The H4 Tail Domain Participates in Intra- and Internucleosome Interactions with Protein and DNA during Folding and Oligomerization of Nucleosome Arrays[∇]

Pu-Yeh Kan,¹ Tamara L. Caterino,² and Jeffrey J. Hayes^{2*}

Department of Biochemistry and Biophysics² and Department of Pathology,¹ University of Rochester, Rochester, New York 14642

Received 22 August 2008/Returned for modification 29 September 2008/Accepted 28 October 2008

The condensation of nucleosome arrays into higher-order secondary and tertiary chromatin structures likely involves long-range internucleosomal interactions mediated by the core histone tail domains. We have characterized interarray interactions mediated by the H4 tail domain, known to play a predominant role in the formation of such structures. We find that the N-terminal end of the H4 tail mediates interarray contacts with DNA during self-association of oligonucleosome arrays similar to that found previously for the H3 tail domain. However, a site near the histone fold domain of H4 participates in a distinct set of interactions, contacting both DNA and H2A in condensed structures. Moreover, we also find that H4-H2A interactions occur via an intra- as well as an internucleosomal fashion, supporting an additional intranucleosomal function for the tail. Interestingly, acetylation of the H4 tail has little effect on interarray interactions by itself but overrides the strong stimulation of interarray interactions induced by linker histones. Our results indicate that the H4 tail facilitates secondary and tertiary chromatin structure formation via a complex array of potentially exclusive interactions that are distinct from those of the H3 tail domain.

Eukaryotic DNA is packaged with core histones and other nonhistone chromosomal proteins through multiple levels of increasingly condensed chromatin structures. Arrays of nucleosomes, which form the primary repeating structure of chromatin, fold into secondary chromatin structures such as the 30-nm-diameter chromatin fiber (21, 37, 45). Chromatin fibers and other secondary structures are further condensed into higher-order tertiary chromatin structures such as the 100- to 130-nm-diameter chromonema observed by Belmont and Bruce (7). While models (solenoid, twisted-ribbon, and crossed-linker) have been proposed for the 30-nm-diameter chromatin fiber, to date no definitive model has been broadly accepted (38). Moreover, only very limited structural information is available with regard to tertiary and higher-order chromatin structures (see references 21, 30, and 45 and see below).

Although the molecular mechanisms behind the formation of secondary and tertiary chromatin structures remain unclear, work employing model systems has shown that *in vitro* reconstituted nucleosome arrays containing only DNA and core histone proteins undergo the same initial salt-dependent condensations as native chromatin (17, 21). In solutions containing physiological concentrations of mono- and divalent cations, nucleosome arrays spontaneously fold into structures with the same hydrodynamic shape as the 30-nm-diameter chromatin fiber and reversibly self-associate into larger assemblies with characteristics of native tertiary chromatin structures (21, 45). Thus, the primary protein determinants defining these structures reside within the core histone proteins. Linker histones promote the salt-dependent folding and condensation of chro-

matin secondary and tertiary structures without altering the fundamental characteristics of these structures (8, 9, 25).

Hydrodynamic studies and electron microscopy observations have revealed that nucleosome arrays lacking the core histone N-terminal tail domains are unable to form 30-nm fibers and do not undergo fiber-fiber association in high-salt solutions (7, 16, 30, 39), indicating that these domains are essential for the formation of secondary and tertiary chromatin structures. Notably, each of the four N-terminal tail domains does not contribute equally to the folding and oligomerization of nucleosome arrays (19). Arrays lacking one or more of the histone tail domains showed characteristic extents of deficiency in both formation of the 30-nm fiber and interarray association, with arrays containing only the H3/H4 tails condensing better than those with only the H2A/H2B tails (39). More detailed characterization of the individual tails indicated that despite being the second-shortest tail overall, the H4 tail provides the largest contribution to stabilizing higher-order chromatin structures (12, 19). In comparison, the H3 tail contributes slightly less to both secondary and tertiary structure formation, while the H2A/H2B tails are the least significant but still important participants in the chromatin condensation processes (19).

Model nucleosome arrays have also been used to elucidate structural aspects of tertiary chromatin structures. Early on, Hansen's group used analytical velocity sedimentation methods to show that formation of self-associated tertiary structures is highly cooperative and reversible, paralleling the behavior of native chromatin (21, 30). They further showed that the initial step in the process involves self-association of monomeric nucleosome arrays (55 S) into oligomeric structures approximately 10 times larger than the arrays (about 500 S), without appreciable accumulation of species of intermediate sizes. As the salt is increased further, these ~500-S structures continue to self-associate into extremely large complexes that sediment

* Corresponding author. Mailing address: Department of Biochemistry and Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642. Fax: (585) 275-6007. E-mail: Jeffrey_Hayes@urmc.rochester.edu.

[∇] Published ahead of print on 10 November 2008.

in excess of $\gg 10,000$ S. These observations provide a rigorous physicochemical basis for a simple microcentrifuge-based assay now routinely used to study self-association (5, 10, 25, 30, 33, 43). Association of linker histones also pushes the equilibrium toward the largest species (8, 30). Interestingly, while subsaturated arrays that have nucleosome-free gaps cannot undergo divalent cation-induced folding, they retain the ability to self-associate into tertiary structures, albeit at higher MgCl_2 concentrations. The formation of such structures in vitro may reflect the continued association of transcriptionally active regions of chromatin in compact chromatin territories in vivo (41).

Chromatin plays an integral role in the regulation of gene expression and other processes typically by restricting access to genomic DNA. Thus, activation of gene expression involves transient decondensation of higher-order chromatin structures, facilitated by specific posttranslational modifications such as lysine acetylation within the histone tail domains or ATP-dependent chromatin remodeling (6, 20). Consistent with their essential roles in chromatin structure, the histone tail domains are the sites of most of the posttranslational modifications related to transcriptional activation, gene silencing, and the formation of heterochromatin (35, 42). Indeed, some posttranslational modifications, such as acetylation, are known to directly alter chromatin structure in preparation for transcriptional activation, presumably by altering interactions of the tail domains (1, 18, 39, 44). For example, hydrodynamic studies have shown that acetylation of the tail domains inhibits folding of nucleosome arrays into secondary and tertiary chromatin structures (18, 40, 43), with acetylation of H2B and H4 having the greatest effect on tertiary structure formation (43).

