Extremophiles (1998) 2:75–81

ORIGINAL PAPER

Divya Soares · Isabel Dahlke · Wen-Tyng Li
Kathleen Sandman · Carina Hethke · Michael Thomm
John N. Reeve

Archaeal histone stability, DNA binding, and transcription inhibition above 90°C

Received, September 25, 1997 / Accepted: December 15, 1997

Abstract The DNA binding and compacting activities of the recombinant (r) archaeal histones rHMfA and rHMfB from *Methanothermus fervidus*, and rHPyA1 from *Pyrococcus* species GB-3a, synthesized in *Escherichia coli*, have been shown to be completely resistant to incubation for 4 h at 95°C in the presence of 1 M KCl. Continued incubation of rHMfA and rHMfB at 95°C resulted in a gradual loss of these activities, and rHMfA and rHMfB lost activity more rapidly at 95°C when the salt environment was reduced to 200 mM KCl. rHPyA1, in contrast, retained full activity even after a 60-h incubation at 95°C in 1 M KCl, and reducing the salt concentration did not affect the heat resistance of rHPyA1. rHPyA1–DNA complexes remained intact at 100°C, and rHPyA1 bound to the template DNA in in vitro transcription reaction mixtures assembled using *Pyrococcus furiosus* components at 90°C. Transcription in vitro from the *P. furiosus gdh* promoter was reduced by rHPyA1 binding, in a manner that was dependent on the histone-to-DNA ratio and on the topology of the DNA template. Transcription from circular templates was more sensitive to rHPyA1 binding than transcription from a linear template, consistent with rHPyA1 binding introducing physical barriers to transcription and causing changes in the topology of circular templates that also reduced transcription.

Key words Protein stability · Salt dependence · Genome stabilization · In vitro transcription

Communicated by K. Horikoshi

D. Soares · W-T Li · K. Sandman · J.N. Reeve (✉)
Department of Microbiology, Ohio State University, Columbus, OH 43210, USA
Tel. +1-614-292-2301; Fax +1-614-292-8120
e-mail: reeve.2@osu.edu

I. Dahlke · C. Hethke · M. Thomm
Institut für Allegemeine Mikrobiologie, Universtität Kiel, 24118 Kiel, Germany

Introduction

All cells must compact their genomic DNA; however, hyperthermophiles face an additional problem, that of heat denaturation. Studies undertaken to address this issue revealed the presence of histones in the hyperthermophile *Methanothermus fervidus* (Krzycki et al. 1990; Sandman et al. 1990) that share a common structure, the histone fold (Arents and Moudrianakis 1995; Ramakrishnan 1995; Starich et al. 1996), and a common ancestry with the eucaryal nucleosome-core histones H2A, H2B, H3, and H4 (Arents et al. 1991; Grayling et al. 1996; Reeve et al. 1997). Eighteen archaeal histone gene sequences have now been established, twelve from hyperthermophiles [*M. fervidus, Pyrococcus* strain GB-3a, *Thermococcus* strain AN1, *Methanococcus jannaschii*, and *Archaeoglobus fulgidus* (Sandman et al. 1994b; Ronimus and Musgrave 1996; Bult et al. 1996; Klenk et al. 1997), three from the thermophile *Methanobacterium thermoautotrophicum* (Tabassum et al. 1992; Smith et al. 1997), and three from a mesophile, *Methanobacterium formicicum* (Darcy et al. 1995). Histones never exist as polypeptide monomers in solution, but form very stable dimers. In *Eukarya*, only histone (H2A + H2B) and (H3 + H4) heterodimers are formed (Arents et al. 1991; Karantza et al. 1995), whereas *M. fervidus* contains both homodimers and heterodimers of HMfA and HMfB (Sandman et al. 1994a). Homogeneous preparations of recombinant (r) HMfA and rHMfB homodimers, obtained by expression of the cloned *hmfA* and *hmfB* genes in *Escherichia coli*, have different DNA binding and compacting properties (Sandman et al. 1994a; Grayling et al. 1996), and the analysis of these *M. fervidus* proteins has now been extended to determine their heat resistances. Comparisons have also been made with the heat resistance of the DNA binding and compacting activities of rHPyA1, synthesized in *E. coli* by expression of the *hpyA1* gene cloned from *Pyrococcus* strain GB-3a (Sandman et al. 1994b).

As a cell-free transcription system is available from the closely related hyperthermophile *Pyrococcus furiosus*

(Hethke et al. 1996), it was also possible to evaluate the effects of rHPyA1 binding to the template DNA on transcription in an homologous system at 90°C. rHPyA1 binding stabilized the double-stranded structure of DNA molecules at 90°C, but reduced transcription in a manner that was dependent on the histone-to-DNA ratio and on the topology of the template.

Materials and methods

hpyA1 subcloning and expression in *E. coli*

The *hpyA1* gene was subcloned on a *Hin*dIII-*Sst*I fragment from pKS391 (Sandman et al. 1994b) into pUC18 (Yanisch-Perron et al. 1985) and polymerase chain reaction (PCR) amplified from the resulting plasmid, pKS392, using the universal sequencing primer #1211 [New England Biolabs (NEB), Beverly, MA, USA] and 5′AAGGATCCCCCATAAATAACTG as primers. The amplified product was digested with *Bam*HI and *Dra*I, ligated with *Bam*HI- and *Eco*RV-digested pLITMUS28 (NEB), and the integrity of the *hpyA1* gene was confirmed by sequencing. The *Bam*H1-*Pst*I fragment that carried *hpyA1* in pLITMUS28 was subcloned into the pT7-7 expression vector, resulting in pDS4.

An ampicillin-resistant pDS4 transformant of *E. coli* B834 (DE3) (Novagen, Madison, WI, USA) was grown aerobically at 37°C in Luria-Bertani medium (Sambrook et al. 1989) that contained 50 μg ampicillin/ml, to an OD_{600} of ~0.6. Isopropyl β-D-thiogalactopyranoside (IPTG) was added (final concentration of 400 μM), and incubation continued for 16 h at 37°C.

