Hyperthermophiles are microorganisms able to grow above 90°C

Many have optimal growth temperature in the 80s

They cannot grow below 60°C
Extending the upper temperature limit for life: 121°C


(A) A1: 10 hours in an autoclave at 121°C followed by 3 days of incubation at 103°C. A2 control

Fe(III) ferric Fe(II) ferrous (magnetite) reduction

(B) Negatively stained electron micrograph and (C) thin section of strain 121; (S) single layer cell envelope, (CM) and cytoplasmic membrane. Bar, 1 µm.

(D) Time for cells of strain 121 to double at different temperatures.

High temperature eukaryotes

- *Cyanidium caldarium* (red algae), up to 45°C
- Fungi, up to 60°C
- *Alvinella pompejana* (polychaete), burrows on hydrothermal vent chimneys, up to 60°C (81°C)

Tree of Life

Bacteria

- Purple bacteria
- *Cyanobacteria*
- *Flavobacteria*
- *Spirochetes*
- *Thermotogales*

Archaea

- *Euryarchaeota*
- *Thermoproteus*
- *Pyrococcus*

Eukarya

- Animals
- Plants
- Fungi

LUCA

(Woese et al., 1990)
High temperature environments encompass a large variety of ecosystems:

- Hot springs
- Alkaline hot springs
- Solfatara fields
- Soda lakes
- Alaskan oil fields
- Abyssal hydrothermal vents

Diversity of Habitats

Fig. 1. Map of known hydrothermal vent biogeographic provinces and major mid-ocean ridges. Orange, Pink, western Pacific; green, northeast Pacific; blue, East Pacific Ridge; yellow, Azores; red, Mid-Atlantic Ridge; orange, Indian Ocean.

Cross section of a deep sea hydrothermal vent

- Black smoker vent (360°C)
- Sea water: [H]SO₄²⁻ = O₂
- Reduced fluids: H₂S, Fe²⁺, Mn⁴⁺
- Anoxic chemosynthesis: yields carbohydrates and methane
- Aerobic chemosynthesis: yields carbohydrates and sulfur

\[ \text{H}_2\text{S} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{CH}_4\text{O}_2 + \text{H}_2\text{SO}_4 \]

\[ 2\text{CO}_2 + 4\text{H}_2 \rightarrow \text{C}_2\text{H}_4\text{O}_2 + 2\text{H}_2\text{O} \]
Vents communities

Giant tube worms showing the sheath (white) and plume (red) of the worm body.
Mussel beds and elemental sulfur deposits

Optical Tweezers

Fig. 3. Isolation of a single cell from a mixed culture using a laser microscope. Schematic drawing, inspired with permission from Speleomicrobiologie Verlag, R. Hubscher, 1981. (A) A single cell is trapped optically within the focus of the laser beam.
(B) The single cell is separated from the mixed culture into the ninth compartment. (1) Microslide, (2) tube, (3) needle, (4) syringe, (5) cutting incision, (6) mixed culture, and (7) objective.

(Huber and Stetter, 2001)
A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont

Electron microscopy and fluorescence light microscopy of the Nanoarchaeum equitans-Ignicoccus sp coculture

Scale bar, 10 µm

Sulfur-based Metabolism
- **Autotrophs**: cellular carbon is obtained by fixing CO₂ (sulfur-reducing archaea, methanogens)
- **Heterotrophs**: cellular carbon is obtained from organic compounds (sulfur-reducing archaea)

Sulfur biogeochemical cycle

<table>
<thead>
<tr>
<th>a. continental; m, shallow marine</th>
<th>d. deep sea</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-) growth in absence of S&lt;sup&gt;2-&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

(Adams, 1995)
**Sulfur Metabolism**

**Sulfur oxidation**

\[ S^0 \text{ (e- donor)} + O_2 \text{ (e- acceptor)} + H_2O \rightarrow H_2SO_4 \text{ (product)} \]

*These organisms are aerobic, and either autotrophs or heterotrophs: Sulfolobus*, Acidianus*

**Sulfur reduction**

\[ H_2 \text{ (e- donor)} + S^0 \text{ (e- acceptor)} \rightarrow H_2S \text{ (product)} \]

*These organisms are anaerobic autotrophs: Igneococcus, Pyrobaculum*, Pyrodictium*, Methanopyrus*

\[ \text{e-: electron; * facultative heterotrophs} \]

**Sulfur respiration (reduction)**

organics (e- donor) + S° (e- acceptor) \(\rightarrow\) \(\text{H}_2\text{S + CO}_2 \text{ (products)}\)

*These organisms are anaerobic heterotrophs: Thermoproteus, Pyrodictium, Desulfurococcus*

Archaeoglobus: reduces sulfate, produces hydrogen

**Fermentation**

Carbohydrates and peptides \(\rightarrow\) short fatty acids chains, organic acids, \(\text{CO}_2\) and \(\text{H}_2\) as major products

*These organisms are anaerobic heterotrophs: Pyrococcus, Pyrodictium, Thermotoga*