While interactions of the basic core histone tail domains with DNA have been well documented, evidence indicates that these domains also interact with protein targets in condensed chromatin. Quantitative analysis of surface charge density during salt-dependent folding of nucleosome arrays indicated that some of the tails participate in interactions with other histones, possibly involving tail-tail interactions in condensed structures (16). Indeed, a region in the N-terminal tail of H4 (amino acids [aa] 16 to 25) was found to contact a surface of the H2A/H2B dimer between highly ordered nucleosome core particles within a crystal lattice (11, 27). Additional evidence for this H4 tail-H2A interaction was later obtained with in vitro cross-linking experiments and interpreted as supporting a two-start model for the 30-nm fiber (13). Hydrodynamic studies showed that a deletion within the H4 tail near this region (aa residues 14 to 19) as well as the introduction of a single acetylated lysine residue into this region inhibited salt-dependent folding and self-association of model nucleosome arrays (12, 33). Furthermore, recent studies have indicated that nonallelic H2A variants change the ability of nucleosome arrays to fold and condense through alterations in this H4 tail-H2A interaction (15, 49).

Although the core histone tail domains are critical regulators of chromatin structure and play an essential role in the control of transcription and other processes, details of the molecular interactions of the tails and the mechanisms by which posttranslational modifications regulate tail structures and interactions remain poorly understood. Since the H4 tail plays a central role in defining higher-order chromatin struc-

tures, we investigated interactions of this domain during formation of secondary and tertiary chromatin structures with model nucleosome arrays. We find that the H4 tail mediates long-range interfiber interactions similar to those previously documented for the H3 tail domain (25). However, in contrast to the H3 tail domain, a region of the H4 tail near the histone fold domain exhibits interarray contacts to both DNA and protein targets in condensed chromatin structures. Moreover, linker histones strongly stimulate interarray interactions, but this stimulation is abrogated by acetylation within the tail domain. These results extend our understanding of critical long-range interactions of the H3 and H4 tail domains as well as provide new insights into the mechanisms underlying formation of higher-order chromatin structures.

MATERIALS AND METHODS

Preparation of core and linker histone proteins. Recombinant H3 and H4 were expressed in bacterial cells, and tetramers were purified as previously described (36) with modifications. Briefly, cells from 50-ml cultures expressing either protein were pelleted and resuspended in 10 ml of 10 mM EDTA, 0.5% Triton X-100 and placed on ice for 30 min. Ice-cold HCl (10 M) was added to a final concentration of 0.4 M and the solution was mixed by vortexing, followed by a 30-min incubation on ice. The sample was centrifuged at 13,000 rpm for 30 min, the supernatant was discarded, and the pellet was resuspended in 5 ml of 8 M urea. A second acid extraction was performed by adding an equal volume of 0.8 M HCl and centrifuging at 13,000 rpm for 30 min, with the protein in the soluble fraction. The supernatant was neutralized by the addition of 0.4 ml of 10 M NaOH. H3/H4 tetramers were formed by mixing acid-extracted H3 with H4 in a 1:1 ratio and dialyzing against 2 M NaCl/Tris-EDTA (TE), 1 mM dithiothreitol overnight. Refolded tetramers were diluted three times with TE and applied to a Biorex 70 ion-exchange column (the typical column size was 1 ml), washed extensively with 0.6 M NaCl/TE, and eluted with 2 M NaCl/TE. Purified H3/H4 tetramers were modified directly in storage buffer with a twofold excess of 4-azidophenacylbromide (APB) at room temperature for 1 h in the dark as described previously (26, 47). The efficacy of APB was periodically tested by UV irradiation of modified H3/H4 tetramers, which produces intratetramer protein-protein cross-links (unpublished results). All manipulations with APB-modified proteins and reconstitutes were carried out in the dark. H2A/H2B dimers and linker histone H1 were expressed and purified as described previously (23). Modification of H2A G2C with fluorescein-5-maleimide was carried out in a 1:1 molar ratio.

Expression of ^3H -labeled H4 (*H4) proteins. Recombinant H4 G2C and H4 V21C, which contain a single cysteine substituted for amino acids 2 and 21, respectively, were prepared based on *Xenopus laevis* major H4 as previously described (48). pET3a vectors containing sequences coding for these proteins were transformed into BL21(DE3) by standard methods. Transformed *Escherichia coli* was grown overnight in 5 ml of minimal medium and then transferred into 50 ml of fresh minimal medium. At an optical density at 600 nm of ~ 0.6 , expression was induced by isopropyl- β -D-thiogalactopyranoside, then 2.5 mCi of [^3H]lysine was added 20 min after induction and the cells were grown for another 3 h (46). Incorporation of [^3H]lysine into recombinant H4 was examined by analyzing bacterial proteins on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), soaking the gel in 1 M salicylic acid for 15 min, drying the gel, and performing detection by exposure to X-ray film at -70°C .

Acetylation of core histone proteins. Purification of the Piccolo v55 histone acetyltransferase (HAT) complex and acetylation reactions were performed as described previously (43). Briefly, components of the Piccolo complex were expressed in transformed BL21(DE3) cells and purified by nickel column chromatography. Activities of the Piccolo complex preparations vary in each purification, and trial serial titrations were necessary to optimize conditions for the acetylation reaction. The acetylation reactions were performed at 30°C in reaction buffer (25 mM Tris, pH 8.0, 0.1 mM EDTA, 150 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, and 10 mM sodium butyrate) with a sufficient amount of acetyl-coenzyme A (10-fold the H4 protein concentration) as a substrate. Briefly, 20 μl of 25 μM (H3/H4)₂ was mixed with 10 μl of purified Piccolo v55 HAT complex and 1 μl of 5 mM acetyl-coenzyme A in a total volume of 200 μl . The extent of H4 acetylation was determined by Triton-acid-urea gel electrophoresis as described previously (43). Typically complete tetraacetylation of the H4 tail also resulted in some monoacetylation of H3.

