Linker Histone H1 Modulates Nucleosome Remodeling by Human SWI/SNF

Received for publication, August 14, 2003, and in revised form, September 22, 2003
Published, JBC Papers in Press, September 25, 2003, DOI 10.1074/jbc.M30903200

Aruna Ramachandran, Mahera Omar‡, Peter Cheslock§, and Gavin R. Schnitzler¶

From the Department of Biochemistry, Tufts University School of Medicine, Boston, Massachusetts 02111

Chromatin, a combination of nucleosomes and linker histones, inhibits transcription by blocking polymerase movement and access of factors to DNA. ATP-dependent remodeling complexes such as SWI/SNF and RSC alter chromatin structure to increase or decrease this repression. To further our understanding of how human SWI/SNF (hSWI/SNF) “remodels” chromatin we examined the octamer location, nature, and template specificity of hSWI/SNF-remodeled mononucleosomes when free or bound by linker histone H1. We find that, in the absence of H1, hSWI/SNF consistently moves nucleosomes to DNA ends, regardless of template sequence. On some sequences the repositioned histone octamer appears to be moved ~45 bp off the DNA edge, whereas on others it appears to be normal, suggesting that the nature of the remodeled nucleosome can be influenced by DNA sequence. By contrast, in the presence of histone H1, hSWI/SNF slides octamers to more central positions and does not promote nucleosome movement off the ends of the DNA. Our results indicate that the nature and position of hSWI/SNF products may be influenced both by DNA sequence and linker histone, and shed light on the roles of H1 and hSWI/SNF in modulating chromatin structure.

The basic unit of chromatin is the nucleosome core particle, composed of two copies each of the four core histones H2A, H2B, H3, and H4, which wrap ~146 bp of DNA into a left-handed superhelix. The presence of nucleosome cores on DNA blocks access to sequence-specific DNA binding factors by steric inhibition, with histone-DNA contacts occluding regulatory sites. It also inhibits movement of RNA polymerase II (1, 2). In addition to core histones, the chromatin of metazoan somatic cells also contains linker histones, such as H1, which are present at a ratio of approximately one per nucleosome. H1 binds to the nucleosome core, protecting ~10 bp of both the entering and exiting DNA, to form a structure termed the chromatosome. H1 is primarily thought to be involved in transcriptional repression. For instance, in Xenopus development the expression of H1 causes the repression of oocyte-specific 5S rDNA genes (3, 4). However, H1 has also been shown to be involved in transcriptional activation at certain promoters (5–7). H1 binding is known to promote the folding of chromatin into more compact structures, decrease nucleosome mobility, influence nucleosome positions, reduce the binding of some sequence-specific factors, inhibit core histone tail acetylation, and inhibit certain activities of chromatin remodeling complexes (8–10). It is unclear, however, exactly how each of these effects is related to transcriptional regulation.

One of the mechanisms cells use to modulate the repressive effects of chromatin, is to employ a family of ATP-dependent chromatin remodeling complexes. Each of these complexes has important functions in the activation and/or repression of different subsets of genes. Mutations in genes encoding remodeling complex components often result in alterations of the chromatin structure of target genes in vivo (11–13). One activity that appears to be shared by all remodeling complexes is the ability to translationally reposition histone octamers on DNA (14). Different complexes, however, can produce differentially positioned products (which is true even for some complexes that contain the same catalytic ATPase subunit) (15). Nucleosome repositioning away from or over regulatory sites in chromatin is likely to be an important aspect of remodeling complex function, and differences in repositioning specificity likely contribute to the unique functions of each complex. Several important questions about octamer repositioning by ATP-dependent remodeling complexes remain unanswered, however, such as the importance of DNA sequence and structure in determining remodeled octamer positions and the effect of linker histones on repositioning.

In addition to the translational repositioning of normal nucleosomes, many remodeling complexes (particularly members of the SWI/SNF subfamily) are capable of creating stable, structurally altered nucleosomal products (11, 16). This is evidenced by a reduction of the number of nucleosome-constrained negative supercoils, without apparent nucleosome loss, on circular polynucleosome templates (11, 17), and by the reduced stability of remodeled polynucleosomal arrays to surface deposition in atomic force microscopy studies (18). Specific altered forms of mononucleosomes have also been characterized. The first such product to be identified is a structurally altered non-covalent mononucleosome dimer (11, 16). In addition, recent reports have shown that yeast and human SWI/SNF complexes can create structurally altered mononucleosome monomers in which both entering and exiting DNA ends appear to be held by the same histone octamer to create a loop structure (19, 20). Altered nucleosomal products could be important for SWI/SNF complex function, because altered dimers have been shown to be more accessible than normal nucleosomes to sequence specific transcription and recombination factors (21, 22). The ability of certain complexes to form struc-
H1 Modulates Octamer Repositioning by hSWI/SNF

