Archaeal Histone Tetramerization Determines DNA Affinity and the Direction of DNA Supercoiling*

The histone fold, three α-helices (α1, α2, and α3) separated by two β-strand loops (L1 and L2), was first identified in the four nucleosome core histones and shown to direct their higher order structures at sites responsible for histone fold dimer-dimer interactions. Almost all of these variants have decreased affinity for DNA. They have also lost the flexibility of the wild type archaeal histones to wrap DNA into a negative or positive supercoil depending on the salt environment; they wrap DNA into positive supercoils under all salt conditions. The histone folds of the archaeal histones, HMfA and HMfB, from Methanothermus fervidus are almost identical, but (HMfA)2 and (HMfB)2 homodimers assemble into tetramers with sequence-dependent differences in DNA affinity. By construction and mutation of HMfA+HMfB and HMfB+HMfA histone dimer fusions, the structure formed at the histone dimer-dimer interface within an archaeal histone tetramer has been shown to determine this difference in DNA affinity. Therefore, by regulating the assembly of different archaeal histone dimers into tetramers that have different sequence affinities, the assembly of archaeal histone-DNA complexes could be localized and used to regulate gene expression.

DNA binding and the topology of DNA have been determined in complexes formed by >20 archaeal histone variants and archaeal histone dimer fusions with residue replacements at sites responsible for histone fold dimer-dimer interactions. Almost all of these variants have decreased affinity for DNA. They have also lost the flexibility of the wild type archaeal histones to wrap DNA into a negative or positive supercoil depending on the salt environment; they wrap DNA into positive supercoils under all salt conditions. The histone folds of the archaeal histones, HMfA and HMfB, from Methanothermus fervidus are almost identical, but (HMfA)2 and (HMfB)2 homodimers assemble into tetramers with sequence-dependent differences in DNA affinity. By construction and mutation of HMfA+HMfB and HMfB+HMfA histone dimer fusions, the structure formed at the histone dimer-dimer interface within an archaeal histone tetramer has been shown to determine this difference in DNA affinity. Therefore, by regulating the assembly of different archaeal histone dimers into tetramers that have different sequence affinities, the assembly of archaeal histone-DNA complexes could be localized and used to regulate gene expression.

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1 The abbreviations used are: α1, α2, and α3, histone fold α-helices 1, 2, and 3; L1 and L2, histone fold β-strand loops 1 and 2; 4HB, four α-helix bundle(s); ssa, sonicated salmon sperm; %T, total concentration of acrylamide and N, N′-methylenebisacrylamide; %C, percent total acrylamide concentration (T) that is N, N′-methylenebisacrylamide; EM, electron microscopy.

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constructed and report here the results of a detailed investigation of DNA binding and complex formation by a much wider spectrum of HMB variants with residue substitutions introduced specifically at the histone fold sites predicted to participate in HMB tetramer stabilization. Single and multiple substitutions have been made for His-49 and/or Leu-62 and Leu-62, the latter two residues being the HMB structural homologs in α2 and α3, respectively, of Cys-110 and Leu-126 in H3 and Tyr-63 and Leu-79 in dTAF 42 (see Fig. 1A). These hydrophobic residues also interact to contribute stability to the 4HBs at the centers of (H3 + H4) and dTAF 42-dTAF 62 tetraters, respectively (2, 18). The results obtained are consistent with residues at these locations in HMB contributing to DNA affinity through tetramer stabilization. Unexpectedly, the results have also revealed that these residues determine whether the resulting HMB tetramer has the ability to wrap DNA in either a negative or positive supercoil. By construction and mutagenesis of HMfA+HMfB and HMfB+HMfA histone heterodimers, we have also demonstrated directly that it is the structure formed at the site of histone fold dimer:dimer interaction that establishes the difference in DNA affinity exhibited by HMfA versus HMB tetraters.

