Intra- and Inter-nucleosomal Protein-DNA Interactions of the Core Histone Tail Domains in a Model System

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Summary:

The core histone tail domains are key regulators of eukaryotic chromatin structure and function and alterations in the tail-directed folding of chromatin fibers and higher order structures are the probable outcome of much of the post-translational modifications occurring in these domains. The functions of the tail domains are likely to involve complex intra- and inter-nucleosomal histone-DNA interactions, yet little is known about either the structures or interactions of these domains. Here we introduce a method for examining inter-nucleosome interactions of the tail domains in a model dinucleosome and we determine the propensity of each of the four N-terminal tail domains to mediate such interactions in this system. Using a strong nucleosome ‘positioning’ sequence, we reconstituted a nucleosome containing a single histone site-specifically modified with a photoinducible crosslinker within the histone tail domain, and a second nucleosome containing a radio-labeled DNA template. These two nucleosomes were then ligated together and crosslinking induced by brief UV irradiation under various solution conditions. After crosslinking, the two templates were again separated so that crosslinking representing inter-nucleosomal histone-DNA interactions could be unambiguously distinguished from intra-nucleosomal crosslinks. Our results show that the N-terminal tails of H2A and H2B, but not of H3 and H4, make internucleosomal histone-DNA interactions within the dinucleosome. The relative extent of intra- to inter-nucleosome interactions was not strongly dependent on ionic strength. Additionally, we find that binding of a linker histone to the dinucleosome increased the association of the H3 and H4 tails with the linker DNA region.
Introduction

Within the basic repeating subunit of eukaryotic chromatin known as the nucleosome, the nucleosome core is comprised of two copies of each of the core histones H2A, H2B, H3 and H4, and 147 bps of DNA. Approximately 75% of the core histone protein mass is organized into a ‘spool’ onto which the nucleosome core DNA is tightly wrapped (1,2). This spool is formed primarily by the histone fold motif within each protein and additional structural elements adjacent to these domains (1,3). The structural details of these domains and their interactions with DNA have been well described (2-7). Each nucleosome also includes a stretch of linker DNA, which joins nucleosome cores together in native chromatin, and, in most cases, a single linker histone (8). Arrays of nucleosomes are compacted into chromatin fibers of about 30 nm in diameter; these fibers are further condensed into poorly defined higher order structures (9,10).

About 25% of the total mass of the core histones is contained within the N-terminal tail domains, which were initially defined by their sensitivity to proteases (8,11). These domains are highly basic and bind DNA in vitro and in native chromatin (12-15). In addition, evidence suggests that these domains also participate in protein-protein interactions between histones in the fully condensed chromatin fiber (3,7,16). Despite the perception of the tails as being ‘unstructured’, biochemical and biophysical studies indicate they adopt defined structures and make specific interactions in chromatin (17-20). The tail domains are not required for assembling or maintaining the structure of the nucleosome core and removal of the tails results in only marginal changes in the hydrodynamic shape, stability and DNA wrapping within the nucleosome (21-23). However, the tail domains are essential for folding of oligonucleosomal arrays into native 30 nm chromatin fibers and are probably required for efficient assembly of fibers into higher order structures (9,24-26). Subsets of tails can promote partial folding capability and intermolecular association, suggesting that the tails have overlapping functions and the H4 tail appears to be the most critical for salt-dependent folding (27-29). However, the requirement for the tail domains, and particularly the H4 tail, for complete folding of oligonucleosomes into the fully folded chromatin fiber suggests the tails mediate specific molecular interactions during chromatin folding (9,16,29). Although it has not been directly demonstrated, it is generally assumed that at least some of these interactions
include inter-nucleosomal contacts (3,7,9,16). However, the complex array of structures and interactions of the tail domains in chromatin remains relatively undefined.

In higher eukaryotes native chromatin also contains approximately one linker histone (histone H1) bound to each nucleosome (8,30). Nearly all linker histones contain an ~80 amino acid residue protease-resistant globular domain which directs binding of these proteins to the exterior of the nucleosome (31,32). Although linker histones bind with high affinity and positive cooperativity to naked DNA, they exhibit preferential binding to reconstituted nucleosomes \textit{in vitro} (33). This binding stabilizes the wrapping of DNA about the histone octamer and directs the association of an extremely basic ~100 residue C-terminal tail which helps neutralize electrostatic repulsion caused by packing the polyanionic DNA backbone within the chromatin fiber (31,34-36). Thus linker histones generally stabilize the core histone tail-dependent intrinsic ability of nucleosome arrays to fold into chromatin fibers (36). Notably, it has been shown that the binding of linker histones can directly alter some of the interactions of the core histone tail domains within individual nucleosomes (18,37).