EXPERIMENTAL PROCEDURES

DNA Fragments—The 215-bp original (+) template, 215-bp +100, 265-bp, and 315-bp DNA templates were all generated by PCR using the plasmid pXP10 (27) as the template. The 215-bp original fragment was amplified using primers at the EcoRI and DdeI sites, at positions −79 and +137, respectively, relative to the start site of transcription of the SS rRNA gene. The 265-bp long, was derived from pXP10 using an upstream primer at −181 and a downstream primer at +34. The 265-bp template was generated using an upstream primer at −128 and the downstream DdeI primer at +137; the 315-bp fragment was amplified using the upstream −181 primer and the downstream DdeI (+137) primer. The 215-bp GSE4 template was generated from the pSSGE4 plasmid (28), using primers at positions −177 and +36 relative to the E4 promoter start site. The DNA fragments were radiolabeled by 5′ kinase end-labeling either the upstream or downstream primer before adding it to the PCR reaction. Full-length PCR products were purified from agarose gels using the QIAquick gel extraction kit (Qiagen), ethanol-precipitated, and resuspended in TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) at 2 A. Ramachandran and G. R. Schnitzler, unpublished observations.

Protein Purification—H1 used in this study was purified from HeLa cells by hydroxylapatite chromatography, essentially as described (29). The H1 preparation appeared to be from HeLa cell core histones (32) in 220 volumes of dialysis buffer (100 mM Tris-HCl, 0.2 mM phenylmethylsulfonyl fluoride; 0.4 mM mg/ml BSA, 60 mM KCl, 5 mM MgCl2, 0.6 mM DTT, 0.12 mM PMSF, and, where indicated, 2 mM ATP plus 2 mM additional MgCl2 (ATP chelates Mg2+), and the additional MgCl2 maintains the free Mg2+ concentration at 5 mM). Differences in these conditions are described in the figure legends (e.g. the nucleosome concentration in the syn experiments to be 100 pm or below). Thus, stoichiometric H1 binding often required H1:nucleosome ratios of greater than one as indicated by gel shift and chromatosome stop appearance, Fig. 2. ~300 ng of hSWI/SNF was then added, and reactions were incubated for 1 h at 30 °C and stopped by stripping hSWI/SNF and H1 from the template using competitor polymonosomes (0.9–1.8 μg) and plasmid DNA (1–2 μg). We found that identical results were seen whether H1 was preincubated with nucleosome cores before SWI/SNF addition or added at the same time as SWI/SNF. The reactions were resolved by EMSA on 5% acrylamide gels in 0.5× TBE at 4 °C for ~2.5 h after which the gel was dried and subjected to PhosphorImager analysis (AP BioTech). An alternative method for the formation of chromatosomes is to add H1 midway through the assembly process (at NaCl concentrations between 0.6 to 0.7 M). This method could not be used for studies of nucleosomes at specific positions, because the gel mobility shift due to binding H1 prevented the separation of differentially positioned H1-bound nucleosomes by EMSA and 2) treatment of any singly positioned nucleosome at 0.7 M NaCl caused decoulization of the octamer in the three positions favored during assembly (e.g. see Ref. 36 and data not shown). We do find, however, that chromatosomes formed by this method show hSWI/SNF-dependent repositioning similar to those induced by a H1 addition in repositioning buffer. For nucleosomal analysis, nucleosomes were incubated for the indicated times at 60 °C in the absence of hSWI/SNF or stop solution and resolved by EMSA as above. Modifications to these general methods are noted in the figure legends. We have found that there is little change in hSWI/SNF activities when 0.1% Nonidet P-40 is added (Refs. 21 and 37 and data not shown).

Micrococcal Nuclease Analyses—For Fig. 2, the indicated concentrations of H1 were titrated into 25-μl reactions containing 0.3 ng of purified, positioned mononucleosomes (83 pm) in standard remodeling conditions (above), except that the buffer contained 0.1% Nonidet P-40, and 30 mM KCl. The tubes were incubated at room temperature for 10 min, which CaCl2 was added to a final concentration of 1 mM, followed immediately by the addition of the 0.59 unit of MNase (Sta., St. Louis, MO). The reaction was stopped after 5 min by the addition of 25

