hSWI/SNF Disrupts Interactions between the H2A N-Terminal Tail and Nucleosomal DNA†

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ABSTRACT: We have employed a site-specific core histone−DNA cross-linking approach to investigate the mechanism of hSWI/SNF remodeling of a nucleosome. Remodeling results in the complete loss of canonical contacts between the N-terminal tail of H2A and DNA while new interactions are detected between this domain and DNA near the center of the original nucleosome. The data are consistent with a model in which remodeling results in the unraveling of a region of DNA from the edge of the nucleosome, leading to a repositioning of the H2A/H2B dimer to a noncanonical position near the center of the remodeled complex. Additionally, we find that prior cross-linking of the H2A N-terminal region to nucleosomal DNA does not restrict hSWI/SNF remodeling of the remainder of the nucleosome. Thus, disruption of both H2A−DNA interactions near the edge of the nucleosome is not an obligatory step in remodeling of the remainder of the complex.

In general, chromatin structure poses a serious impediment to the binding of many trans-acting factors to eukaryotic DNA. For example, assembly of DNA sequence elements with core histone proteins into the nucleosome results in conformational and steric constraints that are incompatible with simultaneous DNA binding by most trans-acting factors (*1*, *2*). These competing histone−DNA interactions reduce the association constant of sequence-specific DNA binding proteins by a factor of 10^2-10^5, dependent upon the location within the nucleosome (*3*).

Eukaryotic cells contain ATP-dependent chromatin "remodeling" activities which serve to perturb histone−DNA interactions and facilitate the binding of sequence-specific trans-acting factors (*4*, *5*). Members of the SWI/SNF family of proteins were originally identified genetically as required for the activated transcription of a variety of genes (*6*−*9*). The SWI/SNF complex and related complexes have been biochemically purified from several organisms, including humans, and are able to catalyze the ATP-dependent remodeling of histone−DNA interactions within the nucleosome in vitro (*4*, *5*, *10*−*14*).

The mechanism by which SWI/SNF-type complexes remodel the nucleosome is not known and is currently under investigation (*15*−*21*). Nucleosomes as well as free DNA stimulate the ATPase activity of the SWI2/SNF2 subunit within this complex (*10*, *11*, *19*). It has been shown that individual nucleosomes that have been remodeled by SWI/SNF are maintained in the disrupted state even after removal of ATP and/or SWI/SNF itself (*15*, *17*). SWI/SNF remodeling of nucleosomes results in an increased susceptibility of nucleosomal DNA to digestion by DNase I or restriction endonucleases, a loss of the ability to constrain DNA supercoils, and a relaxed rotational positioning of the DNA on the surface of the histone octamer (*10*, *11*, *19*). Remarkably, significant histone−DNA interactions can still be detected within a remodeled nucleosome (*17*, *18*, *20*). Under certain conditions, remodeling results in the generation of a structure which has similar hydrodynamic properties and composition to a dinucleosome (*18*, *20*). Interestingly, a very recent report provides evidence for a SWI/SNF remodeling-induced loss of approximately 40 bp worth of histone−DNA interactions from the ends of the remodeled nucleosome (*21*).

Two nonmutually exclusive models may be proposed based on the current data: First, the histone octamer itself may be unwound, such that the DNA now adopts a more linear path but maintains the relative location and number of canonical histone−DNA interactions. This may occur by disruption of the sequential four-helical bundles (indicated by colon) linking together the essentially linear chain of dimers which comprise the histone octamer: H2A/H2B:H4/H3:H3/H4:H2B/H2A (*22*, *23*). Second, the structure of the histone octamer may be maintained with the activity of the SWI/SNF complex causing significant disruption of histone−DNA interactions. Both models could result in a relaxation of rotational positioning constraints with respect to DNA orientation on the histone surface, a decrease in the extent of DNA supercoils constrained within the nucleosome, and a decrease in the overall stability of histone−DNA interactions. The latter model is supported by recent nonspecific cross-linking experiments which suggest that disruption of histone−histone interactions is not required for remodeling

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(*17*). The former model is supported by body-labeled DNase I digestion experiments which indicate that significant, regularly spaced histone−DNA interactions persist after remodeling has occurred (*17*).

