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Archaeal Genome Organization and Stress Responses: Implications for the Origin and Evolution of Cellular Life

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ABSTRACT

For DNA to be used as an informational molecule it must exist in the cell on the edge of stability because all genomic processes require local controlled melting. This presents mechanistic opportunities and problems for genomic DNA from hyperthermophilic organisms, whose unpackaged DNA could melt at optimal temperatures for growth. Hyperthermophiles are suggested to employ the novel positively supercoiling topoisomerase enzyme reverse gyrase (RG) to form positively supercoiled DNA that is intrinsically resistant to thermal denaturation. RG is presently the only archaeal gene that is uniquely found in hyperthermophiles and therefore is central to hypotheses suggesting a hypothermophilic origin of life. However, the suggestion that RG has evolved by the fusion of two pre-existing enzymes has led to hypotheses for a lower temperature for the origin of life. In addition to the action of topoisomerases, DNA packaging and the intracellular ionic environment can also manipulate DNA topology significantly. In the Euryarchaeota, nucleosomes containing minimal histones can adopt two alternate DNA topologies in a salt-dependent manner. From this we hypothesize that since internal salt concentrations are increased following an increase in temperature, the genomic effects of temperature fluctuations could also be accommodated by changes in nucleosome organization. In addition, stress-induced changes in the nucleoid proteins could also play a role in maintaining the genome in the optimal topological state in changing environments. The function of these systems could therefore be central to temperature adaptation and thus be implicated in origin of life scenarios involving hyperthermophiles. Key Words: Archaeal histones—Genome packaging—Genome topology—Stationary phase. Astrobiology 2, 241–253.

INTRODUCTION

DNA SEQUENCE COMPARISONS of genes encoding the small subunit rRNA component of ribosomes (16S rRNA in prokaryotes and 18S rRNA in eukaryotes) have revealed that cellular life can be placed into one of three domains: Bacteria, Archaea, or Eucarya (Woese, 1987; Woese *et al.,* 1990). Archaea are morphologically and metabolically similar to Bacteria, but their genes involved in the information processing systems, including transcription, translation, DNA replication, and recombination, most closely resemble eucaryal orthologues (Rivera *et al.,* 1998). However, the eucaryal-like nature of the informational genes in Archaea could also be the result of dif-

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ferential rates of evolution for metabolic and informational processing genes (Forterre and Phillipe, 1999). The phylogenetic trees based on 16S rRNA sequences also highlight two phenotypically diverse groups in the archaeal domain: the Euryarchaeota and the Crenarchaeota (Woese *et al.*, 1990). One of the major differences between them is that most euryarchaeal species, like all of the Eucarya, have a histone-containing, nucleosome-based DNA condensation system (Ronimus and Musgrave, 1996a; Sandman and Reeve, 2001). This observation, together with the existence of a minimal eucaryal information processing system, predicts that the archaeal and eucaryal domains shared an important evolutionary history subsequent to the universal ancestor stage (Olsen and Woese, 1997).

For many biologists, an interesting outcome of phylogenetic trees is that they predict that the last universal common ancestor was a hyperthermophilic archaeon because these organisms are the closest extant organisms to the root of these trees. However, a vast body of work has cast doubt on the validity of these trees, particularly the position of the root (Forterre and Phillipe, 1999). Doolittle (1999) goes further and argues for a net-like rather than a tree-like structure to describe prokaryotic evolution, describing prokaryotic taxa as being "imprecisely bounded and ephemeral." In 1998, Woese proposed a genetic annealing model for early cellular life in which life originated as a genetically plastic progenote. He described this by saying "the universal ancestor is not a discrete entity. It is, rather a diverse community of cells that survives and evolves as a biological unit." This harks back to the suggestion of Reanney (1974), several decades ago, that we should more realistically view prokaryotes as one global superorganism within which genes are exchanged at different frequencies. The extent and importance of horizontal transfer in early evolution are, in part, demonstrated by the mosaic nature of the genomes of extant prokaryotes. For example, the fully sequenced genome of the deeply rooted thermophilic bacterium *Thermotoga maritima* has shown that 24% of its genes have greater sequence similarity with archaeal genes than any other bacterial or eucaryal gene and that these genes are in defined clusters with conserved gene order and have an atypical mol percent G+C compared with the rest of the genes in the genome (Nelson *et al.*, 1999). However, Forterre and Phillipe (1999) have argued that the mosaicism found in many genomes may be due to differential gene loss or nonorthologous replacement.

The potential nature of the last universal common ancestor can also be predicted by understanding the physical conditions that could have provided environments for the development and evolution of cellular life. The discovery of deep-sea hydrothermal vents (Corliss *et al.*, 1979) has provided such an environment, and the physiology of the Archaea and Bacteria that inhabit hydrothermal vents continues to challenge our preconceptions about the physical limits of conventional life and Darwin's suggestion, found in a private letter to Hooker, of a "warm little pond" for the site of the origin of life itself. Investigations of hyperthermophilic microorganisms have formed the basis of the "hot" origin of life for a number of reasons other than because phylogenetic studies place them close to the root of the universal tree of life. Firstly, they exist in conditions expected to have been common during formation of the earth. Secondly, they could have survived meteorite impacts at the earth's surface. And finally, many Archaea have novel resistance mechanisms, such as extreme radiation resistance, that could have been used to protect them in these extreme environments (Peak *et al.*, 1995; Gerard *et al.*, 2001).

Comparative genomic studies of prokaryotes have shown that only one gene, encoding a type I topoisomerase enzyme, reverse gyrase (RG), is uniquely found in hyperthermophiles. RG is a topoisomerase that has the unique potential to use ATP to produce positively supercoiled DNA (Kikuchi and Asai, 1984), and it has been correlated with the presence of positively supercoiled plasmid and viral DNA in hyperthermophilic organisms. Positively supercoiled DNA is suggested to have greater resistance to thermal denaturation than the negatively supercoiled DNA found in all other cells (Lopez-Garcia and Forterre, 2000). However, both the formation of archaeal nucleosomes and the extreme intracellular salt concentrations also have a significant affect on the structural stability of DNA (Anderson and Bauer, 1978; Ronimus and Musgrave, 1996b).

