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REVIEW

Suzette L. Pereira · John N. Reeve

Histones and nucleosomes in Archaea and Eukarya: a comparative analysis

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Abstract Archaeal histones from mesophilic, thermophilic, and hyperthermophilic members of the Euryarchaeota have primary sequences, the histone fold, tertiary structures, and dimer formation in common with the eukaryal nucleosome core histones H2A, H2B, H3, and H4. Archaeal histones form nucleoprotein complexes in vitro and in vivo, designated archaeal nucleosomes, that contain histone tetramers and protect approximately 60 base pairs of DNA from nuclease digestion. Based on the sequence and structural homologies and experimental data reviewed here, archaeal nucleosomes appear similar, and may be homologous in evolutionary terms and function, to the structure at the center of the eukaryal nucleosome formed by the histone $(H3+H4)_2$ tetramer.

Key words Hyperthermophile · *Methanothermus fervidus* · Histone evolution · Genome structure · Nucleosome positioning

Introduction

The three biological domains, the Bacteria, Archaea and Eukarya, were proposed on the basis of small subunit ribosomal RNA (ssu-rRNA) sequences, with the deepest phylogenetic branch separating the bacterial lineage from an archaeal/eukaryal lineage (Woese et al. 1990). Now, with complete microbial genome sequences available, it is apparent that many proteins that participate in genetic information storage, replication, and expression also have sequences and functions conserved in the Archaea and Eukarya that are not conserved in Bacteria (Brown and Doolittle 1997; Smith et al. 1997), and this includes the presence of histones (Reeve et al. 1997a; Zlatanova 1997). High-resolution structures have been established for archaeal and eukaryal histones (Starich et al. 1996; Decanniere et al. 1996; Luger et al. 1997a; Zhu et al., in manuscript), and here we compare these structures and the structure predicted for the archaeal nucleosome with the structure of the eukaryal nucleosome (Luger et al. 1997a). The data reviewed have been accumulated primarily through in vitro and in vivo studies of HMfA and HMfB, histones A and B from the hyperthermophilic archaeon *Methanothermus fervidus* (Sandman et al. 1990, 1994; Grayling et al. 1996a).

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S.L. Pereira · 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

The histone fold, and a comparison of histone fold primary sequences

The four eukaryal nucleosome core histones, H2A, H2B, H3, and H4, the archaeal histones HMfA and HMfB from *M. fervidus* and HFoB from the mesophile *Methanobacterium formicicum*, eukaryal transcription factors, and components of the eukaryal transcription initiation complex have all been shown to contain the histone fold (Arents and Moudrianakis 1995; Ramakrishnan 1995; Starich et al. 1996; Decanniere et al. 1996; Kim et al. 1996; Xie et al. 1996; Mermelstein et al. 1996; Zhu et al., 1998). In this structure, two short α-helices (α1 and α3) with ~3 helical turns flank a longer, 8-turn-containing α-helix (α2), from which they are separated by short β-strand loops (L1 and L2). The histone fold is stabilized by dimer formation (Grayling et al. 1995; Karantza et al. 1995). Folded histone monomers have not been observed, but they form very stable dimers in which the two α2s are antiparallel, positioning the L1 of each monomer adjacent to the L2 of the second monomer, resulting in two short regions of β-bridge structure. These structures are illustrated in Fig. 1, and identified for the monomers of one of the two archaeal histone dimers that are shown above an alignment of the

