Thermophile

Thermophile

- "Thermophile" is derived from the Greek: *thermotita*, meaning heat, and Greek: *philia*, love

- A **thermophile** is an organism—a type of extremophile—that thrives at relatively high temperatures, between 41 and 122 °C (106 and 252 °F).

INTRODUCTION

- The activities of microorganisms including growth are greatly affected by the chemical and physical state of their environment.Many environmental factors can be considered.

- Temperature is probably the most important environmental factor affecting the growth and survival of microorganisms.

- The minimum and maximum temperatures for growth vary greatly among different microorganisms and usually reflect the temperature range and average temperature of their habitats.

Cardinal Temperatures

- For every microorganism there is a minimum temperature below which growth is not possible.

- An optimum temperature at which growth is most rapid.

- A maximum temperature above which growth is not possible.

- These three temperatures, called the **cardinal temperatures,** are characteristic for any given microorganism.

Cardinal Temperatures

- The maximum growth temperature of an organism reflects the temperature above which denaturation of one or more essential cell components, such as a key enzyme, occurs.

- An organism’s minimum temperature may well be governed by membrane functioning; that is, if an organism’s cytoplasmic membrane stiffens to the point that it no longer functions properly in transport or can no longer develop or consume a proton motive force, the organism cannot grow.

- The growth temperature optimum reflects a state in which all or most cellular components are functioning at their maximum rate and is typically closer to the maximum than to the minimum.

