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# DNA repeats and archaeal nucleosome positioning

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**Abstract** — Archaeal histones, homologs of the eucaryal nucleosome core histones, have been identified in the Euryarchaeota. They assemble as tetramers with dsDNA to form archaeal nucleosomes that resemble the central structure of the eucaryal nucleosome formed by the histone $(H3\text{-}H4)_2$ tetramer. Eucaryal and archaeal nucleosomes assemble preferentially on DNA molecules that best accommodate the severe bends found within these structures, and here we discuss the relationships between archaeal and eucaryal nucleosomes, repeating DNA sequences, and nucleosome positioning. © 1999 Éditions scientifiques et médicales Elsevier SAS

**nucleosome / genome packaging / archaea / histones**

## 1. Eucaryal histones and nucleosome structure

In all cells, DNA is compacted for accommodation within a relatively small volume, but must remain sufficiently dynamic for transcription and DNA replication to occur. DNA compaction and accessibility are balanced within a protein-DNA complex known as chromatin. Although eucaryal chromatin has hierarchical levels of organization, the basic repeating unit is the nucleosome that wraps ≈ 146 bp of DNA in ≈ 1.65 negative toroidal supercoils around an octamer core formed by two copies each of histones H2A, H2B, H3, and H4 [37]. In the absence of DNA, these exist as (H2A-H2B) and (H3-H4) heterodimers, and (H3-H4) dimers associate to form stable $(H3\text{-}H4)_2$ tetramers. In the nucleosome core, a central $(H3\text{-}H4)_2$ tetramer is flanked by (H2A-H2B) dimers [1], and most eucaryal nuclei also contain linker histone H1 (or variant H5) that associates with the core particle. H1 participates in the formation of higher order chromatin structure and directs the DNA trajectory as it enters and exits the core particle [24, 34].

Histones H2A, H2B, H3, and H4 share little amino acid sequence similarity but have a conserved tertiary structure known as the histone fold [1, 2]. The histone fold has a long central α-helix flanked and separated from two shorter α-helices by short loops that contain β-strand structures [1] *(figure 1A, B)*. Histones interact to form dimers in a head-to-tail fashion primarily through hydrophobic interfaces; although each forms the histone fold structure, core histones pair only with their specific partners, H2A with H2B and H3 with H4, and they do not form homodimers. Within the histone octamer, dimers contact DNA at both paired β-strand loop regions and at the paired N-termini [37]. The eucaryal core histones also have N- and C-terminal sequences that extend from the histone fold that participate in higher order chromatin assembly and contain sites for posttranslational modifications (reviewed in [38]).

The DNA helix is distorted as it is wrapped around the histone core; there are local regions of extreme bending, and the helical repeat is reduced from 10.5 bp/turn in solution [47] to an average of 10.2 bp/turn in the nucleosome [37]. As discussed below, nucleosome assembly is facilitated by DNA sequences that readily acc-