Purification of rHMfA, rHMfB, and rHPyA1

The procedures used to generate and purify rHMfA and rHMfB have been described previously (Sandman et al. 1995). Following the IPTG-induced synthesis of rHPyA1, *E. coli* B834 (pDS4) cells were harvested, resuspended in 100 mM NaCl, 50 mM Tris-HCl, 2 mM Na_2HPO_4 (pH 8), and ruptured by passage through a French pressure cell. DNase I (20 μg/ml) and $MgCl_2$ (5 mM final concentration) were added to the supernatant obtained after centrifugation at 30000 *g* for 30 min at 4°C, and 100000 *g* for 90 min. The mixture was incubated for 2 h at 37°C, solid NaCl added to a final concentration of 3 M, and the precipitate that formed after incubation for 10 min at 95°C was removed by filtration through a 0.45-μm Millipore membrane. The filtrate was dialyzed overnight at 4°C against 20 mM K_3-citrate, 50 mM Tris-HCl (pH 8), and loaded onto a HiTrap heparin-Sepharose column (Pharmacia Biotech, Uppsala, Sweden) equilibrated with 20 mM K_3-citrate, 50 mM Tris-HCl (pH 8). Proteins were eluted from the column using a linear gradient of 20–200 mM K_3-citrate in 50 mM Tris-HCl (pH 8), and an aliquot of each fraction was subjected to tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie brilliant blue staining. rHPyA1 migrated through 15% (w/v) polyacrylamide gels to a region unoccupied by *E. coli* proteins, which facilitated the identification of rHPyA1-containing column fractions.

Electrophoretic mobility-shift assays (EMSA)

Archaeal histone–DNA complexes, formed with DNA molecules longer than ~1.8 kbp, migrate faster through 0.8% agarose gels during electrophoresis at 1 V/cm than do the protein-free DNA molecules alone (Sandman et al. 1990). Changes in the electrophoretic mobility of *Eco*RI-linearized pBR322 molecules were used to assay the effects of extended periods of incubation at 95°C on the DNA-compacting activities of rHMfA, rHMfB, and rHPyA1. This EMSA was also used to document rHPyA1 binding to the pLUW479 template DNA in in vitro transcription reaction mixtures assembled at 90°C (Hethke et al. 1996)

Archaeal histone binding to shorter DNA molecules results in complexes that migrate with reduced electrophoretic mobilities through native polyacrylamide gels (Grayling et al. 1997). A 117-bp DNA molecule was generated from pUC19 by PCR, using as primers the #1211 and #1201 forward and reverse sequencing primers (NEB), and rHPyA1 binding to this molecule was assayed by gel retardation. Electrophoresis was through 8% T, 1.3% C polyacrylamide gels run at 8 V/cm in 0.5 X Tris-borate-ethylene diaminetetraacetate (EDTA) buffer at room temperature (Sambrook et al. 1989). DNA and DNA–rHPyA1 complexes were visualized within gels by ethidium bromide staining.

In vitro transcription

Construction of the template, pLUW479, and the primer extension and electrophoresis assays used to identify and quantitate transcripts initiated in vitro from the *P. furiosus gdh* (glutamate dehydrogenase) promoter on this template have been described in detail (Hethke et al. 1996; Hausner et al. 1996). Transcription reaction mixtures contained 1 μg of template DNA, 80 mM hydroxyethylpiperazine ethanesulfonic acid (HEPES) (pH 6.5), 2.5 mM $MgCl_2$, 300 mM KCl, 10 mM dithiothreitol, 35 ng recombinant *Pyrococcus* TATA-binding protein (aTBP), 12 ng recombinant *Pyrococcus* transcription factor IIB (aTFB), 2.5 μl of phenyl-Sepharose purified *Pyrococcus* RNA polymerase (RNAP), and increasing amounts of rHPyA1. Transcription, initiated by the addition of rNTPs, was allowed to proceed for 15 min at 90°, and then terminated by adding an equal volume of 0.6 M Na acetate. The RNA products were phenol extracted, and reverse transcriptase used to quantitate transcripts initiated at the *gdh* promoter. Primer extension products, generated by reverse transcriptase with a [^{32}P]-end-labeled primer complementary to positions +75 through +94 of a *gdh* transcript, were quantitated by autoradiography and scanning densitometry (Hethke et al. 1996).

Results

DNA compaction and heat resistance of DNA-compacting activities

rHMfA and rHMfB have primary sequences that are 84% identical to each other, and that are 59% and 56% identical, respectively, to the sequence of rHPyA1 (Fig. 1). The nucleoprotein complexes formed by rHMfA, rHMfB, and rHPyA1 with linear pBR322 molecules, all migrate faster during electrophoresis through agarose gels than do pBR322 molecules alone, although each of these histone–DNA complexes has a different mobility. At the protein-to-DNA ratios that give maximum gel-shifts, rHMfB-containing complexes migrate faster than rHMfA-containing complexes which, in turn, migrate faster than rHPyA1–containing complexes (Figs. 2, 3).

Using this EMSA, the effects of extended periods of incubation at 95°C on the DNA-compacting activities of the three recombinant histones were determined. All three proteins retained full activity after a 4-h incubation at 95°C in buffer containing 1 M KCl, but rHMfB preparations began to lose activity after only 2 h, and rHMfA preparations after 4 h at 95°C when the salt concentration was reduced to 200 mM KCl. In contrast, rHPyA1 lost no activity after 4 h at 95°C in 200 mM KCl, and formed complexes that migrated faster than the complexes formed by control, unheated rHPyA1 preparations. The basis for this apparent increase in compaction remains obscure, although it could reflect a gradual loss of *E. coli* DNA fragments that bound to rHPyA1 during the purification of the protein from *E. coli*.