In an effort to better understand the mechanism of SWI/SNF remodeling of chromatin, we have employed a site-directed photochemical cross-linking procedure to precisely map interactions between a histone N-terminal tail domain and DNA before and after remodeling. We probed two positions at opposite ends of the N-terminal tail domain of H2A and found that canonical DNA contacts made by the tail are completely lost upon remodeling. Interestingly, we find that outermost residues of the tail contact new positions along the DNA after remodeling, near the dyad of the original nucleosome. These data support models which include significant rearrangement of histone−DNA interactions upon remodeling. In addition, the ability of the hSWI/SNF complex to disrupt nucleosomes in which the H2A N-terminal tail was cross-linked to DNA prior to remodeling was investigated in order to determine if disrupting these interactions is an obligatory step in the remodeling of the nucleosome. We find that disruption of histone−DNA interactions is inhibited only in regions near the cross-linked site while the rest of the nucleosome is still efficiently remodeled by hSWI/SNF. This suggests that nucleosome remodeling by hSWI/SNF does not proceed by a sequential mechanism which first requires disruption of both H2A/H2B−DNA interactions near the periphery of the nucleosome.

EXPERIMENTAL PROCEDURES

DNA Fragment. All experiments employed the 152 bp *Eco*RI−*Rsa*I fragment containing a *Xenopus borealis* somatic 5S RNA gene derived from plasmid pXP10 (*24*). This fragment was radiolabeled at the 5′ or 3′ ends of the *Eco*RI site on the 'top' or 'bottom' strands, respectively, as noted in the figure legends by standard techniques (*25*).

Preparation of Core Histones and Modification with 4-Azidophenacylbromide (APB). *Xenopus laevis* H2A and H2B and cysteine-substituted mutants H2AG2C and H2AA12C were expressed in bacterial cells and purified as preformed dimers as described (*25*). Approximately 5 nmol of fully reduced H2AG2C or H2AA12C was reacted with a 2-fold molar excess of 4-azidophenacylbromide (APB, Sigma) as described (*25*). 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 and Glycerol Gradient Isolation of Nucleosomes. Native core histones H2A/H2B and H3/H4 were prepared from chicken erythrocyte nuclei, and nucleosomes were reconstituted as described (*24*) except the reconstitution was scaled down 10-fold. Reconstitution with the 5S DNA fragment used in this study yields nucleosomes in which the dyad axis of symmetry (−3) is positioned near the start site for transcription of the 5S gene (+1) (*24*). Reconstitutions were loaded directly onto a 10 mL 5−30% glycerol gradient and nucleosomes sedimented at 34 000 rpm for 18 h. Fractions containing purified nucleosomes were identified by running a small portion of the samples on 0.7% agarose 1/2× TBE gels (*24*).

hSWI/SNF Reactions. The human SWI/SNF complex was prepared as described (*20*, *27*). Typical 10 μL analytical remodeling reactions included 1−2 ng of nucleosomes, 4−400 ng of hSWI/SNF (see legend to Figure 1), 12 mM HEPES, pH 7.9, 60 mM KCl, 7 mM $MgCl_2$, 15% glycerol, 0.6 mM DTT,[1] and 60 μM EDTA with or without 4 mM ATP (*11*). Reactions were incubated at 30 °C for 30 min. After the reaction was complete, 0.2 unit of DNase I was added and incubated at room temperature for 5 min. Samples were ethanol precipitated and loaded onto sequencing gels. For preparative 100 μL reactions, 40 ng of nucleosomes and 400 ng of SWI/SNF were used in the same buffer conditions used for the analytical reactions. Nucleosome complexes were irradiated for 30 s with a VWR LM20E transilluminator with the ultraviolet light source set at 365 nm after incubating with SWI/SNF and directly loaded onto SDS denaturing gels. The gel was exposed to Kodak XO-Mat AR film for 2 h, and the cross-linked complexes were gel isolated. DNA from cross-linked complexes was purified, and sites of cross-linking were visualized on autoradiographs of sequencing gels as described (*25*).