1 The abbreviations used are: hSWI/SNF, human SWI/SNF; BSA, bovine serum albumin; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; EMSA, electrophoretic mobility shift assay; Exo III, exonuclease III; ISWI, imitation switch; MNase, micrococcal nuclease.
Histone H1 Causes hSWI/SNF to Move Nucleosomes to Central Positions—Because H1-containing chromatin is likely to be the *in vivo* substrate for mammalian remodeling complexes, it is critical to understand how H1 affects the remodeling reaction. Previous studies have examined the effect of H1 on yeast and human SWI/SNF-mediated changes in the DNase pattern of mononucleosomes and enhancement of restriction enzyme accessibility on mononucleosomes and polynucleosomal arrays (9, 10). To better understand how hSWI/SNF might modulate the structure of H1-containing chromatin in *in vivo*, we wished to determine the effect of H1 on hSWI/SNF-facilitated nucleosome repositioning and altered dimer formation. Unlike the core histones, H1 can bind specifically to preformed mononucleosome cores at physiological salt concentrations (34–36). This allowed us to study the effect of hSWI/SNF on single-position nucleosomes bound by H1 (see “Experimental Procedures”). We titrated HeLa cell H1 into reactions containing purified, positioned nucleosomes to establish conditions for chromatosome formation. Stoichiometric binding of H1 to cores causes a distinct gel mobility shift (e.g. see Refs. 34 and 36), which we observe at between 50 and 85 pmol H1 (Fig. 2B, compare Core band to Chromatosome (Chrom.) band). Similar results were seen for each of the input nucleosome positions U, M, and L (data not shown). Properly bound H1 protects ~10 bp of DNA on either side of the nucleosome core from digestion with MNase, resulting in a “chromatosome stop” band of ~166 bp compared with the “nucleosome core stop” band at ~146 bp (e.g. see Refs. 36 and 45). We observe a chromatosome stop band at between 43 and 85 pmol H1 (Fig. 2C). At higher concentrations, multiple H1 proteins can bind to each nucleosome resulting in a greater mobility shift, observed as a smear toward the wells (*asterish in Fig. 2B*) and protection of a larger DNA fragment from MNase (*uncut band in Fig. 2C*).

We find that H1 can dramatically alter the octamer positions of hSWI/SNF-remodeled mononucleosomes. Remodeling reactions were carried out in the presence of increasing concentrations of H1, followed by addition of competitor DNA and polynucleosomes (which removes both hSWI/SNF and H1, allowing the resolution of nucleosome core positions). Control
reactions showed that incubation of nucleosomes with H1 alone, or in the presence of hSWI/SNF without ATP, followed by removal of H1 to competitor DNA resulted in no change in nucleosome positions (Fig. 2A, compare lanes 1 and 2, and data not shown). In the presence of both hSWI/SNF and ATP, we found that reactions that contained H1 at 50 pM or above showed an increase in slow migrating bands (primarily U, but also some M), loss of the input low band (L), and no generation of fast migrating remodeled low (RL) and lowest (RL) products (Fig. 2A, compare lanes 3, 11, and 19 with lanes 6, 14, and 22). This suggested that H1 could alter nucleosome repositioning by hSWI/SNF. Similar results were obtained regardless of the position of the input nucleosome (Fig. 2A, panels labeled Lower, Middle, and Upper). This effect appears to be due to the specific binding of H1 to nucleosome cores, because the alteration of hSWI/SNF products, appearance of the chromatosome stop, and H1 binding to cores by EMSA all occur at the same H1 concentrations (between 50 and 85 pM H1; Fig. 2, compare...
A–C). Similar effects on repositioning were also seen at H1 concentrations up to 350 pM, indicating that additional nonspecific interactions of H1 with template nucleosomes did not prevent the chromatosome-specific effect (Fig. 2A). Much higher concentrations of H1 (1.2 nM and above) did inhibit all nucleosome repositioning by SWI/SNF. At these concentrations, however, H1 induces template aggregation under our reaction conditions, resulting in the formation of a pellet after a brief microcentrifugation (“pelleting assay” essentially as per Ref. 46, data not shown).

At concentrations of up to 120 pM, H1 did not significantly inhibit the formation of bands at the position of altered nucleosome dimers (Fig. 2A, “Δ”). Dimer mobility was somewhat less in the presence of H1, however, suggesting perhaps that the mobility of dimers can be influenced by octamer positions of constituent mononucleosomes. Although repositioning was not inhibited by H1 concentrations up to 350 pM, dimer formation was inhibited at H1 concentrations over 120 pM, suggesting that these two hSWI/SNF activities are differentially sensitive to nonspecific H1 binding (Fig. 2A).

Previous studies have shown that some aspects of chromatin remodeling can be inhibited by H1, although they did not specifically examine the effect of H1 on nucleosome sliding or dimer formation (9, 10). To determine if H1 inhibits either of these hSWI/SNF activities, we examined the time course of remodeling for L input nucleosomes at a moderately low H1 concentration of 51 pM, allowing us to observe the formation of H1-preferred U and M as well as non-H1-preferred RI products in the same remodeling reaction. We found that there was relatively little octamer movement up to 10 min, at which time both H1-preferred (U and M) and non-H1-preferred (RI) products begin to accumulate (Fig. 2D, lane 7). RI also begins to accumulate at this time in reactions lacking H1 (Fig. 2D, lane 6). The rate of formation of putative altered dimers (Di) was also not significantly inhibited by H1. Together, these results indicate that histone H1 binding to form a chromatosome does not inhibit the rate of repositioning and altered dimer formation by hSWI/SNF on mononucleosomes, but, instead, alters the preferred octamer position resulting from hSWI/SNF action.