Reconstitution and analysis of nucleosome arrays. Nucleosome array DNA templates, containing 12 tandem repeats of a 208-bp 5S rRNA sequence from *Lytechinus variegatus*, were reconstituted with core histone proteins by salt dialysis. Core histone proteins were mixed with DNA in a 1.2:1 ratio in 2 M NaCl/TE, followed by dialysis against 1 M NaCl/TE for 4 h, 0.75 M NaCl/TE for 3 h, and finally 10 mM Tris, 0.1 mM EDTA, and 0.1 mM EGTA overnight. The level of histone saturation was examined by EcoRI digestion and analysis of nucleosome and free DNA bands on nucleoprotein gels as described previously (25). The efficiency of self-association of nucleosome arrays into oligomeric structures in the presence of divalent cations is dependent on the level of saturation and was also used to gauge the extent of nucleosome occupancy (30). The 35-mer nucleosome array was reconstituted by the same method using a DNA fragment containing 35 tandem 5S rRNA gene repeats and core histone proteins purified from chicken erythrocytes as described previously (24).

UV-induced cross-linking and identification of interarray cross-linking. The 35-mer nucleosome array (8.1 μ l, 0.2 mg/ml) was mixed with 8.1 μ l of 12-mer nucleosome arrays (0.04 mg/ml) reconstituted with *H4 and APB-modified recombinant H4, followed by addition of 1.8 μ l of 10 $\times$ MgCl₂ containing 10 mM Tris to attain final MgCl₂ concentrations of 0 to 8 mM. The sample was placed inside a Falcon 5-ml polystyrene tube and enclosed in a 15-ml Pyrex 9820 glass tube. The sample was irradiated for 1 min at 365 nm on a VMR LM-20E light box. The irradiated samples were then adjusted to 0.01% SDS and 0.2 μ g/ml ethidium bromide (EtBr), and the samples were separated on a 0.7% agarose gel (0.5 $\times$ Tris-borate-EDTA, 0.01% SDS, 0.2 μ g/ml EtBr). After UV photography, the gels were soaked in 45% methanol and 10% acetic acid, then 1 M sodium salicylate for 15 min, and then dried. The gels were exposed to Kodak MR film at -70°C for 1 to 2 weeks.

H4 tail-H2A cross-linking. The 12-mer nucleosome arrays or mononucleosomes (18 μ l, 0.08 mg/ml) reconstituted with wild-type H2A or H2A G2C-fluorescein and *H4 V21C-APB were mixed with 2 μ l of 10 $\times$ MgCl₂, and the samples were cross-linked as described above. The irradiated samples were directly analyzed on 15% SDS-PAGE. The fluorescent signals derived from H2A G2C-fluorescein were recorded by fluorography. The location of radioactivity of samples due to *H4 V21C-APB was monitored as described in the interarray cross-linking experiment. In competition experiments, the LANA and LRS (scrambled) peptide (10) were added to a 5- to 10-fold molar excess over nucleosome concentrations.

Linker histone binding assay. Purified recombinant *Xenopus* H1^o was mixed with 8.1 μ l of a 35-mer nucleosome array (0.2 mg/ml) and 8.1 μ l of a 12-mer nucleosome array (0.04 mg/ml) in an approximately 1:1 H1 protein/nucleosome ratio as determined by SDS-PAGE with standards calibrated by amino acid analysis in the presence of 50 mM NaCl on ice for 3 h. The exact amount of H1 required to saturate the nucleosome arrays was empirically determined by centrifugation of H1-bound arrays at 13,000 rpm for 15 min in a microcentrifuge in the presence of 1.3 mM MgCl₂ to precipitate oversaturated arrays (8). The stoichiometries of H1-bound 12-mer and 35-mer arrays were further confirmed by SDS-PAGE and a comparison to native chromatin samples.

RESULTS

The histone tail domains are thought to mediate internucleosome and interarray interactions during formation of higher-order chromatin structures (21, 30). Since, as previously demonstrated, the H3 tail domain participates in long-range interarray interactions during MgCl₂-induced folding and self-association of model nucleosome arrays (25), we wished to determine whether the H4 tail domain participates in similar interactions. To this end, model 12-mer nucleosome arrays were reconstituted with *H4 that was also site-specifically modified with a photoactivatable cross-linking agent at a specific residue within the H4 tail domain. We first incorporated the photoactivatable cross-linker near the N-terminal end of the H4 tail by modification of the mutant H4 G2C (Fig. 1A) with APB, followed by reconstitution of *H4 G2C-APB with native H3, H2A, H2B, and the 208-12 DNA template into a 12-mer nucleosome array. The saturation of reconstituted templates was confirmed by EcoRI digestion to liberate individual

FIG. 1. Detection of interarray H4 tail-DNA interactions from a position near the N terminus of the H4 tail domain. (A) Scheme of histone H4 showing the location of cysteine substitution (H4 G2C) and the site of APB-cross-linker attachment. The related site on the H3 tail domain (H3 T6C) investigated previously is also shown. (B) The 12-mer nucleosome arrays reconstituted with *H4 G2C-APB were mixed with 35-mer arrays, and the extent of the MgCl₂-dependent interarray H4 tail-DNA cross-linking was determined. Cross-linking reactions were carried out in 0, 2, 4, 6, and 8 mM MgCl₂ (lanes 2 to 6, respectively) as indicated. The sample in lane 1 was incubated in 10 mM Tris without UV irradiation. Upper panel, EtBr-stained gel; lower panel, autoradiograph of the same gel. (C) Interarray cross-linking of the H4 tail domain was quantified and plotted as the fraction of total cross-linking at each MgCl₂ concentration (diamonds). For comparison, data previously obtained with *H3 T6C-APB (squares) are also shown. (D) Chromatin compaction decreases intraarray cross-linking efficiency of H4 G2C-APB. The relative extent of cross-linking to the 12-mer template at various MgCl₂ concentrations (25) (C) was determined and normalized to cross-linking in the absence of salt.

nucleosomes and by MgCl_2 -induced self-association assays as previously described (24).