Histone tails undergo numerous post-translational modifications, which either directly or indirectly alter chromatin structure to facilitate transcriptional activation or repression or other nuclear processes (38-41). Thus modifications within these domains are an end-point to many signal-transduction pathways directed to the cell’s nucleus (42). While some modifications generate recognition sites for the binding of specific proteins such as the heterochromatin-associated protein HP1 to the tails (43,44), other modifications can directly alter chromatin structure (45,46). For example acetylation of lysine residues results in a reduction of positive charge and it has been demonstrated that mutation-based alterations in overall charge can mimic the essential function(s) of acetylation in a histone tail domain in vivo (47). This non-specific charge effect is perhaps due to a reduced affinity or interaction of the tail with DNA in chromatin, leading to an alteration of chromatin structure. On the other hand, UV-laser crosslinking studies suggest that acetylation does not lead to a loss of tail-DNA interactions (13) and other critical posttranslational modifications such as methylation do not lead to an alteration of overall charge in the tail domains. Thus some modifications are likely to directly evoke distinct changes in tail structure and interactions (48). However, a detailed
understanding of the mechanism(s) by which posttranslational modifications of the histone tails leads to altered functional states of chromatin must await an understanding of the basic structures and interactions of the tail domains.

Here we introduce a novel dinucleosome system for investigation of core histone tail-DNA interactions. This system allows unambiguous identification of intra- and inter-nucleosomal interactions between the histone tail domains and DNA under a variety of conditions, including those thought to induce folding of the linker DNA in dinucleosomes (49-51). We find that a subset of the tail domains contact the DNA of the adjacent nucleosome while the H3 and H4 tails participate in only intra-nucleosomal interactions. Interestingly, the relative extent of inter-nucleosomal interactions does not exhibit a detectable dependence on ionic strength, but binding of a linker histone increases the association of H3 and H4 tails with the linker DNA region.
Experimental procedures

**DNA fragments**

DNA templates for nucleosomes 1 and 2 were derived from plasmid pXbs-1 containing a *Xenopus borealis* somatic 5S rRNA gene (52). PCR techniques were used to add Hind III and Dra III sites to the ends of the nucleosome 1 DNA template using primers 5’CAG TAA GCT TGA TTC CCG GGC TTG TTT TCC TGC CTG3’ and 5’CAT GTC TAG AGA TCC CGG GAC TAC ACG AGG TCC CAT CCA AGT AC3’ and Dra III and Xba I sites to nucleosome 2 using primers 5’CAG TAA GCT TGA TTC ACC TCG TGC TTG TTT TCC TGC CTG3’ and 5’CAT GTC TAG AGA TCC CGG GAC TAC GGT CTC CCA TCC AAG TAC3’ (Fig. 1). The resulting nucleosome 1 is 205 bps, and nucleosome 2 is 209 bps. Ligation of the two templates at the asymmetric Dra III site creates a tandem repeat of 5S sequences with a repeat of 210 bps. The two templates were cloned into pBluescript plasmids pBSXN1 and pBSXN2. DNA fragments from each were prepared in large quantity by first digesting 10 mg of pBSXN1 or pBSXN2 with Hind III or Xba I, respectively, dephosphorylation with calf intestinal phosphatase (New England Biolabs) and filling in the 5’ overhang with Klenow DNA polymerase in the presence of 100 µM each of dATP, dGTP, dTTP and ddCTP for pBSXN1 or dCTP, dTTP, dGTP and ddATP for pBSXN2. The plasmid was then cut with Dra III and the released template DNAs isolated on preparative 1.2% agarose gels, and recovered by electro-elution. Following phenol extraction and repeated ethanol precipitation, the pellet was dissolved in 10 mM Tris (pH 8.0) and stored frozen at –20°C until needed. For each dinucleosome reconstitution radioactively labeled nucleosome template 1 or 2 was prepared by digesting 10 mg of either parent plasmid with Hind III or Xba I, respectively, dephosphorylation with calf intestinal phosphatase (New England Biolabs) and filling in the 5’ overhang with Klenow DNA polymerase in the presence of 500 µCi (α-32P)dATP, and 100 µM each of dGTP, dTTP and ddCTP for pBSXN1 or dCTP, dTTP, dGTP and ddATP for pBSXN2. The plasmid was then cut with Dra III and the released template DNAs isolated on preparative 1.2% agarose gels, and recovered by electro-elution. Following phenol extraction and repeated ethanol precipitation, the pellet was dissolved in 10 mM Tris (pH 8.0) and stored frozen at –20°C until needed. For each dinucleosome reconstitution radioactively labeled nucleosome template 1 or 2 was prepared by digesting 10 mg of either parent plasmid with Hind III or Xba I, respectively. The free DNA ends were dephosphorylated as above and the DNA stored frozen. For each labeling 100 µg of either digested plasmid was incubated with Klenow in the presence of 500 µCi (α-32P)dATP, and 100 µM each of dGTP, dTTP and ddCTP (α-32P)dCTP, dTTP, dGTP, ddATP for pBSXN2) for 15 min at room temperature, then 100 µM cold dATP (dCTP for pBSXN2) was added for an additional 10 min. The labeled template was isolated on a 6% acrylamide gel by standard techniques and stored in TE.
Preparation of native and APB-modified histones.

