A Distinct Switch in Interactions of the Histone H4 Tail Domain upon Salt-dependent Folding of Nucleosome Arrays*

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Background: The core histone tail domains are essential for formation of native chromatin structures, but interactions are poorly understood.

Results: H4 tail-DNA intra-nucleosome contacts are lost upon nucleosome array condensation, but intra-array, inter-nucleosome DNA contacts are not detected.

Conclusion: The multiple functions of the H4 tail require targeted distinct interactions within condensed chromatin.

Significance: These results illuminate how the H4 tail stabilizes higher-order chromatin structures.

The core histone tail domains mediate inter-nucleosomal interactions that direct folding and condensation of nucleosome arrays into higher-order chromatin structures. The histone H4 tail domain facilitates inter-array interactions by contacting both the H2A/H2B acidic patch and DNA of neighboring nucleosomes (1, 2). Likewise, H4 tail-H2A contacts stabilize array folding (3). However, whether the H4 tail domains stabilize array folding via inter-nucleosomal interactions with the DNA of neighboring nucleosomes remains unclear. We utilized defined oligonucleosome arrays containing a single specialized nucleosome with a photo-inducible cross-linker in the N terminus of the H4 tail to characterize these interactions. We observed that the H4 tail participates exclusively in intra-array interactions with DNA in unfolded arrays. These interactions are diminished during array folding, yet no inter-nucleosome, intra-array H4 tail-DNA contacts are observed in condensed chromatin. However, we document contacts between the N terminus of the H4 tail and H2A. Installation of acetylation mimics known to disrupt H4-H2A surface interactions did not increase observance of H4-DNA inter-nucleosomal interactions. These results suggest the multiple functions of the H4 tail require targeted distinct interactions within condensed chromatin.

The eukaryotic genome is assembled into extremely long strings of nucleosomes (nucleosome arrays), which are folded and condensed into higher-order chromatin structures. This folding is critical for the compaction of DNA within the nucleus as well as the regulation of gene expression and other nuclear processes. The inter-nucleosome interactions involved in folding and stabilization of chromatin higher-order structures are still relatively uncharacterized; however, elucidating these interactions is important for understanding how chromatin structure is regulated.

The core histone tail domains are essential for the folding of nucleosome arrays into secondary chromatin structures such as the 30-nm diameter chromatin fiber and for association of arrays into tertiary chromatin structures (4, 5). Removal of the histone tails by trypsin proteolysis eliminates the ability of oligonucleosomal arrays to form maximally folded structures in vitro, even at divalent ion concentrations well above those encountered in physiological conditions (6–8). Elimination of the H3/H4 tails has a greater impact on the stability of higher-order structures than removal of the H2A/H2B tails (9–11), indicating a hierarchy in the contributions to chromatin structures. Likewise, posttranslational modifications within the core histone tail domains regulate the stability of higher-order chromatin structures in conjunction with nuclear processes. For example, acetylation of specific lysine residues within each of the tails is typically associated with transcriptionally active regions of the genome (12, 13) and can lead to specific recruitment of trans-acting factors and other activities (14, 15). Importantly, acetylation also directly alters the ability of the core histone tail domains to mediate folding and condensation of chromatin. However, the precise interactions of the tail domains and how acetylation alters these interactions remains undefined.

Previous work from our laboratory and others indicates that the histone H3 tails interact primarily with the DNA of the nucleosome from which they project, forming intra-nucleosomal interactions, when model nucleosome arrays exist as extended “beads-on-a-string” structures (16, 17). Such structures are thought to model unfolded chromatin fibers, as would be transiently found in the vicinity of active promoters in vivo. Importantly, the H3 tails rearrange to form primarily inter-nucleosomal interactions with the DNA of neighboring nucleosomes when model nucleosome arrays are condensed in the presence of multivalent cations, such as Mg2+ (17). Moreover, a fraction of these inter-nucleosome contacts were found to consist of long range inter-array interactions. Such interactions facilitate self-association of model nucleosome arrays into large 400–600-nm diameter tertiary chromatin structures in vitro (7).