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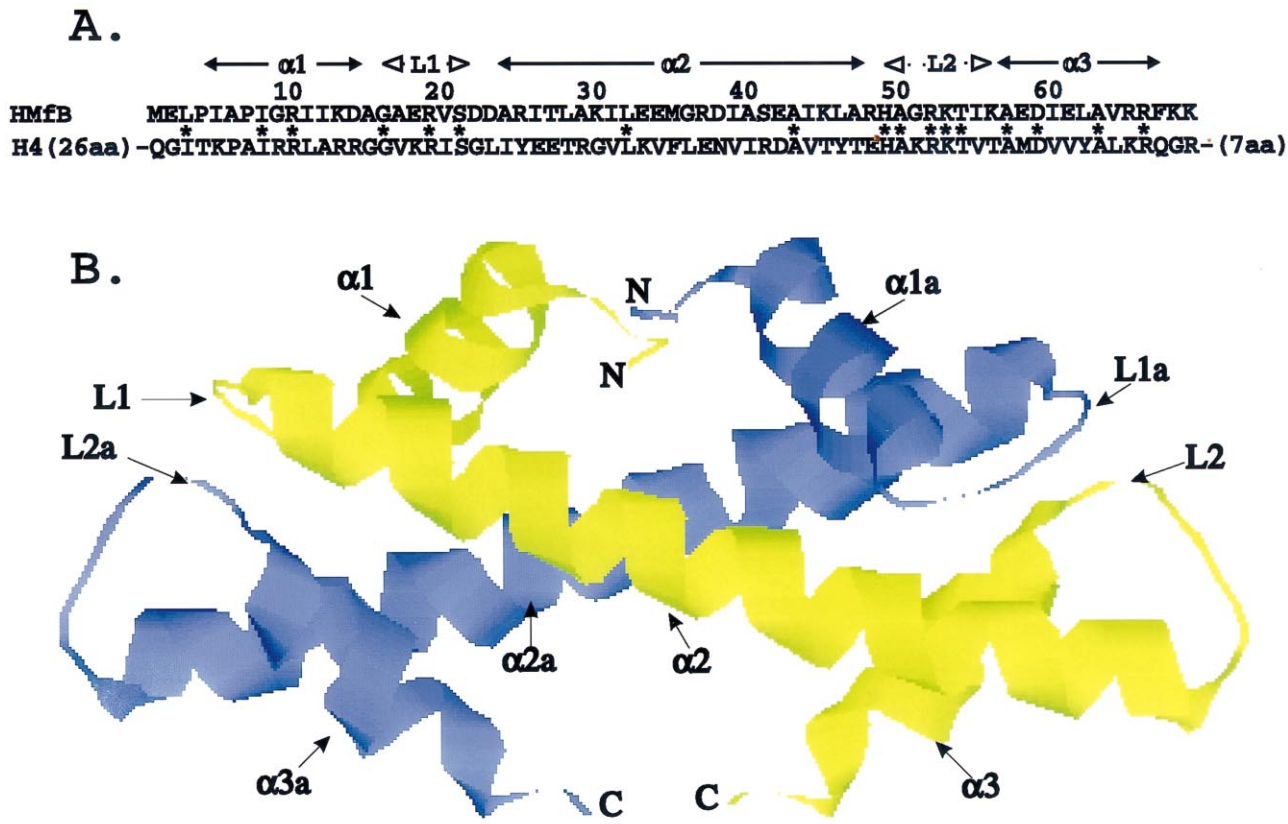


**Figure 1. A.** Alignment of amino acid sequences of HMfB with the histone fold region of eucaryal core histone H4. The positions and lengths of the α-helices (α1, α2, α3) and loop regions (L1, L2) are indicated, and identical conserved residues are marked (*). The number of residues preceding and following the histone fold region of H4 are indicated in parentheses. **B.** Structure of the $(HMfB)_s$ homodimer [61], with one polypeptide in green and the other in purple. The amino (N) and carboxyl (C) termini are labeled.

ept the constraints required for nucleosome formation.

## 2. Archaeal histone HMf and archaeal nucleosomes

The genomes of procaryotes are much smaller than those of Eucarya, but Archaea and Bacteria must still solve a DNA packaging problem. They have similarly-sized ≈ 2–10 Mbp genomes complexed in vivo with proteins but, with few exceptions, archaeal and bacterial chromosomal proteins are not related [15, 51]. Procaryotic chromosomal proteins are often categorized as 'histone-like' because, like the eucaryal histones, they are generally abundant, small, basic, and bind DNA with little sequence specificity, but the majority share no primary, secondary, or tertiary structural features in common with the eucaryal core histones [35, 55]. To date, the only procaryotic homologs of the eucaryal core histones are the HMf family of archaeal histones found in the Euryarchaeota. The archetype, HMf, isolated from the hyperthermophilic methanogen *Methanothermus fervidus* (Histone from *Methanothermus fervidus*), was originally found as a mixture of homodimers and heterodimers of two nearly identical ≈ 7.5-kDa polypeptides designated HMfA and HMfB [33, 50]. Since then, ≈ 30 archaeal histones have been identified [46]. They have very similar amino acid sequences that are ≈ 45% similar to the

consensus amino acid sequences of the four eucaryal core histones. Each eukaryal core sequence is more similar to archaeal histone sequences than to those of the other core histones, consistent with all these proteins having evolved from a common ancestor that existed prior to the divergence of the Archaea and Eucarya [21].

