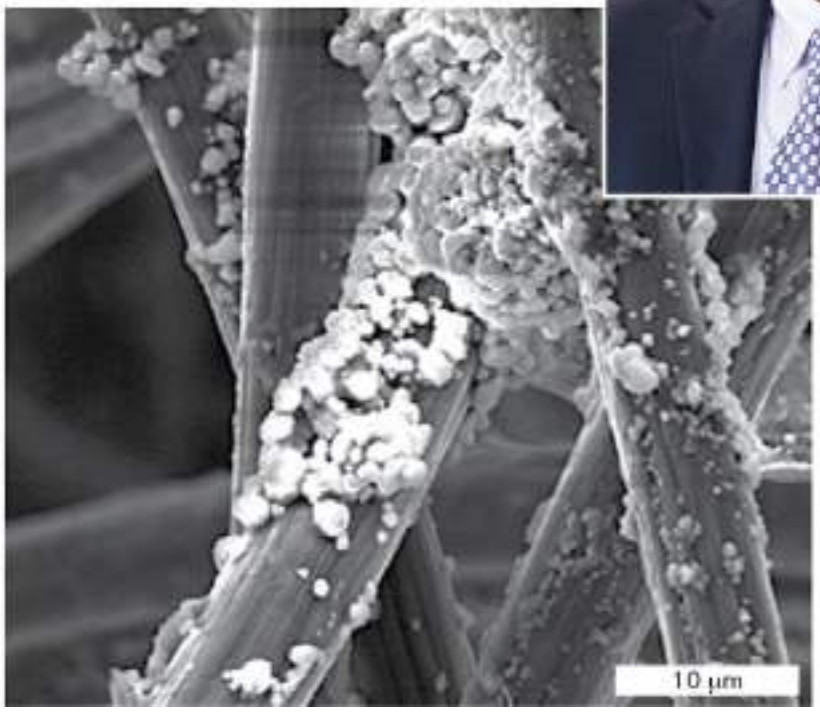
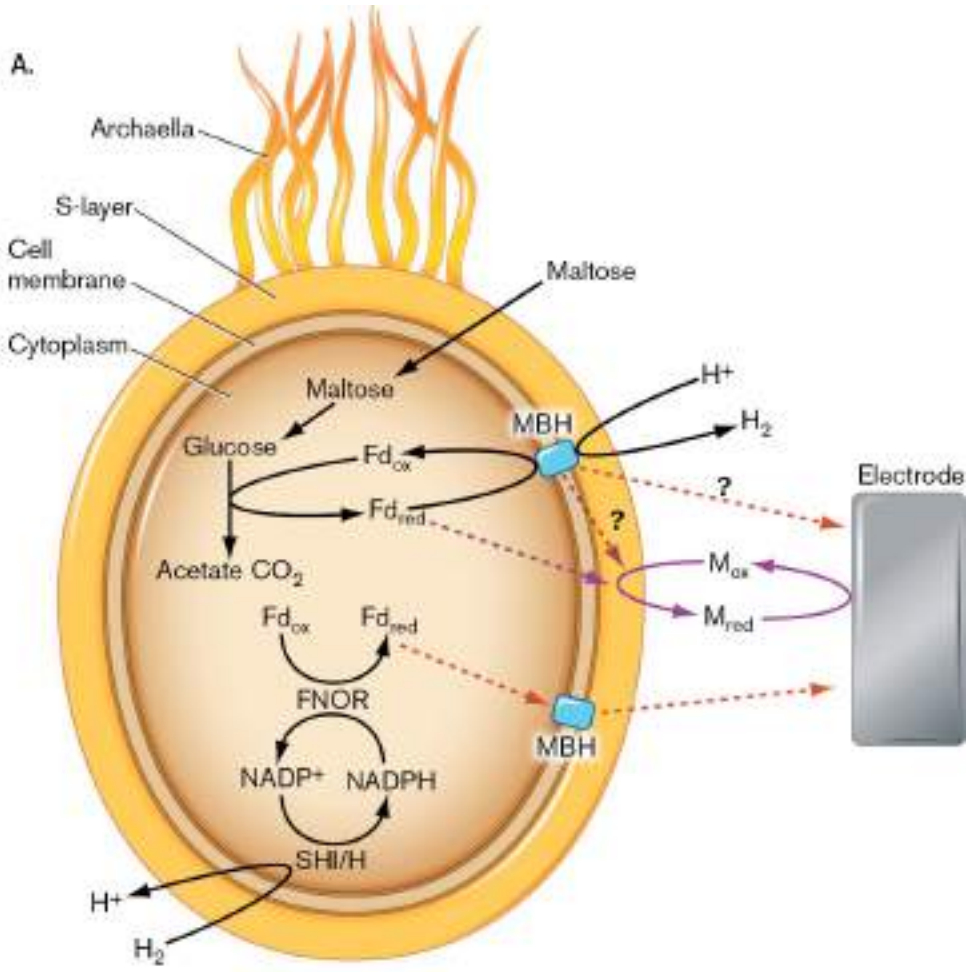