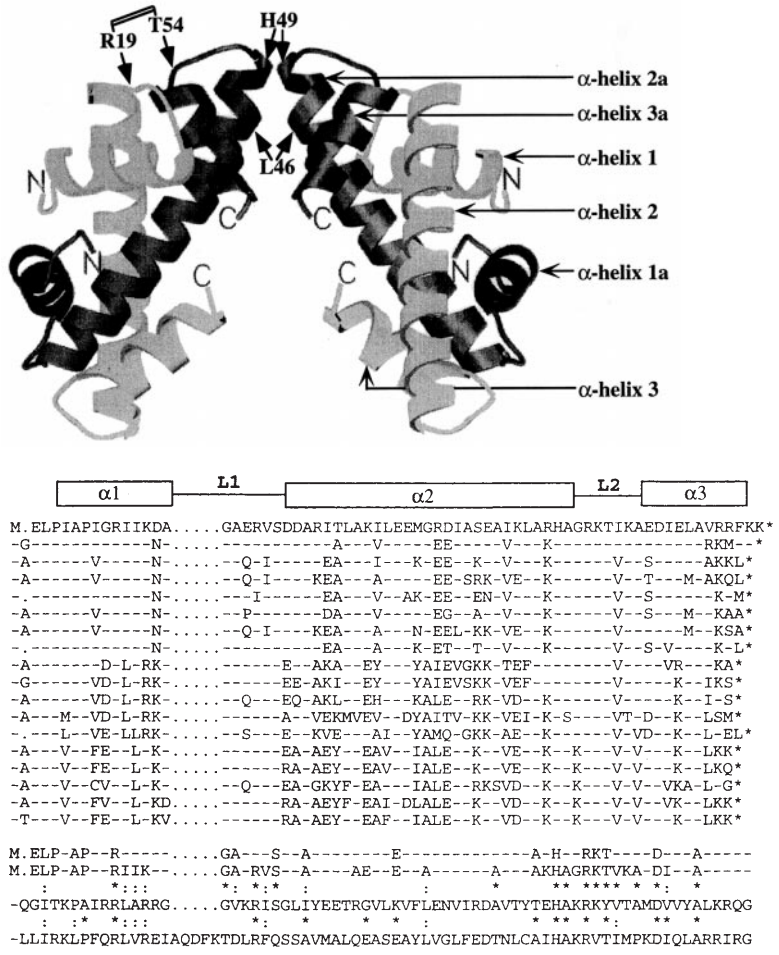

Fig. 1. Two $(HMfB)_2$ dimers are positioned as an archaeal histone tetramer above an alignment of all available archaeal histone sequences. The α-helices of the two monomers in one dimer are labeled 1, 2, 3 and 1a, 2a, and 3a, and in the alignment identical residues are indicated (–). Archaeal histone consensus sequences are shown by the residues present in all archaeal histones (100%), or by the more prevalent residue at positions that are occupied by one of only two residues (*+1 Diff.*). These are aligned above the sequences of the histone fold regions of *Xenopus* histones H4 and H3 with identical (*) or conserved (:) residues in the consensus archaeal and H4 or H3 sequences indicated. The locations of the five residues present in H3 and H2B, that are absent in H4, H2A, and the archaeal histones, and the absence of the A1/G1 residue in the archaeal B histones, are indicated by *dots* (.). R45(H4)-T118(H3) interactions position the R45 side chain into the minor groove of the DNA wrapped around a nucleosome (Luger et al. 1997a). The structurally homologous residues in HMfB are R19 and T54, and the location of one of the four (R19 − T54) pairs and of the two H49 and L46 residues predicted to participate in HMfB tetramer formation are indicated (see Fig. 2)

18 available archaeal histone sequences (Grayling et al. 1996a; Reeve et al. 1997b).

The archaeal histones contain 66–69 amino acid residues with sequences that are 60%–90% identical in pairwise alignments. Nineteen positions contain the same residue, and 17 additional positions contain only 1 of 2 alternative residues, in all the archaeal histones (Fig. 1). The common ancestry of the archaeal histones and the histone fold regions of the eukaryal nucleosome core histones is apparent from an alignment of archaeal histone consensus sequences with the sequences of residues 27–93 and 60–131 that form the histone folds of *Xenopus* histones H3 and H4, respectively (Fig. 1). Although the eukaryal histones have additional N-terminal and C-terminal sequences that extend beyond the histone fold, sequences that are not present in the archaeal histones, these extensions are not absolutely essential for nucleosome assembly. They extend outside the nucleosome to interact with regulatory proteins, provide the targets for histone acetylation, and participate in higher-order chromatin assembly (Wan et al. 1995; Ling et al. 1996; Luger et al. 1997a), regulatory functions of the eukaryal histones that apparently have not evolved in Archaea.

