Human SWI/SNF Nucleosome Remodeling Activity Is Partially Inhibited by Linker Histone H1[†]

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Received June 12, 2000; Revised Manuscript Received July 18, 2000

ABSTRACT: The physical structure and the compact nature of the eukaryotic genome present a functional barrier for any cellular process that requires access to the DNA. The linker histone H1 is intrinsically involved in both the determination of and the stability of higher order chromatin structure. Because histone H1 plays a pivotal role in the structure of chromatin, we investigated the effect of histone H1 on the nucleosome remodeling activity of human SWI/SNF, an ATP-dependent chromatin remodeling complex. The results from both DNase I digestion and restriction endonuclease accessibility assays indicate that the presence of H1 partially inhibits the nucleosome remodeling activity of hSWI/SNF. Neither H1 bound to the nucleosome nor free H1 affected the ATPase activity of hSWI/SNF, suggesting that the observed inhibition of hSWI/SNF nucleosome remodeling activity depends on the structure formed by the addition of H1 to nucleosomes.

Eukaryotic DNA is structurally organized into chromatin. The basic unit of chromatin is the nucleosome, which consists of DNA wrapped around an octamer of the core histone proteins. Micrococcal nuclease digestion of histone H1 depleted chromatin gives rise to the core particle, which is defined as 146 base pairs (bp) of DNA wrapped 1.8 turns around the octamer. The chromatosome contains, in addition to the core histones, one molecule of histone H1 and an additional 20 bp of DNA (*1*). The packaging of cellular DNA into chromatin serves to compact the large genome into a small volume; however, the tight packaging presents an obstacle to any cellular mechanism that requires access to the DNA.

Histone H1 plays a major role in the higher order structure of chromatin. It has been shown to be involved in chromatin compaction; however, the function of histone H1, even after decades of research, is not completely understood (reviewed in refs *2*−*5*). Generally, histone H1 has been viewed as a protein that plays a repressive role in global transcription, yet in some cases it also has been shown to selectively affect transcription of specific genes (reviewed in ref *4* and references cited therein). The exact position of H1 on the nucleosome is controversial; it has been reported to occupy different positions on different substrates (reviewed in refs *4*−*7*). Regardless of the exact location of H1 on the nucleosome, H1 has been shown to stabilize the nucleosome by restricting the sliding of the DNA with respect to the core histones (*8*−*11*).

Recently, there has been a considerable amount of effort to identify, purify, and characterize large protein complexes that alter the structure of chromatin. One class of these complexes uses the energy of ATP hydrolysis to alter nucleosome structure (reviewed in refs *3* and *12*−*16*). The 2 MDa yeast SWI/SNF complex is the prototype of the ATP-dependent chromatin remodeling complexes and is composed of 11 different polypeptide subunits; the ATPase activity is conferred by the SWI2/SNF2 subunit (*17*−*20*). The human SWI/SNF (hSWI/SNF) complexes contain either the hBRM (hSNF2α) or the BRG1 (hSNF2β) homologues of the yeast SWI2/SNF2 ATPase subunit (*21*−*23*). Both in vitro and in vivo data suggest that SWI/SNF complexes affect transcriptional regulation by antagonizing chromatin-mediated repression. In vitro, both the purified yeast and mammalian SWI/SNF complexes use the energy of ATP hydrolysis to disrupt nucleosome structure, facilitating the binding of transcription factors or cleavage by restriction nucleases on both mononucleosomes (*18*, *24*−*26*) and on nucleosome arrays (*27*, *28*). Genetic studies in yeast have shown that ySWI/SNF is required for the transcriptional induction of a subset of yeast genes and for the function of a variety of sequence-specific transcriptional activators in vivo (*29*−*32*). The hSWI/SNF complex is likewise associated with gene activation in vivo. For example, the hSWI/SNF complex is associated with the hormone-bound glucocorticoid receptor (GR) and is required for GR-mediated gene activation and for GR-dependent changes in chromatin structure in vivo (*33*). Additionally, hSWI/SNF proteins cooperate with CEBPβ to induce specific myeloid gene expression (*34*) and facilitate the activation of the hsp70 stress response gene (*35*).

One possible explanation for how cellular machinery gains access to genomic DNA is for the nucleosomes to be transiently mobile (*36*−*38*). ATP-dependent remodeling complexes have been implicated in increasing nucleosome mobility on the DNA (*39*−*42*). In light of the evidence that histone H1 stabilizes the nucleosome against sliding along

[†] This work was supported by Grant GM56244 from the NIH and by a Scholar Award from the Leukemia and Lymphoma Society to A.N.I.

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10.1021/bi001330z CCC: $19.00
Published on Web 08/24/2000

the DNA (*8*−*11*), we investigated the effects that H1 has on hSWI/SNF nucleosome remodeling activity. All previous studies investigating the remodeling of activity of SWI/SNF complexes have used substrates lacking histone H1. We hypothesized that the stabilizing effect of H1 might inhibit the chromatin remodeling activity of hSWI/SNF. To test this hypothesis, we assembled both nucleosomes and nucleosomes with histone H1 (nucleosomes + H1) using the same DNA fragment and subjected them to hSWI/SNF-mediated remodeling. Nucleosome remodeling activity was monitored using DNase I digestion and restriction enzyme accessibility assays. Our results demonstrate that nucleosomes + H1 are remodeled more slowly than nucleosomes lacking H1.