FIGURE ERA 19.1 ■ *Pyrococcus furiosus* colonizes an electrode. A. *Pyrococcus furiosus* cells with tuft of archaella (colorized SEM). **B.** *P. furiosus* forms biofilm on carbon fibers of an anode (SEM). **Inset:** Ramaraja Ramasamy, University of Georgia.

Source: Narendran Sekar et al. 2017. *Biotechnol. Bioeng.* **114** :1419.

EYE OF SCIENCE/SCIENCE SOURCE
N. SEKAR ET AL. 2017. *BIOTECHNOL BIOENG* . **114** :1419–1427
RAMARAJA P. RAMASAMY

How does *P. furiosus* generate electricity? In its natural environment, this archaeon can oxidize both organic and inorganic molecules (discussed in Chapter 14). Cytoplasmic enzymes remove electrons from sugar molecules or from H_2 gas (**Fig. ERA 19.2A**). These electrons are donated to a specific membrane-bound oxidoreductase (hydrogenase) of an electron transport system (in **Fig. ERA 19.2A** , a proposed protein complex MBH). The electron transport system then transfers electrons to cytochromes that can extend outside the cell to reach external metals—or, in the fuel cell, an electrode. The electrode then mediates a current with a circuit that ultimately returns electrons to the microbial suspension. The design of such a fuel cell was shown in Figure 14.23 . Remarkably, the *P. furiosus* fuel cell was able to operate at 90°C.


A.
Archaella
S-layer
Cell membrane
Cytoplasm
Maltose
Maltose
Glucose
Acetate CO_2
Fd_{ox}
Fd_{red}
MBH
H^+
H_2
?
?
Electrode
M_{ox}
M_{red}
Fd_{ox}
Fd_{red}
FNOR
NADP+
NADPH
SHI/H
MBH
H^+
H_2

B.

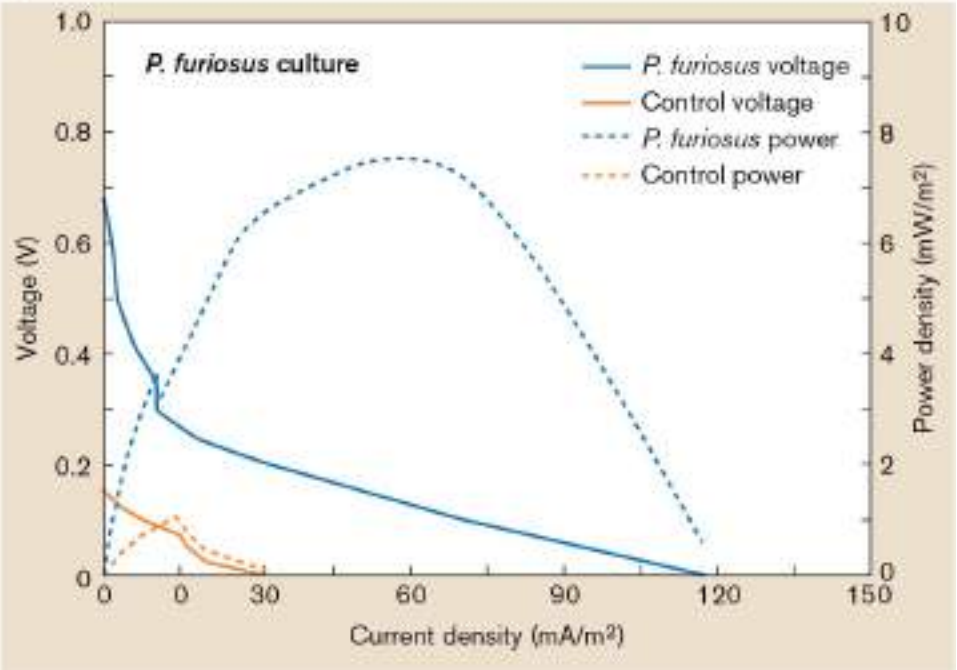

P. furiosus culture
P. furiosus voltage
Control voltage
P. furiosus power
Control power
Voltage (V)
Power density (mW/m²)
Current density (mA/m²)
1.0
0.8
0.6
0.4
0.2
0
10
8
6
4
2
0
0
30
60
90
120
150