Archaeal histones form two groups, based on their N-terminal residues. The N-formyl-methionyl residue is removed during the maturation of archaeal A-histones, which therefore have an alanyl or glycyl residue (A1/G1) at position 1 (Sandman et al. 1995). Archaeal B-histones retain the N-terminal methionyl residue (M1) but lack a homolog of the A1/G1 residue of the A-histones, and therefore position 2 is the same in both A and B histones. Residues E2, L3 and P4 are, in fact, completely conserved in all archaeal histones (Fig. 1). Eukaryal histones H3 and H2B contain five adjacent amino acid residues that are not present in H4, H2B, nor in the archaeal histones and, because of these additional residues, α1 and L1 are longer in H3 and H2B than in H4 and H2A, which results in asymmetric (H3+H4) and (H2A+H2B) dimers (Luger et al. 1997a). Apparently, an H3/H2B histone lineage separated from an H4/H2A/archaeal histone lineage before the H4/H2A and archaeal histone lineages diverged, or convergent evolution has deleted the same five residue positions in H4, H2A, and the archaeal histones or inserted five residues into H3 and H2B (Thatcher and Grovosky 1994) (see Fig. 1).

Histone dimers

Histones exist as dimers in solution, but whereas the eukaryal histones form exclusively (H2A+H2B) and

(H3+H4) heterodimers, archaeal histones form both homodimers and heterodimers (Sandman et al. 1994, 1995). Monomer–monomer interactions that maintain the eukaryal histones in the dimer configuration have been identified between hydrophobic residues positioned along the antiparallel aligned α2s and between residues in adjacent L1 and L2 regions (Figs. 1 and 2) (Luger et al. 1997a). Specific residues at these sites must direct the formation of (H2A+H2B) and (H3+H4) heterodimers and prevent the formation of other heterodimers and homodimers. The archaeal histones also have hydrophobic residues at these α2 positions, and some of the structurally homologous sites in the archaeal and eukaryal L1 and L2 regions contain identical residues (Figs. 1 and 2). Recombinant (r) archaeal histones are synthesized in *Escherichia coli* as soluble homodimers (Sandman et al. 1995), whereas expression of individual *Xenopus* histone encoding genes in *E. coli* results in insoluble inclusion bodies (Luger et al. 1997b). Because dimer formation is required to stabilize the histone fold (Karantza et al. 1995), these inclusion bodies presumably contain unfolded monomers that accumulate in the absence of their heterodimer partner.

When $(rHMfA)_2$ and $(rHMfB)_2$ homodimers are mixed, (rHMfA+rHMfB) heterodimers form spontaneously, and both $(HMfA)_2$ and $(HMfB)_2$ homodimers and (HMfA+HMfB) heterodimers exist in vivo. As the two homodimers have different DNA-binding properties, they and the (HMfA+HMfB) heterodimer may have different biological roles in vivo (Sandman et al. 1994; Grayling et al. 1996a). Identical residues are present in HMfA and HMfB at most of the α2, L1, and L2 sites that are predicted to interact to form histone dimers, consistent with forming both homodimers and heterodimers. The residues at these sites are, in fact, conserved to a large extent in all the archaeal histones, indicating that most archaeal homodimers and potential heterodimers could be formed. *Methanococcus jannaschii* has five histone-encoding genes (Bult et al. 1996), providing the potential for 15 different histone dimers and considerable opportunity for the evolution of functional differences. Consistent with this predicted promiscuity in archaeal histone dimer formation, mixing $(rHMfB)_2$ and $(rHFoB)_2$ homodimers results in the spontaneous formation of (rHMfB+rHFoB) heterodimers (K. Sandman, personal communication), heterodimers that therefore contain monomers encoded by genes cloned from different Archaea, and which have never existed in vivo.

Histone tetramers, octamers, and nucleosome assembly

The protein core of the eukaryal nucleosome is a wedge-shaped histone octamer (Arents and Moudrianakis 1993).