Although there was no loss of DNA-compacting activity during a 4-h incubation at 95°C in the presence of 1 M KCl,

```
                |-helix I-|          |---------helix II---------|        |-helix III-|
HPyA1      MGELPIAPVDRLIRKAGAERVSEEAAKILAEYLEEYAIEVSKKAVEFARHAGRKTVKAEDIKLAIKS*
           1         10        20        30        40        50        60        69
HMfA       MGELPIAPIGRIIKNAGAERVSDDARIALAKVLEEMGEEIASEAVKLAKHAGRKTIKAEDIELARKMFK*
HMfB       M-ELPIAPIGRIIKDAGAERVSDDARITLAKILEEMGRDIASEAIKLARHAGRKTIKAEDIELAVRRFKK*
HMtA1      MAELPIAPVGRIIKNAGAQRISDDAREALAKILEEKGEEIAKEAVKLAKHAGRKTVKASDIELAAKKL*
HMtA2      MAELPIAPVGRIIKNAGAQRISDDAKEALAKALEEMGEEISRKAVELAKHAGRKTVKATDIEMAAKQL*
HMtB       M-ELPIAPIGRIIKNAGAEIVSDDAREALAKVLEAKGEEIAENAVKLAKHAGRKTVKASDIELAVKRM*
HFoA1      MAELPIAPVGRIIKNAGAPRVSDDARDALAKVLEEMGEGIAAEAVKLAKHAGRKTVKASDIEMAVKAA*
HFoA2      MAELPIAPVGRIIKNAGAQRISDDAKEALAKALEENGEELAKKAVELAKHAGRKTVKAEDIEMAVKSA*
HFoB       M-ELPIAPIGRIIKNAGAERVSDDAREALAKALEEKGETIATEAVKLAKHAGRKTVKASDVELAVKRL*
HPyA2      MAELPIAPVDRLIRKAGAQRVSEQAAKLLAEHLEEKALEIARKAVDLAKHAGRKTVKAEDIKLAIRS*
HAN1A1     MAELPIAPIDRLIRKAGAERVSEDAAKALAEYLEEYAIEVGKKATEFARHAGRKTVKAEDVRLAVKA*
HAfA       MAELPMAPVDRLIRKAGAERVSADAVEKMVEVLEDYAITVAKKAVEIAKHSGRKTVTADDIKLALSM*
HAfB       M-ELPLAPVERLLRKAGASRVSEDAKVELAKAIEEYAMQIGKKAAELAKHAGRKTVKVDDIKLALREL*
MJ0168     MAELPVAPFERILKKAGAERVSEAAAEYLAEAVEEIALEIAKEAVELAKHAKRKTVKVEDIKLALKK*
MJ0932     MAELPVAPFERILKKAGAERVSRAAAEYLAEAVEEIALEIAKEAVELAKHAKRKTVKVEDIKLALKQ*
MJ1258     MAELPVAPCVRILKKAGAQRVSEAAGKYFAEALEEIALEIARKSVDLAKHAKRKTVKVEDVKAALRG*
MJECL17    MAELPVAPFVRILKKDGAERVSRAAAEYFAEAIEDLALEIAKEAVDLAKHAKRKTVKVEDVKLALKK*
MJECL29    MTELPVAPFERILKKVGAERVSRAAAEYLAEAFEEIALEIAKEAVDLAKHAKRKTVKVEDIKLALKK*
```

Fig. 1. Alignment of the archaeal histone amino acid sequences. The HPyA1 and A2 sequences are from *Pyrococcus* GB-3a (Sandman et al. 1994b); HMfA and B from *M. fervidus* (Sandman et al. 1996); HMtA1, A2, and B from *Methanobacterium thermoautotrophicum* ΔH (Tabassum et al. 1992; Smith et al. 1997); HFoA1, A2, and B from *Methanobacterium formicicum* (Darcy et al. 1995); HAN1A1 from *Thermococcus* Strain AN1 (Ronimus and Musgrave 1996); HAfA and B from *Archaeoglobus fulgidus* (Klenk et al. 1997); and the MJ sequences from *Methanococcus jannaschii* (Bult et al. 1996). The three α-helical regions that form the histone fold (Arents and Moudrianakis 1995; Starich et al. 1996) are indicated *above* the HPyA1 sequence

