BIOPHYSICS AND COMPUTATIONAL BIOLOGY


The authors note that, due to a printer’s error, references 41–50 appeared incorrectly. The corrected references follow.


DeVELOPMENTAL BIOLOGY


The authors note that, due to a printer’s error, references 25–29 appeared incorrectly. The corrected references are:


PHYSICS


The authors note: “Our paper unfortunately missed reference to an earlier suggestion of the T6 structure (43). This work entitled ‘A hypothetical dense 3,4-connected carbon net and related B2-C and CN2 nets built from 1,4-cyclohexadienoid units’ by M. J. Bucknum and R. Hoffmann was published in J Am Chem Soc 116:11456–11464 (1994), where the electronic structure of a hypothetical 3,4-connected tetragonal allotrope of carbon is discussed. The results in this article are consistent with what we find. The same group had also suggested a metallic carbon structure (44) that was published in J Am Chem Soc 105:4831–4832 (1983), which we also missed to cite. We thank Prof. Hoffmann for bringing these papers to our attention.”

The complete references appear below.


www.pnas.org/cgi/doi/10.1073/pnas.1223851111

CELL BIOLOGY


The authors note that, due to a printer’s error, references 25–29 appeared incorrectly. The corrected references are:


www.pnas.org/cgi/doi/10.1073/pnas.1223494111
Structural insights into the histone H1-nucleosome complex

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Edited by S. Walter Englander, The University of Pennsylvania, Philadelphia, PA, and approved October 22, 2013 (received for review August 6, 2013)

Linker H1 histones facilitate formation of higher-order chromatin structures and play important roles in various cell functions. Despite several decades of effort, the structural basis of how H1 interacts with the nucleosome remains elusive. Here, we investigated Drosophila H1 in complex with the nucleosome, using solution nuclear magnetic resonance spectroscopy and other biophysical methods. We found that the globular domain of H1 bridges the nucleosome core and one 10-base pair linker DNA asymmetrically, with its α3 helix facing the nucleosomal DNA near the dyad axis. Two short regions in the C-terminal tail of H1 and the C-terminal tail of one of the two H2A histones are also involved in the formation of the H1-nucleosome complex. Our results lead to a residue-specific structural model for the globular domain of the Drosophila H1 in complex with the nucleosome, which is different from all previous experiment-based models and has implications for chromatin dynamics in vivo.

Eukaryotic genomic DNA is packaged into chromatin through association with positively charged histones to form the nucleosome, the structural unit of chromatin (1–3). The nucleosome core consists of an octamer of histones with two copies of H2A, H2B, H3, and H4, around which ~146 bp of DNA winds in ~1.65 left-handed superhelical turns (4). At this level of the DNA packaging, chromatin resembles a beads-on-a-string structure, with the nucleosome core as the beads and the linker DNA between them as the strings (5). At the next level of DNA packaging, H1 histones bind to the linker DNA and the nucleosome to further condense the chromatin structure (6, 7). H1-mediated chromatin condensation plays important roles in cellular functions such as mitotic chromosome architecture and segregation (8), muscle differentiation (9), and regulation of gene expression (10, 11).

Linker H1 histones typically are ~200 amino acid residues in length, with a short N-terminal region, followed by a ~70–80-amino acid structured globular domain (gH1) and a ~100-amino acid unstructured C-terminal domain that is highly enriched in Lys residues. H1 stabilizes the nucleosome and facilitates folding of nucleosome arrays into higher-order structures (12–15). gH1 alone confers the same protection from micrococcal nuclease digestion to the nucleosome as the full-length H1 does (16). The N-terminal region of H1 is not important for nucleosome binding (16, 17), whereas the C terminus is required for H1 binding to chromatin in vivo (18, 19) and for the formation of a stem structure of linker DNA in vitro (17, 20, 21).

