Both DNA and Histone Fold Sequences Contribute to Archaeal Nucleosome Stability

Kathryn A. Bailey§, Frédéric Marc, Kathleen Sandman, and John N. Reeve‡

From the Department of Microbiology, Ohio State University, Columbus, Ohio 43210

Received for publication, October 17, 2001, and in revised form, December 18, 2001

The roles and interdependence of DNA sequence and archaeal histone fold structure in determining archaeal nucleosome stability and positioning have been determined and quantitated. The presence of four tandem copies of TTTAAAGCCG in the polylinker region of pLITMUS28 resulted in a DNA molecule with increased affinity (ΔΔG of ~700 cal mol⁻¹) for the archaeal histone HMfB relative to the polylinker sequence, and the dominant, quantitative contribution of the helical repeats of the dinucleotide TA to this increased affinity has been established. The rotational and translational positioning of archaeal nucleosomes assembled on the (TTTAAAGCCG)₄ sequence and on DNA molecules selectively incorporated into archaeal nucleosomes by HMfB have been determined. Alternating A/T- and G/C-rich regions were located where the minor and major grooves, respectively, sequentially faced the archaeal nucleosome core, and identical positioning results were obtained when such molecules were incorporated into a nucleosome, the alternating A/T- and G/C-rich regions were positioned where the minor and major grooves were compressed, respectively, as they sequentially faced the histone core (7). However, now with high resolution structures available, it is known that the nucleosome is only quasi-symmetric and that the wrapped DNA is also distorted into regions of under- and overwinding and has sites with non-ideal base pair stacking (1–3). As might be anticipated, to accommodate these additional distortions considerably more complex “rules” have recently been established for nucleosome positioning sequences, based primarily on the sequences of both natural and synthetic DNA molecules selectively incorporated into nucleosomes in vitro by octamer binding (10–12). Consistent with the designed molecules, these selected molecules also have A/T- and G/C-rich regions alternating in phase with the helical repeat as a dominant feature, but most also have multiple short A-tracks (A₃–₄) on one DNA strand and an overabundance of TA dinucleotides at ~10-bp intervals, features that introduce elements of sequence complexity. For example, the mouse genomic DNA fragments isolated with the highest histone octamer affinity have sequences that contain 10-bp repeats that conform to a consensus TATAAAGCCGC motif (11). Subsequently, these molecules have been shown to exhibit exceptional flexibility, both in bending and in accommodating changes to the helical twist (13, 14). In this regard, it is noteworthy that TA is the least stable dinucleotide, and TATA is one of the most flexible tetranucleotides (15, 16). Consistent with exploiting these sequence-determined structural properties, TA steps have been localized ±15 bp from the central dyad of positioned nucleosomes, specifically at the sites at which the base pair stacking is necessarily most distorted (17). Presumably, this use of TA dinucleotides minimizes the energy costs of nucleosome assembly.

A complicating feature in extending these analyses is that the eukaryotic histone octamer contains four different histones, H2A, H2B, H3, and H4. This makes it difficult to interpret such nucleosome positioning sequences in terms of individual histone-DNA interactions. In addition, because the octamer-
assembly of DNA into nucleosomes in vitro is very salt-dependent, the assembly technology used has been raised as an issue in quantitative data interpretation (12). Fortunately, in contrast, archaeal nucleosome assembly in vitro occurs with only one archaeal histone and occurs spontaneously under almost all solution conditions, providing a much simpler alternative experimental system for parallel and comparative studies (18–20). The histone folds of archaeal histones and the eukaryotic nucleosome core histones are almost identical, but unlike their eukaryotic counterparts, archaeal histones do not have N- and C-terminal amino acid sequences extending from this fold (21, 22). Archaeal nucleosomes resemble the structure formed by the (H3+H4)4 tetramer at the center of the eukaryotic nucleosome. Both contain a histone tetramer circumscribed by ~85 bp of DNA but which makes direct histone fold contacts with only ~60 bp of sequential DNA (1, 23). In both structures, the DNA can be wrapped in either a negative or positive toroidal supercoil (24–26), and both tetramers recognize and respond to nucleosome positioning sequences (27–29). Consistent with this homology, the DNA molecules selected into archaeal nucleosomes by HMfB, the most extensively studied archaeal histone, originate from Methanothermus fervidus, have short A-tracts and an overabundance of AA (30, 31). The histone tetramer positioned where the minor and major grooves, respectively, face the histone core. The importance of the TA dinucleotide position is apparently determined by differences in the C-terminal region of HMfB. Even more surprising, this difference in affinity is apparently determined by differences in the C-terminal region of archaeal nucleosomes by HMfB. Even more surprising, this difference in affinity is apparently determined by differences in the C-terminal region of archaeal nucleosomes. M. fervidus also contains HMfA, a second archaeal histone with a primary sequence 84% identical to that of HMfB, and based on high resolution crystal structures HMfA and HMfB dimers have almost identical histone folds (22, 31). However, HMfA does not bind similarly with high affinity to the DNA molecules selectively incorporated into archaeal nucleosomes in vitro by HMfB. Even more surprising, this difference in affinity is apparently determined by differences in the C-terminal region of helix 3, a region of the histone fold predicted to be buried within the nucleosome core with no direct contacts to the DNA (1–3, 20, 21, 32).