RESULTS

Reconstituted Nucleosomes Containing Site-Specifically-Modified H2A Are Competent Substrates for hSWI/SNF Remodeling in Vitro. To determine the effects of hSWI/SNF remodeling on nucleosomal DNA contacts made by the H2A N-terminal tail, we placed a photochemical cross-linking probe at either end of this domain. Mutant H2A proteins were prepared in which either the 2nd (H2AG2C) or the 12th amino acid residue (H2AA12C) was mutated to cysteine and modified with the cross-linking probe APB (see Experimental Procedures). Previous cross-linking studies with these proteins have shown that the H2A N-terminal tail contacts a stretch of about 10 bp of nucleosomal DNA located 40 bp to either side of the dyad axis of symmetry (*25*). We first demonstrated that reconstituted nucleosomes containing APB-modified H2A, native histones H2B, H3, and H4, and the labeled *Xenopus* 5S DNA fragment were competent substrates for remodeling. Previous work has shown that the regular 10−11 bp DNase I cleavage ladder from digestion of native nucleosomes is disrupted by the ATP-dependent SWI/SNF activity, indicating perturbation of rotationally phased histone−DNA interactions (*10*, *11*, *19*). As expected, the DNase I digestion of 5S nucleosomes incubated with hSWI/SNF in the absence of ATP produced a clear 10 bp ladder characteristic of a rotationally phased nucleosome (Figure 1A, lane 1). In contrast, incubation with hSWI/SNF in the presence of ATP caused a loss of the regular array of histone−DNA interactions, indicating that hSWI/SNF can disrupt a nucleosome containing the *X. borealis* 5S DNA fragment and APB-modified H2A (Figure 1A, lane 2). Titrations showed that 4 ng of SWI/SNF was sufficient to disrupt 2 ng of 5S nucleosomes (Figure 1B). This is equivalent to approximately 1 hSWI/SNF complex per 5 nucleosomes and is consistent with previous reports that SWI/SNF can act catalytically (*20*, *28*).

SWI/SNF Remodeling of Interactions between the H2A N-Terminal Tail and DNA. We next determined the fate of

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

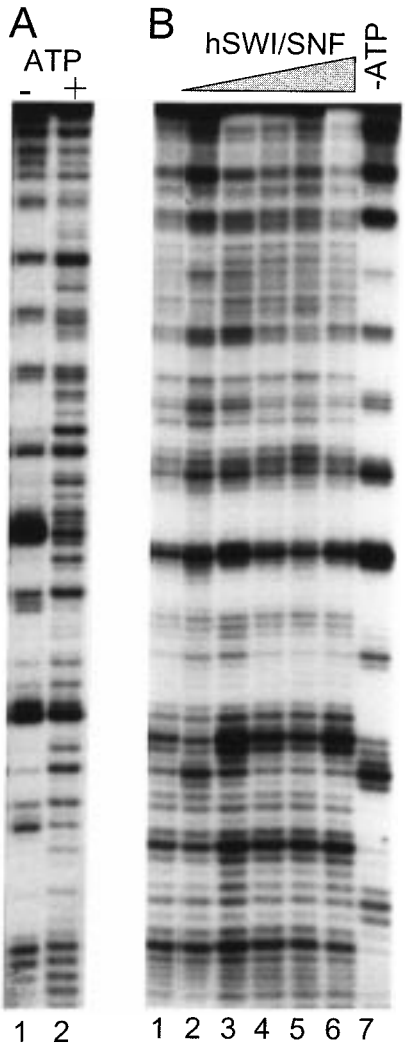

FIGURE 1: The hSWI/SNF complex efficiently remodels 5S nucleosomes. (A) 5S nucleosomes containing APB-modified H2A are competent substrates for hSWI/SNF remodeling. Approximately 2 ng of 5S nucleosomes was incubated with ~200 ng of hSWI/SNF in glycerol-containing buffer, as described under Experimental Procedures. Lanes 1 and 2 show the DNase I footprint of 5S nucleosomes incubated with hSWI/SNF in the absence and presence of ATP, respectively. Nucleosomes contained 5S DNA labeled at the 5′ end of the *Eco*RI site. (B) Titration of hSWI/SNF activity on 5S nucleosomes. Lane 1 shows the DNase I digestion pattern obtained with naked 5S DNA. Lanes 2−6 show the DNase I footprint of 5S nucleosomes (~2 ng) after incubation with 4, 20, 40, 100, 200, and 400 ng of hSWI/SNF, respectively, in the presence of ATP. Lane 7 is the footprint obtained after incubation with 200 ng of hSWI/SNF in the absence of ATP. Nucleosomes were reconstituted with 5S DNA labeled at the 3′ end of the *Eco*RI site.

interactions between the H2A N-terminal tail and DNA when the nucleosome was disrupted by hSWI/SNF. Nucleosomes were prepared containing APB-modified H2A as described above and ^{32}P-labeled DNA. These nucleosomes were incubated with hSWI/SNF in the absence or presence of ATP, and then the samples were irradiated with UV light. The nucleosomes were denatured and loaded onto an SDS gel, and the DNA was visualized by autoradiography (Figure 2). DNA that was cross-linked to protein migrated more slowly than free DNA on these gels. Interestingly, SWI/SNF remodeling had no appreciable effect on the total amount of cross-linked species for nucleosomes containing H2AG2C-APB (lanes 2 and 3) while cross-linking was reduced