Compared with the H3 tail domain, inter-nucleosome interactions of the H4 tail in condensed chromatin are not as well characterized. As originally observed in an x-ray crystal structure of a nucleosome core (18) and later in solution assays (1, 3), a region within the H4 tail contacts a protein surface of closely
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The H4 tail interacts with DNA in condensed chromatin. Cross-linking studies show the H4 tail contacts DNA within extended arrays (1). However, H4 tail-DNA contacts are significantly diminished upon chromatin folding/concentration, in contrast to the H3 tail (1). A portion of the H4 tail contacts to DNA in condensed chromatin are comprised of inter-array interactions, presumably mediating array self-association (1). However, the extent to which the remaining intra-array contacts are comprised of intra-nucleosome versus inter-nucleosome interactions remains undefined.

To address this question, we developed a novel method to quantify intra- and inter-nucleosome interactions of the histone H4 tail domain. We find that the histone H4 tail domain contacts DNA in an exclusively intra-nucleosomal manner when the nucleosome arrays exist as extended fibers. Surprisingly, our data indicate that the H4 tail domain does not participate in inter-nucleosomal, intra-array interactions with DNA in folded, self-associated arrays. Installation of acetylation mimics within the histone H4 tail did not result in detectable inter-nucleosomal interactions with DNA. These data are consistent with a model in which the histone H4 tail rearranges from intra-nucleosomal interactions with DNA in extended arrays to primarily inter-nucleosomal interactions with the H2A/H2B acidic pocket and inter-array interactions with both DNA and the acidic pocket in highly condensed chromatin structures.

EXPERIMENTAL PROCEDURES

Expression and Purification of Histone Proteins—Coding sequences for *Xenopus borealis* H3 containing a cysteine to alanine substitution at position 110 (H3C110A) and H4 harboring a glycine to cysteine substitution at position 2 (H4G2C) were subcloned into pET expression plasmids (Stratagene), and proteins were expressed in *Escherichia coli* containing the expression plasmids for H4G2C, H4G2CK16Q (containing an additional single lysine → glutamine substitution at residue 16), and H4G2CKQ-4 (containing four lysine → glutamine substitutions at positions 5, 8, 12, and 16), which were grown to an optical density of 0.6 at 600 nm. Expression of H4G2C was induced with isopropyl-β-D-thiogalactopyranoside. Twenty minutes after induction, 2.5 ml (2.5 mCi) [3H]lysine was added to the culture, and the cells were grown for an additional 4 h. The [3H]H4G2C was purified from cells and dialyzed into tetramer as described above.

The specific activity of [3H]H4G2C was determined by excising the H4 band from a Coomassie stained 18% SDS-PAGE gel, crushing the excised slice in a microfuge tube with a plastic pestle and suspension in scintillation fluid (Beckman Coulter). A region of the gel with no band and an unlabeled H4 band were also recovered to determine background. After an overnight incubation to eliminate mechanical scintillation, the samples were subjected to scintillation counting for [3H] detection (20 min per vial) in an LS 6000 C scintillation counter (Beckman Coulter).

APB Modification—Purified and reduced H3/H4G2C tetraters were modified with a 5-fold molar excess of APB (Sigma-Aldrich) at 25 °C for 45 min. Reactions were terminated by freezing on dry ice. The presence of a viable APB modification was confirmed by UV irradiation of the modified tetramer on a VNR LM-20E light box in a 15 ml Pyrex 9820 glass tubes at 365 nm for 2 min, which yields a characteristic H4-H4 cross-linked band on a Coomassie-stained 18% SDS-PAGE gel (data not shown). All reactions were carried out, and modified proteins were kept in the dark.

