STRATEGIES OF HYPERTHERMOPHILES IN NUCLEIC ACIDS ADAPTATION TO HIGH TEMPERATURE

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Summary

Nucleic acids are heat-labile macromolecules. At temperatures typical for hyperthermophiles, they are susceptible to denaturation (unwinding of DNA double helices and RNA hairpins) and chemical degradation (hydrolysis). Stable RNAs are protected against denaturation in hyperthermophiles by a high GC content and extensive nucleotide modifications which stabilize their secondary and tertiary structures. Methylation of 2'OH can also be involved in the protection of stable RNA against hydrolysis of the phosphodiester bonds. In contrast to RNA, intracellular DNA is intrinsically stabilized against global denaturation by the existence of topological links between the two DNA strands, explaining why the genomic DNA of hyperthermophiles is not specifically GC rich. However, DNA is susceptible to depurination and cytosine deamination at high temperature. Furthermore, depurination can induce DNA breakage and subsequent denaturation. The extent of depurination in hyperthermophiles *in vivo* is not known, but the rate of depurination and subsequent denaturation could be reduced by high salt concentrations (as it is the case *in vitro*) and/or polyamines or specific binding proteins (but this remains to be demonstrated). Hyperthermophilic Archaea are

highly radioresistant, indicating very efficient DNA repair mechanisms. However, DNA repair proteins detected to date in hyperthermophiles are not different from those existing in mesophilic organisms, with few exceptions (such as DNA polymerase that can check for uracile in DNA). In contrast, all hyperthermophiles contain a unique enzyme, reverse gyrase, which can modify DNA topology. Comparative genomic analysis reveals that reverse gyrase is the only protein that is specific for hyperthermophiles. Although the precise role of reverse gyrase *in vivo* is still unknown, this enzyme is probably essential for life at extremely high temperatures. This implies that a major problem that hyperthermophiles have to solve is to maintain the DNA double helix functional at high temperature.

1. Introduction

At the end of the 1960s, it was believed that life couldn't exist above 70–75 °C, because of the instability of the DNA molecules at such temperatures. Not only does the DNA double helix unwind at temperatures above 80 °C in laboratory conditions, but these temperatures induce various chemical modifications that are highly mutagenic, such as depurination or cytosine deamination. The discovery of hyperthermophiles led to a reevaluation of these early conceptions, since it is now clear that organisms can manage to protect their nucleic acids, both DNA and RNA, against the deleterious effects of temperature, at least up to 113 °C. The mechanisms designed by natural selection in hyperthermophiles to deal with this problem are especially interesting to study, since they are directly relevant to the problem of the putative link between hyperthermophiles and a hot origin of life. Is it possible to imagine a hot primordial life based on RNA, despite the intrinsic instability of RNA at high temperature? How old are the specific mechanisms observed in hyperthermophiles for thermoprotection? Are they adaptation devices or primitive features? The studies already done in this area of research strongly suggest that hyperthermophiles used a combination of diverse strategies to prevent both thermodenaturation (opening of the DNA double helix and RNA hairpins) and thermodegradation (base modifications or cleavage of the phosphodiester bond). We will see that these strategies are clearly secondary adaptations, indicating that present-day hyperthermophiles are most likely not direct descendent of hypothetical primitive ones, but have originated from less thermophilic ancestors.

2. The General Problem of Nucleic Acid Stability at High Temperature

Denaturation of the DNA double helix at high temperatures is such a common experience for molecular biologists that its challenges the imagination of scientists first in contact with hyperthermophiles. *A priori*, stabilization of DNA duplex and RNA hairpins can be partly achieved by increasing the GC content of the molecule, since GC base pairs (with three hydrogen bonds) are more stable than AU or AT base pairs (with only two hydrogen bonds). However, this strategy has limitation, since a melting temperature (T_m) of 110 °C would require more than 95% GC for a linear DNA in physiological salt conditions! We will see that the strategy of increasing the GC content is used by RNA, but that, for topological reasons, it is not required in double-stranded DNA. Another trick to stabilize nucleic acids could be to enhance the internal salt concentration, as the T_m of DNA or RNA double helices increases with the ionic strength, due to the screening of the negative charges associated to the phosphate-sugar