The globular domain structures of avian H5 (22) and budding yeast Hho1 (23), which are both H1 homologs, have been determined at atomic resolution and show similar structures. In addition, numerous studies have indicated that gH1/gH5 binds around the dyad region of the nucleosome (14, 24), leading to many conflicting structural models for how the globular domain of H1/H5 binds to the nucleosome (SI Appendix, Fig. S1) (24–26). These models are divided into two major classes, symmetric and asymmetric, on the basis of the location of gH1/gH5 in the nucleosome. In the symmetric class, gH1/gH5 binds to the nucleosomal DNA at the dyad and interacts with both linker DNAs (16, 17, 27, 28). In the asymmetric class, gH1/gH5 binds to the nucleosomal DNA in the vicinity of the dyad axis and to 10 bp (27, 29–32) or 20 bp (19, 29, 33, 34) of one linker DNA, or is located inside the DNA gyres, where it interacts with histone H2A (35). In addition, Zhou and colleagues also characterized the orientation of gH5 in the gH5-nucleosome complex (29). The use of nonuniquely positioned nucleosomes and indirect methods may have contributed to the differences in these models (SI Appendix, Fig. S1).

Multidimensional nuclear magnetic resonance (NMR), and in particular methyl-based NMR, provides a direct approach to the structural characterization of macromolecular complexes (36, 37). We have previously assigned chemical shifts of the methyl groups of the side chains of residues Ile, Leu, and Val in the core histones (38) and the backbone amides in the disordered histone tails (39), which provide the fingerprints for investigating the interactions between H1 and the nucleosome. Here, we used NMR, along with several other methods, to determine the location and orientation of the globular domain of a stable mutant of Drosophila H1 on a well-positioned nucleosome.

Results
Histone Regions Involved in the Formation of the H1–Nucleosome Complex. To identify the nucleosome-binding regions of H1, we first used a gel shift assay to examine the binding of several H1 fragments, which contain gH1 and various lengths of C-terminal regions, to the nucleosome centered on 167 bp DNA with the Widom “601” sequence (40), which can uniquely position the

Significance
Linker H1 histones control the accessibility of linker DNA between two neighbor nucleosomes to DNA-binding proteins and regulate chromatin folding. We investigated the structure of the H1–nucleosome complex through a combination of multidimensional nuclear magnetic resonance spectroscopy, site-directed mutagenesis-isothermal-titration calorimetry and computational design/modeling. The results lead to a unique structural model for the globular domain of H1 in complex with the nucleosome that contains residue-level information and have implications for the dynamics of chromatin in vivo. In addition, our approach will be useful for testing the hypothesis that the globular domain of H1 variants might have distinct binding geometries within the nucleosome, and thereby contribute to the heterogeneity of chromatin structure.
nucleosome. To be consistent with later NMR experiments, we used a stable mutant of H1 in which four residues in the hydrophobic core of gH1 are replaced with the corresponding residues in gH5 (22) (Fig. 1 A and B and SI Appendix, Fig. S2). This stable H1 mutant is necessary to allow NMR experiments to be conducted at the high temperature (35 °C) required to observe the methyl groups in the H1-nucleosome complex (38). In this study, H1 refers to this stable mutant unless specified otherwise. We found that all fragments of H1 shift the position of the nucleosome in the agarose gel (Fig. 1C). In particular, the fragment containing residues 37–211 shifted the nucleosome as efficiently as H137–256. Using isothermal titration calorimetry (ITC), we further showed that H137–256, H137–211, and the wild type full-length H1256 have similar binding affinities for the nucleosome (SI Appendix, Fig. S3 and Table S1). Therefore, to avoid signal overlaps in NMR spectra (SI Appendix, Fig. S4 A), we chose H137–211 for subsequent NMR studies. Using 15N/13C-labeled H137–211, we assigned the observable residues of H137–211 in complex with the nucleosome (SI Appendix, Fig. S4B). The unobserved residues of H137–211, which are presumably folded into the nucleosome, include gH1 (45–118), residues 19NASAKKEK125 immediately following the globular domain, and the Lys-rich region 164KKPKAKKAVAT174 in the middle of the C-terminal tail (Fig. 1 D and E). We further demonstrated that H137–211 and the wild-type full-length H1256 used the same regions to bind to the nucleosome (SI Appendix, Fig. S4C). Similar results were observed when the nucleosome with 208 bp DNA was used (SI Appendix, Fig. S4D), indicating that H1 binds within the 10 bp of the linker DNAs that enter/exit the nucleosome core particle.