**EXPERIMENTAL PROCEDURES**

*Archaeal Histone Purification and α-Helix 3 Exchange*

Recombinant HMfA and HMfB were synthesized in Escherichia coli JM105, purified, and quantitated as previously described (18, 32, 33). A QuikChange kit (Stratagene, La Jolla, CA) was used to change the sequences of codons 61–63 of hmfA and hmfB, cloned in the expression plasmids pKS595 and pKS232, from GAATAGCT and GAAGTACGA to GAGCTAGCT and GAGCTAGCA, introducing an Nhel site (GCTA-GC) into both genes without changing the encoded amino acid sequence (ELA) and generating plasmids pKS597 and pKS600. Oligonucleotides with the sequences 5′-CTAGCAGGAAAATGTTCAATAAGATCTA and 3′-GTGCTTTTTCAAGTTATCTAGTCCGA and 5′-CTAGCGGTGCGAGAAATTTAATAA and 3′-GTCAGGCTTTTAAATTCCCTTTTATTCCGA, and 5′-CTAGCGGTGCGAGAAATTTAATAA and 3′-GTCAGGCTTTTAAATTCCCTTTTATTCCGA, were annealed to obtain da2 DNA molecules with single-stranded Nhel and HindIII half-site extensions that encoded the restriction of HMfA and HMfB, C-terminal to residue 62. Aliquots of pKS597 and pKS600 plasmids DNAs were digested with Nhel plus HindIII, and ligation with the DNA molecule encoding the appropriate C-terminal sequence resulted in plasmids pKS603 and pKS604. These plasmids were transformed into *E. coli* JM105, and isopropyl-β-D-thiogalactopyranoside (400 μM) was added to exponentially growing cultures of the transformants to induce the synthesis of HMfA/B and HMfA/αβ. These proteins were purified, quantitated, and their CD spectra measured using an AVIV model 62A-DA spectrophotometer (Aviv, Lakewood, NJ) as previously described for HMfA and HMfB (33–35). Archaeal histone monomer solution concentrations are cited in terms of dimers, and the apparent NC values reported for archaeal nucleosome assembly are given in terms of histone tetramers.

**DNA Constructions and Labeling**

*Plasmid Construction and Labeling* 

- **MM201 and MM301—Plasmid pMM201 and pMM301** 
  - Purified by Qiagen maxi prep kits (Valencia, CA) and digested with EcoRI plus PstI. 
  - The products were labeled at the EcoRI half-sites using [γ-32P]ATP in exchange reactions catalyzed by T4 polynucleotide kinase (Invitrogen) (37) and separated by electrophoresis through 8% T, 0.11% C polyacrylamide gels run at 8 V/cm (1 hr) at 4°C. 
  - EcoRI plus PstI digestion released 175-bp and 115-bp molecules, which were used to transform pMM201 and pMM301, from pMM201 and pMM301, respectively, which were then digested with Sau3AI, separated by gel electrophoresis, and visualized by autoradiography. 
  - The gel from which these molecules were excised, crushed, and incubated overnight at 37°C in 0.5 mM ammonium acetate, 2 mM EDTA, 0.1% (w/v) SDS. 
  - The gel fragments were removed by centrifugation, and the eluted DNA was precipitated from the supernatant by addition of 2 volumes of ethanol, ethanol-washed and redissolved in TE (10 mM Tris/HCl, 1 mM EDTA, pH 7.5). 

**Ltitmus28**

- (TTTTAAGCCG)2, (TTTTAAGCCG)4, and (TTTTAAGCG)6—Preparations of plasmid pLITMUS28 (New England Biolabs, Beverly, MA) were purified from *E. coli* DH5α using Qiagen maxi prep kits and digested with BseHI plus Acc65I. This released a molecule with a 95-bp ds region from the polylinker region (designated Litmus28) that was [γ-32P]ATP and T4 polynucleotide kinase, gel-purified, and used as a control DNA that lacked TTTAAAGCCG sequences. 

**Oligonucleotide Construction**

- (TTTTAAGCCG)2, (TTTTAAGCCG)4, and (TTTTAAGCG)6—Preparations of plasmid pLITMUS28 (New England Biolabs, Beverly, MA) were purified from *E. coli* DH5α using Qiagen maxi prep kits and digested with BseHI plus Acc65I. This released a molecule with a 95-bp ds region from the polylinker region (designated Litmus28) that was [γ-32P]ATP and T4 polynucleotide kinase, gel-purified, and used as a control DNA that lacked TTTAAAGCCG sequences. 

**Oligonucleotide Construction**

- Aliquots (1 μM) of oligonucleotides with the sequences 5′-CTCGTTGATGACCTTTAAGCGG-TGCAGGAACTCTCGCTCGAG, 20 μM dNTPs, 50 mM NaCl, 10 mM MgCl2, 50 mM Tris/HCl (pH 8). These reaction mixtures were placed at 95°C for 2 min, 47°C for 2 min, and 37°C for 5 min before 3 units of the Klenow fragment of DNA polymerase (Invitrogen) were added, and incubation was continued for 30 min at 37°C. 

**DNA Constructions and Labeling**

- (TTTTAAGCCG)2, (TTTTAAGCCG)4, and (TTTTAAGCG)6—Preparations of plasmid pLITMUS28 (New England Biolabs, Beverly, MA) were purified from *E. coli* DH5α using Qiagen maxi prep kits and digested with BseHI plus Acc65I. This released a molecule with a 95-bp ds region from the polylinker region (designated Litmus28) that was [γ-32P]ATP and T4 polynucleotide kinase, gel-purified, and used as a control DNA that lacked TTTAAAGCCG sequences. 