Here we propose that the ability of archaeal nucleosomes to form both positive and negative supercoils in a salt-dependent manner (Musgrave *et al.*, 2000) and a novel stress response in hyperthermophilic Archaea (Dinger *et al.*, 2000) give us additional clues to how these organisms can

adapt their genome topology to changing environments and act as an alternate system to RG, allowing hypotheses for hyperthermophilic origins of life to be revisited.

MATERIALS AND METHODS

Reference strains

Pyrococcus abyssi GE5 was obtained from Patrick Forterre, University of Paris XI, Paris, France, and *Thermococcus zilligii* was obtained from the Thermophile Research Unit, University of Waikato, Hamilton, New Zealand.

Culturing of T. zilligii *and* P. abyssi

T. zilligii was grown in 850-mL volumes, in 1.0-L Schott bottles as previously described (Klages and Morgan, 1994), and *P. abyssi* cells were cultivated in "YPS" medium (Erauso *et al.,* 1993). The OD_{600} of the samples was measured and converted to cell density by multiplying the OD_{600} by a conversion factor empirically determined by cell counting as described (Koch, 1994).

Isolation of RNA

Total RNA was isolated using TRIZOL LS Reagent (GibcoBRL, Life Technologies, Ltd.), according to the manufacturer's instructions. RNA was recovered after phenol (pH 4.0)/chloroform and chloroform extraction, then precipitated and resuspended in RNase-free water, and stored at −70°C. The concentration and quality of RNA were estimated by absorbance (A_{260}, A_{260}/A_{280}, and A_{260}/A_{230}) measurement.

Northern blot procedures

For northern blots, total RNA samples were denatured by incubation for 15 min at 65°C in an equal volume of a solution containing 20% formalin, 50% formamide, and 0.1 *M* sodium 3-(*N*-morpholino)propanesulfonic acid (MOPS) and then separated by electrophoresis through 2% (wt/vol) agarose gels containing 2.2 *M* formaldehyde, 20 m*M* MOPS, and 8 m*M* sodium acetate at 4.2 V cm^{-1}. After washing the gels for 30 min in 10× saline–sodium citrate (SSC), the RNA was transferred to Hybond$^+$ nylon membranes by capillary blotting for 12 h in 10× SSC. Nucleic acids were UV-cross-linked to the membrane by irradiating the membrane for 45 s with an UV transilluminator. Membrane hybridization was performed essentially as described by Dinger and Musgrave (2000).

Electroblotting from polyacrylamide gels onto PVDF membranes for N-terminal sequencing

Electroblotting of proteins from polyacrylamide gels onto a retentive membrane was used for western blotting and N-terminal protein sequencing (Ausubel *et al.,* 1998). For N-terminal sequencing Trans-Blot membranes (Bio-Rad) were used to transfer proteins from sodium dodecyl sulfate-containing polyacrylamide electrophoresis gels.

The transfer procedure was as described by Ausubel *et al.* (1998). After blotting, the gel was stained with silver or Coomassie Brilliant Blue to check transfer efficiency and photographed. The PVDF membrane was stained with 10% (wt/vol) amido black (naphthol blue black 10B, Sigma) in 10% (vol/vol) acetic acid, destained with 10% acetic acid/30% ethanol, and then drip-dried. The desired band on the membrane was excised and sent for protein N-terminal sequencing. Sequencing was carried out using Edman degradation chemistry on an Applied Biosystem Procise 492 protein sequencer at the Sequencing Facility, Auckland University, Auckland, New Zealand.

Radioactive labeling of DNA probes and ladders

DNA probes were prepared from polymerase chain reaction (PCR) products by random primer labeling using the Rediprime™ II DNA labeling system (Amersham Pharmacia Biotech), following the manufacturer's instructions. The labeled DNA was denatured by adding 7.32 μL of 10 *M* NaOH 10 min prior to use. Single-stranded oligonucleotides were end-labeled using T4 polynucleotide kinase as described by Sambrook *et al.* (1989), stored at 4°C, and used within 3 months of preparation.

Preparation of archaeal histone proteins

Native HTz was purified as previously described (Ronimus and Musgrave, 1996b). Recombinant HMfA and HMfB were purified from *Escherichia coli* as described (Sandman *et al.,* 1990), and recombinant MkaH was purified from *E. coli* as described (Fahrner *et al.,* 2001).

Preparation of DNA substrates

pUC18 plasmid was isolated by alkaline lysis (Sambrook *et al.,* 1989) and CsCl density gradient centrifugation followed by treatment with proteinase K (Sigma), phenol/chloroform extraction, and ethanol precipitation. Relaxed pUC18 plasmids were produced by treating negatively supercoiled plasmid DNA with Topo V (Fidelity Systems) at 70°C or 85°C or with wheat germ Topo I (BRL) at 37°C followed by phenol/chloroform extraction and ethanol precipitation. pUC18 topoisomers of different superhelical densities were produced by relaxation in the presence of ethidium bromide as previously described (Pugh *et al.,* 1989) except that Topo V was used. Positively supercoiled pUC18 control topoisomers were produced by incubation of negatively supercoiled pUC18 DNA with RG (Kikuchi and Asai, 1984).

DNA topology assays

Topology assays and two-dimensional gels to determine the handedness of topoisomers were performed as described (Musgrave *et al.,* 2000).

Gel visualization and photography

Nucleic acids, stained with ethidium bromide, were visualized under UV light, with a TFx-35 UV transilluminator (Life Technologies). The gels were photographed using a COHU high-performance CCD camera and printed on a Sony UP-D890 Digital Graphic Printer. A digital imaging program, Scion Image (release beta 2 12/5/97, Scion Corp.) was used for manipulation of the images.