Construction, Preparation, and Radiolabeling of DNA Templates—A 204-base pair (bp) "N1" DNA fragment containing a nucleosome positioning sequence from a *Xenopus borealis* 5 S rRNA gene, was prepared from the pBSXN1 plasmid as...
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described previously (23). A 2.5-kb DNA fragment containing 12 tandem repeats of a *Lytechinus variegatus* 5 S rRNA gene was prepared from the p12-5S-C1 plasmid as described previously (24) except the Klenow/fill-in step was omitted, and the vector sequence was also cleaved with FspI before gel isolation. Briefly, the plasmid was cleaved with BamHI, followed by treatment with phosphatase to block ligation at this site. The reaction was stopped by addition of 1/100 volume of 10% SDS and extracted twice with phenol:chloroform (1:1, EMD Millipore) followed by ethanol precipitation. The DNA was digested with FspI and AlwNI (New England Biolabs) to cleave the remainder of the plasmid into shorter fragments, easily resolved from the 12-mer template, followed by ethanol precipitation. Finally, the 12-mer DNA fragment was released by digestion with DraIII and purified by electrophoresis and recovery from preparative 0.7% agarose gels. The 2.5-kb 12-mer DNA template containing the complimentary asymmetric DraIII DNA overhang and dephosphorylated BamHI end and the 204-bp N1 fragment were resuspended in TE and stored at −20°C. Both fragments contained one blunt end and one complimentary asymmetric DraIII DNA overhang for unique ligation (17). For inter-nucleosome cross-linking experiments, an internal radiolabel was added to the 12-mer DNA template by first cleaving the p12-5S-C1 plasmid with BglII (New England Biolabs) at a single site 13 bp downstream from the DraIII site, treating with calf intestinal phosphatase (New England Biolabs), extraction with phenol:chloroform (1:1), and ethanol precipitation. The digested and dephosphorylated plasmid (200 µg) was incubated with 5 µl (50 units) T4 phosphonucleotide kinase (New England Biolabs) and 10 µl (1.5 mCi, high concentration) [γ-^32P]ATP in 1× T4 phosphonucleotide kinase buffer in a 200-µl final volume at 37°C for 30 min. One µl of 10 mM ATP was then added, and the reaction was incubated for another 15 min. The labeled plasmid was precipitated with ethanol, resuspended in 200 µl of TE, and the plasmid recircularized by incubation with T4 DNA ligase (6000 units) (New England Biolabs) in 1 ml of 1× ligase buffer at room temperature overnight. The DNA was precipitated with ethanol and resuspended in 200 µl of TE. The 12-mer template containing an internal radiolabel was isolated as described above.

A second mononucleosome DNA template was prepared by PCR amplification of the 601 positioning sequence using the primer (GGCCGCAAGCTTCCGGGCCCCGGCCACAGGT- GCCG) containing an XbaI and DraIII recognition sequence and the primer (CGCCCGTGCTAGAGCGGGCCCGGCAC- GAGGTTGAT) containing a DraIII plus HindIII recognition sequence to generate a 255-bp fragment. The amplified fragment was digested with XbaI and HindIII to release a 241-bp monosome “DC” (for DraIII Center) template with asymmetric DraIII sites at each end. The fragment was purified by preparative agarose gel electrophoresis, ligated into a PBS sk(+) plasmid, and amplified in DH5α cells. Typically, 200 µg of the DC insert was prepared from 10 mg of the plasmid by digestion with DraIII to generate a 204-bp DC DNA fragment with an asymmetric overlap at each end, allowing ligation to two 12-mer oligonucleosome arrays with complementary ends.

Preparation and Analysis of Mononucleosomes, 13 Nucleosome (13-mer) Arrays, and 25 Nucleosome (25-mer) Arrays—Reconstitution of N1 and DC mononucleosomes containing APB-modified H4G2C and 12-mer arrays was carried out by serial salt dialysis as described previously (1, 17). DTT was omitted from reconstitutions with APB-modified proteins. Reconstituted mononucleosomes were purified over 7–20% sucrose gradients. Samples from fractions were exposed to UV light as described above to check for viable H4-DNA cross-linking. Labeled 12-mer templates were diluted with a 30-fold excess of unlabeled 12-mer template before reconstitution. All procedures with APB-modified proteins were performed under low light conditions. MgCl2 self-association assays were performed on all reconstituted arrays to ensure saturation (25).

Ligation of N1 mononucleosomes to 12-mer arrays to form 13-mer arrays was carried out with a 2-fold molar excess of mononucleosomes. Typically, 80 ng of purified N1 nucleosomes, 415-ng 12-mer arrays, and 1 µl (400 units) of T4 DNA ligase were combined in ligation buffer lacking DTT (0.2 µg BSA, 1 mM ATP, 10 mM MgCl2, 0.25 mM EDTA, and 10 mM Tris, pH 8.0) in a final volume of 50 µl. Twenty of these reactions were set up and allowed to incubate in the dark at room temperature for 4 h. Typically, 75–100% of the 12-mer array was ligated into a 13-mer array. Two YM-50 filters (Microcon) were placed in the provided 1.5-ml microfuge tubes and washed with 400 µl of TEN buffer (10 mM Tris, 0.25 mM EDTA, 2.5 mM NaCl) by centrifugation at 3,000 × g for 15 min in a microfuge. The 20 ligation reactions were combined, and 500 µl was placed in each washed filter, and the samples were centrifuged at 3,000 × g for 15 min. The eluate was discarded, and the concentrated samples (~20 µl) were diluted in the filter tubes with 400 µl of TEN buffer. The centrifugation and dilution was repeated two additional times. After the final centrifugation, the filters containing the sample were placed inverted into clean Eppendorf tubes and centrifuged at 1,000 × g for 7 min to collect ~50 µl of the concentrated 13-mer arrays in TEN buffer. The collected sample was diluted to 250 µl in TEN buffer. Typically, 70–80% of ligated arrays were recovered. All procedures were performed under low light conditions.