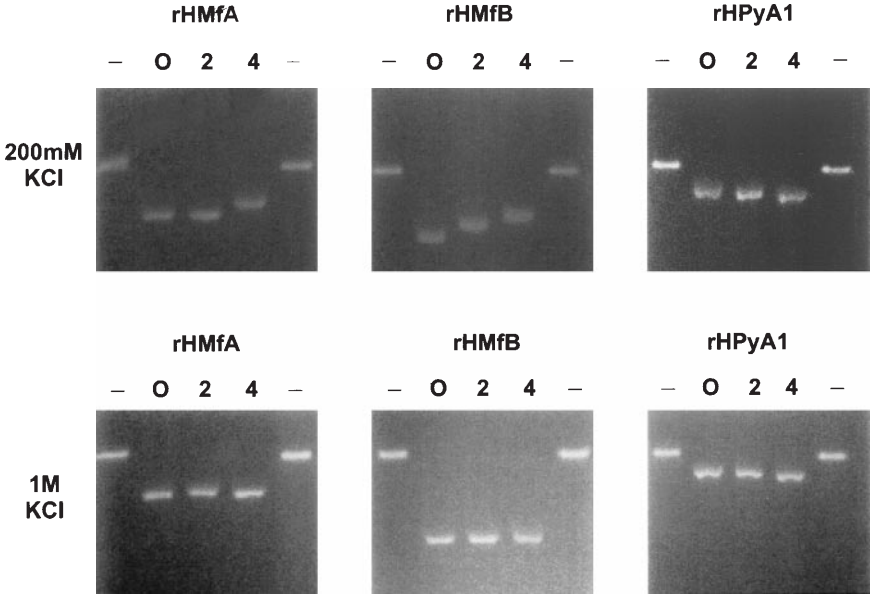

Fig. 2. Mobility shift assays of heat-treated rHMfA, rHMfB, and rHPyA1. Aliquots of rHMfA, rHMfB, and rHPyA1 were incubated at 95°C for 0, 2, and 4 h in 25 mM potassium phosphate buffer (pH 7) that contained either 200 mM KCl (*upper gels*) or 1 M KCl (*lower gels*). Nucleoprotein complexes that formed during a subsequent incubation with linear pBR322 for 15 min at 20°C were subjected to agarose gel electrophoresis. Control tracks (-) contained protein-free linear pBR322 molecules

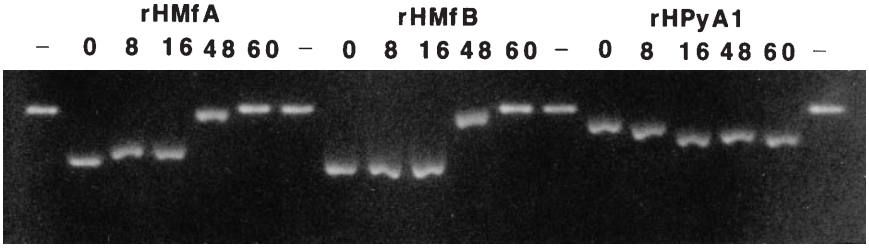

Fig. 3. Mobility shift assays of heat-treated rHMfA, rHMfB, and rHPyA1. Aliquots of rHMfA, rHMfB, and rHPyA1 were incubated at 95°C for 0, 8, 16, 48, and 60 h in 25 mM potassium phosphate buffer (pH 7) that contained 1 M KCl, allowed to bind to linearized pBR322 for 15 min at 20°C, and the nucleoprotein complexes formed were subjected to agarose gel electrophoresis. Control tracks (-) contained protein-free, linear pBR322 molecules

rHMfA and rHMfB did eventually lose all activity after incubation for 2–3 days in 1 M KCl at 95°C. Remarkably, however, even after 60 h at 95°C, rHPyA1 showed no loss of activity, and the apparent improvement in DNA-compacting ability continued for the first 16 h of incubation at 95°C (Fig. 3).

rHPyA1 stabilizes double-stranded DNA at 100°C

The ability of rHPyA1 to maintain the double-stranded (ds) DNA configuration of DNA was investigated using a 117-bp fragment, PCR-amplified from pUC19. rHPyA1–DNA complexes formed at temperatures below the melting temperature of the DNA remained intact when incubated at 100°C for 10 min (Fig. 4). In the absence of rHPyA1, the strands of the DNA molecule separated at 100°C, and the subsequent addition of rHPyA1 to the single-stranded (ss) DNA molecules did not result in a gel shift. Stable histone–DNA complexes were formed regardless of whether the rHPyA1 preparation had or had not been incubated for 10 min at 100°C before mixing with the ds DNA.

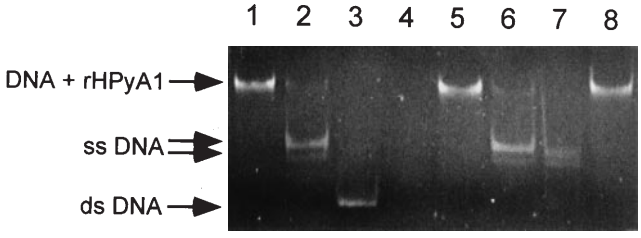

Fig. 4. Electrophoretic mobilities of DNA and HPyA1–DNA complexes through an 8% T, 1.3% C native polyacrylamide gel. *Track 1*, complexes formed by incubating an 117-bp DNA molecule with rHPyA1 at room temperature; *track 2*, complexes formed when the DNA molecule was first incubated at 100°C for 10 min before mixing with rHPyA1; *track 3*, the 117-bp ds DNA molecule alone; *track 4*, rHPyA1 alone (does not stain); *track 5*, complexes formed at room temperature with rHPyA1 preincubated at 100°C for 10 min before mixing with the DNA; *track 6*, complexes formed at room temperature with rHPyA1 preincubated at 100°C for 10 min before mixing with DNA molecules that had also been preincubated at 100°C for 10 min; *track 7*, DNA molecules incubated at 100°C for 10 min; *track 8*, DNA–HPyA1 complexes formed at room temperature (as in track 1) but then incubated at 100°C for 10 min before the electrophoresis

rHPyA1 binds to the template DNA and inhibits transcription in vitro at 90°C

As in *Eukarya*, transcription initiation in *Archaea* requires the assembly, at the promoter, of a preinitiation complex (Langer et al. 1995; Hausner et al. 1996; Thomm 1996; Quresh et al. 1997; Reeve et al. 1997). Assembly at 90°C, of preinitiation complexes containing the *P. furiosus* RNA polymerase (RNAP), archaeal TATA-binding protein (aTBP), and archaeal transcription factor B (aTFB) resulted in a substantial reduction in the electrophoretic mobility of circular, negatively-supercoiled template molecules, but only a marginal reduction in the mobility of relaxed pLUW479 molecules. Regardless of topology, addition of rHPyA1 increased the electrophoretic mobility of these preinitiation-complex-containing template DNAs (Fig. 5), demonstrating that rHPyA1 bound to this template DNA at 90°C under conditions consistent with in vitro transcription. The effects of rHPyA1 binding on transcription at 90°C initiated at the *P. furiosus gdh* promoter were therefore determined. As the histone-to-DNA ratio was increased, transcription decreased; however, the extent of this