RESULTS

Recently it has become clear that in eukaryotes chromatin and the packaging of DNA in nucleosomes are intimately involved in the control of gene expression (Wu and Grunstein, 2000). Also, the discovery of histone acetyltransferases, histone deacetylases, and other chromatin remodeling complexes, which can specifically modify the structure of chromatin, has suggested that eukaryotic chromatin is structurally dynamic (Jenuwein and Allis, 2001). The structural plasticity of chromatin is also evidenced by the production of nucleosomes containing H2A.Z, a novel histone that promotes nucleosome instability when incorporated into chromatin, and the ability of nucleosomes to wrap DNA in two opposite configurations forming positive and negative toroidal supercoils (Hamiche *et al.,* 1996; Hamiche and Richard-Foy, 1998; Santisteban *et al.,* 2000). We have suggested that archaeal nucleosomes are also structurally plastic because of their ability to form both positive and negative supercoils in a salt-dependent manner and, in *T. zilligii,* possibly by direct modifications to the nucleoid (Dinger and Musgrave, 2000; Musgrave *et al.,* 2000).

To examine the effect that archaeal chromatin has on global gene expression control we have tested stationary-phase changes in the archaeal nucleoid and have examined the level of transcripts of the histone genes and genes encoding nucleoid proteins at various growth phases in *P. abyssi,* another member of the Thermococcales. To test whether both the *P. abyssi* histone genes (*hpa1* and *hpa2*) and the stress response genes (*srbPa1* and *srbPa2*) were being transcribed in a growth phase-dependent manner, as was found for the homologous genes in *T. zilligii* (Dinger, 1998), total RNA samples were isolated from cells at different growth phases as depicted in Fig. 1. The RNA samples shown in Fig. 2 were subject to northern blotting and hybridization analysis. Individual duplicate N^+ membranes containing total RNA samples were hybridized with one of the probes listed in Table 1. Figure 2 shows that RNA degradation was minimal, as indicated by the equivalent intensities and lack of smearing of the 23S and 16S rRNA bands for each RNA sample.

The northern hybridization results for *hpa1, hpa2,* and *srbPa1/srbPa2* are shown in Figs. 3–5, respectively. A single transcript of 206 bp for *hpa1* and *hpa2* and two transcripts of the predicted sizes of *srbPa1* (585 bp) and *srbPa2* (417 bp) were detected. Southern blots were hybridized at the same time in the same hybridization tube, with the same probe used for northern hybridization as a control for the integrity of the probes used and to confirm the *P. abyssi* genome sequence annotation (Figs. 3B and 4B). The northern hybridization autoradiographs were quantified, and the relative level of each transcript was determined in relation to the level of 23S rRNA in each sample from Fig. 2 (Figs. 3C, 4C, and 5B). The intensity of *hpa1* and that of *hpa2* did not show any significant change at any of the points in the growth phase that were tested.

In contrast, the level of both *srbPa1* and *srbPa2*

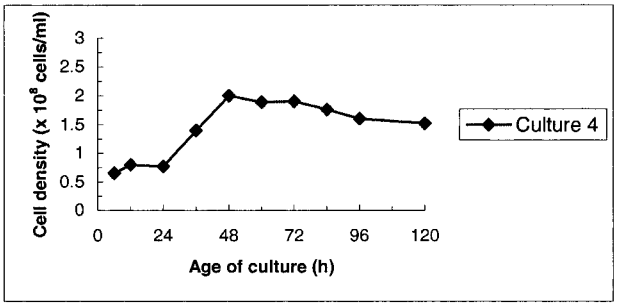

FIG. 1. Growth curve of a representative batch culture of *P. abyssi* cells grown in "YPS" medium at 80°C. The cell density of the culture versus culture age is indicated.

transcripts increased significantly in stationary-phase cells, indicative of induction of *srbPa1* and *srbPa2* gene transcription in these cells. Both *srbPa1* and *srbPa2* transcripts were able to be detected using the *srbPa1* gene probe because these two genes are ~40% identical, but because a single probe was used the relative levels of *srbPa1* and *srbPa2* could not be determined.

Three other genes of *P. abyssi* (*srcPa, srdPa,* and *srePa,* described as encoding stationary-phase response proteins in *T. zilligii*) were expressed constitutively (results not shown). Constitutive expression of these genes was surprising because *T. zilligii* and *P. abyssi* are both members of a same archaeal family, the Thermococcales, and each contain the same suite of highly conserved stationary-phase stress genes (Dinger and Musgrave, 2000). The significantly different expression of the histone and stationary-phase stress genes in these species suggests that the response to stationary-phase stress in these organisms is significantly different. The results also suggest that the depletion of nucleosomes is not a significant factor in the control of global gene expression in *P. abyssi,* as has been suggested for *T. zilligii.*

The dynamic behavior of archaeal nucleosomes has been demonstrated by the ability of the archaeal histones to wrap DNA in both positive and negative supercoils in a salt-dependent manner. This is also seen in Fig. 6, which shows the toroidal wrapping of a number of different histones at a salt concentration in which both positive and negative topoisomers are produced. The results for the HMf histone from *Methanothermus fervidus* and the HTz histone from *T. zilligii* show that, at a salt concentration of 150 m*M*, negative supercoils are produced at protein/DNA mass ratios below ~0.8 (lanes a–e for HMfB, lanes a–f for HMfA+B and HTz). Furthermore, positive supercoils are produced at protein/DNA mass ratios above this (lanes f–j for HMfB and lanes g–j and g–i for HMfA+B and HTz, respectively). We describe the ability of the archaeal nucleosome to adopt two conformations as "nucleosome flipping" in order to suggest that one conformation can be converted to the other, as is seen in the production of both conformations in the same tube. This is most evident in lane g of Fig. 6, in which topoisomers of pUC18 are produced by the binding of the HTz histone isolated from *T. zilligii.* In contrast, only negative supercoils were produced using the histone MkaH from *Methanopyrus kandleri.* MkaH is an unusual archaeal histone in that it is two histone monomers fused in a single polypeptide (Fahrner *et al.,* 2001). We have proposed that this fusion prevents the rearrangement of the monomers that are necessary for the structural changes that allow two nucleosome conformations to occur (Musgrave *et al.,* 2000). Models aimed at explaining the significance of nucleosomal flipping are presented in Figs. 7 and 8. In 1987, Liu and Wang proposed that the tracking of a macromolecule along a DNA molecule would result in the production of positive supercoils in front of the advancing complex and that the equivalent number of negative