**EXPERIMENTAL PROCEDURES**

Site-directed Mutagenesis, Construction of Recombinant Histone Fusions, and Purification of Recombinant Archael Histones

Mutations were introduced into hmfB as previously described (25) using the QuikChange™ procedure (Stratagene, La Jolla, CA) with mutagenic oligonucleotide primers (sequences available on request) purchased from Ransom Hill Biosciences (Ramona, CA). The presence of the desired mutation(s) was confirmed by DNA sequencing. The mutated gene was introduced into the pKK223-3-based expression system developed for recombinant HMB synthesis (29), and E. coli JM105 was transformed with the expression plasmids. To construct mutated gene fusions, a mutagenic oligonucleotide primer was annealed with aliquots (400 ng) of this DNA under low salt (10 mM Tris-HCl (pH 8), 2 mM NaCl, 50 mM NaCl, or 50 mM potassium glutamate) or high salt (10 mM Tris-HCl (pH 8), 1 mM EDTA, 350 mM potassium glutamate) conditions for 30 min at 37 °C. The complexes formed were exposed to the NCE and proteinase K, and the topologies of the resulting deproteinized DNA molecules were determined by one- and two-dimensional agarose gel electrophoresis as previously described (35).

**RESULTS**

**DNA Topology Assays**

Relaxed circular pUC18 DNA molecules were generated from negatively supercoiled molecules by incubation with a DNA nicking-closing extract (NCE) prepared from chicken blood (34). Increasing amounts of an archaeal histone were incubated with aliquots (400 ng) of this DNA under low salt (10 mM Tris-HCl (pH 8), 2 mM NaCl, 50 mM NaCl, or 50 mM potassium glutamate) or high salt (10 mM Tris-HCl (pH 8), 1 mM EDTA, 350 mM potassium glutamate) conditions for 30 min at 37 °C. The complexes formed were exposed to the NCE and proteinase K, and the topologies of the resulting deproteinized DNA molecules were determined by one- and two-dimensional agarose gel electrophoresis as previously described (35).

**Electron Microscopy**

Glutaraldehyde-fixed archaeal histone-DNA complexes were prepared and visualized by EM as previously described (36, 37). Using a 1313-bp DNA molecule designated fragment M (38), length measurements were made of histone-free DNA molecules and of a minimum of 50 molecules derived from one to four clearly distinct archaeal histone-DNA complexes. Based on the apparent differences in length, the length of DNA assembled per complex was calculated.

**Electrophoretic Mobility Gel-shift Assays**

**Agarose Gel-shift Assays—Archaeal histone binding results in DNA compaction, and complexes formed with DNA molecules >2 kbp migrate faster during agarose gel electrophoresis than the histone-free DNA molecule (29, 33). Using this assay, HMB variants unable to bind DNA gave no gel shift. However, some variants, including L46I, L46V, H49A, L62I, and L62V, gave reduced gel shifts, suggesting the formation of less compact structures than those formed by the wild type HMB (25). To pursue this apparent correlation with tetramer formation, HMB variants were generated with a range of substitutions at one or more of these sites. All of these variants, L46A, L46C, H49D, H49E, H49N, H49K, L62A, L62C, L62M, L62Q, L62R, L62W, L64A+H49A, L64A+L62A, H49A+L62A, and L64A+H49A+L62A, formed complexes with linear pBR322 DNA, but in all cases the complexes formed migrated more slowly through agarose gels than complexes formed by wild type HMB (Fig. 1B). In some cases, the magnitude of the gel shift decreased at higher histone to DNA ratios, consistent with additional histone binding but no additional compaction. Three variants (L46F, H49D, and L62Y) were reported previously (25) to give wild type gel shifts. This was confirmed for the L46F (Fig. 1B) and L62Y variants but not for the H49D variant, which in repeated experiments gave a slightly reduced gel shift. Wild type gel shifts were also observed with several additional variants constructed with residue substitutions introduced at adjacent locations (K45A, K45E, A50G, R66I, R66M).