Recombinant wild type and mutant H2A, H2B, H2AG2C and H2B2C were prepared and purified as preformed dimers as described (19,53). Coding sequences for *Xenopus* H3T6CC110A (containing cysteine and alanine codon substitutions at amino acid positions 6 and 110, respectively) and H4G6C (containing a cysteine codon at position 6) were constructed and cloned into pET3 expression plasmids. The individual core histones and mutant proteins were expressed individually in bacteria then H2A/H2B dimers purified as described previously (53) and H3/H4 tetramers prepared via the following procedure. As these proteins were present in insoluble forms in traditionally prepared lysates, we first determined the mass of proteins within each bacterial cell culture then mixed cultures containing roughly equal amounts of H3 and H4, H3T6CC110A and H4, or H3C110A and H4G6C. A pellet containing both proteins (from one combined liter of culture) was suspended in 100ml P1 buffer (50 mM Tris, 10 mM EDTA, 100µg/ml RNase, pH8.0), then cells lysed with equal volume of P2’ buffer (0.2 M NaOH, 0.4% Triton-100) in the presence of 0.4mM PMSF, 8mM DTT at room temperature for an hour. The lysate was dialyzed against 2M NaCl TE buffer (pH8.0, 10 mM β-mercaptoethanol) at 4°C for at least 16 hours, with three buffer exchanges. The sample was centrifuged at 15,000g for 30 min, and the pellet discarded. For each 10 ml of supernatant 30 ml of TE and 100 µl 1M DTT were added then 1ml of exchange resin added per ml of solution (Biorex 70, 50-100 mesh, 50% slurry). Proteins were allowed to bind the resin for 4 hours at 4°C. The resin was washed extensively with 0.6M NaCl in TE and H3/H4 tetramer eluted with 2M NaCl in TE. Fractions containing the tetramer were sonicated to reduce the size of contaminating nucleic acids and the BioRex resin purification repeated. The fractions containing H3/H4 tetramers were mixed with 50 µl hydroxyapatite resin per ml (BioRad Laboratories, 50% slurry) for 30 minutes at 4°C, and the resin removed by centrifugation. This process was repeated 3 times to eliminate final traces of contaminating nucleic acids (54). The proteins were checked by SDS PAGE and trial reconstitutions. APB modification of H2AG2C, H2B2C, H3T6C and H4G6C was carried out as previously described (19,55).

*Preparation of model dinucleosomes.*
Nucleosomes 1 and 2 were prepared separately then ligated together. In most cases one nucleosome was reconstituted on a cold DNA template and the second reconstituted on a radioactively end-labeled template prepared as described above. Nucleosome reconstitution was carried out by a standard salt dialysis procedure (53). Typically, 6.6 µg of unlabeled or ~3 µg of labeled template fragment, respectively, was mixed with, 15 µg H2A/H2B, 15 µg H3/H4, and 40 µg linearized Bluescript plasmid with blunt ends (as competitor) were used for each reconstitution. Due to slight variations of different preparations of DNA fragments and APB modified histone mutants, the ideal ratio was empirically determined. Reconstituted mononucleosomes were loaded onto 10 ml 5%-20% sucrose gradients prepared in 10 mM Tris (pH 8.0) 1 mM EDTA (Beckerman 41 Ti rotor, 34 kRPM, 18 hours). Fractions containing purified mononucleosomes were identified by analysis in 0.7% agarose nucleoprotein gels. The nucleosome fractions were concentrated 10-fold by centrifugation through a Microcon YM-50 filter membrane. The ligation reactions typically contained 50 µl (5 or 2.5 pMol of unlabeled or labeled nucleosomes, respectively) each of nucleosomes 1 and 2, 20 µg BSA, and 3,200 Unit T4 Ligase (New England Biolabs) were kept at room temperature for 30 min in total volume of 160 µl, followed by the addition of 20 µl 10 mM ATP and 20 µl 20 mM Mg2+ for additional 60 minutes. The ligation product was purified by sucrose gradient centrifugation as above except for 15 hours at 4°C.

Restriction enzyme digestion and hydroxyl radical footprinting of nucleosomes.

Sucrose gradient purified mononucleosomes and dinucleosomes were subjected to restriction enzyme digestion as described (56). Dinucleosomes used for hydroxyl radical footprinting were first subjected to buffer exchange to 10 mM Tris, (pH8.0) 1 mM EDTA by Microcon YM-50 concentrator to eliminate sucrose.