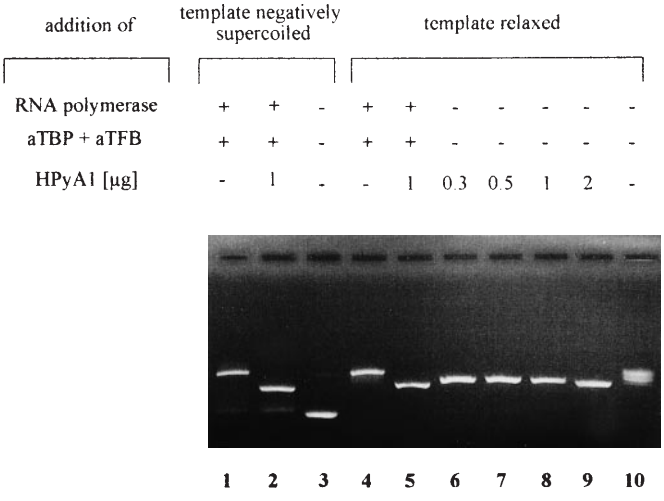

Fig. 5. Mobility shift assay of rHPyA1 binding to pLUW479 template DNA under in vitro transcription conditions. rHPyA1, RNA polymerase, archaeal TATA-binding protein (*aTBP*), and archaeal transcription factor B (*aTFB*) were incubated with pLUW479 DNA for 15 min at 90°C, and the complexes formed were then subjected to agarose gel electrophoresis. Addition of a protein is indicated by +. In *tracks 1–3*, the DNA was covalently closed and negatively supercoiled; in *tracks 4–7*, the DNA was covalently closed but topologically relaxed

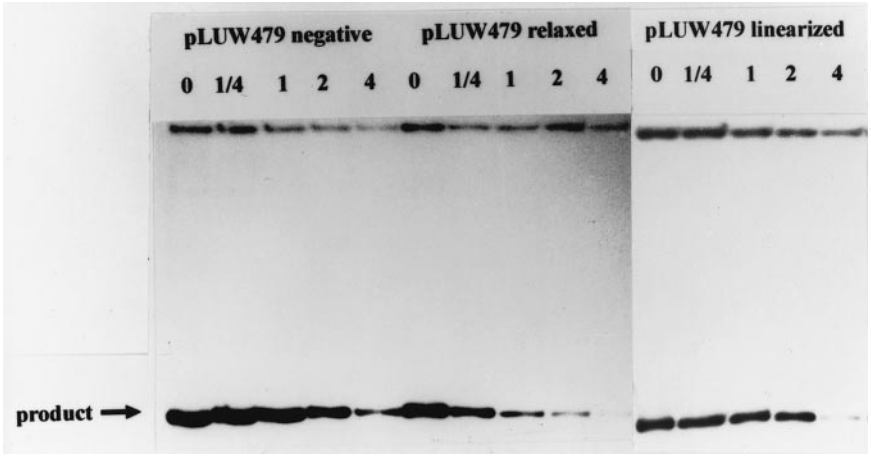

Fig. 6. Effects of rHPyA1 on transcription in vitro. Increasing amounts of rHPyA1 were added to preinitiation complexes that contained pLUW479 as the template DNA (histone : DNA mass ratios of 1 : 4, 1 : 1, 2 : 1, and 4 : 1 indicated *above* the corresponding tracks as *1/4, 1, 2,* and *4*), and transcription was then initiated and allowed to proceed for 15 min at 90°C. Transcripts initiated at the *gdh* promoter were identified and quantitated by primer extension (Hethke et al. 1996). As indicated, the template DNA was initially either circular and negatively supercoiled, circular and relaxed, or linearized by *Bam*HI digestion

decrease was dependent on the topology of the template (Figs. 6,7). At a histone-to-DNA mass ratio of 1:1, transcription from a relaxed, circular template DNA was reduced by ~70%, and transcription from the same template, when negatively-supercoiled, was reduced by ~10%, but there was no reduction in transcription when the template was supplied as a linear DNA molecule (Fig. 7).

Discussion

The DNA binding and compacting activities of rHMfA, rHMfB, and rHPyA1 are remarkably heat resistant. In the presence of 1 M KCl, all three proteins retained full DNA-compacting activity for >16 h at 95°C, and rHPyA1 retained this activity for >60 h at 95°C (Figs. 2, 3). This inherent heat resistance of rHMfA and rHMfB is, however, salt dependent. When incubated in the presence of only 200 mM KCl, rHMfB preparations began to lose activity after only 2 h, and rHMfA preparations after 4 h at 95°C. This may reflect the inactivation of unfolded molecules, as rHMfA and rHMfB do exhibit partial unfolding in 200 mM KCl, and this is less for rHMfA than for rHMfB (Grayling et al. 1995) consistent with rHMfA retaining activity longer at 95°C in 200 mM KCl than rHMfB. *M. fervidus* cells contain ~1 M K^+ (Hensel and König 1988), and therefore it is not surprising that the folded structure of *M. fervidus* proteins is stabilized by a high salt environment. However, the cytoplasm of *Pyrococcus* species similarly contains ~600 mM K^+ (Scholtz et al. 1992), but rHPyA1 exhibits no loss of activity when incubated at 95°C in 200 mM KCl, and circular dichroism studies have confirmed that rHPyA1 has the same α-helical content in 200 mM and 1 M KCl (W-T. Li, J.N. Reeve and J.W. Shriver; unpublished results).

