Archaeal histones: structures, stability and DNA binding

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Abstract
Structures, stability and DNA-binding properties have been established for archaeal histones from mesophiles, thermophiles and hyperthermophiles. Most archaeal histones are simply histone folds that are stabilized by dimer formation. Archaeal histones and the histone folds of the eukaryotic nucleosome core histones share a common ancestry and bind and wrap DNA similarly using conserved residues. The histone-fold residues that stabilize dimer–dimer interactions within an archaeal histone core contribute to determining archaeal histone–DNA affinity.

Introduction
Nucleosomes form the primary level of nuclear DNA compaction in almost all eukaryotes. In all cases, they contain essentially the same four histones, H2A, H2B, H3 and H4 [1], always arranged as (H2A + H2B)-(H3 + H4)2-(H2A + H2B) heterodimers in an octamer core, with ≈146 bp of DNA wrapped in ≈1.65 negatively supercoiled circles [2]. The nucleosome was apparently therefore present in the last common ancestor of all eukaryotes, and its immediate ancestor presumably existed in whatever prokaryotic complex gave rise to the first eukaryotic nucleus. There are no histone-fold-containing proteins in Bacteria [3], but histones are present in the euryarchaeal branch of the Archaea, suggesting the likely prokaryotic origin of the histone fold [4]. When histones were discovered in Archaea in 1990 [5], it was consistent with the close relationship already established for the archaeal and eukaryotic basal transcription machineries [4,6]. Since then, archaeal and eukaryotic DNA replication and repair systems have also been shown to have many features in common that differ from their bacterial counterparts [7].

The histone fold
The histone fold is formed by three α-helices (short α1, long α2, short α3) separated by two short β-strand loops (L1, L2), and is only stable in a histone fold–histone fold dimer configuration [8,9]. In a dimer, the two α2s are anti-parallel aligned with the L1-L2a (where a denotes a component of the second histone fold) and L1a-L2 loops paired at each end of the α2-α2a alignment. The α1 and α3 helices are folded back on the same side of α2, and side chains from all six α-helices contribute to the hydrophobic dimer core ([2]; Figure 1A).

Archaeal histone variants
Approx. 30 archaeal histone sequences have been established (http://www.biosci.ohio-state.edu/~microbio/Archaealhistones/Alignments/alignments.html) and most are 65–69 amino acids in length. These sequences fold into structures that are just histone folds [10,11] whereas the eukaryotic nucleosome core histones have additional sequences that extend N- and/or C-terminal from their histone folds [1,2]. These extensions carry almost all the residues that are the targets for the post-translation modifications that regulate eukaryotic chromatin structure and gene expression [12]. Three archaeal histones, HMvA, MM0929 and MJ1647 from three methanococcal species, Methanococcus voltae, Methanococcus maripaludis and Methanococcus jannaschii, respectively, have ≈30 residue C-terminal extensions. The sequences of these extensions are related, but do not show any obvious sequence relationship to the eukaryotic histone extensions. Construction of MJ1647Δ, a recombinant variant of MJ1647 that lacked the C-terminal extension, revealed that the extension increased the structural stability of (MJ1647)2 homodimers but reduced their ability to form stable complexes with DNA [13].

Two archaeal histones, HMk in Methanopyrus kandleri and HHb in Halobacterium halobium, have sequences that fold into two histone folds linked tandemly by peptides with the sequences VEGVEDDGE and DTAPDRRGDL, respectively. The crystal structure of HMk has confirmed that the two histone folds interact to form a histone-fold dimer (Figure 1C), and therefore that HMk is a histone-fold dimer within one polypeptide chain [14].

Residues responsible for archaeal histone dimer stability
When exposed to low pH or high temperature, archaeal histone dimers unfold directly to random coil monomers...
without detectable folded-monomer intermediates. As unfolding is 100% reversible, complete thermodynamic descriptions of dimer stability can be obtained [8,9]. Consistent with their mesophile versus hyperthermophile origins, (HFoB)2 from *Methanobacterium formicicum* and (HMfB)2 from *Methanothermus fervidus* have maximum free energies of unfolding of 7.2 and 14.6 kcal/mol, respectively, equating to *T*° values (calculated unfolding temperatures for 1 M solutions) of 76 and 114 °C. The HFoB and HMfB sequences differ at 15 locations (Figure 1C), eight of which are in α2. These include A31 and K35 in HFoB versus I31 and M35 in HMfB, residues that are located near the centre of the hydrophobic core where α2-α2a interact most closely (Figure 1A). HFoB and HMfB variants with reciprocal residue substitutions at these and several other α2 and α3 locations were constructed and their free energies of unfolding determined [15]. The thermal unfolding data accumulated from these laboratory engineered variants were fully consistent with the conclusion from crystal structures that archaeal histone fold stability, and therefore dimer stability, results primarily from intermolecular interactions of buried hydrophobic residues positioned along the aligned faces of α2 and α2a.

