Molecular Cell, Vol. 16, 439–452, November 5, 2004, Copyright ©2004 by Cell Press

DNA Instructed Displacement of Histones H2A and H2B at an Inducible Promoter

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Summary

Regulation of gene expression requires dynamic changes in chromatin, but the nature of these changes is not well understood. Here, we show that progesterone treatment of cultured cells leads to recruitment of progesterone receptor (PR) and SWI/SNF-related complexes to Mouse Mammary Tumor Virus (MMTV) promoter, accompanied by displacement of histones H2A and H2B from the nucleosome containing the receptor binding sites, but not from adjacent nucleosomes. PR recruits SWI/SNF to MMTV nucleosomes in vitro and facilitates synergistic binding of receptors and nuclear factor 1 to the promoter. In nucleosomes assembled on MMTV or mouse rDNA promoter sequences, SWI/SNF catalyzes ATP-dependent sliding of the histone octamer followed only on the MMTV promoter by displacement of histones H2A and H2B. In MMTV nucleosome arrays, SWI/SNF displaces H2A and H2B from nucleosome B and not from the adjacent nucleosome. Thus, the outcome of nucleosome remodeling by SWI/SNF depends on DNA sequence.

Introduction

Regulation of gene expression in eukaryotes is usually associated with increased nuclease sensitivity of chromatin over gene regulatory regions such as enhancers and promoters. These changes reflect the action of chromatin-remodeling complexes that make the DNA more accessible for transcription factors by either modifying the core histone tails or by using the energy of ATP hydrolysis to change the chromatin structure. There is a large variety of such ATP-dependent chromatin-remodeling complexes, which contain a catalytic subunit with ATPase activity and a variable number of additional subunits with poorly defined functions. In general, the mechanism of action of these chromatin-remodeling complexes has been studied with artificial promoters or chimeric DNA constructs. Based on these studies, a variety of mechanisms have been proposed for nucleosome remodeling, including sliding of the histone octamer (Fazzio and Tsukiyama, 2003; Kassabov et al., 2002; Jaskelioff et al., 2000 ; Whitehouse et al., 1999), looping out of the DNA (Kassabov et al., 2002), partial or total displacement of core histones (Boeger et al., 2003; Bruno et al., 2003; Reinke and Horz, 2003), changes in DNA topology (Gavin et al., 2001), and the formation of dinucleosome transition states (Schnitzler et al., 1998). The outcome of these processes is a remodeled structure with altered histones-DNA interactions (Kassabov et al., 2003). Despite these detailed mechanistic studies, little attention has been paid to the possible influence of the nucleotide sequence of the nucleosomal DNA on the mechanism or the outcome of the remodeling process. Moreover, with a few exceptions (Fazzio and Tsukiyama, 2003; Reinke and Horz, 2003), these studies have been made in vitro. Very little is known about the action of chromatin remodeling enzymes in the chromatin context within intact cell or about the influence of the DNA sequence on the remodeling process.

To start investigating these issues, we have chosen to study gene activation by steroid hormones, because suitable model systems are available in cultured cells and cell-free systems. Steroid hormones regulate gene expression by virtue of their binding to intracellular receptors, which in turn interact with hormone responsive elements (HREs) and other transcription factors at promoter or enhancer regions of inducible genes and activate transcription by recruitment of coactivator complexes and the general transcriptional machinery (Beato et al., 1995). It is well established that gene induction by glucocorticoids and estrogens depends on the action of ATP-dependent remodeling complexes (Muchardt and Yaniv, 1993; Yoshinaga et al., 1992), which are recruited to the responsive genes upon hormone treatment (Nie et al., 2000; Wallberg et al., 2000) and facilitate access of transcription factors to the promoters.