Ligation of DC mononucleosomes to 12-mer arrays to form 25-mer arrays was carried out in a similar manner. The ligation reactions typically contained 20 ng of gradient-purified DC nucleosomes, 500 ng of 12-mer arrays, and 1 µl (400 units) of T4 DNA ligase in ligation buffer lacking DTT in a final volume of 50 µl. Buffer exchange and array concentration was performed as described above. MgCl2 self-association assays were performed on ligated arrays to ensure saturation persisted through buffer exchange.

Cross-linking Reactions—Typically 8 µl (~0.3 µg) of 13-mer arrays containing 3H-labeled H4G2C-APB within the N1 nucleosome were mixed with an equal volume of buffer containing MgCl2 to generate final concentrations indicated in the figure legends and incubated at 25°C for 10 min. The samples were irradiated for 0.5, 1, or 2 min on a VMR LM-20E light box in 15-ml Pyrex 9820 glass tubes to induce cross-linking. Samples were digested post-irradiation where indicated with either 10 units of HhaI, BglIII, or DraIII in 20 µl of final volume for 2 h at 37°C, and digestion was terminated by the addition of SDS. Total cross-linking was analyzed by running samples on a 0.7% SDS-agarose gel. The gels were soaked in 45% methanol and...
10% acetic acid, followed by 1 m sodium salicylate for 15 min, and then the gels were dried and exposed to Kodak BioMax MS film for 3 months at −80 °C. Cross-linking was also determined by ethidium staining the gels and exciting DNA bands with a razor blade along with equal-sized sections from the rest of the lane. The gel pieces were gel crushed and suspended in 700 μl of scintillation fluid (Beckman Coulter). The samples were allowed to stand overnight, and 3H content was determined by scintillation counting as described above.

Arrays containing an internal 32P label were incubated in the presence or absence of MgCl2, irradiated to induce cross-linking, and digested with BglII (New England Biolabs) as described above. The samples were run on 0.7% SDS-agarose gels, and the gels were dried and analyzed by phosphorimaging (GE Healthcare). To identify inter-nucleosomal cross-linking after UV exposure, the samples were digested with 1 μl (20 μl) of EcoRI (New England Biolabs), with half of the samples undergoing additional cleavage with 20 units of DraIII, in appropriate reaction buffer. Samples were treated with native gel loading solution containing 1% SDS and run on a 7% SDS-agarose gel, and products were analyzed by ethidium bromide staining and a phosphorimaging device. Lane profile analysis was conducted using the volume tools in ImageQuant.

**RESULTS**

The H4 tail domain contacts an acidic patch comprised of histones H2A and H2B on the surface of neighboring nucleosomes to stabilize folding of nucleosome arrays into secondary chromatin structures (3). In addition, the H4 tail participates in long range inter-array contacts to DNA and the acidic patch to promote formation of higher-order tertiary chromatin structures (1, 19). We hypothesized that the H4 tail also participates in localized inter-nucleosome, intra-array interactions with the DNA of neighboring nucleosomes to direct folding of the chromatin fiber and other secondary chromatin structures, similar to those documented for the histone H3 N-terminal tail domain (17). To test this hypothesis, we created a mononucleosome (N1) that contained an H4 site-specific modification at residue 2 with the photo-inducible cross-linker 4-azidophenacyl bromide (H4G2C-APB) (Fig. 1A). Upon brief UV illumination, this moiety generates cross-links to any DNA or protein in the immediate vicinity of the modified residue. The N1 nucleosomes were ligated to 12-mer nucleosome arrays containing native histones to create 13-mer oligonucleosome arrays (Fig. 1B). The ligated arrays undergo MgCl2 induced self-association between 2 and 4 mM (Fig. 1C, top), indicating nucleosome saturation. In addition, EcoRI digestion liberates individual nucleosomes from the array and little free DNA (data not shown). Note that in our model system inter-nucleosome interactions by a tail domain can include both short-range intra-array and longer-range inter-array contacts, with the former likely important for array folding, whereas the latter mediates array self-association. Importantly, in these experiments, H4 tail DNA inter-array cross-linking is not observed due to the presence of an excess of unlabeled nucleosome arrays.