UV-induced crosslinking and identification of crosslinking bands.

Gradient purified dinucleosomes were subjected to buffer exchange as above then 32 µl (~0.1 pmole) was rapidly mixed with 4µl BSA (1µg/µl), and 4ul concentrated NaCl solutions. The mix was placed into the bottom of a Falcon 5ml polystyrene tube, which was inside a 15ml Pyrex No. 9820 glass tube. The sample was irradiated with 365nm UV light generated by a VMR LM-20E light box for 1 min. The sample was then mixed with 5µl 1M DTT, 2µl 10% SDS, 2µl 1.5 µg/µl calf thymus DNA, and incubated at 37°C for
10 min, followed by adding 85 µl ddH2O and standard ethanol precipitation. The pellet was dissolved in 30 µl 10 mM Tris, pH 8.0 then 13 µl of the sample was digested with DraIII, in a total volume of 30 µl at 37°C for 3 hours. Another 13 µl portion was diluted to 30 µl with ddH2O and mock-digested at 37°C for the same time. The samples then mixed with SDS and loaded onto a 6% acrylamide gel containing 0.5 X TBE an run at 100V for 14 hours before gel drying and phosphorimager analysis.
Results

Assembly of the dinucleosome.

In order to assess potential internucleosomal interactions of the core histone tail domains, we assembled a model dinucleosome in which one nucleosome contained native histones and a radiolabeled DNA fragment while the second contained one histone with a photoactivatable crosslinking probe located within the tail domain. To assemble the dinucleosome, the two individually reconstituted nucleosomes were ligated together (Fig. 1). In order to ensure proper orientation upon ligation, we placed an asymmetric recognition sequence for the restriction enzyme Dra III at appropriate ends of the DNA templates (Fig 1) and polished the opposite ends (filled in with a terminal dideoxy nucleotidate and dephosphorylated) to prevent self ligation (see Methods; Fig. 2A). We found that efficient ligation of the nucleosome containing the radioactive required this nucleosome to be present at least 10 nM concentration and a 2-3-fold excess of the unlabeled nucleosome (Fig. 2). Ligated dinucleosomes were isolated by centrifugation through sucrose gradients to remove any unligated material or material not containing a full complement of core histones.

Characterization of the model dinucleosome.

Purified ligated dinucleosomes migrate through sucrose gradients and in agarose gels as expected based on migration of mononucleosomes and native dinucleosome controls (Fig. 2B and data not shown). Also, similar to other dinucleosomes (57), our ligated dinucleosome showed a two-step shift when bound by increasing amounts of histone H1 (Fig. 2C). To further check integrity, gradient purified dinucleosomes were subjected to hydroxyl radical footprinting and restriction enzyme digestion assays. By labeling different ends of the dinucleosome, we compared the hydroxyl radical footprinting patterns of each end of the template with the respective mononucleosomes. As shown in Fig. 3, the cleavage patterns from nucleosome 1 and nucleosome 2 in the model dinucleosome were very similar to corresponding mononucleosomes, indicating that the ligation process did not alter nucleosome rotational orientation or position or select for minor populations of mononucleosomes with alternative positioning. Mapping of nucleosome positions in the mono and model dinucleosome via restriction enzyme site protection assays substantiated the footprinting results (Fig. 4). Again, the extents of
protection at each site in the mono- and dinucleosomes were similar, indicating the mononucleosome positions were not significantly altered when ligated into dinucleosomes.

The H2A N-terminal tail makes intra-nucleosomal DNA contacts in a dinucleosome.

To assess contacts to DNA made by the H2A tail in the model dinucleosome, we attached a photoactivatable crosslinking probe to a position near the tip of H2A N-terminal tail (H2AG2C-APB) in nucleosome 2, while the Hind III end of nucleosome 1 was radioactively labeled. Crosslinking was initiated as described in the Methods and the extent of intra- versus inter-nucleosomal contacts to DNA by the H2A tail measured as the relative abundance of crosslinked species before and after DraIII digestion. Before DraIII digestion, a strong crosslinking band was observed above the full-length dinucleosome template band, dependent on UV irradiation (Fig. 5, lanes 1 and 2). This band represents both intra- and inter-nucleosomal crosslinking to DNA by the tail of H2A (Fig. 5, graphic). Digestion with Dra III reveals that approximately 15%-20% of the total crosslinking is due to inter-nucleosomal DNA binding by the H2A N-terminal tail (Fig. 5, lane 10).