FIGURE 19.2 ■ *Pyrococcus furiosus* generates electric current. A. Electron transfer pathways of *Pyrococcus furiosus* . Black solid arrows represent redox pathways of the wild type *P. furiosus* . Colored arrows represent possible extracellular electron transfer pathways to the electrode. Fd_{ox} = oxidized form of ferredoxin; Fd_{red} = reduced form of ferredoxin; MBH = membrane-bound hydrogenase; SHI/II = soluble hydrogenase I and II; FNOR = ferredoxin $NADP^+$ oxidoreductase; M_{ox} and M_{red} = oxidized and reduced forms of mediators, respectively. **B.** Voltage and power density achieved by *P. furiosus* as a function of current density.

Source: Narendran Sekar et al. 2017. *Biotechnol. Bioeng.* **114** :1419.

The current measured from the *P. furiosus* fuel cell was 0.2–0.4 mA (milliamps) for up to six hours. This current produced a voltage of 0.2–0.4 V, for a power density of up to 7 mW/m^2 (milliwatts per square meter; **Fig. ERA 19.2B**). While this amount of electricity is small, it represents an early step toward generating a source of power that operates under formidable conditions.

Further Exploration

How might the fuel cell be made more efficient to produce greater power? Which would make a better substrate, hydrogen gas or sugars?

Sekar, Narendran, Chang-Hao Wu, Michael W. W. Adams, and Ramaraja P. Ramasamy. 2017. Electricity generation by *Pyrococcus furiosus* in microbial fuel cells operated at 90° C. *Biotechnology and Bioengineering* **114** :1419.

