Linker DNA and H1-Dependent Reorganization of Histone−DNA Interactions within the Nucleosome†

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ABSTRACT: We have employed a site-directed photochemical cross-linking procedure to precisely map interactions between nucleosomal DNA and the C-terminal tail of core histone H2A. We find that this tail has the potential to contact multiple sites within the nucleosome and that these contacts are dependent upon the configuration of the complex. This tail contacts DNA near the dyad axis within nucleosome core particles but rearranges to a site near the edge of the nucleosomal DNA when linker DNA is present. Moreover, in the presence of linker histone H1 the contacts near the edge of the nucleosome but not at the dyad are further rearranged. In addition, we present further evidence for the suggestion that the binding of linker histone causes a subtle but global change in core histone−DNA interactions within the nucleosome [Usachenko, S. I., Gavin, I. M., and Bavykin, S. G. (1996) *J. Biol. Chem. 271*, 3831−3836].

The nucleosome is the fundamental structural unit of chromatin and is comprised of ∼160 base pairs of DNA wound twice about an octamer of core histone proteins (*1*). Approximately 75% of the core histone protein mass is organized into structured "histone one-fold" domains which form the spool onto which the DNA is wrapped (*2*−*4*). The remaining ∼25% of the mass of the core histones consists of the "tail" domains. These domains are located at the N-terminal portion of all four core histone proteins and the C-terminus of histone H2A and are generally defined by their sensitivity to proteases (*5*). Proteolytic removal of the tail domains does not drastically alter the conformation or hydrodynamic properties of individual nucleosomes, and the tails do not play a role in nucleosome positioning or the correct assembly of nucleosomes in vitro (*6*−*8*). However, these domains are required for folding of oligonucleosomal arrays into the more compact 30 nm fibers and perhaps for assembly of these fibers into higher order structures (*9*−*11*).

The tail domains contain a high proportion of basic amino acid residues, several of which have been shown to contact DNA within chromatin complexes (*12*, *13*). The tails are also the sites of most of the posttranslational modifications which occur within the core histone proteins (*1*, *14*). Although the function of most of these modifications remain undefined, it is clear that they are important for many nuclear processes which involve chromatin (*14*, *15*). For example, acetylation of lysine within the tail domains has been shown to play a direct role in transcription (*16*, *17*). The core histone tails also may be directly involved in mediating transcription factor access to cognate sites within nucleosomal DNA, either by direct steric effects, by modulation of nucleosome stability, or by effects of tail modifications on linker histone binding (*6*, *18*, *19*). In addition to interactions with DNA, the tails also mediate regulatory functions via direct interactions with proteins such as the Tup1/Ssn6 transcription factor complex, the Sir2p/Sir3p complex involved in telomeric silencing in yeast, the nucleosome remodeling factor NURF, and perhaps other histones within the condensed chromatin fiber (*20*−*22*). Thus the tail domains are crucial end points for signal transduction within the cell nucleus (*14*−*17*).

Unfortunately, the molecular details of the interactions of the tail domains within various biologically relevant chromatin structures are not well defined. In the absence of DNA these domains are disordered (*2*). In chromatin complexes which contain DNA the tails are bound in low ionic strength solutions but are released in moderate salt conditions (0.3−0.4 M monovalents) to adopt random coil conformations in solution (*23*−*25*). A recent high-resolution crystal structure of a nucleosome core reveals that about one-third of the tail domains adopt unique and defined positions within these complexes (*4*). Cross-linking studies suggest that the remainder of these domains may be bound at multiple distinct sites within mononucleosomes (*13*, *26*). Furthermore, studies of nucleosomal arrays suggest that the array of molecular contacts made by the tail domains in monosomes is significantly rearranged, perhaps to linker DNA and internucleosomal sites, as extended arrays condense into the 30 nm chromatin fiber (*27*, *28*).