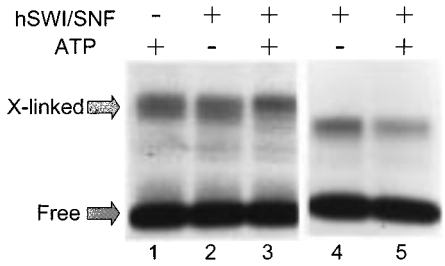

FIGURE 2: Effect of hSWI/SNF activity on the production of covalently cross-linked species. Nucleosomes were prepared as in Figure 1, incubated with hSWI/SNF in the absence or presence of ATP, and irradiated with UV light, and cross-linked species were separated on SDS gels as described under Experimental Procedures. Lane 1, UV-irradiated nucleosomes incubated without hSWI/SNF; lanes 2−5, nucleosomes cross-linked after incubation with hSWI/SNF in the absence (lanes 2 and 4) and presence (lanes 3 and 5) of ATP, respectively. Samples from nucleosomes containing H2AG2C-APB or H2AA12C-APB are shown in lanes 1−3 or 4−5, respectively.

approximately 40% in nucleosomes containing H2AA12C-APB (lanes 4 and 5). We found that the cross-linked species from remodeled nucleosomes exhibited a slightly different mobility and appearance than those from the control samples (Figure 2; cf. lanes 1 and 2 with lane 3 for H2AG2C, lane 4 with lane 5 for H2AA12C). This suggests that the hSWI/SNF caused a structural alteration of the nucleosome that resulted in the formation of a different set of cross-linked species.

We next determined the effect of hSWI/SNF remodeling on the actual positions of cross-links within the nucleosomal DNA. The cross-linked DNA was extracted from an SDS gel (Figure 2) and treated with heat and alkali to cause strand breakage at the sites of cross-linking. The DNA was then run on sequencing gels and the cross-linked position identified by autoradiography (*23*). We first determined the effects of remodeling on cross-linking to the 12th amino acid residue in H2A, located at the innermost position of the N-terminal tail, adjacent to the structured domain of this protein (*22*, *23*). Nucleosomes containing H2AA12C-APB were reconstituted, cross-linking was carried out before and after nucleosome remodeling by hSWI/SNF, and cross-linking sites were identified on sequencing gels as described above. Consistent with previous results, the major site of cross-linking found in nucleosomes containing H2AA12C-APB was detected around position +39 in 5S DNA, and the position of this cross-link was unchanged when nucleosomes were incubated with hSWI/SNF in the absence of ATP (Figure 3, lane 1; *25*). However, in the presence of ATP, hSWI/SNF activity resulted in the loss of almost all of the cross-linking at +39 (Figure 3, lane 2). We were unable to identify the location of any significant new sites of cross-linking made by H2AA12C-APB after the remodeling, perhaps because the new cross-links are much more dispersed along the DNA or occur at sites which do not result in significant base-catalyzed strand breakage. In addition, the reduction of cross-linking efficiency we find for this position as a result of the remodeling may represent a loss of a majority of the species which yield specific signals on the sequencing gels (Figure 2, lane 5).

Next we monitored the fate of cross-links formed between the second amino acid residue of H2A and DNA upon SWI/SNF remodeling. Consistent with results from the SDS gel,