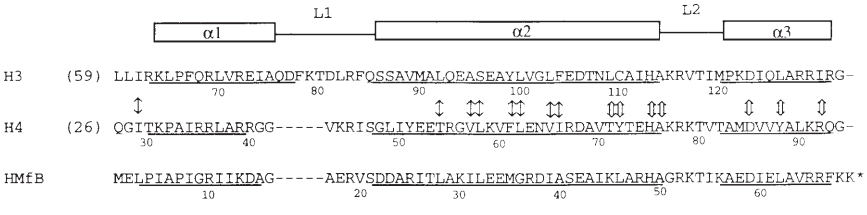

Fig. 2. Residues involved in histone dimer and tetramer formation. *Xenopus* histone H3 and H4 residues that interact to direct the formation of a (H3+H4) dimer and a $(H3+H4)_2$ tetramer are listed in the table adjacent to the residues located at the structurally homologous positions in HMfB. The residues at these positions in the other archaeal histones are listed under Natural Variants (see Fig. 1), with (–) indicating positions at which the HMf residue is present in all the archaeal histones. The H3 and H4 residues that interact to form dimers (↕) and tetramers (⇕) are identified between the alignment of the *Xenopus* H3 and H4 histone fold sequences. The α1, α2, α3, L1, and L2 regions are indicated in the diagram above the alignment, with the α-helical sequences underlined. Hyphens identify the locations of the five residues present in H3 that are not present in H4 nor in the archaeal histones. The histone fold is stabilized by a conserved intramolecular L2–α3 interaction formed, as listed in the table, between R116 and D123 in H3, R78 and D85 in H4, and R52 and D59 in all the archaeal histones

Interaction	H3	H4	HMfB	Natural Variants
↕ α2-α2 dimer	L92	T54	L28	M, F
	A95	V57	I31	A, V, H, Y
	S96	L58	L32	V, I, F
	Y99	F61	M35	I, L, N, Y, K
	L100	L62	G36	A
	L103	V65	I39	V, L
	F104	I66	A40	G, S
L1-L2 dimer	R83	R45	R19	I
	T118	T80	T54	-
⇕ α2-α3 tetramer	L109	T71	K45	D, E
	C110	Y72	L46	I, F
	H113	H75	H49	-
	A114	A76	A50	S
	L126	Y88	L62	A, M
	I130	R92	R66	G, A, S, M, E, Q, K
L2-α3 monomer	R116	R78	R52	-
	D123	D85	D59	-

Two (H3+H4) dimers assemble to form a $(H3+H4)_2$ tetramer that recognizes nucleosome positioning signals and binds to chromosomal DNA to initiate nucleosome assembly. The core is completed by the addition of an (H2A+H2B) dimer on each side of the $(H3+H4)_2$ tetramer, and 146 bp of DNA are wrapped in 1.65 negative superhelical turns around the surface of this core to complete the nucleosome (Wolffe 1992; Luger et al. 1997a). (H2A+H2B) dimers do not form $(H2A+H2B)_2$ tetramers, but do form [(H2A+H2B)+(H4+H3)] tetramers when attached to the nucleosome core. The histone octamer is therefore formed by three overlapping tetramers, arranged [(H2A+H2B)+(H4+H3)]–[(H4+H3)+(H3+H4)]–[(H3+H4)+(H2B+H2A)], with the mid-point of the nucleosome particle, designated the position of dyad symmetry, located at the interface of the two H3 monomers in the center of the $(H3+H4)_2$ tetramer (Luger et al. 1997a).

Histone tetramer formation

Histone dimers assemble into histone tetramers by forming a four-helix bundle through interactions between residues in the C-terminal halves of two α2s and two α3s (see Fig. 1). In an $(H3+H4)_2$ tetramer, only H3 α-helices participate whereas α-helices from both H4 and H2B interact to form the [(H2A+H2B)+(H4+H3)] tetramers that assemble to complete the nucleosome core. The residues at these sites of interaction must determine the specificity of tetramer formation, and residues in α2 and α3 of *Xenopus* H3 and H4 histones that participate in tetramer formation are identified and listed together with the residues at the structurally homologous positions in the archaeal histones in Fig. 2. As there is very little variation in these residues in the different archaeal histones (Figs. 1 and 2), it seems likely that most pairs of archaeal histone homodimers and heterodimers could assemble into tetramers.

