
# Morphology, Physiology, biochemistry & Diversity of domain Archaea

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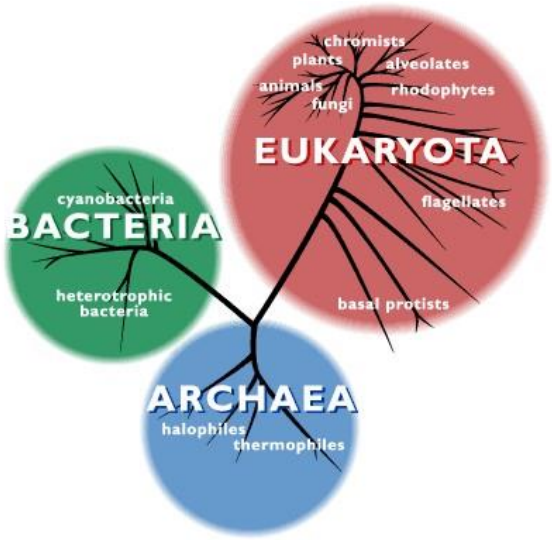
# Introduction to the Archaea

The Domain Archaea wasn't recognized as a major domain of life until quite recently. Until the 20th century, most biologists considered all living things to be classifiable as either a plant or an animal. But in the 1950s and 1960s, most biologists came to the realization that this system failed to accomodate the fungi, protists, and bacteria. By the 1970s, a system of Five Kingdoms had come to be accepted as the model by which all living things could be classified. At a more fundamental level, a distinction was made between the prokaryotic bacteria and the four eukaryotic kingdoms (plants, animals, fungi, & protists). The distinction recognizes the common traits that eukaryotic organisms share, such as nuclei, cytoskeletons, and internal membranes.

The scientific community was understandably shocked in the late 1970s by the discovery of an entirely new group of organisms the Archaea. Dr. Carl Woese and his colleagues at the University of Illinois were studying relationships among the prokaryotes using DNA sequences, and found that there were two distinctly different groups. Those "bacteria" that lived at high temperatures or produced methane clustered together as a group well away from the usual bacteria and the eukaryotes. Because of this vast difference in genetic makeup, Woese proposed that life be divided into three domains: Eukaryota, Eubacteria, and Archaebacteria. He later decided that the term Archaebacteria was a misnomer, and shortened it to Archaea.




Further work has revealed additional surprises, which you can read about on the other pages of this exhibit. It is true that most archaeans don't look that different from bacteria under the microscope, and that the extreme conditions under which many species live has made them difficult to culture, so their unique place among living organisms long went unrecognized. However, biochemically and genetically, they are as different from bacteria as you are.

Although many books and articles still refer to them as "Archaebacteria", that term has been abandoned because they aren't bacteria they're Archaea. There are three main groups of Archaea: extreme halophiles, methanogens, and hyperthermophiles.

Archaeans include inhabitants of some of the most extreme environments on the planet. Some live near rift vents in the deep sea at temperatures well over 100 degrees Centigrade. Others live in hot springs, or in extremely alkaline or acid waters. They have been found thriving inside the digestive tracts of cows, termites, and marine life where they produce methane. They live in the anoxic muds of marshes and at the bottom of the ocean, and even thrive in petroleum deposits deep underground.

Some archaeans can survive the dessicating effects of extremely saline waters. One salt-loving group of archaea includes *Halobacterium*, a well-studied Archaean. The light-sensitive pigmentbacteriorhodopsin gives *Halobacterium* its color and provides it with chemical energy. Bacteriorhodopsin has a lovely purple color and it pumps protons to the outside of the membrane. When these protons flow back, they are used in the synthesis of ATP, which is the energy source of the cell. This protein is chemically very similar to the light-detecting pigment rhodopsin, found in the vertebrate retina.

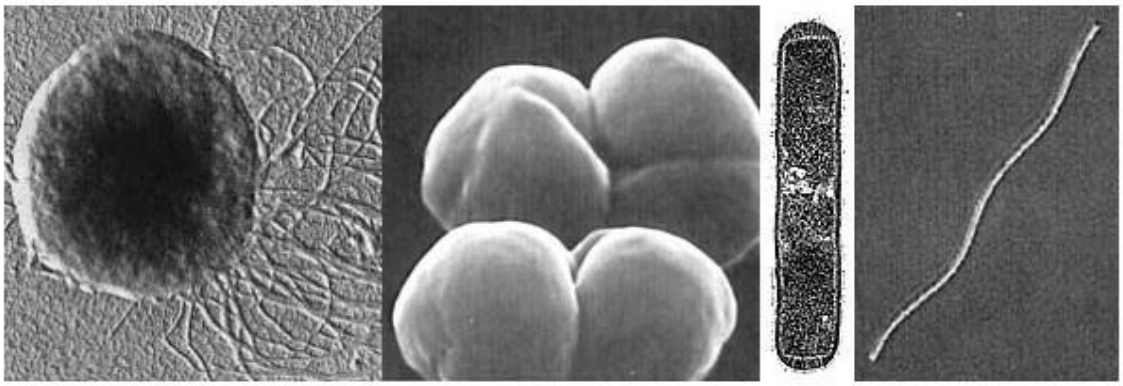
Archaeans may be the only organisms that can live in extreme habitats such as thermal vents or hypersaline water. They may be extremely abundant in environments that are hostile to all other life forms. However, archaeans are not restricted to extreme environments; new research is showing that archaeans are also quite abundant in the plankton of the open sea. Much is still to be learned about these microbes, but it is clear that the Archaea is a remarkably diverse and successful clade of organisms.

The hot springs of Yellowstone National Park, USA, were among the first places Archaea were discovered. At left is Octopus Spring, and at right is Obsidian Pool. Each pool has slightly different mineral content, temperature, salinity, etc., so different pools may contain different communities of archaeans and other microbes. The biologists pictured above are immersing microscope slides in the boiling pool onto which some archaeans might be captured for study.

# Morphological features of Archaea

Archaea are tiny, usually less than one micron long (one one-thousandth of a millimeter). Even under a high-power light microscope, the largest archaeans look like tiny dots. Fortunately, the electron microscope can magnify even these tiny microbes enough to distinguish their physical features.

Archaea organisms are so small would not have much variety of shape or form, but in fact archaeal shapes are quite diverse. Some are spherical, a form known as coccus, and these may be perfectly round or lobed and lumpy. Some are rod-shaped, a form known as bacillus, and range from short bar-shaped rods to long slender hair-like forms. Some oddball species have been discovered with a triangular shape, or even a square shape like a postage stamp.



**Basic Archaeal Shapes** : At far left, *Methanococcus janaschii*, a coccus form with numerous flagella attached to one side. At left center, *Methanosarcina barkeri*, a lobed coccus form lacking flagella. At right center, *Methanothermus fervidus*, a short bacillus form without flagella. At far right, *Methanobacterium thermoautotrophicum*, an elongate bacillus form.

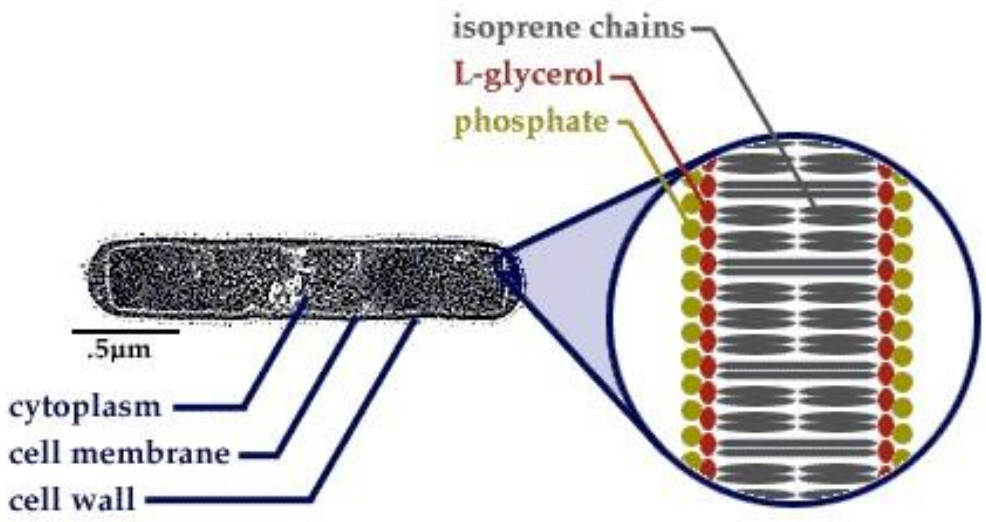
Structural diversity among archaeans is not limited to the overall shape of the cell. Archaea may have one or more flagella attached to them, or may lack flagella altogether. The flagella are hair-like appendages used for moving around, and are attached directly into the outer membrane of the cell. When multiple flagella are present, they are usually attached all on one side of the cell. Other appendages include protein networks to which the cells may anchor themselves in large groups.

Like bacteria, archaeans have no internal membranes and their DNA exists as a single loop called a plasmid. However, their tRNAs have a number of features that differ from all other living things. That RNA molecules (short for "transfer RNA") are important in decoding the message of DNA and in building proteins. Certain features of tRNA structure are the same in bacteria, plants, animals, fungi, and all known living things except the Archaea. There are even features of archaeal tRNA that are more like eukaryotic critters than bacteria, meaning that Archaea share certain features in common with you and not with bacteria. The same is true of their ribosomes, the giant processing molecules that assemble proteins for the cell.

While bacterial ribosomes are sensitive to certain chemical inhibiting agents, archaeal and eukaryotic ribosomes are not sensitive to those agents. This may suggest a close relationship between Archaea and eukaryotes.

As with other living things, archaeal cells have an outer cell membrane that serves as a barrier between the cell and its environment. Within the membrane is the cytoplasm, where the living functions of the archeon take place and where the DNA is located. Around the outside of nearly all archaeal cells is a cell wall, a semi-rigid layer that helps the cell maintain its shape and chemical equilibrium. All three of these regions may be distinguished in the cells of bacteria and most other living things, but when you take a closer look at each region, you find that the similarities are merely structural, not chemical.

In other words, Archaea build the same structures as other organisms, but they build them from different chemical components. For instance, the cell walls of all bacteria contain the chemical peptidoglycan. Archaeal cell walls do not contain this compound, though some species contain a similar one. Likewise, archaea do not produce walls of cellulose (as do plants) or chitin (as do fungi). The cell wall of archaeans is chemically distinct.