MATERIALS AND METHODS

Purification of Histone Proteins. Chicken core particles were prepared following a previously described procedure (*43*). HeLa octamers and histone H1 were purified from asynchronously growing HeLa cells as described previously (*44*).

Purification of the hSWI/SNF Complex. hSWI/SNF complex A was purified from HeLa cells as previously described (*25*, *45*).

Nucleosome and Nucleosome + H1 Assembly. Purified DNA fragments were assembled into nucleosomes by salt dilution after incubation with purified HeLa octamers or by exchange with purified chicken core particles as described previously (*24*, *46*, *47*). For Figure 3, the *Mlu*I−*Eco*RI fragment of pTPGAL4$_1$HXh, a derivative of pTPT (*48*) in which the *Hin*dIII−*Xho*I fragment of pTPT was replaced by an oligonucleotide containing a single GAL4 binding site, was used. For all other experiments, a 216-bp *Eco*RI−*Dde*I fragment from pXP10 (*49*) was uniquely end labeled at the *Eco*RI site using Klenow *E. coli* polymerase (NEB) and [α-^{32}P]dATP (NEN). Following removal of unincorporated [α-^{32}P]dATP via a Sephadex G-50 spin column, the DNA was EtOH precipitated and dissolved in 10 μL of a 20 mM Tris, pH 7.5/1 M NaCl mixture containing either 10 μg of chicken core particles or 8.2 μg of purified HeLa octamers (quantified by Bradford assay using BSA as a standard) plus 5 μg of *Hae*III-digested pUC19 DNA. Nucleosomes + H1 were assembled in parallel with nucleosomes by adding histone H1 during the dilution process when the NaCl concentration of the reaction reached 0.6 M. The assembled nucleosomes and nucleosomes + H1 were purified from the nonassembled material by sedimentation in a 5%−30% glycerol gradient. Fractions of the glycerol gradient were analyzed by electrophoresis on a 0.5× TBE/5% polyacrylamide gel (29:1 bis) for 30 min at 150 V. To demonstrate that there was no proteolysis of any of the histone proteins during the assembly process, nucleosomes and nucleosomes + H1 were assembled, TCA precipitated, and electrophoresed on a 18% SDS−polyacrylamide gel at 35 mA for 1 h (Figure 1B).

Micrococcal Nuclease Digestions. Micrococcal nuclease (MNase) digestions were performed by incubating 21 fmol of nucleosomes or nucleosomes + H1 in a 25 μL total volume of 10 mM Tris pH 7.5, 1 mM EDTA, 1.8% glycerol, and 12 μg/mL BSA with 2.5 or 5.0 × 10^{-3} unit of MNase (Worthington) for 1, 3, or 5 min. The reactions were started by adding $CaCl_2$ to 5 mM and were stopped by taking a 4 μL aliquot from the reaction and mixing it with 50 μL of 1× TE containing 0.1 μg/μL tRNA and 50 μL of phenol/chloroform/isoamyl alcohol. Following extraction, the DNA was EtOH precipitated, dried, and then boiled in 5 μL of formamide before being electrophoresed on an 8% sequencing gel. The dried gel was exposed to a Molecular Dynamics phosphor screen. DNA size markers were created by digesting the end-labeled 216-bp *Eco*RI/*Dde*I fragment of pXP10 with *Hae*III, *Eco*RV, and *Sca*I to produce bands of 22, 144, 110, and 153 bp.

DNase I Assay. DNase I assays were performed as previously described (*24*) with minor alterations. The DNase I reactions were incubated for 2 min with hSWI/SNF and ATP and then for 3 min with DNase I at room temperature before being stopped by addition of 80 μL of a mixture of 10 mM EDTA and 50 ng/μL proteinase K (PNase K). The stopped reactions were phenol/chloroform/isoamyl alcohol extracted, EtOH precipitated, and dried, and the DNA pellet was dissolved and boiled in 4 μL of formamide containing 0.05% bromophenol blue dye. Samples were then loaded onto an 8% sequencing gel and electrophoresed at 75 mA.

Antibody Inhibition. The antibody inhibition reactions followed previously described DNase I digestion assays (*24*). The reaction components (buffer, salts, and hSWI/SNF) were preincubated for 10 min with preimmune serum or αBRG1 polyclonal antisera (*48*) prior to the addition of nucleosomes. The reactions were then incubated for an additional 25 min, after which time 0.2 unit of DNase I was added for 2 min. The reactions were stopped with EDTA and prepared for electrophoresis as described above.

EcoRV Restriction Accessibility Assay. Twenty-one femtomoles of nucleosomes was incubated in a 25 μL total volume containing 10 mM HEPES (pH 7.9), 3 mM $MgCl_2$, 2 mM $CaCl_2$, 15 mM KCl, 11 fmol of hSWI/SNF, and 20 units of *Eco*RV for 2 min prior to addition of 5 μL of 20 mM ATP that started the remodeling reaction. Reactions were timed and stopped at indicated time points by taking 4 μL of the reaction and mixing it with 50 μL of 10 mM EDTA containing 0.1 μg/μL tRNA. The samples were then extracted with an equal volume of phenol/chloroform/isoamyl alcohol, ethanol precipitated, dried, dissolved in 5 μL of formamide with dye, boiled for 5 min, and electrophoresed for 0.5 h at 150 V on 8 cm × 10 cm, denaturing 5% polyacrylamide gel (29:1 bis, 7 M urea). Band densities of no fewer than three separate experiments were quantified using ImageQuant software (Molecular Dynamics) and plotted using Excel (Microsoft). Initial rates of the remodeling reactions were determined by calculating the initial slope of a nonlinear regression curve using the equation $f = a(1 - e^{-bx})$ fit to the data with $R^2 \geq 0.995$ using SigmaPlot (SPSS Inc.) curve fitting software.