The arrays were then mixed with a fivefold excess of 35-mer nucleosome arrays containing unlabeled native histones. The samples were incubated in 0 to 8 mM MgCl_2 to induce various extents of array oligomerization (21) and then briefly exposed to UV irradiation to induce cross-linking. The cross-linking products formed between *H4 and the DNA templates were analyzed on SDS-agarose gels, with interarray interactions of the H4 tail domain indicated by cross-linking to the 35-mer DNA template. As expected, only intraarray H4 tail-DNA cross-links were observed in the absence of MgCl_2 , as indicated by the UV-dependent covalent cross-linking of *H4 to the 12-mer template and the lack of radiolabeled H4 comigrating with the 35-mer template in the gel (Fig. 1B, lanes 1 and 2). However, upon elevation of MgCl_2 concentrations to levels sufficient to induce array self-association (4 to 8 mM), detectable amounts of H4 were covalently cross-linked to the 35-mer array DNA template, indicating that a fraction of H4 tails from the 12-mer array contacted the DNA of the 35-mer array (Fig. 1B, lanes 4 to 6). In 4, 6, and 8 mM MgCl_2 , the fraction of H4 cross-linked to the 35-mer DNA template increased from 12% to 18% to 24% of the total cross-linked H4, respectively, similar to the extent of interarray cross-linking observed for the N-terminal end of the H3 tail using the mutant *H3 T6C (25) (Fig. 1A and C). As with the H3 tail, the extent of interarray cross-linking appears to reach a maximum value in 6 to 8 mM MgCl_2 , correlating with the point at which fully saturated arrays reach 100% self-association (21, 31).

We also noticed that increases in interarray interactions by the N-terminal region of the H4 tail domain during salt-dependent condensation of the nucleosome arrays were accompanied by a distinct loss of cross-linking to the 12-mer array. For example, at 8 mM MgCl_2 , cross-linking of the H4 tail to the 12-mer template drops to about 35% of the value observed in the absence of salt (Fig. 1D). By comparison, cross-linking of the N-terminal end of the H3 tail domain remained fairly constant for all levels of MgCl_2 , at about 80% of the value observed in the absence of salt. This result suggests that intraarray contacts of the H4 tail domain are lost upon salt-dependent condensation of the arrays, perhaps in favor of interarray contacts to the 35-mer template (Fig. 1C) or other targets (see below).

Previous work showed that the extent of interarray interactions detected for the H3 tail domain decreased as the probe was moved closer to the histone fold domain (25). We therefore tested whether the extent of interarray interaction is dependent upon location within the H4 tail domain by generating 12-mer arrays containing *H4 V21C-APB, in which the cross-linker is located near the histone fold domain of H4 (Fig. 2A). In contrast to an equivalent position in the H3 tail domain (Fig. 2A), arrays containing *H4 V21C-APB exhibited a significant fraction of total cross-links to the 35-mer DNA template in elevated MgCl_2 , similar to that observed for the N-terminal end of the H4 tail domain (Fig. 1C and 2B and C). However, it is important to note that cross-linking of *H4 V21C-APB to the DNA templates occurred with a much lower efficiency compared to that observed with other positions in either the H3 or H4 tail domains. For example, quantification of Fig. 2C shows that about $1\% \pm 0.3\%$ of H4 V21C-APB was

FIG. 2. Regions within the H3 and H4 tails near the histone fold domain exhibit distinctly different patterns of interactions. (A) A scheme of the H4 protein showing the site near the H4 histone fold domain (H4 V21C) modified by APB. The location of an analogous site in the H3 tail domain (H3 V35C) previously investigated is also shown. (B) The 12-mer nucleosome arrays were reconstituted with *H4 V21C-APB or *H3 V35C-APB and analyzed for interarray tail-DNA cross-linking as described in the legend to Fig. 1B. (C) Interarray cross-linking for regions near the histone fold domains in H4 (diamonds) and H3 (squares) was quantified and plotted as described in the legend to Fig. 1.

cross-linked to the 12-mer template, while about $20\% \pm 5\%$ H4 G2C-APB or H3 T6C-APB (25) was cross-linked to this template.

Although many factors can affect cross-linking efficiency, one possible explanation for the reduced histone-DNA contacts by *H4 V21C-APB could be that this region of the H4 tail also interacts with non-DNA targets in chromatin. Indeed, residue 21 in the H4 tail is located within a domain postulated to contact a surface of H2A on an adjacent nucleosome in condensed chromatin (11, 12, 27). To test the possibility of H4 tail-H2A interactions during folding and self-association, we reconstituted nucleosomal arrays containing fluorescently tagged H2A (H2A G2C-fluorescein) and *H4 V21C-APB as described in the experiments above. Previous experiments suggested that H4-H2A internucleosomal contacts take place within the 30-nm fiber structure, which is formed in 2 mM MgCl_2 (13, 31). Thus, the 12-mer arrays were UV irradiated in

FIG. 3. A region within the H4 tail interacts with H2A in both an intra- and internucleosomal fashion. The 12-mer nucleosome arrays reconstituted with H2A G2C-fluorescein and *H4 V21C-APB were incubated in 0, 1, 2, and 4 mM MgCl₂ and then UV-irradiated (lanes 2 to 5). The sample in lane 1 was incubated in 10 mM Tris without UV irradiation. Cross-linking products observed by Coomassie blue staining (A), fluorography (B), and autoradiography (C) are shown. (D) Quantification of H2A-H4 cross-linked species in nucleosome arrays (squares) and mononucleosomes (diamonds). (E) Specific competition of H4-H2A cross-linking with LANA peptide. Cross-linking of arrays was carried out in the presence of the LANA or LRS peptide (10), and the extent of cross-linking was quantified and plotted relative to that in the absence of peptide. Errors represent ± 1 standard deviation. (F and G) H4-H2A cross-linking in mononucleosomes analyzed by Coomassie blue staining (F) and fluorography (G).

the presence of 0, 1, 2, or 4 mM MgCl₂ to generate extended arrays, moderately folded arrays, completely array folded arrays, and folded arrays with 70 to 80% of self-association, respectively. The cross-linking products were then analyzed by both fluorography and autoradiography to allow monitoring of fluorescently tagged H2A and *H4, respectively. In the absence of irradiation, four protein bands representing the four core histone proteins appeared on the Coomassie blue-stained SDS-PAGE gel (Fig. 3A, lane 1). Upon UV irradiation, two additional bands appeared on the gel above the four core histone proteins (Fig. 3A, lanes 2 to 5). Control experiments with native H2A showed that the upper band (marked H2A-X) was due to spurious cross-linking of H2A G2C-fluorescein to another histone, most likely itself (results not shown). The lower band (marked H2A-H4) was dependent upon APB modification of H4 and comigrated with a band in the fluorograph (Fig. 3B) and autoradiograph (Fig. 3C) of the gel, indicating

that this band contains both H2A and H4. An additional band with a much lower intensity was also observed in the Coomassie blue-stained gel and autoradiograph but not in the fluorograph (Fig. 3C), perhaps indicating a small amount of cross-linking of the H4 tail to another core histone (the H4-X band). Notably, the intensity of the H4 tail-H2A cross-linking band increased as the MgCl₂ concentration increased from 0 mM to 4 mM (Fig. 3D). Moreover, the intensity of this band was decreased by competition with a peptide (LANA) that is known to bind to the H2A pocket on the surface of the nucleosome and displace the H4 tail (10) but significantly less so by a peptide of identical composition but scrambled sequence (Fig. 3D). These data suggest that this band represents interaction of the H4 tail domain with the H2A pocket previously observed in crystal structures of nucleosome core particles (27). These results are consistent with previous reports that the H4 tail binds to a pocket on the surface of H2A, generating an

internucleosomal interaction to enhance folding of nucleosome arrays (10, 13, 33, 49).