A well-characterized system for the study of gene regulation by glucocorticoids or progesterone is the Mouse Mammary Tumor Virus (MMTV) promoter in which the first HREs were identified (Beato et al., 1995). In cells, the MMTV promoter is covered by positioned nucleosomes of which the structure and dynamics have been intensively studied in vivo and in vitro. The positioned nucleosome B, which covers the region containing the HREs and the binding site for nuclear factor 1 (NF1), allows binding of progesterone receptor (PR)

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only to two of the five HREs and precludes binding of NF1, which is possible on MMTV promoter sequences assembled on positioned H3/H4 tetramers (Spangenberg et al., 1998). We have shown that the MMTV promoter assembled in minichromosomes with a *Drosophila* embryo extract requires an ATP-dependent remodeling event when activated by PR (Di Croce et al., 1999). This remodeling event is essential for the synergistic activation of the promoter by PR and NF1, a physiological hallmark of this promoter (Beato et al., 1995). Here, we show that after progesterone induction of breast cancer cells carrying a chromosomally integrated copy of the MMTV promoter, a Brg1-containing complex is recruited along with PR to the MMTV sequences. Concurrently, histone H2A and H2B are depleted from the promoter nucleosome B containing the HREs, but not from the nucleosomes located immediately upstream. Purified SWI/SNF complex from *Saccharomyces cerevisiae* catalyzes the ATP-dependent remodeling of mononucleosomes assembled on either MMTV promoter or on a DNA fragment of similar size derived from the mouse ribosomal promoter (rDNA). Moreover, in the presence of competitor DNA, binding of the PR to the exposed HREs recruits the SWI/SNF complex to the MMTV promoter, which remodels the nucleosome and catalyzes synergistic binding of NF1 and PR. Therefore, we can reproduce in a defined system with unmodified core histones the initial events that take place during progesterone activation of the MMTV promoter organized in chromatin. Although SWI/SNF catalyzes sliding of the histone octamer in recombinant mononucleosomes MMTV and rDNA promoter fragments, the outcome of the remodeling depends on the sequence of DNA: on MMTV promoter sequences, but not on rDNA sequences, both H2A/H2B dimers are displaced. In an MMTV nucleosomes array, in the absence of added factors, SWI/SNF displaces histones H2A and H2B from nucleosome B, but not from the adjacent MMTV nucleosome, which is also remodeled as judged by restriction enzyme accessibility. Thus, the outcome of the remodeling process is determined by yet-unknown features of the DNA sequence.

Results and Discussion

Progesterone Induction of the MMTV Promoter Is Accompanied by Depletion of H2A and H2B from the Promoter

The chromatin structure of the MMTV promoter is known to be remodeled during hormone induction (Truss et al., 1995). To study this process after progesterone treatment, we analyzed the recruitment of transcription factors and ATP-dependent remodeling complexes in T47D-MTVL breast cancer cells, which carry a single copy of the MMTV promoter integrated in their nuclear genome (Truss et al., 1995). In chromatin immunoprecipitation (ChIP) experiments with specific antibodies, we detected recruitment of the progesterone receptor (PR) to the MMTV promoter 30 min after hormone induction (Figure 1A). Binding of PR to the promoter is accompanied by recruitment of Brg-1-containing complexes, the coactivator Src-1 (Figure 1A), and RNA polymerase II (data not shown), indicative of an activation of transcription.

In a search for the molecular mechanism of chromatin remodeling, we analyzed the stoichiometry of core histones in the promoter nucleosome after digestion of chromatin with MNase (MN) to mono- and dinucleosome size. ChIP experiments with polyclonal antibodies specific for individual core histones and directed against their globular domains (Angelov et al., 2000) showed that 30 min after hormone induction, histone H4 content remained unchanged, but the amount of histone H2A crosslinked to the MMTV promoter was decreased (Figure 1B, top). With polyclonal antibodies to H2B, a similar decrease in immunoprecipitated nucleosome B sequences was observed (data not shown and Figure 1C, top left, lanes 3 and 4). We found no changes in core histone content on the GAPDH gene promoter (the control) upon hormone treatment (Figure 1B, bottom). These results suggest that histones H2A and H2B are displaced from the promoter nucleosome B upon hormone induction.