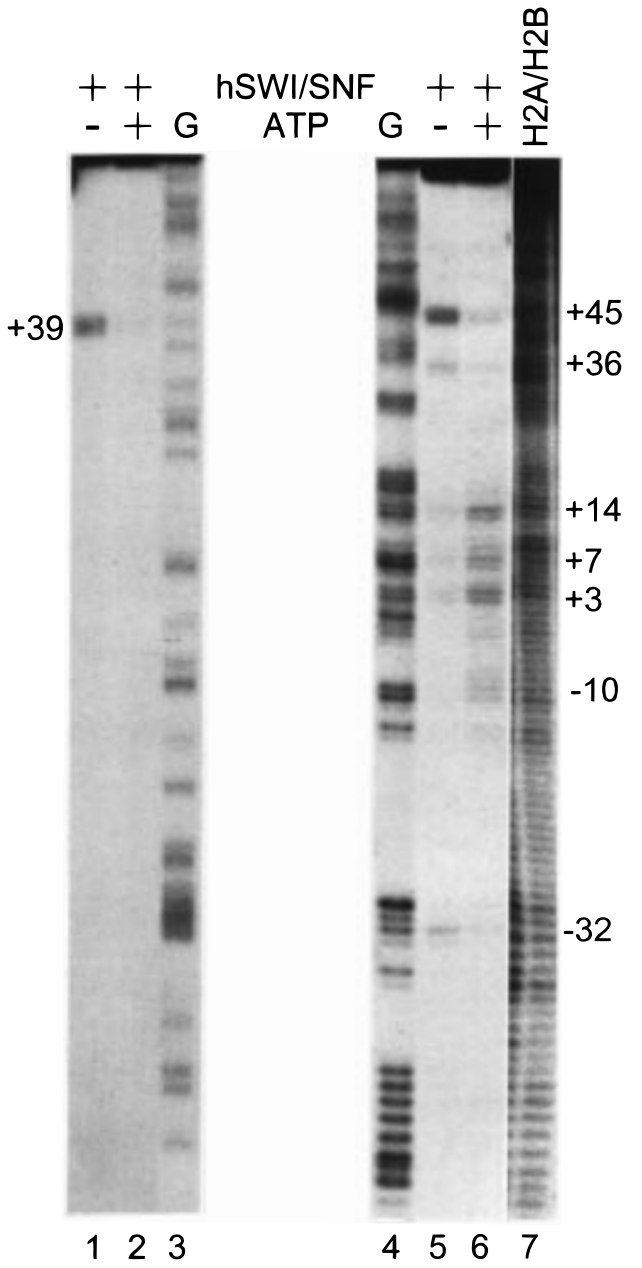

FIGURE 3: Specific interactions between the H2A N-terminal tail and nucleosomal DNA are disrupted upon remodeling by hSWI/SNF. Nucleosomes were reconstituted with APB-modified H2AG2C or H2AA12C, incubated with hSWI/SNF in the absence or presence of ATP, and irradiated, and sites of cross-linking were analyzed by autoradiography of sequencing gels as described under Experimental Procedures. Lanes 1 and 2 or lanes 5 and 6 show the positions of DNA cross-links from complexes containing H2AA12C-APB or H2AG2C, respectively. Lanes 1 and 5 or lanes 2 and 6 were cross-linked in the absence or presence hSWI/SNF remodeling activity, respectively as indicated. Lane 7 shows the cross-linking pattern obtained when the H2A/H2B dimer is bound directly to naked 5S DNA. The 'bottom' or 'top' strands of the 5S DNA fragment (*25*) were radioactively labeled either at the 5′ (lanes 1−3) or at the 3′ (lanes 4−7) end of the *Eco*RI site, respectively. Lanes 3 and 4 show G-specific reaction markers. Numbers indicate the location along the 5S sequence of major sites of cross-linking.

we found that incubation of nucleosomes containing H2AG2C-APB in the presence of hSWI/SNF but without ATP resulted in no change in the position of the cross-links compared to mock-incubated controls and previous mapping experiments (Figure 3, lane 5; ref *24*). Cross-links between this position in the tail and DNA were found at +45, +36, and −32, as expected. However, when nucleosomes were incubated with hSWI/SNF + ATP, significant changes in the cross-linking pattern were detected. The previously observed cross-links were drastically diminished, and new cross-links at +14, +7, +3, and −10 appeared (Figure 3, lane 6). The new cross-links were located in the vicinity of the dyad axis of the original nucleosome and indicate that the position of the histone H2A N-terminal tail has been drastically changed upon remodeling. (Note trace amounts of these cross-links observed in the absence of ATP may be due to trace ATP found in the SWI/SNF preparation or residual SWI/SNF activity.) Importantly, we find that cross-links formed when the H2AG2C-APB/H2Bwt dimer is bound directly to naked 5S DNA do not recapitulate the pattern found in the remodeled complex (Figure 3, lane 7). These results suggest that hSWI/SNF remodeling repositions the H2A N-terminal tail to a nonrandom but noncanonical location(s) within the disrupted nucleosome.