Interestingly, although the nucleosome array is expected to exist as a completely extended structure with little or no internucleosome interactions in the absence of MgCl_2 (25, 30, 31), cross-linking between H4 and H2A was detectable under these conditions (Fig. 3A, B, and C, lane 1). This raised the possibility that some H4 tail-H2A interactions might be intranucleosomal, especially in the fully extended arrays. To test this possibility, we repeated the cross-linking experiment with mononucleosomes reconstituted with *H4 V21C-APB and H2A G2C-fluorescein. The results of these assays revealed detectable cross-linking between the H4 tail and H2A within the mononucleosomes (Fig. 3F and G). Control experiments (not shown) indicated that the extent of cross-linking was not dependent on nucleosome concentration, supporting the notion that the cross-links are due to intranucleosome interactions. Moreover, the cross-linking signal detected in mononucleosomes did not increase upon elevation of MgCl_2 , as was observed with the arrays (Fig. 3E). These results suggest that the H4 tail can contact the H2A pocket of its own nucleosome.

Acetylation of the histone tails can have a direct effect on higher-order chromatin structures, possibly by altering intra- or internucleosome interactions of the core histone tail domains (2, 18, 33, 40). Four lysines within the H4 tail domain can be acetylated, and hyperacetylated H4 is found in transcriptionally active chromatin (20). To examine the effect of acetylation of the H4 tail domain on tail-DNA and protein interactions, $(\text{H3/H4})_2$ tetramers were incubated with the Piccolo v55 HAT complex (32, 43), which resulted in quantitative tetraacetylation of H4 within the complex (Fig. 4A and B, lane 2). Consistent with published data (43), we found that arrays containing tetraacetylated *H4 G2C-APB exhibited a reduction in the propensity to undergo salt-dependent self-association, with an Mg_{50} for unacetylated and H4-acetylated arrays of 3.5 ± 0.5 mM and 5.2 ± 0.5 mM, respectively (plots not shown; see reference 42). However, cross-linking experiments with nucleosome arrays containing unacetylated and acetylated *H4 G2C-APB showed that acetylation had little effect on the extent of interarray interactions in 6 to 8 mM MgCl_2 (Fig. 4D).

Interactions of the core histone tail domains may also be directly affected by the association of linker histones, which greatly stabilize the formation of higher-order chromatin structures (8, 37). We previously showed that binding of linker histone H1 greatly stimulated interarray contacts of the H3 tail in low (0 to 2 mM) MgCl_2 concentrations (25). To determine whether H4 tail interarray interactions are also influenced by H1, linker histone was incorporated into 12-mer and 35-mer arrays before cross-linking in 50 mM NaCl as described previously (25) (Fig. 5A, lanes 7 to 12). Cross-linking experiments with no linker histone were performed in the presence of 50 mM NaCl as a control (Fig. 5A, lanes 1 to 6). In agreement with previous results (25), we observed a reduction of *H4-mediated interarray cross-linking in the presence of 50 mM NaCl alone, possibly due to competition between Na^+ and Mg^{2+} for binding to the chromatin (Fig. 5B). Nevertheless, association of the linker histone greatly stimulated H4 tail-mediated interarray cross-linking such that these long-range interactions were observed even in 2 mM MgCl_2 (Fig. 5A and B). However, the presence of tetraacetylated H4 in the array

FIG. 4. Effect of H4 acetylation on H4 tail-DNA interarray interactions. The 12-mer array was prepared with tetraacetylated *H4, and interarray cross-linking was analyzed as described in the legend to Fig. 1. (A) A scheme of H4 showing the four sites of acetylation in the tail domain. (B) A Triton-acid-urea gel showing H4 tetraacetylated and H3 monoacetylated by HAT Piccolo. Lane 1, nonacetylated H3/H4; lane 2, highly acetylated H3/H4; lane 3, moderately acetylated H3/H4 revealing various extents of H4 acetylation. (C and D) Analysis of interarray cross-linking mediated by control (squares) and tetraacetylated (diamonds) *H4 G2C-APB. Experimental conditions and data analysis were as described in the legend to Fig. 1.

greatly dampened the stimulation of interarray interactions by H1 (Fig. 5C), suggesting that acetylation overrides H1's stimulation of long-range tail interactions.

DISCUSSION

The H4 tail plays a primary role in promoting the formation of higher-order chromatin structures (13, 19). Here, we demonstrate that this domain mediates internucleosomal and interarray interactions during folding and self-association of model nucleosome arrays into higher-order chromatin structures via direct contacts with both DNA and protein targets (Fig. 6). We previously demonstrated that nearly all cross-links formed by the N-terminal region of the H3 tail domain in fully folded and condensed arrays were representative of internucleosomal contacts (48). While the fraction of H4 interactions represented by intra- versus internucleosome interactions in folded and condensed chromatin remains to be determined, it is interesting to note that both the H3 and H4 tail domains

FIG. 5. Acetylation overrides H1 enhancement of interarray interactions of the H4 tail domain. (A) Arrays incubated in the absence (lanes 1 to 6) or presence (lanes 7 to 12) of H1 were assayed for interarray H4 tail-DNA cross-linking. The cross-linking assay was carried out as described in the legend to Fig. 1 except that each sample also contained 50 mM NaCl to facilitate H1 binding. (B) Interarray cross-linking in the absence (circles) and presence (diamonds) of H1 was quantified and plotted as indicated. Quantification of cross-linking for arrays in buffer without NaCl (squares) is shown for reference. Panel C is as described in panel B except that arrays contained acetylated H4.

exhibit the same extent of interarray interactions in these fully condensed structures, with about 1/4 of tail interactions represented by bridging interactions between arrays (Fig. 1). Thus, our results indicate that the N-terminal ends of both the H3 and H4 tails facilitate folding and condensation of chromatin

FIG. 6. Summary of H3 and H4 tail interactions. Intra-array and interarray interactions with DNA and histones detected for two positions in either tail domain are as indicated.

by mediating long-range interactions between nucleosomes and arrays (Fig. 6).

