Temperature adaptation in Extremophiles

Unit-III, Paper-Zoo-103

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Who are Thermophiles?

- Eukaryotes generally do not survive temperatures > 60°C . The amoeba Echinamoeba thermarum, found in hot springs in many places on the globe, grows optimally at 50°C and thus is one of the few truly thermophilic eukaryotes.
- Bacteria and Archaea can be classified according to their optimal growth temperature as follows: mesophiles (T_{opt} 20–45°C), thermophiles (T_{opt} 45–80°C), and hyperthermophiles (T_{opt}> 80°C)
- thermophiles are further subdivided into **thermotolerant**, which grow optimally at **mesophilic** temperatures but have maximum growth temperatures < 50°C, **thermophiles** (T_{opt} 50–70°C), and **extreme thermophiles** (T_{opt} > 70°C).
- TYPES OF THERMAL ENVIRONMENTS: Terrestrial, Nonanthropogenic Environments, Marine Environments, Subsurface Environments (that includes petroleum reservoirs and geothermally heated lakes and aquifers), Anthropogenic Environments (that includes household compost piles and water heaters and industrial process environments and thermal effluent from power plants), temporary environments and mesobiotic environments (various environments, such as animal droppings, manure piles, orcompost, temporarily heated by biodegradation of organic material).

Thermophiles?

- Thermophilic organisms are a subgroup of extremophiles which are defined as having an optimum growth temperature above 45°C for moderate thermophiles, above 65°C for extreme thermophiles, and above 80°C for hyperthermophiles.
- These are *Pyrococcus*, *Pyrobaculum*, and *Methanopyrus*, are able to grow optimally at temperatures as high as 100 to 105˚C (even higher in vegetative state or under high pressure).
- The hyperthermophilic genera are mostly Archaea, except for *Thermotoga* and *Aquifex* genus that belong to Bacteria.
- They thrive in very hot terrestrial habitats such as geysers, hot springs, and hot sediments of volcanic eruptions or near deep-sea hydrothermal vents and undersea volcanoes.
- The moderate thermophiles can be found almost everywhere, while most extreme thermophiles are found essentially in moderately hot environments (for details, see other chapters in this volume).

Thermophiles?

- extreme thermophiles and hyperthermophiles are exclusively unicellular organisms lacking internal membranes, including nucleus and organelles.
- Extreme thermophiles and hyperthermophiles cannot live at temperatures below 60°C, their macromolecules (DNA, RNA, and proteins/enzymes), and are composed of the same “building blocks” as those in mesophilic and psychrophilic organisms, and adapted to function only within a certain range of temperatures.

Effect of Tempt. On DNA/RNA of thermophiles

- chemical cleavages of the N-glycosidic bond between the sugar moiety and a base (especially with guanine and adenine, i.e., purines)
- hydrolytic deaminations of cytosine and adenine to uracil and hypoxanthine
- It has been estimated that up to 1,000 purine bases are liberated after incubation for 1h in vitro at 70°C, i.e., about 0.025% of the bases, in fact about 0.1% of the total purines in DNA
- at elevated temperatures, both depurination and cytidine/adenine in DNA
- In tRNA, and rRNA, the naturally occurring modified purines N7-methylguanosine, wybutosine, and the pyrimidine dihydrouridine are highly thermolabile where these modified bases are the target sites for chemical thermodegradation.
- At temperatures higher than 50°C and in the presence of Mg^{2+} ions, RNA undergoes the spontaneous hydrolysis of 3’ to 5’ phosphodiester bonds (due to the presence of a hydroxyl group in the 2’ position of every ribose that promotes the intramolecular cleavage of the phosphodiester bond)

Effect of Tempt. On DNA/RNA of thermophiles

- Despite the intrinsic potentiality of nucleic acids to degrade at elevated temperatures, many hyperthermophiles can survive at very high temperatures approaching or even surpassing the boiling point of water. The strategies adopted to protect its nucleic acids by thermophiles are-