H2A is the only core histone with a C-terminal domain that shares much of the same characteristics of the N-terminal tails. For example, the majority of this tail is not resolved in the crystal structure of the histone octamer or in the structure of a nucleosome core particle (*2*, *4*). Likewise, this tail potentially makes multiple sets of interactions within different chromatin complexes. Cross-linking experiments indicate that the C-terminal tail contacts DNA near the dyad axis in nucleosome core particles, which lack linker DNA (*29*). However, in chromatin complexes which contain linker DNA this tail contact is severely diminished, suggesting a rearrangement of the tail to another position within the

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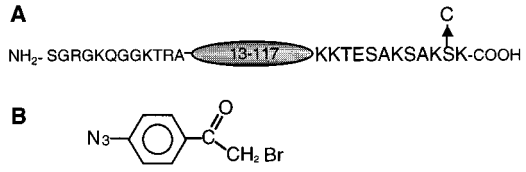

FIGURE 1: (A) Schematic of cysteine substitution within the C-terminal domain of H2A. (B) Chemical structure of 4-azidophenacyl bromide (APB).

nucleosome (*29*). Further, the position of the H2A C-terminal tail domain also may be affected by the binding of linker histones within chromatin. This tail emanates from the nucleosome very near a proposed binding site for the globular domain of linker histone (*30*, *31*). Cross-linking experiments have shown that the C-terminal half of H2A is located near the H1 globular domain in chromatin (*32*). This suggests that H1 might influence the structural characteristics or molecular contacts of the H2A C-terminal tail through direct interactions.

Here we have precisely defined the complex molecular interactions made by the C-terminal tail domain of H2A within three nucleosomal complexes which differ with regard to the presence or absence of "linker"[1] DNA and linker histone. The precise location of the H2A C-terminal tail was determined by a high-resolution photochemical mapping technique (*26*). Consistent with previous findings we find that the C-terminal tail of H2A contacts DNA at the dyad axis of the nucleosome in the absence of linker DNA (*29*) but in the presence of linker DNA this tail rearranges to contact DNA near the periphery of the nucleosome. We also find that the tail−DNA contacts near the periphery of the nucleosome but not near the dyad are altered by the binding of linker histone. Furthermore, evidence is provided to support the suggestion that H1 binding causes a subtle but global change in contacts between histones and DNA (*33*).

EXPERIMENTAL PROCEDURES

DNA Fragments. Either the 152 bp *Eco*RI−*Rsa*I fragment or the 215 bp *Eco*RI−*Dde*I fragment containing a *Xenopus borealis* somatic 5S RNA gene was derived from plasmid pXP10 (*34*). These fragments were radiolabeled at the 5′ or 3′ ends of individual strands as noted in the figure legends by standard techniques (*35*).

Preparation of Core Histones and Modification with 4-Azidophenacyl Bromide (APB). *Xenopus laevis* H2A, H2B, and cysteine-substituted mutants H2AS128C (see Figure 1A) and H2AA12C (*26*) were expressed in bacterial cells and purified as preformed dimers as described (*35*). Approximately 5 nmol of fully reduced H2AS128C or H2AA12C was reacted with a 2-fold molar excess of 4-azidophenacyl bromide (APB, Sigma) as described (*26*). Complete modification was confirmed by subsequent reaction of a portion of the sample with a molar excess of ^{14}C-labeled *N*-ethylmaleimide (NEM) as described (*26*).

Reconstitution of Nucleosome Complexes and Linker Histone H1 Addition. Native core histones H2A/H2B and H3/H4 were prepared from chicken erythrocyte nuclei, and nucleosomes were reconstituted as described (*35*). Upon reconstitution, the 5S DNA fragments used in this study yield nucleosomes in which the dyad axis of symmetry is positioned near the start site for transcription of the 5S gene (+1) (*34*). *X. laevis* histone H1° was prepared and bound to reconstituted nucleosomes as described (*35*).

Photo-Cross-Linking and Mapping Cross-Links. Approximately 100 pmol of reconstituted nucleosomes was separated from naked DNA on preparative 0.7% agarose gels and visualized by exposure of the wet gel to film for 3 h. Gel slices containing nucleosome complexes were then irradiated for 30 s with a VWR LM20E Transilluminator ultraviolet light source set at 365 nm. Nucleosomes were eluted from the agarose gel slice and loaded onto a preparative SDS−PAGE[2] denaturing gel and run at 80 mA for 4 h. The gel was exposed to Kodak XO-Mat AR film for 3 h, and the cross-linked complexes were gel isolated. DNA from irradiated complexes was purified, and sites of cross-linking were visualized on autoradiographs of sequencing gels as described (*26*).