In order to substantiate the above results we placed the both the radioactive label and H2AG2C-APB within the same nucleosome (Fig. 6, schematic). Again, UV irradiation results in the generation of a band corresponding to crosslinked species (Fig. 6, lane 2). After DraIII digestion, the majority (approximately 80%) of the crosslinked band is converted to a slower-migrating crosslinked species (Fig. 6, lane 9). In addition, we note that increases in salt concentration did not significantly alter the fraction of the total crosslinks that were inter-nucleosomal, but did reduce the overall efficiency of crosslinking (Figs. 5 and 6, lanes 10-16 and 9-14, respectively). Although the basis for this reduction has not been completely defined, it is due to in part to a moderate effect of salt on the efficiency of the crosslinking reaction as a similar effect of ionic strength on crosslinking is observed with mononucleosomes (C.Z. and J.H., unpublished results).

APB H2AA12C does not participate in inter-nucleosomal interactions.

We next determined the extent to which a position near the first α-helix in H2A is able to mediate inter-nucleosome interactions. This position is located at the junction of the H2A
N-terminal tail and the histone fold domain, near the inside of the superhelical DNA gyre, and therefore is less likely to participate in inter-nucleosomal DNA binding (3,5-7,58). Previous experiments have shown that irradiation of mononucleosomes containing H2AA12C-APB results in crosslinks to DNA located approximately 40 bps to either side of the nucleosome dyad, consistent with the known structure of this complex (3,19). Similar to the previous experiment the Hind III end of nucleosome 1 in the dinucleosome was radiolabeled, and H2AA12C-APB was incorporated into nucleosome 2. Upon UV irradiation a band indicating crosslinking between H2AA12C-APB and DNA was generated but DraIII digestion showed that the observed crosslinks were virtually entirely due to intra-nucleosomal interactions with DNA (Fig 7, compare lanes 2 and 9). As previously observed, the total extent of crosslinking was reduced as the NaCl concentration was raised to 100 mM (Fig. 7, lanes 2-7). To confirm this result, in a second experiment we put both the radioactive label and H2AA12C-APB in the same nucleosome. In this case, after DraIII digestion, nearly 100% of the crosslinking was converted to the slower migrating species (Fig. 7, lanes 15-18).

The H2B N-terminal tail makes inter-nucleosomal contacts with DNA. To probe potential internucleosomal interactions by the N-terminal tail domain of H2B, we radiolabeled the Hind III end of nucleosome 1, and placed H2B2C-APB in nucleosome 2. Protein-DNA crosslinking bands were generated upon UV irradiation under different salt concentrations (Fig. 8, lanes 2-8). Upon separation of the two nucleosome templates by DraIII digestion, protein crosslinked to mononucleosome DNA was observed, indicating that H2B N-terminal tail participates in inter-nucleosomal interactions (Fig. 8, lanes 10-16). Again, we noticed that the relative proportion of H2B inter-nucleosomal interactions did not change upon increasing ionic strength of the solution.

H3 and H4 tails did not make inter-nucleosomal interactions in the model dinucleosome. We probed for internucleosomal interactions by the N-terminal tail domains of H3 and H4 to DNA in manner identical to that used for H2A and H2B. Strong protein-DNA crosslinking to the dinucleosome template was observed when the crosslinking probe was placed near the tip of either the H3 or H4 tail in nucleosome 2 and the template of nucleosome 1 contained a radioactive label. However, when the individual nucleosome
templates were separated via DraIII digestion, no protein crosslinking to the DNA of nucleosome 1 was observed, regardless of the ionic strength tested (Figs. 9 and 10, lanes 6-7 and 8-11, respectively). These results suggest that H3 and H4 tails do not interact with the DNA of the neighboring nucleosome in our model dinucleosome system.

**Effect of linker histone on tail interactions.**

Previously we and others showed that the association of linker histones with the nucleosome affects the interactions of some of the histone tail domains with DNA in mononucleosomes (18,37). To investigate potential effects of H1 binding on internucleosome interactions in our model dinucleosome, we incubated templates with H1 and repeated the crosslinking experiments. H1 was carefully titrated with respect to dinucleosome concentration up to two molecules of H1 per dinucleosome template (Fig. 2C). The binding of linker histone did not induce the formation of bands corresponding to internucleosomal crosslinking by the H3 and H4 tail domains (Fig 9, lanes 8-10; Fig. 10, lanes 12-14). However, H1 binding did induce the formation of crosslinked species refractory to digestion by DraIII (Fig. 9, lanes 8-10, Fig. 10, lanes 8-11). Such bands are likely due to the formation of crosslinks in and around the cognate DNA site for DraIII in the linker DNA. Interestingly, the binding of H1 had little or no apparent effect on the total extent of internucleosomal or linker DNA interactions by the H2A or H2B N-terminal tail domains (data not shown). We note however that since DraIII did not cut the crosslinked dinucleosome band completely even in the absence of linker histone in the H2A experiments (Fig. 5, lanes 10-16), it is possible that minor H1-dependent increases in crosslinking would not be detected. In this case, presumably some crosslinking of the H2A tail to the linker DNA in the absence of H1 binding prevents Dra III cutting.
Discussion
Our results support the emerging view that different histone tails have different structural roles in chromatin (9,59). We find that in our model dinucleosome the N-terminal tails of H2A and H2B make detectable interactions with the DNA of a neighboring nucleosome, while the tails of H3 and H4 apparently make exclusively intra-nucleosomal histone-DNA interactions. If interactions within our model system resemble those found within native chromatin, our results imply that dislocation of H2A/H2B dimers, such as has been suggested to occur in transcriptionally active chromatin (60,61) would lead to a loss of inter-nucleosomal interactions and a drastic loosening of compact chromatin structure (62). It has been shown that loss of only one or two sets of such contacts within a much larger array of nucleosomes significantly destabilizes folding of the array into a condensed higher-order structure (63). This is consistent with the relatively high rate of transcription-coupled turnover of the H2A/H2B dimer in vivo, compared to the (H3/H4)2 tetramer (64-66).