The 13-mer arrays were incubated in low-salt solutions to ensure the arrays were in an extended conformation and then exposed to UV light. Cross-linking products were analyzed on SDS-agarose gels, which dissociate all non-cross-linked proteins from the 13-mer DNA. To analyze cross-linked products, we first employed 3H-labeled H4-APB in the experiment, and...
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found that upon UV irradiation, radiolabeled histone H4 co-migrates with the 13-mer DNA, indicating that the H4 tail domains within N1 are in contact with the array DNA template (Fig. 2A). Additionally, non-cross-linked free $[^{3}H]H4$ was detected as a faster migrating species, distinct from the 13-mer and 12-mer DNA bands (Fig. 2A). To further characterize cross-linking by the H4 tail domain, we next inserted the $^{32}P$ radiolabel into the 12-mer DNA template at the DraIII site before reconstitution and ligation to the N1 template (Fig. 2B, top, red star). Note that the HhaI site at the end of the array is also radiolabeled in this experiment, which allows for the ligation of arrays to form a 26-mer in addition to the 13-mer. Upon UV exposure of the 13-mer and 26-mer nucleosome arrays, a shift in both templates is observed on the SDS-agarose gel, indicating H4-tail cross-linking (Fig. 2B, compare lanes 1 and 2). Cleavage at either HhaI or BglII restriction sites downstream of the labeled DraIII site releases the N1 nucleosome DNA along with the radiolabel (Fig. 2B, lane 3). H4-N1 DNA cross-linking within the extended arrays was clearly evident by observation of a UV-dependent shift in the released N1 DNA band (Fig. 2B, bottom, compare lanes 3 and 6 with lanes 4 and 7, respectively). However, little or no UV-dependent shift in the position of the residual 12-mer or 24-mer templates is evident in the gel. These results suggest that the H4 tail cross-links primarily to N1 DNA within the extended 13-mer array.

We next determined whether H4 tail-DNA interactions are altered by salt-dependent folding of the 13-mer arrays. Incubation of the nucleosome arrays in 8 mM MgCl$_2$ forms immitation of condensed chromatin fibers and higher-order structures (10). In these experiments, arrays containing radiolabel were mixed with an excess of unlabeled arrays to eliminate observable cross-links due to inter-array cross-linking by the H4 tail domain. The condensed arrays were irradiated then digested as described above to liberate the N1 nucleosome. MgCl$_2$-induced folding of the nucleosome arrays caused a decrease in the extent of H4-N1 DNA intra-nucleosome cross-linking, from 46% of cross-linked N1 DNA to 16% (HhaI digestion) (Fig. 2B, lanes 4 and 5). Similar results were obtained when BglII was used to separate the N1 nucleosome from the 12-mer array (Fig. 2B, lanes 7 and 8). These results suggest that the H4 tails form intra-nucleosomal interactions with DNA in extended arrays and that these interactions are diminished upon folding and condensation of the array. In the case of the H3 tail domain, a similar reduction in intra-nucleosome interactions is accompanied by an increase in H3 tail DNA inter-nucleosome contacts (24). However, in the MgCl$_2$-condensed arrays, a UV-dependent shift in the position of the remaining 12-mer template (HhaI) or 24-mer template (BglII) was not apparent on the gel. However, as cross-linking-dependent shifts in the 12-mer template band may be difficult to visualize in this experiment, we employed a modified approach to more accurately detect potential inter-nucleosomal H4-DNA interactions.