Cross-Linking within Nucleosome Core Particles. Five ug of calf thymus DNA was reconstituted with native histones and the APB-modified H2A mutants. Reconstituted material was then digested with 0.02 unit of micrococcal nuclease in the presence of 2 mM $CaCl_2$ at 37 °C for 10 min as described (*26*). The sample was separated on a 0.7% agarose gel (*34*), and the gel was then irradiated with UV light as above. The core particle band was identified by ethidium bromide staining and brief UV illumination, the cross-linked core DNA was purified and 5′ end labeled, and sites of cross-linking were detected as above.

RESULTS

We wished to map the interactions of the C-terminal tail of H2A in three different chromatin complexes: a nucleosome core particle, a nucleosome containing linker DNA, and an H1−nucleosome complex. To map these interactions, we attached a photoactivatable cross-linking probe to the end of the C-terminal tail of H2A. A mutant protein was generated by substituting the penultimate residue within H2A for cysteine to generate H2AS128C (Figure 1). The free sulfhydryl group within H2AS128C was modified to completion with the heterobifunctional cross-linking reagent 4-azidophenacyl bromide (APB) (*36*). The modified cysteine is able to cross-link to any nucleosomal DNA in the vicinity upon UV irradiation (*26*). Nucleosomes were then reconstituted with APB-modified H2A, wild-type histones H2B, H3, and H4, and a uniquely end-labeled DNA fragment. The APB modification and UV irradiation procedure did not cause any alteration in nucleosome conformation or loss of proteins from the complex as judged by several criteria. For example, the cross-linking procedure had no effect on the characteristic mobility of these complexes through a nucleoprotein gel or on the hydroxyl radical footprint of the nucleosome or on the ability of nucleosomes to preferentially bind a single molecule of linker histone (Figure 2A, results not shown).

[1] The term "linker" DNA as used here denotes DNA sequences outside of the classically defined nucleosome core region in mononucleosomal complexes.

[2] Abbreviations: EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; IPTG, isopropyl β-D-thiogalactopyranoside.

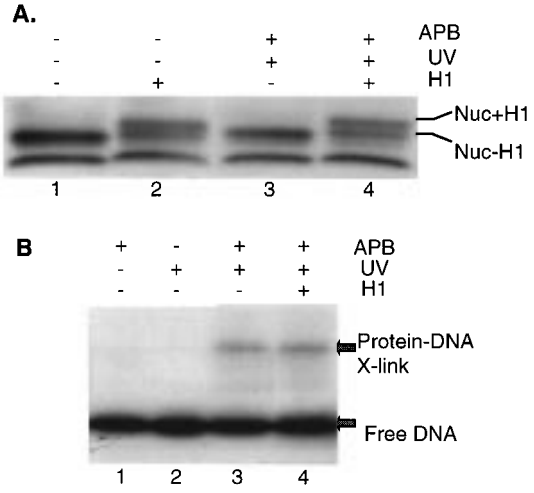

FIGURE 2: (A) Nucleoprotein gel analysis of reconstituted nucleosomes. Lanes 1 and 2: nucleosomes or H1−nucleosome complexes, respectively, containing wild-type H2A were directly loaded onto the gel. Lanes 3 and 4: nucleosomes or H1−nucleosome complexes, respectively, containing APB-modified H2A were UV irradiated and then loaded onto the gel. The positions of the nucleosome and the H1−nucleosome complex are indicated. (B) Protein−DNA cross-linking is dependent upon APB modification and UV irradiation. Nucleosomes containing unmodified or modified H2A and labeled DNA in the presence or absence of H1 were irradiated with UV light, and products were separated by SDS−PAGE. An autoradiograph of the gel is shown. Lanes: 1, unirradiated APB-modified nucleosomes; 2, UV-irradiated wild-type nucleosomes; 3, APB-modified and UV-irradiated nucleosomes; 4, APB-modified and UV-irradiated nucleosomes in the presence of H1. The positions of the naked DNA band and protein−DNA cross-linked products are indicated.