All archaeal histones have histidinyl-49 (H49) and aspartyl-59 (D59) residues that are the structural homologs of H113 and D123, and H75 and D85 in eukaryal histones H3 and H4, respectively (Fig. 1). In a $(H3+H4)_2$ tetramer, intermolecular hydrogen bonds form between the two H113s and D123s, and in a [(H3+H4)+(H2B+H2A)] tetramer there is an intermolecular bond between H75 of H4 and E90 of H2B (Luger et al. 1997a). Based on these established eukaryal structures and the conservation of residues, all archaeal histone tetramers are likely to contain two α2–α3 intermolecular hydrogen bonds formed between H49–D59 residues. Similarly, based on structure conservation, $[(rHMfB)_2+(rHMfB)_2]$ tetramers are predicted to contain a four-helix bundle stabilized by hydrophobic interactions that involve L46 and A47 in α2, and L62 and A63 in α3, and as similar or identical residues are present at these sites in most archaeal histones (see Figs. 1 and 2), there seems to be considerable potential for promiscuity in archaeal histone tetramer formation.

Sites of histone–DNA interactions

X-ray diffraction studies of *Xenopus* nucleosome crystals revealed that each histone dimer interacts with ~2.5 consecutive helical turns of the DNA, and specific interactions were identified between residues preceding, and in the α1s, in L1 and in L2 with phosphate oxygens and deoxyribose moieties of the DNA backbone. Arginine side chains are inserted into the minor groove of the DNA at 10 of the 14 locations that the minor groove faces the nucleosome core (Luger et al. 1997b). The archaeal histones have identical or similar residues at most positions that are structural homologs of these sites, consistent with a conserved mechanism of DNA binding and wrapping (Fig. 1; Table 1). The archaeal histones lack a homolog of R63 that precedes α1 in H3, but otherwise most have arginines at the positions required for side chain insertions into the minor groove. All the archaeal histones except HMtB (Tabassum et al. 1992) have, for example, R19 in L1, which is positioned adjacent to T54 in L2 of the second monomer in a dimer (Fig. 1). These are structural homologs of R83 and T118 and of R45 and T80 in *Xenopus* histones H3 and H4, respectively, and in the *Xenopus* nucleosome, the side chains of R83 (H3) and R45 (H4) are positioned in the minor groove through

Table 1. Residues in the histone fold regions of *Xenopus* histones H3 and H4 that participate in histone–DNA interactions compared with the residues at the structurally homologous locations in HMfB and in all other archaeal histones

Residue location	Residue in H3	Residue in H4	Residue in HMfB	Natural archaeal histone variants[a]
Preceding α1	R63	T31	L3	–
	R69	R36	R10	–
α1	R72	R39	K14	R
	L65	P32	A6	–
	P66	A33	P7	–
L1	R83	R45	R19	I[b]
L2	K115	K77	G51	K[c]

[a] Sites at which the same residue is conserved in all available archaeal sequences (see Fig. 1) are indicated (–).
[b] I19 occurs in only one archaeal histone, HMtB (Tabassum et al. 1992; Smith et al. 1997).
[c] K51 occurs in the *Methanococcus jannaschii* histone sequences (Bult et al. 1996).

L1–L2 intermolecular bonds made between R83(H3)-T80 (H4) and R45(H4)-T118 (H3). Based on this residue and structure conservation, L1–L2 inter-molecular interactions between R19 and T54 almost certainly position the R19 side chain into the minor groove of the DNA wrapped around an archaeal nucleosome, and four such R19–T54 interactions will occur in almost all archaeal histone tetramers (Fig. 1).

Nuclease protection and histone content of archaeal nucleosomes

Although archaeal histones are dimers in solution (Grayling et al. 1995), chemical cross-linking studies revealed the presence of tetramers in archaeal histone–DNA complexes assembled in vitro (Grayling et al. 1996b). These complexes protected ~60 bp of DNA from micrococcal nuclease (MN) digestion, and sequencing the protected fragments demonstrated that archaeal histones bound and assembled complexes in vitro at preferred sites (Grayling et al. 1997). Essentially the same results have also been obtained for archaeal histone–DNA complexes, designated archaeal nucleosomes, assembled in vivo (Pereira et al. 1997). As shown in Fig. 3, MN digestion of DNA–protein complexes, cross-linked in vivo by exposure of *M. fervidus* cells to formaldehyde, generates fragments of *M. fervidus* genomic DNA that are ~60 bp, and multiples ~60 bp in length. Analysis of the proteins in these complexes reveals the presence of only archaeal histones, and cross-linked tetramers are the highest histone oligomer detected (Fig. 3). When visualized by electron microscopy, archaeal nucleosomes resemble eukaryal nucleosomes (Sandman et al. 1990; Takayanagi et al. 1992); however, they appeared to be separated by nucleosome-free regions in spreads of *Methanobacterium thermoautotrophicum* genomic DNA (Pereira et al. 1997) and therefore not to be as tightly packed as nucleosomes in eukaryal chromatin. Quantitative immunoblotting revealed that HMt histones constitute ~0.9% of the total soluble protein in *M. thermoautotrophicum* (Pereira 1997), significantly less than the approximately 4% of total soluble protein constituted by the HMf histones in *M. fervidus*, but still sufficient for about one HMt tetramer per 100 bp of the 1.75-Mbp *M. thermoautotrophicum* genome (Smith et al. 1997).