**Basic Archaeal Structure** : The three primary regions of an archaeal cell are the cytoplasm, cell membrane, and cell wall. Above, these three regions are labelled, with an enlargement at right of the cell membrane structure. Archaeal cell membranes are chemically different from all other living things, including a "backwards" glycerol molecule and isoprene derivatives in place of fatty acids.

The most striking chemical differences between Archaea and other living things lie in their cell membrane. There are four fundamental differences between the archaeal membrane and those of all other cells:

(1) chirality of glycerol.

(2) ether linkage.

(3) isoprenoid chains.

(4) branching of side chains.

(5) Arranging phospholipids as Tetra ethers to form Monolayer of cell membrane.

(6) Formation of Cyclopropane and Cyclohexane Carbon rings by Isoprenoid side chains.

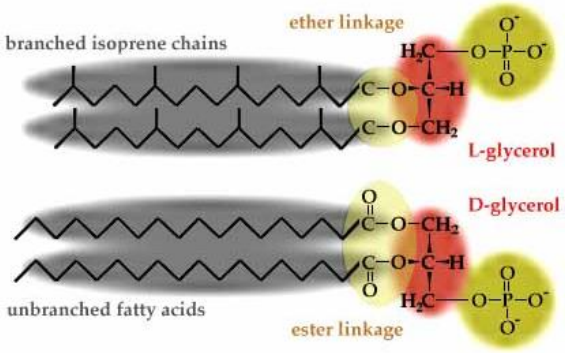
These may sound like complex differences, but a little explanation will make the differences understandable.

## 1. Chirality of glycerol

The basic unit from which cell membranes are built is the phospholipid. This is a molecule of glycerol which has a phosphate added to one end, and two side chains attached at the other end. When the cell membrane is put together, the glycerol and phosphate end of the molecules hang out at the surface of the membrane, with the long side chains sandwiched in the middle. This layering creates an effective chemical barrier around the cell and helps maintain chemical equilibrium.

The glycerol used to make archaeal phospholipids is a stereoisomer of the glycerol used to build bacterial and eukaryotic membranes. Two molecules that are stereoisomers are mirror-images of each other. This can be describe using simple example.

Ex. Put your hands out in front of you, palms up. Both hands are oriented with fingers pointing away from you, wrists toward you, and with palms upwards. However, your thumbs are pointing different directions because each hand is a mirror image of the other. If you turn one hand so that both thumbs point the same way, that one will no longer be palm-up.



This is the same situation as the stereoisomers of glycerol. There are two possible forms of the molecule that are mirror images of each other. It is not possible to turn one into the other simply by rotating it around. While bacteria and eukaryotes have D-glycerol in their membranes, archaeans have L-glycerol in theirs. This is more than a geometric difference. Chemical components of the cell have to be built by enzymes, and the "handedness" (chirality) of the molecule is determined by the shape of those enzymes. A cell that builds one form will not be able to build the other form.

**2. Ether linkage.**

When side chains are added to the glycerol, most organisms bind them together using an ester linkage. The side chain that is added has two oxygen atoms attached to one end. One of these oxygen atoms is used to form the link with the glycerol, and the other protrudes to the side when the bonding is done. By contrast, archaeal side chains are bound using an ether linkage, which lacks that additional protruding oxygen atom. This gives the resulting phospholipid different chemical properties from the membrane lipids of other organisms.

**3. Isoprenoid chains.**

The side chains in the phospholipids of bacteria and eukaryotes are fatty acids, chains of usually 16 to 18 carbon atoms. Archaea do not use fatty acids to build their membrane phospholipids. Instead, they have side chains of 20 carbon atoms built from isoprene.

Isoprene is the simplest member of a class of chemicals called terpenes. By definition, a terpene is any molecule belt by connecting isoprene molecules together, rather like building with Lego® blocks. Each isoprene unit has a "head" and a "tail" end (again like a Lego® block), but unlike their toy counterparts, isoprene blocks can be joined in many ways. A head can be attached to a tail or to another head end, and tails can be similarly joined. The immense variety of terpene compounds that can be built from simple isoprene units include beta-carotene (a vitamin), natural and synthetic rubbers, plant essential oils (such as spearmint), and steroid hormones (such as estrogen and testosterone).

**4. Branching of side chains.**

Not only are the side chains of achaeal membranes built from different components, but the chains themselves have a different physical structure. Because isoprene is used to build the side chains, there are side branches off the main chain. The fatty acids of bacteria and eukaryotes do not have these side branches (the best they can manage is a slight bend in the middle), and this creates some interesting properties in archaeal membranes.

**5. Arranging phospholipids as Tetra ethers to form Monolayer of cell membrane**

In some Archaeal species, isoprene side chains are joined together to form Tetra ether structures. This can mean that the two side chains of a single phospholipid can join together, or they can be joined to side chains of another phospholipid on the other side of the membrane.

The chemical nature of these types of molecules can be describe as "Bolaamphiphile". No other group of organisms can form such transmembrane phospholipids. So in this case Lipid bilayer is replaced by a monolayer. This arrangement may make their membranes more rigid and better able to resist harsh environments. For example, the lipids in Ferroplasma are of this type, which is thought to aid this organism's survival its highly acidic habitat.

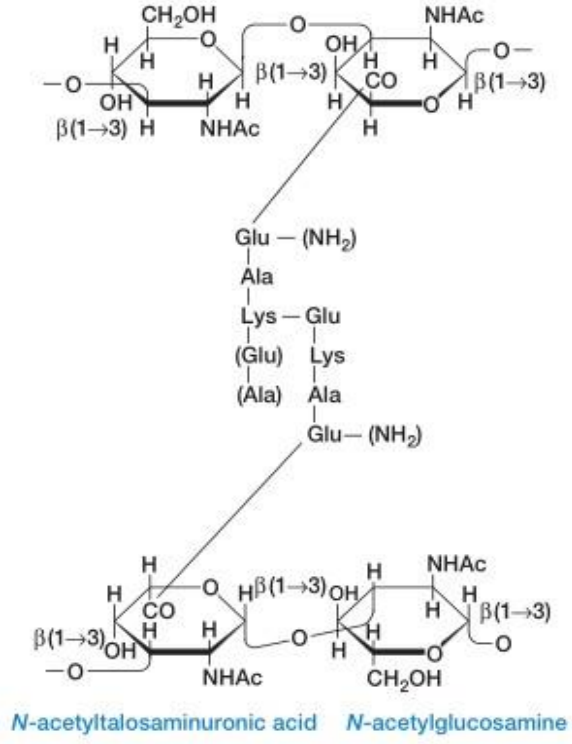
6. **Formation of Cyclopropane and Cyclohexane Carbon rings by Isoprenoid side chains**.

Side branches is their ability to form carbon rings. This happens when one of the side branches curls around and bonds with another atom down the chain to make a ring of five carbon ams. Such rings are thought to provide structural stability to the membrane, since they seem to be more common among species that live at high temperatures. They may work in the same way that cholesterol does in Eukaryotic cells to stabilize the membrane.

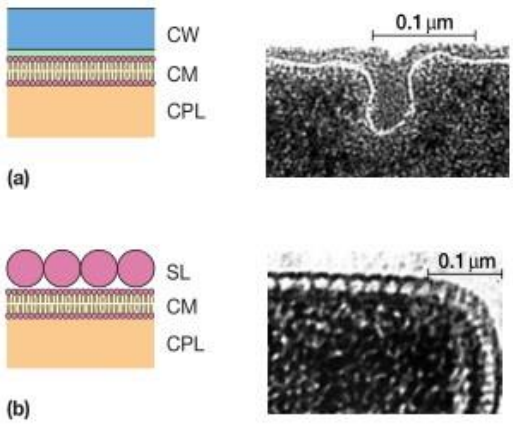
## Archaeal cell wall –

Before they were distinguished as a unique domain of life, the Archaea were characterized as being either gram positive or gram negative. However, their staining reaction does not correlate as reliably with a particular cell wall structure as does the Gram re- action of Bacteria. Archaeal wall structure and chemistry differ from those of the Bacteria. Archaeal cell walls lack peptidoglycan and also exhibit considerable variety in terms of their chemical make-up. Some of the major features of archaeal cell walls are described in this section. Many archaea have a wall with a single, thick homogeneous layer resembling that in gram-positive bacteria These archaea often stain gram positive. Their wall chemistry varies from species to species but usually consists of complex heteropolysaccharides. For example, *Methanobacterium* and some other methane-generating archaea (methanogens) have walls containing **pseudomurein**, a peptidoglycan-like polymer that has L-amino acids instead of D-amino acids in its cross-links, N-acetyltalosaminuronic acid instead of N-acetylmuramic acid, and β (1→3) glycosidic bonds instead of β (1→4) glycosidic bonds. Other archaea, such as Methanosarcina and the salt-loving Halococcus, contain complex polysaccharides similar to the chondroitin sulfate of animal connective tissue.

Many archaea that stain gram negative have a layer of glycoprotein or protein outside their plasma membrane. The layer may be as thick as 20 to 40 nm. Sometimes there are two layers an electron-dense layer and a sheath surrounding it. Some methanogens (*Methanolobus*), salt-loving archaea (*Halobacterium*), and extreme thermophiles (*Sulfolobus, Thermoproteus*, and *Pyrodictium*) have glycoproteins in their walls. In contrast, other methanogens (*Methanococcus, Methanomicrobium*, and *Methanogenium*) and the extreme thermophile *Desulfurococcus* have protein walls.




**The Structure of Pseudomurein**. The amino acids and amino groups in parentheses are not always present. Ac represents the acetyl group.