**Oligonucleotide Construction**

- Aliquots (1 μM) of oligonucleotides with the sequences 5′-CTCGTTGATGACCTTTAAGCGG-TGCAGGAACTCTCGCTCGAG, 20 μM dNTPs, 50 mM NaCl, 10 mM MgCl2, 50 mM Tris/HCl (pH 8). These reaction mixtures were placed at 95°C for 2 min, 47°C for 2 min, and 37°C for 5 min before 3 units of the Klenow fragment of DNA polymerase (Invitrogen) were added, and incubation was continued for 30 min at 37°C. The ds DNA molecules generated were digested with BamHI plus EcoRI, and after gel purification, the products were ligated with BamHI plus EcoRI-digested pLITMUS28 and transformed into *E. coli* DH5α. Plasmids designated pTA2, pTA4, and pTA6 were isolated from ampicillin-resistant transformants, sequenced to confirm the constructions, and then digested with BseHI plus Acc65I, BseHI plus AatII, and BseHI plus BamHI, respectively, to generate molecules with ds regions of 95 bp, 93 bp, and 89 bp that contained (TTTTAAGCCG)2, (TTTTAAGCCG)4, and (TTTTAAGCG)6, located at the same position within the pLITMUS28 polylinker (see Fig. 2A). These molecules were [γ-32P]labeled at the BseHI half-site using [γ-32P]ATP and T4 polynucleotide kinase and gel-purified.

1 The abbreviations used are: ds, double-stranded; CD, circular dichroism; MN, micrococcal nuclease; nt, nucleotide(s); ss, sonicated salmon sperm; % T, total concentration of acrylamide and N,N'-methylenebisacrylamide; % C, percentage of total acrylamide concentration (T) that is N,N'-methyleylenbisacrylamide; TBE, Tris borate/EDTA; MES, 2-(N-morpholino)ethanesulfonic acid.
gusted with BssHII plus AarII to obtain molecules with 93-bp ds regions with (TTTTGAACCGC)\(_n\), (TTTGAACGCG)\(_n\), (AAATTTGCCG)\(_n\), or (TAAATGCG)\(_n\), positioned identically within the plLTMS28 polylinker. These molecules were \(^{32}\)P-labeled at the BssHII half-site and gel-purified.

**PCR Amplifications and Labeling**

Some DNA molecules used in gel-shift assays and all of the DNA molecules used for rotational and translational positioning studies were generated by PCR-amplification from plasmid template DNA using Taq DNA polymerase (Invitrogen). These molecules were end-labeled by the incorporation of one \(^{32}\)P-\(^5\)P-end-labeled primer and/or labeled internally by the incorporation of \(^{32}\)P-\(^5\)P-dATP during the extension reactions. \(^{32}\)P-labeled molecules (111 bp containing (TTTTAAGCGC)\(_n\), (TTTTGACGGCG), (TTTGAACGCG), (AAATTTGCCG), and (TAAATGCG)) were amplified from pTA, pTG, pGc, pAT, and pTAT, respectively, using primers with the sequences 5'-CATCGCGCACCTGTCG-3' and 5'-CATCGCGCGCACCGTACGTC-3' labeled by the incorporation of \[^{32}\]P\-

**Hydroxyl Radical Footprinting**

Archaeal nucleosomes (assembled by incubation of 250 ng of HMb or HMB with 100 ng of the (TTTTAAGCGC)\(_n\)-containing DNA, clone 1 or 20 DNA at 25 °C for 20 min) were exposed to 10 mM Fe(NH\(_4\))\(_2\)(SO\(_4\))\(_2\), 20 mM Tris acetate (pH 8.8) for 25 min at 37 °C. The resulting DNA was precipitated with ethanol and redissolved in TE. The hydroxyl radicals were generated by the addition of 100 mM KCl, 50 mM Tris/HCl, and 15 mM CaCl\(_2\) to 1 mM DNA, Histone Fold Sequences, and Archaeal Nucleosomes.

**RESULTS**

Archaeal Nucleosome Assembly on Curved versus Non-curved DNA Molecules—Archaeal nucleosomes, like eukaryotic nucleosomes, were shown previously by electron microscopy to assemble preferentially at sites on DNA molecules that were intrinsically curved (38). To pursue this observation, a comparison was made of archaeal nucleosome assembly on two DNA molecules, designated MM201 and MM301 (36), which contained similar repetitive sequences, (AAATTTGCCG)\(_n\) and (TTTTAAGCGC)\(_n\), respectively, but have very different intrinsic curvature (36). The sequences of both molecules conform to the \([\langle\{AT\}n\rangle\langle\{NN\}G\langle\{C\}3\rangle\langle\{NN\}n\rangle\) nucleosome positioning motif, but MM201 is very highly curved whereas MM301 is only slightly curved (36). As expected for a highly curved molecule, MM201 had a very anomalous electrophoretic mobility, whereas MM301 had only a slightly anomalous mobility (Fig. 1), but HMB assembled preferentially into archaeal nucleosome on MM301. Under HMB-limited conditions, only MM301 molecules were incorporated into archaeal nucleosomes in reaction mixtures that contained an equimolar mixture of MM201 and MM301 (Fig. 1, lane 3). Intrinsic DNA curvature alone did not therefore equate to preferential assembly into an archaeal nucleosome, and as observed for eukaryotic nucleosomes (7), this therefore equate to preferential assembly into an archaeal nucleosome, and as observed for eukaryotic nucleosomes (7), this second DNA strand similarly end-labeled by \(^{32}\)P-primer incorporation. Hydroxyl cleavage reactions were terminated by the addition of thiouria (200 mM final concentration), and after electrophoresis through 8% T, 0.1% C polyacrylamide gels run at 5 V/cm in TBE, the hydroxyl radical-treated complexes were located by autoradiography. The bands in the gel that contained these complexes were excised, crushed, and incubated overnight at 25 °C on 0.5 μm ammonium acetate, 2 mM EDTA, 0.1% (v/v) methanol, dried, and used to expose x-ray films resulting in autoradiograms from which band intensity profiles were obtained by laser-scanning densitometry.