To determine whether the histone core of the nucleosome interacts with H137–211, we compared the methyl spectra of the nucleosome histones in the absence or presence of H137–211. We found that H137–211 did not perturb the methyl spectra (SI Appendix, Fig. S5A), indicating that H137–211 and the histone core are not in direct contact. We then tested whether the C-terminal tails of H2A and the N-terminal tails of H3 interact with H137–211 as they are close to the linker DNA (Fig. 2A). The peak intensities of the C-terminal residues (119–122) of the 15N-labeled H2A decreased by about half in the H137–211 or the wild-type full-length H1256, with little changes in their chemical shifts (Fig. 2 B–E), whereas those in 15N-labeled H3 and the N-terminal tails of 15N-labeled H2A remain unchanged in both peak intensities and chemical shifts (Fig. 2 B–E and SI Appendix, Fig. S5 B and C). Similar results were observed for H137–211 in complex with the nucleosome array, which has a longer linker DNA (SI Appendix, Fig. S6). These results indicate that one of the two disordered H2A C-terminal tails in the nucleosome and two short regions in the disordered C-terminal tails of the wild-type full-length H1256 became folded upon binding of H1 to the nucleosome.

**Determination of the Orientation of gH1 on the Nucleosome by PRE.** We next performed paramagnetic relaxation enhancement (PRE) experiments to determine the orientation of gH1 in the H137–211 nucleosome complex. In the PRE experiments, residues immediately outside the two ends of gH1 (P44 and A119) and on the surface (L60, A83, and K109) were chosen to minimize the perturbation to the gH1 structure (Fig. 1B). These residues were each individually mutated to Cys and linked to the paramagnetic compound [(S-2,5,5-tetramethyl-2,5-dihydro-1H-pyrrolyl-3-yl) methyl methanethiosulfonate] (MTSL) or [(S-methanethiosulfonyletaminomethylenediamine-N,N,N’N’-tetraacetic acid) (MTS-EDTA-Mn2+)] through a disulfide bond. Binding of paramagnetic spin-labeled H137–211 reduced the peak intensities of the observable backbone amide 1H-N signals in the nucleosome in a manner dependent on distance from the paramagnetic center (42). The peak intensities of residues in the C-terminal tails of H2A were only weakly affected by MTSb but were strongly affected by MTS-EDTA-Mn2+ (Fig. 3 A–D and SI Appendix, Fig. S7), consistent with the anticipated PRE effects from the two types of spin labels (42). The intensity changes in the observable residue T119 in H2A, which is near the folded region of H2A, indicate that the gH1 structure (Fig. 1B) is folded into the nucleosome, and that the nucleosome is not in direct contact with the histone core (42). We then tested whether the C-terminal tails of H2A and the N-terminal tails of H3 interact with H137–211 as they are close to the linker DNA (Fig. 2A). The peak intensities of the C-terminal tails of H2A were only weakly affected by MTSb but were strongly affected by MTS-EDTA-Mn2+ (Fig. 3 A–D and SI Appendix, Fig. S7), consistent with the anticipated PRE effects from the two types of spin labels (42). The intensity changes in the observable residue T119 in H2A, which is near the folded region of H2A, indicate that the gH1 structure (Fig. 1B) is folded into the nucleosome, and that the nucleosome is not in direct contact with the histone core (42). We then tested whether the C-terminal tails of H2A and the N-terminal tails of H3 interact with H137–211 as they are close to the linker DNA (Fig. 2A). The peak intensities of the C-terminal residues (119–122) of the 15N-labeled H2A decreased by about half in the H137–211 or the wild-type full-length H1256, with little changes in their chemical shifts (Fig. 2 B–E), whereas those in 15N-labeled H3 and the N-terminal tails of 15N-labeled H2A remain unchanged in both peak intensities and chemical shifts (Fig. 2 B–E and SI Appendix, Fig. S5 B and C). Similar results were observed for H137–211 in complex with the nucleosome array, which has a longer linker DNA (SI Appendix, Fig. S6). These results indicate that one of the two disordered H2A C-terminal tails in the nucleosome and two short regions in the disordered C-terminal tails of the wild-type full-length H1256 became folded upon binding of H1 to the nucleosome.