To better reveal and map potential intra-array interactions, we internally labeled the array template at the BglII site, so that a radiolabel remains within the 12-mer DNA after N1 removal by DraIII cleavage (Fig. 3A). In addition, to observe the position within the array of any inter-nucleosome cross-linking, we took advantage of the EcoRI restriction sites between every nucleosome within the 13-mer array. Because EcoRI digestion was carried out on the chromatin, where a fraction of the sites are blocked by positioned nucleosomes, a ladder of DNA products is generated (Fig. 3B). In the absence of DraIII cleavage, the smallest labeled DNA band corresponds to the free N1 DNA,
followed by a fragment consisting of the N1 DNA + the DNA of the first nucleosome of the array (A1), with bands of higher molecular weight containing increasing numbers of successive repeats from the array (Fig. 3B). Under these conditions, both intra- and inter-nucleosome cross-links will result in a shift in position of the labeled DNA fragments on the gel. Additional digestion with DralII removes the N1 template from all fragments while leaving the label with the array nucleosomes. Thus, only inter-nucleosome, intra-array cross-links would result in a shifted band on the gel after DralII cleavage. The appearance of a cross-linked N1 DNA band indicates the histone H4 tails participate in intra-nucleosomal contacts to DNA within the extended array (Fig. 3B, lane 2). Importantly, although 46% of N1 DNA is cross-linked in extended arrays, cross-linking is diminished to 16% of N1 DNA after MgCl₂-dependent folding of the 13-mer array (Fig. 3B, compare lanes 2 and 4), reaffirming that a majority of H4 tail intra-nucleosomal interactions are lost within condensed chromatin. Importantly, upon DralII digestion and removal of the N1 nucleosome from all labeled DNA fragments, little to no cross-linked H4-DNA bands are observed in condensed arrays (Fig. 3B, compare lanes 4 and 8). This indicates that the cross-links observed in lane 4 are largely intra-nucleosomal and few, if any, inter-nucleosome, intra-array H4 tail-DNA contacts are detectable in the condensed array.

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To eliminate potential artifacts due to the location of the N1 nucleosome at the end of the 13-mer array, we next placed the nucleosome containing the APB-modified H4 at the center of a 25-nucleosome array. We generated a mononucleosome (DC), similar to N1 but with asymmetric DralII sites located on both sides of the 601-nucleosome positioning sequence, allowing ligation between two 12-mer arrays (Fig. 4A). After digestion with EcoRI, a ladder of products is observed as with the 13-mer arrays; however, the appearance of multiple bands between the main ladder of products is due to an increased number of potential cleavage products from the 25-mer array as compared with the 13-mer array (Fig. 4B). As observed with the 13-mer arrays, intra-nucleosomal H4-DNA cross-links to the single DC nucleosome are observed after UV exposure when the 25-mer arrays are in an extended conformation, and cross-links are diminished as the chromatin is condensed with MgCl₂ (Fig. 4, B, lanes 1–5, and C). Importantly, after DralII excision of the DC nucleosome, no H4-DNA inter-nucleosomal cross-linked bands are observed within the 25-mer arrays (Fig. 4B, lanes 6–10), indicating that the position of the nucleosome has no effect on the H4 N-terminal tail interactions.

Our cross-linking data indicate that the H4 tail rearranges from intra-nucleosome contacts to DNA in extended chromatin to largely non-DNA interactions in the condensed array. It is known that the H4 tail contacts an acidic patch on the surface of H2A/H2B of neighboring nucleosomes in salt-condensed nucleosome arrays (1, 18, 26). It is possible that H4 tail interactions with the H2A acidic pocket preclude inter-nucleosome interactions with DNA. To test this, we took advantage of previous work demonstrating that acetylation of the H4 tail domain abrogates interactions with the acidic pocket and incorporated histone H4 tail mutants with lysine → glutamine substitutions at either residue 16 (H4G2CK16Q) or at residues 5, 8, 12, and 16 (H4G2CKQ-4) into the DC nucleosomes. Glutamine has been shown to at least partially mimic the effect of lysine acetylation within the H4 tail domain on salt-dependent folding and condensation of nucleosome arrays (1, 25). The 25-mer arrays containing these DC nucleosomes were subjected to the same folding, UV exposure, and restriction enzyme digestions described previously. However, neither the arrays containing the H4G2CK16Q-DC nucleosome nor the H4G2CKQ-4-DC nucleosome (Fig. 5, A and B, respectively) showed any increase in inter-nucleosomal H4-DNA interactions, suggesting that disruption of any H4-protein inter-nucleosomal interactions does not result in increased inter-nucleosome contacts to DNA by the N terminus of the H4 tail domain. Notably, a decrease in intra-nucleosomal interactions for H4G2CKQ-4-DC mutants within the extended array was observed, suggesting that the acetylation mimics in these positions may disrupt H4-DNA interactions overall, leading to the lack of observable H4-DNA inter-nucleosomal interactions.
The region within H4 shown to interact with the H2A/H2B acidic patch includes residues 16–25 within the tail domain (18), whereas in our experiments, the cross-linker is attached to residue 2. This raises the possibility that regions of the H4 tail other than residues 16–25 might also interact with the acidic patch or other sites on the protein surface of the nucleosome and supersede tail-DNA interactions. To determine whether such interactions occur, we prepared arrays containing H4G2C-APB and fluorescein-labeled H2A and examined potential cross-linking by fluorography. Interestingly, we observed cross-linking between APB-modified residue 2 within the H4 tail domain and H2A (Fig. 6) in a manner similar to that previously observed with arrays containing H4V16C-APB (1), suggesting that a region of the H4 tail nearby or including residue 2 interacts with the H2A acidic pocket in condensed chromatin.