Glossary

Figure 14.23

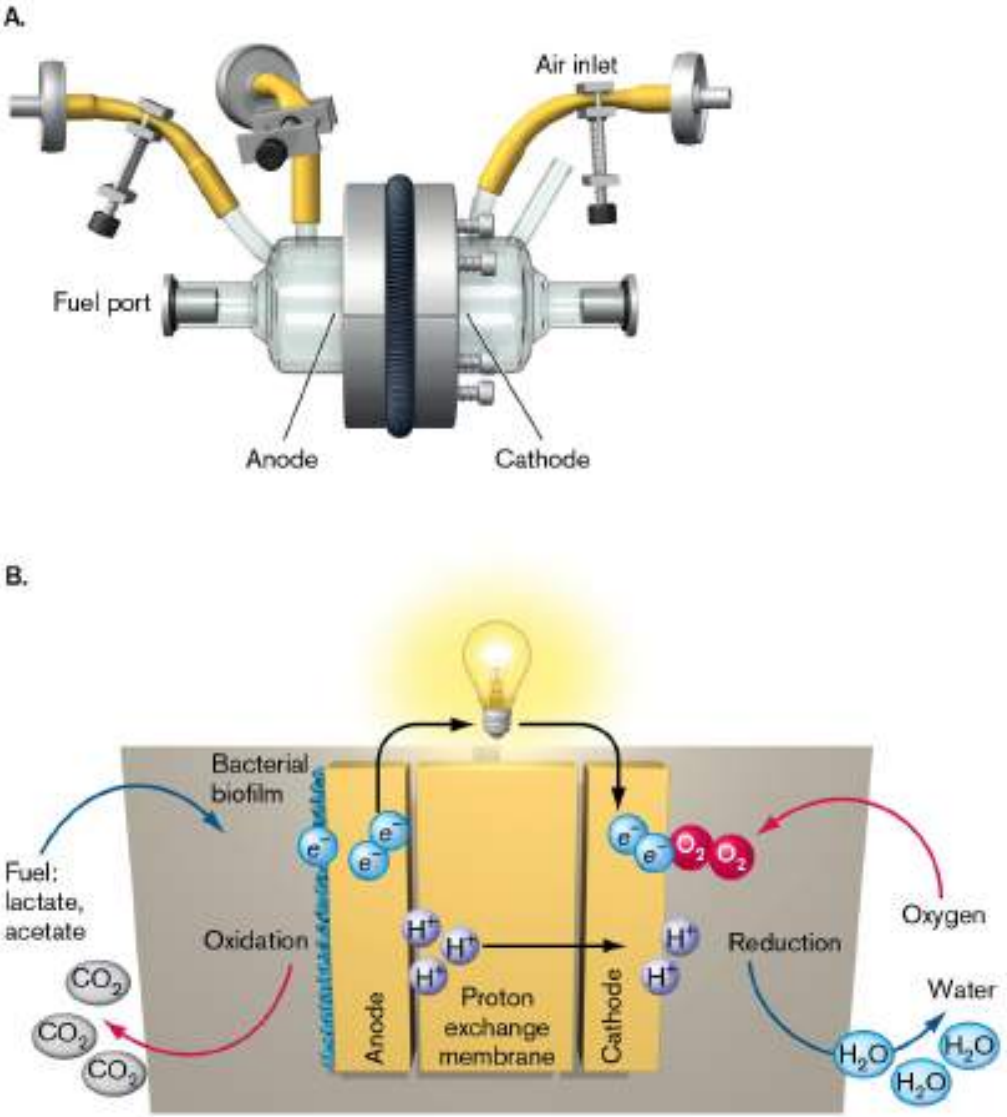


FIGURE 14.23 ■ A microbial fuel cell. A. A bacterial fuel cell. B. Reaction cycle of a bacterial fuel cell.

Source: Part B modified from MURI Microbial Fuel Cell Project, University of Southern California (http://mfc-muri.usc.edu).

CHAPTER REVIEW

Review Questions

1. What distinctive structures are seen in the archaeal cell membrane and envelope?
2. Which aspects of archaeal genetics resemble the genetics of bacteria, and which aspects have more in common with eukaryotes?
3. Compare and contrast diverse members of the TACK superphylum.
4. Outline the genetic phylogeny and key traits of these groups of archaea: Haloarchaea, methanogens, Thermococcales, Thermoplasmatales.
5. What are some specific physiological adaptations found in hyperthermophiles? Halophiles? Extreme acidophiles?
6. Outline three different specific types of mutualism involving an archaeal symbiont.
7. Explain how different archaea contribute to cycling of nitrogen and sulfur in ecosystems.
8. Explain what is known and what is unknown about the following groups of archaea: marine TACK archaea; marine benthic anaerobes; soil and plant root–associated archaea. What kinds of experiments may reveal additional traits of these organisms?
9. How do methanogens interact metabolically with communities of bacteria, within a host animal or within a soil environment?

Thought Questions

1. What approaches can you use to discover previously unknown deeply branching groups of archaea? Explain the strengths and limitations of each method.
2. Why do you think we have found no archaea that are pathogens of animals or plants?
3. Why do you think methanogens appear in many branches among different groups, whereas Haloarchaea branch as a single group?

Key Terms

- Aigarchaeota (779)
- Anaerobic Methane-Oxidizing Euryarchaeota (ANME) (788)
- archaellum (766)
- bacteriorhodopsin (771 , 794)
- barophile (773)
- Bathyarchaeota (769)
- black smoker (777)
- cannula (778)
- Desulfurococcales (768 , 773)
- electron bifurcation (790)
- Euryarchaeota (769)
- gas vesicle (793)
- Geoarchaeota (768)
- Haloarchaea (791)
- halorhodopsin (794)
- hamus (798)
- histone (766)
- isoprenoid (765)
- Korarchaeota (778)

- Lokiarchaeota (772)
- methane gas hydrate (788)
- methanogen (784)
- methanogenesis (784)
- Nanoarchaeota (772)
- pseudopeptidoglycan (pseudomurein) (786)
- reverse gyrase (767)
- Sulfolobales (768 , 774)
- syntrophy (785)
- tetraether (766)
- Thaumarchaeota (769)
- Thermoplasmatales (796)
- Thermoproteales (768)

Recommended Reading

Bang, Corinna, Katrin Weidenbach, Thomas Gutsmann, Holger Heine, and Ruth A. Schmitz. 2014. The intestinal archaea *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* activate human dendritic cells. *PLoS One* **9** :e99411.

Bolduc, Benjamin, Daniel P. Shaughnessy, Yuri I. Wolf, Eugene Koonin, Francisco F. Roberto, et al. 2012. Identification of novel positive-strand RNA viruses by metagenomic analysis of archaea-dominated Yellowstone hot springs. *Journal of Virology* **86** :5562–5573.