ATPase Assay. ATPase assays were performed as described (*50*). The reactions were started by mixing 1.5 μL of 6.6 fmol of hSWI/SNF in 40 mM Tris, pH 8.0, 10 mM $MgCl_2$, 0.4 mM DTT, 0.2% Tween-20, 10% glycerol, 200 μg/mL BSA, 100 μM ATP, and 10 nCi/μL [γ-^{32}P]ATP with 1.5 μL of 96 nM cofactor and incubating at 37 °C for the indicated amount of time. Cofactors were the 216-bp *Eco*RI/*Dde*I fragment of pXP10 and nucleosomes and nucleosomes + H1 assembled with the same fragment. To achieve concentrations of nucleosomes and nucleosomes + H1 that were high enough to reach optimum levels of ATPase

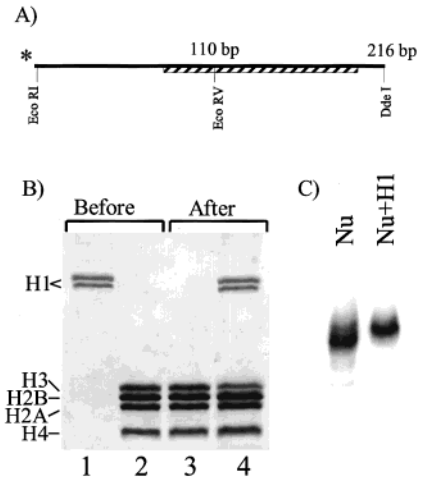

FIGURE 1: (A) Diagram of the *Eco*RI/*Dde*I fragment used to assemble both nucleosomes (Nu) and nucleosomes + H1 (Nu+H1). The hashed box designates the location of the *Xenopus* 5S rDNA coding sequence. (B) SDS−18% polyacrylamide gel of histones prior to assembly (Before) and following assembly and glycerol gradient purification (After): H1 purified from asynchronously growing HeLa cells (lane 1), core histones from chicken erythrocyte core particles (lane 2), assembled nucleosomes (Nu, lane 3), and assembled nucleosomes + H1 (Nu+H1, lane 4). (C) Native 5% polyacrylamide gel demonstrating that the assembled nucleosomes + H1 have an H1-dependent shift.

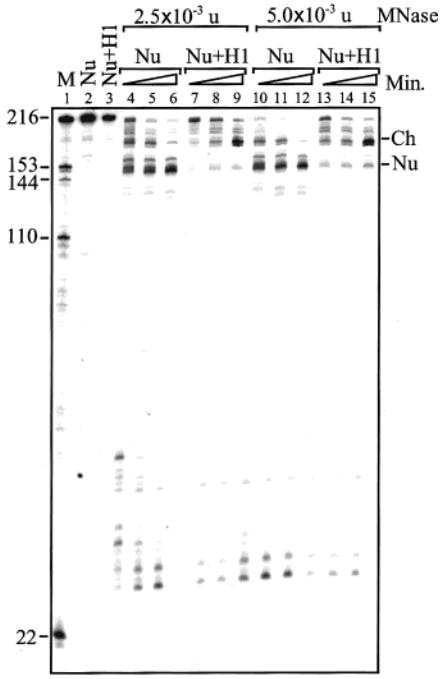

FIGURE 2: MNase digestion of nucleosomes and nucleosomes + H1. Nucleosomes (Nu) and nucleosomes + H1 (Nu+H1) were digested with 0 (lanes 2 and 3), 2.5×10^{-3} (lanes 4−9), or 5.0×10^{-3} (lanes 10−15) unit of MNase for 1, 3, or 5 min. Size markers in bp (M) and nucleosome (Nu) and chromatosome (Ch) stops are indicated.

activity, both nucleosomes and nucleosomes + H1 were not gradient purified prior to use in this assay. Portions of the reactions were stopped by spotting 0.8 μL onto a TLC plate (PEI−cellulose F, EM Science). The TLC plate was developed using 0.75 M KH_2PO_4, pH 3.5, liquid phase and exposed to a phosphor screen for visualization and quantitation.