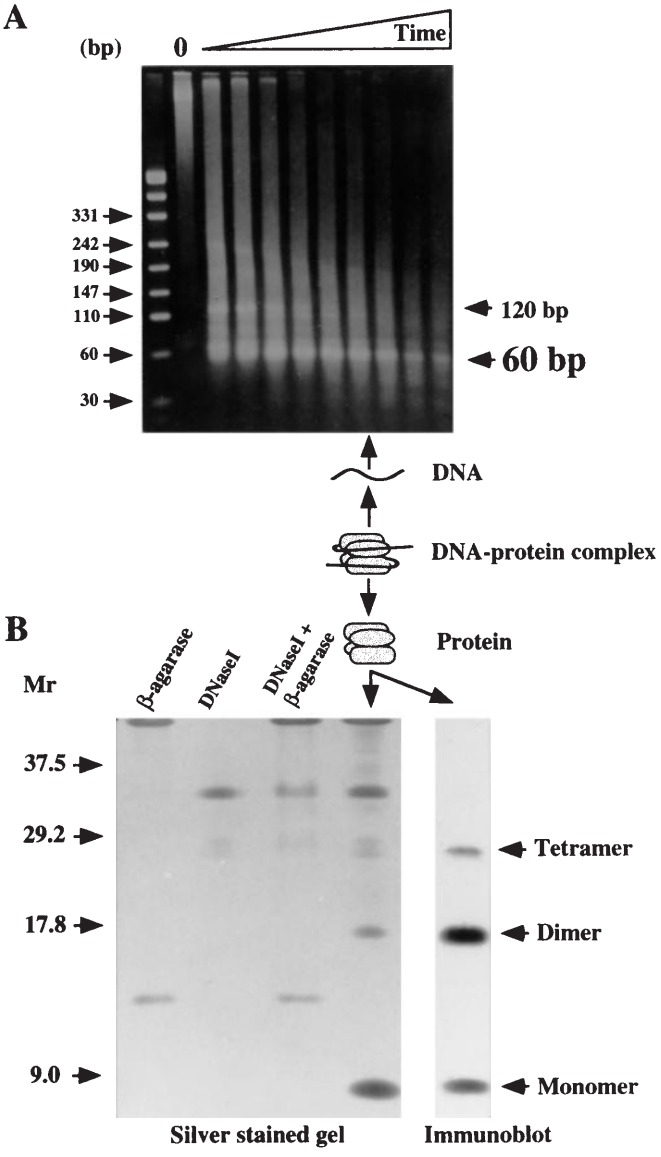

Fig. 3A,B. DNA and protein content of archaeal nucleosomes. **A** An electrophoretic separation of DNA fragments protected from micrococcal nuclease (MN) digestion in nucleoprotein complexes isolated following in vivo formaldehyde fixation in *Methanothermus fervidus* cells. The control tracks contained size standards and undigested, cross-linked nucleoprotein complexes (*O*). **B** Electrophoretic separation and silver staining of the proteins isolated from the complexes, indicated above in **A**, that protected ~60-bp fragments from MN digestion. Purification of these proteins involved incubations with β-agarase and DNase I and, as revealed by the control tracks, small amounts of the polypeptides in these reagents remained as contaminants in the experimental material. An immunoblot of the purified proteins, generated using anti-HMf antibodies, is shown adjacent to the stained gel