We also uncovered an interesting difference between the H3 and H4 tail domains when we compared interactions of sites in the tails near the histone fold domains of these proteins. While the region near the histone fold domain in H3 appears to exclusively contact DNA of its own nucleosome, regardless of array conformation (25, 48), a similar region in the H4 tail domain exhibited a switch to partial interarray interactions to a level similar to that observed for the end of the H4 tail in fully condensed arrays when measured as a fraction of total cross-links (Fig. 2). This difference between the interior regions of the H3 and H4 tail could be due to the different paths by which these tail domains exit the nucleosome. While the H4 tail exits the nucleosome over/under the top/bottom DNA superhelical turns, the H3 tail follows a path between the DNA superhelical gyres to the exterior of the nucleosome (11, 22, 28). Therefore, if nucleosomes contact one another via their flat faces, as has been proposed (29, 34, 38, 42), the inner region of the H4 tail is in a much better position to make internucleosome contacts to DNA compared to the same region in the H3 tail.

Importantly, while we do detect interarray interactions between the interior of the H4 tail domain and DNA, the efficiency of cross-linking to DNA is significantly less than that detected from a site near the N-terminal end of the H4 tail (see above). Moreover, this region of the H4 tail domain (aa 16 to 25) has been shown to contact a pocket on the surface of H2A in crystals of nucleosome core particles and to form disulfide cross-links between nucleosomes with H2A in solution studies of nucleosome arrays containing specific cysteine substitution mutants of these proteins (13, 27). A 6-aa acidic patch (E56, E61, E64, D90, E91, and E92) on H2A forms multiple contacts with K16, R19, K20, and R23 on the H4 tail (11, 27), and H2A variants, which have a less-extensive acidic patch (e.g., H2A.Z and H2A.Bbd, respectively), have been shown to alter the propensity of nucleosome arrays to form higher-order chromatin structures, presumably through alterations in this H2A-H4 tail interaction (15, 49). Consistent with these observations, we find robust cross-linking between the interior region of the H4 tail and H2A, which increases significantly upon salt-dependent folding of the arrays (Fig. 3). Importantly, we find that cross-linking between H4 and H2A is competed by the LANA

peptide, previously shown to bind strongly to the H2A pocket and displace the H4 tail but significantly less so by a peptide of similar composition but scrambled sequence (Fig. 3D). Thus, our results are consistent with a model whereby interaction of the H4 tail with the H2A pocket stimulates intramolecular folding of nucleosome arrays, while interarray interactions with DNA facilitate self-association, perhaps in an exclusive manner (10, 13, 15, 33, 49) (Fig. 6). For example, some H4 tails may contact H2A to facilitate the formation of secondary chromatin structures, while others may participate in longer-range interarray contacts to DNA to mediate formation of tertiary structures. This model is supported by studies showing that LANA peptide-mediated displacement of H4 from the H2A pocket stimulates self-association of arrays, presumably because the H4 tail is more available to contact interarray DNA targets (10), and the observation that alterations in the interaction between H2As and the H4 tail appear to exert opposite effects on chromatin folding and array self-association (15, 49).

Interestingly, we also detected H4 tail-H2A interactions in low-salt (10 mM Tris) conditions where the nucleosome arrays exist in fully extended conformations, with little opportunity for internucleosomal contacts (13, 31). We considered the possibility that these interactions represented previously undocumented intranucleosomal interactions. Indeed, the existence of intranucleosomal H4 tail-H2A interactions is supported by our cross-linking experiments with mononucleosomes (Fig. 3). Such interactions may be relevant in regions where chromatin is transiently decondensed due to nucleosome eviction or post-translational modification (21). A recent study of the histone variant H2A.Bbd also suggested that the H4 tail might interact with the H2A pocket in an intranucleosomal manner (49). Nucleosome arrays containing H2A.Bbd exhibited an unusually extended conformation in low salt, suggesting that the DNA was partially unwrapped from the edges of the nucleosome, consistent with studies showing H2A.Bbd nucleosomes protect less than 147 bp of DNA from nuclease digestion (4, 14). Moreover, it was found that a restored acidic patch on the H2A.Bbd surface works together with the H4 tail to maintain an intact 29 S (normally extended) chromatin conformation in low salt in which the nucleosomes contain DNA that is fully wrapped around the histone octamers (49). These data, together with our cross-linking results, suggest that the H4 tail participates in intranucleosome contacts with the acidic patch to stabilize wrapping of DNA in the nucleosome. It is unclear whether this intranucleosome interaction competes with internucleosome interactions of the H4 tail facilitating formation of higher-order chromatin structures.

We also found that 12-mer arrays reconstituted with tetra-acetylated H4 showed a lower tendency to self-associate in high $MgCl_2$ concentrations, consistent with previous work (43). However, interarray interactions as detected by our cross-linking method did not appear to be reduced. Work by Hansen and colleagues has shown that self-association of arrays into tertiary structures occurs via a multistep process, with initial formation of soluble intermediates of ~ 500 S in lower $MgCl_2$, which then associate into complexes of thousands of S in higher $MgCl_2$, which can be rapidly sedimented in a microcentrifuge (21, 30). Our cross-linking results suggest that interarray interactions of the H4 tail domain occur during this first step and that the extent of these interactions is not appreciably

altered by acetylation of the tail domain. However, acetylation of the tail does appear to reduce the propensity of subsequent steps in the self-association process, such that higher levels of $MgCl_2$ are required to achieve the same extent of sedimentable material. Thus, our results are consistent with the idea that tertiary chromatin structure formation is a multistep process, with the H4 tail domain making long-range interarray contacts to DNA during an initial step of this process.