RESULTS

Nucleosomes were assembled on a 216-bp DNA fragment containing a *Xenopus* 5S rDNA coding sequence (*51*) by the salt dilution method (Figure 1A). Nucleosomes + H1 were assembled in parallel reactions by adding purified HeLa histone H1 during the assembly process (Figure 1B). To demonstrate that the relative histone concentrations of assembled nucleosomes and nucleosomes + H1 were correct and that there was no proteolytic digestion of the histone proteins during the nucleosome assembly reaction, the purified histone proteins used in the assembly reaction as well as equal amounts of nucleosomes and nucleosomes + H1 after glycerol gradient purification were electrophoresed on an 18% SDS gel (Figure 1B). Both nucleosomes and nucleosomes + H1 were purified from the nonassembled material on 5%−30% glycerol gradients. To demonstrate the formation of both nucleosomes and nucleosomes + H1, the peak fractions of the glycerol gradient were electrophoresed on a 5% native polyacrylamide gel (Figure 1C). The nucleosomes + H1 migrated more slowly than the nucleosomes in the gel, which is indicative of a histone H1-bound nucleosome (*1*). To further demonstrate that H1 was incorporated into the nucleosomes, we performed micrococcal nuclease (MNase) stop assays (Figure 2). Nucleosomes and nucleosomes + H1 were digested with MNase for increasing amounts of time. MNase readily digests DNA that is not protected by the histone proteins; thus, the digested nucleosomes will produce a DNA fragment of approximately 146 bp and nucleosomes + H1 will generate a DNA fragment that is approximately 20 bp longer (*1*, *51*). Digestion of our assembled nucleosomes with MNase produced products that migrate between the 144- and 153-bp markers while the products from the digestion of nucleosome + H1 were about 20 bp longer. These results are in complete agreement with a previous report that used the same DNA substrate (*51*). We note that the nucleosomes digested with MNase produce a minor stop at a position that corresponds to the same position as the "chromatosome stop" (∼166 bp). By increasing the time of digestion this minor nucleosome stop diminishes concomitantly with the appearance of the nucleosome stop (∼146 bp). In contrast, digestion of nucleosome + H1 samples over the same time frame yields predominately the chromatosome stop band of ∼166 bp. Together, the results from the MNase stop assay and mobility shift assay indicate that H1 is properly incorporated onto the nucleosomes.

The chromatin remodeling activity of SWI/SNF complexes is commonly demonstrated by a DNase I accessibility assay, in which rotationally phased nucleosomes are digested with DNase I in the presence or absence of SWI/SNF (*18*, *24*, *25*). Naked DNA is cleaved by DNase I in more or less a random fashion; however, DNA that is bound to a surface, such as the core histones of a nucleosome, is partially protected from cleavage due to the exclusion of the DNase I. The 10-bp twist of DNA in the nucleosome produces a structure where the DNA is protected from the DNase I every 10 bp; therefore, DNase I will digest a population of rotationally phased nucleosomes to give a 10-bp periodic cleavage pattern. Any alterations to the position of the DNA relative to the core histones in a nucleosome will be visually detectable as an alteration in the 10-bp cleavage pattern; the

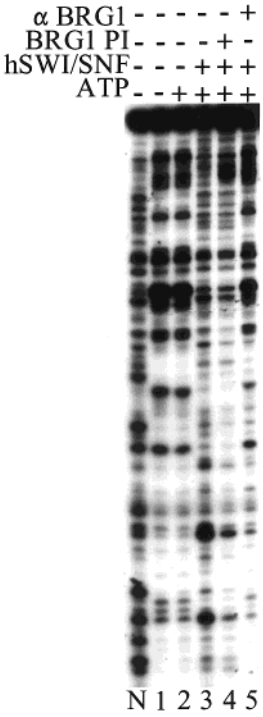

FIGURE 3: hSWI/SNF remodeling activity is inhibited by preincubation with αBRG1 antisera. Rotationally phased nucleosomes were subjected to DNase I digestion. Rotationally phased nucleosomes (lane 1) were mixed with ATP alone (lane 2) or with ATP and hSWI/SNF (lane 3). Reactions in lanes 4 and 5 were preincubated with preimmune antisera (BRG1 PI, lane 4) or αBRG1 antisera (lane 5). N is naked DNA.

DNase I digestion pattern changes to a more naked DNA-like digestion pattern.

The hSWI/SNF complex was partially purified from HeLa cell extracts using published protocols (*25*). Whether the nucleosome remodeling activity was due to a BRG1 or hBRM based complex was not determined previously (*24*, *25*, *45*). We performed antibody inhibition reactions to demonstrate that the nucleosome remodeling activity present in hSWI/SNF fraction A contains BRG1 (Figure 3). DNase I reactions containing hSWI/SNF were preincubated for 10 min with either preimmune sera or αBRG1 antisera. The results show a difference in the DNase I digestion pattern between naked DNA, nucleosomal DNA (lane1), and disrupted nucleosomes (lane 3). The 10-bp cleavage pattern of the rotationally phased nucleosomes essentially remained unchanged when hSWI/SNF was preincubated with αBRG1 antisera (lane 5) but was disrupted when hSWI/SNF was preincubated with preimmune sera (lane 4). These results demonstrate that, even though the hSWI/SNF fraction is not homogeneous, the nucleosome disruption activity present in hSWI/SNF fraction A is predominantly the result of complexes containing the BRG1 ATPase subunit.