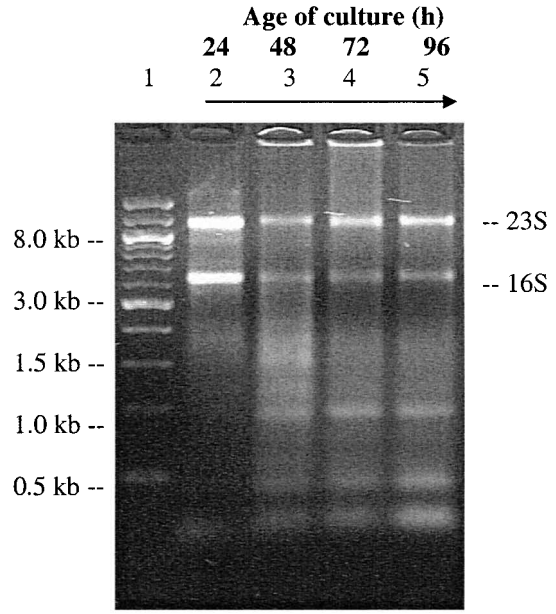

FIG. 2. Total RNA samples extracted from *P. abyssi* cells at various stages of growth, resolved by TBE agarose gel electrophoresis. Lane 1 contains a 1-kb DNA ladder. Lanes 2–5 each contain 2 μg of total RNA, isolated from cells at the times indicated.

TABLE 1. PRIMERS USED FOR PCR AMPLIFICATION OF *P. ABYSSI* GENE PROBES USED IN NORTHERN ANALYSES

HPa1-F	ATGGGAGAGTTG CCAATT
HPa1-R	TCAGCTCTTAATA GCCAA
HPa2-F	ATGGCTGAGTTGC CAATT
HPa2-R	GTCAGCTCCTAAT TGCAA
SrbPa1-F	GTGATGCATATGA GGATCGCGGTTCC AACTAA
SrbPa1-R	AAAAAGGCTCTTC CGCACCACCACCA GCCGTAGAGCCACC

The oligonucleotide primers listed were used to PCR-amplify the histone and stress response protein B genes from *P. abyssi* genomic DNA. PCR products were purified, labeled with γ-^{33}P, and used as a probes for the *P. abyssi hpa* and *srb* transcripts in northern hybridizations using total RNA extracted from *P. abyssi* cells of various ages. The start and stop codons of the respective open reading frames are indicated in boxes, and restriction recognition sites are underlined.

supercoils would be produced behind. This could be envisaged to occur when DNA is replicated or transcribed by the tracking of DNA or RNA polymerase. The production of positive supercoils ahead of a DNA tracking complex is expected to modulate gene expression by decreasing the stability of nucleosomes because nucleosomes have been shown to be unstable on positively supercoiled DNA templates *in vitro*. Therefore, transcriptionally mediated supercoiling is thought to be another way in which the repressive effects of nucleosomes on transcription could be overcome by clearing nucleosomes from in front of the advancing RNA polymerase. This could also contribute to polar effects, when the transcription of one gene is seen to affect the expression of a gene

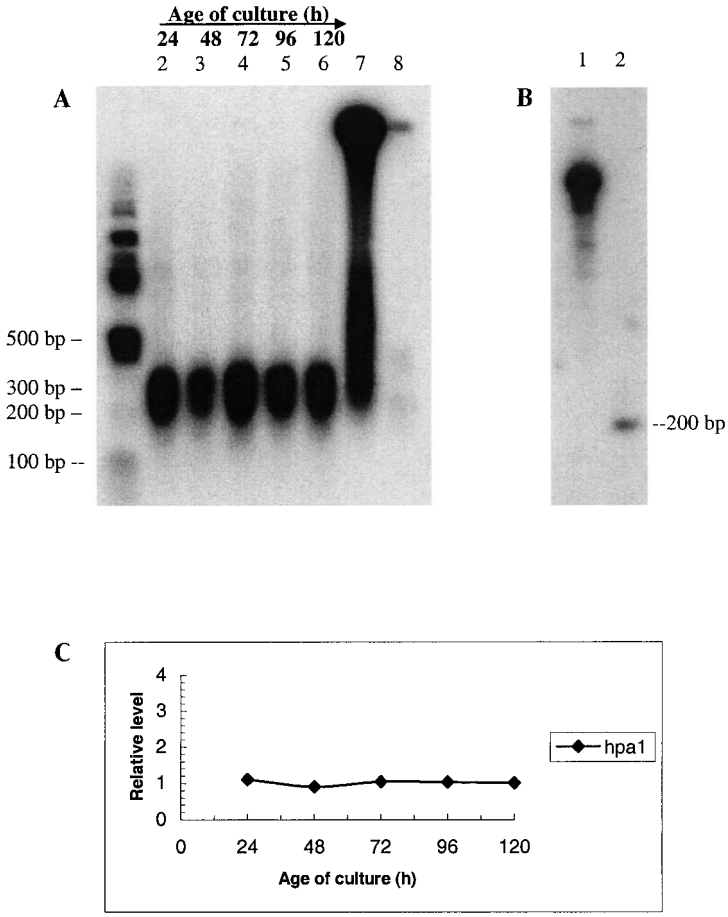

FIG. 3. A: Northern hybridization of total RNA from *P. abyssi* cells after various periods of growth, with the *hpa1* gene probe. Lane 1 contains 1.5 μg of γ-^{33}P-5′ end-labeled DNA marker, with sizes indicated. Lanes 2–6 each contain 10 μg of total RNA from cells of the ages indicated. Lane 7 contains 1 ng of *hpa1* PCR product, and lane 8 contains 1 ng of *hpa2* PCR product, as a positive control. **B:** Southern hybridization controls for *hpa1.* Lane1 contains 3 μg of *Sac*I-digested *P. abyssi* genomic DNA, and lane 2 contains 0.5 ng of the *hpa1* PCR product. **C:** Levels of *hpa1* mRNA relative to the corresponding 23S rRNA levels versus culture age. Levels were determined by densitometric analysis of the northern blots shown in (A) and were compared with the level of 23S rRNA stained with methylene blue.