Effects of Cross-Linking H2A Tails to DNA on Subsequent Remodeling. The experiments described above imply that the H2A tail is moved with respect to the DNA during the remodeling reaction catalyzed by SWI/SNF. We next asked if disruption of these H2A N-terminal tail−DNA contacts is an obligatory step in the global remodeling of the nucleosome. In this experiment, nucleosomes containing APB-modified H2A were first irradiated with UV light to cross-link the H2A N-terminal tails to DNA, effectively immobilizing these domains within a subpopulation of nucleosomes. The cross-linked nucleosomes then were incubated with hSWI/SNF and ATP and subsequently treated with DNase I. After digestion, cross-linked nucleosomal DNA was separated from un-cross-linked DNA on a preparative SDS gel (see Figure 2), and the DNase I digestion patterns of the DNA that had been cross-linked prior to the SWI/SNF reaction were compared to the DNase pattern of un-cross-linked nucleosomal DNA from the same reaction (see Figure 4). An important control was to show that the cross-linking did not affect the DNase I digestion pattern of the nucleosome in the absence of SWI/SNF; we observed the canonical 10 bp ladder with these cross-linked, unremodeled nucleosomes (Figure 5, lanes 1 and 4). This indicated that the cross-linked species was generated from complexes representative of the bulk population of nucleosomes and, moreover, the cross-linking apparently did not alter the structure of the nucleosome.

We next determined if the presence of the cross-links had any effect on the ability of hSWI/SNF to remodel nucleosomes. Cross-links were made between the 2nd position (Figure 5, lane 3) or the 12th position of H2A and DNA (lane 6) prior to remodeling. Nucleosomes not cross-linked during the irradiation were efficiently remodeled by hSWI/SNF, as expected (Figure 5, lanes 2 and 5). Interestingly, treatment of cross-linked nucleosomes with SWI/SNF led to substantial remodeling, as evidenced by changes in the DNase pattern (Figure 5, lanes 3 and 6). However, a close inspection of the digestion patterns of the unremodeled (lanes 1 and 4), remodeled (lanes 2 and 5), and cross-linked/remodeled samples (lanes 3 and 6) reveals that the digestion pattern of short regions near the sites of cross-linking (see numbers) within the latter is similar to that of the unremodeled nucleosomes. Thus, the presence of the cross-links appears to inhibit remodeling only within about 5−10 bp of

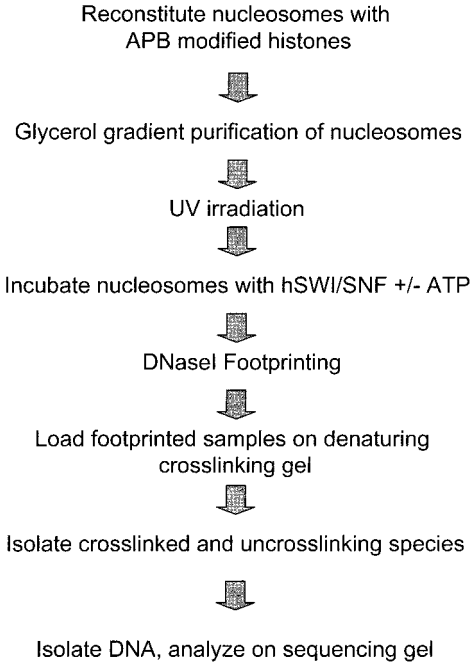

FIGURE 4: Scheme for determination of the effect of cross-links between H2A and nucleosomal DNA on the ability of hSWI/SNF to remodel the nucleosome.

the cross-link, but does not inhibit remodeling of the bulk of the nucleosome.

DISCUSSION

We have used a site-directed cross-linking method to probe histone−DNA interactions in a nucleosome before and after remodeling by hSWI/SNF. We find that SWI/SNF completely disrupts canonical interactions between the H2A N-terminal tail region and nucleosomal DNA while new interactions are detected between this tail domain and DNA near the dyad of the unremodeled complex. In addition, we find that disruption of these interactions is not a prerequisite for remodeling of the remainder of the nucleosome. These results suggest that the mechanism of hSWI/SNF involves a simultaneous perturbation of overall histone−DNA contacts which includes a drastic rearrangement of the H2A/H2B dimer.