Mapping of Nucleosome Positions Using Exonuclease III—

Our results indicate that hSWI/SNF, in the absence of H1, causes mononucleosomes to move to the ends of the DNA. However, the EMSA assays used above cannot reveal which end the nucleosome is on. They also cannot reveal whether the products, in the presence or absence of H1, were structurally normal nucleosomes. To answer these questions, we used exonuclease III digestion to map the nucleosome boundaries of input nucleosomes and all major hSWI/SNF products. Exo III is a 3′ to 5′ exonuclease that requires a double-stranded DNA template. On bare DNA it will chew in from each 3′-end until no double-stranded DNA is left, producing a mixture of single-stranded fragments about half the length of the original DNA (see Fig. 3, A and B, lanes 1 and 2). The presence of a nucleosome will block Exo III digestion, and the size of the resulting protected fragment is indicative of nucleosome position. When the top strand of a double-stranded DNA is 5′-end-labeled, the length of the protected DNA will define the right edge of the nucleosome (see Fig. 3B, diagram). Conversely, 5′ labeling the lower strand and digesting with Exo III will define the left edge of the nucleosome (see Fig. 3A, diagram). Theoretically, for a normal nucleosome the distance between these marks will be 146 bp. In practice, however, the distance between these marks is often 10 to 20 bp shorter, because the propensity of DNA at the edge of the nucleosome to unpeel from the octamer surface can facilitate Exo III overdigestion (39).

To map octamer positions before and after remodeling, nucleosomes were assembled onto the 215-bp SS rDNA template with either the top (upstream/EcoRI end) or the bottom (downstream/Ddel end) strands 5′-labeled by PCR, using the respective end-labeled primers. Isolated upper, middle, and lower nucleosome bands from each assembly were subjected to hSWI/SNF remodeling in the presence or absence of histone H1, after which hSWI/SNF and H1 were removed to competitor DNA, and the products separated by EMSA. Bands for each major hSWI/SNF product and controls were excised from each lane and subjected to Exo III digestion, and the resulting DNA fragments were resolved by denaturing urea-PAGE. For each nucleosome species, the location of principal cutting sites from the top-labeled and bottom-labeled templates were then used to determine the major translational position of the nucleosome on the template. For instance, for the control Lower band, the 145 bp between the top strand cut (at +66) and the bottom strand cut (at −79) maps the primary octamer position of this EMSA band to the left edge of the nucleosome (see Fig. 3, A and B, lane 5, and C, top right). Similarly, the primary positions of the control Middle and Upper bands map further from this edge, toward the middle of the fragment. Note that, some bands (notably +66 for top strand-labeled templates and −6 for bottom strand-labeled templates, see “Δ” symbol) appeared to be favored sites for Exo III digestion of nucleosomes and were generated, to some degree, from all controls and products. These favored sites do not result from octamer repositioning during electrophoresis, because when each band is eluted from the gel its position in subsequent EMSA analysis is unchanged (data not shown). These sites were deemed to be real nucleosome boundaries only if they were the most intense Exo III product and were −146 bp away from the primary cutting site on the other strand. In general, we found paired major top and bottom strand sites to be separated by 136–145 bp, consistent with the presence of a normal nucleosome, and a moderate degree of Exo III overdigestion. Control Middle nucleosomes, however, appeared to have only 120 bp between major cutting sites. Additional experiments using tiritations of Exo III indicate that the first major cut on the bottom strand is really at +87 (dashed arrow in Fig. 3C; data not shown) but that this is followed by a rapid subsequent digestion to the Exo III-favored site at +66. MNase digestion also indicates that control Middle nucleosomes protect the normal −146 bp of DNA (see Fig. 4A below). The positions measured for control nucleosome are consistent with other reports on the location of the Xenopus somatic 5S ribosomal DNA nucleosome positioning sequences, which are expected to localize the nucleosome at or near the upstream edge of our template (26, 47).

H1-favored hSWI/SNF Products Are Similar to Control Upper and Middle Nucleosomes—Incubation with histone H1 in the absence of hSWI/SNF, followed by removal of H1 to competitor DNA, did not alter the nucleosome positions for any of the control nucleosomes, as observed by EMSA and Exo III digestion analysis (Fig. 3, A and B, compare lanes 5 and 6, Fig. 2A, and data not shown). When the bands produced by hSWI/SNF action in the presence of H1 (primarily U, as well as some M, Fig. 2A) were treated with Exo III, it revealed a cutting pattern very similar to control Upper and Middle nucleosomes, respectively (Fig. 3C, and compare lanes 3 and 9 in Fig. 3, A and B, and data not shown). Combined with the EMSA experiments, these results indicate that hSWI/SNF and H1, together, tend to result in movement of the histone octamer to the more central of the positions favored by the 5S nucleosome positioning sequences, but do not otherwise alter the nucleosome.