The histone fold has been firmly established for rHMfB (Starich et al. 1996), and circular dichroism (CD) and preliminary nuclear magnetic resonance (NMR) studies indicate that rHMfA and rHPyA1 have very similar structures (Grayling et al. 1995; W-T. Li, K. Sandman, J.N. Reeve, J.W. Shriver, W. Zhu, G. Lee, and M.F. Summers, unpublished results). The extreme heat resistance of rHPyA1, when compared with rHMfA and rHMfB, should therefore be explicable in terms of this structure, and the differences in their primary sequences (Fig. 1), but this explanation is not readily apparent. *Pyrococcus* proteins have more aromatic residues than their *M. fervidus* homologs (Zwickl et al. 1990; Hess et al. 1995) and, consistent with this, rHPyA1 has aromatic residues, Y31, Y35, and F46, at positions that contain valine, methionine, and leucine residues in rHMfA, and isoleucine, methionine, and leucine residues in rHMfB. However, the folded structure of a variant of rHMfB constructed with tyrosine residues at positions 31 and 35 is no more heat resistant than that of rHMfB (W-T. Li, K. Sandman and J.N. Reeve, unpublished results). High temperatures accelerate the deamination of asparagine residues, which may result in protein inactivation (Querol et al. 1996), and asparagine residues are notably absent from rHPyA1, but this is also the case for rHMfB, and rHMfA contains only one asparagine residue (Fig. 1).

Minimizing the volume of unoccupied space within the hydrophobic core of a protein should confer structural stability (Pace 1992; Richards and Lim 1994; Querol et al. 1996). However, except for the three aromatic residues, the R-groups of the residues that contribute to the rHPyA1 core are not consistently larger than their rHMfA or rHMfB counterparts. Substituting larger isoleucine residues for valine residues is often reported to increase heat resistance (Pace 1992; Kotsuka et al. 1996) but, paradoxically, rHPyA1 has valine residues at positions 9, 39, and 44, positions occupied by isoleucine residues in rHMfB. As surface-located ionic interactions should be reduced by increasing the K^+ concentration, the increased stability of rHMfA and rHMfB in high salt may result from the elimination of unfavorable ionic interactions. These proteins do have large numbers of charged residues, 50 per rHMfA dimer and 54 per rHMfB dimer, most of which are surface located, but rHPyA1 dimers, which are not detectably stabilized by a high salt environment, similarly have 50 charged residues. All three histones, as expected for DNA-binding proteins, are predicted to have a net positive charge under in vivo conditions.

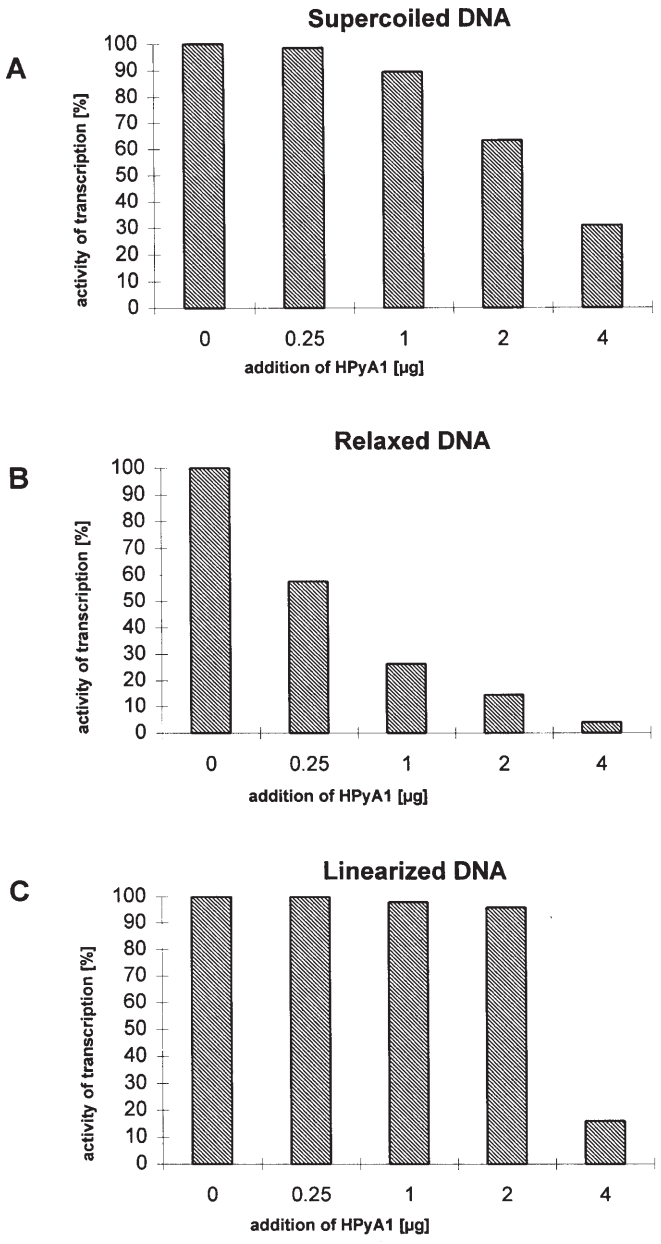

Fig. 7. Quantitative effects of rHPyA1 on transcription in vitro. The transcripts identified in Fig. 6 were quantitated by scanning densitometry, and the amount of transcript synthesized at each histone:DNA mass ratio is expressed as a percentage of the amount of transcript synthesized in the absence of rHPyA1. The topology of the DNA template is indicated *above* the corresponding graph

rHPyA1 binding prevents the denaturation of ds DNA at high temperatures (Fig. 4), consistent with a role in stabilizing the *Pyrococcus* GB-3a genome at growth temperatures of ~100°C. This binding, however, inhibited transcription in vitro (Figs. 6, 7) which obviously poses a problem that must be overcome in vivo. Archaeal histone binding to DNA is readily reversible and dynamic (Krzycki et al. 1990), and HPyA1 molecules presumably migrate away from sites of active transcription to sites of inactivity or, as in eucaryal systems (Kornberg and Lorch 1992; Puerta et al. 1993; Wolffe 1994), archaeal RNAPs may transcribe through histone–DNA complexes in vivo. Recent studies have revealed that template topology is an important parameter for *gdh* transcription in vitro in the *Pyrococcus* system: negatively supercoiled molecules are better templates than relaxed, circular DNA molecules, and positive supercoiling inhibits transcription (C. Hethke, M. Thomm and P. Forterre, unpublished results). As the extent of *gdh* transcription inhibition by rHPyA1 binding was also template-topology dependent, it appears that archaeal histone binding might reduce transcription both by causing a detrimental change in template topology and by the introduction of physical barriers. Consistent with this, the introduction of histone–DNA complexes into a linear template, as opposed to a circular template, does not change the template's overall topology, and transcription from the linear template was least sensitive to rHPyA1 binding. Although rHPyA1 binding did inhibit transcription from the linear template, this only occurred at a high rHPyA1:DNA ratio (Fig. 7) and presumably resulted only from the introduction of physical barriers.