Homology of HMf and eucaryal histones is further supported by the structures established for $(HMfB)_2$ homodimers from *M. fervidus* and $(HFo)_2$ homodimers from the mesophile *Methanobacterium formicicum* [61, 69]. These structures are essentially only histone folds without N- or C-terminal extensions *(figure 1B)*. When superimposed, the α-carbon atoms of the $(HMfB)_2$ homodimer and the histone fold region of a $(H3\text{-}H4)_2$ heterodimer had a root-mean-square deviation of only 2.1Å [39].

HMf was isolated in a search for extrinsic factors that would help stabilize the 33% mol G + C *M. fervidus* genome against thermal denaturation in its growth range of 70–95° C [33, 69]. The *M. fervidus* cytoplasm contains > 300 mM tripotassium 2′, 3′ cyclic diphosphoglycerate ($K_3$cDPG), which stabilizes *M. fervidus* enzymes in vitro and may help maintain the double-stranded configuration of the genomic DNA in vivo [21, 28]. HMf probably also helps stabilize the *M. fervidus* genome, as it has been shown to increase the melting temperature of double-stranded DNA (dsDNA) by > 20°C [33] and has been localized to the *M. fervidus* nucleoid [7, 44]. HMf forms archaeal nucleosomes in vivo [44] that visibly resemble eucaryal nucleosomes [43, 50] but contain ≈ 85 bp DNA wrapped around an HMf tetramer in contrast to 146 bp wrapped around a histone octamer [4, 21, 37, 44]. Based on homologies with eucaryal histones, HMf dimers are predicted to bind DNA through similarly paired β-strand regions and the paired ends of helix I *(figure 1B)* [2, 37, 61].

At low histone to DNA ratios, rHMfA and rHMfB assembly into archaeal nucleosomes on relaxed circular DNAs introduced negative superhelicity, whereas at higher histone to DNA ratios, positive superhelicity was introduced [42]. HMfB generated more highly supercoiled molecules than rHMfA, but archaeal nucleosome formation was detectable with rHMfA at lower histone to DNA ratios. The electrophoretic mobility of linear DNA > 2 kbp through agarose gels is increased relative to the protein-free DNA by both rHMfA and rHMfB binding, but the rHMfB-DNA complexes migrate faster than rHMfA-DNA complexes, indicating that rHMfB forms a more compact structure [52].

There is ≈ one HMf tetramer per ≈ 67 bp genomic DNA in *M. fervidus* cells [44, 63]; however, the relative amounts of HMfB and HMfA differ with the growth phase [42, 52], with HMfA predominating during exponential growth and HMfB in stationary phase. This observation suggests that these proteins may have different roles in vivo, possibly related to their different DNA affinities and supercoiling activities. As HMfA and HMfB form both homodimers and heterodimers, *M. fervidus* could have archaeal nucleosomes with six different tetramer cores, each of which might have different effects on DNA packaging and topology. To date, all archaeal species with histones have more than one histone-encoding gene, and *Methanococcus jannaschii* has five [10], providing the opportunity to form many different homo- and heterodimers which could play different roles in gene regulation.

Archaeal histones have sequences most similar to histone H4 sequences *(figure 1A)*, and the archaeal nucleosome appears to be similar, and possibly homologous, to the $(H3\text{-}H4)_2$ tetramer complex. $(H3\text{-}H4)_2$ tetramers recognize nucleosome positioning signals and initiate nucleosome formation, organizing the central ≈ 120 bp of DNA and directly wrapping and protecting ≈ 73 bp from micrococcal nuclease (MN) digestion [26, 27]. Nucleosome positioning signals are recognized by the $(H3\text{-}H4)_2$ tetramer in the absence of (H2A-H2B) and do not require their N- and C-terminal extensions [16, 27, 60]. The octamer-containing eucaryal nuclesome wraps DNA in negative supercoils, but recently it has been shown that $(H3\text{-}H4)_2$ tetramers can also wrap DNA in positive supercoils [25] as established earlier for archaeal nucleosomes [42].