To assay for the formation of covalent cross-links between protein and DNA within reconstituted nucleosomes, SDS was added to irradiated complexes to dissociate noncovalently bound proteins from DNA, and the components were separated on SDS−PAGE gels. Nucleosomes reconstituted with radioactively end-labeled DNA and only wild-type histones yielded only a single band on an autoradiograph of the gel corresponding to naked DNA, even after UV irradiation of these complexes (Figure 2B, lanes 1 and 2). This indicates that the irradiation alone does not cause spurious protein−DNA cross-link formation. However, nucleosomes containing an APB modification in the C-terminal tail of H2A yielded a higher molecular weight complex on the gel which was dependent upon UV irradiation of the sample (Figure 2B, lane 3). Interestingly, the yield of the H2AS128C−DNA cross-link was also not affected by the binding of H1 to the nucleosomes (Figure 2B, lane 4, but see below).

We next wished to map the location of cross-links between the C-terminal tail of H2A and nucleosome DNA within the three complexes mentioned above. As described in Experimental Procedures, a two-step approach was used to enrich for cross-linked complexes, and the sites of cross-linking were mapped on sequencing gels. We first analyzed cross-linking within nucleosome core particles reconstituted with the 152 bp 5S DNA fragment. Reconstitution with this fragment yields a complex nearly identical to enzymatically produced nucleosome core particles in that there is no linker DNA flanking a centrally positioned nucleosome core (see schematic, Figure 3A) (*37*). Cross-links detected on the top and bottom strands of the 152 bp fragment are shown in the

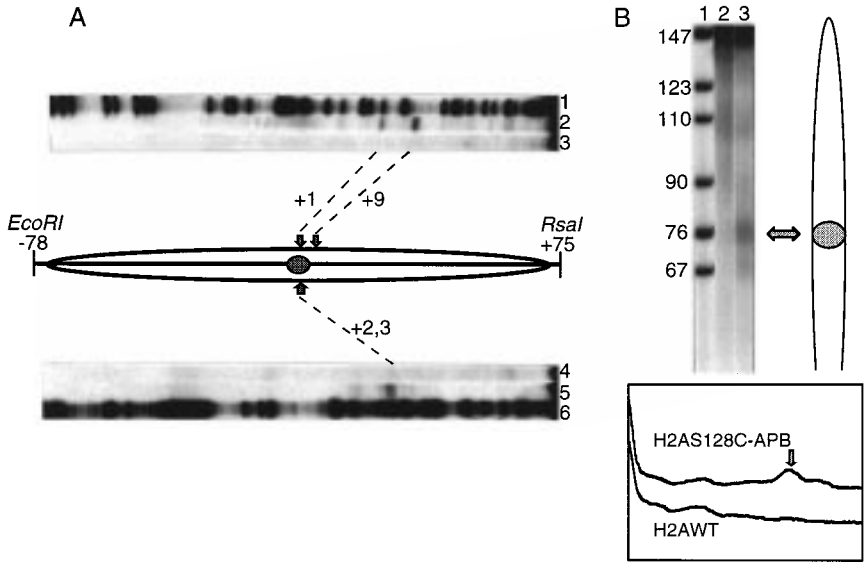

FIGURE 3: Location of cross-links between APB-modified H2AS128C and DNA in nucleosome core particles. (A) Cross-linking in nucleosomes reconstituted with the 152 bp *Eco*RI−*Rsa*I 5S DNA fragment and core histones including APB-modified H2AS128C (see schematic, center). Cross-linking was carried out and the DNA analyzed on sequencing gels as described in Experimental Procedures. (Top) Location of cross-links to the top strand of DNA within reconstituted nucleosome cores. Lanes: 1, G-specific markers; 2, cross-linking sites of H2AS128C−APB within the nucleosome core; 3, un-cross-linked DNA from H2AS128C−APB containing nucleosomes. (Bottom) Location of cross-links to the bottom strand of DNA within reconstituted nucleosome cores. Lanes: 4 un-cross-linked DNA from H2AS128C−APB containing nucleosomes; 5, cross-linking sites of H2AS128C−APB within the nucleosome core; 6, G-specific markers. Top and bottom strands were independently radioactively end-labeled at the *Eco*RI site (see Experimental Procedures). (B) Location of cross-linked sites with random sequence nucleosome core particles containing APB-modified H2AS128C. Cross-linked products from core particles were prepared and analyzed by sequencing gel electrophoresis and autoradiography as described in Experimental Procedures. (Top) Autoradiograph of products from core particles containing wtH2A (lane 2) and H2AS128C (lane 3). Lane 1 shows a *Msp*I digest of pBR322 as a marker. The top (symmetrical) half of the nucleosome core is shown, and the dyad symmetry axis (filled circle) is indicated. The cross-linking signal is indicated (arrow). (Bottom) Densitometric scans of lanes from the gel in panel A.

