Basis of thermophily and thermostolerance in prokaryotes

Much variation among species
What is the upper temperature limit for life?

• Cells require water, but above 100°C water is steam (steam above 94° at 7000 ft in Yellowstone)

• Life at temperatures >100°C restricted to hydrothermal vents of sea floor (where pressure increases boiling point)

• Temperature limit of life is dictated by stability of monomers! ATP and NAD+ hydrolyse rapidly at ~120°C and have a half life of 30 min

• Estimated that upper temperature limit for life is 130-140°C (but no organisms known near this limit)
Hyperthermophilic Archaea and Microbial Evolution:

• Have Archaea adapted to extreme environments or co-evolved and flourished with such extremes during earth’s formation, that is, are they relics of ancient life?

• 16S rRNA sequencing and sequence analysis suggests the latter, but ?. The Aquifex and Thermotoga groups of the domain Bacteria, are also hyperthermophiles, have slower “evolutionary clocks”. Thus they evolved at a slower rate than did the Archaea – a fast clock means adaptation.

• Life under extremes are under strong evolutionary pressure to maintain the specialized genes essential for their survival and, therefore, beyond a certain point, additional genetic changes would not be of any benefit (see Pyrodictium).
Hydrogen as a primitive energy source

• Many *Archaea* grow using H$_2$ as an electron donor with S°, NO$_3$-, CO$_2$, or Fe$^{3+}$ as electron acceptors under anaerobic, high temperature and dark subsurface conditions (primordial early earth conditions with protection from UV radiation) (e.g. H$_2$ + S° $\Rightarrow$ H$_2$S, H$_2$ + HNO$_3$- $\Rightarrow$ HNO$_2$- + H$_2$O, 4H$_2$ + CO$_2$ $\Rightarrow$ CH$_4$ + 2H$_2$O, H$_2$ + 2 Fe$^{3+}$ $\Rightarrow$ 2 Fe$^{2+}$ + 2 H$^+$)

• Physiological relic of ancient metabolic schemes?

• Diversity of H$_2$ oxidizing hyperthermophilic Archaea (and Bacteria); also many mesophilic H$_2$ oxidizers, and “modern” aerobic types using O$_2$ as electron acceptor

• Hydrothermal vents or deep subsurface waters of Earth suggested as locations for the origins of life (by some biologists)
HEAT TOLERANCE OF NUCLEIC ACIDS: ADAPTATIONS
DIFFICULT TO SUMMARIZE SINCE NUCLEIC ACID SEQUENCES AND
STRUCTURES SO DIVERSE THAT STRATEGIES DIFFER SIGNIFICANTLY

1. INTRINSIC DIFFERENCES IN STRUCTURE OF THE NUCLEIC ACIDS
2. DEPENDENCE ON EXTRINSIC INTERACTIONS WITH OTHER
BIOMOLECULES
3. DEPENDENCE ON NUMEROUS ENZYMES THAT DETECT AND REPAIR DNA
DAMAGE OR CONSTANTLY RENEW FUNCTIONAL RNA MOLECULES

Plasmid DNA more resistant to heat (circular)

G:C base pairs more resistant; high G+C content is response to high temp.

Additional Na⁺, K⁺, Mg²⁺ protect DNA

Various polyamines stabilize DNA & RNA

Reverse gyrase in hyperthermophiles: local protection of DNA against
depurination, single and double stranded breakages common at high temp.

Family of specific thermostable proteins: chromatin, and a family of
small DNA-binding proteins (e.g. chaperonins)
DNA thermostability (continued)

• Cytoplasm of hyperthermophilic methanogens contains large amounts of potassium cyclic 2,3-diphosphoglycerate which prevents some damage (depurination)

• Reverse DNA gyrase introduces positive supercoils which are thought to enhance thermostability

• High magnesium concentrations that stabilize the DNA by neutralizing the phosphates

• Other proteins that increase the stability of DNA (*Sulfolobus* has a small heat stable binding protein Sac7d which binds to a minor groove that increases melting temperature of DNA by ~40°C - in phylum Crenarchaeota)

• Phylum Euarchaeota contains highly basic histone-like proteins that wind and compact DNA into nucleosome-like structures

ETC.
Figure 1. Strategies for thermostabilization of nucleic acids. In boxes are mentioned the various factors that allow a thermophilic organism to protect their nucleic acids against the deleterious effect of heat. A clear distinction between the giant extended macromolecule DNA and the more compact smaller RNA molecules has to be made. For details, see text.
Thermostability of Proteins in thermophiles

• Amino acid composition and structural features are not very unusual in most thermophiles, but may have a larger proportion of charged amino acids on the molecule surface (glutamate, arginine, lysine).

• More ionic interactions (salt bridges) on surface of the protein (attraction of + and - charged components); H bonds increase

• Highly hydrophobic cores of proteins that reduce tendency to unfold from tertiary structure (non-polar amino acids tend to aggregate to minimize their collective interaction with surrounding polar water molecules).

• Chaperonins (one type of heat shock protein) refold partially denatured proteins and help fold newly synthesized proteins; protect other proteins from heat denaturation.

• Therefore a combination of inherent properties of the proteins plus protection by chaperonins impart thermal resistance (but they lose “flexibility” at lower temperatures) (much still unknown)
Pyrodictium has the HSP (chaperonin) known as THERMOSOME

- Can be up to 80% of cell dry weight
- Helps cells survive above their growth limit (108°C)
- Can survive autoclaving 1 hour at 121°C
Mostly saturated f. a. in thermophiles-
higher melting temperature

Cell membrane of most Bacteria & Eukarya

Light area =
glycerol portion of phospholipid (hydrophilic)

Dark area =
region of fatty acids (hydrophobic)

Mostly saturated f. a. in thermophiles-
higher melting temperature
Major lipids of *Archaea* and the structure of archaeal membranes

(a) Glycerol diether

(b) Diglycerol tetraether
Ester linkage

(a) Bacteria & Eukarya

Ether linkage

(b) Most Archaea

Isoprene

(c) R group for most Archaea (4 linked isoprenes=phytanyl)
Figure 3. Core structures of phospholipids in bacteria and tetraether lipids in *Archaea*. (A) Diacylglycerol in bacteria; and the archaeal tetraether lipids; (B) caldarachaeol; (C) isocaldarachaeol; (D) calditiglycerocaldarachaeol; and (E) crenarchaeol.
The general structure of a sterol

(a) Eukarya contain sterols in membranes

(b) structure of the hopanoid diploptene

(c) many Bacteria contain hopanoids, not in Archaea