Previously, both site-directed and general crosslinking techniques have been used to map interactions of the core histone tail domains (67-70). However, much of this work has been done with either single nucleosomes or with native chromatin where inter-nucleosomal tail interactions were difficult or impossible to quantify. Our system allows unequivocal identification of inter-nucleosomal interactions. Radiolabeled mononucleosome template covalently crosslinked to histone protein can only arise from internucleosome interaction of a tail domain with DNA of the adjoining nucleosome. Such interactions, representing communication between adjacent nucleosomes, were not detected for histone H3 or H4 N-terminal tails, yet these tails, and particularly the H4 tail, are clearly required for formation of the fully condensed chromatin fiber (27-29). Interestingly, recent results suggest that the H3 tail does not contribute significantly to the stability of the folded chromatin fiber and that the predominant contribution of the H4 tail appears to coincide with a region thought to interact with an acidic patch on the surface of the H2A/H2B dimer, perhaps in an adjacent nucleosome (3,29). These results are consistent with the our observation suggesting that these tails do not make contacts to the DNA of an adjacent nucleosome in chromatin. In addition, it is unclear if H3 or H4 tails would make contacts between successive nucleosomes along the DNA strand (N to N+1)
or if these tails would interact preferentially with nucleosomes beyond the nearest
neighbor (N to N+2, N+3...). Indeed, dependent on the actual structure of the chromatin
fiber, nucleosome N may be physically closest to nucleosomes N+2 or N+3 (71). These
observations are also consistent with the idea that the H3/H4 tails are the primary
mediators of intra-nucleosomal DNA accessibility (56,72).

It has been well established that raising the monovalent cation concentration in a
solution can effect condensation or compaction of an extended array of nucleosomes into
a form resembling a contacting-zig-zag or ‘10-nm’ structure (8,9,73-75). Furthermore,
even in the absence of linker histones, addition of small amounts of divalent cations (i.e.
Mg^{2+}) can induce further compaction of oligonucleosomes to a structure with the same
hydrodynamic shape as a fully condensed ~30 nM chromatin fiber (9,73). In addition,
some studies suggest that chromatin fragments as small as dinucleosomes can undergo
some amount of salt-dependent condensation, perhaps related to the condensation of
oligonucleosomes (49-51). Given the role of the histone tail domains in fiber compaction
(24,25,27), we hypothesized that one or more of the tails may exhibit an alteration in the
extent of inter-nucleosome interactions upon transition to more condensed states.
However, in our system the relative proportion of inter-nucleosome interactions did not
increase with increasing NaCl (or upon the addition of Mg^{2+}, data not shown) at
concentrations reported to cause condensation of the dinucleosome (49-51). It is possible
that although the conformation of the dinucleosome changes, the interactions of histone
tails with DNA do not rearrange in such a way resulting in increasing inter-nucleosome
tail-DNA interactions. Alternatively, it is possible that adjacent nucleosomes do not come
in close proximity in native chromatin, as has been proposed for some models of the 30
nm chromatin fiber (see above) (71). In addition, some of the tail domains may
participate in inter-nucleosomal protein-protein interactions in condensed chromatin
(9,76). In this regard, we note that two grooves in the nucleosome protein surface have
been proposed to bind histone tails from adjacent nucleosome in native chromatin, based
on interactions observed in X-ray crystal structures of nucleosome cores or clusters of
histone mutations interrupting silencing (3,7,76). Experiments in progress assessing inter-
nucleosomal histone tail-protein interactions will address these issues.
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Figures

FIG.1. Experimental strategy. Regions of DNA templates encompassed within the nucleosome core regions are indicated by ovals; the asymmetric Dra III site is indicated by the arrowheads. A typical configuration of radiolable (asterix) and crosslinker in a tail domain (line and filled circle) is shown. After Dra III digestion, only inter-nucleosomal interactions will be detected as protein covalently crosslinked to radiolabeled DNA.