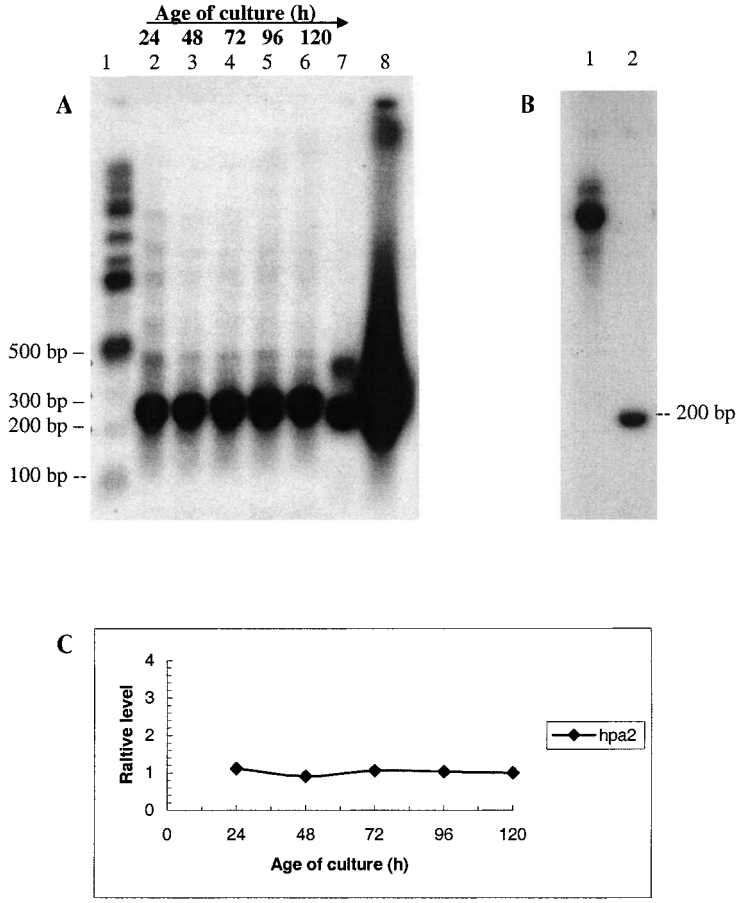

FIG. 4. **A:** Northern hybridization of total RNA from *P. abyssi* cells after various periods of growth, with the *hpa2* gene probe. Lane 1 contains 1.5 μg of γ-^{33}P-5′ end-labeled DNA marker, with sizes indicated. Lanes 2–6 each contains 10 μg of total RNA from cells of the ages indicated. Lane 7 contains 1 ng of *hpa1* PCR product, and lane 8 contains 1 ng of *hpa2* PCR product as positive controls. **B:** Southern hybridization controls for *hpa2*. Lane 1 contains 3 μg of *Sac*I-digested *P. abyssi* genomic DNA, and lane 2 contains 0.5 ng of *hpa2* PCR product. **C:** Relative levels of *hpa2* mRNA to the corresponding 23S rRNA levels versus their culture ages. The mRNA levels were determined as described for Fig. 3.

downstream. In Fig. 7 we propose a topology sensing model in which the ability for nucleosomes to flip from one conformation to another could negate the topological effects produced by protein tracking. Flipping from a negative to a positive supercoil within the archaeal nucleosome could minimize the positive supercoils produced ahead of a tracking complex, and, conversely, flipping in the opposite direction could maximize it. The former change could be used by the cell to minimize the topological effects produced by transcription or replication, whereas the latter change could maximize the ability of the advancing complex to clear nucleosomes ahead of it and hence modify the topology of downstream genes. A temperature sensing model is proposed in Fig. 8 in which nucleosome flipping could be used by the cell to accommodate changes in the temperature of its surrounding environment. Many hyperthermophilic archaeal species contain concentrations of salts in their cytoplasm as high as 1 *M*, and increased internal salt concentrations are correlated with increased growth temperatures in archaeal cells (Hensel and Konig, 1988; Kurr *et al.*, 1991). Since the wrapping of DNA in archaeal nucleosomes is sensitive to salt concentration (Musgrave *et al.*, 2000) and since negative supercoils are substituted for positive supercoils as the salt concentration is increased, we envisage that nucleosome flipping could be utilized by the cell to bring about a rapid change in the topology of genomic DNA when cells are required to adapt to different growth temperatures. The ability of both archaeal and eucaryal nucleosomes to wrap DNA in both positive and negative supercoils is also another indicator of the shared evolutionary history of the Euryarchaeota and eukaryotes. Nucleosome flipping of the eucaryal H3/H4 core tetramer also suggests that the evolution of the eucaryal nucleosome, by the addition of flanking H2A/H2B dimers on each side, has stabilized the octamer in a negative orientation. This may be required to limit its structural flexibility, given the seemingly unlimited posttranslational modifications that are possible for eucaryal histone proteins and the