Acetylation does appear to have a profound effect on the ability of H1 to stimulate interarray interactions by both the H3 (25) and H4 tail domains (Fig. 5). H1 might either directly interact with the tail domains, redirecting them to interarray locations, or indirectly increase interarray contacts by stabilizing formation of higher-order chromatin structures. Importantly, H1 enhanced interarray interactions of both the H3 and H4 tail domains without altering the maximum extent of interarray contacts generated by these tails observed in the absence of H1. This result is consistent with the idea that H1 stabilizes but does not appreciably alter the final condensed structure (8, 9). Interestingly, acetylation of either the H3 or H4 tail domain appears to override this stimulatory effect of H1, suggesting that the effects of this posttranslational modification are dominant over linker histones in regulating the stability of higher-order chromatin structures. It will be interesting in future work to determine whether this effect is due to acetylation of specific lysine residues or requires a simple threshold level of acetylation within the tail domains.

In conclusion, our results underscore the complex and diverse interactions of the H4 tail domain and are consistent with the idea that the tails perform multiple independent functions in chromatin (21). We have detected intranucleosome interactions of the H4 tail with H2A, consistent with a role for the H4 tail in stabilizing wrapping of DNA around the nucleosome (3, 49) and internucleosome and interarray contacts with both H2A and DNA, consistent with a role of the H4 tail in stabilizing secondary and tertiary chromatin structures (10, 19, 49) (Fig. 6). Moreover, our results highlight the fact that individual histone tail domains participate in distinct sets of interactions. For example, a region near the histone fold domain in the H3 tail interacts exclusively with DNA in an intranucleosomal fashion, while an analogous region in the H4 tail interacts both intra- and internucleosomally with both DNA and an acidic pocket on the surface of H2A (Fig. 6). It will be interesting in future experiments to dissect the contributions of each of these interactions to various states of chromatin structure.

ACKNOWLEDGMENTS

We thank Song Tan for the Piccolo expression construct and Karolin Luger for the LANA and LRS peptides.

This work was supported by NIH grant GM52426.

REFERENCES

1. Allan, J., N. Harborne, D. C. Rau, and H. Gould. 1982. Participation of the core histone tails in the stabilization of the chromatin solenoid. *J. Cell Biol.* **93**:285–297.
2. Annunziato, A. A., L.-L. Y. Frado, R. L. Seale, and C. L. F. Woodcock. 1988. Treatment with sodium butyrate inhibits the complete condensation of interphase chromatin. *Chromosoma* **96**:132–138.
3. Ausio, J., F. Dong, and K. E. van Holde. 1989. Use of selectively trypsinized nucleosome core particles to analyze the role of the histone “tails” in the stabilization of the nucleosome. *J. Mol. Biol.* **206**:451–463.
4. Bao, Y., K. Konesky, Y. J. Park, S. Rosu, P. N. Dyer, D. Rangasamy, D. J. Tremethick, P. J. Laybourn, and K. Luger. 2004. Nucleosomes containing