The topology of DNA in hyperthermophilic *Archaea* is very sensitive to growth conditions and changes in stress situations (Charbonnier and Forterre 1994; López-Garcia and Forterre 1997) and, consistent with this, transcription initiation in hyperthermophiles appears to be finely tuned to template topology. With purified RNAP, aTBP, aTFB, and histones all available from *Pyrococcus* species (Sandman et al. 1994b; Wettach et al. 1995; Hausner et al. 1996; Hethke et al. 1996), this central feature of the molecular biology of hyperthermophiles can now be investigated directly using homologous components, under in vitro conditions that simulate the intracellular milieu in *Pyrococcus* cells growing at ~100°C.

Acknowledgments This work was supported by NIH grant GM53185, a grant from the Deutsche Forschungsgemeinschaft, and NATO collaborative research grant CRG950733.

References

Arents G, Burlingame RW, Wang B-C, Love WE, Moudrianakis EN (1991) The nucleosomal core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left-handed superhelix. Proc Natl Acad Sci USA 88:10148–10152

Arents G, Moudrianakis EN (1995) The histone fold: a ubiquitous architectural motif utilized in DNA compaction and protein dimerization. Proc Natl Acad Sci USA 92:11170–11174

Bult CJ, White O, Olsen G, Zhou L, Fleischmann RD, Sutton GG, Blake JA, FitzGerald LM, Clayton RA, Gocayne JD, Kerlavage AR, Dougherty BA, Tomb J-F, Adams MD, Reich CI, Overbeek R, Kirkness EF, Weinstock KG, Merrick JM, Glodek A, Scott JL, Geoghagen NSM, Weidman JF, Fuhrmann JT, Nguyen D, Utterback TR, Kelley JM, Peterson JD, Sadow PW, Hanna MC, Cotton MD, Roberts KM, Hurst MA, Kaine BP, Borodovsky M, Klenk H-P, Fraser CM, Smith HO, Woese CR, Venter JC (1996) Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii.* Science 273: 1058–1073

Charbonnier F, Forterre P (1994) Comparison of plasmid DNA topology among mesophilic and thermophilic eubacteria and archaebacteria. J Bacteriol 176:1251–1259

Darcy TJ, Sandman K, Reeve JN (1995) *Methanobacterium formicicum*, a mesophilic methanogen, contains three Hfo histones. J Bacteriol 177:858–860

Grayling RA, Becktel WJ, Reeve JN (1995) Structure and stability of histone HMf from the hyperthermophilic archaeon *Methanothermus fervidus*. Biochemistry 34:8441–8448

Grayling RA, Sandman K, Reeve JN (1996) Histones and chromatin in hyperthermophilic Archaea FEMS Microbiol Rev 18:203–213

Grayling RA, Bailey KA, Reeve JN (1997) DNA binding and nuclease protection by the HMf histones from the hyperthermophilic archaeon *Methanothermus fervidus.* Extremophiles 1:79–88

Hausner W, Wettach J, Hethke C, Thomm C (1996) Two transcription factors related with the eucaryal transcription factors TATA-binding protein and transcription factor TFIID direct promoter recognition by an archaeal RNA polymerase. J Biol Chem 271:30144–30148

Hensel R, König H (1988) Thermoadaptation of methanogenic bacteria by intracellular ion concentration. FEMS Microbiol Lett 49:75–79

Hess D, Krüger K, Knapp A, Palm P, Hensel R (1995) Dimeric 3-phosphoglycerate kinases from hyperthermophilic Archaea. Cloning, sequencing and expression of the 3-phosphoglycerate kinase gene of *Pyrococcus woesei* in *Escherichia coli* and characterization of the protein. Structural and functional comparison with the 3-phosphoglycerate kinase of *Methanothermus fervidus.* Eur J Biochem 233:227–237

Hethke C, Geerling ACM, Hausner W, de Vos WM, Thomm M (1996) A cell-free transcription system for the hyperthermophilic Archaeon *Pyrococcus furiosus*. Nucleic Acids Res 24:2369–2376

Karantza V, Baxevanis AD, Freire E, Moudrianakis EN (1995) Thermodynamic studies of the core histones: ionic strength and pH dependence of H2A–H2B dimer stability. Biochemistry 34:5988–5996

Klenk H-P, Clayton RA, Tomb J-F, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD, Richardson DL, Kerlavage AR, Graham DE, Krypides NC, Fleischmann RD, Quackenbush J, Lee NH, Sutton GG, Gill S, Kirkness EF, Dougherty BA, McKenny K, Adams MD, Loftus B, Peterson S, Reich CI, McNeil LK, Badger JH, Glodek A, Zhou L, Overbeek R, Gocayne JD, Weidman JF, McDonald L, Utterback T, Cotton MD, Spriggs T, Artiach P, Kaine BP, Sykes SM, Sadow PW, D'Andrea KP, Bowman C, Fujii C, Garland SA, Mason TM, Olsen GJ, Fraser CM, Smith HO, Woese CR, Venter JC (1997) The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature 390:364–370