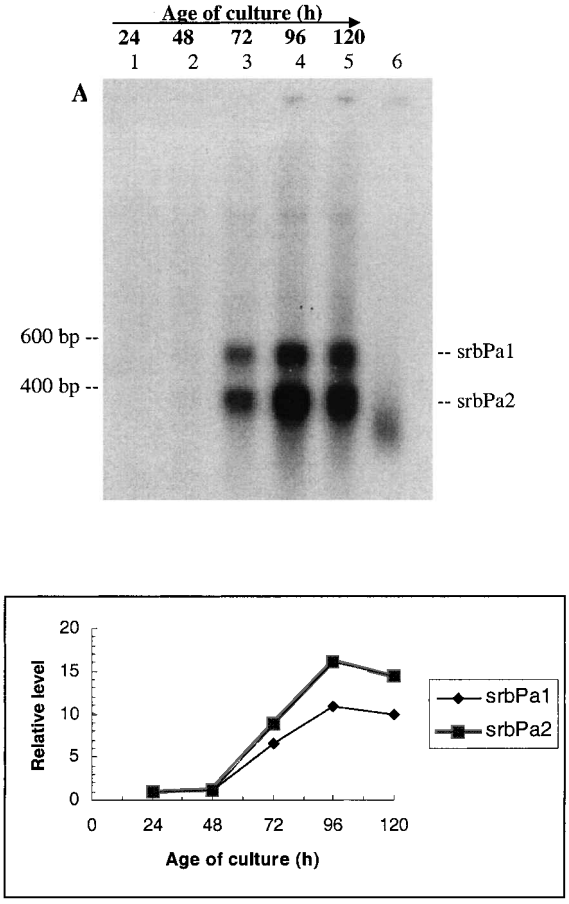

FIG. 5. **A:** Northern hybridization of total RNA from *P. abyssi* cells after various periods of growth, with the *srbPa1* gene probe. Lanes 1–5 each contain 10 μg of total RNA corresponding from cells of the ages indicated. Lane 6 contains 1 ng of *srbPa1* PCR product as a positive control. **B:** Relative levels of *srbPa1* and *srbPa2* mRNA to the corresponding 23S rRNA levels versus their culture ages. The mRNA levels were determined as described in Fig. 3.

fact that topoisomerases capable of increasing the superhelicity of DNA have not been found in the Eucarya.

DISCUSSION

RG is a type I DNA topoisomerase that has the unique ability to use ATP to drive the production of positive supercoils. It is proposed that the positive supercoils produced by RG result from the tracking of the enzyme along the DNA in a helicase-like fashion and the consequent removal of negative supercoils by its topoismerase I-like activity, resulting in the production of net positive supercoils in the DNA (Declais *et al.*, 2000). This view of RG is influenced by its low ATP requirement, and by the observation that the two domains can function separately (Collin *et al.*, 1988; Collin, 1990; Declais *et al.*, 2000). RG has been at the center of many "hot origin" hypotheses for the origin of cellular life because it is, to date, the only protein that is exclusively found in hyperthermophilic species and is thus seen to be essential to life at high temperature. However, since RG is considered to be a modern enzyme, constructed by the fusion of a topoisomerase I and a DNA helicase (Declais *et al.*, 2000), it has been proposed that life could not have evolved at the temperatures inhabited by hyperthermophilic microorganisms. In "lower temperature" origin hypotheses, cellular life is proposed to have evolved both up temperature as well as down temperature (Forterre, 1995). However, because of the lack of a robust genetic system in hyperthermophilic organisms, it has yet to be proven that RG is essential for life at all, let alone essential for hyperthermophily. Also, positively supercoiled DNA could be produced in a number of other ways in the absence of RG. Firstly, the negative wrapping of DNA by archaeal histones, as seen in Fig. 6, produces positive supercoils in nucleosome-free DNA. Secondly, positive supercoils can be produced by the removal of negative supercoils by a conventional type I topoisomerase. Thirdly, as suggested here, positive wrapping could also be the result of nucleosome flipping caused by an increase in intracellular salt concentrations or the selective removal of negative supercoils by a conventional type I topoisomerase following the tracking of a complex along the DNA. These methods could have been used to stabilize DNA molecules in the early evolution of cellular life at temperatures currently inhabited by hyperthermophiles in the absence of RG. RG is thus proposed to be a modern solution to the DNA stability problem in hyperthermophiles. In addition to modulating topology and the production of inherently stable positive supercoils in nucleosome-free DNA, the wrapping of DNA in nucleosomes has a significant effect on the temperature stability of double-stranded DNA in its own right. The melting temperature of DNA is increased by 20°C by the formation of HTz histone nucleosomes *in vitro* (Ronimus and Musgrave, 1996b). The melting temperature of DNA can also be increased significantly by charge neutralization resulting from an increase in the ionic strength of the cytoplasm or an increase in the concentration of basic proteins, peptides, or other

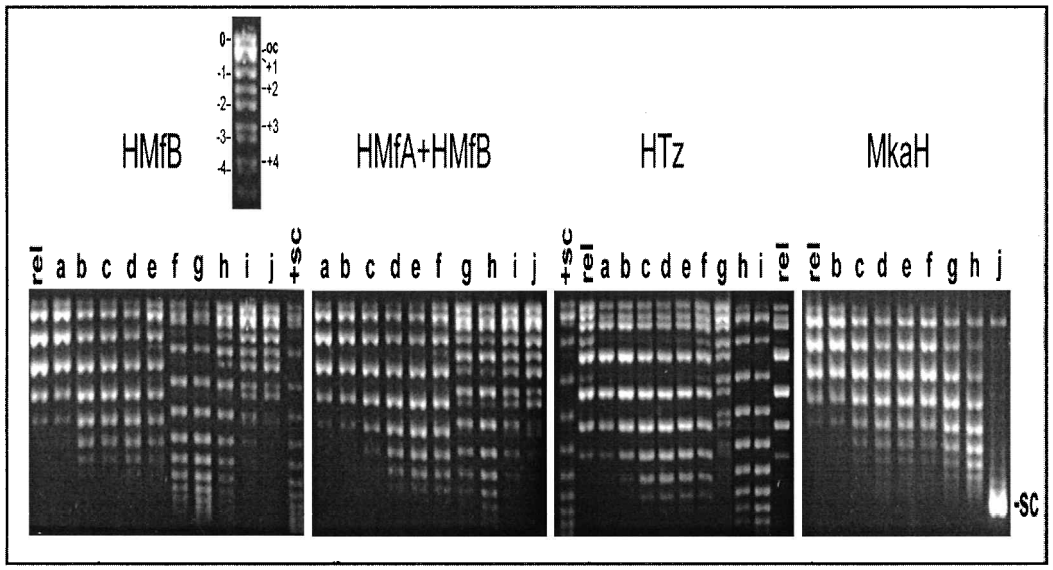

FIG. 6. DNA topology assays of the archaeal histones: recombinant HMfB homodimer from *M. fervidus*, recombinant HMfA/B heterodimers, HTz native heterodimer from *T. zilligii*, and native MkaH from *M. kandleri*. pUC18 (150 ng) relaxed at 70°C in 150 m*M* potassium glutamate was incubated in these conditions with archaeal histone proteins at protein/DNA mass ratios of (a) 0:1, (b) 0.1:1, (c) 0.15:1, (d) 0.3:1, (e) 0.6:1, (f) 0.8:1, (g) 1:1, (h) 1.5:1, and (i) 2:1. Topoisomerase V (20 U) was added, and the incubation was continued for 15 min. Reaction mixes were incubated with 1% sodium dodecyl sulfate for 2 min at 90°C and treated with 25 μg/ml proteinase K for 30 min at 40°C. Topoisomers were separated by 1.5% agarose gel electrophoresis at 1 V/cm for 16 h with buffer circulation. Lanes labeled "rel" are untreated substrate molecules, and those labeled +SC are pUC18 positively supercoiled topoisomer controls.