**Fig. 1.** The globular domain and two discrete regions of linker histone H1 bind to the nucleosome. (A) Sequence of H1, highlighting the globular domain (cyan), the quadruple mutations (green and bold), and the C-terminal regions (red) that are involved in the binding of the nucleosome, and secondary structures. (B) The structural model of the globular domain, which is modeled using the B chain of the gH5 globular domain structure as the template (22). The mutated residues are shown as green sticks. The balls indicate the positions for spin labeling, except that positions 44 and 119 outside the gH1 are indicated with residues 45 and 118 in gH1, respectively. (C) Gel shift assay results for different fragments of gH1. The nucleosome contains 167 bp DNA centered with the 601 sequence. (D) Overlay of 1H–15N HSQC spectra of H1 in free form (blue) and in complex with the nucleosome (black). In this experiment, the ratio of H137–211 to the nucleosome in the complex is ~0.7. The disappearance of peaks indicates that many residues in H137–211 become folded on binding of the nucleosome. (E) Deviation of 1H chemical shifts of observable residues of H1 in complex with the nucleosome from random coil values.
the nucleosome, and spin-labeling effects on the methyl groups in gH1 in the absence or presence of the nucleosome. Using the assigned methyl groups, we examined the PRE effects on the methyl groups of gH1 with MTSL labeled at position T119 of H2A or at position K37 of H3 in the nucleosome (Fig. 24). The residues at these positions are disordered in the nucleosome but are close to the nucleosome core, with relatively fixed locations. The spin labels have little effects on the chemical shifts of the methyl groups in gH1, indicating that they do not perturb the structure of the gH1-nucleosome complex (Fig. 3 F–G). The spin-label at position 119 of H2A has large PRE effects on the methyl groups of L97, V98, and V99 at the C-terminal region of the α3 helix of gH1, whereas the spin-label at position 37 of H3 strongly affected not only these methyl groups but also L60 in loop 1 and L103 and I104 in the C-terminal region of the β1 strand (Fig. 3 F–I).

Because there are two H3 K37 residues in the nucleosome structure that are separated by ~65 Å (Fig. 24), the observations that methyl groups in one region of gH1 are strongly affected by the MTSL labels at the H3 K37 sites further indicates that gH1 binds to the nucleosome asymmetrically. This is also consistent with the finding of only one of the two H2A C-terminal tails in the nucleosome on H137–211 binding (Fig. 2 B and C). Furthermore, as MTSL spin labels in gH1 have only small effects on the backbone amide NMR signals from the disordered H2A T119 (Fig. 3 A), gH1 must be distant from this disordered H2A C-terminal tail in the H137–211 nucleosome complex. Therefore, the large PRE effects on some methyl groups of gH1 from MTSL labels at H2A T119 (Fig. 3 F) indicate that gH1 is close to the folded H2A C-terminal tail in the H1–nucleosome complex.