**DISCUSSION**

The histone H4 tail plays several important roles in the formation and stabilization of higher-order chromatin structures. Our data support the idea that there are independent and distinct mechanisms by which the H4 tail, and indeed other tail domains, mediate array folding and array oligomerization (27). Similar to the H3 tail, we find that the H4 tail exclusively participates in intra-nucleosome contacts to DNA within extended, unfolded arrays. However, in contrast to the H3 tail domain, the H4 tail participates in few, if any, inter-nucleosome, intra-array contacts to DNA to stabilize array folding, consistent with significant H4 tail-H2A interactions in fully folded and self-associated nucleosome arrays (3, 26). Thus, the
H3 and H4 N-terminal tails are involved in precisely defined but distinct interactions to direct array folding into secondary structures and to stabilize formation of tertiary chromatin structures.

We find that the H4 tail contacts DNA in an intra-nucleosomal fashion for both the end-positioned N1 and center-positioned DC nucleosomes when the oligonucleosome arrays are in an extended structure in a low salt environment. As arrays undergo folding and self-association by raising the MgCl2 concentration, we observe a diminution in intra-nucleosomal interactions, but not a concomitant appearance of H4-DNA inter-nucleosomal interactions. Previous work showed that ~25% of the H4 tail contacts to DNA in MgCl2-condensed arrays consist of intra-array, H4-DNA interactions, whereas 75% are inter-array (1). Moreover, this work also showed that salt-dependent folding and condensation of arrays results in an ~70% reduction in total contacts to DNA by the H4 tail (1). Given that the H4 tail does not make inter-nucleosome, intra-array contacts to DNA, our data imply that the intra-array H4 tail-DNA contacts documented previously (1) were nearly all intra-nucleosomal, likely resembling the interactions observed in fully extended beads-on-a-string arrays in low salt solutions. The data is consistent with a model whereby the H4 tail N terminus rearranges from primarily intra-nucleosome interactions with DNA in extended structures to a complicated set of interactions in fully condensed chromatin including 1) intra-nucleosome contacts to DNA (Ref. 1 and this work), 2) inter-nucleosome, intra-array contacts to DNA in the 30-nm fiber structure (Refs. 18 and 26 and this work), and 3) inter-nucleosome, inter-array contacts to DNA in self-associated oligomers (Fig. 7) (1). In contrast, the H3 tail domain undergoes a transition from primarily intra-nucleosome to exclusively inter-nucleosome interactions with DNA upon array folding and condensation (24).

Our data also suggest that a region within the H4 tail other than the residues 16–25 can interact with the acidic patch on the surface of H2A/H2B. Our data show that the very N terminus of the H4 tail does not participate in inter-nucleosome, intra-array contacts to DNA in condensed arrays, in contrast to contacts made by the H3 tail domain. Moreover, we find cross-linking to H2A when the photoactivated cross-linker is attached to residue 2 with the H4 tail (Fig. 6), in a manner similar to that observed when the cross-linker was placed at residue 21 (1). A primary feature of interaction of several proteins with the acidic surface of H2A involves insertion of an arginine side chain into a pocket in this region (28). It is therefore possible that the N terminus of the H4 tails participates in similar interactions with the H2A/H2B acidic pocket, perhaps facilitated by insertion of Arg-3 within the H4 tail into the pocket. In addition, alternative H4 tail-protein interactions are possible, as others have noted that the H4 tail domain might interact with other tails, including other H4 tails (26, 29).

Given the apparent propensity for the H4 tail to bind the H2A/H2B acidic patch, we tested whether mimics of acetylated lysine would abrogate H4 tail-patch interactions and lead to detectable H4 tail-DNA inter-nucleosomal cross-linking. Previous work has shown that acetylation of H4K16 reduces interactions with the acidic patch and inhibits salt-dependent condensation of arrays (19, 30). However, we found that installation of (H4G2CK16Q) and tetra-(H4G2CKQ-4) acetylation mimics did not increase H4-DNA inter-nucleosomal interactions within the folded 25-mer array. It is possible that the mimics interfere with H4 tail-H2A/H2B acidic pocket interactions and are altered in a way that diminishes folding and self-association (3, 19) but that does not eliminate such interactions. Alternatively, it is possible that potential H4-DNA inter-nucleosome interactions themselves are disrupted by these modifications, as suggested by the observation of a decrease in the intra-nucleosomal interactions for the H4G2CK16Q and tetra-(H4G2CKQ-4) acetylation mimics compared to the acetylated wild type (1).