Several different ATP-dependent chromatin remodeling complexes have been shown to promote nucleosome mobility on DNA (*39*−*42*). In contrast, histone H1 has been shown to stabilize the nucleosome structure by suppressing the translational sliding of nucleosomal DNA relative to the core histones (*8*−*11*). Thus it seems possible that histone H1 might stabilize the nucleosome and interfere with hSWI/SNF-mediated chromatin remodeling. To test this hypothesis, nucleosomes and nucleosomes + H1 were subjected to DNase I digestion after being incubated with increasing amounts of hSWI/SNF in the presence or absence of ATP (Figure 4). In the absence of ATP or hSWI/SNF the patterns of DNase I digestion on nucleosomes and nucleosomes + H1 were identical, indicating that the addition of H1 to the nucleosome did not alter the rotational phasing or the translational position of the nucleosomal DNA (Figure 4, compare lanes 1−4). This observation is in agreement with those who have used the *Xenopus* 5S coding sequence to position nucleosomes (*51*, *52*) and those who have used a different DNA sequence (*53*, *54*). The control lanes also indicated that hSWI/SNF, in the absence of ATP, was unable to disrupt nucleosomes or nucleosomes + H1 (lanes 3 and 4). In the presence of ATP, hSWI/SNF was able to disrupt both the nucleosomes and nucleosomes + H1 although to different degrees. The differences in remodeling appear as more intense DNase I cleavage patterns in the nucleosome lanes when compared to the nucleosome + H1 lanes (Figure 4, compare nucleosome lanes 6−8 to the nucleosome + H1 lanes 9−11). In the experiment shown in Figure 4, the remodeling reactions were stopped after 3 min to capture the most visible difference in remodeling between nucleosomes and nucleosomes + H1. Increasing the reaction time allowed remodeling of nucleosomes and nucleosome + H1 to proceed to completion, which made visualization of the differences more difficult (data not shown). To demonstrate the differences between the digestion patterns for nucleosomes and nucleosomes + H1 more clearly, lanes 8 and 11 were scanned and the plots overlaid. It is apparent from the overlaid scans that the areas under the peaks produced from DNase I digestion of nucleosomes are greater than those from nucleosome + H1 (marked by arrows). Scans of lanes 6, 7, 9, and 10 were also prepared, and the respective bands were overlaid and compared as described; the results were similar except that the peak areas were smaller due to the diminished band density (data not shown).

Qualitative changes in the remodeling activity of hSWI/SNF can be readily monitored by DNase I digestion assays; however, quantitative differences in the remodeling activity of hSWI/SNF are more difficult to demonstrate by the DNase I assay. To more rigorously determine the difference in the ability of hSWI/SNF to remodel nucleosomes and nucleosome + H1, we used a restriction accessibility assay that makes use of the unique *Eco*RV restriction site inside the 5S rDNA coding sequence of the template (Figure 1A). Our initial experiments demonstrated that *Eco*RV was occluded from its restriction site but that the site became more accessible for cleavage with the addition of hSWI/SNF and ATP to the reaction (data not shown and Figure 5B). Subsequently, nucleosomes and nucleosome + H1 were incubated with *Eco*RV and hSWI/SNF in the absence or presence of ATP for increasing amounts of time (Figure 5). The full-length and digested DNA products were separated on a 5% denaturing polyacrylamide gel (Figure 5A). The results show that the 110-bp restriction product accumulates faster in the gel where nucleosomes (middle gel) were digested when compared to the gel where nucleosome + H1 were digested (bottom gel), indicating that the *Eco*RV restriction site becomes available more rapidly on the nucleosome substrate than it does on the nucleosome + H1 substrate. This experiment demonstrates that the presence of histone H1 inhibits the rate at which hSWI/SNF can remodel the nucleosomes to make the *Eco*RV site accessible.