**Important H1 Residues for Nucleosome Binding.** We used site-directed mutagenesis and ITC to identify gH1 residues that are important for the binding of H137–211 to the nucleosome. Positively charged Lys residues on the surface of gH1 were each mutated to Ala, and the effects of the mutations on the dissociation equilibrium constant (Kd) were measured by ITC (Fig. 4 A and SI Appendix, Table S1). Mutation of each of the six residues (K91, K96, K102, K107, and K116) showed larger effects (> a factor of 2.5) than others. These mutations are located on two distinct surfaces on nearly opposite sides of the gH1 structure (Fig. 4 B). One surface includes K91 and K96 in the α3 helix. The other surface includes residues K91 at the C-terminal region of the α3 helix and residues K102, K107, and K116 in the two β strands. In addition, ITC experiments showed that gH1 binding to the nucleosome was ~12 times weaker than H137–211 (SI Appendix, Fig. S9). These results are consistent with the NMR observation that residues 119 ASAKKEK215 and 118 KKKPAKK214 in the middle of the C-terminal tail of H137–216 are folded.

**A Structural Model for the gH1–Nucleosome Complex.** Taking all the data together, our experimental results suggest that gH1 uses the two positively charged surfaces to bridge the nucleosome core and the linker DNA asymmetrically. gH1 is close to one of the two H2A C-terminal tails and one of the two H3 N-terminal tails, with its α3 helix facing the nucleosome core. Using these restraints and the HADDOCK program (43), we docked the gH1 onto the nucleosome by forcing the K91 and K95 residues in the α3 helix of gH1 to interact with the nucleosomal DNA near the dyad region and the K58, K102, K107, and K116 residues to interact with one of the nearby 10-bp linker DNAs (SI Appendix, Fig. S10). After initial rigid body docking and subsequent energy minimization that allows contacting residues to adopt different conformations, the calculated low-energy structures were clustered. We found that one of the clusters was most consistent with all of the experimental results (Fig. 5 A and B and SI Appendix, Fig. S10 D), including the mutation effects on the binding affinity between H137–211 and the nucleosome (Fig. 5 C), PRE effects on H2A T119 in the nucleosome by spin labels in gH1 (Fig. 5 D), and the region that includes K91 and K95 in the nucleosome in complex with mutant H1 and the region that include K118, E120, and K122 of the H2A tails in the complex (Fig. 5 E).

**Figs. 2. Asymmetric binding of H137–211 to the nucleosome.** (A) Nucleosome structure, highlighting residues H2A T119 and H3 K37. The DNA structure is taken from the tetra-nucleosome structure with 10 bp DNA at both entry and exit regions (44): H2A is in orange, H3 is in blue, and H4 is in green. (B) Overlay of 1H–15N HSQC spectra of the nucleosome with 15N-labeled H2A in the absence (black) or the presence (orange) of mutant H137–211. (C) Same as in B except the wild-type full-length H1–256 (magenta) was used instead of mutant H137–211. Note that only strong peaks in the spectra are shown (SI Appendix, Fig. S7). In this experiment, the ratio of H137–211 to the nucleosome is 1.0. The dashed lines indicate that the volumes of the peaks in the boxes are integrated together. (D) Ratios of peak volumes of T119, K121, and the region that include K118, E120, and K122 of the H2A tails in the nucleosome in complex with mutant H137–211 to those in the free nucleosome. (E) Same as in D except that the wild-type full-length H1–256 was used instead of mutant H137–211. The asterisks in B–E indicate that the peaks in the dashed box were integrated together when the peak volumes were measured.
and PRE effects on the methyl groups of gH1 by spin labels at H2A T119 (Fig. 5E) and H3 K37 (Fig. 5F). In the model, although gH1 directly interacts with only one linker DNA, it is very close to the other linker DNA (Fig. 5B and SI Appendix, Fig. S10E). A slight shift of this linker DNA toward the nucleosome core could also lead to its interaction with gH1. Therefore, it is possible that gH1 may interact with both DNA linkers, one strongly and the other weakly (SI Appendix, Fig. S6A).

The C-Terminal Tail of Histone Variant H2A.Z Disfavors H1 Binding.