Cardinal Temperatures

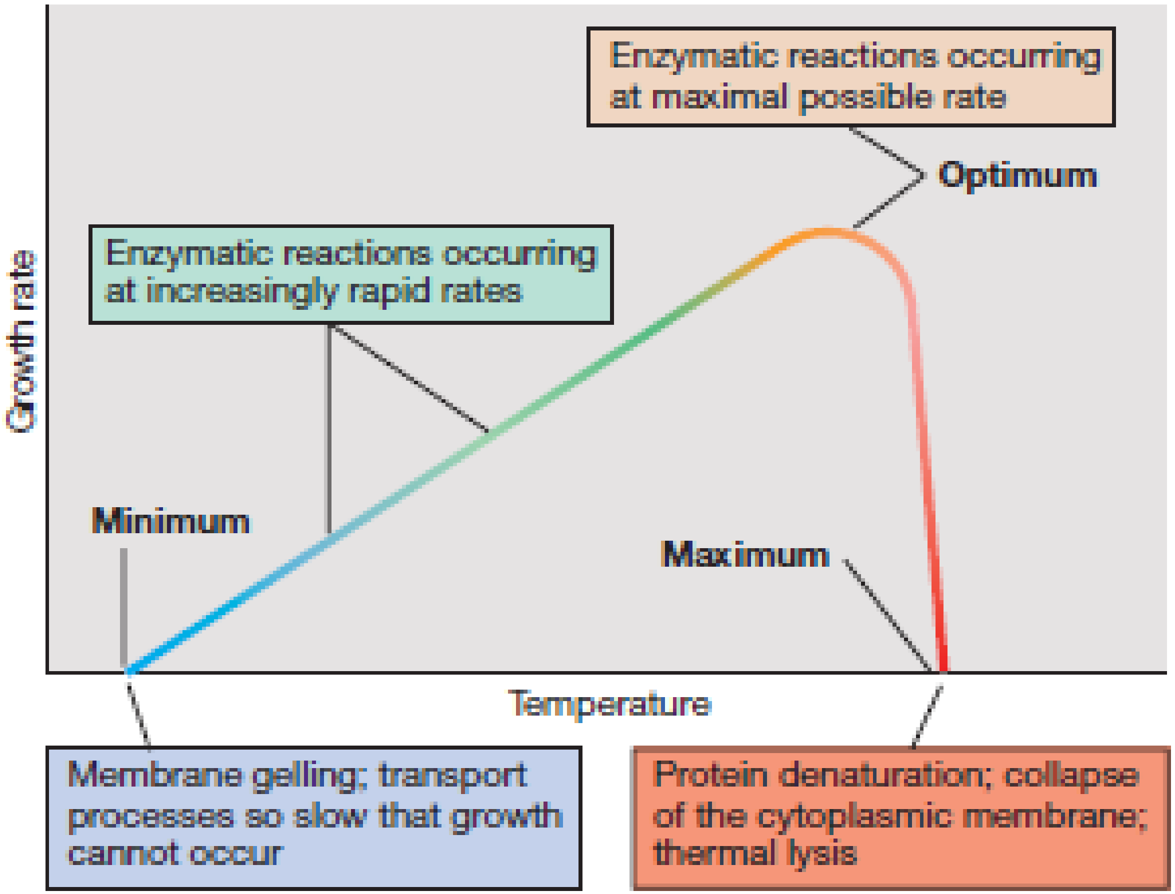

Figure 5.18 **The cardinal temperatures: minimum, optimum, and maximum.** The actual values may vary greatly for different organisms

Classification

- Thermophiles can be classified in various ways. One classification sorts these organisms according to their **optimal growth temperatures**:

i.Moderate thermophiles: 50–64 °C

ii.Extreme thermophiles 65–79 °C

iii.Hyperthermophiles 80°C &above

Classification of Microorganisms by Temperature Requirements

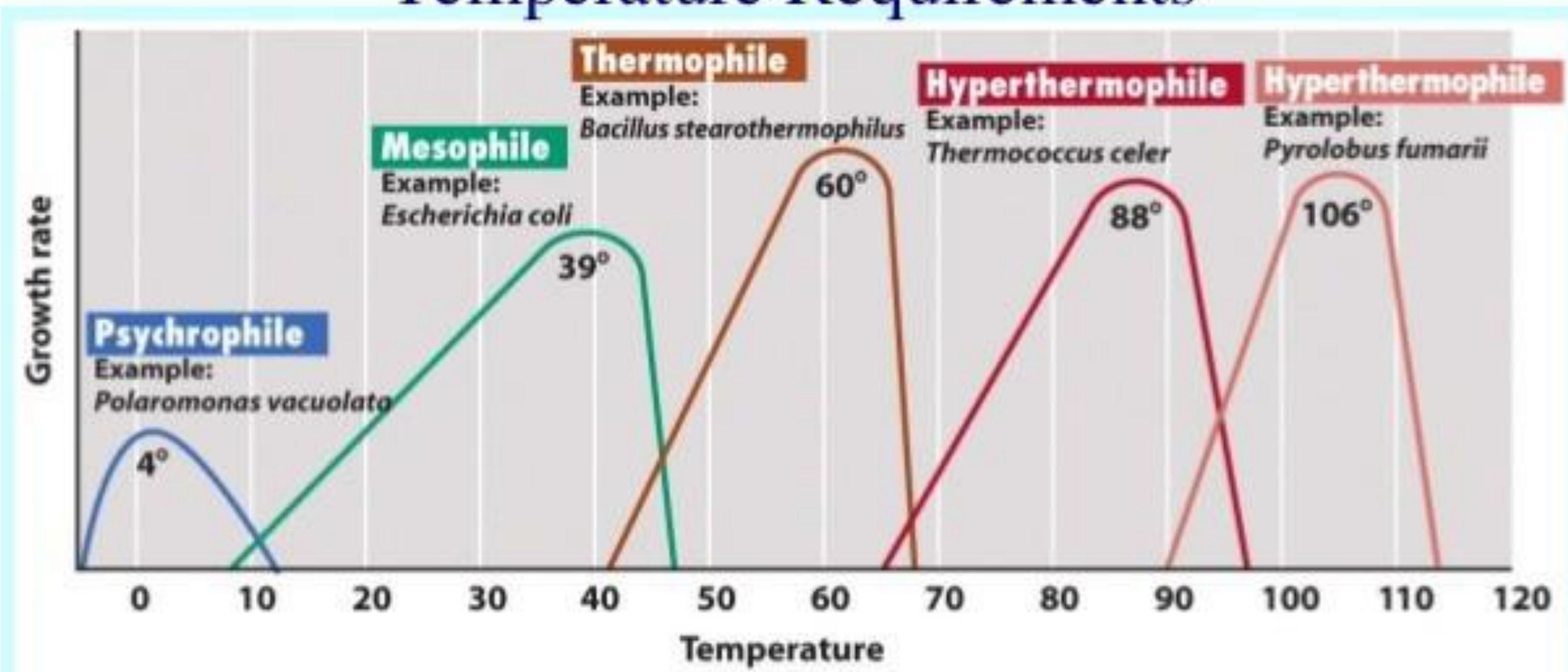

Figure 6-17 Brock Biology of Microorganisms 11/e

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ANCESTRAL ORGANISMS

- Several phyla branch off early in the phylogenetic tree of Bacteria. Although phylogenetically distinct, these groups are unified by their ability to grow at very high temperatures (hyperthermophily).

- Organisms such as ***Aquifex*** **and** ***Thermotoga*** grow in hot springs that are near the boiling point. The early branching of these phyla on the phylogenetic tree is consistent with the widely accepted hypothesis that the early Earth was much hotter than it is today.

- Assuming that early life form were hyperthermophiles, it is not surprising that their closest living relatives today would also be today world hyperthermophiles.

- Interestingly, the phylogenetic trees of both Bacteria and Archaeaare in agreement here; hyperthermophiles such as ***Aquifex, Methanopyrus, and Pyrolobus*** lie near the root of their respective phylogenetic trees.

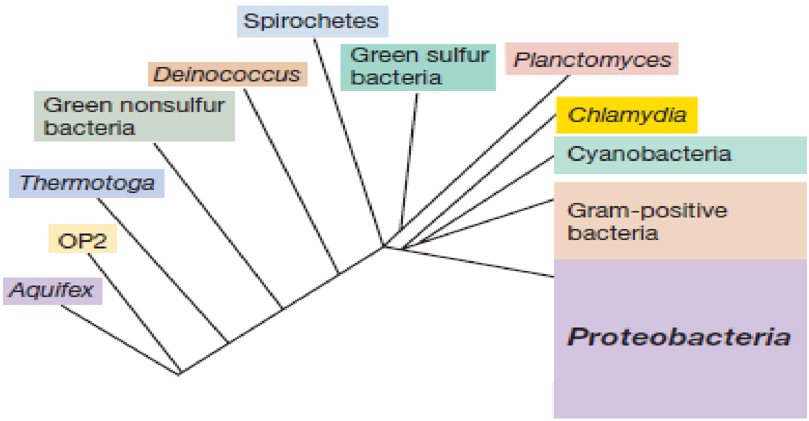


Figure 2.19 Phylogenetic tree of some representative *Bacteria*. The *Proteobacteria* are by far the largest phylum of *Bacteria* known. The lineage on the tree labeled OP2 does not represent a cultured organism but instead is an rRNA gene isolated from an organism in a natural sample. In this example, the closest known relative of OP2 would be *Aquifex*. Many thousands of other environmental sequences are known, and they branch all over the tree. Environmental sequences are also called *phylotypes*, and the technology for deriving them is considered in Section 22.4.

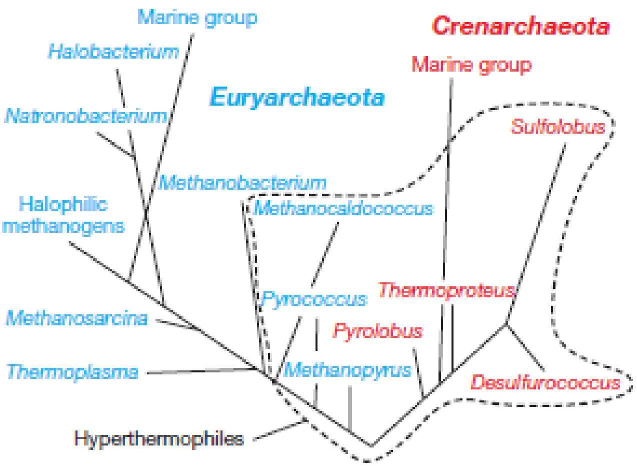


Figure 2.28 Phylogenetic tree of some representative *Archaea*. The organisms circled are hyperthermophiles, which grow at very high temperatures. The two major phyla are the *Crenarchaeota* and the *Euryarchaeota*. The "marine group" sequences are environmental rRNA sequences from marine *Archaea*, most of which have not been cultured.