EM visualization has demonstrated that the agarose gel shift assay reports the net effect on electrophoretic mobility of complexes formed at multiple locations along a long DNA (36, 37). However, complex formation by a single archaeal histone tetramer can be detected and apparent Kd values measured by using a conventional polyacrylamide gel retardation assay with an archaeal histone molecule incubated with aliquots of this DNA (24, 25, 27). All of the L46F, H49A, and Leu-62 variants were so assayed using an 80-bp DNA molecule that contained the 60-bp clone 20 sequence, which has inherently high affinity for HMB (26). Examples of the results obtained are shown in Fig. 1C. All of the variants formed complexes with this DNA but, in many cases, only at much
**A.** Increasingly detailed views of the 4HB formed by the H3 histone folds in an (H3 + H4) tetramer at the center of a nucleosome. The 4HB is viewed from above the central dyad of the nucleosome in the lower section of the left panel and in the right panel. To gain this perspective, the complex illustrated in the upper section of the left panel was rotated by ~90° toward the reader. Below the illustrations, the histone fold sequences of HMfB, histone H3, and dTAFII42 are aligned with the regions forming α-helices (α1, α2, α3), loops (L1, L2), and the residues in HMfA that differ from those in HMfB identified above the HMfB sequence. The C-terminal residue of HMfA is Lys-68, and the absence of an equivalent of Lys-69 in HMfB is indicated by the symbol #. The locations of five residues that are present in the eukaryotic histone folds that have no archaeal histone counterparts are indicated by hyphens. Conserved residues are identified by asterisks. The side chains of the H3 residues boxed in the sequence alignment are shown in the illustrations above, positioned and interacting as predicted to contribute to tetramer stabilization. The histone fold homologs in dTAFII42 (2) and HMfB (22) are listed.

**B.** Agarose gel electrophoresis of the complexes formed by incubation of linear pBR322 DNA (50 ng) with 0 (−), 15, 30, 45, 65, and 105 ng of HMfB flanked by the complexes formed by the HMfB variants indicated assembled at the same histone to DNA ratios.

**C.** Polyacrylamide gel electrophoresis of the complexes formed by incubating 0.1 ng of 32P-labeled clone 20 DNA (80 bp) plus 1 ng of sss DNA with 0 (−), 0.1, 0.3, 0.5, 1, 3, and 10 ng, or 10, 30, 50, 100, and 300 ng of HMfB or of the HMfB variants listed.

**FIG. 1.** Histone fold tetramer-DNA complex, histone fold sequences, and gel-shift assays. A, increasingly detailed views of the 4HB formed by the H3 histone folds in an (H3 + H4) tetramer at the center of a nucleosome. The 4HB is viewed from above the central dyad of the nucleosome in the lower section of the left panel and in the right panel. To gain this perspective, the complex illustrated in the upper section of the left panel was rotated by ~90° toward the reader. Below the illustrations, the histone fold sequences of HMfB, histone H3, and dTAFII42 are aligned with the regions forming α-helices (α1, α2, α3), loops (L1, L2), and the residues in HMfA that differ from those in HMfB identified above the HMfB sequence. The C-terminal residue of HMfA is Lys-68, and the absence of an equivalent of Lys-69 in HMfB is indicated by the symbol #. The locations of five residues that are present in the eukaryotic histone folds that have no archaeal histone counterparts are indicated by hyphens. Conserved residues are identified by asterisks. The side chains of the H3 residues boxed in the sequence alignment are shown in the illustrations above, positioned and interacting as predicted to contribute to tetramer stabilization. The histone fold homologs in dTAFII42 (2) and HMfB (22) are listed. B, agarose gel electrophoresis of the complexes formed by incubation of linear pBR322 DNA (50 ng) with 0 (−), 15, 30, 45, 65, and 105 ng of HMfB flanked by the complexes formed by the HMfB variants indicated assembled at the same histone to DNA ratios. C, polyacrylamide gel electrophoresis of the complexes formed by incubating 0.1 ng of 32P-labeled clone 20 DNA (80 bp) plus 1 ng of sss DNA with 0 (−), 0.1, 0.3, 0.5, 1, 3, and 10 ng, or 10, 30, 50, 100, and 300 ng of HMfB or of the HMfB variants listed.
higher histone to DNA ratios than required for complex formation by wild type HMfB. At the highest ratios tested (>500 histone variant tetramers/DNA molecule), only a small percentage of the DNA was assembled into complexes by the H49D and H49E variants (Fig. 1C), and these variants failed to form detectable complexes when incubaed with other ~80-bp DNA molecules that lacked inherently high affinity for HMfB.