We previously have shown that the N-terminal tail of histone H2A contacts the nucleosomal DNA ~40 bp away from the dyad (*25*). Interestingly, cross-links by H2AG2C-APB at the end of the tail are replaced by new interactions with DNA near the center of the original nucleosome. The distance between the cross-links within SWI/SNF-remodeled and native nucleosomes suggests it is unlikely that only the H2A tail domain was rearranged but rather that the entire H2A is displaced from its usual site and 'moved' a considerable distance. We estimate the distance from the original tail binding site to the new sites to be ~80 Å (*23*, *25*). Thus, even if fully extended, the 12 residue H2A N-terminal tail would be unable to reach these new sites within a native nucleosome. Moreover, since the 12th position within H2A is immediately adjacent to the structured domain of this protein (*22*, *23*) and since H2A is intimately dimerized with histone H2B, it is likely that the position of the entire H2A/H2B dimer has been significantly altered.

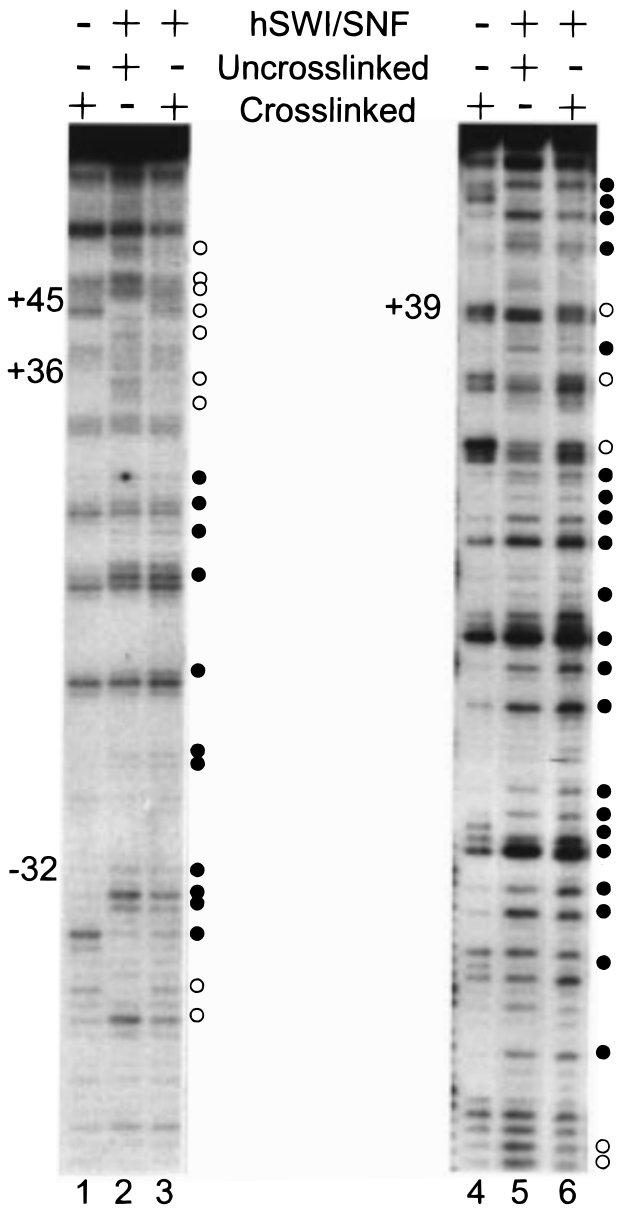

FIGURE 5: Prior cross-linking of H2A to DNA does not inhibit hSWI/SNF remodeling of the nucleosome. Nucleosomes were prepared containing either H2AG2C-APB or H2AA12C-APB and irradiated, and the extent of remodeling within cross-linked and un-cross-linked nucleosomes was determined by DNase I cleavage as outlined in Figure 4. Lanes 1 and 4 shows the DNase I cleavage pattern of cross-linked nucleosomes incubated with SWI/SNF in the absence of ATP while samples in lanes 2, 3, 5, and 6 were incubated with hSWI/SNF in the presence of ATP. Patterns from un-cross-linked nucleosomes (lanes 2 and 5) or nucleosomes cross-linked prior to remodeling (lanes 3 and 6) are as indicated. Bands in the DNase I pattern that indicate SWI/SNF-dependent disruption of histone−DNA contacts in both the un-cross-linked and the cross-linked nucleosomes are marked by filled circles. SWI/SNF-dependent changes in bands in the un-cross-linked nucleosomes that do not appear in the cross-linked nucleosomes are indicated by open circles. Numbers indicate the location of major cross-linking sites as identified in Figure 3. Nucleosomes in lanes 1−3 were assembled with H2AG2C-APB and 5S DNA radioactively end-labeled on the 'bottom strand' at the 3′ end of the *Eco*RI site while those in lanes 4−6 were assembled with H2AA12C-APB and 5S DNA radioactively end-labeled on the 'top strand' at the 5′ end of the *Eco*RI site (*25*).