We note that it is formally possible that inter-nucleosome interactions of the H4 tail are present but not captured by our cross-linking approach. However, we feel this is unlikely as the H4 tail domain readily cross-links at several positions within mononucleosomes,3 within nucleosomes in extended chromatin.

3 K. J. Murphy and J. J. Hayes, unpublished data.

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**FIGURE 6.** N terminus of the H4 tail domain contacts H2A in condensed nucleosome arrays. 12-mer nucleosome arrays containing H4G2C-APB and H2A/G2C-fluorescein were incubated in the absence or presence of MgCl2, as indicated, and cross-linked with UV light as described in Ref. 1. Samples were analyzed by SDS-PAGE, followed by fluorography and Coomassie staining. A small amount of a fluorescent cross-linking product is observed in the absence of UV irradiation (asterisk).
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tin fibers (this work), and to DNA of neighboring arrays in self-associated structures (1). Moreover, an identical modification in the H3 tail results in observable inter-nucleosome cross-linking (17). Additionally, considering the high sensitivity of the cross-link detection methods used here, these observations suggest any H4 tail-DNA interactions are likely to be detected. We also note that cross-links are likely to represent the appropriate proportion of molecular interactions as the lifetime of the UV-generated nitrene biradical is on the order of nanoseconds, far shorter than the rate of molecular rearrangements (31).

The idea that there are independent and distinct mechanisms by which the H3 and H4 tails facilitate array folding versus array oligomerization is supported by work in the field. Arrays containing hybrid nucleosomes in which the H3/H4 histone tails are lacking are unable to form the fully folded 30-nm fiber, at best achieving a contacting zigzag structure in the highest MgCl2 concentrations possible before array oligomerization (9). These arrays, however, are still able to self-associate, albeit at a higher divalent cation concentration than necessary to drive self-association for wild type arrays. The H4 tail is most important in driving self-association reactions, as experiments utilizing oligonucleosome arrays in which the histone tails have been removed individually show that the arrays most resistant to oligomerization are those lacking H4 tail domains (11). Moreover, cysteine cross-linking studies indicate an inter-array interaction occurs between a position on the H4 tail and the H2A/H2B acidic patch in folded and self-associated arrays (26). However, when H4V21C is cross-linked in an intra-array fashion to H2AE64C, the propensity of the arrays to undergo MgCl2-dependent self-association is greatly reduced. This suggests that individual H4 tail domains participate in either intra-array or inter-array interactions. Collectively, these results demonstrate a need for the histone H4 tails in both folding and self-association and fits with our data indicating that the H4 tail likely mediates folding and self-association through multiple interactions that change as the chromatin folds.

It should also be noted that in this work, we investigated H4 contacts in fully expanded and fully condensed model chromatin structures. In fully condensed structures, either intra- and inter-array functions of the tails could take precedence. For example, the H4 tail may participate in intra-array, inter-nucleosome interactions to stabilize array folding in the absence of inter-array interactions. This may occur at intermediate levels of condensation or under conditions where higher-order array-array interactions are suppressed. Indeed, evidence exists for competition folding and self-association promoting interactions/functions of the H4 tail domain (32).

In summary, our data shed light on not only how the H4 tail domains interact with DNA arrays throughout folding and oligomerization, but also where these interactions take place. Taken together with prior work, these results indicate that the N terminus of the H4 tail participates in a wide array of interactions in condensed chromatin, including 1) intra-nucleosome contacts to DNA to stabilize wrapping of DNA within the nucleosome (33), 2) inter-nucleosome contacts to the H2A acidic pocket (3, 19) (and lack of inter-nucleosome contacts to DNA, as shown in this work) to stabilize folding of the chromatin fiber, and 3) inter-nucleosome contacts to DNA (1) and protein (26) to stabilize array-array self-association to form higher-order chromatin structures. These interactions reflect the contributions of the histone H4 tail to either folding or oligomerization and help explain how folding and oligomerization can occur independently.

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