Hyperthermophiles

- Hyperthermophiles were first discovered by Thomas D. Brock in 1965, in hot springs in Yellowstone National Park, Wyoming.

- At present- about 90 species of hyperthermophilic archaea and bacteria are known. **Most hyperthermophilic organism – Archea *Pyrolobus fumarii***- *can thrive upto 113 ℃*.Archea *Methanopyrus kandleri- can thrive upto 122 ℃*.

- Other- *Geogemma barosii (Strain 121)*

- *Nanoarchaeum equitans, Thermus aquaticus, Pyrococcus furiosus.*

Hyperthermophiles

- A **hyperthermophile** is an organism that thrives in extremely hot environments—from 60 °C upwards. An optimal temperature for the existence of hyperthermophiles is above 80 °C.
- Hyperthermophiles are often within the domain Archaea, although some bacteria are able to tolerate temperatures of around 100 °C, as well.
- Some bacteria can live at temperatures higher than 100 °C at large depths in sea where water does not boil because of high pressure.
- Many hyperthermophiles are also able to withstand other environmental extremes such as high acidity or high radiation levels. Hyperthermophiles are a subset of extremophiles.

Molecular 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.

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.

- Thermostable proteins typically do display some structural features that likely improve their thermostability. 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 bolsters the protein together and work against unfolding.

Protein Folding and Thermostability

- Finally, solutes such as **di-inositol phosphate, diglycerol phosphate, and mannosylglycerate** are produced at high levels in certain hyperthermophiles, and these may also help stabilize their proteins against thermal degradation

- 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

SALT BRIDGE

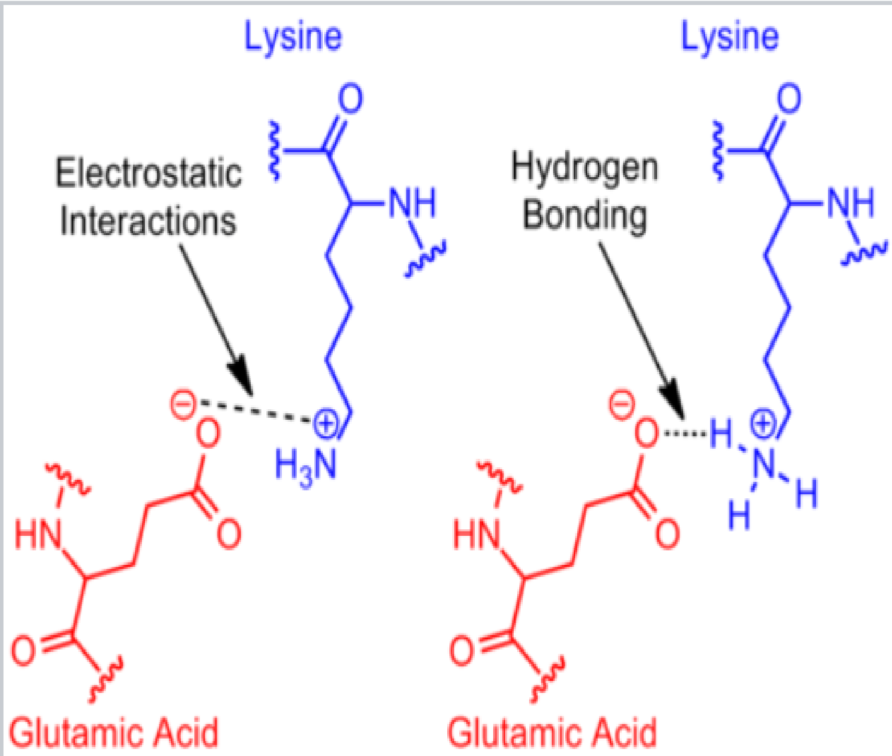


Figure 1. Example of salt bridge between amino acids glutamic acid and lysine demonstrating electrostatic interaction and hydrogen bonding

- A salt bridge is a non-covalent interaction between two ionized sites.

- It has two components: a hydrogen bond and an electrostatic interaction. In a salt bridge, a proton migrates from a carboxylic acid group to a primary amine or to the guanidine group in Arg.