top and bottom of Figure 3A, respectively. Two prominent sites of cross-linking are detected on the top strand, at positions +1 and +9 relative to the transcription start site of the 5S gene (Figure 3A, lane 2). These sites are located very near the dyad axis of symmetry of the nucleosome at position ~−3 (*37*, *38*). Un-cross-linked DNA isolated in the same experiment showed only minor bands due to background resulting from the alkaline base elimination procedure (Figure 3A, lane 3). The intensity of these bands varies between experiments (results not shown). Cross-links to the bottom strand of the 5S fragment are found at positions +2 and +3, consistent with results from the top strand (Figure 3A, lane 5). These results indicate that the C-terminal tail of H2A cross-links to DNA near the dyad axis within the reconstituted nucleosome core particles, consistent with results obtained by general cross-linking of native core particles (*29*).

To confirm that our results with reconstituted nucleosome cores were not influenced by sequence selectivity of the reagent or by sequence-specific structural effects, we repeated the experiments with a standard preparation of nucleosome core particles containing random DNA sequences (*1*, *26*). Either wtH2A or H2AS128C−APB was reconstituted with bulk calf thymus DNA into nucleosomes, and core particles were prepared by careful digestion with micrococcal nuclease. Core particles were then UV irradiated, and the position of the cross-links was identified as with the 5S nucleosome cores. Analysis of core particles containing APB-modified H2AS128C revealed a clear cross-linking signal near the dyad axis, in agreement with the cross-linking data from the 5S nucleosome (Figure 3B, lane 3). Core particles containing wild-type H2A show no detectable signal near the dyad (Figure 3B, lane 2). Note that due to the heterogeneity of DNA lengths within the core particle preparation the cross-linking signal is distributed over a wider range of positions than with 5S nucleosomes.

Previously, Usachenko et al. (*29* and references therein) have shown that contacts to nucleosomal DNA near the dyad axis by the C-terminal tail of H2A were only observed in the absence of linker DNA. To further investigate this rearrangement, we repeated the cross-linking experiment in the presence and absence of linker DNA flanking one side of the nucleosome core. Nucleosomes were reconstituted onto a 215 bp DNA fragment identical to the 152 bp fragment used above except for an additional 63 bp of DNA appended to one side of the nucleosome core region DNA (Figure 4, schematic). The position of the nucleosome on this DNA fragment is identical to that found on the 152 bp fragment (*37*; see below). Mapping of the cross-links between H2AS128C−APB and the *top* strand of the 215 bp DNA fragment reveals that the cross-links previously detected near the dyad axis are drastically diminished as a result of the addition of the linker DNA (Figure 4, lane 3). Additionally, we found new sites of cross-linking to this strand located near the periphery of the nucleosome predominantly at position +70 (cf. Figure 3A, lane 2, and Figure 4, lane 3). Surprisingly, analysis of the *bottom* strand shows the cross-linking pattern on the longer fragment is virtually the same as the 152 bp *Eco*RI−*Rsa*I fragment. The major sites of cross-linking to the bottom strand still occur at positions +2 and +3, near the nucleosome dyad (Figure 4, lane 6). Thus the positions of cross-links to the top and bottom strands are differentially affected by the addition of linker DNA to one end of the nucleosome.