**Number and Sequence of Repeats That Facilitate Archaeal Nucleosome Assembly**—Archaeal nucleosome assembly occurs spontaneously in vitro with DNA molecules of >85 bp (23).
not result in increased affinity for HMfB relative to the 93-bp control. Litmus28 DNA generated from the same polylinker region without an insert. However, the presence of four and six copies resulted in DNA molecules with much higher affinities for HMfB (Fig. 2A). In addition to intrinsic shape, MM201 differs from MM301 in lacking TA dinucleotides repeated at 10-bp intervals. To evaluate this feature as a facilitator of archaean nucleosome assembly, DNA molecules were generated with four copies of variants of the MM301 motif that lacked TA steps. All these molecules had reduced affinities for HMfB relative to the molecule containing four copies of the MM301 motif (Fig. 2B). Simply replacing the A at position 4 with G reduced the affinity for HMfB more than 3-fold, decreasing the apparent $K_d$ for HMfB tetramers from 35 ± 5 to 104 ± 6 nM (Fig. 2C and Table I). For comparison, four copies of the mouse genomic high affinity motif, TATAAACGCC (13, 14), were also identically positioned in the pLITMUS28 polylinker, and the 93-bp DNA molecule amplified from this construct did have increased affinity for HMfB (apparent $K_d$ of 72 ± 6 nM relative to the Litmus28 control and the MM301 TA-less variants. However, the affinity of this molecule for HMfB was still 2-fold lower than that conferred by four copies of TTTAAGCCG, the MM301 motif (Fig. 2B, and C and Table I).

### Archaeal Nucleosome Positioning by (TTTAAAGCCG)$_4$—Hydroxyl radical footprinting was used to determine the rotational positioning of the (TTTAAAGCCG)$_4$ sequence when assembled into an archaeal nucleosome. By comparing the hydroxyl radical cleavage patterns of this DNA in solution and when assembled into an archaeal nucleosome, it was apparent that the four A/T-rich regions, and specifically the four TA steps, were protected from hydroxyl radical cleavage when the molecule was assembled in an archaeal nucleosome (Fig. 3A). Virtually identical patterns of protection and cleavage were observed with archaean nucleosomes assembled by using HMFIA or HMfB, and as reported for the mouse genomic (TATAAACGCC)$_4$ sequence, most notably the CC dinucleotides, were cleaved preferentially by the hydroxyl radical both in the presence and absence of histones. These molecules must therefore adopt similar structures, in terms of preferred sites for histone nucleosomal positioning.
Fig. 3. Archaeal nucleosome positioning by (TTTAAAGCCG)_4. A, archaeal nucleosomes were assembled on the (TTTAAAGCCG)_4-containing DNA, using either HMfA or HMfB, and exposed to hydroxyl radical cleavage. The DNA products were separated by electrophoresis under denaturing conditions, and the dried gel was used to generate the autoradiogram from which band intensity profiles were obtained by laser-scanning densitometry. The control lanes contained aliquots of the DNA that were (+) or were not (-) exposed to the hydroxyl radical in the absence of the archaeal histone. Regions protected from hydroxyl radical cleavage by assembly into an archaeal nucleosome are connected to the corresponding hydroxyl radical footprint. B, archaeal nucleosomes assembled containing HMfB and 32P-labeled-(TTTAAAGCCG)_4 DNA were exposed to MN, and samples were removed at increasing times from 1 to 120 min. The MN digestion products were separated by polyacrylamide gel electrophoresis and visualized by autoradiography. Control lanes contained an aliquot of the DNA incubated without (-) or with (+) MN for 2 min in the absence of HMF. C, aliquots of a population of ~60-bp MN-protected DNA molecules, generated as illustrated in B, were exposed to BstHI (Bsa), EcoRI (E), BamHI (B), or Ncol (N) digestion. The products were separated by electrophoresis under denaturing conditions, visualized by autoradiography, and quantitated by scanning densitometry of the autoradiogram. Control lanes contained an aliquot of the ~60-bp population (+) and single-stranded size standards. Quantification of the BamHI digestion products revealed that >60% of the ~60-bp MN-protected molecules contained the region between the two triangles positioned above the sequence in A.

accumulation of ~60-bp MN-protected molecules (Fig. 3B). A population of these ~60-bp molecules was isolated and aliquots subjected to digestion by different restriction enzymes. Electrophoresis through denaturing gels revealed that the ~60-bp molecules actually had single strands ranging in length from 55 to 61 nt, with the majority being 57 ± 1 nt. Approximately 60% of the ~60-bp molecules were cut by BamHI and ~3% by EcoRI, but there was no detectable cleavage by any of the other restriction enzymes for which restriction sites were originally present in the DNA that flanked the (TTTAAAGCCG)_4 sequence (Fig. 3A). BamHI digestion generated predominantly one large product with single strands of 49 and 44 nt (Fig. 3C), and therefore the majority, but not all, of the archaeal nucleosomes assembled on the (TTTAAAGCCG)_4-containing DNA positioned such that they protected the same 55–61-bp region from MN digestion (Fig. 3A). This encompassed a 61-nt TTTAAAGCCG sequence and the BamHI restriction site. Because the single strands of the BamHI restriction product differed in length by 5 nt (49 and 44 nt), the ~60-bp MN-protected substrate molecules must have had one 3’-terminal unpaired nucleotide.