**Topology Assays**—Archaeal histones form complexes in which the DNA is constrained alternatively in a negative or positive supercoil (35, 40). Under low salt conditions (~50 mM K⁺) and at low histone to DNA ratios, complex formation on relaxed circular pUC18 molecules results in negative DNA supercoiling, but this changes to positive DNA supercoiling with additional histone binding (Fig. 2A). This change from negative to positive supercoiling occurs only under low salt conditions. Under higher salt conditions (>300 mM K⁺), more similar to the high salt content of the *M. fervidus* cytoplasm (41), HMfB binding to relaxed pUC18 DNA molecules results in only negative supercoiling of the DNA at all histone to DNA ratios (40). The topology of the pUC18 DNA in complexes formed by the Leu-46, His-49, and Leu-62 variants was determined under both low and high salt assembly conditions. Only the L46F, L62V, and L62Y variants retained the ability to form complexes in which the DNA was either negatively or positively supercoiled. In contrast, the L46A, L46C, L46I, L46Q, L46V, H49A, H49D, H49E, H49K, L62A, L62C, L62I, L62Q, L62R, L62W, L46A+L62A, L46C+L62C, L46A+H49A, H49A+L62A, and L46A+H49A+L62A variants were never observed to form complexes in which the DNA was negatively supercoiled. At all histone to DNA ratios and under both low and high salt conditions, these variants only formed complexes in which the pUC18 DNA was positively supercoiled (Fig. 2).

**FIG. 2.** Topology assays of HMfB and His-49 variants. A. agarose gel electrophoretic separation of the topoisomers of pUC18 generated by incubation of relaxed, circular pUC18 DNA (400 ng) with 0 (−), 20, 40, 80, 120, 160, 240, and 320 ng of HMfB or of the HMfB H49A variant under low salt conditions. In the lower gels, aliquots of the reaction products indicated by the arrows were analyzed by two-dimensional agarose gel electrophoresis with ethidium bromide present in the second dimension to facilitate the separation of negative (−ve) and positive (+ve) supercoiled topoisomers. B. agarose gel electrophoretic separation of the topoisomers generated under high salt conditions by incubation of pUC18 DNA (400 ng) with 0 (−), 20, 40, 80, 160, 240, and 400 ng of HMfB, HMfB/H49A, or HMfB/H49E. Two-dimensional electrophoretic separations of the topoisomers in aliquots of the reaction products, indicated by the arrows, are shown adjacent to the one-dimensional separations.