hSWI/SNF Products Formed in the Absence of H1 Differ from Input Nucleosomes—Exo III mapping of hSWI/SNF products in
the absence of H1 revealed two striking results. First, the remodeled Lower band ("RL", which comigrates with control lower, "L", by EMSA) had a histone octamer primarily positioned on the downstream end of the DNA, in contrast to the control Lower nucleosome where the octamer was positioned at the upstream end (Fig. 3; and Fig. 3, A and B, compare lanes 5 and 7). This indicates that hSWI/SNF does not simply move a nucleosome to the nearest DNA end but that it can move an octamer that was positioned on one end up to 70 bp to the opposite end. By contrast, the remodeled lowest band (RL) gave full protection of the bottom strand (suggesting a nucleosome positioned on the upstream end) but only protected 100 bp of the top strand from Exo III (Fig. 3; and Fig. 3, A and B, lane 8). This was not due to overdigestion by Exo III, because the same result was observed at several concentrations of enzyme (data not shown). These results indicate that the remodeled lowest band is an altered nucleosome in which the histone octamer has either been shifted off the DNA edge by H1 to 40 to 45
of DNA from MNase digestion. Accordingly, when control Middle and Lower bands are digested with MNase the principle product is ~146 bp (Fig. 4A, lanes 2 and 3). The protected fragment from the hSWI/SNF-remodeled lower product (RL) was also ~146 bp, indicating that the histone:DNA contacts in this product are essentially normal (Fig. 4A, lane 4). The purified remodeled lowest product (RL), however, protected only ~100 bp of DNA from MNase digestion (Fig. 4A, lanes 4 and 5), consistent with the Exo III mapping results. The observation that 100 bp of DNA in RL is protected from both exonuclease and endonuclease action suggests that this length of DNA is in relatively tight association with histone. The size of this protected region is also consistent with that observed in cross-linking studies of a similar product formed by yeast SWI/SNF (19).

**All hSWI/SNF Products Appear to Contain the Full Complement of Core Histones—Tetramer nucleosomes containing only histones H3 and H4, and hexamers lacking one copy of H2A and H2B, run faster than octamer particles in EMSAs (48).** To test whether the remodeled hSWI/SNF products (RL and RL) were complete octamers, scaled-up 215-bp 5S rDNA nucleosome assemblies were treated with hSWI/SNF in the presence or absence of ATP, and nucleosome core products resolved by EMSA (Fig. 4B, upper panels). Note that hSWI/SNF products of the same mobilities as previously observed were formed even though these reactions contained 34 nm nucleosomes that were in 2.4-fold excess over hSWI/SNF. We also see similar repositioning effects when nucleosomes (as HeLa poly nucleosomes) are in ~20-fold excess over hSWI/SNF (data not shown). This indicates that hSWI/SNF acts catalytically to reposition nucleosomes to DNA ends and that this result is unaffected by the hSWI/SNF:nucleosome ratio. The region containing mononucleosome bands from each lane was then cut out, and the gel slice was rotated 90° (Fig. 4B, top panels), before being directly loaded onto a 17% SDS-polyacrylamide gel to resolve the core histones and DNA (Fig. 4B, bottom panels). The competitor polynucleosomes used to remove hSWI/SNF run at the top of the EMSA gel and are absent from the mononucleosome region isolated. The SDS-PAGE gel of the control reaction in the absence of ATP, where remodeling did not occur, showed the four core histone bands at the U, M, and L positions, but no bands in the remodeled lowest position (bottom left panel). Bands for all four core histones were also observed for the remodeled Lower band (RL) at similar relative intensities to controls (bottom right panel), showing that even though the histones in this product have been moved to the end of the template opposite the nucleosome positioning sequences, their stoichiometry is unchanged. The remodeled lowest band also appears to contain all four core histones. However, because of the relatively low abundance of RL in this scaled-up reaction, we cannot rule out the possibility that this product is a histone octamer, i.e. it has lost one H2A/H2B dimer. We think that this is unlikely, however, because re-addition of H2A and H2B dimers did not restore band RL to the L gel shift position, as has been shown for other hexamer particles (data not shown (48)). This conclusion is also supported by the recent observation that a similar product can be formed by yeast SWI/SNF action even when the histones in octamers have been cross-linked to each other with dimethyl suberimidate (19). Together, these data suggest that the remodeled lowest band is a monomeric nucleosomal species that contains a complete histone octamer but has altered histone:DNA contacts and that the ability to create this type of altered product is evolutionarily conserved in the SWI/SNF subfamily of remodeling complexes.