Archaeal Nucleosome Positioning by in Vitro Selected Molecules—DNA molecules selected previously by their preferential incorporation into archaeal nucleosomes by HMfB were cloned and sequenced (30). They all had fixed 25-bp sequences (25R and 25L) flanking different 60-bp sequences, and by aligning these variable 60-bp sequences, a consensus sequence was generated that conformed to the [(A/T)_n(N/G/C)_nNN)_n nucleosome positioning motif (Ref. 30 and Fig. 4). Many of these molecules have TA dinucleotides repeated at ~10-bp intervals. In this regard they resemble the (TTTAAAGCCG)_4- and (TATTAAAGCCG)_4-repeats, but they differ in not having reiterated sequences or inverted repeat symmetry. This lack of symmetry seems noteworthy because these are molecules that were selectively incorporated by HMfB homodimers into archaeal nucleosomes that seem likely to have a symmetric homotetramer histone core. Based on conforming to the [(A/T)_n(N/G/C)_nNN)_n motif, it was expected that these molecules would assemble into archaeal nucleosomes with the alternating A/T- and G/C-rich regions located where the minor and major grooves faced the nucleosome core (30). Hydroxyl radical footprinting of archaeal nucleosomes assembled using clones 1 and 20 as representative DNA molecules, and either HMfB or HMfA, generated results entirely consistent with this prediction. When assembled into archaeal nucleosomes, the AT-rich regions present in these DNA molecules at ~10-bp intervals, which in many cases included a TA dinucleotide, were protected from hydroxyl radical cleavage (Fig. 4). As illustrated by the consensus sequence shown in Fig. 4, the majority of the 60-bp HMB-selected sequences have a poly(A) tract centered around position 20, and this region of both clone 1 and 20 DNAs was almost completely protected from hydroxyl radical cleavage when assembled into an archaeal nucleosome.

HMfA Assembly of HMfB-selected DNA Molecules into Archaeal Nucleosomes—Although most of the HMfB-selected molecules have 60-bp central sequences that conform generically to the [(A/T)_n(N/G/C)_nNN) DNA, they differ in not having reiterated sequences or inverted repeat symmetry. This lack of symmetry seems noteworthy because these are molecules that were similarly optimized for archaeal nucleosome assembly by HMfA. Surprisingly, this was not the case (Fig. 5). Although HMfA and HMfB have very similar high resolution structures (22), and all the DNA-binding residues identified by mutagenesis in HMfB (32) are conserved in HMfA, HMfA had affinities for clone 1 and 20 DNAs that were ~2-fold and ~20-fold lower than the affinities of HMfB for these molecules (Table II). However, as noted above, HMfA did assemble these DNAs into archaeal nucleosomes with the same rotational positioning as HMB (Fig. 4).

Role of α-Helix 3 in Archaeal Nucleosome Stability—The sequences of HMfA and HMfB are 84% identical but do differ in three consecutive positions, RKM versus VRR, very near the C terminus (Fig. 6). When the crystal structures of the HMfA and HMfB dimers were superimposed, these differences apparently caused a slight difference in the histone fold orientation of α-helix 3 relative to α-helix 2 (22). However, based on the structure of the (H3+H4) tetramer within the eukaryotic nucleosome core (1, 19, 21), these C-terminal residues were predicted to be buried inside the archaeal nucleosome histone tetramer core. They were expected to contribute to a four-helix bundle that forms the dimer-dimer interface at a site that has no direct contact with the DNA (Fig. 6). Nevertheless, a difference in the dimer-dimer interface might result in HMfA and HMfB tetramers with structures and/or shapes sufficiently different to require different DNA sequences for optimum nucleosome assembly. To test this hypothesis, HMfA/B-α3 and HMfB/ A-α3 variants were constructed. In these variants, the C-terminal residues of α-helix 3 of HMfA were replaced by their
counterparts from HMfB, and vice versa. Based on ellipticity measurements at 222 nm (ε222), under identical solution conditions at 25 °C, HMfA/B-α3 and HMfB/A-α3 had ~90% and ~75% of the α-helical content of HMfA and HMfB, respectively, and both formed complexes with DNA with features characteristic of the α-helix 3 donor (Fig. 7). It has been well established that archaeal nucleosome assembly on DNA molecules of >2 kbp results in complexes that migrate faster during agarose gel electrophoresis than the DNA molecule alone and that, under saturating conditions, such complexes formed by HMfB migrate faster than those formed by HMfA (33). This difference in mobility was transferred with the α-helix 3 residues. Complexes formed by HMfA/B-α3 with linear pBR322 DNA migrated faster than those formed by HMfA, and complexes
The (A/T)₃NN(G/C)₃NN Motif—Neither intrinsic curvature nor a sequence that conformed to the generic (A/T)ₙNN(G/C)ₙNN nucleosome positioning motif (6, 7) guaranteed preferential incorporation into an archaeal nucleosome. Incubation of HMB with an equimolar mixture of MM201 and MM301 resulted in the preferential incorporation of MM301 into archaeal nucleosomes, even though MM301 has much less intrinsic curvature than MM201 (Fig. 1). The difference in the affinities of MM201 and MM301 for HMB could therefore reflect a difference in intrinsic writhe, or the structure of MM301 may more readily accommodate the distortions needed for archaeal nucleosome assembly. In this regard, the MM301 motif (TATTAAGCCG) but not the MM201 (AAATTTGCCG) contains TA, the most easily distorted dinucleotide (15), and as few as 40 bp of a TA-containing repetitive sequence were sufficient to result in preferential incorporation of a DNA molecule into an eukaryotic nucleosome (6). Consistent with this, the presence of four copies of the 10-bp MM301 motif resulted in a molecule with increased affinity for HMB relative to the control Litmus28 DNA (Fig. 2A and Table I). Based on differences in apparent $K_d$ values calculated from polyacrylamide gel-shift data, the difference in free energy ($\Delta G$) of HMB assembly into an archaeal nucleosome containing the MM301 motif versus assembly using Litmus28 polylinker sequence was 702 ± 150 cal mol⁻¹. In contrast, when the TA dinucleotides in this sequence were changed to TGs, there was only a marginal difference in the free energy of assembly of HMB into an archaeal nucleosome ($\Delta G = 59 ± 88$ cal mol⁻¹) relative to assembly using the Litmus28 control. Given these values, each of the four 10-bp MM301 motifs contributed $\sim 175 ± 38$ cal mol⁻¹ (702 ± 150) relative to assembly using the Litmus28 control. Given these values, each of the four 10-bp MM301 motifs contributed $\sim 175 ± 38$ cal mol⁻¹ (702 ± 150) relative to assembly using the Litmus28 control. Given these values, each of the four 10-bp MM301 motifs contributed $\sim 175 ± 38$ cal mol⁻¹ (702 ± 150) relative to the increased stability of an archaeal nucleosome containing (TATTAAGCCG)ₙ, relative to an archaeal nucleosome containing Litmus28. Similarly, for comparison, each helical turn of a high affinity TA-containing repetitive molecule was calculated to contribute $\sim 200$ cal mol⁻¹ to eukaryotic nucleosome stability (6), and substitution of G for A at the critical TA step located at position $\sim 15$ relative to the central dyad decreased eukaryotic nucleosome stability ($\Delta G$) by $\sim 480$ cal mol⁻¹ (17). As anticipated, the TA dinucleotides in this sequence were positioned where the minor groove faced the archaeal nucleosome core (Fig. 2), and although they contributed predominantly to the archaeal nucleosome stability, the flanking sequences apparently also played a role. The presence of four copies of the mouse genomic TATAAAGCCG motif also resulted in increased affinity for HMB relative to the control Litmus28 DNA, but archaeal nucleosomes assembled using this TA-containing repeat were less stable than those assembled using (TATTAAGCCG)ₙ, the MM301 repeat. The presence of (TATTAAGCCG)ₙ resulted in a $\Delta G$ of 276 ± 109 cal mol⁻¹ (Table I) or a $\Delta G$ of only $\sim 69 ± 27$ cal mol⁻¹ per