- Typical salt bridges involve Lys or Arg as the bases and Asp or Glu as the acids.

- **Of all the non-covalent interactions, salt bridges are among the strongest**.

Chaperonins

- Hyperthermophilic Archaea have special classes of chaperonins that function only at the highest growth temperatures.

- for example, In cells of *Pyrodictium abyssi* , 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).

Chaperonins

- 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.

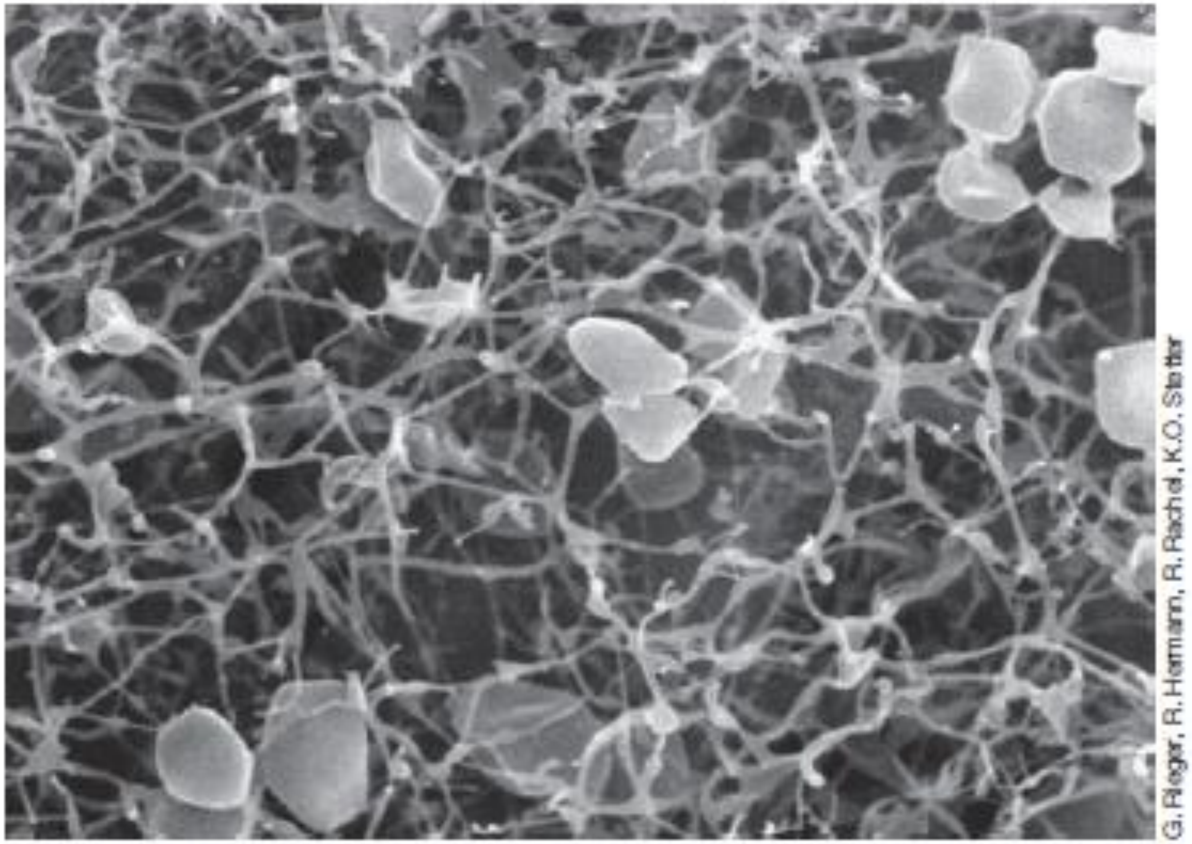


Figure 19.26 ***Pyrodictium abyssi*, scanning electron micrograph.** *Pyrodictium* has been studied as a model of macromolecular stability at high temperatures. Cells are enmeshed in a sticky glycoprotein matrix that binds them together.

DNA Stability at High Temperatures

- Various mechanisms keeps DNA from melting at high temperatures.
- One such mechanism is increased 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; prevents chemical damage to DNA, such as depurination or depyrimidization 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.