**HMfA versus HMfB Histone Fold Tetramer Formation and DNA Affinity**—Both the α3 domain-swap evidence (27) and the L46F, L62V, and L62Y variants. Under low salt conditions, the HMfB affinity for the clone 20 sequence. To further investigate this, the HMfAB and HMfBA histone heterodimer fusions were constructed and H49E substitutions introduced into either the HMfA or HMfB component. (HMfB)₂ homodimers with an H49E substitution in both monomers were known to be essentially incapable of forming complexes with this DNA (Fig. 1C). However, with only one H49E substitution, it was reasoned that the histone dimer fusion variants HMfAB(H49E), HMfA(H49E)/B, HMfBA(H49E), and HMfB(H49E)/A might still bind DNA. To do so, two such molecules would interact to form a histone fold tetramer stabilized by a 4HB formed by the histone folds that retained the wild type His-49 residues. If the assembly of this structure determined the overall affinity of the resulting histone fold tetramer for DNA, then the HMfAB(H49E) and HMfB(H49E)/A variants would form complexes with HMfA-like affinity. The HMfA(H49E)/B and HMfBA(H49E) variants, in contrast, would have higher HMfB-like affinity for the clone 20 DNA. This experiment is illustrated in Fig. 3. The results obtained with both the wild type heterodimer fusion controls, the heterodimer fusion variants, and the HMfAA and HMfBB homodimer fusions are shown in Fig. 4. As anticipated, the HMfAA and HMfBB homodimer fusions formed complexes with DNA affinities, electrophoretic mobilities, and topologies essentially the same as those formed by (HMfA)₂ and (HMfB)₂, homodimers, respectively. The wild type HMfAB and HMfBA heterodimer fusions also had DNA affinities and formed complexes with mobilities and topologies essentially the
same as those formed by (HMfB)$_2$ and HMfBB. However, the introduction of an H49E substitution into the HMfB component resulted in HMfAB(H49E) and HMfB(H49E)A fusion variants assembled 4HBs as illustrated, stabilized by interactions (see B=B and A=A) that involved the histone folds that retained the wild type His-49 residues. The wild type HMfBA fusion apparently assembled a 4HB involving the α2 and α3 helices of the HMfB histone folds (B=B blue), but as illustrated in the box three additional 4HB assemblies are theoretically possible involving the α2 and α3 helices of only the HMfA histone folds (A=A) or involving helices from both the HMfA and HMfB histone folds (A=B, B=A). With the addition of a third HMfBA(H49E) or HMfB(H49E)A molecule, several alternative 4HB assemblies are possible (A=A, A=B, B=B, and/or B=A) that would involve either one His-49 and one Glu-49, or two Glu-49 residues as illustrated. To facilitate visualization, the added third molecule is shown displaced from the real circular configuration. In all cases, the resulting tetramer wraps the DNA molecule in a positive supercoil, whereas the further polymerization of wild type HMfBA molecules under physiologically relevant high salt conditions (41) results in negative supercoiling (Fig. 4C).

The electron micrographs show (a) fragment-M DNA molecules (1313 bp; Ref. 38) and the complexes formed by incubation of HMfBA with this DNA (b) at 1:3, (c) 1:2, and (d) 1:1 mass ratios. Arrows in panel b indicate kinks introduced into the DNA resulting from partial circularization of the DNA around an (HMfBA)$_2$ histone fold tetramer. At higher HMfBA to DNA ratios, the DNA is fully circularized (c), and the number of complexes formed on each DNA molecule increases (d). Based on measurements of 177 fragment-M molecules with from 0 to 4 clearly discernable fully circularized complexes, the assembly of one complex resulted in an apparent length reduction of 91 ± 5 bp. Bar is 100 nm.
FIG. 4. Gel shift, affinity, and topology assays of histone fold fusions. A, agarose gel electrophoresis of complexes formed by incubating linear pBR322 DNA (50 ng) with 0 (−), 25, 50, 75, 100, 150, and 200 ng of HMfA, HMfB, or the histone fusion listed. The presence of an H49E substitution in the HMfA or HMfB component of a histone fusion is indicated by a gray oval superimposed over the corresponding A or B. HMfA forms complexes that migrate more slowly than those formed by HMfB (33, 37) as shown by the dotted lines. An adjacent comparison of the electrophoretic mobilities of samples of all the complexes formed at saturating histone to DNA ratios is provided at the lower right. B, polyacrylamide gel electrophoresis of the complexes formed by incubation of 0.1 ng of 32P-labeled clone 20 DNA (110 bp) plus 1 ng of ssDNA with 0 (−), 0.1, 0.5, 1, 5, 10, and 25 ng of the archaeal histone listed. The apparent $K_d$ values calculated in terms of histone fold tetramer binding to this DNA, determined from at least three repetitions of each experiment (27), are listed in the Table with S.D. C, agarose gel electrophoretic separation of the pUC18 topoisomers generated in complexes formed under low and high salt conditions by assembly of 0 (−), 80, 160, 240, 320, 400 ng of HMfBA or HMfBA(H49E) on relaxed pUC18 DNA (400 ng). Two-dimensional electrophoretic separations of the topoisomers in aliquots of the reaction products indicated by the arrows are shown adjacent to the one-dimensional separations.
tone variants resulted in complexes in which the pUC18 DNA was only positively supercoiled at all histone to DNA ratios and under both low and high salt assembly conditions (Fig. 4C).