**DISCUSSION**

The simplest explanation for the widespread conservation of the eukaryotic nucleosome is that this complex, in essentially its current configuration, existed in the last common ancestor of all eukaryotes. A likely prokaryotic origin for the nucleosome became apparent with the discovery of archaeal histones. All the evidence accumulated since supports that archaeal histones are ancestral and structural homologs of their eukaryotic nucleosome counterparts and that the archaeal nucleosome resembles the structure formed at the center of the eukaryotic nucleosome by DNA wrapped around the histone (H3 + H4), tetramer (19, 20, 23, 30). Both structures have a histone tetramer core that recognizes positioning signals, directly contacts $\sim 60$ bp, and wraps $\sim 85$ bp of DNA alternatively in either a positive or negative toroidal supercoil (24, 25, 26). However, unlike the eukaryotic histones, archaeal histones form soluble homodimers in solution that assemble spontaneously in vitro into archaeal nucleosomes. The experiments reported here were undertaken to investigate and quantify single archaeal nucleosome assembly and positioning in reaction mixtures that contained the minimal components, one histone and a defined DNA molecule only $\sim 100$ bp in length. The results obtained are, overall, fully consistent with the results from previous eukaryotic nucleosome assembly studies (6, 7, 10, 11, 12, 17). However, we have also obtained results that reveal an unexpected sequence specificity in archaeal histone-DNA interactions, results that hint at a previously unsuspected structural basis for nucleosome positioning that could be exploited to regulate gene expression.

**TABLE II**

| Histone | DNA   | $K_d$ (nM) | $\Delta G$ (cal mol⁻¹) |
|---------|-------|------------|-----------------------|
| HMB     | Litmus28 | 115 ± 13  | 88 cal mol⁻¹         |
|         | Clone 1 | 20 ± 2    | 1032 ± 117           |
|         | Clone 20| 2.7 ± 0.5 | 2213 ± 161           |
| HMFA    | Litmus28 | 94 ± 8    | 119 ± 110            |
|         | Clone 1 | 53 ± 11   | 461 ± 176            |
|         | Clone 20| 53 ± 7    | 461 ± 99             |
| HMB/A-o3| Litmus28 | 490 ± 100 | 855 ± 171           |
|         | Clone 1 | 214 ± 19  | 367 ± 109            |
|         | Clone 20| 98 ± 7    | 94 ± 106             |
| HMFA/B-o3| 207 ± 25 | 347 ± 129 |                     |
|         | Clone 1 | 45 ± 2    | 553 ± 87             |
|         | Clone 20| 6 ± 1     | 1742 ± 153           |

$^a$ $K_d$ values calculated on the basis of an archaeal histone tetramer. Average and standard deviations values are shown based on at least three separate experiments.

$^b$ $\Delta G$ is the $\Delta G$ value of the experimental sequence minus the $\Delta G$ value of 9428 ± 62 cal mol⁻¹ calculated from the apparent $K_d$ value ($\sim KT \ln(K_d)$) of HMB tetramer assembly into an archaeal nucleosome on the Litmus28 control DNA.
DNA, Histone Fold Sequences, and Archaeal Nucleosomes

Fig. 6. Sequence alignment and structure of an archaeal nucleosome containing clone 1 DNA. The sequence of clone 1 DNA protected from MN digestion by HMfB incorporation into an archaeal nucleosome is shown on the DNA strands assembled in the predominant rotational and translational positions established for clone 1 DNA. The sequences of HMfA and HMfB are shown aligned with the sequence of the histone fold of Xenopus H4 with regions that form the α-helices 1, 2, and 3 (α1, α2, and α3) and loops 1 and 2 (L1 and L2) of the histone folds identified. The heavy overline and blue shading indicate the C-terminal residues that were exchanged to generate HMfA/β-α3 and HMfB/α3. Conserved DNA-binding residues are boxed (32), and the side chains of these residues (Arg-10, Arg-19, and Lys-53) are shown in the figure in stick format on the surface of each HMfB monomer positioned appropriately for DNA binding.