Earlier studies have shown that the nucleosome containing histone variant H2A.Z affects H1 binding (44), and the C-terminal tail of H2A.Z may play a role in the reduced binding affinity (45). The amino acid sequence of the very C-terminal region (119TEKKA23) of H2A is different from corresponding residues (123EETVQ27) of H2A.Z. To test the role of the C-terminal tail of H2A.Z in H1 binding, we measured the binding affinity of H137–211 to the nucleosome containing H2A with the last six residues replaced by the corresponding residues of H2A.Z. We found that the binding was reduced to an undetectable level by ITC (SI Appendix, Fig. S11), whereas deletion of the last five residues of H2A increased $K_D$ by a factor of $\sim$10. These results further confirm the earlier NMR observation that one of the C-terminal tails of H2A is involved in the formation of the H1–nucleosome complex.

Discussion

Our structural model of the gH1–nucleosome complex differs from all previous experiment-based models in either the location or orientation of gH1/H5 or the length of linker DNA involved. The observation that MTSL labels at H2A T119 or H3 K37 have large PRE effects on the methyl groups in only one region of gH1 clearly shows that the gH1 binds to the nucleosome asymmetrically. For a symmetric binding, large PRE effects on methyl groups of gH1 from spin labels at H3 K37 are not expected because they are separated by more than 20 Å (SI Appendix, Fig. S12A). It is interesting to note that Zhou and coworkers have also found that gH5 binds to the nucleosome complex asymmetrically, involving 10-bp linker DNA (SI Appendix, Fig. S12A). However, the orientation of gH5 in their model is opposite to that of gH1 in ours: The $\alpha_3$ helix of gH5 in their model binds to the linker DNA instead of the nucleosomal DNA, as in our model (SI Appendix, Fig. S12B and C). One possibility for the differences among various models of the gH1/H5–nucleosome complex might be that there are multiple gH1/H5 binding modes for the nucleosome. Another possibility is that earlier models are

![Fig. 3. Location and orientation of gH1 on the nucleosome determined by PRE effects. (A–D) PRE effects of spin labeling of H137–211 at positions 44, 60, 83, 109, and 119 on the amide protons of H2A C-terminal tail residues T119 and K122 and N-terminal R3 and K5 residues in the nucleosome. (E–G) Methyl-spectra of gH1 in complex with the nucleosome (red) and the nucleosomes with MTSL labels at H2A T119 (orange) and H3 K37 (blue), respectively. (H and I) Bar graphs showing PRE effects on the methyl groups of gH1 with MTSL label at H2A T119 (orange) and H3 K37 (blue), respectively.

![Fig. 4. Effects of mutations in gH1 on the binding affinity of H137–211 to the nucleosome. (A) Effects of mutation of surface residues in gH1 on the binding affinity between H137–211 and the nucleosome. (B) Structural illustration of the distribution of the gH1 residues whose Ala mutations lead to a large decrease in binding affinity.](image-url)
Derived from less-controlled systems, using indirect methods (SI Appendix, Fig. S1).

Among several computation-based models of the gH1/H5–nucleosome complex (30–32, 46), two of them show similarities to our model in terms of both location and orientation of gH1 in the complex (30, 31). In addition, our model is consistent with several other experimental observations. For example, the two C-terminal tails of H2A in the *Xenopus* nucleosome cross-link to different positions of the DNA in the nucleosome on addition of H1 (47). *Calf* H1 is cross-linked to the C-terminal region of H2A (48). The location of gH1 in our model is very close to the disordered negatively charged C-terminal tail of HMGN2 in complex with the nucleosome, consistent with its role in inhibiting the binding of H1 to the nucleosome (38). Binding of H1 to the 10-bp linker DNA that neighbors the nucleosome core particle has been used to explain the preferential “out-of-phase” population of AT bases in the region (31, 49). The identification of two major discrete DNA-binding surfaces of gH1 in our mutation studies is in excellent agreement with earlier mutation studies of the histone H5 globular domain (50) and *Mouse* H1*, using fluorescence recovery after photo-bleaching methods (19) (SI Appendix, Fig. S13). In addition, within the limit of the resolution, the tetra-nucleosome crystal structure can accommodate gH1 in our model (41) with only small clashes with the linker DNA that connects the neighboring nucleosomes (SI Appendix, Fig. S14), suggesting that a small structural rearrangement of the linker DNA could allow H1 to condense chromatin to the two-start zigzag nucleosome high-order structure (7, 51).