- the histone variant H2A.Bbd organize only 118 base pairs of DNA. *EMBO J.* **23**:3314–3324.
5. Barski, A., S. Cuddapah, K. Cui, T. Y. Roh, D. E. Schones, Z. Wang, G. Wei, I. Chepelev, and K. Zhao. 2007. High-resolution profiling of histone methylations in the human genome. *Cell* **129**:823–837.
 6. Becker, P. B., and W. Horz. 2002. ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem.* **71**:247–273.
 7. Belmont, A. S., and K. Bruce. 1994. Visualization of G1 chromosomes: a folded, twisted, supercoiled chromonema model of interphase chromatid structure. *J. Cell Biol.* **127**:287–302.
 8. Carruthers, L. M., J. Bednar, C. L. Woodcock, and J. C. Hansen. 1998. Linker histones stabilize the intrinsic salt-dependent folding of nucleosomal arrays: mechanistic ramifications for higher-order chromatin folding. *Biochemistry* **37**:14776–14787.
 9. Carruthers, L. M., and J. C. Hansen. 2000. The core histone N termini function independently of linker histones during chromatin condensation. *J. Biol. Chem.* **275**:37285–37290.
 10. Chodaparambil, J. V., A. J. Barbera, X. Lu, K. M. Kaye, J. C. Hansen, and K. Luger. 2007. A charged and contoured surface on the nucleosome regulates chromatin compaction. *Nat. Struct. Mol. Biol.* **14**:1105–1107.
 11. Davey, C. A., D. F. Sargent, K. Luger, A. W. Maeder, and T. J. Richmond. 2002. Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 Å resolution. *J. Mol. Biol.* **319**:1097–1113.
 12. Dorigo, B., T. Schalch, K. Bystrycky, and T. J. Richmond. 2003. Chromatin fiber folding: requirement for the histone H4 N-terminal tail. *J. Mol. Biol.* **327**:85–96.
 13. Dorigo, B., T. Schalch, A. Kulangara, S. Duda, R. R. Schroeder, and T. J. Richmond. 2004. Nucleosome arrays reveal the two-start organization of the chromatin fiber. *Science* **306**:1571–1573.
 14. Doyen, C. M., F. Montel, T. Gautier, H. Menoni, C. Claudet, M. Delacour-Larose, D. Angelov, A. Hamiche, J. Bednar, C. Faivre-Moskalenko, P. Bouvet, and S. Dimitrov. 2006. Dissection of the unusual structural and functional properties of the variant H2A.Bbd nucleosome. *EMBO J.* **25**:4234–4244.
 15. Fan, J. Y., D. Rangasamy, K. Luger, and D. J. Tremethick. 2004. H2A.Z alters the nucleosome surface to promote HP1 α -mediated chromatin fiber folding. *Mol. Cell* **16**:655–661.
 16. Fletcher, T. M., and J. C. Hansen. 1995. Core histone tail domains mediate oligonucleosome folding and nucleosomal DNA organization through distinct molecular mechanisms. *J. Biol. Chem.* **270**:25359–25362.
 17. Garcia-Ramirez, M., F. Dong, and J. Ausio. 1992. Role of the histone “tails” in the folding of oligonucleosomes depleted of histone H1. *J. Biol. Chem.* **267**:19587–19595.
 18. Garcia-Ramirez, M., C. Rocchini, and J. Ausio. 1995. Modulation of chromatin folding by histone acetylation. *J. Biol. Chem.* **270**:17923–17928.
 19. Gordon, F., K. Luger, and J. C. Hansen. 2005. The core histone N-terminal tail domains function independently and additively during salt-dependent oligomerization of nucleosomal arrays. *J. Biol. Chem.* **280**:33701–33706.
 20. Grunstein, M. 1997. Histone acetylation in chromatin structure and transcription. *Nature* **389**:349–352.
 21. Hansen, J. C. 2002. Conformational dynamics of the chromatin fiber in solution: determinants, mechanisms, and functions. *Annu. Rev. Biophys. Biomol. Struct.* **31**:361–392.
 22. Harp, J. M., B. L. Hanson, D. E. Timm, and G. J. Bunick. 2000. Asymmetries in the nucleosome core particle at 2.5 Å resolution. *Acta Crystallogr. D* **56**:1513–1534.
 23. Hayes, J. J., and K. M. Lee. 1997. In vitro reconstitution and analysis of mononucleosomes containing defined DNAs and proteins. *Methods* **12**:2–9.
 24. Kan, P. Y., and J. J. Hayes. 2007. Detection of interactions between nucleosome arrays mediated by specific core histone tail domains. *Methods* **41**:278–285.
 25. Kan, P. Y., X. Lu, J. C. Hansen, and J. J. Hayes. 2007. The H3 tail domain participates in multiple interactions during folding and self-association of nucleosome arrays. *Mol. Cell. Biol.* **27**:2084–2091.
 26. Lee, K. M., and J. J. Hayes. 1998. Linker DNA and H1-dependent reorganization of histone-DNA interactions within the nucleosome. *Biochemistry* **37**:8622–8628.
 27. Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent, and T. J. Richmond. 1997. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389**:251–260.
 28. Luger, K., T. J. Rechsteiner, A. J. Flaus, M. M. Waye, and T. J. Richmond. 1997. Characterization of nucleosome core particles containing histone proteins made in bacteria. *J. Mol. Biol.* **272**:301–311.
 29. Robinson, P. J., L. Fairall, V. A. Huynh, and D. Rhodes. 2006. EM measurements define the dimensions of the “30-nm” chromatin fiber: evidence for a compact, interdigitated structure. *Proc. Natl. Acad. Sci. USA* **103**:6506–6511.
 30. Schwarz, P. M., A. Felthaus, T. M. Fletcher, and J. C. Hansen. 1996. Reversible oligonucleosome self-association: dependence on divalent cations and core histone tail domains. *Biochemistry* **35**:4009–4015.
 31. Schwarz, P. M., and J. C. Hansen. 1994. Formation and stability of higher order chromatin structures. Contributions of the histone octamer. *J. Biol. Chem.* **269**:16284–16289.
 32. Selleck, W., I. Fortin, D. Seriwattayawong, J. Cote, and S. Tan. 2005. The *Saccharomyces cerevisiae* Piccolo NuA4 histone acetyltransferase complex requires the enhancer of polycomb A domain and chromodomain to acetylate nucleosomes. *Mol. Cell. Biol.* **25**:5535–5542.
 33. Shogren-Knaak, M., H. Ishii, J. M. Sun, M. J. Pazin, J. R. Davie, and C. L. Peterson. 2006. Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science* **311**:844–847.
 34. Staynov, D. Z., and Y. G. Proykova. 2008. The sequentiality of nucleosomes in the 30 nm chromatin fibre. *FEBS J.* **275**:3761–3771.
 35. Strahl, B. D., and C. D. Allis. 2000. The language of covalent histone modifications. *Nature* **403**:41–45.
 36. Thiriet, C. 2004. Analysis of chromatin assembled in vivo using exogenous histones in *Physarum polycephalum*. *Methods* **33**:86–92.
 37. Thoma, F., T. Koller, and A. Klug. 1979. Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J. Cell Biol.* **83**:403–427.
 38. Tremethick, D. J. 2007. Higher-order structures of chromatin: the elusive 30 nm fiber. *Cell* **128**:651–654.
 39. Tse, C., and J. C. Hansen. 1997. Hybrid trypsinized nucleosomal arrays: identification of multiple functional roles of the H2A/H2B and H3/H4 N-termini in chromatin fiber compaction. *Biochemistry* **36**:11381–11388.
 40. Tse, C., T. Sera, A. P. Wolffe, and J. C. Hansen. 1998. Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. *Mol. Cell. Biol.* **18**:4629–4638.
 41. Tumber, T., G. Sudlow, and A. S. Belmont. 1999. Large-scale chromatin unfolding and remodeling induced by VP16 acidic activation domain. *J. Cell Biol.* **145**:1341–1354.
 42. van Holde, K. E. 1989. Chromatin. Springer Verlag, New York, NY.
 43. Wang, X., and J. J. Hayes. 2008. Acetylation mimics within individual core histone tail domains indicate distinct roles in regulating the stability of higher-order chromatin structure. *Mol. Cell. Biol.* **28**:227–236.
 44. Wang, X., and J. J. Hayes. 2007. Site-specific binding affinities within the H2B tail domain indicate specific effects of lysine acetylation. *J. Biol. Chem.* **282**:32867–32876.
 45. Woodcock, C. L., and S. Dimitrov. 2001. Higher-order structure of chromatin and chromosomes. *Curr. Opin. Genet. Dev.* **11**:130–135.
 46. Yang, Z., C. Zheng, C. Thiriet, and J. J. Hayes. 2005. The core histone N-terminal tail domains negatively regulate binding of transcription factor IIIA to a nucleosome containing a 5S RNA gene via a novel mechanism. *Mol. Cell. Biol.* **25**:241–249.
 47. Zheng, C., and J. J. Hayes. 2003. Intra- and inter-nucleosomal protein-DNA interactions of the core histone tail domains in a model system. *J. Biol. Chem.* **278**:24217–24224.
 48. Zheng, C., X. Lu, J. C. Hansen, and J. J. Hayes. 2005. Salt-dependent intra- and internucleosomal interactions of the H3 tail domain in a model oligonucleosomal array. *J. Biol. Chem.* **280**:33552–33557.
 49. Zhou, J., J. Y. Fan, D. Rangasamy, and D. J. Tremethick. 2007. The nucleosome surface regulates chromatin compaction and couples it with transcriptional repression. *Nat. Struct. Mol. Biol.* **14**:1070–1076.