DNA Stability at High Temperatures

- A unique protein found only in hyperthermophiles is responsible for DNA stability in these organisms. All hyperthermophiles produce a special DNA topoisomerase called **reverse DNA gyrase**.

- This enzyme introduces positive supercoils; in contrast to the negative supercoils introduced by DNA gyrase present in all other prokaryotes.

- Positive supercoiling stabilizes DNA to heat and thereby prevents the DNA helix from spontaneously unwinding.

- The noticeable 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.

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 *Halobacterium*. 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.

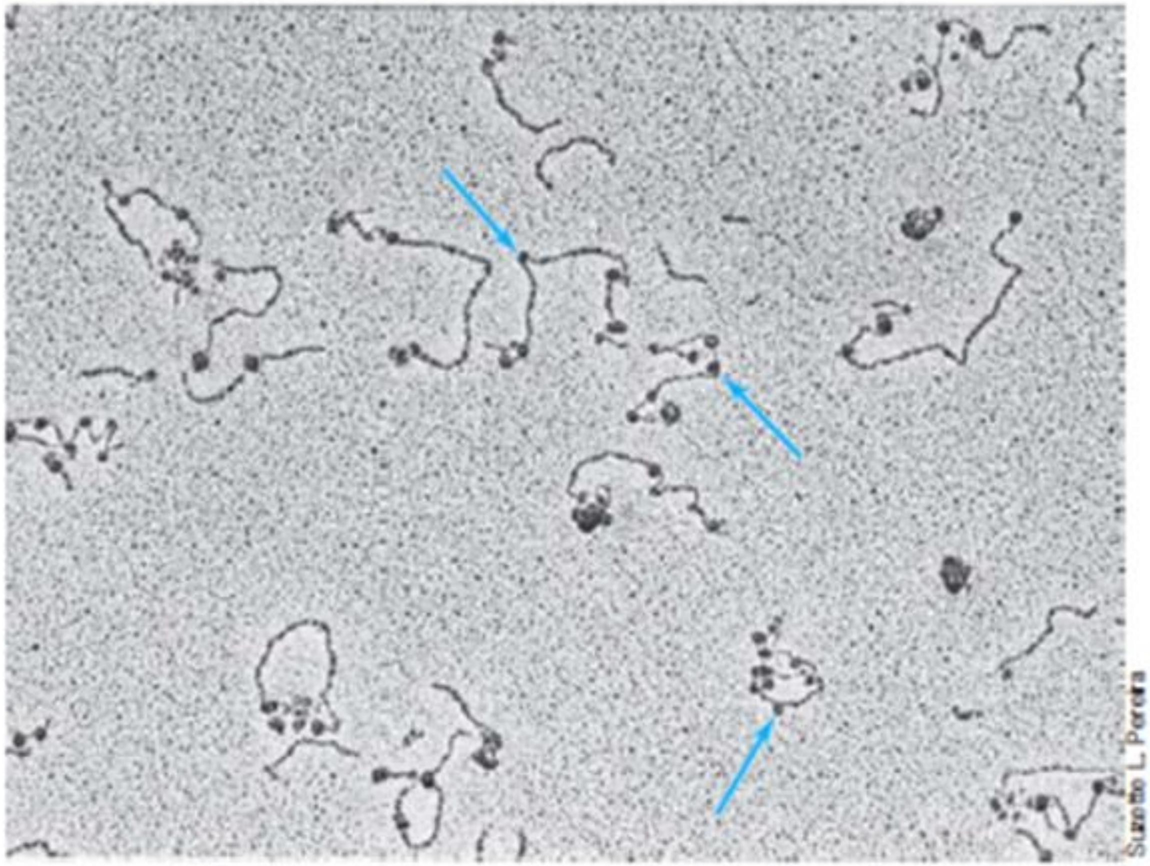


Figure 19.27 Archaeal histones and nucleosomes. Electron micrograph of linearized plasmid DNA wrapped around copies of archaeal histone Hmf (from the hyperthermophilic methanogen *Methanothermus fervidus*) to form the roughly spherical, darkly stained nucleosome struc-

Membrane Stability at High Temperatures

- Thermophiles typically have lipids rich in saturated fatty acids. This feature allows the membranes to remain stable and functional at high temperatures.