Kornberg RD, Lorch Y (1992) Chromatin structure and transcription. Annu Rev Cell Biol 8:563–587

Kotsuka T, Akanuma S, Tomuro M, Yamagishi A, Oshima T (1996) Further stabilization of 3-isopropylmalate dehydrogenase of an extreme thermophile, *Thermus thermophilus*, by a suppressor mutation method. J Bacteriol 178:723–727

Krzycki JA, Sandman K, Reeve JN (1990) Purification and characterization of histone HMf from the hyperthermophilic archaebacterium *Methanothermus fervidus*. In: Heslot H, Davies J, Florent J, Bobichon L, Durand C, Penasse L (eds) Proceedings, sixth international symposium on the genetics of industrial microorganisms. Société Français de Microbiol, Strasbourg, pp 603–610

Langer D, Hain J, Thuriaux P, Zillig W (1995) Transcription in Archaea: similarity to that in Eucarya. Proc Natl Acad Sci USA 92:5768–5772

López-Garcia P, Forterre P (1997) DNA topology in hyperthermophilic archaea: reference states and their variation with growth phase, growth temperature, and temperature stresses. Mol Microbiol 23:1267–1279

Pace CN (1992) Contribution of the hydrophobic effect to globular protein stability. J Mol Biol 226:29–35

Puerta C, Hernández F, Gutiérrez C, Piñeiro M, López-Alarcón, Palacián E (1993) Efficient transcription of a DNA template associated with histone $(H3 \cdot H4)_2$ tetramers. J Biol Chem 268:26663–26667

Querol E, Perez-Pons JA, Mozo-Villarias A (1996) Analysis of protein conformational characteristics related to thermostability. Protein Eng 9:265–271

Quresh SH, Bell SD, Jackson SP (1997) Factor requirements for transcription in the archaeon *Sulfolobus shibatae*. EMBO J 16:2927–2936

Ramakrishnan V (1995) The histone fold: evolutionary questions. Proc Natl Acad Sci USA 92:11328–11330

Reeve JN, Sandman K, Daniels CJ (1997) Archaeal histones, nucleosomes and transcription initiation. Cell 89:999–1002

Richards FM, Lim WA (1994) An analysis of packing in the protein folding problem. Quart Rev Biophys 26:423–498

Ronimus RS, Musgrave DR (1996) A gene, *han1A*, encoding an archaeal histone-like protein from the *Thermococcus* species AN1: homology with eukaryal histone consensus sequences and the implication for delineation of the histone fold. Biochim Biophys Acta 1307:1–7

Sambrook J, Maniatis T, Fritsch EF (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Plainview, NY

Sandman K, Krzycki JA, Dobrinski B, Lurz R, Reeve JN (1990) HMf, a DNA-binding protein isolated from the hyperthermophilic archaeon *Methanothermus fervidus*, is most closely related to histones. Proc Natl Acad Sci USA 87:5788–5791

Sandman K, Grayling RA, Dobrinski B, Lurz R, Reeve JN (1994a) Growth-phase-dependent synthesis of histones in the archaeon *Methanothermus fervidus* Proc Natl Acad USA 91:12624–12628

Sandman K, Perler FB, Reeve JN (1994b) Histone-encoding genes from *Pyrococcus*: evidence for members of the HMf family of archaeal histones in a non-methanogenic archaeon. Gene 150:207–208

Sandman K, Grayling RA, Reeve JN (1995) Improved N-terminal processing of recombinant proteins synthesized in *Escherichia coli* Bio/Technology 13:504–506

Scholz S, Sonnenbicher J, Schäfer W, Hensel R (1992) Di-myo-inositol-1,1′-phosphate: a new inositol phosphate isolated from *Pyrococcus woesei.* FEBS Lett 306:239–242

Smith DR, Doucette-Stamm LA, DeLoughery C, Lee H, Dubois J, Aldredge T, Bashirzadeh R, Blakely D, Cook R, Gilbert K, Harrison D, Hoang L, Keagle P, Lumm W, Pothier B, Qiu D, Spadafora R, Vicaire R, Wang Y, Wierzbowski J, Gibson R, Jiwani N, Caruso A, Bush D, Safer H, Patwell D, Prabhakar S, McDougall S, Shimer G, Goyal A, Pietrokovski S, Church G, Daniels CJ, Mao J, Rice P, Nölling J, Reeve JN (1997) Complete genome sequence of *Methanobacterium thermoautotrophicum* strain ΔH: functional analysis and comparative genomics. J Bacteriol 179:7135–7155

Starich MR, Sandman K, Reeve JN, Summers MF (1996) NMR structure of HMfB from the hyperthermophile, *Methanothermus fervidus*, confirms that this archaeal protein is a histone. J Mol Biol 255:187–203

Tabassum R, Sandman KM, Reeve JN (1992) HMt, a histone-related protein from *Methanobactrium thermoautotrophicum* ΔH. J Bacteriol 174:7890–7895

Thomm M (1996) Archaeal transcription factors and their role in transcription initiation. FEMS Microbiol Rev 18:159–171

Wettach J, Gohl HP, Tschochner H, Thomm M (1995) Functional interaction of yeast and human TATA-binding proteins with an archaeal RNA polymerase and promoter. Proc Natl Acad Sci USA 92:472–476

Wolffe A (1994) Transcription: in tune with the histones. Cell 77:13–16

Yanisch-Perron C, Vieira J, Messing J (1985) Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene 33:103–119

Zwickl P, Fabry S, Bogedain C, Haas A, Hensel R (1990) Glyceraldehyde-3-phosphate dehydrogenase from the hyperthermophilic archaebacterium *Pyrococcus woesei*: characterization of the enzyme, cloning and sequencing of the gene, and expression in *Escherichia coli.* J Bacteriol 172:4329–4338