Brumfield, Susan K., Alice C. Ortmann, Vincent Ruigrok, Peter Suci, Trevor Douglas, et al. 2009. Particle assembly and ultrastructural features associated with replication of the lytic archaeal virus *Sulfolobus* turreted icosahedral virus. *Journal of Virology* **83** :5964–5970.

Da Cunha, Violette, Morgan Gaia, Arshan Nasir, and Patrick Forterre. 2018. Asgard archaea do not close the debate about the universal tree of life topology. *PLoS Genetics* **14** :e1007215.

DasSarma, Priya, Regie C. Zamora, Jochen A. Müller, and Shiladitya DasSarma. 2012. Genome-wide responses of the archaeon *Halobacterium* sp. strain NRC-1 to oxygen limitation. *Journal of Bacteriology* **194** :5530.

DeMaere, Matthew Z., Timothy J. Williams, Michelle A. Allen, Mark V. Brown, John A. E. Gibson, et al. 2013. High level of intergenera gene exchange shapes the evolution of haloarchaea in an isolated Antarctic lake. *Proceedings of the National Academy of Sciences USA* **110** :16939–16944.

Golyshina, Olga V., and Kenneth N. Timmis. 2005. *Ferroplasma* and relatives, recently discovered cell wall-lacking archaea making a living in extremely acid heavy metal-rich environments. *Environmental Microbiology* **7** :1277–1288.

Heimerl, Thomas, Jennifer Flechsler, Carolin Pickl, Veronika Heinz, Benjamin Salecker, et al. 2017. A complex endomembrane system in the archaeon *Ignicoccus hospitalis* tapped by *Nanoarchaeum equitans* . *Frontiers in Microbiology* **8:** 1072.

López-García, Purificación, Yvan Zivanovic, Philippe Deschamps, and David Moreira. 2015. Bacterial gene import and mesophilic adaptation in archaea. *Nature Reviews. Microbiology* **13** :447–456.

Perras, Alexandra K., Gerhard Wanner, Andreas Klingl, Maximilian Mora, Anna K. Auerbach, et al. 2014. Grappling archaea: Ultrastructural analyses of an uncultivated, cold-loving archaeon, and its biofilm. *Frontiers in Microbiology* **5** :397.

Pester, Michael, Christa Schleper, and Michael Wagner. 2011. The Thaumarchaeota: An emerging view of their phylogeny and ecophysiology. *Current Opinion in Microbiology* **14** :300–306.

Sauder, Laura A., Katja Engel, Jennifer C. Stearns, Andre P. Masella, Richard Pawliszyn, et al. 2011. Aquarium nitrification revisited: Thaumarchaeota are the dominant ammonia oxidizers in freshwater. *PLoS One* **6** :e23281.

Sivabalasarma, Shamphavi, Hanna Wetzel, Phillip Nußbaum, Chris van der Does, Morgan Beeby, et al. 2021. Analysis of cell-cell bridges in *Haloferax volcanii* using electron cryo-tomography reveal a continuous cytoplasm and S-layer. *Frontiers in Microbiology* **11** :612239.

Skennerton, Connor T., Karuna Chourey, Ramsunder Iyer, Robert L. Hettich, Gene W. Tyson, et al. 2017. Methane-fueled syntrophy through extracellular electron transfer: Uncovering the genomic traits conserved within diverse bacterial partners of anaerobic methanotrophic archaea. *mBio* **8** :e00530-17.

Spang, Anja, Eva F. Caceres, and Thijs J. G. Ettema. 2017. Genomic exploration of the diversity, ecology, and evolution of the archaeal domain of life. *Science* **357** :eaaf3883.

Vanderhaeghen, Sonja, Christophe Lacroix, and Clarissa Schwab. 2015. Methanogen communities in stools of humans of different age and health status and co-occurrence with bacteria. *FEMS Microbiology Letters* **362** :fnv092.

Zaremba-Niedzwiedzka, Katarzyna, Eva F. Caceres, Jimmy H. Saw, Disa Bäckström, Lina Juzokaite, et al. 2017. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* **541** :353–358.

Glossary

Thermoproteales
: A clade of the phylum Thermoproteota, thermophilic archaea including hyperthermoacidophiles, in the TACK superphylum.

Aigarchaeota
: A phylum of archaea in the TACK superphylum that contains filamentous aerobic hyperthermophiles in hot springs.