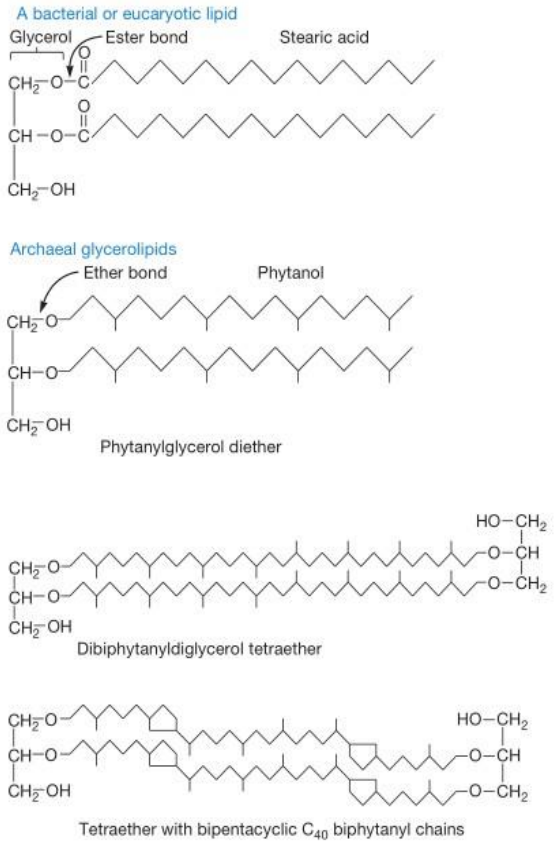

**Cell Envelopes of Archaea**. Schematic representations and electron micrographs of (a) *Methanobacterium formicicum*, and (b) *Thermoproteus tenax*.CW,cell wall;SL,surface layer;CM,cell membrane or plasma membrane;CPL,cytoplasm.

## Archaeal cell membrane –

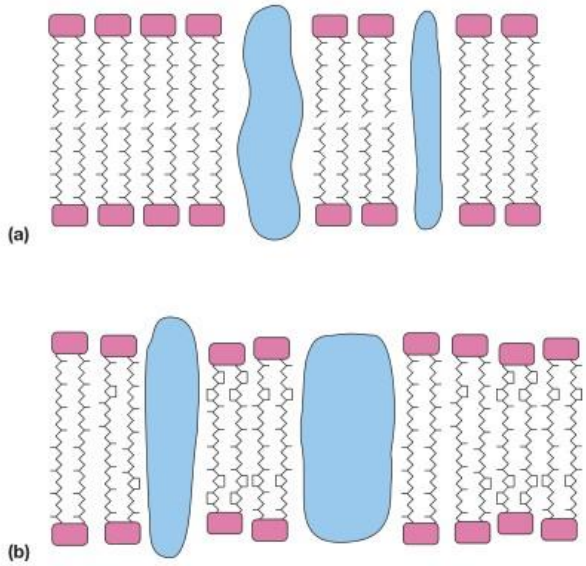
One of the most distinctive features of the Archaea is the nature of their membrane lipids. They differ from both Bacteria and Eucarya in having branched chain hydrocarbons attached to glycerol by ether links rather than fatty acids connected by ester links. Sometimes two glycerol groups are linked to form an extremely long tetraether. Usually the diether hydrocarbon chains are 20 carbons in length, and the tetraether chains are 40 carbons. Cells can adjust the overall length of the tetraethers by cyclizing the chains to form pentacyclic rings.

Phosphate-, sulfur- and sugar-containing groups can be attached to the third carbons of the diethers and tetraethers, making them polar lipids. These predominate in the membrane, and 70 to 93% of the membrane lipids are polar. The remaining lipids are non- polar and are usually derivatives of squalene. Despite these significant differences in membrane lipids, the basic design of archaeal membranes is similar to that of Bacteria and eucaryotes there are two hydrophilic surfaces and a hydrophobic core. When $C_{20}$ diethers are used, a regular bilayer membrane is formed. When the membrane is constructed of $C_{40}$ tetraethers, a monolayer membrane with much more rigidity is formed. As might be expected from their need for

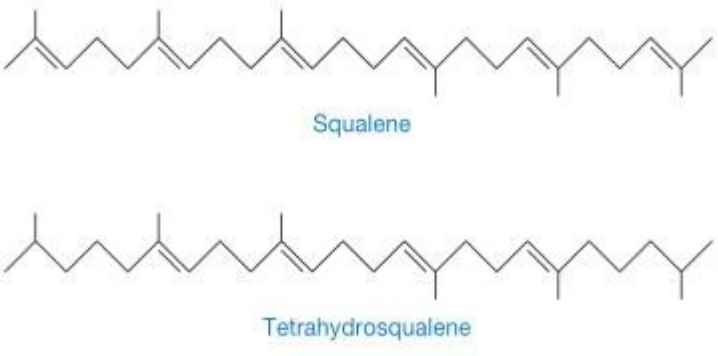
stability, the membranes of extreme thermophiles such as *Thermoplasma* and *Sulfolobus*, which grow best at temperatures over 85°C, are almost completely tetraether monolayers. Archaea that live in moderately hot environments have a mixed membrane containing some regions with monolayers and some with bilayers.




**Archaeal Membrane Lipids**- An illustration of the difference between archaeal lipids and those of Bacteria. Archaeal lipids are derivatives of isopranyl glycerol ethers rather than the glycerol fatty acid esters in Bacteria. Three examples of common archaeal glycerolipids are given.




**Examples of Archaeal Membranes**- (a) A membrane composed of integral proteins and a bilayer of $C_{20}$ diethers. (b) A rigid monolayer composed of integral proteins and $C_{40}$ tetraethers.




**Nonpolar Lipids of Archaea** - Two examples of the most predominant nonpolar lipids are the $C_{30}$ isoprenoid squalene and one of its hydroisoprenoid derivatives, tetrahydrosqualene.

## Archaeal flagella –

The archaeal flagellum is a unique motility apparatus distinct in composition and likely in assembly from the bacterial flagellum. Gene families comprised of multiple flagellin genes co-transcribed with a number of conserved, archaeal-specific accessory genes have been identified in several archaea. However, no homologues of any bacterial genes involved in flagella structure have yet been identified in any archaeon, including those archaea in which the complete genome sequence has been published. Archaeal flagellins possess a highly conserved hydrophobic N-terminal sequence that is similar to that of type IV pilins and clearly unlike that of bacterial flagellins. Also unlike bacterial flagellins but similar to type IV pilins, archaeal flagellins are initially synthesized with a short leader peptide that is cleaved by a membrane-located peptidase. With recent advances in genetic transfer systems in archaea, knockouts have been reported in several genes involved in flagellation in different archaea. In addition, techniques to isolate flagella with attached hook and anchoring structures have been developed. Analysis of these preparations is under way to identify minor structural components of archaeal flagella. This and the continued isolation and characterization of flagella mutants should lead to significant advances in our knowledge of the composition and assembly of archaeal flagella.

# Molecular biological features of Archaea

Some features of archaeal genetics are similar to those in the Bacteria, while others more closely resemble the Eucarya. The genomes of some archaea are significantly smaller than those of many bacteria. For instance, while the genome of *Bacillus subtilis* is 4.20 million base pairs (Mb), the crenarchaeote *Pyrobaculum aerophilum* genome is 2.22 Mb and that of *Methanobacterium thermoautotrophicum*, a euryarchaeote, is 1.75 Mb. A sign of archaeal diversity is the variation in G + C content, from about 21% to 68%. To date, it appears that the Archaea have few plasmids.

Comparative genomics between the completely sequenced genomes of archaea, bacteria, and eucaryotes show several apparent trends. First, about 30% of all genes shared exclusively between archaea and eucaryotes encode proteins involved in transcription, translation, or DNA metabolism. In contrast, a large number of the genes shared only between Bacteria and Archaea are involved in metabolic pathways. In addition, there is evidence for horizontal gene transfer between these two domains, especially between thermophilic bacteria and archaea. The small number of genes found in all three domains does not seem to fit any specific pattern.

Archaeal DNA replication appears to be a complex mixture of eucaryotic and procaryotic features. Like Bacteria, most archaea have circular chromosomes with a single origin of replication, and replication appears to be bidirectional. However, in archaeal genomes that have been sequenced, the replication origin is flanked by genes encoding the eucaryotic-

like initiation protein Cdc6/Orc1 and at least a few archaea have multiple origins. While it was originally thought that archaeal replication proteins were uniformly eukaryotic – like, further genome analysis reveals that some replication proteins are similar to those bacteria, while still others are uniquely archaeal. Some archaeal chromosomes differ from Bacteria in having eucaryotic-like histone proteins that bind DNA to form nucleosome-like structures.

Transcription in the Archaea likewise blends bacterial and eucaryotic features. Archaeal RNApolymerases consist of at least 10 subunits that are highly homologous to eucaryotic subunits. Also, like eucaryotic nuclear RNA polymerase, archaeal RNA polymerases do not efficiently recognize promoter regions without the aid of additional proteins. Instead, promoter recognition is dependent on at least two eucaryotic-like proteins: the TATA-box-binding protein (TBP) and transcription factor B (TFB). It is therefore not surprising that many archaeal promoters are similar to certain eucaryotic promoters, possessing a TATA box (a 7-bp sequence found about 25 bp before the transcriptional start site) preceded by a purine rich region called the B responsive element (BRE). In eucaryotes, the BRE is the site to which transcription factor IIB binds. It is thought that archaeal TFB and TBP bind the BRE region of DNA as a prerequisite for the assembly of RNA polymerase sub-units prior to the initiation of transcription. However, archaeal mRNA appears to be similar to bacterial mRNA in that it is polycistronic and there is no evidence for mRNA splicing.

Finally, the translational machinery in the Archaea is unique. Unlike both Bacteria and eucaryotes, the T+C arm of archaeal tRNA lacks thymine and contains pseudouridine or 1-methylpseudouridine. The archaeal initiator tRNA carries methionine as does the eucaryotic initiator tRNA. Although archaeal ribosomes are 70S, similar to bacterial ribosomes, electron microscopy studies show that their shape is quite variable and sometimes differs from that of both bacterial and eucaryotic ribosomes. They resemble eucaryotic ribosomes in their sensitivity to the antibiotic anisomycin and insensitivity to chloramphenicol and kanamycin. Furthermore, their elongation factor2 reacts with diphtheria toxin like the eucaryotic EF-2 does.

Like archaeal protein synthesis, archaeal protein secretion has both bacterial and eucaryotic features. All three domains have signal recognition particles (SRPs) that target new proteins to translocation sites, but the archaeal SRP differs from those in the other two domains. As in the Bacteria, the archaeal SRP binds to the signal sequence of a pre protein and can direct it to the Sec-dependent protein secretion pathway for transport through the plasma membrane. The archaeal Sec-dependent pathway proteins, however, more closely resemble those of the eucaryotic pathway than the bacterial proteins. After the pre protein is moved across the membrane, its signal sequence is removed by a signal peptidase that resembles a subunit of the eucaryotic peptidase.

# Significance of gene transfer in Archaea

Archaea are genetically distinct from bacteria and eukaryotes, but are poorly understood: many of the genes that Archaea encode are of unknown function. Transcription and translation in archaea resemble the same processes more closely in eukaryotes than in bacteria, with the archaean RNA polymerase and ribosomes being very close to their equivalents in eukaryotes.