## 3. Eucaryal nucleosome positioning

Nucleosome positioning regulates gene expression and participates in higher order chromatin organization. Nucleosome positions have two parameters: the translational position refers to the location of the histone octamer along a DNA molecule, and the rotational position describes which face of the DNA helix contacts the histone core [5]. In addition to DNA sequence, histone competition with other DNA binding proteins, the position of neighboring nucleosomes and higher order chromatin structure must also play roles in nucleosome placement in vivo. The sequence 'rules' that govern nucleosome positioning continue to be refined [11, 36, 64]; although it is well established that inherently curved and inherently flexible DNA sequences direct nucleosome assembly. Presumably these sequences most readily accept the DNA distortion associated with nucleosome formation at a lower free energy cost than other DNA sequences [64].

### 3.1. Curved DNA

Nucleosomes assemble preferentially on some intrinsically curved regions of DNA, both in vitro and in vivo [13, 30]. Several models predict and explain sequence-dependent DNA curvature (reviewed in [14, 41]), with a common theme being the repetition of short sequences that introduce structural deformations in phase with the ≈ 10.5 bp/turn helical repeat, which results in global DNA curvature [14, 18]. For example, $(A)_{4–6}$ tracts repeated in phase with the DNA helical repeat are classic models for curved DNA [40], although some repeating G/C-rich elements such as GGGCCC also cause curvature [9].

In vitro competitive reconstitution assays revealed that eucaryal nucleosomes preferentially assembled at two curved sites in plasmid $pJGC_1$/svt. One region contained *Crithidia fasiculata* DNA with 16 $(A)_{4–6}$ tracts repeated in phase with the DNA helix, and had a five- to sevenfold higher probability of being assembled into nucleosomes than adjacent uncurved DNA. The second region was from the simian virus 40 (SV40) terminus of replication, which had a two- to fivefold higher probability of assembling in nucleosomes than flanking uncurved vector DNA [23, 30, 32]. The SV40 DNA did not, however, contain phased $(A)_{4–6}$ tracts, but did have a curve of ≈ 200° [23, 30].

SELEX (systematic evolution of ligands by exponential enrichment) procedures have been used to select DNA molecules with high affinities for histone octamer cores. The most abundant group of selected high affinity molecules had multiple tracts of three or more consecutive A nucleotides. They had slight intrinsic curvature and form nucleosomes more stable than the starting population of bulk nucleosomal DNA [67].

### 3.2. The 5′-$(G/C)_3$NN$(A/T)_3$NN-3′ repeat element

As DNA is wrapped around the histone core, the face of the helix in contact with the core is compressed, resulting in alternating compressions of the major and minor DNA grooves at ≈ 5-bp intervals *(figure 2)*. When DNA molecules containing alternating short A/T-rich and G/C-rich sequences were assembled into nucleosomes, these A/T and G/C regions were positioned at minor and major groove compression sites, respectively [17]. Based on this observed rotational positioning, DNA fragments containing the repeating motif 5′-$(G/C)_3$NN$(A/T)_3$NN-3′ would be expected to favor nucleosome assembly as anisotropically flexible wedges favoring alternating major and minor groove compression sites are present every ≈ 5 bp [57, 58]. Consistent with this prediction, synthetic DNA molecules containing the 5′-$(G/C)_3$NN$(A/T)_3$NN-3′ repeat exhibit stronger nucleosome positioning than all known natural positioning sequences, and have up to 100-fold higher affinity for nucleosome assembly than bulk genomic nucleosomal DNA [57]. DNA molecules with the highest affinities contain this motif repeated at a frequency that is less than the helical repeat of DNA in solution, consistent with nucleosomal DNA being overwound relative to free DNA [58]. Also, as predicted, these fragments assemble with a defined rotational position