Our results suggest that hSWI/SNF remodeling results in a large change in the location of the H2A/H2B dimer relative

to the DNA. This might result from either a movement of the DNA, a movement of H2A/H2B, or both. We do not know if hSWI/SNF activity has caused an equally drastic change in the contacts between the H3/H4 tetramer and the nucleosomal DNA. However, the observation that H2A/H2B cross-links made after remodeling resemble those made by H2A/H2B dimers added directly to unremodeled nucleosomes (data not shown; *30*) suggests that significant residual H3/H4 tetramer−DNA interactions persist in the vicinity of the center of the remodeled complex and influence the position of the dislocated H2A/H2B dimer. This is consistent with recent results indicating that SWI/SNF remodeling occurs without complete displacement of histones and that the remodeled complex contains regularly spaced histone−DNA interactions detectable by DNase I digestion (*17*, *18*, *20*). In addition, a very recent report provides evidence for a loss of approximately 40 bp worth of histone−DNA interactions from remodeled nucleosomes while the total mass of core histones remains unchanged (*21*). The loss of these contacts may be related to the drastic change in the location of interactions between H2A and nucleosomal DNA that are observed in the present work.

Alternatively, it is possible that the observed changes in cross-linking are due to the SWI/SNF-dependent induction of alternate translational positions of the histone octamer on the DNA. Because of the short length of the DNA fragment used in the reconstitution, to account for the observed changes the histone octamer would have to adopt largely asymmetric translational positions along the DNA that would result in the loss of approximately 30−40 bp of DNA−histone interactions from the upstream end of the nucleosome. The energetic cost of the loss of these contacts [∼20 kcal/mol (ref *29*)] could be offset by the hydrolysis of approximately 2.5 ATPs (∼8 kcal/mol) per remodeled nucleosome. However, translational repositioning would not necessarily cause the differential change in the efficiency of cross-linking between the 2nd and 12th positions of H2A and DNA observed upon remodeling (Figure 2). Additionally, prior cross-linking of these positions to DNA, which presumably would eliminate or drastically inhibit translational mobility, does not have any detectable effect on the extent of remodeling by SWI/SNF (Figure 5).

These results are consistent with a model in which the highly basic histone tail domains are responsible for tethering the displaced H2A/H2B dimer to the nucleosome after remodeling by hSWI/SNF. We find that the efficiency of cross-linking for the position at the end of the tail (2C) is unaffected by remodeling while the cross-linking efficiency at the 12th position, near the structured domain, is significantly reduced, suggesting the latter has moved some distance away from nucleosomal DNA. Indeed, Mirzabekov and coworkers have shown that after transcriptional activation of the hsp70 promoter, cross-linking of the central structured domain of the H2A/H2B dimer to DNA was markedly diminished while cross-linking of the N-terminal tail domains was maintained (*31*).

The hSWI/SNF and related complexes utilize the energy of ATP hydrolysis to generate a dynamic, somewhat unstable intermediate which can then revert back into a canonical nucleosome (*17*, *18*, *20*, *28*). Our results suggest that the H2A/H2B dimer is displaced from its original position upon SWI/SNF remodeling but still contacts DNA via the tail domains. Thus, the reversibility of SWI/SNF remodeling may be facilitated by retention of the H2A/H2B dimer near its original position within the remodeled nucleosome (*18*, *20*).

We find that the disruption of the H2A/H2B dimer−DNA interactions is not required for remodeling the remainder of histone−DNA contacts within the nucleosome (Figure 5). This implies remodeling does not occur via a mechanism which includes obligatory disruption of both H2A/H2B dimers. However, the reconfiguration of the H2A/H2B dimers without their eviction in trans may facilitate factor binding by favorably exposing DNA binding sites in vivo (*1*, *11 19*). It is interesting that cross-linking H2A to the DNA inhibits disruption of the nucleosome near the cross-linked site (Figure 5). The SWI/SNF complex is about 10 times the size of a single nucleosome (*13*), and it has been shown that SWI/SNF binds both naked DNA and the nucleosome (*18*, *20*, *32*). Although the details remain to be elucidated, it is possible that SWI/SNF interacts with DNA throughout the entire nucleosome, independently disrupting all histone−DNA interactions.

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