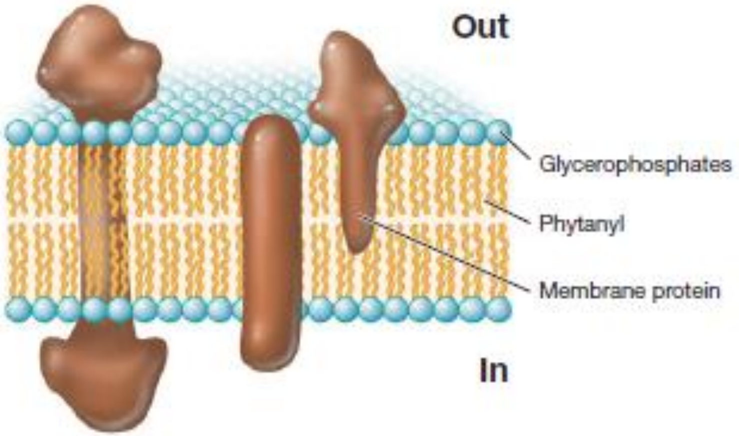
- Saturated fatty acids form a stronger hydrophobic environment than do unsaturated fatty acids, which helps account for membrane stability.

- The cytoplasmic membrane of Archaea can be constructed of either glycerol diethers, which have 20-carbon side chains (the 20-C unit is called a phytanyl group), or diglycerol tetraethers, which have 40-carbon side chains. In the tetraether lipid, the ends of the phytanyl side chains that point inward from each glycerol molecule are covalently linked.

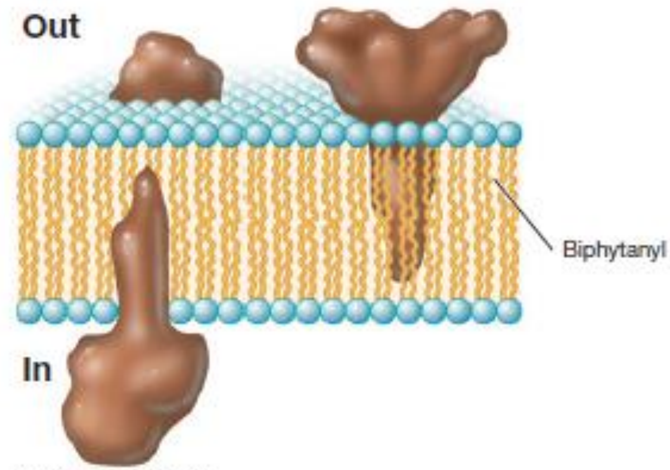
Membrane Stability at High Temperatures

- In addition, however, the architecture of the cytoplasmic membranes of hyperthermophiles takes a unique twist: the membrane forms a lipid monolayer rather than a lipid bilayer.

- This structure prevents the membrane from melting (peeling apart) at the high growth temperatures of hyperthermophiles.



(d) Lipid bilayer



(e) Lipid monolayer

Utilization of thermophilic microorganisms

- Contamination risk is very low
- High temperature allows to work with higher substrates concentrations due to viscosity decrease & solublity increase
- Less studied, so many new & undiscovered enzymes for researchers to find
- Produces thermostable enzymes-thermozymes
- Culture Thermostability
- Purification of recombinant enzymes is simplified (thermaltreatment) as other will simply denature and open up

Bst polymerase

- Isolated from ***Bacillus stearothermophilus.***

- This enzyme having heat-resistant property and a **strand-displacement type or helicase like DNA polymerase activity**, which synthesizes a new DNA strand while dissociating the hydrogen bond of the double stranded template DNA by itself.

- Since the strand-displacement DNA polymerase does not require dissociation of double-stranded DNA by its characteristics, DNA can be synthesized at a constant temperature, and the synthesis is not inhibited by the secondary structure of DNA.

- Optimum functional temperature is **60-65 C** and get easily **inactivated at temperatures** above 80°C.

- Suitable for synthesis of DNA strands having high GC content