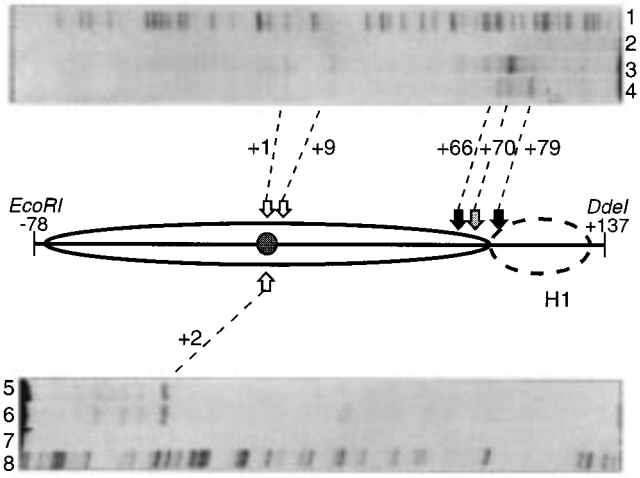

FIGURE 4: Location of cross-links between APB-modified H2AS128C and nucleosome DNA in the presence of linker DNA and linker histone H1. Samples were reconstituted with the 215 bp *Eco*RI−*Dde*I 5S DNA fragment and analyzed as in Figure 3. Note that reconstitution with this fragment results in extranucleosomal linker DNA on one end of the nucleosome core (see schematic, center). (Top) location of cross-links to the top strand of DNA. Lanes: 1, G-specific markers; 2, un-cross-linked DNA from H2AS128C−APB containing nucleosomes; 3 and 4, cross-linking sites of H2AS128C−APB within nucleosomes and H1−nucleosome complexes, respectively. (Bottom) Location of cross-links to the bottom strand of DNA. Lanes: 5 and 6, cross-linking sites of H2AS128C−APB within H1−nucleosome and nucleosome complexes, respectively; 7, un-cross-linked DNA from H2AS128C−APB containing nucleosomes; 8, G-specific markers.

We next asked if the binding of linker histone to the nucleosome has any influence on any of the H2A C-terminal tail−DNA contacts identified above. H1 binds preferentially to nucleosomes assembled onto the 215 bp 5S DNA fragment over naked 5S DNA and yields a discrete complex containing one H1 per nucleosome (*37*). When H1 was bound to the nucleosomes containing APB-modified H2AS128C before irradiation, the cross-links detected to the top strand at position +70 were clearly reduced and new signals were detected at positions +79 and a minor position at +66 (Figure 4, lane 4). Interestingly, the cross-links to the bottom strand located in the vicinity of the dyad were not altered by the binding of H1 (Figure 4, cf. lanes 3 to 4 and 5 to 6).

Since we detected the H1-dependent change in the DNA contacts made by the C-terminal tail of H2A, we next investigated if H1 binding had any effect on core histone−DNA interactions at another location within the nucleosome. We previously have shown that the 12th amino acid residue of H2A, located at the innermost position of the N-terminal tail of this protein, cross-links to DNA at a single position about 40 bp from the dyad within the nucleosome and that these contacts are not affected by the presence or absence of linker DNA (*26*). This latter result also confirms the conservation of nucleosome position between the two DNA fragments (see above). The results presented in Figure 5A show that the position of H2AA12C−APB cross-links to nucleosomal DNA was unaltered by the presence of H1. Cross-linking to the top strand was detected at position +39 within 5S nucleosomes with or without linker histone H1 (Figure 5, cf. lanes 3 and 4). Interestingly, although the position of the cross-links did not change, we found that the