Effect of Tempt. on DNA/RNA & protection mechanism in Thermophiles

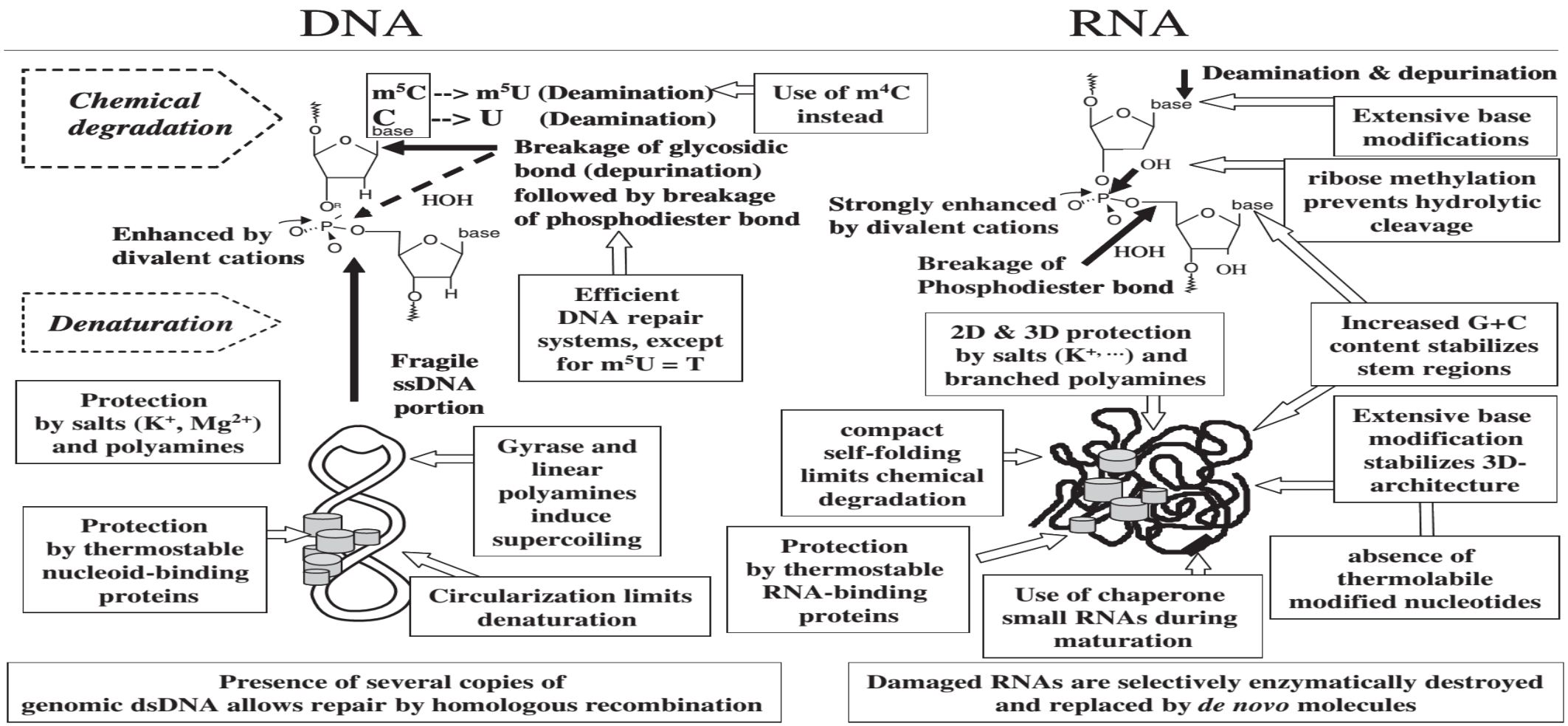


Figure 1. Strategies for thermostabilization of nucleic acids. In boxes are mentioned the various factors that allow a thermophilic organism to protect their nucleic acids against the deleterious effect of heat. A clear distinction between the giant extended macromolecule DNA and the more compact smaller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