---

**Superoxide reductase**

\[ \text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^\cdot \]

\[ \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} \]

Model for detoxification of reactive oxygen species in *P. furiosus* SOD uses electrons from reduced NAD(P) by way of rubredoxin (Rd) and oxidoreductase (NROR) to reduce superoxide to hydrogen peroxide, then reduced to water by peroxidases.

\[ \text{XH}_2\text{, unknown organic electron donor. In bold, enzymes and proteins purified from } P. \text{ furiosus; others are hypothetical, based on genome sequence analyses.} \]

(Jenney et al., 1998)
Overview

- Membrane thermostability
- Protein thermostability
- Genome Stability
- DNA repair mechanisms

How can hyperthermophiles maintain the integrity of their macromolecules at temperatures where the chemical degradation of DNA and proteins is greatly accelerated?

Archaeal Lipids
Factors Influencing Protein Thermostability

- Extrinsic factors:
  - inorganic salts
  - organic solutes
  - Heat shock proteins/chaperonins
  - pressure

- Intrinsic factors
  - mosaic of strategies

Distribution of Organic Solutes in Thermophiles

<table>
<thead>
<tr>
<th>MANNOSYLGLYCERATE</th>
<th>ARCHEA</th>
<th>BACTERIA</th>
</tr>
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<tbody>
<tr>
<td>Pyrococcus furiosus</td>
<td>Pyrococcus furiosus</td>
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<td>Pyrococcus aerophilus</td>
<td>Pyrococcus aerophilus</td>
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<td>Thermococcus stetteri</td>
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<td>Methanothermus fervidus</td>
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<td>Rhodothermus marinus</td>
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<td>Thermotoga maritima</td>
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<tr>
<td>Archaeoglobus fulgidus</td>
<td>Archaeoglobus fulgidus</td>
<td></td>
</tr>
<tr>
<td>Aquifex pyrophilus</td>
<td>Aquifex pyrophilus</td>
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<th>MANNOSYLGLYCERITER</th>
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<table>
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<tr>
<th>CYCLIC-2,3-BIPHOSPHOGLYCERATE</th>
<th>ARCHEA</th>
<th>BACTERIA</th>
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</thead>
<tbody>
<tr>
<td>Methanobacterium</td>
<td>Methanobacterium</td>
<td></td>
</tr>
</tbody>
</table>

(Santos and Da Costa, 2001)
Chaperones

- Class of complex high molecular weight proteins (~1000 kDa)
- Bind to polypeptides and proteins and facilitate their correct folding through ATP hydrolysis
- Facilitate the translocation of proteins through membranes
- Promote the formation of multiprotein assemblies
- Found in the 3 domains of life

### Table 2: The classes of Hps in the three Domains

<table>
<thead>
<tr>
<th>Hsp</th>
<th>Bacteria</th>
<th>Eukarya</th>
<th>Archaea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp100s</td>
<td>ChpA, ChpB, HtpU</td>
<td>HSP100</td>
<td>Absent*</td>
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<tr>
<td>Hsp60s</td>
<td>HtpA</td>
<td>HSP60, HSP83</td>
<td>Absent</td>
</tr>
<tr>
<td>Hsp70s</td>
<td>DnaK</td>
<td>HSP70, HSC70</td>
<td>Hsp70*</td>
</tr>
<tr>
<td>Hsp90s</td>
<td>DnaJ</td>
<td>TCP-1</td>
<td>TCP2, Thermosome</td>
</tr>
</tbody>
</table>

*ChpA homologues are found in Methanococcus maripaludis, Thermotoga maritima, and Thermococcus kodakarensis. Hsp70s are absent from most of the hyperthermophiles and in all of the hypothermophiles, except possibly Aeropyrum pernix. In which a putative thermophilic Hsp70 has been identified. TCP-1, 1 complex protein 1, TCP2, thermophilic factor 2.

FIG 2. SDS-15% PAGE analysis of thermal protection of E. coli crude extracts by Pfu-sHSP at 105°C. Lanes 1-8: crude extracts without Pfu-sHSP and lanes 1-2 and lanes 3-4 show the crude extracts without overexpression of Pfu-sHSP heated to 105°C for 20, 30, and 40 min, respectively; lanes 5-8 show crude extracts with overexpressed Pfu-sHSP heated to 105°C for 20, 30, and 40 min, respectively.
Structural basis of Thermostability in hyperthermophilic proteins, or "there's more than one way to skin the cat"

- Increased number of ion pairs (salt bridges), ion pair networks
- Increased hydrogen bonding
- Increased secondary structure formation and stability
- Better packing or rather decreased surface to volume ratio
- More hydrophobic residues
- Increased rigidity

So everything is important everywhere!