Although Archaea only have one type of RNA polymerase, its structure and function in transcription is similar to that of the eukaryotic RNA polymerase II, with similar protein assemblies (the general transcription factors) directing the binding of the RNA polymerase to a gene's promoter. However, other archaean transcription factors are closer to those found in bacteria. Post-transcriptional modification is simpler than in eukaryotes, since most archaean genes lack introns, although there are many introns in their transfer RNA and ribosomal RNA genes, and introns may occur in a few protein-encoding genes. This is all to say there are many similarities in the genes shared between Archaea and the other domains of life, suggesting there was a transfer of genetic material between the domains of life. This phenomenon is described as horizontal gene transfer.

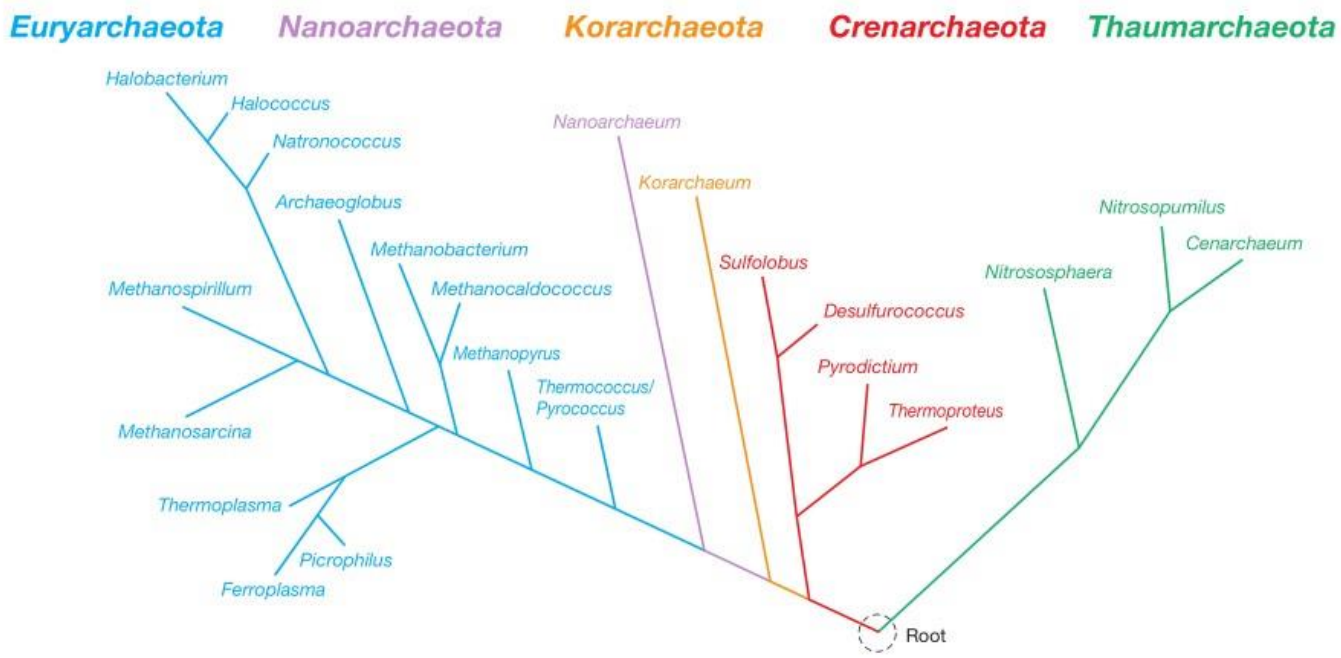
Horizontal gene transfer (HGT) refers to the transfer of genes between organisms in a manner other than traditional reproduction. Also termed lateral gene transfer, it contrasts with vertical transfer, the transmission of genes from the parental generation to offspring via sexual or asexual reproduction. HGT has been shown to be an important factor in the evolution of many organisms, including bacteria, plants and humans.

Archaea show high levels of horizontal gene transfer between lineages. Some researchers suggest that individuals can be grouped into species-like populations given highly similar genomes and infrequent gene transfer to/from cells with less-related genomes, as in the Archaea genus Ferroplasma. On the other hand, studies in Halorubrum found significant genetic transfer to/from less-related populations. These gene transfers are identified by sequencing the DNA of various Archaea species; through the similarities and differences of the DNA of the different types of Archaea it is determined if the gene was perfectly transferred or from a common ancestor. The elucidation of this can be controversial.

How genetic material can move from one Archaea to another is poorly understood. In bacteria the natural ways in which this occurs is through either bacterial conjugation or viral transfer, also known as transduction. Conjugation is where two (sometimes distantly related) bacteria transfer genetic material by direct contact. Transduction occurs when a virus "picks up" some DNA from its host and when infecting a new host, moves that genetic material to the new host. It is thought that conjugation can occur in Archaea, though unlike bacteria the mechanism is not well understood. As well Archaea can be infected by viruses. In fact, Archaea can be infected by double-stranded DNA viruses that are unrelated to any other form of virus and have a variety of unusual shapes, including bottles, hooked rods, or teardrops. Taken together it is clear that gene transfer happens in Archaea, and probably is similar to horizontal gene transfer seen in the other domains of life.

# Introduction to the diversity of Archaea

The domain Archaea consists of seven major phyla, only five of which contain species described on the basis of cultivated strains. Most described species fall within the phyla *Crenarchaeota* and *Euryarchaeota*, while only a handful of species have been described for the *Nonoarchaeota, Korarchaeota* and *Thaumarchaeota.* A phylogenetic tree of Archaea is shown in below. The tree, based on comparative sequences of ribosomal proteins.




Detailed phylogenetic tree of the Archaea based on comparisons of ribosomal proteins from sequenced genomes. Each of the five archaeal phyla is indicated in a different color. The *Korarchaeota* and *Nanoarchaeota* are each represented by only a single known species.

The exact ancestry of these groups remains a contentious issue, and phylogenetic trees constructed from 16S ribosomal RNA gene sequences often conflict with those made using other genomic loci. The evolutionary history of the Archaea ancient and complex, involving horizontal gene transfers within and between phyla. Common traits shared by all Archaea include their ether linked lipids, their lack of peptidoglycan in cell walls, and their structurally complex RNA polymerases, which resemble those of Eukarya. But beyond this Archaea show enormous phenotypic diversity. Archaea include species that carry out chemoorganotrphic or chemolithotrophic metabolisms and both aerobic and anaerobic species are common.

Chemoorganotrophy is widespread among Archaea, and fermentations and anaerobic respirations are common. Chemolithotrophy is also well established in the Area, with $H_2$ being a common electron donor, and with ammonia oxidation found among species of *Thaumarchaeota.*

**Anaerobic respiration**, especially forms employing elemental sulfur (S) as an electron acceptor, is prevalent among the Archaea, especially *Crenarchaeota.*

**Aerobic respiration** occurs widely in *Thaumarchaeota* and is common among a few groups of *Euryarchaeota* but is characteristic of only a few species of *Crenarchaeota.*

*Euryarchaeota* that conserve energy from the production of methane. Methanogenesis is a globally important process that is uniquely arceal. Archaea are also well known for containing many species of Extremophiles, including species that are Hyperthermophiles, Halophiles and Acidophiles. However, a great many species in the *Euryarchaeota* and most *Thaumarchaeota* are not extremophiles and are found in soils, sediments, oceans, lakes, in association with animals, and even in the human gut.

## *Euryarchaeota*

*Euryarchaeota* comprise a large and physiologically diverse group of Archaea. This phylum includes methanogens as well as many genera of extremely halophilic (salt-loving) Archaea. As a study in physiological contrasts, these two groups are remarkable: Methanogens are the strictest of anaerobes while extreme halophiles are primarily obligate aerobes. Other groups of euryarchaeotes include the hyperthermophiles, *Thermococcus* and *Pyrococcus,* the *hyperthermophilic* methanogen *Methanopyrus,* and the cell wall–less *Thermoplasma*, an organism phenotypically similar to the mycoplasmas.

### 1. Extremely halophilic Archaea

Extremely halophilic Archaea, often called the "haloarchaea," are a diverse group that inhabits environments high in salt. Here include *Halobacterium, Haloferax,* and *Natronobacterium.* These include naturally salty environments, such as solar salt evaporation ponds and salt lakes, and artificial saline habitats such as the surfaces of heavily salted foods, for example, certain fish and meats. Such salty habitats are called hypersaline. The term extreme halophile is used to indicate that these organisms are not only halophilic, but that their requirement for salt is very high, in some cases at levels near saturation. An organism is considered an extreme halophile if it requires 1.5 M (about 9%) or more sodium chloride (NaCl) for growth. Most species of extreme halophiles require 2–4 M NaCl (12–23%) for optimal growth. Virtually all extreme halophiles can grow at 5.5 M NaCl (32%, the limit of saturation for NaCl), although some species grow very slowly at this salinity. Some phylogenetic relatives of extremely halophilic Archaea, for example species of *Haloferax* and *Natronobacterium*, are able to grow at much lower salinities, such as at or near that of seawater (about 2.5% NaCl); nevertheless, these organisms are phylogenetic relatives of other extreme halophiles.

## ➢ Taxonomy and physiology of extremely halophilic Archaea

Besides the term haloarchaea, these Archaea are sometimes called "halobacteria," because the genus *Halobacterium* was the first in this group to be described (prior to the discovery of Archaea) and is still the best-studied representative of the group. *Natronobacterium, Natronomonas*, and their relatives differ from other extreme halophiles in being extremely alkaliphilic as well as halophilic. As befits their soda lake habitat, natronobacteria grow optimally at very low $Mg^{2+}$ concentrations and high pH (9–11). Haloarchaea stain gram-negatively, reproduce by binary fission, and do not form resting stages or spores. Cells of the various cultured genera are rod-shaped, cocci, or cup-shaped. Cells of Halo- quadratum are square in shape and are only about 0.1 μm thick. *Haloquadratum* also forms gas vesicles that allow it to float in its salty hypersaline habitat, probably as a means to be in contact with air since most extreme halophiles are obligate aerobes.