Rule 1: Use of High G+C Content in RNA, but Not in DNA

- an estimation of 5% increment in GC content in the base-paired regions of tRNA brings about a 1.5°C rise in the melting temperature, with a theoretical upper limit of about 87°C for a poly(C)–poly(G) double helical structure under normal salt concentration. For instance, tRNAs and tRNA genes from extreme thermophilic and hyperthermophilic organisms reveals that all stems of the cloverleaf are almost exclusively constituted of G:C base pairs.
- Also a linear correlation has been demonstrated between the optimal growth temperature and the GC content in the secondary structure of rRNAs
- The bias for G-C-rich stems in tRNA and rRNA, together with the preference of certain dinucleotides in coding sequences of mRNAs of hyperthermophiles, promotes the need for optimizing the translation process (efficiency and accuracy) at high temperature.

Rule 2: Stabilization of Nucleic Acid Structures by Small Ligand Binding

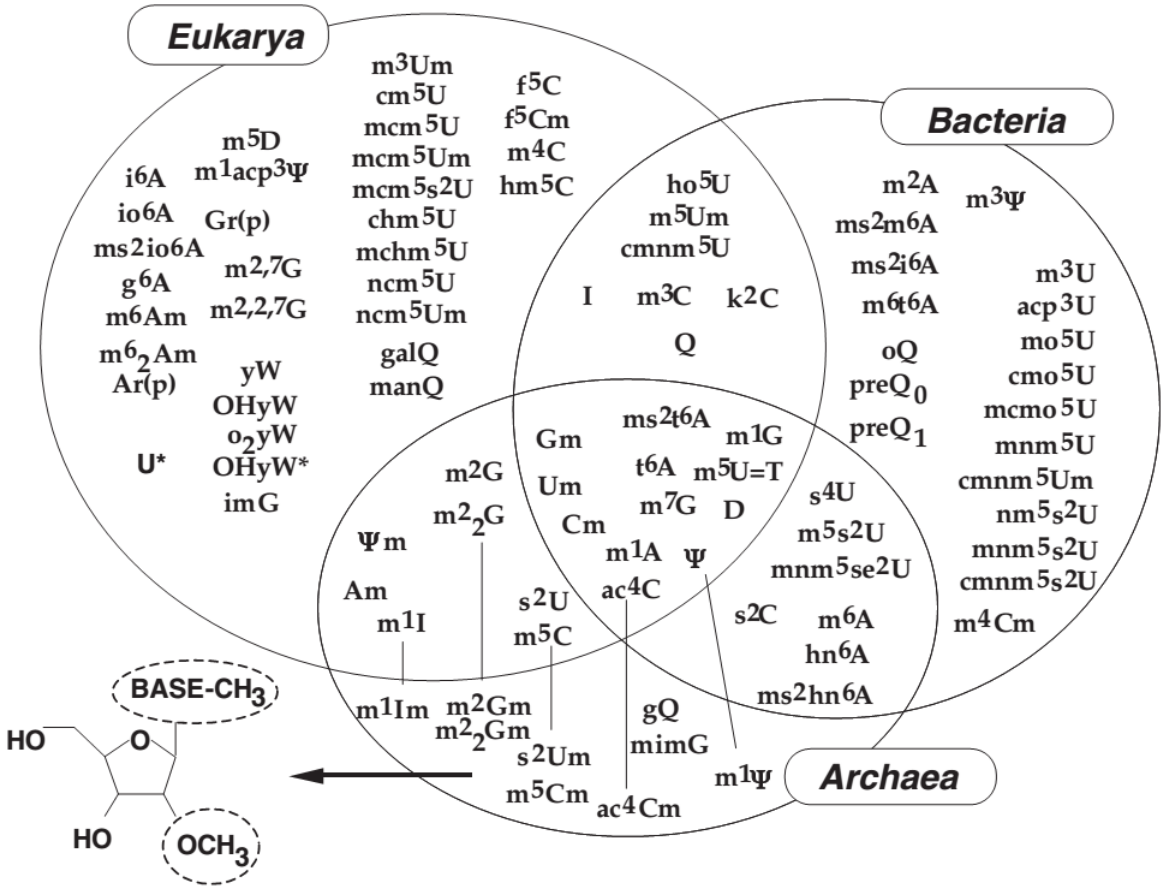
- High concentrations of monovalent cations Na+, K+, and Mg2+ protect dsDNA against chemical thermodegradation of the phophodiester bonds.
- High concentrations of these monovalent cations reduce also the spontaneous chemical degradation of RNA.
- These monovalent cations stabilize the conformation of both DNA and RNA against thermodenaturation.
- Intracellular K+ concentration of certain hyperthermophilic euryarchaeota is much higher than that in mesophilic organisms.
- higher melting temperatures are observed when longer polyamines are added to tRNA or DNA in vitro, and in case of tRNAs, the highest melting temperature was recorded in the presence of a branched quaternary polyamine tetrakis(3-aminopropyl)ammonium. For instance, attachment of linear polyamines $NH_3^+[(CH_2)_{3\text{-}4}NH_2^+]_{x=1\text{-}4}(CH_2)_{3\text{-}4}NH_3^+$ Or branched (ternary or quaternary) polyamines, can stabilize DNA and RNA.