Archaeal nucleosome positioning and in vivo footprints

Positioned nucleosomes participate both negatively and positively in regulating eukaryal gene expression. They inhibit transcription by blocking access to promoters, but also stimulate transcription initiation by appropriately positioning and displaying enhancer sequences (Wolffe 1992, 1994; Kornberg and Lorch 1995; Beato and Elsfeld 1997). Consistent with archaeal nucleosome positioning, Southern blots of the DNA in archaeal nucleosomes, formaldehyde fixed in vivo, revealed the presence of most but not all *M. fervidus* genomic sequences. Some sequences were significantly enriched, most notably sequences from the stable 7S and 16S rRNA-encoding regions, whereas sequences from the *mcr* operon that encodes methyl coenzyme M reductase I (MRI) were undetectable (Pereira et al. 1997). This is

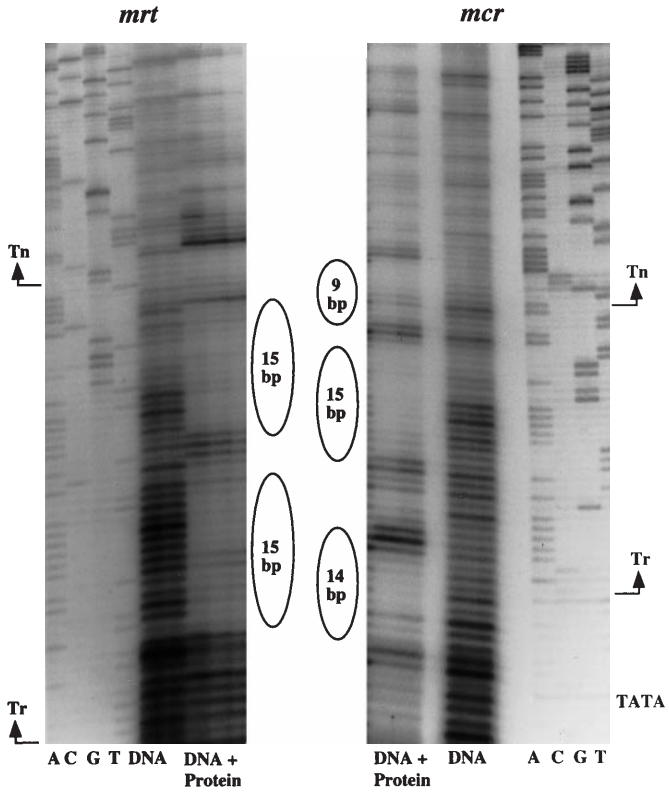

Fig. 4. In vivo footprints of regulatory regions upstream of the *mrt* (*left*) and *mcr* (*right*) operons in *M. fervidus*. As indicated by the ovals, following in vivo formaldehyde cross-linking, footprints are observed downstream from the *mrt* and *mcr* TATA-box promoter elements, and between the sites of transcription (*Tr*) and translation (*Tn*) initiation in *M. fervidus* (Pereira 1997). These nuclease-protected regions are shorter than the 60–70 bp predicted for protection by a positioned archaeal nucleosome, consistent with sites at which transcription factors might bind to direct the H_2-dependent regulation of transcription of the *mrt* and *mcr* operons (Reeve et al. 1997a). The control tracks contain digests of protein-free DNA (*DNA*) and sequencing ladders (*A,C,G,T*) used as size standards

intriguing because *mcr* transcription occurs at very high levels, although it is replaced by transcription of the *mrt* operon that encodes an isoenzyme, MRII, when the gaseous growth substrates, H_2 plus CO_2, are supplied at high levels (Reeve et al. 1997a). The *mcr* and *mrt* operons are differentially regulated by substrate availability, but this regulation does not appear to involve positioned archaeal nucleosomes. Sequences from upstream of the *mrt* operon were associated with HMf in vivo (Pereira et al. 1997), and following formaldehyde fixation of *M. fervidus* cells growing under conditions of *mrt* and *mcr* transcription, in vivo footprints are obtained in both upstream regulatory regions, but these are shorter than the 60 bp predicted for a positioned archaeal nucleosome (Fig. 4) (Pereira 1997). Presumably, these footprints identify sites at which transcription factors bind, some of which are probably responsible for the substrate-dependent regulation of *mcr* and *mrt* transcription.