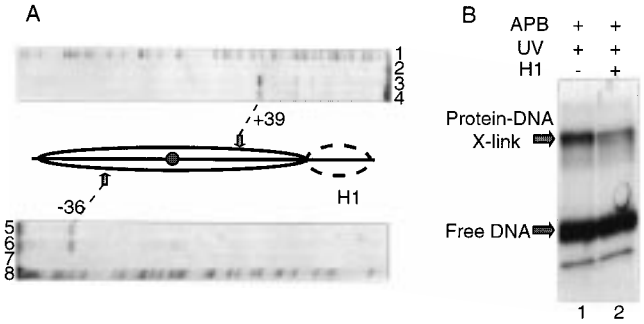

FIGURE 5: Cross-linking between the N-terminal tail of H2A and nucleosome DNA in the presence of linker DNA and linker histone H1. (A) Location of DNA cross-links within nucleosomes containing APB-modified H2AA12C. Samples were reconstituted with the 215 bp *Eco*RI−*Dde*I 5S DNA fragment and analyzed as in Figure 3. (Top) Location of cross-links to the top strand of DNA. Lanes: 1, G-specific markers; 2, un-cross-linked DNA from H2AA12C−APB containing nucleosomes; 3 and 4, cross-linking sites of H2AA12C−APB within nucleosomes and H1−nucleosome complexes, respectively. (Bottom) Location of cross-links to the bottom strand of DNA. Lanes: 5 and 6, cross-linking sites of H2AA12C−APB within H1−nucleosome and nucleosome complexes, respectively; 7, un-cross-linked DNA from H2AA12C−APB containing nucleosomes; 8, G-specific markers. (B) Efficiency of cross-linking between the N-terminal tail of H2A and nucleosome DNA is reduced by the presence of H1. Nucleosomes and H1−nucleosome complexes containing APB-modified H2AA12C were isolated by preparative nucleoprotein gel electrophoresis and irradiated with UV, and the yield of the protein−DNA cross-links was quantitated by SDS−PAGE and autoradiography as in Figure 2B. Lanes 1 and 2 show the cross-linked products from UV-irradiated nucleosomes and H1−nucleosome complexes, respectively.

efficiency of the cross-linking between the 12th position of H2A and nucleosomal DNA was dramatically reduced in the presence of histone H1. Phosphorimager analysis of the higher molecular weight band representing the cross-linked protein−DNA complex shows that the efficiency of cross-linking is reduced approximately 4-fold in the presence of H1 (Figure 5b, cf. lanes 1 and 2). No decrease in C-terminal tail cross-linking efficiency was detected (Figure 2b, lane 4), indicating a general quenching of the cross-linking reaction has not occurred.

DISCUSSION

We have used a site-specific photochemical cross-linking technique to investigate interactions of the C-terminal tail of H2A in several chromatin contexts. Our results confirm the results of Usachenko et al. (*29*) by demonstrating the interaction of the H2A C-terminal tail with DNA near the dyad in nucleosome core particles (which lack linker DNA and H1). We extend these results by demonstrating that linker DNA-dependent reorganization of this tail results in new interactions between the C-terminal tail and DNA near the edge of the nucleosome. In addition, we find that the binding of H1 causes a detectable rearrangement of C-terminal tail contacts near the edge of the nucleosome but has no effect on contacts remaining near the dyad. Last, H1 did not affect the location of DNA−protein interactions between a position in the N-terminal tail of H2A but did cause a substantial change in the efficiency of cross-link formation between this position and DNA. These results indicate that chromatin configuration and association of H1 can have profound effects on the structure and DNA contacts made by the core histone tail domains.

FIGURE 6: A model for reorganization of the C-terminal domain of H2A. Parts A, B, and C show the position of the H2A C-terminal tail in nucleosome core particles (no linker DNA), in the presence of linker DNA, and in the presence of H1, respectively. The dark column represents the histone octamer, and the tube wrapped around is the DNA. Amino acid residues within the C-terminal tail of H2A are shown emanating out from the histone octamer (gray circles). The APB-modified residue is shown in white. The globular domain of H1 is shown (light gray sphere) inside the top DNA gyre (*30*, *31*) while amino acid residues within the N- and C-terminal domain of H1 (light gray circles) are pictured extending to presumed binding sites in back and in front of this domain, respectively.

The C-terminus of H2A contacts DNA near the dyad axis within nucleosome cores reconstituted with a 152 bp DNA fragment and within core particles containing random DNA sequences (Figure 3). Thus the lack of linker DNA, i.e., DNA beyond the core region, allows the C-terminal tail of H2A to extend out to the center of the nucleosome core and bind DNA near the dyad axis (Figure 6A), in perfect agreement with previous cross-linking studies on nucleosome core particles (*29*). These results are also consistent with a recent X-ray crystal structure of a nucleosome core particle (*4*). Although the precise location of the H2A C-terminal tail was not resolved in this structure, a simple extrapolation from those H2A residues included in the molecular model shows that the remaining undefined residues in this tail could easily span the distance to the dyad axis.