Many other extremely halophilic Archaea also produce gas vesicles. Most species of extreme halophiles lack flagella, but a few strains are weakly motile by flagella that rotate to propel the cell forward. The genomes of *Halobacterium* and *Halococcus* are unusual in that large plasmids containing up to 30% of the total cellular DNA are present and the GC base ratio of these plasmids (near 60% GC) differs significantly from that of chromosomal DNA (66–68% GC). Plasmids from extreme halophiles are among the largest naturally occurring plasmids known.

Most species of extremely halophilic Archaea are obligate aerobes. Most haloarchaea use amino acids or organic acids as electron donors and require a number of growth factors such as vitamins for optimal growth. A few haloarchaea oxidize carbohydrates aerobically, but this capacity is rare; sugar fermentation does not occur. Electron transport chains containing cytochromes of the a, b, and c types are present in *Halobacterium*, and energy is conserved during aerobic growth via a proton motive force arising from electron transport. Some haloarchaea have been shown to grow anaerobically, as growth by anaerobic respiration linked to the reduction of nitrate or fumarate has been demonstrated in certain species.

## ➢ Water Balance in extreme halophiles

Extremely halophilic Archaea require large amounts of NaCl for growth. Detailed salinity studies of *Halobacterium* have shown that the requirement for $Na^+$ cannot be satisfied by any other ion, even the chemically related ion potassium ($K^+$). However, cells of *Halobacterium* need both $Na^+$ and $K^+$ for growth, because each plays an important role in maintaining osmotic balance.

To do so in a high-solute environment such as the salt-rich habitats of *Halobacterium*, organisms must either accumulate or synthesize solutes intracellularly. These solutes are called compatible solutes. These compounds counteract the tendency of the cell to become dehydrated under conditions of high osmotic strength by placing the cell in positive water

balance with its surroundings. Cells of *Halobacterium*, however, do not synthesize or accumulate organic compounds but instead pump large amounts of $K^+$ from the environment into the cytoplasm. This ensures that the concentration of $K^+$ inside the cell is even greater than the concentration of $Na^+$ outside the cell. This ionic condition maintains positive water balance.

The *Halobacterium* cell wall is composed of glycoprotein and is stabilized by $Na^+$. Sodium ions bind to the outer surface of the *Halobacterium* wall and are absolutely essential for maintaining cellular integrity. When insufficient $Na^+$ is present, the cell wall breaks apart and the cell lyses. This is a consequence of the exceptionally high content of the acidic (negatively charged) amino acids aspartate and glutamate in the glycoprotein of the *Halobacterium* cell wall. The negative charge on the carboxyl group of these amino acids is bound to $Na^+$; when $Na^+$ is diluted away, the negatively charged parts of the proteins tend to repel each other, leading to cell lysis.

- **Halophilic Cytoplasmic Components**

Like cell wall proteins, cytoplasmic proteins of *Halobacterium* are highly acidic, but it is $K^+$, not $Na^+$, that is required for activity. This makes sense because K+ is the predominant cation in the cytoplasm of cells of *Halobacterium.* Besides having a high acidic amino acid composition, halobacterial cytoplasmic proteins typically contain lower levels of hydrophobic amino acids and lysine, a positively charged (basic) amino acid, than proteins of nonhalophiles. This is also to be expected because in a highly ionic cytoplasm, polar proteins would tend to remain in solution whereas nonpolar proteins would tend to cluster and perhaps lose activity. The ribosomes of *Halobacterium* also require high KCl levels for stability, whereas ribosomes of nonhalophiles have no KCl requirement.

Extremely halophilic Archaea are thus well adapted, both internally and externally, to life in a highly ionic environment. Cellular components exposed to the external environment require high $Na^+$ for stability, whereas internal components require high $K^+$. With the exception of a few extremely halophilic members of the Bacteria that also use KCl as a compatible solute, in no other group of prokaryotes do find this unique requirement for such high amounts of specific cations.

## 2. Methanogenic Archaea

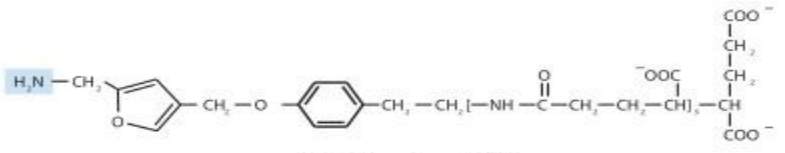
Many *Euryarchaeota* are methanogens, microorganisms that produce methane ($CH_4$) as an integral part of their energy metabolism (methane production is called methanogenesis). Methanogens are strict anaerobes that obtain energy by converting $CO_2$, $H_2$ ,formate, methanol, acetate, and other compounds to either, methane or methane and $CO_2$ .They are autotrophic when growing on $H_2$ and $CO_2$ . This is the largest group of archaea. There are five orders (*Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales, and Methanopyrales*) and 26 genera, which differ greatly in overall shape, 16SrRNA sequence,

cell wall chemistry and structure, membrane lipids, and other features. For example, methanogens construct three different types of cell walls. Several genera have walls with pseudomurein; other walls contain either proteins or heteropolysaccharides. It should be noted that although almost all archaea in these orders are methanogens, methanotrophs (i.e., organisms that use methane as a carbon and energy source) have recently been discovered in the *Methanosarcinales.*

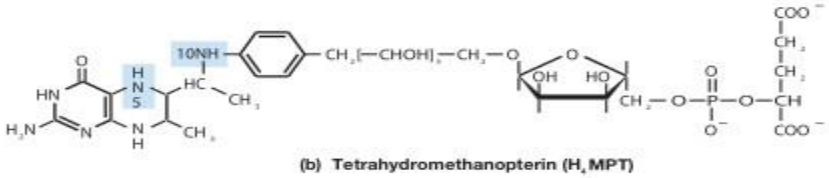
One of the most unusual methanogenic groups is the class *Methanopyri*. It has one order*, Methanopyrales*, one family and a single genus, *Methanopyrus*. This hyperthermophilic, rod-shaped methanogen has been isolated from a marine hydrothermal vent. *Methanopyrus kandleri* has a temperature minimum at 84°C and an optimum of 98°C; it will grow at temperatures up to 110°C. *Methanopyrus* occupies the deepest and most ancient branch of the euryarchaeotes. Perhaps methanogenic archaeal ancestors were among the earliest organisms. They certainly seem well adapted to living under conditions similar to those presumed to have existed on a young Earth.

As might be inferred from the methanogens' ability to produce methane anaerobically, their metabolism is unusual. These prokaryotes contain several unique cofactors: tetrahydromethanopterin($H_4$MPT),methanofuran(MFR),coenzymeM(2mercaptoethanesulfoni -c acid), coenzyme $F_{420}$, and coenzyme $F_{430}$.The first three cofactors bear the $C_1$ unit when $CO_2$ is reduced to $CH_4$ . $F_{420}$ carries electrons and protons, and $F_{430}$ is a nickel tetrapyrrole serving as a cofactor for the enzyme methyl-CoM methylreductase. It appears that ATP synthesis is linked with methanogenesis by electron transport, proton pumping, and a chemiosmotic mechanism. Some methanogens can live autotrophically by forming acetyl-CoA from two molecules of $CO_2$ and then converting the acetyl-CoA to pyruvate and other products.

Methanogens thrive in anoxic environments rich in organic matter: the rumen and intestinal system of animals, freshwater and marine sediments, swamps and marshes, hot springs, anoxic sludge digesters, and even within anaerobic protozoa. Methanogens often are of ecological significance. The rate of methane production can be so great that bubbles of methane sometimes rise to the surface of a lake or pond. Rumen methanogens are so active that a cow can belch 200 to 400 liters of methane a day.



(a) Methanofuran (MFR)



(b) Tetrahydromethanopterin ($H_4$MPT)

**Table 20.2 Selected Characteristics of Representative Genera of Methanogens**

| Genus | Morphology | % G + C | Wall Composition | Gram Reaction | Motility | Methanogenic Substrates Used |
|---|---|---|---|---|---|---|
| Order *Methanobacteriales* | | | | | | |
| *Methanobacterium* | Long rods or filaments | 32–61 | Pseudomurein | + to variable | − | $H_2 + CO_2$, formate |
| *Methanothermus* | Straight to slightly curved rods | 33 | Pseudomurein with an outer protein S-layer | + | + | $H_2 + CO_2$ |
| Order *Methanococcales* | | | | | | |
| *Methanococcus* | Irregular cocci | 29–34 | Protein | − | + | $H_2 + CO_2$, formate |
| Order *Methanomicrobiales* | | | | | | |
| *Methanomicrobium* | Short curved rods | 45–49 | Protein | − | + | $H_2 + CO_2$, formate |
| *Methanogenium* | Irregular cocci | 52–61 | Protein or glycoprotein | − | − | $H_2 + CO_2$, formate |
| *Methanospirillum* | Curved rods or spirilla | 47–52 | Protein | − | + | $H_2 + CO_2$, formate |
| Order *Methanosarcinales* | | | | | | |
| *Methanosarcina* | Irregular cocci, packets | 36–43 | Heteropolysaccharide or protein | + to variable | − | $H_2 + CO_2$, methanol, methylamines, acetate |

## 3. Thermoplasmatales

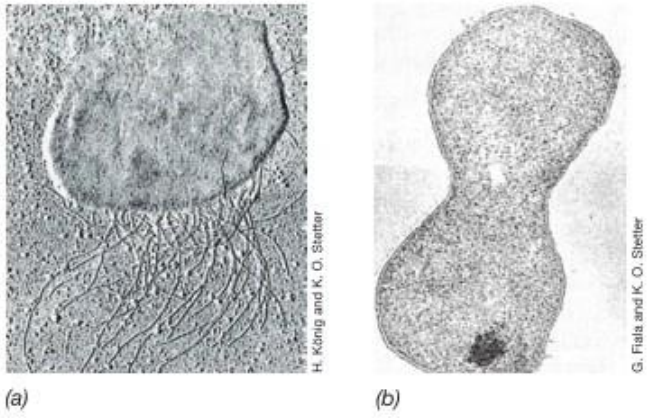
A phylogenetically distinct line of Archaea contains Thermophilic and extremely acidophilic genera*: Thermoplasma, Ferroplasma, and Picrophilus*. These prokaryotes are among the most acidophilic of all known microorganisms, with *Picrophilus* being capable of growth even below pH 0. Most are thermophilic as well. These genera also form their own taxonomic order within the *Euryarchaeota*, the Thermoplasmatales. They resemble the mycoplasmas.