Structure of the archaeal nucleosome

Archaeal nucleosomes appear to be similar, and possibly homologous, to the structure that is formed by the $(H3+H4)_2$ tetramer at the center of the eukaryal nucleosome. Archaeal and H4 histone sequences are most similar (Fig. 1); $(H3+H4)_2$ tetramers form spontaneously, and bind to DNA forming stable complexes in the absence of H2A and H2B that protect ~73 bp and footprint ~80 bp (Dong and van Holde 1991; Hayes et al. 1991; Wolffe 1992). Within the nucleosome, the $(H3+H4)_2$ tetramer contacts primarily the central 70 bp, although it does also make contacts with base pairs close to the DNA entry and exit sites of the nucleosome (Luger et al. 1997a), and hydroxyl radical footprinting indicates contacts are made with a region ~120 bp. Apparently, therefore, the $(H3+H4)_2$ tetramer protects only the 70–80 bp that minimally circumscribe this structure, but forms a complex which involves a longer region of DNA. Archaeal nucleosomes similarly contain a histone tetramer, and protect ~60 bp of DNA from nuclease digestion; however, the length of DNA incorporated into these structures in vivo may be significantly longer than that protected from nuclease digestion in vitro.

The direction of DNA wrapping around archaeal nucleosomes in vivo has yet to be determined. Archaeal histone binding to circular DNA molecules in vitro, at low histone to DNA ratios, introduces negative superhelicity that spontaneously and reversibly becomes positive superhelicity when the histone to DNA ratio is increased (Musgrave et al. 1991). The basis for this is unclear, and this phenomenon appeared to indicate a fundamental difference from the $(H3+H4)_2$ tetramer-containing structure. Recently, however, it was proposed that a small change in the dimer–dimer interface within an $(H3+H4)_2$ tetramer would result in a shift from negative to positive DNA wrapping, and that this dimer–dimer reorientation should be reversible and depend on the topological tension in the DNA flanking the $(H3+H4)_2$ tetramer-containing structure (Hamiche et al. 1996). Adding more archaeal histones to a circular DNA introduces more archaeal nucleosomes, and therefore increases the topological tension in the regions of a circular DNA molecule that remain nucleosome free. The switch observed from negative to positive helicity is therefore consistent with the torsion-dependent dimer–dimer reorientation model, and substituting bulkier, less mobile residues at the sites of archaeal histone tetramer formation (see Figs. 1 and 2) might still allow the assembly of archaeal nucleosomes with negatively wrapped DNA, but inhibit the dimer–dimer reorientation and therefore prevent the switch to positive wrapping.

Did histones and eukaryal transcription evolve in a hyperthermophile?

Packing a long DNA molecule into a much shorter cell must have been a problem faced, and solved, early during cellular evolution. All Eukarya employ the same histones and

nucleosome-based wrapping to solve this problem, and therefore these basic features of this system presumably evolved in an ancestor of all Eukarya. This could have been an ancestor faced with the problem of inhabiting a high temperature environment that may have developed DNA wrapping by histones initially to maintain the integrity of its double-stranded DNA genome. This solution to the heat denaturation problem would have brought the bonus of genome compaction, but would have also required the evolution of mechanisms to replicate and access genes wrapped around a histone core. Solving these problems possibly resulted in the DNA replication proteins, TATA-box-containing promoters, TATA-box-binding protein (TBP), and transcription initiation factors that are conserved in Archaea and Eukarya (Brown and Doolittle 1997; Qureshi et al. 1997; Reeve et al. 1997b) but which are not found in Bacteria. The absence of histones (Schmid 1990; Hayat and Mancarella 1995) and the TATA-box-based system of transcription initiation in Bacteria could be explained by the bacterial and archaeal lineages having diverged before the coevolution of these systems (Woese et al. 1990; Olsen et al. 1994), but this argument does not explain the lack of histones in Crenarchaeota (Zlatanova 1997). Members of this archaeal lineage do have TATA-box-containing promoters and employ TBP and TFIIB to initiate transcription (Qureshi et al. 1997), and therefore either genome compaction by histone wrapping and the TATA-box-based system of transcription initiation evolved separately, or histones must have been lost and replaced by the structurally unrelated DNA-binding proteins that do exist in Crenarchaeota (Bohrmann et al. 1994; Grayling et al. 1996a,b).

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