**Remodeled Lowest Cannot Be Further Repositioned by hSWI/SNF—**The accumulation of the remodeled lowest product as a
result of hSWI/SNF action might indicate either 1) that it is a terminal product that can no longer be acted upon by hSWI/SNF or 2) that it is merely one of the most highly favored remodeled positions and thus accumulates at any given point in the ongoing remodeling reaction. To test these models, the hSWI/SNF-generated Rl nucleosome was purified and again subjected to hSWI/SNF action. In the absence of hSWI/SNF or ATP, no change in the mobility or percent of bare DNA in Rl fractions was observed, even after incubation at 30 °C for up to 24 h (Fig. 5A, lane 1, and data not shown). Thus, despite protecting only ~100 bp of DNA from nuclease, Rl did not appear to be inherently unstable. Even when treated with hSWI/SNF and ATP, no change in Rl band mobility was observed, suggesting that hSWI/SNF was not capable of moving the histone octamer from this position (Fig. 5A, lanes 2 and 3). Interestingly, hSWI/SNF appears capable of forming altered dimer-like products from Rl (Fig. 5A, lane 3, “Di.”), indicating that the absence of translational movement of Rl was due to complete inhibition of hSWI/SNF enzyme activity. For control nucleosomes, H1 and hSWI/SNF together promote movement to the center of the template (Upper and Middle bands) and block the formation of Rl (Fig. 5B, lanes 1–3, and see Fig. 2A). Thus, if Rl was still an effective substrate for hSWI/SNF, its translocation ~45 bp off the upstream end of the template was dependent on DNA sequences, then deletion of 100 bp of DNA from the downstream end and addition of 100 bp to the upstream end would generate a template on which hSWI/SNF action would force the octamer to the middle of the template (the +100 template, see Fig. 6A). If formation of the altered nucleosome Rl was also dependent on these sequences, then the centrally located nucleosome that was formed would be altered. Also, if the position to which hSWI/SNF moved the octamer in the presence of H1 was sequence-dependent, we would expect hSWI/SNF and H1 to now generate fast migrating nucleosomes with octamers bound to the downstream end of the +100 template. Alternatively, if repositioning was primarily due to non-sequence-dependent structural properties of DNA ends, remodeling of the +100 template would give similar results to remodeling of the original +0 template.

We generated this +100 template by PCR from the pXP10 plasmid (which contains additional upstream 6S rDNA sequence), assembled it into mononucleosomes and isolated each of the three major assembly products from EMSA bands. Each of these was then remodeled by hSWI/SNF in the presence or absence of H1 (Fig. 6B). Our results show that, in the absence of H1, hSWI/SNF generates primarily fast migrating bands on this template (+100L), whereas in the presence of H1 hSWI/SNF generates primarily slow moving bands (+100U, +100M). This suggests that, on short mononucleosome templates at least, DNA ends strongly influence the hSWI/SNF-favored position of nucleosome cores and chromatosomes. Remodeling of the +100 template, however, did not generate any gel shift band migrating faster than control nucleosomes, suggesting that the formation of altered nucleosomes might depend on DNA sequence, as was also indicated by the observation that Rl only formed on the upstream end of the 215+0 template.

To further examine the sequence specificity of hSWI/SNF-dependent repositioning, we generated mononucleosomes from a 215-bp non-homologous sequence. Recently, we used atomic force microscopy and restriction enzyme accessibility assays to characterize nucleosome repositioning by hSWI/SNF on the polynucleosomal template 5S5E4. Those studies indicated that a nucleosome core protecting the unique XbaI restriction site (within the 430-bp promoter/transcription cassette of the 2.5-kb construct) was moved away by hSWI/SNF, resulting in a stable increase in XbaI digestion (18). We used PCR to generate an end-labeled 215-bp template surrounding this XbaI site (G5E4) and assembled this into nucleosomes. The two major assembly products (G5E4 U and G5E4 L, Fig. 6C, lanes 1 and 4) were eluted from EMSA bands. Exo III mapping of the most abundant of these assembly products (U), revealed a centrally positioned octamer with the pseudodad near the XbaI site, suggesting that the preferred octamer position on this sequence is the same for mononucleosomes and polynucleosomes (Ref. 18 and data not shown). The results of hSWI/SNF remodeling of this template show that, similar to the 5S 215+0 template, treatment of either input band with hSWI/SNF in the absence of H1 results in an accumulation of the lower band (G5E4 RL) and also an even faster migrating lowest band (Fig. 6C, G5E4 RL). The appearance of a “Remodeled lowest” band suggests that formation of altered products is not unique to the upstream edge of the 5S 215+0 template. Treatment of either input band with both hSWI/SNF and H1 also had a similar effect on G5E4 as it did on the 5S rDNA template, causing hSWI/SNF to move nucleosomes to the slowly migrating band position, U. These results indicate that end positioning in the absence of H1 and more central
positioning in the presence of H1 are sequence-independent properties of hSWI/SNF action.