positively charged species. However, considerations of the *in vivo* stability of DNA must be considered with some caution, as its cellular concentration cannot be approached *in vitro.* For example, a bacterial chromosome must be condensed 1,000-fold to fit inside the cell, resulting in a cellular concentration of DNA of between 14 and 34 μg/mL (Bohrmann *et al.,* 1991).

The ability of archaeal histones to wrap DNA in two alternate topologies, positive and negative toroidal supercoils, allows archaeal nucleosomes to be structurally dynamic. Initially we suggested that positive toroidal supercoils might be necessary to counteract the affect that cytoplasms containing high salt might have on the cellular DNA in hyperthermophiles (Musgrave *et al.,* 1991, 1992). We reasoned that positive wrapping would result in an increase in the negative superhelicity of free DNA and that this wrapping would have the opposite effect to that exerted by intracellular salt. At low salt concentrations all archaeal histones wrap DNA in positive toroidal supercoils. However, as the salt concentration is increased, the wrapping becomes both positive and negative in the same reaction and possibly on the same molecule. This is evidenced by the positively supercoiled molecules produced at low protein/DNA mass ratios and negatively supercoiled molecules produced at higher protein/DNA mass ratios in the topology assay shown in Fig. 6. At 150 m*M* potassium glutamate a switch from positive to negative wrapping occurs with a very small increase in added protein. We suggest here that this structural plasticity could be used by the cell to modulate the topology of chromosomal DNA in a rapid and energetically efficient way. We propose that this "nucleosome flipping" could allow Archaea to rapidly adjust the topology of their genome in response to environmental changes. Because Archaea have been shown to increase their cytoplasmic salt concentrations in response to temperature increases (Hensel and Konig, 1988; Kurr *et al.,* 1991) we propose here that nucleosome flipping could also result from such an increase. The result of this would be to switch the toroidal wrapping in the nucleosomes from positive to negative as the temperature increased. This would cause the free DNA to become more positively supercoiled, a result that is similar to the action of RG, and consequently the free DNA would be less prone to denaturation. An example of a drastic topologi-

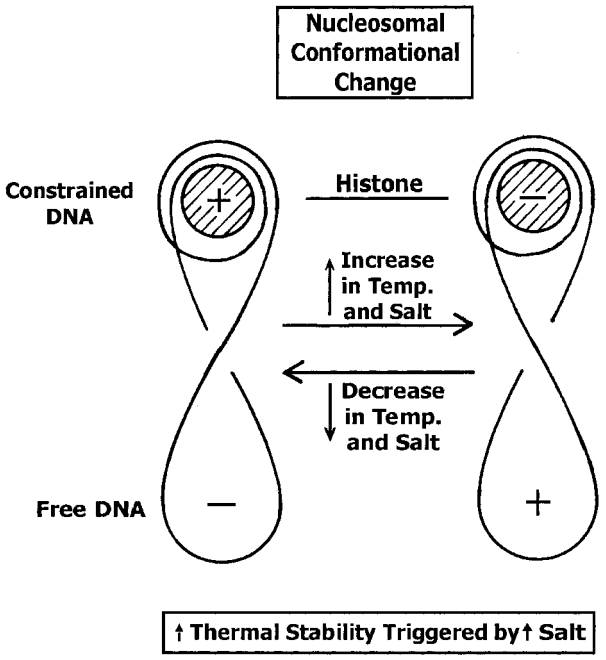

FIG. 7. Topology sensing via protein tracking-induced conformational change of the DNA in archaeal nucleosomes. A model is presented to propose that archaeal nucleosome conformational changes could modulate the topological changes that occur in DNA as a result of the tracking of a protein complex. Archaeal nucleosomes, depicted as half-shaded circles, can wrap the DNA in positive and negative toroidal supercoils. Negative and positive plectonemic supercoils are produced in the free DNA by protein tracking along the DNA. Conformational changes in the mode of wrapping of the DNA by the archaeal nucleosomes, from positive to negative on the left and negative to positive on the right, would negate the plectonemic supercoils in the free DNA.

cal change brought about by a change in DNA packaging is the positive wrapping of the DNA found in the spores of *Bacillus subtilis.* Positive wrapping, as opposed to the negative wrapping of bacterial chromosomal proteins found in vegetative cells, is induced by the small acid-soluble spore proteins (SASPs). This wrapping is suggested to account for the resistance of spore DNA to irradiation by UV light because SASP/DNA complexes *in vitro* prevent the production of DNA-damaging thymine dimers (Nicholson *et al.,* 1991; Griffith *et al.,* 1994).

Another potential function of nucleosome flipping could be to compensate for changes in genome topology that accompany DNA replication and transcription as described in Fig. 7. Transcriptionally mediated supercoiling occurs because the mass of the RNA transcript with its attached ribosomes is too great to be rotated around the DNA helix during transcription. Therefore, for transcription to proceed the DNA must effectively rotate, resulting in the production of supercoils in the DNA (Liu and Wang, 1987; Wu *et al.,* 1988). Because DNA strands are not broken, the positive supercoils produced ahead of RNA polymerase result in an equal number of negative supercoils behind the complex. A change in the toroidal supercoiling in archaeal nucleosomes from negative to positive would decrease the positive superhelicity ahead of tracking complex, and *vice versa.* With eucaryal nucleosomes it has been proposed that the positive supercoils produced ahead of RNA polymerase could help to remove nucleosomes form the DNA, leading to more efficient elongation of the transcript. This model is based on the instability of nucleosomes on positively supercoiled DNA and thermodynamics suggesting that formation of negatively wrapped nucleosomes on negatively supercoiled DNA is favored because of the effective reduction in net negative supercoils. In Eucarya, the dynamic nature of the nucleosome is modulated by remodeling. However, it appears that archaeal nucleosomes are not subject to the same remodeling because archaeal histones do not have the required N-terminal tails, and, in *T. zilligii,* histones with altered mass have not been isolated (Dinger *et al.,* 2000).