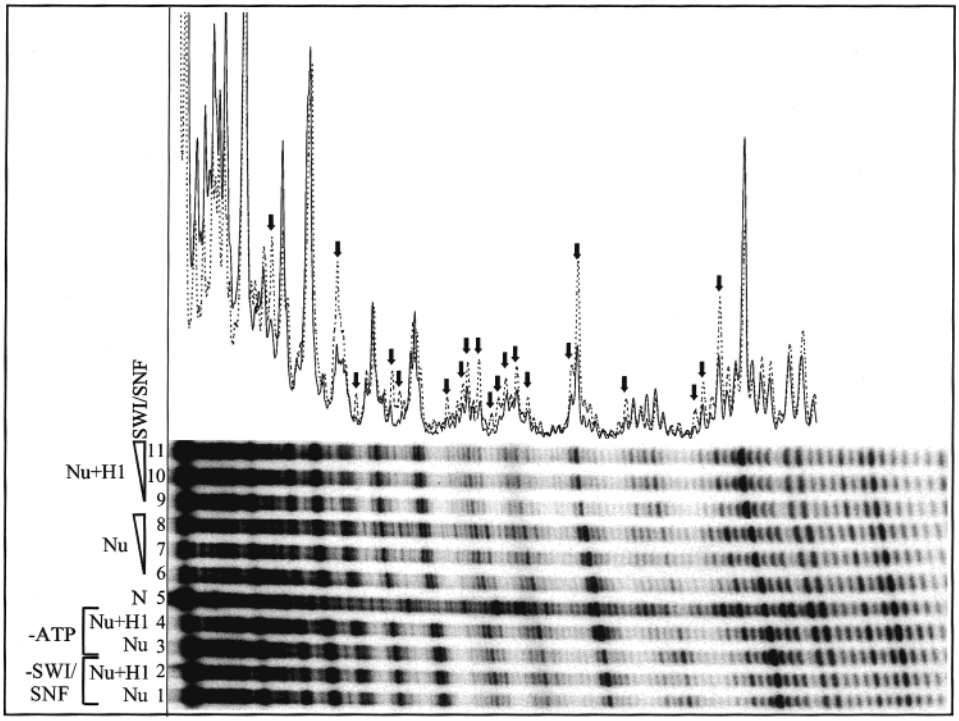

FIGURE 4: H1 partially inhibits hSWI/SNF-mediated nucleosome remodeling demonstrated by DNase I digestion of rotationally phased nucleosomes and nucleosomes + H1. Nucleosomes (Nu) and nucleosomes + H1 (Nu+H1) were digested with DNase I in the presence of increasing concentrations of hSWI/SNF (lanes 6−11). The molar ratio of substrate to hSWI/SNF used was 4:1, 2:1, and 1:1 in lanes 6−8 and 9−11, respectively. DNase I digestion patterns of rotationally phased nucleosomes exhibit a characteristic 10 bp repeat (lanes 1−4) when compared to digested naked DNA (N, lane 5). To quantify the differences in DNase I digestion between Nu and Nu+H1, scans of lanes 8 (dotted) and 11 (solid) were overlaid. For control experiments, both Nu and Nu+H1 were digested with DNase I without ATP or hSWI/SNF (lanes 1−4).

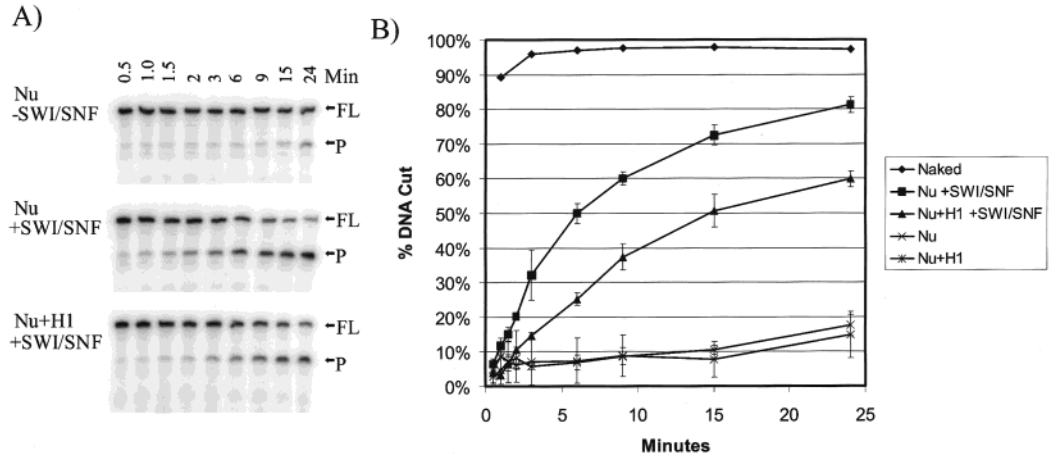

FIGURE 5: *Eco*RV endonuclease accessibility assay demonstrates that chromatosomes are remodeled more slowly than are nucleosomes. (A) Nucleosomes (Nu) and nucleosomes + H1 (Nu+H1) were incubated with *Eco*RV in the presence of a 2:1 molar ratio to hSWI/SNF for the time indicated. The 110 bp cleaved product (P) was separated from the 216 bp substrate on a denaturing 6% polyacrylamide gel. (B) The band intensities of the full-length DNA (FL) and the product (P) were quantified and plotted. Control experiments include the digestion of naked DNA and reactions with no hSWI/SNF.

To determine the difference in the remodeling rates, the band densities of the full-length and digested products of no fewer than three separate experiments were quantified and plotted (Figure 5B). The initial rates of the remodeling reactions were determined by fitting the data with a nonlinear regression curve and calculating the initial slope of that curve. The initial slopes indicated that hSWI/SNF remodels 10% of the nucleosomes in the reaction every minute. The nucleosomes + H1 are remodeled more than 2-fold less efficiently, with only 4.8% of the nucleosomes + H1 being remodeled per minute. In our reactions there was a 2:1 molar ratio of substrate to hSWI/SNF. Calculating the rates on a mole basis, one hSWI/SNF complex can remodel one nucleosome every 5.2 min or one nucleosome + H1 every 10.9 min. Control experiments demonstrated that *Eco*RV digested naked DNA completely in less than 3 min, while in the absence of hSWI/SNF or ATP the nucleosomes and nucleosome + H1 were not digested to any great extent; the *Eco*RV site on both substrates became accessible at a rate of 0.3% of the substrate in the reaction per minute (Figure 5B and data not shown). The difference in the remodeling rates between the nucleosomes and nucleosome + H1 implies