**Archaeal histone residues responsible for DNA binding**

In the eukaryotic nucleosome [2], residues from each histone-fold dimer contact the DNA at three separate sites over ≈28 bp. Arginine and lysine residues on the surface of α1, an arginine in L1, and a lysine and threonine in L2 participate directly in DNA binding, and arginine, lysine and threonine residues occupy the same histone fold positions in most archaeal histones. Consistent with these residues being directly responsible for DNA binding, almost all of

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**Figure 1** (A) Histone-fold dimer, (B) tetramer-containing complexes and (C) alignment of archaeal histone sequences

In (C) Solid lines connect the C-terminal extension of MJ1647 [13] and the two histone-fold domains of HMk [14]. The shaded regions identify the α1, α2 and α3 regions of histone folds. Introduction of a stop codon (R69*) generated the MJ1647Δ variant [13]. Open-head arrows identify α2 residues that contribute to stabilizing histone-fold dimers [2,10,11,14], and closed-head arrows identify α2 and α3 residues that interact to form a four-α-helix bundle (4HB) in a histone-fold tetramer [2,20,21]. In (B), the darker shading identifies the region of α3 exchanged in (HMfA)2 versus (HMfB)2 domain-swap experiments [20].
the HMfB variants constructed with residue substitutions introduced at these locations had reduced gel shifts [16]. They also had decreased DNA affinities based on changes in DNA ellipticity measured at 275 nm and on DNA ligase circularization assays of histone-bound DNA. Consistent with each histone-fold dimer having three independent DNA-binding sites, a combination of three substitutions, one at each of these locations was needed to generate a HMfB variant with absolutely no detectable ability to bind DNA [16]. Two HMfB variants, E18K and G51K, have been characterized that have increased DNA affinity. Apparently the addition of positively charged lysine residues results in additional DNA binding but the complexes formed have reduced flexibility [17]. The ends of the bound DNA were much less available for ligation and the DNA was wrapped only in a negative supercoil. In contrast, wild-type HMfB forms complexes in which the DNA can be constrained in a positive or a negative supercoil [18].

Archaeal histone residues that stabilize dimer–dimer interactions also determine DNA affinity

Archaeal histones are dimers in solution but must polymerize further to bind DNA [19]. Substitutions for L46 and H49 in α2, and for L62 in α3, generated HMfB variants with reduced DNA affinities even though these residues do not directly contact the DNA [16]. Rather, they stabilize the assembly of four α-helix bundles (4HBs) that form the interface between adjacent HMfB dimers in an archaeal histone core. The stability of this assembly apparently therefore also contributes to the strength of DNA binding. By constructing histone-fold domain-swap variants, the difference in DNA affinity of (HMfA)2 and (HMfB)2 for the same DNA sequence was shown to be embodied in α3. Presumably, this similarly reflects the involvement of the different α3s in dimer–dimer assembly [20,21].
Stability and DNA binding by archaeal histone fusion variants

HMfAA and HMfBB homodimer fusions, and HMfAB and HMfBA heterodimer fusions were constructed [21] by linking the HMfA and/or HMfB sequences using peptides with sequences based on the HMk and HHb linker peptides (see above). CD measurements revealed that these fusions have folded structures very similar to their unlinked archaeal histone dimer equivalents (Figures 2A–2C) but that they remained folded at temperatures at which the unlinked dimers unfolded (Figure 2D). Linking the two histones must, in effect, increase the ‘dimer’ concentration and so increase dimer stability. The DNA binding, compaction and supercoiling properties of the homodimer fusions were identical to those of the corresponding (HMfA)2 and (HMfB)2 homodimers. The DNA interaction properties of the HMfAB and HMfBA heterodimer fusions confirmed that the 4HBs formed between two fusion dimers in a histone fold tetramer core determined the DNA affinity of that core [21].

Regulation by archaeal histones

Archaea contain different numbers of histones that are apparently synthesized differentially depending on growth conditions [22,23]. Given this observation, and that different archaeal histones can have different affinities for the same DNA sequence, it seems likely that localized archaeal histone assembly could directly regulate, or could contribute to the co-regulation of gene expression, replication and/or recombination in vivo. Consistent with this, electron microscopy has revealed that histone-containing complexes are separated by histone-free regions in archaeal genomic DNA [24,25]. Most archaeal regulators described to date compete as repressors with TATA-box binding protein (TBP) and/or transcription factor B (TFB) for binding to the TATA-BRE (TFB-responsive element) region, or with RNA polymerase for the site of transcription initiation [4,6], and archaeal histones could also directly participate in such binding competitions. Alternatively, they could inhibit or stimulate transcription by binding, wrapping and distorting the DNA helix at, or adjacent to, sites of transcription initiation.

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References

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