The presence of linker DNA severely attenuates at least some of the interactions we detected between the C-terminal tail and DNA near the dyad (*29*). Concomitantly, new contact sites are found near the periphery of the nucleosome core. The data indicate a partial rearrangement of the H2A C-terminal tails from a binding site near the dyad axis in the absence of linker DNA to a position near the periphery of the nucleosome when linker DNA is present (Figure 6B, see below). This finding is consistent with previous results and supports the view that the H2A C-terminal tail interacts with linker DNA in chromatin (*39*). Interestingly, we found that C-terminal tail cross-linking to the bottom strand of 5S DNA was unaltered as a result of the addition of the linker DNA or H1 binding (Figure 4, lanes 5 and 6). Our interpretation is that the C-terminal tails emanating from the top and bottom halves of the nucleosome are responsible for the contacts mapped to the top and bottom strands of DNA near the nucleosome core dyad, respectively. In our experiments, the additional linker DNA is added to only one end of the nucleosome (corresponding to the top half of the nucleosome in Figure 6) while the other end remains truncated at the edge of the nucleosome core in the bottom half of the structure (Figure 6A,B). Thus, the additional linker DNA in our complexes only affects the nearest C-terminal tail and does not affect the position of the tail emanating from the bottom half of the nucleosome (Figure 6A,B).

H1 binds nucleosomes reconstituted onto 5S DNA in an asymmetric manner such that the globular domain of this protein contacts DNA near the periphery of the top half of the nucleosome core DNA (Figure 6C) (*30*, *31*). In further support of this model, we detected differential effects of the binding of histone H1 binding on each of the two H2A C-terminal tails. After addition of H1 the H2A C-terminal tail−DNA contacts detected to the top strand at a position ∼70 bp away from the dyad were changed to ∼80 and ∼65 bp away from the dyad. However, cross-linking near the dyad was not affected by H1 binding. These results indicate that the binding of H1 perturbs the position of only the H2A C-terminal tail emanating from the top half of the reconstituted nucleosome, which is closest to the binding site of H1 (Figure 6C).

How does H1 cause the rearrangement of H2A C-terminal tail contacts near the edge of the nucleosome? A portion of the beginning of the H2A C-terminal tail occupies a position very near the H1−globular domain binding pocket (*30*, *31*). Thus H1 binding may slightly alter the position of this tail by direct interaction with the H1 globular domain. Alternatively, the long C-terminal tail of H1 may bind the linker DNA in a position near to or overlapping that adopted by the H2A C-terminal tail (Figure 6C). Functional evidence for this interaction may be found in studies which show that the tails are important for mediating H1-dependent repression of transcription factor access to cognate sites in chromatin complexes (*19*).

The 12th amino acid residue within H2A is located at the innermost position within the N-terminal tail of this protein, just near the structure domain (*2*−*4*). Thus cross-linking at this position may be a good indicator of conformational changes within the histone octamer. This residue previously has been shown to cross-link to a single position about ∼40 bp away from the dyad axis in the presence or absence of linker DNA (*26*). Interestingly, we find that although H1 binding to the nucleosome has no effect on the *position* of this cross-link (Figure 5A, lanes 3 and 4), the *efficiency* of cross-linking is significantly decreased upon H1 binding (Figure 5B, lanes 1 and 2). Previous results from a general cross-linking method have shown that H1 binding causes a subtle and global change in the strengths of several histone−DNA contacts within the nucleosome (*33*). Our results support this suggestion that H1 binding influences the integrity of a wide variety of core histone−DNA interactions (*33*). The exact molecular basis for these effects awaits further definition of the binding of linker histones within chromatin complexes. In addition, the core histone tail domains are involved in multiple and complex functions within eukaryotic chromatin. The array of molecular interactions made by the N-terminal tails is thought to change as unfolded nucleosome arrays undergo one folding and condensation (*28*). Thus a particular arrangement of tail interactions might be mechanistically important for the maintenance of different functional states of the chromatin fiber (*27*). Posttranslational modifications or interactions with nuclear proteins such as the TUP1/SSN6 complex or the Sir2p/Sir3p complexes may serve to alter or construct a defined tail arrangement (*20*−*22*). Molecular contacts made by the C-terminal tail of H2A in complexes with true linker DNA such as reconstituted arrays of nucleosomes or after defined posttranslational modifications are presently under investigation.

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