**End Positioning Still Occurs on Longer Templates**—The tendency of hSWI/SNF to move nucleosomes to DNA ends might be expected to be weaker if the ends were further from the original octamer position. Thus, we reasoned that, on longer mononucleosome templates, preferred internal positions for remodeled octamers might be observed. We used PCR to extend the 5S rDNA 215-bp template 50 or 100 bp upstream without changing the downstream end (265 and 315 templates, see Fig. 6A) and assembled these templates into mononucleosomes. For 265, we isolated each of the three major assembly bands (U, M, and L) by EMSA. The 315 template is long enough to allow dinucleosome formation during assembly, and we used glycerol gradient ultracentrifugation to separate the mixture of 315 mononucleosomes from both dinucleosomes and bare DNA. As for the 215 template, treatment of these longer mononucleosomes with hSWI/SNF resulted primarily in the accumulation of fast migrating bands and the loss of slow migrating, centrally positioned, input bands (Fig. 7A, lanes 3, 6, 9, and 12, black arrows). Some of these remodeled bands migrated faster than any of the bands from assembly, suggesting the genera-
H1 Modulates Octamer Repositioning by hSWI/SNF

**DISCUSSION**

**hSWI/SNF Moves Mononucleosomes Toward DNA Ends**—Our findings establish that the tendency of hSWI/SNF to move octamers to DNA ends is a general property of the complex, independent of initial octamer position, DNA sequence, and template length. Together with other studies, this suggests that the movement of nucleosomes toward DNA ends is a common property of all members of the SWI/SNF class of ATP-dependent remodeling complexes (18, 19, 26, 51). hSWI/SNF action may favor end-positioned octamers, because the complex recognizes ends and specifically moves nucleosomes toward them. Alternatively, ends may be favored because either 1) they are the lowest energy binding sites for remodeled nucleosomes or 2) end-positioned nucleosomes can no longer be productively repositioned by the complex. This latter possibility may well be the case for the remodeled lowest product, which appears to be resistant to further repositioning by hSWI/SNF (Fig. 5).

Previous studies show that hSWI/SNF can also reposition central nucleosomes on polynucleosomal arrays (18) and repositioning in the absence of DNA ends is likely to be important for the transcriptional regulatory functions of hSWI/SNF in vivo. The observed tendency toward end positioning on mononucleosomes indicates that fully understanding the nature and specificity of repositioning by hSWI/SNF will require analysis of nucleosomes far from DNA ends, such as at the center of polynucleosomal arrays. The present work, however, does suggest some likely characteristics of hSWI/SNF-dependent nucleosome repositioning in vivo. For instance, the comparison of hSWI/SNF-remodeled nucleosomes to thermally repositioned nucleosomes indicates that repositioning by hSWI/SNF can ignore nucleosome positioning sequences. In this way, hSWI/SNF action may oppose that of other remodeling complexes,
like the ISWI-containing NURF (Nucleosome Remodeling Factor) complex, which has been shown to move nucleosomes to heat treatment favored sites (42). Repositioning away from naturally favored sequences could be important for the attenuation of transcription after removal of an activating signal, in that nucleosomes moved to non-natural sites might snap back to their original, preferred positions over time, re-establishing a repressive chromatin state.

**Generation and Position of Altered Nucleosome Monomers**—We find that some hSWI/SNF products appear to be complete histone octamers that have moved about 45 bp off the edge of the DNA, resulting in DNA:histone contacts that protect only 100 bp of DNA from exonuclease and endonuclease digestion. A very similar product was seen in site-specific cross-linking studies of the mononucleosomal products of yeast SWI/SNF (19) and in MNase digestion studies of hSWI/SNF-remodeled mononucleosomes (20). A less dramatically altered product that protects only about 125 bp of DNA from MNase digestion is also created by the ISWI ATPase (52). Intriguingly, previous studies with yeast and human SWI/SNF found that altered nucleosomes were formed on both ends of the template. Using a somewhat longer, 215-bp template, however, we find that hSWI/SNF generates normal nucleosomes on one end of the DNA and “off the edge” nucleosomes on the other. This indicates that, although a tendency toward end positioning is a sequence-independent property of hSWI/SNF, the nature of the repositioned products can be influenced by underlying DNA sequence or sequence-dependent features. It has been proposed that the altered product formed by yeast and human SWI/SNF is stabilized by having exiting DNA wrap back onto vacated sites on the histone octamer surface, forming a loop of DNA between the normal sites for entering and exiting DNA. If this were the case for RI, there would be no place for exonuclease III to enter the structure, and only full-length DNA would be recovered. Alternatively, the DNA that wraps back to form a loop might be in relatively weak or dynamic association with the histone octamer, allowing Exo III to push past this looped DNA to give the 100-bp protection that we observe. The formation of an intramolecular DNA loop may also help to explain why the RI species is so stable, despite having only about 100 bp of Exo III- and MNase-resistant histone-DNA contacts.