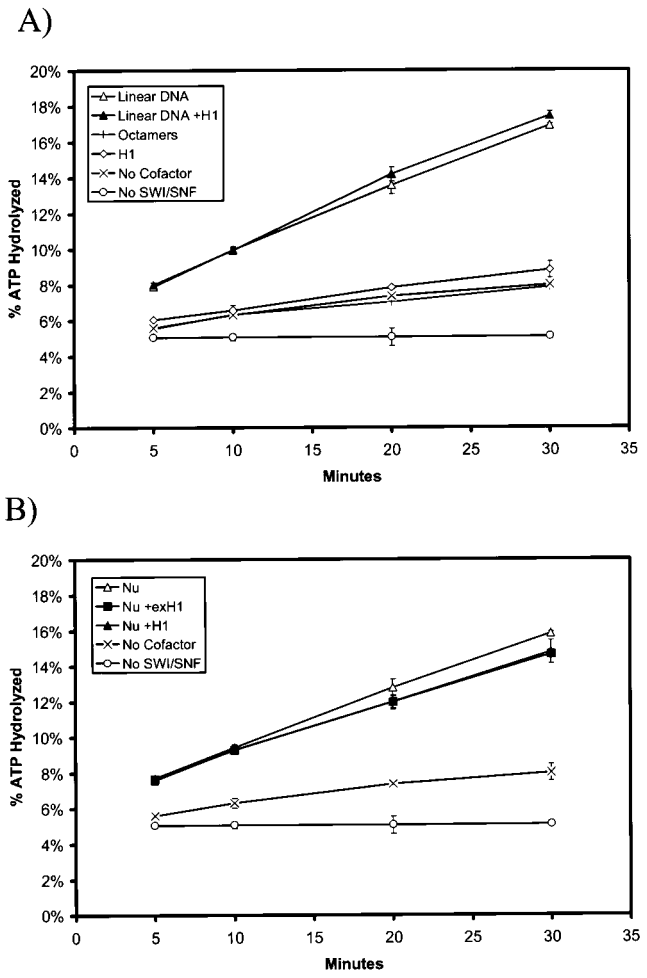

FIGURE 6: Histone H1 incorporated into a nucleosome or free in solution does not inhibit the ATPase activity of hSWI/SNF. (A) DNA alone (open triangle) stimulates ATP hydrolysis. Addition of free H1 (filled triangle) to the reaction does not affect the rate. Neither H1 alone (open diamond) nor histone octamers (+) stimulate the rate of ATP hydrolysis above the basal level (×) for hSWI/SNF. (B) Nucleosomes (open triangle) stimulate ATP hydrolysis by hSWI/SNF; however, H1 added exogenously to the reaction (filled square) or assembled into nucleosomes + H1 (filled triangle) does not inhibit the rate of ATP hydrolysis.

that histone H1 confers to the nucleosome some degree of stability against the remodeling activity of hSWI/SNF.

It has been previously demonstrated that the remodeling activity of the hSWI/SNF complex relies on the hydrolysis of ATP (*25*). It is plausible that the inhibition seen in the remodeling assays could be due to some interaction of H1 with the hSWI/SNF complex and have little to do with the structure formed when H1 binds to a nucleosome. To assess whether H1 alone can inhibit the activity of hSWI/SNF, we analyzed the ATPase activity of the hSWI/SNF complex in the presence of different cofactors containing or lacking H1. The ATPase activity of SWI/SNF family members from different species can be stimulated with a variety of DNA cofactors (*18*, *25*, *55*). We determined that stimulation of the ATPase activity of hSWI/SNF by linear DNA remained unaltered upon the addition of equal molar H1 (Figure 6A). We also demonstrated that the ATPase activity of hSWI/SNF was not stimulated by core histones or histone H1 alone, since the rate of ATP hydrolysis in the presence of these proteins was identical to that of hSWI/SNF alone. Addition of nucleosomes or nucleosomes + H1 stimulated the ATPase activity of hSWI/SNF to the same degree as naked DNA. Furthermore, H1 added exogenously to the nucleosome-stimulated reaction had no effect (Figure 6B). In all of the experiments shown, we used reactions containing 48 nM cofactor because this was the lowest concentration of cofactor needed to maximally stimulate the ATPase activity of hSWI/SNF. Lower concentrations of cofactors (from 24 to 1 nM) gave comparable results, though the rates of ATP hydrolysis were diminished (data not shown). These results indicate that H1 incorporated into a nucleosome or free in the reaction did not inhibit the ability of hSWI/SNF to hydrolyze ATP. This strongly suggests that the inhibition of remodeling monitored in the previous assays is due to the structure that H1 confers to the nucleosome.

DISCUSSION

The structure of chromatin is a barrier for any cellular process that requires access to genomic DNA. Large protein complexes can use the energy of ATP hydrolysis to disrupt chromatin structure (*3*, *12*, *13*, *15*, *16*) and may function by loosening the histone−DNA contacts. Loss of these contacts might then make the nucleosomes more mobile (*39*−*42*). One antagonist to nucleosome mobility is the linker histone H1 (*8*−*11*). We have demonstrated by both DNase I and restriction enzyme accessibility assays that the hSWI/SNF chromatin remodeling activity is reduced on nucleosomes that contain H1. Because the presence of histone H1 is the only difference between the two substrates and because H1 does not inhibit the ATPase activity of hSWI/SNF, we postulate that addition of H1 to the nucleosome forms a structure that is more resilient to the remodeling activity of hSWI/SNF.

The observation that hSWI/SNF hydrolyzes ATP at the same rate in the presence of either nucleosomes or nucleosomes + H1 yet remodels nucleosomes + H1 more slowly than nucleosomes suggests that ATP hydrolysis may be functionally uncoupled from the remodeling of chromatin structure. Thus the stimulation of ATPase activity is apparently insensitive to the addition of H1 even though the presence of H1 partially inhibits the consequence of the ATP hydrolysis reaction. Observations uncoupling ATPase and nucleosome remodeling activities have been made previously for both yeast and human SWI/SNF complexes. Boyer et al. found that the ATPase activity of yeast and human SWI/SNF complexes, as well as of other classes of ATP-dependent remodeling enzymes, could be stimulated by array templates assembled with H3−H4 tetramers, yet these templates were inefficiently remodeled or not remodeled at all (*56*). We conclude that ATP hydrolysis by these enzymes, while necessary for remodeling, is not necessarily sufficient to alter all chromatin templates. The actual structure of the chromatin template or the manner in which SWI/SNF binds to the substrate appears to play a role as well.