**DISCUSSION**

**Histone Fold Tetramer Formation and DNA Affinity**—Based on homology with eukaryotic histone folds (see Fig. 1A), Leu-46, His-49, Asp-59, and Leu-62 were identified as residues likely to stabilize a 4HB that formed the dimer:dimer interface in an 2(HMfB) tetramer. To bind DNA, (HMfB) dimers must form a tetramer (23, 24), and consistent with reduced ability to form a stable tetramer, almost all of the Leu-46, His-49, and Leu-62 variants investigated had reduced affinity for an 80-bp DNA (Fig. 1C). The three variants with wild type affinity had large conservative side chain substitutions (L46F and L62Y) or a cysteine (L46C) residue at precisely the location of Cys-110 in histone H3 (42). Presumably, the side chains of these residues must still provide fully effective hydrophobic stabilizing interactions within the 4HB formed between the two (HMfB) dimers. The His-49 variants were consistently the most defective in DNA binding, suggesting that the histidine-aspartate interactions lost by these variants are of predominant importance in stabilizing a histone fold tetramer. If this is correct, the presence of a histidine near the C terminus of a2 could predict the likelihood of that histone fold participating in stabilizing a histone fold tetramer. The hTAFII31 human homolog of dTAFii42 does, for example, have such a histidine that could stabilize (hTAFII31+ hTAFII80) tetramers, whereas the predicted yeast homolog, yTAFI17, lacks an appropriately positioned histidine, arguing against (yTAFI17+yTAFI80) tetramer stabilization (3, 6–8). The aspartate in a3 is conserved in virtually all histone folds (42), but because this is also required to stabilize the histone fold monomer (18, 22), the presence of this residue is more a predictor of a histone fold than of tetramerization.

Each histone fold dimer binds the DNA at three locations in the nucleosome over an ~28-bp region (18–21) and, as this predicts, complexes containing an archaeal histone tetramer protect ~60 bp from micrococcal nuclease digestion (43). Based on EM measurements, complexes in which the DNA is fully circularized involve a minimum of ~90 bp (Fig. 3), but with additional archaeal histone polymerization, complexes are formed that protect not only ~90 but also ~120 and ~180 bp (44–46). Based on the agarose gel shift results, all the Leu-46, His-49, and Leu-62 variants (Fig. 1B) retained some ability to assemble such larger complexes, but the complexes formed were less compact than those formed by wild type HMfB. In some cases, at relatively high histone to DNA ratios, additional histone binding occurred but resulted in no additional compaction (Fig. 1B).

The affinities of the HMfAB(H49E), HMfA(H49E)B, HMfB(H49E)A, and HMfBA(H49E) fusion variants for clone 20 DNA confirmed that the difference in affinity of HMfA versus HMfB for this DNA sequence reflected a difference at the site of histone fold tetramerization (Fig. 3). Because the HMfB components of the wild type HMfAB and HMfBA fusions would be expected to form histone fold tetramers with higher affinity for the clone 20 sequence than those formed by the HMfA components, it was anticipated and observed (Fig. 4B) that the wild type fusions had HMfB-like affinities for the clone 20 DNA. Their assembly to form complexes with pBR322 DNA with HMfB-like properties was less predictable but is consistent with EM observations. HMfB has been seen to assemble more complexes than HMfA at the same histone to DNA ratios on all DNA molecules so far investigated from ~1 to 5 kbp (36, 39).

HMfB assembly apparently has fewer sequence or structural demands, which results in the assembly of more complexes and therefore in more compaction and in complexes that migrate faster through agarose gels (Fig. 4A).