Rule 2: Stabilization of Nucleic Acid Structures by Small Ligand Binding

- As a matter of fact, higher melting temperatures are observed when longer polyamines are added to tRNA or DNA in vitro, and in case of tRNAs, the highest melting temperature was recorded in the presence of a branched quaternary polyamine tetrakis(3-aminopropyl)ammonium).
- In extreme thermophilic bacteria Thermotoga, Thermomicrobium, and Thermodesulfovibrio and hyperthermophilic archaeons Aeropyrum and Pyrodictium, a large variety of long polyamines (mostly penta-amines and hexa-amines) are produced.

Rule 3: Stabilization of Nucleic Acid Structures by Covalent Modification of Nucleosides

- acetyl function at N^4 in ac4C serves to make the cytidine N^4 proton more acidic, favoring a strong H-bond with guanine opposition of a Watson–Crick base pair. Similarly methylation of guanine & cytosine, m_2Gm and m^5Cm promotes stabilization of RNA.
- the methyl group on 2'-hydroxyl of ribose allows to avoid hydrolysis of the corresponding phosphodiester bond in RNA. It also creates a more hydrophobic micro-environment which could help compaction of RNA and/or interaction with other molecules such as proteins.
- the thermolabile dihydrouridine (D) that obviously cannot form Watson–Crick base pair within a double helix (due to its non-aromatic character) allows more flexibility of the RNA backbone and thereby destabilizes locally the RNA molecules.
- methylation of uridine producing the conserved m5U (thymidine) in position 54 of the T-loop resulted in an increase of the Tm by 6°C (local Tm) in *Escherichia coli*.
- The presence of a thiol group on m^5U54 (s2T54) in in $tRNA^{Trp}$ of Bacillus subtilis, increases the local Tm around the thiol group by 20°C



Hypermodified nucleotides in hyperthermophiles

Figure 2. Phylogenetic distribution of modified nucleosides in RNA from the three domains of life. Abbreviations of modified nucleosides are the conventional ones. For details, including the chemical structures, see in Limbach et al. (1995). Lines point out which ones among the hypermodified nucleosides in *Archaea* correspond to non-ribose methylated counterparts in *Eukarya* or *Bacteria*.

Rule 3: Stabilization of Nucleic Acid Structures by Covalent Modification of Nucleosides

- Methylation of C5 of pyrimidine ring of cytosine promotes the spontaneous hydrolytic deamination of cytosine, especially at high temperatures leading to synthesis of m^5U (deoxy-T),
- in m5C facilitates thyperthermophiles (Bacteria and Archaea) contains exclusively deamination-resistant m^4C residues (in addition to m6A) and no detectable amount of m5C, a high frequency of spontaneous mutation is expected if DNA contains too much m5C.