In addition to the role that the wrapping of archaeal chromosomes in nucleosomes might play in allowing cellular life at high temperatures, we have described a stationary-phase stress response protein that has a phylogenetic profile that mimics that seen for RG (Dinger *et al.,* 2000). This gene, named *srb,* is found in the genomes of both archaeal and bacterial thermophiles. We previously detected SRB protein as a stationary phase-induced acid-soluble protein in the hyperthermophile *T. zilligii* (Dinger *et al.,* 2000). In accord with this, we show here that transcription of the *srbPa* paralogues is also stationary phase-induced in *P. abyssi,* another hyperthermophilic member of the Thermococcales. SRB protein has been crystallized from *Methanobacterium thermoautotrophicum,* and the structure has been used to search for structural homologues. Although highly significant matches were not made, the closest structural homologues all have roles in nucleic acid metabolism or processing, a role that agrees with a GR repeat found in the C-terminus of some SRB proteins (Cort *et al.,* 2000). In addition to testing the transcription of the *srb* genes in stationary-

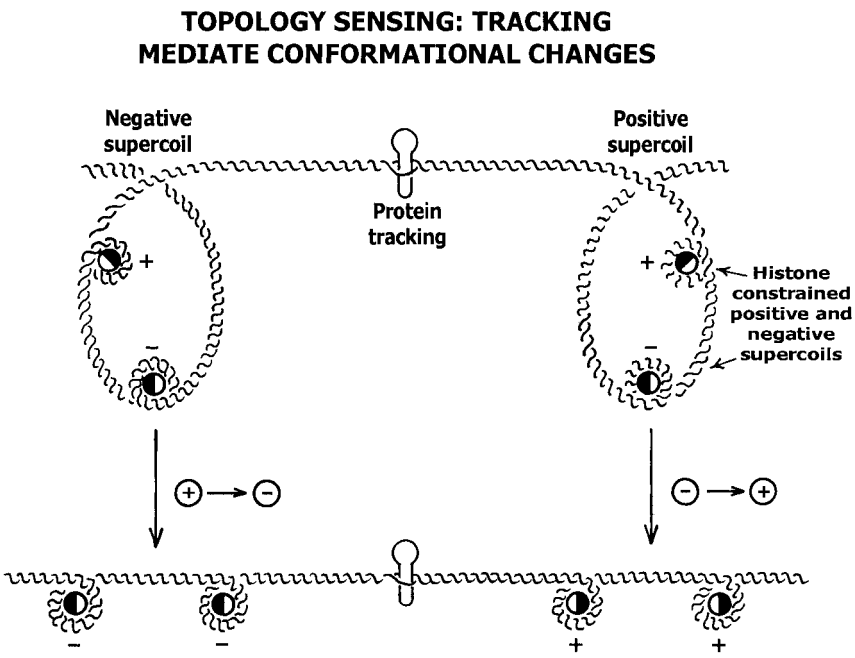

FIG. 8. Temperature sensing via a salt-induced conformational change of the DNA in archaeal nucleosomes. A model is presented to propose that archaeal nucleosome conformational changes could modulate the topological changes that occur in DNA as a result of a change in intracellular salt concentrations induced by a change in temperature of the environment, or another physical parameter that would influence the topology of genomic DNA. Archaeal nucleosomes, depicted as hatched circles labeled (+) or (−), can wrap the DNA in positive and negative toroidal supercoils, respectively. An increase in temperature, salt, or both would cause archaeal nucleosomes to "flip" from a positive toroidal supercoil to a negative toroidal supercoil, and *vice versa.* An increase in temperature would therefore result in the free DNA more positively supercoiled, a response similar to that effected by the topoisomerase RG.

phase cells we have shown that there are contrasting transcription patterns of the histone paralogues in *T. zilligii* and *P. abyssi.* We previously reported that the histone proteins were rapidly depleted from cells in the stationary phase in *T. zilligii* and that the level of the *htz1* gene transcript was decreased as cells entered the stationary phase and was not able to be detected by northern hybridization using stationary-phase cells (Dinger *et al.,* 2000). In this study we show that, in contrast with the similar stationary-phase induction of expression of the *srb* genes, the histone genes (*hpa1* and *hpa2*) are not transcribed in such an obvious stationary phase-repressed manner in *P. abyssi* as was shown for *htz1* and *htz2 (*Zhang, 2001*).* Consistent with this result, the levels of the *sre* gene transcript, which may encode a histone replacement protein, is likewise not stationary phase-induced in *P. abyssi.* From these results we conclude that the nucleiod and hence the topology of genomic DNA is dynamic in the Archaea. Therefore the mechanisms for genome packaging should be considered along with the function of topoisomerases in discussing the role that the physical and genetic stability of the genome plays in cellular life and the evolution of that life. We also conclude that the systems induced to allow Archaea to survive stationary phase show significant diversity given that *T. zilligii* and *P. abyssi* are members of the same archaeal family. This should promote a wider examination of these mechanisms, particularly since the recently described archaeal symbiont *NanoArchaeaum equitans,* whose 16S rRNA sequence suggests it belongs to a new phylum, are found associated predominantly with stationary-phase cells (Huber *et al.,* 2002).

ABBREVIATIONS

MOPS, sodium 3-(*N*-morpholino)propanesulfonic acid; PCR, polymerase chain reaction; RG, reverse gyrase; SASP, small acid-soluble spore protein; SSC, saline–sodium citrate.

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