**H1 Alters hSWI/SNF-favored Nucleosome Positions**—We find that addition of H1 can switch hSWI/SNF action from favoring end positions to favoring central positions. This effect is first observed at H1 concentrations that give a first gel shift band and a chromatosome stop band. Thus, we feel that it is likely to be due to the specific binding of H1 to form chromatosomes. In addition, if H1 were simply inhibiting repositioning by nonspecifically coating the DNA, this should result in inhibition of repositioning from all octamer locations, which we do not see (e.g., L is converted to M and U). Finally, we see similar results when chromatosomes are first formed by adding H1 at the 0.6 M NaCl step during nucleosome assembly, and nucleosome concentrations are kept well above the apparent Kₜ for H1 binding (data not shown). This method of assembly was not ideal, however, because it gives a mixture of upper, middle, and lower nucleosomes, preventing the analysis of hSWI/SNF products from defined nucleosome positions (see “Experimental Procedures,” under “Remodeling Assays”).

One possible explanation for this effect of H1 might be that by binding to about 10 bp of both entering and exiting DNA (as suggested by structural studies (53)) H1 stabilizes nucleosomes that are at least 10 bp in from the edge of the DNA. In support of this, we find that when H1 is added during nucleosome assembly the percentage of low band nucleosomes is reduced (data not shown). The favorable free energy of proper H1 binding, especially at nucleosome positioning sequences, might overwhelm the mechanistic or energetic considerations that promote end positioning by hSWI/SNF. Alternatively, H1 bound to entering and exiting DNA to form a chromatosome might inhibit further repositioning by SWI/SNF (e.g. by blocking access). This latter theory would predict that, in the presence of H1, hSWI/SNF would move an end-positioned nucleosome to the first internal site with enough flanking DNA to allow chromatosome formation. We find, however, that hSWI/SNF action on input lower nucleosomes (at the left edge) generates some middle (about 35 bp from the left edge), but primarily upper nucleosomes (about 45 bp from the left edge), suggesting that nucleosome repositioning in the presence of H1 is not just a matter of incremental sliding until the octamer is far enough from the DNA ends. It is possible, however, that nucleosomes already in H1-preferred positions are refractory to further repositioning by SWI/SNF. This is suggested by the observation that even though hSWI/SNF treatment of the H1-bound lower band generates some middle as well as upper bands, the same treatment of upper generates very little middle band (see Fig. 2A).

We do not see a strong inhibitory effect of H1 on repositioning or altered dimer formation. However, the ability of H1 to alter repositioning specificity might help explain how H1 decreases the effects of SWI/SNF complexes in other assays. H1 was shown to inhibit the ability of hSWI/SNF to alter the DNase digestion pattern and increase the restriction enzyme accessibility of mononucleosomes by about 3-fold (9). If “off the edge” nucleosomes were partially responsible for these changes, the ability of H1 to block RI formation could lead to decreased hSWI/SNF activity in these assays. H1 was also shown to greatly inhibit the ability of yeast and human SWI/SNF to increase restriction enzyme accessibility at a single, central restriction site on polynucleosomes (10). Our results raise the possibility that H1 might alter hSWI/SNF repositioning specificity on polynucleosomes, which could favor octamers positioned over any given restriction site, resulting in decreased accessibility.

The results presented here are the first indication that a non-sequence-specific chromatin-associated factor, H1, can also influence the specificity of nucleosome repositioning. In contrast, a recent study showed that HMGB1, a “linker histone replacement” protein linked to transcriptional activation, increased the rate of repositioning by the ISWI ATPase or two complexes based on it, ACF (ATP-utilizing Chromatin Assembly and Remodeling Factor) and CHRFC (Chromatin Accessibility Complex), but did not change the resulting octamer location (54). Taken together, these observations suggest that different chromatin-associated proteins might regulate transcription by differentially modulating chromatin remodeling complex action. The observation that some remodeling complex activities are inhibited by H1 has suggested a model by which chromatin can be remodeled only if histone H1 is first modified or removed (9, 10). Our work suggests a novel, non-exclusive model for the regulatory interactions between linker histones and remodeling complexes; that H1 may alter the nucleosome structures and/or positions resulting from remodeling complex action. By causing hSWI/SNF (and potentially other chromatin remodelers) to shift nucleosomes to sites on chromatin that differ from those preferred in its absence, H1 could play a critical role in revealing or masking regulatory sequences in order to regulate transcription in vivo.

**Acknowledgments**—We thank Adrienne Boire for purification of H1, Lisa Colgan for help with remodeling assays using the G5E4 template, and Molly Brown for technical support. We also thank Bob Kingston, Geeta Narlikar, Jaya Yodh, and Natalia Ulyanova for their comments on the manuscript.
Aruna Ramachandran, Mahera Omar, Peter Cheslock and Gavin R. Schnitzler

Linker Histone H1 Modulates Nucleosome Remodeling by Human SWI/SNF

J. Biol. Chem. 2003, 278:48590-48601.
doi: 10.1074/jbc.M309033200 originally published online September 25, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M309033200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 54 references, 15 of which can be accessed free at
http://www.jbc.org/content/278/49/48590.full.html#ref-list-1