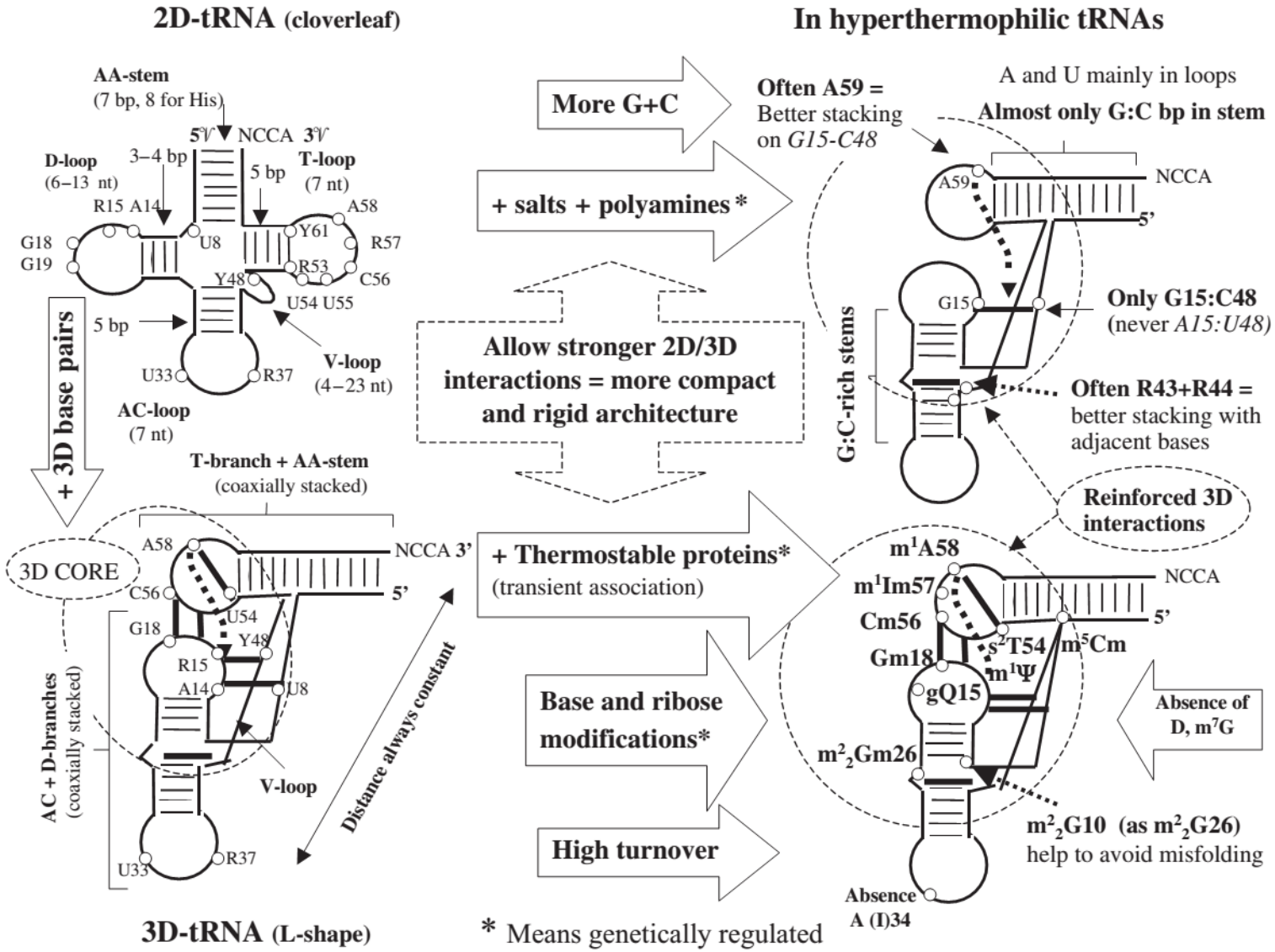


Figure 4. Main factors allowing tRNA molecules to function at high temperatures. On the left part are conventional schematic representations of 2D and 3D structures of tRNA. The remarkable features that are characteristic of a tRNA from a hyperthermophilic organism are indicated on the right side. In the boxes in the central part are indicated the various factors that allow a thermophilic organism to protect their nucleic acids against the deleterious effect of heat. For details, see text. Numbers indicate conventional tRNA positions; letters correspond to bases A, C, G, and U; R for purine, Y for pyrimidine, and N for any base.

Rule 4: Generation of Compact Tertiary Structures

- Increasing the G+C content and the presence of sufficient amount of small cationic ligands allow enhancement of the stability of relatively long stretches of dsDNA and short stems in cellular dsRNA, as well as DNA–RNA complexes (during transcription) and RNA–RNA interaction (during translation).
- in majority of hyperthermophilic archaea and in few hyperthermophilic bacteria, a completely different type of DNA gyrase (called reverse gyrase) is found. This reverse gyrase is a bifunctional enzyme resulting from a fusion between a gene coding for an ATP-dependent “helicase-like” protein and a type I DNA topoisomerase. This enzyme promotes positive supercoiling as well as thermoresistance of genomic DNA at temperatures above 80°C.
- In hyperthermophilic archaeon *Thermococcus kodakaraensis*, reverse gyrase is essential for cell growth only at extreme temperatures such as above 93°C, and not in the range of 80 to 90°C. This reverse gyrase confers local protection of DNA against increasing number of damages (depurination, single and double-stranded breakages, and UV irradiation) due to harsh conditions of life at high temperatures.

Rule 5: Generation of RNP Particles with Thermostable Proteins