Anaerobic Methane-Oxidizing Euryarchaeota (ANME) *or* anaerobic methane oxidizers

A group of archaea in the superphylum Euryarchaeota that oxidize methane. Some, such as the *Methanoperedenaceae* , oxidize methane independently whereas others oxidize methane syntrophically with sulfate-reducing bacteria (SRB).

archaellum *pl.* archaella
: A rotary complex for motility in archaea, analogous to the bacterial flagellum.

bacteriorhodopsin
: A haloarchaeal membrane-embedded protein that contains retinal and acts as a light-driven proton pump; it is homologous to the bacterial proteorhodopsin.

barophile
: Also called *piezophile* . An organism that requires high pressure to grow.

Bathyarchaeota
: A phylum of thermophilic archaea in the TACK superphylum, widely distributed in anoxic soil, water, and marine sediments.

black smoker
: An oceanic thermal vent containing high concentrations of dark minerals such as iron sulfide.

cannula *pl.* cannulae
: A narrow tubule. For *Pyrodictium* species, glycoprotein cannulae interconnect cells at their periplasm.

Desulfurococcales
: A phylum of thermophilic archaea in the TACK superphylum that metabolize sulfur and organic compounds.

electron bifurcation
: A biochemical reaction in which an electron transfer that yields energy is coupled to an electron transfer that consumes energy.

Euryarchaeota
: A major division of Archaea, containing methanogens, halophiles, acidophiles, and thermophiles, as well as soil archaea that are not extremophiles.

gas vesicle
: An organelle that traps gases to increase the buoyancy of aquatic microbes.

Geoarchaeota
: A clade of Thermoproteota, thermophilic archaea including mats at thermal vents, in the TACK superphylum.

Haloarchaea
: A monophyletic clade of Euryarchaeota that contains extremely halophilic archaea, inhabiting high-salt environments. Formerly known as Halobacteria.

halorhodopsin
: A haloarchaeal membrane-embedded protein that contains retinal and acts as a light-driven chloride pump; it is homologous to bacteriorhodopsin.

hamus *pl.* hami
: An archaeal cell appendage with grappling hooks that enable cells to connect with each other, adhere to a surface, and form biofilms.

histone
: A protein that binds eukaryotic DNA and compacts chromosomes in nucleosomes.

isoprenoid
: A condensed isoprene chain, found in archaeal membrane lipids.

Korarchaeota
: A phylum of archaea in the TACK superphylum that contains anaerobic hyperthermophiles in hot springs and deep-sea thermal vents.

Lokiarchaeota
: A phylum in the Asgard superphylum of Archaea that contains deep-sea thermophiles isolated from a thermal vent named Loki's Castle. Formerly known as the Deep-Sea Archaeal Group or Marine Benthic Group B (DSAG or MBGB).

methane gas hydrate
: A crystalline material in which methane molecules are surrounded by a cage of water molecules. This molecular configuration is found in the deep ocean.

methanogen

An archaeon that uses hydrogen to reduce CO_2 and other single-carbon compounds or acetate to methane, yielding energy. Clades of methanogens branch within the superphylum Euryarchaeota.

methanogenesis
: An energy-yielding metabolic process that releases methane, commonly from hydrogen gas and oxidized one- or two-carbon compounds. It is unique to archaea.

Nanoarchaeota
: A phylum in the DPANN superphylum of Archaea that includes very small cells, which are obligate symbionts of vent hyperthermophiles.

pseudopeptidoglycan *or* pseudomurein
: A peptidoglycan-like molecule composed of sugars and peptides that is found in some archaeal cell walls.

reverse gyrase
: An enzyme that adds positive superturns to DNA in addition to the fundamental twist of the DNA helix. Found mainly in archaea, reverse gyrase differs from the gyrase of bacteria and eukaryotes, which adds negative superturns, against the twist.

Sulfolobales
: A phylum of thermophilic archaea in the TACK superphylum that includes sulfur oxidizers.

syntrophy
: Metabolic cooperation between two different species; usually one member releases a product whose removal by the second species enables the pair to metabolize with a negative value of ΔG.

tetraether
: A molecule containing four ether links. An example is found in archaeal membranes, when two lipid side chains form ether linkages with a pair of side chains from the other side of the bilayer.

Thaumarchaeota

A phylum of archaea in the TACK superphylum that includes ammonia oxidizers and symbionts of marine invertebrates

Thermoplasmatales

An order of extreme thermoacidophiles in the Euryarchaeota superphylum.