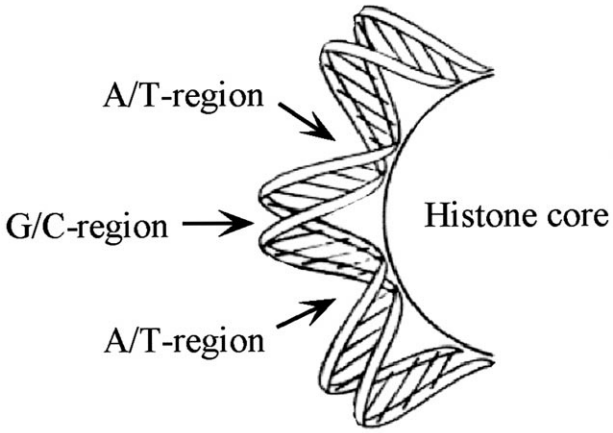


**Figure 2.** Rotational orientation of DNA wrapped around the histone core. DNA wrapping around the histone core is facilitated by the 5'-$(G/C)_3NN(A/T)_3NN$-3' repeat, which places A/T- and G/C-rich regions at sites of minor and major groove compressions, respectively [60].

in nucleosomes with G/C regions at major groove compression sites [57, 58]. In contrast, synthetic DNA fragments containing 5'-$(G/C)_3NN(G/C)_3NN$-3' repeats, sequences which would be expected to favor major groove compressions at sites where minor groove compression are required for nucleosome assembly, are refractory to nucleosome formation in vitro [58].

The 5'-$(G/C)_3NN(A/T)_3NN$-3' repeat element appears to have relevance to nucleosome positioning in vivo as this motif is present in several 5S RNA genes that position nucleosomes in vivo and in vitro [48, 59], and is also present in bulk chicken erythrocyte nucleosomal DNA [17]. The repeat sequence in nucleosomal DNA is present with a periodicity of ≈ 10.17 bp throughout the entire 146 bp of DNA within the nucleosome, although there are some irregularites near the dyad [54]. This motif is also found in DNA regions bent by the bacterial catabolite activator protein (CAP), consistent with these sequences playing a generic role in favoring many protein-induced DNA bending events [19].

### 3.3. CTG repeats

The expansion of trinucleotide tandem repeats (CTG, CGG, or AAG) has been correlated with some human genetic disorders such as fragile X syndrome and Huntington's disease [3], and as these repeats adopt unusual structures, they could be responsible for genetic instability through errors in DNA replication. CTG repeats are also very strong nucleosome positioning elements, and CTG repeat expansion is also associated with increased nucleosome assembly [20, 65]. Competitive reconstitution assays demonstrated that a DNA fragment containing 130 CTG repeats had ≈ ninefold higher affinity for nucleosome assembly than the *Xenopus borealis* 5S RNA gene [65]. Nucleosomes assembled within the *Xenopus laevis* H4 gene at a unique translational position with a $(CTG)_6$ repeat near the nucleosome dyad, and deletion of the $(CTG)_6$ region decreased positioned nucleosome assembly [20]. CTG repeats appear to be more flexible and more highly writhed than random B-DNA, which may facilitate nucleosome assembly at these regions [3, 12].

### 3.4. Dinucleotide repeats

Fourier transform analysis of bulk nucleosomal DNA has revealed that some dinucleotides are present more often than others within 5'-$(G/C)_3NN(A/T)_3NN$-3' repeats. Periodic signals for AA (= TT) dinucleotides occur every ≈ 10.2 bp, with much reduced frequencies of AT or TA dinucleotides at this frequency [54]. A strong repeat signal also exists every ≈ 10.2 bp for GC and GG (= CC), ≈ 5 bp away from the A/T-rich repeats [6, 8, 31]. Consistent with these findings, the affinity of histone octamers for synthetic DNA molecules in vitro differs somewhat depending on the precise sequences that form a 5'-$(G/C)_3NN(A/T)_3NN$-3' repeat [57, 58].