backbone. Some hyperthermophiles indeed exhibit very high intracellular K^+ concentrations, but this is not a general feature. A high polyamine content might play a role in nucleic acid stabilization at high temperature, since these positively charged molecules also increase the T_m of both RNA and DNA *in vitro*. In fact, as described below more complex and diverse strategies are apparently at work in hyperthermophiles to stabilize the secondary and tertiary structures of nucleic acids. Furthermore, the problem of nucleic acids thermodegradation is probably as critical as the problem of thermodenaturation. Both DNA and RNA are susceptible to chemical degradation by hydrolysis at temperature typical for hyperthermophiles. Intracellular water activity is indeed sufficient to trigger thermodegradation as indicated by several lines of evidence: a) highly concentrated DNAs in bacteriophages, spermatozoa, and cell nucleic acids are in the highly hydrated B form; b) DNA is still hydrated in solution containing high concentrations of neutral polymers; and c) photoproducts observed after irradiation *in vivo* by UV irradiation are those that are typical of fully hydrated DNA. From these ancient data, one can concluded that DNA (and probably RNA alike) is most likely fully hydrated *in vivo* and that thermodegradation should be a real problem for hyperthermophiles.

3. Thermoprotection of RNA in Hyperthermophiles

3.1. Protection of RNA against Thermodenaturation

The secondary and tertiary structures of stable RNAs are partly stabilized against thermodenaturation in hyperthermophiles by an increasing GC content. GC rich sequences are usually localized in the stem of putative RNA hairpins, whereas helices are longer and contain fewer bulged nucleotides and other irregularities, which might be destabilizing at high temperature. However, the GC enrichment strategy and modification of helix structure are clearly not sufficient to confer RNA stability in hyperthermophiles. Indeed, although the T_m of native unfractionated tRNA from *Escherichia coli*, *Thermus thermophilus*, *Pyrococcus furiosus*, and *Pyrodictium occultum* are correlated with the optimal growth temperature of the organisms (75, 85.5, 97, and 101.5 °C, respectively), and their increasing GC content, these values are from 10 to 20 °C higher than predicted solely from the GC content alone. The stabilization above T_m values expected from the GC content observed in stable RNAs of thermophiles and hyperthermophiles is due both to a higher level of nucleotide modification and to the presence of novel unique modified nucleosides. The role of modified nucleotides is apparent from the correlation between the optimal growth temperatures of the organisms and the level of modifications in both tRNA and rRNA, as well as the increase in modification for a given organism at different temperatures. For example, 13–15 different forms of post transcriptional modifications were found in the tRNA of several psychrophiles, compared with ~29 known to occur in bacterial mesophiles, and 24–31 known to occur in the hyperthermophilic Archaea. Extensive methylation is a hallmark of the novel modified nucleotides discovered in hyperthermophilic Archaea, some of them being methylated on both the base and the ribose (Figure 1). Up to 67 sites of ribose methylation have thus been indentified in the rRNA of *Sulfolobus*. Methylation is most likely related to thermostabilization, as 20–

25% increased in ribose methylation levels of rRNA is observed when cultures of *Sulfolobus solfataricus* are grown at progressively higher temperatures.