FIG.2. Assembly of the model dinucleosome. (A) Proper ligation of template DNAs. Ligation reactions (total volume of 10 µl in 1× T4 ligase buffer) were incubated at room temperature for 1 hour then loaded onto a 6% acrylamide gel. Samples shown in lanes 1 and 2 contained no ligase, while those in 3, 4, and 5 contained 400 Units T4 DNA ligase. Samples shown in lanes 1 and 3 contained 0.1µg nucleosome 1 template (N1); lanes 2 and 5, 0.2µg nucleosome template 2 (N2); lane 4, 0.1 µg N1 and 0.2 µg N2. (B) Mononucleosomes were efficiently ligated into dinucleosomes. In this experiment nucleosome 1 was radioactively labeled and samples were run in a 0.7% agarose nucleoprotein gel. Lane 1, mononucleosome DNA; lane 2, gradient purified mononucleosome 1; lane 3, ligated dinucleosome DNA; lanes 4 and 5, nucleoprotein products from the ligation reaction before and after gradient purification of the dinucleosome, respectively. (C) Two H1s bind model dinucleosomes. Gradient-purified dinucleosomes were incubated with histone H1 then products resolved on a 0.7% agarose nucleoprotein gel. Lane 1, dinucleosome DNA template shown as a control; lane 2, gradient purified dinucleosomes; lanes 3-6, dinucleosomes incubated with 4.0, 2.0, 1.5 and 1.0 moles of H1 per mole of dinucleosome, respectively.

FIG.3. Hydroxyl radical footprinting of model mono- and di-nucleosomes. Radioactively labeled nucleosomes and naked DNA were prepared, subjected to hydroxyl radical cleavage, and the products analyzed in sequencing gels. Left gel: Footprints of complexes labeled at the Hind III end of nucleosome 1. Lanes 1 and 7, G-reaction markers of dinucleosome and nucleosome 1 DNA, respectively; lanes 2 and 3, dinucleosome DNA treated with hydroxyl radicals or untreated, respectively, lanes 4-6,
hydroxyl radical cleavage patterns of the model dinucleosome and nucleosome 1, and mononucleosome 1 DNA, respectively. Right: Footprints of complexes labeled at the Xba I end of nucleosome 2. Lanes 1 and 6, G-reaction of dinucleosome and mononucleosome DNA, respectively. Lanes 2-5, hydroxyl radical cleavage patterns of dinucleosome DNA, dinucleosomes, mononucleosomes and mononucleosome DNA, respectively.

FIG.4. **Restriction enzyme digestion mapping of a model dinucleosome.** The percentage of uncut DNA template remaining for mono and di nucleosomes was determined for Bbv I, EcoR V, Rsa I and Dra III. Upper left: Hind III end labeled mononucleosome 1. Lower left: Hind III labeled dinucleosomes. Upper right: Xba I end labeled mononucleosome 2. Lower right: Xba I labeled dinucleosomes.

FIG.5. **The tip of H2A tail make inter-nucleosomal contacts to DNA in a dinucleosome.** Dinucleosomes were prepared with a radioactive label at the Hind III end of nucleosome 1 and H2AG2C-APB in nucleosome 2 then irradiated with UV light and crosslinks analyzed on SDS-PAGE as described in the Methods. Samples shown in lanes 1 and 9 are unirradiated controls while samples in 2-8 and 10-16 were irradiated. Samples in lanes 2 and 10 were treated directly from the gradient (i.e. contain sucrose) while samples shown in lanes 3-8 and 11-16 underwent buffer exchange to 10 mM Tris containing 0, 5, 10, 20, 50, 100 mM NaCl, respectively. Samples in lanes 9-16 were digested with Dra III after UV irradiation. Lane 17 contains nucleosome 1 assembled with H2AG2C-APB and irradiated as a mononucleosome crosslinking control. The scheme on the upper side of gel indicates the location of the radiolabel on the DNA (asterix), nucleosomes 1 and 2 (ovals, with numbers below) and the location of the crosslinker-modified histone tail domain (vertical line from oval) within the model dinucleosome. The schemes below indicate the species represented by the bands on the gel. Note that the radiolabeled dinucleosome template in the gel has been stripped of non-covalently bound proteins. The location of the crosslinked protein (slanted bar) is indicated.
FIG. 6. **The tip of H2A tail also contacted intra-nucleosomal DNA in a dinucleosome.** Dinucleosomes were prepared containing a radioactive label at the Xba I end of nucleosome 2 and H2AG2C-APB in nucleosome 2. All samples shown underwent buffer exchange to 10 mM Tris (pH 8.0) 1 mM EDTA after gradient purification. Samples in lanes 1 and 8, are unirradiated controls; lanes 2-7 and 9-14 contained 0, 5, 10, 20, 50, 100 mM NaCl, respectively, and were UV-irradiated. Samples in lanes 8-14 were digested with Dra III after irradiation. Scheme (right) is as in Fig. 5.