The question arises of whether one or the two H2A/H2B dimers are displaced from the MMTV promoter chromatin. Because the DNA used for these ChIP experiments is a mixture of 200 and 400 bp fragments, displacement of a single H2A/H2B dimer because of a remodeling event restricted to the HRE-containing promoter nucleosome B should lead to not more than 25%–33% H2A/H2B depletion. Given the stochastic nature of MMTV induction by steroid hormones (Ko et al., 1990), this is a maximal estimate, as it assumes that all the cells responded to hormone treatment. We have shown previously that only 70% of the progestin-treated cells exhibit DNase I hypersensitivity over nucleosome B and a footprint of NF1 on the MMTV promoter (Truss et al., 1995). On the basis of this percentage, the maximal decrease expected for H2A/H2B content in case of displacement of a single dimer would be 23%. Quantitation of the data in Figure 1B yielded 40% depletion of histone H2A. Therefore, either two adjacent nucleosomes each lose a single H2A/H2B dimer, or alternatively, a single nucleosome loses both dimers.

To distinguish between these two possibilities, we performed ChIP experiments with chromatin digested to mainly mononucleosome size and analyzed H2A and H2B content in the individual nucleosomes with nucleosome-specific PCR primers (Figure 1C). To exclude the possibility that our results could be influenced by epitope masking in the context of chromatin remodeling, we also used a different antibody directed against the C-terminal tail of H2A (α-ctH2A, Cell Signaling). The result with all three antibodies showed that only the promoter nucleosome containing the HREs, nucleosome B, loses histones H2A and H2B upon hormone induction, whereas the two adjacent nucleosomes C and D do not exhibit a decrease in either H2A or H2B content. We conclude that after hormone induction, both H2A/H2B dimers are displaced from the HRE-containing nucleosome.

SWI/SNF Complex Is Recruited to MMTV Promoter Nucleosomes In Vitro and Facilitates Synergistic Binding of Progesterone Receptor and NF1

To test whether the changes in core histones stoichiometry could be catalyzed directly by the SWI/SNF complex, we have investigated whether purified SWI/SNF

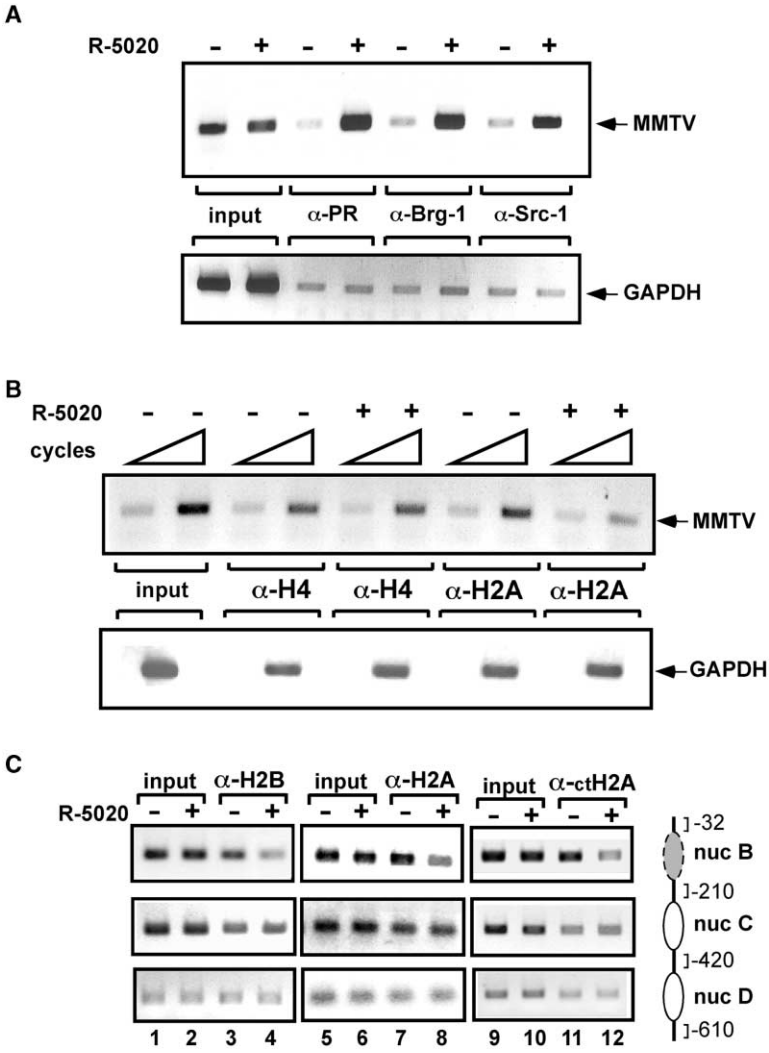


Figure 1. Recruitment of Factors to the MMTV Promoter and Selective Depletion of Histones H2A and H2B from Nucleosome B in Cultured Cells