## 4. *Thermococcus* and *Pyrococcus*

*Thermococcus* is a spherical hyperthermophilic euryarchaeote indigenous to anoxic thermal waters in various locations through- out the world. The spherical cells contain a tuft of polar flagella and are thus highly motile. *Thermococcus* is an obligately anaerobic chemoorganotroph that metabolizes proteins and other complex organic mixtures (including some sugars) with elemental sulfur ($S^{0}$) as electron acceptor at temperatures from 55 to 95°C. *Pyrococcus* is morphologically similar to *Thermococcus.*

Pyrococcus differs from *Thermococcus* primarily by its higher temperature requirements; *Pyrococcus* grows between 70 and 106°C with an optimum of 100°C. *Thermococcus* and *Pyrococcus* are also metabolically quite similar. Proteins, starch, or maltose are oxidized as electron donors, and

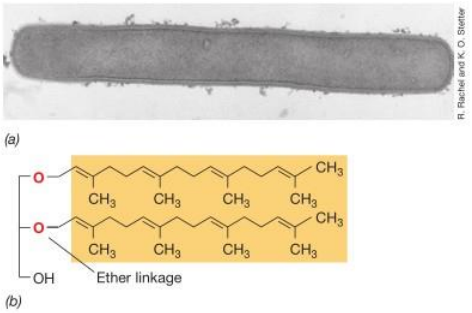
($S^{0)}$ is the terminal electron acceptor and is reduced to hydrogen sulfide ($H_2S$). Both *Thermococcus* and *Pyrococcus* form $H_2S$ when ($S^0$) is present, but form $H_2$ when $S^0$ is absent.




**Spherical hyperthermophilic Euryarchaeota from submarine volcanic areas.** (a) *Thermococcus celer*. Electron micrograph of shadowed cells (note tuft of flagella). (b) Dividing cell of *Pyrococcus furiosus*. Electron micrograph of thin section. Cells of both organisms are about 0.8 μm in diameter.

## 5. *Methanopyrus*

*Methanopyrus* is a rod-shaped hyperthermophilic methanogen. *Methanopyrus* was isolated from hot sediments near submarine hydrothermal vents and from the walls of "black smoker" hydrothermal vent chimneys. *Methanopyrus* shares phenotypic properties with both the hyperthermophiles and the methanogens. *Methanopyrus* produces $CH_4$ only from $H_2 + CO_2$ and grows rapidly for an autotrophic organism (generation time <1 h at 100°C). In special pressurized vessels, growth of one strain of *Methanopyrus* has been recorded at 122°C, the highest temperature yet shown to support microbial growth. *Methanopyrus* is also unusual because it contains membrane lipids found in no other known organism. Recall that in the lipids of Archaea, the glycerol side chains contain phytanyl rather than fatty acids bonded in ether linkage to the glycerol. In *Methanopyrus*, this ether-linked lipid is an unsaturated form of the otherwise saturated dibiphytanyl tetraethers found in all other hyperthermophilic Archaea. These unusual lipids may help to stabilize the cytoplasmic membrane of *Methanopyrus* at its unusually high growth temperatures.




***Methanopyrus.*** *Methanopyrus* grows optimally at 100°C and can make $CH_4$ only from $CO_2 + H_2$. (a) Electron micrograph of a cell of *Methanopyrus kandleri*, the most thermophilic of all known organisms (upper temperature limit, 122°C). This cell measures 0.5 × 8 μm. (b) Structure of the novel lipid of *M. kandleri*. This is the normal ether-linked lipid of the Archaea except that the side chains are an unsaturated form of phytanyl (geranylgeraniol).

## 6. *Archaeoglobales*

Hyperthermophilic Crenarchaeota catalyze anaerobic respirations in which elemental sulfurb(S0) is used as an electron acceptor, being reduced to H 2 S. One hyperthermophilic euryarchaeote, *Archaeoglobus,* can reduce sulfate ($SO_4^{2-}$) and form phylogenetically distinct lineage within the *Euryarchaeota* . Eg: *Archaeoglobus, Ferroglobus*

- ***Archaeoglobus***

*Archaeoglobus* was isolated from hot marine sediments near hydrothermal vents. In its metabolism, *Archaeoglobus* couples the oxidation of $H_2$, lactate, pyruvate, glucose, or complex organic compounds to the reduction of $SO_4^{2-}$ to $H_2S$. Cells of *Archaeoglobus* are irregular cocci and grow optimally at 83°C. *Archaeoglobus* and methanogens share some characteristics. Briefly, this process requires a series of novel coenzymes, and with rare exceptions, these coenzymes have only been found in methanogens. Surprisingly, however, *Archaeoglobus* also contains many of these coenzymes and cultures of this organism actually produce small amounts of $CH_4$. Thus, *Archaeoglobus*, which also shows a rather close phylogenetic relationship to methanogens, may be a metabolically intermediate type of organism, bridging the energy-conserving processes of methanogenesis and other forms of respiration among Archaea. Not surprisingly then, the genome of *Archaeoglobus*, which contains about 2400 genes, shares a number of genes in common with methanogens.

- ***Ferroglobus***

*Ferroglobus* is related to *Archaeoglobus* but is not a sulfate reducer. Instead, *Ferroglobus* is an iron-oxidizing chemolithotroph, conserving energy from the oxidation of $Fe^{2+}$ to $Fe^{3+}$ coupled to the reduction of nitrate ($NO^{3-}$) to nitrite ($NO^{2-}$). *Ferroglobus* grows autotrophically and can also use $H_2$ or $H_2S$ as electron donors in its energy metabolism. *Ferroglobus* was isolated from a shallow marine hydrothermal vent and grows optimally at 85°C.

*Ferroglobus* is interesting for several reasons, but especially for its ability to oxidize $Fe^{2+}$ to $Fe^{3+}$ under anoxic conditions. This process might help explain the origin of the abundant $Fe^{3+}$ in ancient rocks such as the banded iron formations, rocks dated to before the predicted appearance of cyanobacteria on Earth. With organisms like *Ferroglobus*, it would have been possible for $Fe^{2+}$ oxidation to proceed without the need for molecular oxygen ($O_2$) as an electron acceptor. The metabolism of *Ferroglobu*s thus has implications for dating the origin of cyanobacteria and the subsequent oxygenation of Earth. Certain anoxygenic phototrophic bacteria can also oxidize $Fe^{2+}$ under anoxic conditions, and so several anaerobic routes to ancient $F^{3+}$ are possible. This makes it difficult to estimate when cyanobacteria first appeared on Earth and to what degree non phototrophic organisms helped trigger the Great Oxidation Event.

# *Thaumarchaeota*

Early surveys of 16S ribosomal RNA genes from open ocean microbial communities resulted in the shocking conclusion that Archaea were abundant and widespread in the oceans. Ex. *Nitrosopumilus, Nitrososphaera* . At the time, the archaeal domain was considered to contain only extremophiles and obligate anaerobes, and their presence in oxygen-rich temperate and even polar oceanic environments was something of a mystery. Even more remarkable, these novel Archaea were widespread and common in soils all over the world. Phylogenetic analysis of their 16S ribosomal RNA gene sequences initially suggested that this novel group of Archaea was a deeply divergent lineage of the *Crenarchaeot*a, a group of hyperthermophilic Archaea. It was only after genome sequence analysis of the marine nitrifier *Nitrosopumilus maritimus* that it became clear that the *Thaumarchaeota* are a distinct phylum of Archaea. Analyses of genome sequences confirm that *Thaumarchaeota* constitute a unique phylum of Archaea and that they diverged from the primary line of archaeal descent prior to the divergence of *Crenarchaeota* and *Euryarchaeota.*

## ➢ Physiological Characteristics of *Thaumarchaeota*

The physiology of *Thaumarchaeota* remained a mystery until the isolation of *Nitrosopumilus maritimus*. *N. maritimus* grows chemolithotrophically by aerobically oxidizing ammonia ($NH_3$) to nitrite ($NO^{2-}$), the first step in nitrification. This organism uses $CO_2$ as its sole carbon source (autotrophy), as do nitrifying Bacteria. However, unlike ammonia-oxidizing Bacteria such as *Nitrosomonas, N. maritimus* is adapted to life under extreme nutrientlimitation, as would befit an organism indigenous to open ocean waters. *N. maritimus* can grow at NH 3 concentrations that are a hundred times lower than those required by bacterial nitrifiers,and are actually growth inhibited at the higher $NH_3$ concentrations required to support growth of nitrifying species of Bacteria.

Several species of Thaumarchaeota have been isolated and characterized, revealing a number of properties common to this group. Species have been isolated from habitats including theoceans, marine sediment, an estuary, soil, and hot springs. All existing isolates are chemolithotrophic ammonia-oxidizers, and most species, like *N. maritimus*, are able to grow at very low concentrations of $NH_3$. The membranes of all Thaumarchaeota also have a unique lipid called crenarchaeol, a compound limited to species of this phylum. In addition, autotrophy in *Thaumarchaeota* is supported by the 3-hydroxypropionate/ 4-hydroxybutyrate cycle, which further distinguishes archaealnitrifiers from nitrifying Bacteria that employ the Calvin cycle for $CO_2$ fixation. The 3-hydroxypropionate/ 4-hydroxybutyrate cycle also allows for the assimilation of organic carbon, and some archaeal nitrifiers have been shown to assimilate pyruvate during mixotrophic growth. Growth temperatures of *Thaumarchaeota* vary widely, as some species thrive in polar seas while others inhabit hot spring environments up to about 75°C. *Nitrososphaera viennensis,* which represents a lineage of Thaumarchaeota found widely in soils, can grow at a wide range of $NH_3$ concentrations. Like marine species of *Thaumarchaeota, N. viennensis* can grow at low concentrations of $NH_3$, but *N. viennensis* can

also tolerate high levels (up to 10 mM) of ammonium at neutral pH. Hence, *N. viennensis,* and other archaeal nitrifiers, may be active in soils that have fairly high levels of ammonia and in these environments they may compete directly with bacterial nitrifiers. In addition, several species of *Thaumarchaeota*, including *N. viennensis*, possess urease activity. *N. viennensis* can grow with urea as the sole source of energy, hydrolyzing it to ammonia, which is subsequently used as an electron donor.