Fourier analyses of the results of two SELEX experiments have demonstrated that DNA molecules selected in vitro for very high affinity for histone octamers have strong TA dinucleotide signals every ≈ 10 bp, which was not a signal detected previously with statistical significance in bulk eucaryotic genomic or nucleosomal DNAs [6, 54, 64, 68]. Lowary and Widom [36] selected very high affinity DNA molecules for

histone octamers from a pool of chemically synthesized (nonnatural) DNA fragments, and the strongest periodic signal was TA every ≈ 10 bp. A significant but lower signal at ≈ 10 bp intervals also existed for AA (= TT) as well. DNA sequences with high affinities for histone octamers isolated from a library of mouse nucleosomal DNA by SELEX [67] also had a strong occurrence for TA and AA (= TT) every ≈ 10 bp [64]. The lack of periodic occurrences of CA or CG indicates that not all pyrimidine-purine steps favor nucleosome assembly [64].

## 4. Archaeal nucleosome positioning

HMfA and HMfB also associate preferentially with specific DNA sequences, resulting in positioned assembly of archaeal nucleosomes both in vitro and in vivo.

### 4.1. Curved DNA

EM experiments analogous to those described above for eucaryal histones [30] investigated HMf positioning on plasmid pJGC1/svt in vitro [29]. HMf was found to preferentially assemble on the highly-curved *C. fasiculata* region that contained phased $A_{4\text{-}6}$ tracts, binding with ≈ fourfold higher affinity than to the adjacent plasmid DNA, but HMf exhibited only a slight preference for archaeal nucleosome assembly at the curved SV40 DNA [29].

In competition experiments, rHMfA bound preferentially to synthetic, uniformly-curved DNA molecules that contained $A_6$ tracts phased every ≈ 10 bp rather than to zig-zag molecules that contained $A_6$ tracts phased every ≈ 15 bp [22, 45]. MN digestion of rHMfA-containing archaeal nucleosomes assembled in vitro on plasmid DNA, followed by sequencing of the MN-protected DNA fragments, indicated that nucleosome assembly was clustered and localized to regions of the plasmid that exhibited intrinsic curvature [22].

### 4.2. 7S RNA gene

Immunoprecipitation experiments of HMf-DNA complexes cross-linked in situ in *M. fervidus* cells revealed HMf was bound preferentially to localized regions of the *M. fervidus* genome [44]. The 7S RNA-encoding gene was very abundant in these immunoprecipitates, and the 5′ region of the 7S RNA gene was subsequently shown to be specifically protected from MN digestion by assembly in vitro into archaeal nucleosomes. Analysis of the ≈ 64 bp MN-protected fragments demonstrated these HMf-containing archaeal nucleosomes were assembled with a precise translational and rotational position [45]. Although the elements that facilitate this positioning have not been established, this DNA does have short alternating A/T-rich and G/C-rich regions, with the minor grooves of several of the A/T-rich regions oriented towards the nucleosome core [45].

### 4.3. CTG repeats

DNA sequences containing $(CTG)_6$ and $(CTG)_8$ repeats directed the translational positioning of archaeal nuclesome assembly in vitro. Analyses of the ≈ 70 bp DNA fragments that were protected from MN digestion by incorporation of these DNAs into archaeal nucleosomes revealed they were assembled predominantly at two positions, one at each junction of CTG repeats with the adjacent plasmid DNA [53]. $(CTG)_6$ repeats do not, however, occur in the genomes sequenced to date from histone-containing Archaea.