Effect of temperature on DNA stability

The chemical effects are believed to be hydrolytic with:

- Cleavage of N-glycosyl bonds resulting in base deamination and formation of mainly apurinic sites and some apyrimidic sites
- Base deamination, in particular cytosine
- Phosphodiester bond scission resulting in backbone breakage

Lindahl calculated that at 100ºC the hydrolytic effects of temperature are increased by 3 000 folds (Lindahl, 1993)

Strategies for RNA protection

rRNA
65 to 200 nucleosides modified per molecule (1 to 3%)

There is a strong positive correlation between the G+C content of rRNA and the optimal growth temperature (Galtier et al., 1999)

tRNA
4 to 26 nucleosides modified per molecule (4 to 26%)

In hyperthermophiles, mostly methylation with 9/35 hyper-methylated (Henri Grosjean, unpublished)
<table>
<thead>
<tr>
<th>Species</th>
<th>T_{opt} (°C)</th>
<th>Genomic DNA G+C %</th>
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<tbody>
<tr>
<td>Sulfolobus acidocaldarius</td>
<td>80</td>
<td>37</td>
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<td>Archaeoglobus fulgidus</td>
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<td>Methanococcus jannaschii</td>
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<td>Acidianus infernus</td>
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<td>Pyrodictium occultum</td>
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<td>62</td>
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<td>Pyrolobus fumarii</td>
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Strategies for DNA protection

- High intracellular salt concentration
- Histone and histone-like proteins
- DNA topology/Reverse gyrase

High intracellular salt concentration protects DNA

- Physiological concentrations of KCl (1 mM to 2 M) and MgCl₂ (0.25 to 10 mM) protect both double and single-strand DNA against thermodegradation
- Supercoiled plasmid DNA is resistant to denaturation up to 107°C, but is progressively cleaved and then denatured

(from Marguet and Forterre, 94, 98)
Archaeal Histones

- only in Euryarchaeota (Cubonova et al., 2005)
- small, abundant and basic proteins
- bind DNA with no sequence specificity
- protect ~60 bp of DNA from nuclease digestion
- resemble eukaryotic histones in sequence (40% similarity), structure, and recognition of positioning signals

Families of small, abundant, basic DNA binding proteins in Archaea

- Constitute up to 5% total soluble proteins
- Bind DNA tightly without sequence specificity
- Possible role in regulation of DNA packing
- Increases melting temperature of the DNA by ~40°C

Sul7d only in Sulfolobales
Alba found in many thermophilic Archaea

A hot story from comparative genomics: reverse gyrase is the only hyperthermophile-specific protein

<table>
<thead>
<tr>
<th>Organism</th>
<th>Optimal growth temperature (°C)</th>
<th>Presence of reverse gyrase</th>
<th>Presence of DNA gyrase</th>
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<tbody>
<tr>
<td>Pyrococcus abyssi</td>
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<td>Pyrococcus thermoplasticus</td>
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<td>Methanobacterium thermoautotrophicum</td>
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<td>Thermococcus lupuligeris</td>
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<td>Thermococcus piezotolerans</td>
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</tr>
</tbody>
</table>

(Forster, 2002)
Reverse gyrase

helicase  top1
Pyrococcus
Sulfolobus

Reverse gyrase (Topo I)

Tapa I
Tapa V

+ ATP

Tapa III
Tapa V

+ ATP

Tapa IV, VI

- ATP

Gyrase (Topo II)

([Farre et al., 1996; Déclais et al., 2001])

Reverse gyrase has heat-protective DNA chaperone activity independent of supercoiling

Martin Kompans and Daniela Stock

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK


FIG. 3. Growth curves and specific growth rates of the host strain and the Δrgy strain. Open circles represent the host strain, and filled circles represent the Δrgy strain. N.D., not determined; DOD, optical density at 660 nm.
Reverse gyrase has heat-protective DNA chaperone activity independent of supercoiling

Figure 1. Reverse gyrase inhibits thermodegradation of DNA. (A) Degradation of full-length linear 1843 bp DNA quantified by measuring band intensities. Lanes labeled ‘M’ contained molecular weight marker; 2500, 2000 and 1500 bp. (B) Data were fitted to first-order kinetics as indicated by the curves.

Reverse gyrase has heat-protective DNA chaperone activity independent of supercoiling

Figure 6. Hypothetical model for targeted heat protection by reverse gyrase. (D) Hydrolysis reactions are indicated by red arrows, enzymatic repair processes by green arrows. Reverse gyrase is drawn as a blue rectangle.

Genomic Mutation Rate

Standard rate for DNA-based microorganisms: 0.0034 (0.0025–0.0046)

Sulfolobus acidocaldarius genomic mutation rate: 0.0018 (Grogan et al., 2001)

Low Genomic Mutation Rate for Hyperthermophiles
Many Questions Still Remain

- Do Archaea have DNA repair proteins that are too poorly conserved to be identified by sequence comparison?
- Do they have novel type of repair enzymes?
- How are the repair pathways regulated?

Take home message

- Hyperthermophilic Archaea have evolved highly efficient mechanisms to maintain the integrity of their macromolecules.
- Comparative genome sequence analyses give insights into some enzymes and pathways involved in those mechanisms.
- More studies are needed to identify additional enzymes (DNA repair) and regulatory pathways (DNA repair, cell cycle), using genomic and biochemical methods.
- Genetic systems are needed to understand the cellular function of putative proteins.