**Histone Fold Tetramerization and the Direction of DNA Supercoiling**—The DNA appears sharply kinked in complexes formed at low archaeal histone to DNA ratios (Ref. 36 and Fig. 3), consistent with partial circularization around a histone tetramer. The assembly of such complexes by the wild type proteins introduces negative superhelicity into a circular DNA (35), presumably because the DNA is held across the dimer:dimer interface in an orientation that, when extended to the whole circular DNA, results in net negative supercoiling of the molecule (Fig. 2A). The regions of the DNA entering and exiting such a structure do not necessarily interact, but with additional histone binding, the entering and exiting DNA must be forced into close proximity as the DNA is fully circularized. Under low salt conditions, this must overcome DNA-DNA repulsive forces which, based on the experimental results (Fig. 2A), is accomplished by the DNA being wrapped in a positive supercoil. Under higher salt conditions, cation ion binding must shield these repulsive forces, and the negative supercoiling initiated by the initial tetramer-DNA interaction can continue and be extended with additional histone polymerization and DNA circularization (Fig. 2B). Surprisingly, the majority of the Leu-46, His-49, and Leu-62 variants formed complexes in which the pUC18 DNA was only positively supercoiled, regardless of the assembly conditions (Fig. 2, A and B). This result resembles the observations made with (H3+H4) tetramers that had large chemical adducts attached to the Cys-110 residues of the H3 histone folds (10), the homolog of Leu-46 in HMfB. These tetramers could wrap DNA in only a negative or positive supercoil, and it was argued that the bulky chemical adducts blocked the dimer:dimer interface movement needed to switch from negative to positive supercoiling (10, 47). Possibly, the Leu-46, His-49, and Leu-62 variants have similarly lost structural flexibility and now form HMfB tetramers locked in structures that can only direct positive supercoiling. However, with a weakened dimer:dimer interaction, it seems more likely that these variants form tetramers that lack the strength to hold the DNA in a more demanding negative toroidal supercoil. The DNA molecule in an archaeal histone-DNA complex is overwound at ~10 bp/turn, in effect positively supercoiled (27, 44). Positive toroidal supercoiling should therefore result in the absence of a strong countering histone dimer:dimer interaction.

The above explanation for the DNA supercoiling phenomena observed with the wild type proteins predicts that both the wild type HMfAB and HMfBA fusions and variant histone fusions should form complexes at low histone to DNA ratios that introduce negative superhelicity into circular pUC18 DNA. This was observed for the wild type fusions, but the HMfAB(H49E), HMfA(H49E)B, HMfB(H49E)A, and HMfBA(H49E) fusion variants only formed complexes in which the DNA was positively supercoiled, regardless of the assembly conditions (Fig. 3). This result argues that the first histone fold tetramer formed by the assembly of two of these fusion variant molecules is stabilized by a wild type 4HB, but apparently alone this is insufficient to hold the DNA in negative supercoil. Because the complexes formed by the wild type HMfAB and HMfBA fusions do have this ability, the additional His-49 residues present in these complexes located ~2.5 helical turns on either side of the central 4HB (locations occupied by Glu-49 residues in complexes formed by the fusion variants (see Fig. 3)) must also contribute to holding the DNA in a negative supercoil.

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2 F. Marc, K. Sandman, R. Lurz, and J. N. Reeve, unpublished results.
Histone Tetramerization and DNA Affinity

Conclusions—That very similar archaeal histones might form complexes with different properties was suggested by the observation that the ratio of HMFA to HMFB changed in *M. fervidus* cells under different growth conditions (37). The discovery that HMFB had much lower affinity than HMFA for nucleosomes specifically at the centromere (11, Ref. 16) is also consistent with this concept. It is already well established that incorporation of the CENP-A family of histone H3 variants results in nucleosomes that assemble at the centromere (11–17). Although unique functions have not yet been assigned to these nucleosomes, this positioning is entirely consistent with the demonstration here that very similar histone fold dimers can assemble into tetramers with structures so different that they have different DNA affinities. Regulating the assembly of alternative histone fold tetramers could therefore provide a mechanism to regulate gene expression.

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