FIG. 7. **APB-H2AA12C did not yield inter-nucleosomal crosslinks in a model dinucleosome.** Dinucleosomes containing a radioactive label at the Hind III end of nucleosome 1 and H2AA12C-APB in nucleosome 2 were analyzed for crosslinking as described in the Methods. All samples shown underwent buffer exchange to 10 mM Tris (pH 8.0) 1 mM EDTA after gradient purification. Samples shown in lanes 1 and 8 are unirradiated controls; lanes 2-7 and 9-14 contained 0, 5, 10, 20, 50, 100 mM NaCl, respectively, and were UV-irradiated. Samples in lanes 8-14 were digested with Dra III after irradiation. Samples in lanes 16-18 are as above except that the Xba I end of nucleosome 2 DNA was labeled. Lanes 16 and 18 were UV-irradiated and samples in 17 and 18 were digested with with Dra III. Scheme (right) is as in Fig. 5.

FIG. 8. **Inter-nucleosomal H2B tail-DNA crosslinking in a model dinucleosome.** Dinucleosomes were prepared containing a radiolabel at the Hind III end of nucleosome 1 DNA and H2B2C-APB in nucleosome 2 then analyzed for crosslinking as described. Samples shown in lanes 1 and 9 are unirradiated controls while samples in lanes 2-8 and 10-16 were irradiated. Samples in lanes 2 and 10 were taken directly from the gradient fractionation (i.e. contain sucrose) while samples shown in lanes 3-8 and 11-16 underwent buffer exchange 10 mM Tris (pH 8.0) 1 mM EDTA and 0, 5, 10, 20, 50, 100 mM NaCl, respectively. Samples in lanes 9-16 were digested with Dra III after UV irradiation. Mononucleosomes containing H2B2C-APB were analyzed in lanes 17 (no irradiation) and 18 (irradiated) as controls. Scheme (right) is as in Fig. 5.
FIG. 9. Binding of H1 increased H3 tail-linker DNA interactions in the dinucleosome. Dinucleosomes were prepared with a radiolabel at the Hind III end of nucleosome 1 and H3T6C-APB in nucleosome 2 then irradiate and crosslinks analyzed as described. All samples shown underwent buffer exchange to 10 mM Tris (pH 8.0) 1 mM EDTA after gradient purification. Samples in lanes 1 and 6 contain no NaCl; lanes 2-5 and 7-10 contain 50 mM NaCl; lanes 3-5 and 8-10 contain 2.0, 1.5 and 1.0 moles of H1 per mole of dinucleosome, respectively; lanes 6-10 were digested with Dra III after UV irradiation. Scheme (right) is as in Fig. 5.

FIG. 10. APB-H4G6C crosslinking in a model dinucleosome. Dinucleosomes were prepared with a radioactive label at the Hind III end of nucleosome 1 and H4G6C-APB in nucleosome 2. All samples shown underwent buffer exchange to 10 mM Tris (pH 8.0) 1 mM EDTA after gradient purification. Lanes 1 and 8 unirradiated controls; samples in lanes 2-7 and 9-14 contain 20, 50, 100, 50, 50, 50 mM NaCl respectively, and were UV irradiated. Samples in lanes 5-7 and 12-14 contain 2.0, 1.5 and 1.0 moles of H1 per mole of dinucleosome, respectively; lanes 8-14 were digested with Dra III after UV irradiation. Scheme (right) is as in Fig. 5.
Fig. 1

N1

* 

Hind III  Dra III

Ligation

N2

* 

Dra III  Xba I

UV irradiation

Denaturation

Cut with Dra III
Fig. 2
Fig. 3
Hind III  Xba I

Percentage remaining

100%
80%
60%
40%
20%

20%
40%
60%
80%
100%

12

Rsa I

Dra III

Bbv I

Eco RV

Fig. 4
Fig. 5
Fig. 6
Fig. 7
Fig. 8
|       | H1                  |       | H1                  |
|-------|---------------------|-------|---------------------|
| 0     | 0                   | 50    | 50                  |
| 50    | 50                  | 50    | 50                  |
| 50    | 50                  | 50    | 50                  |
| 50    | 50                  | 50    | 50                  |
| 1     | mM NaCl             |
| 2     | -DraIII,+UV         | 6     | +DraIII,+UV         |
| 3     | 0                   | 7     | 50                  |
| 4     | 50                  | 8     | 50                  |
| 5     | 50                  | 9     | 50                  |
| 10    | 50                  | 10    | 50                  |

Fig. 9
Fig. 10