- In case of DNA, chromatin proteins, such as basic histones, histone-like proteins, and a family of small DNA-binding proteins, Sul7d, Sac7, Sis7, Sso7, Alba, MC1, and HU, tightly bind to the nucleic acid. These proteins prevent the free rotation of the two strands around each other and facilitate the compaction of DNA and/or condense linear dsDNA into regular, globular nucleosome.
- However, due to the strong basic character of the histones and most DNA-binding proteins, they can catalyze chain scission at abasic sites by catalyzing a beta-elimination reaction, a drawback that might become a real problem at high temperatures if not appropriately circumvented.
- Like for nucleosomes, interaction of the several ribosomal proteins (which are more numerous in the ribosomes of hyperthermophiles than those of mesophiles) with specific sites on 16S- and 23S-rRNA is essential to protect several exposed phosphodiester bonds from hydrolysis by water but mostly to form stable globular ribosomal RNP particles able to function at high temperatures.
- As far as tRNAs are concerned, transient association with abundant thermostable proteins like elongation factor and aminoacyl-tRNA ligases can also help to protect the nucleic acids at high temperatures.

Rule 6: Controlling DNA Damages by Efficient DNA Repair

- Presence of DNA-glycosylases, suicide DNA-(de)-methyltransferases, endonucleases, 5’ to 3’ exonucleases, DNA-ligase, and polymerases enable the thermophiles to eliminate efficiently any abasic site, deaminated, alkylated, or oxidized base, and radiation-induced cross-link products that may appear accidentally in the DNA of the chromosome, especially at high temperatures.

Rule 7: Eliminating RNA Damages by RNA Turnover

- existence of specific RNA-transglycosylases or editing machinery able to repair abnormal chemically damaged RNAs
- Coupling gene transcription with translation of the nascent mRNA is obviously an advantage for the hyperthermophiles.
- Efficient elimination of abnormal, nonfunctional RNA molecules operated via specific degradative machineries (RNA surveillance-control quality and rapid RNA degradation—RTD—systems) is probably also vital for the thermophiles and hyperthermophiles.