Fig. 7. CD spectra and agarose gel-shift assays of complexes formed by archaeal histones. CD spectra were obtained from 10 μM solutions of the four archaeal histones, as indicated, dissolved in 25 mM MES, 50 mM K2SO4 (pH 7) at 25 °C. Aliquots of linear pBR322 DNA (100 ng) were incubated for 25 min at 25 °C with 0 (−), 25, 50, 75, 100, 150, and 200 ng of each histone. As shown, the products were then separated by agarose gel electrophoresis and visualized by ethidium bromide staining.

TA-containing 10-bp repeat relative to archaeal nucleosome assembly on the Litmus28 DNA.

HMfB-selected Sequences—Most of the DNA molecules selectively incorporated by HMfB into archaeal nucleosomes in vitro have sequences that conform to the (A/T)NN(G/C)NN motif (30). Hydroxyl radical footprinting of clone 1 and 20 DNAs demonstrated that A/T-rich regions, and specifically TA steps, that were centered around positions 20, 30, 40, and 50 within the 60-bp selected sequences were protected from cleavage by incorporation into archaeal nucleosomes (Fig. 4). As in eukaryotic nucleosome footprinting experiments (13, 40), the sequences maximally protected from hydroxyl radical cleavage were displaced by ~2 bp on the two DNA strands, and as expected, identical footprinting patterns were obtained using either HMfA or HMfB. However, in view of their very similar structures and the lack of N- or C-terminal extensions, it was also expected that HMfA and HMfB would have very similar, high affinities for these HMfB-selected DNA molecules, but this was not the case. HMfA had consistently lower affinities for these molecules than HMfB (Table II). HMfB assembly into an archaeal nucleosome using clone 1 and 20 DNAs resulted in ΔΔG values of 1032 ± 117 and 2213 ± 161 cal mol⁻¹, respectively, relative to assembly on the Litmus28 DNA, whereas HMfA assembly using these DNA resulted in ΔΔG values of only 461 ± 176 and 461 ± 99 cal mol⁻¹, respectively, relative to archaeal nucleosome assembly using the control DNA.

Clone 1 and 20 DNAs were isolated after eight rounds of selection by HMfB incorporation into archaeal nucleosomes. The increases in stability conferred by these DNAs on archaeal nucleosomes assembled using HMfB are similar to those reported for eukaryotic nucleosome stabilization (average ΔΔG value of ~1700 cal mol⁻¹) by DNA molecules isolated after nine rounds of selection by octamer incorporation into nucleosomes (10). However, the highest affinity complex investigated, formed by HMfB with clone 20 DNA, had an apparent Kₐ of 2.7 ± 0.5 nM in terms of HMfB tetramers, whereas subnanomolar Kₐ values have been reported for salt-dependent dissociation constants of eukaryotic nucleosome core particles (41).

The Role of α-Helix 3 and Tetramer Formation in Archaeal Nucleosome Positioning—It was previously noted that the orientation of α3 relative to α2 differed slightly in the histone folds of HMfA and HMfB (22) and that this apparently resulted from differences in the C-terminal residues of the two histones (Fig. 6). These C-terminal residues were therefore exchanged, and the resulting variants, HMfB/β-α3 and HMfB/α3, formed complexes with DNA with properties typical of the α3 donor (Figs. 5 and 7 and Table II). The C-terminal regions of these archaeal histones apparently therefore participate in determining how the archaeal nucleosome tetramer interacts with DNA. The decreased affinity of HMfB/α3 relative to HMfB for clones 1 and 20 DNA might be explained trivially by the decreased solution stability of this variant, but this would
not readily explain the changes observed in the agarose gel mobility of the complexes formed by these two proteins with pBR322 DNA. Similarly, it seems very unlikely that a reduced stability argument could explain the increased affinity of HMFαB-α3 relative to HMFα for the HMFαB-selected molecules. Given homology with the (H3+H4)2 tetramer (1–3), it is most likely that a four-helix bundle involving two α2 and two α3 helices forms the dimer-dimer interface at the center of an archaeal nucleosome (Fig. 6) and that this interaction stabilizes and establishes the overall shape of the archaeal histone tetramer core (19, 20). It has been argued that only a slight change at this interface generates histone tetramers that wrap DNA alternatively in either a positive or negative supercoil (20, 25, 26). It is certainly therefore conceivable that differences at this interface could also result in histone tetramers with structures/shapes sufficiently different to require different DNA sequences for optimum nucleosome assembly. If this is correct, then formation of alternative archaeal nucleosome tetramer cores could be used as a mechanism to regulate gene expression. All archaeal histones investigated to date form homodimers as well as heterodimers with other archaeal histones, including histones from other archaeal species (18, 35). If this promiscuity in partnerships extends to tetramer formation, then there is an obvious opportunity to regulate gene expression by assembling different archaeal nucleosome cores with different shapes and, therefore, different sequence and positioning preferences. This could be an especially useful system for regulating gene expression in a species such as *M. jannaschii* with six different archaeal histones (20, 35). It might also have provided the ancient foundation for the evolution of the now widespread use of different histone fold partnerships to assemble multisubunit, regulatory complexes (42).

Acknowledgments—We thank M. Shimizu for providing plasmids pMM201 and pMM301 and D. Soares and J. Widom for technical help and advice.