### 4.4. 5′-$(G/C)_3$NN$(A/T)_3$NN-3′ repeats

Synthetic DNA molecules with the repeat sequence 5′-$(T)_3(A)_3$GCCG-3′ have very high affinity for rHMfB and rHMfA *(figure 3)*. This repeat is consistent with the generic eucaryal nucleosome positioning sequence 5′-$(G/C)_3$NN$(A/T)_3$NN-3′ [54, 58] and contains the TA dinucleotide every ≈ 10 bp as found in the DNA molecules with high affinities for histone octamers selected by SELEX [36, 64, 67]. Insertion of four tandem repeats of the 5′-$(T)_3(A)_3$GCCG-3′ sequence into a 110-bp vector fragment was sufficient for high-affinity binding by rHMfB and rHMfA (K. Bailey and J.N. Reeve, unpublished results).

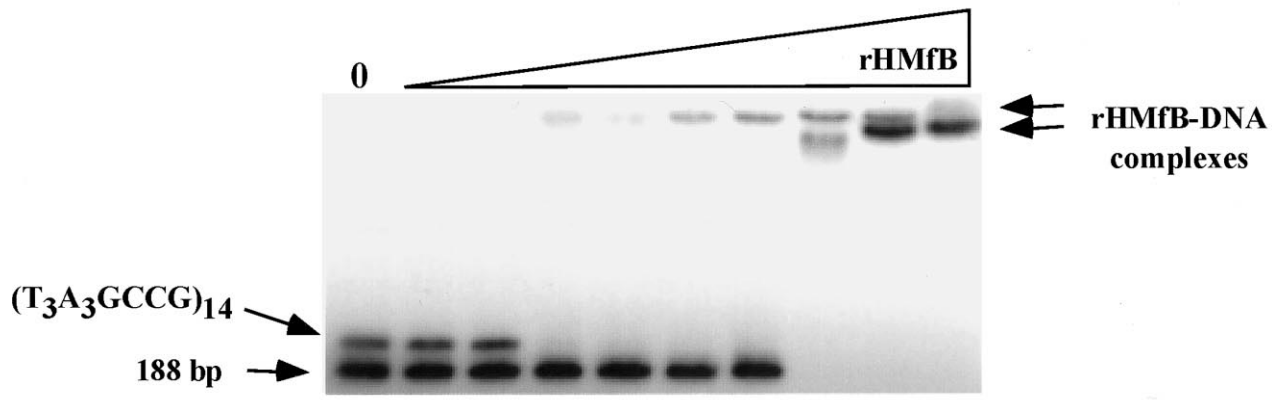


**Figure 3.** Competitive EMSA of rHMfB with $(T_3A_3GCCG)_{14}$ and 188-bp DNA fragment. Equimolar (0.9 nM) amounts of $^{32}$P-labeled $(T_3A_3GCCG)_{14}$ and a $^{32}$P-labeled 188-bp fragment isolated from a *Bam*HI-*Eco*RV digest of pBR322 [56] were incubated with excess unlabeled sonicated herring sperm DNA alone (0) or in the presence of 5, 13, 64, 130, 260, 500, 650, 1 000, 1 300 nM rHMfB. The binding reactions were allowed to reach equilibrium at 25° C, and rHMfB-DNA complexes were separated from free DNA by electrophoresis through 8% polyacrylamide gels [22]. The gels were autoradiographed, revealing that archaeal nucleosome assembly with $(T_3A_3GCCG)_{14}$ occurs at a ≈ tenfold lower rHMfB concentration than with the 188-bp pBR322 fragment.

## 5. Conclusions

DNA molecules that direct the localized assembly of archaeal nucleosomes have repeat sequence features in common with well-characterized eucaryal nucleosomal positioning elements. DNA structure, determined by DNA sequence, must play an important role in positioning, and such repeated sequences as phased oligo(dA) tracts, 5′-$(G/C)_3NN(A/T)_3$NN-3′ and CTG repeats direct both archaeal and eucaryal nucleosome positioning in vitro. Positioning and repositioning of nucleosomes is a central feature of gene expression in Eucarya and it appears likely that this will also be the case in the histone-containing Archaea. The experimental challenge is to document precisely-positioned archaeal nucleosomes in vivo, and to relate their presence or absence to adjacent gene expression.

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