## *Nanoarchaeota*

The *Nanoarchaeota* are represented by a single species, the highly unusual *Nanoarchaeum equitans*. *N. equitans* is one of the smallest cellular organisms known and has the smallest genome among species of Archaea (0.49 Mb). The coccoid cells of *N. equitans* are very small, about 0.4 μm in diameter, and have only about 1% of the volume of *an Escherichia coli* cell. They cannot grow in pure culture and replicate only when attached to the surface of their host organism, Ignicoccus hospitalis, a hyperthermophilic species of *Crenarchaeota* whose name means "the hospitable fireball." *N. equitans* grows to 10 or more cells per *Ignicoccus* cell and lives an apparently parasitic lifestyle, making it the only known archaeal symbiont.

### ➢ *Nanoarchaeum* and Its host

*N. equitans* and its host *Ignicoccus* were first isolated from a submarine hydrothermal vent off the coast of Iceland. However, environmental sampling of 16S ribosomal RNA genes indicates that organisms phylogenetically similar to *N. equitans* exist in other submarine hydrothermal vents and in terrestrial hot springs, so Archaea of this kind are probably distributed worldwide in suitable hot habitats. Like its host *Ignicoccus, N. equitans* grows at temperatures from 70 to 98°C and optimally at 90°C.

The metabolism of *Nanoarchaeum* is not fully understood, but it likely depends on its host for many metabolic functions. *Ignicocus* is an autotroph that uses $H_2$ as an electron donor and $S^0$ as an electron acceptor and so probably supplies *N. equitans* with organic carbon. N. equitans is incapable of metabolizing $H_2$ and $S^0$ for energy, and whether it generates ATP from substances obtained from Ignicoccus or obtains its ATP directly from its host is unknown. The appearance of *N. equitans* cells is typical of Archaea, with a cell wall consisting of an S-layer that overlays what appears to be a periplasmic space.

## *Korarchaeota*

Ribosomal RNA sequences of *Korarchaeota* have been observed in a range of geothermal habitats, both submarine and terrestrial. However, *Korarchaeum cryptofilum*, whose name means "the cryptic filament of youth," is the only characterized species in the phylum *Korarchaeota*.

First observed as a 16S ribosomal RNA gene phylotype recovered from the hot spring named Obsidian Pool in Yellowstone National Park, USA, *K. cryptofilum* has yet to be grown in pure culture, as for *N. equitans*. However, its genome sequence has been determined from metagenomic analyses of an enrichment culture. *K. cryptofilum* is an obligately anaerobic chemoorganotroph and a hyperthermophile, growing at 85°C. Cells are long, thin (<0.2-μm diameter) filaments of variable length, with most filaments being around 15 μm long but some reaching as much as 100 μm. Filaments of *K. cryptofilum* have a tough paracrystalline S-layer, which maintains cell integrity in its extremely hot habitat.

Though *K. cryptofilum* cannot be grown in isolation, its genome sequence provides clues about its lifestyle. *K. cryptofilum* lacks the ability to perform anaerobic respiration (with the possible exception of proton reduction, and lives a fermentative lifestyle. Similar to other archaeal hyperthermophiles, *K. cryptofilum* grows by fermentation of peptides or amino acids. *K. cryptofilum* lacks many core genes in biosynthesis including the ability to synthesize purines, coenzyme A, and several essential cofactors. Presumably *K. cryptofilum* obtains these essential components from its environment. The inability of *K. cryptofilum* to synthesize molecules essential for its own growth may be explained by the evolution of mutual dependence as described by the Black Queen hypothesis. This dependence on other members of the hot spring microbial community may explain why *K. cryptofilum* has proven difficult to obtain in pure culture.

As with the *Nanoarchaeota*, there is some uncertainty about the phylogenetic position of the *Korarchaeota.* The genome of *K. cryptofilum* includes some gene families that share affinity with *Euryarchaeota* and others that share affinity with *Crenarchaeota.* For example, phylogenetic analysis of ribosomal proteins, RNA polymerase subunits, and ribosomal RNA genes indicate affinity between *Crenarchaeota* and *Korarchaeota*. In contrast, genes for cell division, tRNA maturation, and DNA replication and repair indicate affinity between *Euryarchaeota* and *Korarchaeota.* The unique genetic composition of *K. cryptofilum* supports its placement near the base of the archaeal radiation, and future work on this interesting archaeon should clarify its actual phylogenetic position.

# *Crenarchaeota*

As mentioned previously, most of the crenarchaeotes that have been cultured are extremely thermophilic, and many are acidophiles and sulfur dependent. The sulfur may be used either as an electron acceptor in anaerobic respiration or as an electron donor by lithotrophs. Many are strict anaerobes. They grow in geothermally heated water or soils that contain elemental sulfur. These environments are scattered all over the world. Examples are the sulfur-rich hot springs in Yellowstone National Park and the waters surrounding areas of submarine volcanic activity. Such habitats are sometimes called solfatara. These archaea can be very thermophilic and often are classified as hyperthermophiles. The most extreme example was isolated from an active hydrothermal vent in the northeast Pacific Ocean. This is one of three novel isolates that constitute a new genus in the *Pyrodictiaceae* family. Its optimum growth rate is about 105°C, but even autoclaving this microbe at 121°C for one hour fails to kill it! It is strictly anaerobic, using Fe(III) as a terminal electron acceptor and $H_2$ or formate as electron donors.

At present, the *Crenarchaeota* contains 25 genera; two of the better-studied genera are *Thermoproteus* and *Sulfolobus*. Members of the genus Sulfolobus stain gram negative, and are aerobic, irregularly lobed spherical archaea with a temperature optimum around 70 to 80°C and a pH optimum of 2 to 3. For this reason, they are thermoacidophiles, so called because they grow best at acid pH values and high temperatures. Their cell wall contains lipoprotein and carbohydrate. They grow lithotrophically on sulfur granules in hot acid springs and soils while oxidizing the sulfur to sulfuric acid. Oxygen is the normal electron acceptor, but ferric iron may be used. Sugars and amino acids such as glutamate also serve as carbon and energy sources.

Thermoproteusis a long, thin rod that can be bent or branched. Its cell wall is composed of glycoprotein. *Thermoproteus* is a strict anaerobe and grows at temperatures from 70 to 97°C and pH values between 2.5 and 6.5. It is found in hot springs and other hot aquatic habitats rich in sulfur. It can grow organotrophically and oxidize glucose, amino acids, alcohols, and organic acids with elemental sulfur as the electron acceptor. That is, *Thermoproteus* can carry out anaerobic respiration. It will also grow chemolithotrophically using $H_2$ and $S^0$. Carbon monoxide or $CO_2$ can serve as the sole carbon source.

Although the *Crenarchaeota* are notorious for their life at high temperatures and acidic pH, sequence analysis of DNA fragments derived directly from environmental samples reveals that this phylum is more widespread in nature. Recall that only a small fraction of microbes have been grown in culture, so the ability to analyze microbial communities using molecular techniques is an important way to truly understand microbial diversity. Such studies have revealed that the *Crenarchaeota* have significant populations in marine plankton from polar, temperate, and tropical waters. Crenarchaeotes also appear to inhabit rice paddies, soils, freshwater lake sediments, and at least two symbiotic species have been isolated, one from a cold water sea cucumber and another from a marine sponge. As more is learned about these

microbes, our understanding of archaeal phylogeny will no doubt be enhanced and most likely modified.

**Energy-yielding reactions of hyperthermophilic *Archaea***

| *Nutritional class* | *Energy-yielding reaction* | *Metabolic type*[a] | *Example genera*[b] |
|---|---|---|---|
| *Chemoorganotrophic* | Organic compound + $S^0 \rightarrow H_2S + CO_2$ | AnR | *Thermoproteus, Thermococcus, Desulfurococcus, Thermofilum, Pyrococcus* |
| | Organic compound + $SO_4^{2-} \rightarrow H_2S + CO_2$ | AnR | *Archaeoglobus* |
| | Organic compound + $O_2 \rightarrow H_2O + CO_2$ | AeR | *Sulfolobus* |
| | Organic compound $\rightarrow CO_2 + H_2$ + fatty acids | AnR | *Staphylothermus, Pyrodictium* |
| | Organic compound + $Fe^{3+} \rightarrow CO_2 + Fe^{2+}$ | AnR | *Pyrodictium* |
| | Organic compound + $NO_3^- \rightarrow CO_2 + N_2$ | AnR | *Pyrobaculum* |
| | Pyruvate $\rightarrow CO_2 + H_2$ + acetate | AnR | *Pyrococcus* |
| | Peptides | F | *Hyperthermus, Korarchaeum* |
| *Chemolithotrophic* | $H_2 + S^0 \rightarrow H_2S$ | AnR | *Acidianus, Pyrodictium, Thermoproteus, Stygiolobus, Ignicoccus* |
| | $H_2 + NO_3^- \rightarrow NO_2^- + H_2O$ ($NO_2^-$ is reduced to $N_2$ by some species) | AnR | *Pyrobaculum* |
| | $4\,H_2 + NO_3^- + H^+ \rightarrow NH_4^+ + 2\,H_2O + OH^-$ | AnR | *Pyrolobus* |
| | $H_2 + 2\,Fe^{3+} \rightarrow 2\,Fe^{2+} + 2\,H^+$ | AnR | *Pyrobaculum, Pyrodictium, Archaeoglobus* |
| | $2\,H_2 + O_2 \rightarrow 2\,H_2O$ | AeR | *Acidianus, Sulfolobus, Pyrobaculum* |
| | $2\,S^0 + 3\,O_2 + 2\,H_2O \rightarrow 2\,H_2SO_4$ | AeR | *Sulfolobus, Acidianus* |
| | $2\,FeS_2 + 7\,O_2 + 2\,H_2O \rightarrow 2\,FeSO_4 + 2\,H_2SO_4$ | AeR | *Sulfolobus, Acidianus, Metallosphaera* |
| | $2\,FeCO_3 + NO_3^- + 6\,H_2O \rightarrow 2\,Fe(OH)_3 + NO_2^- + 2\,HCO_3^- + 2\,H^+ + H_2O$ | AnR | *Ferroglobus* |
| | $4\,H_2 + SO_4^{2-} + 2\,H^+ \rightarrow 4\,H_2O + H_2S$ | AnR | *Archaeoglobus* |
| | $4\,H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O$ | AnR | *Methanopyrus, Methanocaldococcus Methanothermus* |

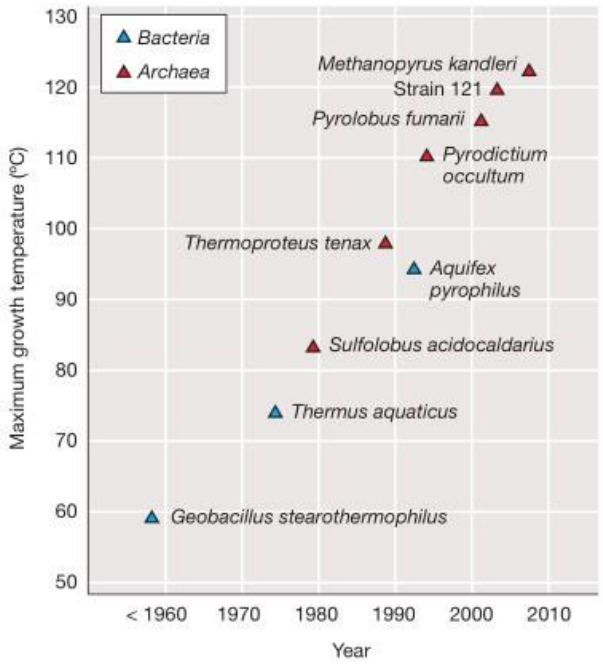
[a]AnR, anaerobic respiration; AeR, aerobic respiration; F, fermentation.