Our data indicate that hSWI/SNF remodels nucleosomes + H1 at slightly less than half the rate that nucleosomes are remodeled. Because histone H1 is a determinant of and promotes the formation of higher order chromatin structure on nucleosomal arrays (*57*−*59*), the 2-fold inhibition of hSWI/SNF activity observed on a mononucleosome template might be additive or perhaps even further amplified on higher order chromatin substrates. At present, we are unable to analyze hSWI/SNF function on nucleosome arrays containing

linker histone due to the requirement of at least 2 mM Mg^{2+} for hSWI/SNF activity (unpublished results) and the propensity of nucleosomal arrays containing linker histone to oligomerize and condense in the presence of Mg^{2+} (*58*). Carruthers et al. reported that a 208-12 nucleosomal array saturated with linker histone H5 sedimented at ~57 S and condensed to a conformation of ~30 nM in 0.65 mM $MgCl_2$. Bulk oligomerization of the template occurred at Mg^{2+} concentrations beyond 0.65 mM (*58*). Further advances in techniques used to analyze hSWI/SNF function and/or assemble nucleosome arrays containing H1 in the presence of Mg^{2+} will be necessary to test the effects of linker histone on hSWI/SNF remodeling on multinucleosome templates.

Our calculations indicate that each hSWI/SNF complex can remodel a nucleosome every 5.2 min. This correlates precisely with the previously reported rate of 4.5 min per nucleosome that was determined for yeast SWI/SNF on an 11-mer nucleosome array template (*27*). The striking similarity in the nucleosome remodeling rate by yeast and human SWI/SNF complexes suggests that these complexes act in mechanistically similar ways. Previous data on both yeast and human SWI/SNF complexes have demonstrated qualitative similarities between the two in that both complexes can alter the DNase I digestion pattern of rotationally phased mononucleosomes, stably maintain the altered pattern after removal of ATP, alter the topology of chromatin assembled on closed circular DNA, and facilitate transcription factor binding to nucleosomal DNA (*18*, *24*, *25*, *42*, *60*). More recent studies comparing the activities of yeast and human SWI/SNF on nucleosomal arrays directly demonstrate that ATPase and nucleosome remodeling rates of these complexes are quantitatively similar, providing strong evidence that the mechanism by which ATP-dependent chromatin altering disruption occurs is well conserved (*56*).

Finally, inhibition of hSWI/SNF chromatin remodeling activity by H1 suggests that remodeling may be productive only on substrates that have lost the stabilizing effect of H1. Thus, hSWI/SNF may be active on a limited subset of genes or loci where the stabilizing effect of H1 is compromised by modification or removal. It has already been demonstrated that the yeast SWI/SNF complex is required for regulation of only a subset of yeast genes (*29*−*32*, *61*). Similarly, the requirement for hSWI/SNF ATPase proteins, BRG1 and hBRM, also appears to be specific to certain genes and specific signaling pathways. For example, BRG1/hBRM interaction with C/EBPβ induces some, but not all, myeloid genes (*34*). Similarly, BRG1 and hBRM contribute to the induction of the hsp70 gene by chemical stress while other genes induced by the same stresses apparently do not require hSWI/SNF (*35*). This line of reasoning implies that hSWI/SNF functions via a targeting mechanism, perhaps being targeted concomitantly with elements or factors that remove or alter the activity of histone H1. Alternatively, as has recently been shown, SWI/SNF complexes could be targeted to specific loci via direct interaction with specific transcriptional regulatory proteins (*34*, *62*−*67*), perhaps at a time following events that modify or displace H1. We suggest that hSWI/SNF is not functioning as a random nucleosome remodeling machine; rather it may function at precise loci that have lost or modified linker histones. In support of this idea, it was recently shown that the activity of P/CAF, a nucleosome acetyl transferase enzyme, is inhibited on H1 containing chromatin (*68*), perhaps suggesting that different classes of chromatin remodeling machines may require loss or modification of linker histones in order to function.

The alteration in chromatin structure observed in assays developed to discern changes in chromatin structure due to ATP-dependent remodeling are all manifested as changes in the position of the DNA relative to the core histones. These changes can be described as rotational or translational changes of the DNA with respect to the core histones or a change in the path as the DNA traverses the surface of the nucleosome (reviewed in ref *12*). As mentioned, H1 has been reported to inhibit the translational sliding of nucleosomal DNA on the histone core. Thus, this could be a possible explanation for the inhibition of hSWI/SNF remodeling activity that we have observed. It is also plausible that the mode of interaction between H1, the DNA, and the core histones prevents or inhibits the rotational changes in the DNA with respect to the core histones or inhibits the DNA from changing its path around the core. Regardless of the mechanism used by hSWI/SNF to disrupt the nucleosome, we have demonstrated that H1 inhibits the hSWI/SNF remodeling activity. Further study to elucidate the function of H1 and its subtypes on mononucleosomes and higher order chromatin substrates and how they function with respect to ATP-dependent and independent chromatin remodeling mechanisms will likely yield insight into how the cell controls and regulates the packaging of its genetic information.

ACKNOWLEDGMENT

We thank K. Knight, C. Peterson, and members of the Peterson laboratory for discussion and comments.

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BI001330Z