REFERENCES

1. Luger, K., Mader, A., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Nature **389**, 251–260
2. Harp, J. M., Hanson, B. L., Timm, D. E., and Bunick, G. J. (2000) *Acta Crystallogr. D* **56**, 1513–1514
3. White, C. L., Suto, R. K., and Luger, K. (2001) *EMBO J.* **20**, 5207–5218
4. Svedrup, J., and Horz, W. (1996) *Curr. Opin. Genet. Dev.* **6**, 164–170
5. Widom, J. (1998) *Annu. Rev. Biophys. Biomol. Struct.* **27**, 285–327
6. Shraiber, T. E., and Crothers, D. M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 7418–7422
7. Shraiber, T. E., and Crothers, D. M. (1990) *J. Mol. Biol.* **216**, 69–84
8. Satchwell, S. C., Drew, H. R., and Travers, A. A. (1986) *J. Mol. Biol.* **191**, 659–675
9. Burkholder, A. M., and Tullius, T. D. (1987) *Cell* **48**, 935–943
10. Widlund, H. R., Cao, H., Simonsson, S., Magnusson, E., Simonsson, T., Nielson, P. E., Kahn, J. D., Crothers, D. M., and Kubista, M. (1997) *J. Mol. Biol.* **267**, 807–817
11. Thirumaresu, A., Lowary, P. T., Widlund, H. R., Cao, H., Kubista, M., and Widom, J. (1999) *J. Mol. Biol.* **288**, 213–229
12. Widlund, H. R., Kuduvalli, P. N., Bengtsson, M., Cao, H., Tullius, T. D., and Kubista, M. (1999) *J. Biol. Chem.* **274**, 31847–31852
13. Roychoudhury, M., Siti, J., Lapham, J., and Crothers, D. M. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97**, 13608–13613
14. Breslauer, K. J., Frank, R., Blocker, H., and Marky, L. A. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 6833–6837
15. Packer, M. J., Donnay, M. P., and Hunter, C. A. (2000) *J. Mol. Biol.* **295**, 85–103
16. Fitzgerald, D. J., and Anderson, J. N. (1999) *J. Mol. Biol.* **293**, 477–491
17. Sandman, K., Grayling, R. A., and Reeve, J. N. (1995) *Bio/Technology* **3**, 504–506
18. Reeve, J. N., Sandman, K., and Daniels, C. J. (1997) *Cell* **89**, 999–1002
19. Sandman, K., and Reeve, J. N. (2000) *Arch. Microbiol.* **173**, 165–169
20. Luger, K., and Richmond, T. J. (1998) *Curr. Opin. Struct. Biol.* **8**, 33–40
21. Decanniere, K., Babu, A. M., Sandman, K., Reeve, J. N., and Heinemann, U. (2000) *J. Mol. Biol.* **303**, 35–47
22. Bailey, K. A., Chow, C. S., and Reeve, J. N. (1999) *Nucleic Acids Res.* **27**, 522–536
23. Musgrave, D. R., Sandman, K. M., and Reeve, J. N. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 10387–10401
24. Hamiche, A., DeLaCasa, F., Donohoue, M. F., Revet, B., and Prunell, A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 7588–7593
25. Hamiche, A., and Richard-Foy, H. (1999) *J. Biol. Chem.* **273**, 9261–9269
26. Dong, F., and van Holde, K. E. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 10596–10600
27. Hayes, J. J., Clark, D. J., and Wolfe, A. P. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 6829–6833
28. Pereira, S. L., and Reeve, J. N. (2000) *J. Mol. Biol.* **298**, 675–681
29. Bailey, A. K., Pereira, S. L., Widom, J., and Reeve, J. N. (2000) *J. Mol. Biol.* **303**, 25–34
30. Sandman, K., Grayling, R. A., Dobrinski, B., Lurz, R., and Reeve, J. N. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91**, 12924–12928
31. Soares, D. J., Sandman, K., and Reeve, J. N. (2000) *J. Mol. Biol.* **297**, 37–47
32. Sandman, K., Bailey, A. K., Pereira, S. L., Soares, D., Li, W-T., and Reeve, J. N. (1999) *Methods Enzymol.* **334**, 116–129
33. Li, W-T., Grayling, R. A., Sandman, K., Edmondson, S., Shriver, J. W., and Reeve, J. N. (1998) *Biochemistry* **37**, 10563–10572
34. Li, W-T., Sandman, K., Pereira, S. L., and Reeve, J. N. (2000) *Extremophiles* **4**, 43–51
35. Shimizu, M., Miyake, M., Kanke, F., Matsumoto, U., and Shindo, H. (1995) *Biochim. Biophys. Acta* **1264**, 330–336
36. Sambrook, J., Maniatis, T., and Fritsch, E. F. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory Press, Plainview, NY
37. Howard, M. T., Sandman, K., Reeve, J. N., and Griffith, J. D. (1992) *J. Bacteriol.* **174**, 7841–7847
38. Bailey, K. A. (2000) *DNA Interactions of the Archaeal Histone HMf from the Hyperthermophilic Methanogen Methanothermus Fervidus*, Doctoral dissertation, The Ohio State University, Columbus, OH
39. Fitzgerald, D. J., and Anderson, J. N. (1998) *Nucleic Acids Res.* **26**, 2526–2535
40. Gottesfeld, J. M., and Luger, K. (2001) *Biochemistry* **40**, 10927–10933
41. Gangloff, Y.-G., Romier, C., Thuault, S., Wertén, S., and Davidson, I. (2001) *Trends Biochem. Sci.* **26**, 250–257

Downloaded from http://www.jbc.org/ by guest on July 24, 2018
Both DNA and Histone Fold Sequences Contribute to Archaeal Nucleosome Stability
Kathryn A. Bailey, Frédéric Marc, Kathleen Sandman and John N. Reeve

J. Biol. Chem. 2002, 277:9293-9301.
doi: 10.1074/jbc.M110029200 originally published online December 21, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M110029200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 12 of which can be accessed free at
http://www.jbc.org/content/277/11/9293.full.html#ref-list-1