## Evolution and Life at high temperatures of Archaea

Most of the hyperthermophiles discovered so far are species of Archaea and some grow near to what may be the upper temperature limit for life. Here we consider the major factors that likely define the upper temperature limit for life and the biological adaptations of hyperthermophiles that permit them to exist at the exceptionally high temperatures of 100°C and higher. We end with a discussion of the importance of hydrogen ($H_2$ ) metabolism to the biology of hyperthermophiles.

## ➢ What Is the Upper Temperature Limit for Life?

How high a temperature can hyperthermophiles withstand? Over the past several decades, the known upper temperature limit for life has been pushed higher and higher with the isolation and characterization of new species of thermophiles and hyperthermophiles. For some time, the record holder was *Pyrolobus fumarii*, with its upper temperature limit for growth of 113°C. The current record holder, Methanopyrus, however, has pushed the limit somewhat higher, with the ability to grow at 122°C and to survive substantial periods at even higher temperatures. Given the trend over the past several years, one can predict that Archaea even more hyperthermophilic than Methanopyrus may inhabit hydrothermal environments but have yet to be isolated. Indeed, many experts predict that the upper temperature limit for prokaryotic life is likely to exceed 140°C, perhaps even 150°C, and that the maximum temperature allowing survival but not growth is even hotter yet.




Thermophilic and hyperthermophilic prokaryotes. The graph gives the species that were, in turn, the record holders for growing at the highest temperature, from before 1960 to the present.

## ➢ Archaeal adaptations to Life at high temperature

Because all cellular structures and activities are affected by heat, hyperthermophiles are likely to exhibit multiple adaptations to the exceptionally high temperatures of their habitats. Here we briefly examine some adaptations employed by hyperthermophiles to protect their proteins and nucleic acids at high temperatures.

## 1. protein Folding and thermostability

Because most proteins denature at high temperatures, much research has been done to identify the properties of thermostable proteins. Protein thermostability derives from the folding of the molecule itself, not because of the presence of any special amino acids. Perhaps surprisingly, however, the amino acid composition of thermostable proteins is not particularly unusual except perhaps in their slight bias for increased levels of amino acids that promote alpha-helical secondary structures. In fact, many enzymes from hyperthermophiles contain the same major structural features in both primary and higher-order structure as their heat-labile counterparts from organisms that grow best at much lower temperatures.

Thermostable proteins typically do display some structural features that likely improve their thermosstability. These include having highly hydrophobic cores, which decrease the tendency of the protein to unfold in an ionic environment, and more ionic interactions on the protein surfaces, which also help hold the protein together and work against unfolding. Ultimately, it is the folding of the protein that most affects its heat stability, and noncovalent ionic bonds called salt bridges on a protein's surface likely play a major role in maintaining the biologically active structure. But, as previously stated, many of these changes are possible with only minimal changes in primary structure (amino acid sequence), as seen when thermostable and heat-labile forms of the same protein are compared.

## 2. Chaperonins: assisting proteins to remain in their Native State.

Earlier we discussed a class of proteins called chaperonins that function to refold partially denatured proteins. Hyperthermophilic Archaea have special classes of chaperonins that function only at the highest growth temperatures. In cells of *Pyrodictium abyssi* , for example, a major chaperonin is the protein complex called the thermosome. This complex keeps other proteins properly folded and functional at high temperature, helping cells survive even at temperatures above their maximal growth temperature. Cells of *P. abyssi* grown near its maximum temperature (110°C) contain high levels of the thermosome. Possibly because of this, the cells can remain viable following a heat shock, such as a 1-h treatment in an autoclave (121°C). In cells experiencing such a treatment and then returned to the optimum temperature, the thermosome, which is itself quite heat-resistant, is thought to refold sufficient copies of key denatured proteins that *P. abyssi* can once again begin to grow and divide. Thus, due to chaperonin activity, the upper temperature limit at which many hyperthermophiles can survive is higher than the upper temperature at which they can grow. The "safety net" of chaperonin activity probably ensures that cells in nature that briefly experience temperatures above their growth temperature maximum are not killed by the exposure.

## 3. DNA Stability: Solutes, reverse Gyrase, and DNa-Binding proteins.

What keeps DNA from melting at high temperatures? Various mechanisms are known to contribute. One such mechanism increases cellular solute levels, in particular potassium ($K^+$) or compatible organic compounds. For example, the cytoplasm of the hyperthermophilic methanogen *Methanopyrus* contains molar levels of potassium cyclic 2,3-diphosphoglycerate. This solute prevents chemical damage to DNA, such as depurination or depyrimidization (loss

of a nucleotide base through hydro- lysis of the glycosidic bond) from high temperatures, events that can lead to mutation. This compound and other compatible solutes, such as potassium di-myo-inositol phosphate, which protects against osmotic stress, and the polyamines putrescine and spermidine, which stabilize both ribosomes and nucleic acids at high temperature, help maintain key cellular macromolecules in hyperthermophiles in their active forms.

A unique protein found only in hyperthermophiles is responsible for DNA stability in these organisms. All hyperthermophiles produce a special DNA topoisomerase called reverse DNAgyrase. This enzyme introduces positive supercoils into the DNA of hyperthermophiles. Positive supercoiling stabilizes DNA to heat and thereby pre- vents the DNA helix from spontaneously unwinding. The notice- able absence of reverse DNA gyrase in prokaryotes whose growth temperature optima lie below 80°C strongly suggests a specific role for this enzyme in DNA stability at high temperatures.

Species of *Euryarchaeota* also contain highly basic (positively charged) DNA-binding proteins that are remarkably similar in amino acid sequence and folding properties to the core histones of the Eukarya. Archaeal histones from the hyperthermophilic methanogen *Methanothermus fervidus* have been particularly well studied. These proteins wind and compact DNA into nucleosome-like structures and maintain the DNA in a double-stranded form at very high temperatures. Archaeal histones are found in most *Euryarchaeota,* including extremely halophilic Archaea, such as Halo- bacterium. However, because the extreme halophiles are not thermophiles, archaeal histones may have other functions besides DNA stability, in particular in assisting in gene expression by opening the helix to allow for transcriptional proteins to bind.

**4. Lipid and ribosomal RNA Stability**

How have the lipids and the protein-synthesizing machinery of hyperthermophiles adjusted to high temperatures? Virtually all hyperthermophilic Archaea synthesize lipids of the dibiphytanyl tetraether type. These lipids are naturally heat-resistant because the phytanyl units forming each half of the membrane structure are covalently bonded to one another; this yields a lipid monolayer membrane instead of the normal lipid bilayer. This structure resists the tendency of heat to pull apart a lipid bilayer constructed of fatty acid or phytanyl side chains that are not covalently bonded.

A final point on molecular adaptations to life at high temperatures is that of the base composition of ribosomal RNAs. Ribosomal RNAs are key structural and functional components of the ribosome, the cell's protein-synthesizing apparatus. Hyperthermophilic species of both Bacteria and Archaea show as much as a 15% greater proportion of GC base pairs in their small ribosomal subunit RNAs compared with organisms that grow at lower temperatures. GC base pairs form three hydrogen bonds compared to the two of AU base pairs, and thus the higher GC content of the ribosomal RNAs should confer greater thermal stability on the ribosomes of these organisms and this should assist protein synthesis at high temperatures. By contrast to ribosomal RNAs, the GC content of genomic DNA of

hyperthermophiles is often rather low, which suggests that the thermal stability of ribosomal RNA might be an especially significant factor for life under hyperthermophilic conditions.

## Introdustrial application of Archaea

### ➢ Extremophiles as a source of novel enzymes for industrial application

- Extremophilic microorganisms are adapted to survive in ecological niches:
  - high temperatures,
  - extremes of pH,
  - high salt concentrations
  - high pressure.
- These microorganisms produce unique biocatalysts which operateunder extreme conditions
- Selected extracellular-polymer-degrading enzymes and other enzymes could be used in food, chemical and pharmaceutical industries and in environmental biotechnology.

(amylases, pullulanases, cyclodextrin glycosyltransferases, cellulases, xylanases, chitinases, proteinases, esterases, glucose isomerases, alcohol dehydrogenases and DNA-modifying enzymes )

### ➢ Biotechnological applications of archaeal biomasses

- Biological methanogenesis is applied to the anaerobic treatment of
  - sewage sludge,
  - agricultural, municipal and industrial wastes
- Methanogens are a group of microorganisms that obtain energy for growth from the reaction leading to methane production.
- Many bioreactor configurations have been exploited

  to increase the efficiency of anaerobic digestion,

  such as,
  - the rotating biological contactor,
  - the anaerobic baffle reactor
  - the upflow anaerobic sludge blanket reactor,
  - several large-scale plants are in operation

- **Archaeal enzymes of biotechnological interest**

- Natural and modified archaeal enzymes present huge possibilities for industrial applications
- Many archaeal enzymes involved in carbohydrate metabolism - special interest to the industrial biotechnology sector.
- The starch processing industry can profit from the exploitation of thermostable enzymes.
- Another promising application of hyperthermophilic archaeal enzymes is in trehalose production.
- Several other polymer-degrading enzymes isolated from archaea could play important roles in the chemical, pharmaceutical, paper, pulp or waste treatment industries. (xylanases and cellulases)
- Some archaeal metabolites have potential industrial applications.

  (proteins, osmotically active substances, exopolysaccharides and special lipids )
- Archaeal lipids have been proposed as monomers for bioelectronics

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