Our finding that two discrete regions in the C-terminal tail of H1, 119ASAKKEK125 and 164KPKAKKAVAT174, are involved in nucleosome binding is consistent with the earlier experimental observations that the C-terminal tail of H1 plays an important role in nucleosome binding and chromatin structure condensation (18, 20, 21). In particular, our results support the conclusions that specific regions in the C-terminal tail of H1, rather than the distribution of positively charged residues, are responsible for interaction with linker DNA (17, 18, 52, 53). In the case of human H1.5, it has been shown that the seven residues following the globular domain, 121PKAKKAG127, are necessary for the formation of the linker DNA stem structure (17). Six of these seven residues are conserved in the 164KPKAKKAVAT174 region of the C-terminal tail of *Drosophila* H1. Our results show that NMR provides a powerful tool for identifying specific nucleosome-binding regions in the C-terminal tail of H1.

It has been shown that the H2A.Z-containing nucleosome is typically present at regions flanking the nucleosome-free region (54), near the DNA double-strand break (55), and at the boundary of euchromatin and heterochromatin, which prevents spread of heterochromatin regions (56). In general, these chromatin regions are less condensed and more dynamic. Our finding that the very last several residues at the H2A.Z C-terminal tail inhibit the binding of H1 to the H2A.Z nucleosome provides a possible mechanistic explanation for the dynamic features of the H2A.Z-enriched chromatin regions.

Finally, our approach provides an experimental tool for testing the hypothesis that the globular domains of individual H1 variants might have distinct binding geometries within the nucleosome that contribute to the heterogeneity of chromatin structure (9, 19, 57).

**Materials and Methods**

**Design of a Stable Mutant of gH1 and Preparation of Samples.** The gH1 structure model was built using homology modeling and the structure of gH5. Protein design programs were used to select the mutation (VS3I, SS6A, CB1V, and A9TL) that stabilizes gH1 structure. The *Drosophila* H1 gene was synthesized and inserted into the pET42b vector. Mutations were made using the QuickChange kit. Proteins were expressed in *Escherichia coli* and purified using chromatography. MTSL or MTS-EDTA was linked to the protein, with the target site mutated to Cys. Nucleosomes were reconstituted by stepwise salt dialysis in the absence of reducing agent, followed by HPLC to remove free DNA and immature nucleosomes (SI Appendix, Materials and Methods and SI Appendix, Fig. S15).

**NMR, PRE, and ITC Experiments.** Isotope-labeled H1 mixed with the nucleosome, or vice versa. The spin-labeled H1 was mixed with nucleosomes containing 15N-labeled histones or vice versa. 1H–15N HSQC or 1H–13C heteronuclear multiple quantum correlation (HMOC) spectra were recorded. The NMR peak
intensities or volumes were measured. ITC experiments were performed on a VP–ITC microcalorimeter (Microcal). The dissociation constant ($K_d$) and the stoichiometry of binding ($n$) were determined by fitting the observed binding curves to the model with independent $n$ and $K_d$ values (SI Appendix, Materials and Methods).

Docking Calculation. We docked gH1 to the 167-bp DNA obtained from the tetra-nucleosome structure using HADDOCK program. Residues Lys91 and Lys95 in gH1 were forced to interact with the nucleosomal DNA near the dyad, the K58, K102, K107, and K106 residues were forced to interact with the nearby linker DNA. A cluster of structures that is most consistent with the experimental data were selected as the final model (SI Appendix, Materials and Methods).

ACKNOWLEDGMENTS. We thank Dr. Jemima Barrowman for editing the manuscript. This work is supported by the intramural research program of National Cancer Institute and National Institutes of Health (NIH) (to Y.B.) and by the National Institute of General Medical Sciences of the NIH (R01GM085003, to Y.Z.).