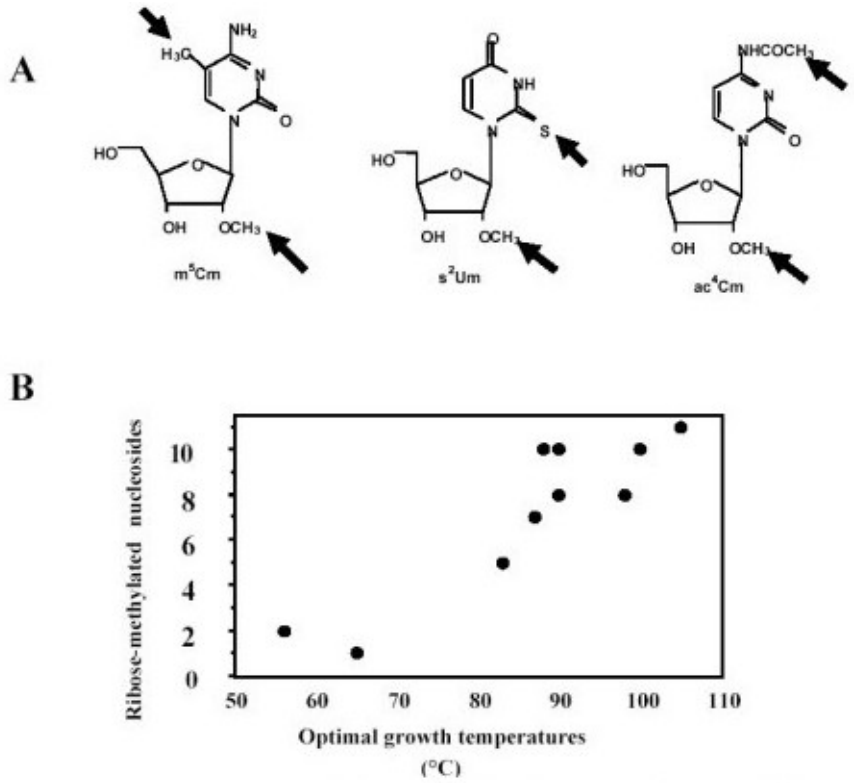

Figure 1. A) Structure of three modified nucleosides discovered in archaeal tRNA; m5Cm, 2'-O-methyl-5 methyl cytosine; s2Um, 2'-O-methyl-thiouridine; ac4Cm, 2'-O-methyl-4 acetyl cytosine. B) Correlation between the optimal growth temperatures of various Archaea and the number of 2'-O methylated nucleosides in their tRNA.

The role of nucleotide modifications in stable RNA of thermophiles and hyperthermophiles is not only to prevent unfolding of the hairpins, but to stabilize the molecule in a correct conformation and possibly to protect against thermodegradation (see below). In particular, both ribose methylation in 2' and thiolation of m^5U (two current modifications observed in thermophiles) increase the rigidity of the nucleoside by favoring the C3'-*endo* configuration of ribose. This restricts conformational flexibility of the sugar by stabilizing the A-type helical conformation of RNA. Cytidine acetylation in ac^4C has the same type of structural effect and the additional methylation in ac^4Cm (which is unique to hyperthermophilic Archaea) increases it further, such that ac^4Cm is the most rigid nucleoside thus far analyzed. As in the case of proteins, RNA modifications increasing rigidity would produce a molecule with the optimal flexibility at high temperature. Interestingly, the only modified base whose amount is reduced in hyperthermophile is dihydrouridine, which is precisely involved in the maintenance of the conformational flexibility of RNA. Again, an opposite trends is observed between hot and cold lover organisms, and dihydrouridine turned out to be exceptionally abundant in the tRNA of three psychrophiles recently tested .

Nucleotide modifications in hyperthermophiles are performed by methylases that are homologous to their bacterial/and or eukaryal counterparts. In particular, ribose 2'-0-methylation in *Archaea* is performed by homologues of the eukaryal fibrillarin/Nop56/58 complex, guided by homologues of eukaryal C/D box snoRNAs that hybridize with target sequences near methylation sites. The archaeal snoRNAs are smaller than their eukaryal counterparts and contain two guide sequences instead of one, two features that could be related to thermoadaptation. Indeed, the number of genes that are detectable in currently available archaeal genomes correlates with the optimal growth temperature. 56 and 29 snoRNA-like genes have been detected in *Pyrococcus furiosus* and *Sulfolobus acidocaldarius*, respectively. The large number of proteins in the ribosomes of hyperthermophilic *Archaea*, and the methylation system (methylase and guide RNA) could possibly act as chaperones in ribosome formation at high temperature. In *Sulfolobus*, sequences that are complementary to snoRNA guides have also been detected in tRNA, 7S RNA and the RNA component of RNAse P, indicating that stabilization by 2'O methylation is a general strategy of RNA stabilization in this hyperthermophile.

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DNA repair enzymes from hyperthermophiles.]

Biographical Sketch

Patrick Forterre, has been working on various aspects of DNA metabolism since the 1970s. He started working on *E. coli* DNA replication at the Institut Jacques Monod in Paris and focused on the role of DNA gyrase in this process. He then moved to the study of archaeal DNA polymerases and topoisomerases in the mid eighties. A major contribution was the identification of reverse gyrase as a type I DNA topoisomerase and the discovery of positive supercoiling *in vivo*, in collaboration with Michel Duguet. He defended his PhD in 1985, under the supervision of Dr. A.M. De Recondo. P. Forterre set up his own laboratory in 1989 at the Institut of Genetic and Microbiology (University Paris

XI or Paris-Sud) where he got a Professor position. From that time, his laboratory has been mainly working on DNA topology and DNA topoisomerases in Archaea with emphasize on the problem of DNA stability in hyperthermophiles. The two major discoveries made during this period were the identification of a new family of type II DNA topoisomerase present in both Archaea and Eukarya and the first identification of an archaeal origin of replication.

Further information can be found at http://www-archbac.u-psud.fr/.