(A) T47D-MTVL cells, carrying one stably integrated copy of the luciferase reporter gene driven by the MMTV promoter (Truss et al., 1995), were deprived of progestins for 48 hr prior to treatment with either 10^{-8} M R-5020 (a synthetic progesterone analog) or ethanol (EtOH) for 30 min. Cells were then harvested, crosslinked in 1% formaldehyde, and sonicated. The fragmented chromatin (30 μg) was subjected to immunoprecipitation with antibodies against PR, Brg-1, or SRC-1. The precipitated DNA fragments were subjected to PCR analysis to test for the presence of sequences corresponding to the MMTV promoter (top, 27 cycles) and the GAPDH gene (bottom, 30 cycles). Input material (1%) is shown for comparison.

(B) T47D-MTVL cells were subjected to ChIP assays with specific antibodies against H4 or H2A as in (A), but instead of sonication, the cells were permeabilized with lysolecithin (palmitoyl) and treated with micrococcal nuclease to generate mainly mononucleosome and some dinucleosome fragments as source of chromatin. PCR analysis was performed with primers corresponding to sequences from the MMTV promoter nucleosome B (top, 25 and 30 cycles) and the GAPDH gene (bottom, 28 cycles).

(C) T47D-MTVL cells were subjected to ChIP assays as described in (B) with specific antibodies against H2B, H2A, and the C-terminal tail of H2A (α-ctH2A). PCR analysis was performed with primers corresponding to sequences from the MMTV promoter nucleosomes B, C, and D.

complex is recruited by PR to MMTV nucleosomes in vitro and have analyzed the ATP-dependent effects of the complex on MMTV promoter nucleosomes. A 232 bp fragment of the MMTV promoter (Figure 2A), containing the HREs and the NF1 binding site, adopts two main translational positions when assembled into nucleosomes with core histone octamers made with recombinant histones expressed in *E. coli* (Spangenberg et al., 1998; Vicent et al., 2002). In both populations, the core histones protect the cleavage sites for SacI near the nucleosome pseudodyad and over the hidden HREs and the HinfI site closer to the 3′ end of the nucleosome and over the NF1 binding site (Figure 2A). Incubation of these nucleosomes with purified SWI/SNF and ATP made both sites more accessible for cleavage by the corresponding restriction enzyme particularly near the nucleosome border (Figure 2B). Moreover, the nucleosomal 10 bp DNase I cleavage pattern was partly lost (Figure 2C, compare lanes 4 or 5 with lane 6), and cleavage became more similar to that of free DNA (Spangenberg et al., 1998). These results indicate that purified SWI/SNF, in an ATP-dependant process, remodels MMTV promoter nucleosomes assembled with unmodified recombinant histones.

SWI/SNF-related remodeling complexes have been shown to interact with estrogen receptor (Ichinose et al., 1997) and glucocorticoid receptor (Wallberg et al., 2000). We therefore tested whether PR recruits SWI/SNF to MMTV nucleosomes in vitro with an experimental strategy that successfully demonstrated recruitment of SWI/SNF-remodeling activity by the Gal4-VP16 activator (Yudkovsky et al., 1999). When nucleosomes are incubated with SWI/SNF and an excess of competitor DNA, no remodeling is observed, as indicated by the lack of SacI cleavage (Figure 2D, compare lanes 2 and 3). However, when the nucleosomes are preincubated with PR, there is an increase in SacI cleavage upon addition of SWI/SNF and ATP (Figure 2D, lane 4). This increase requires the exposed HREs 1 and 4 and is eliminated by mutation of these two sites (Figure 2D, lanes 6–10). Because we used a defined system, recruitment of the remodeling complex likely takes place by direct protein-protein interactions.

We next investigated the consequences of SWI/SNF-mediated remodeling for PR binding by mobility shift assays. Whereas only the exposed HRE-1 was recognized in the absence of remodeling (Figure 3A, lane 3) (Vicent et al., 2002), SWI/SNF appeared to facilitate

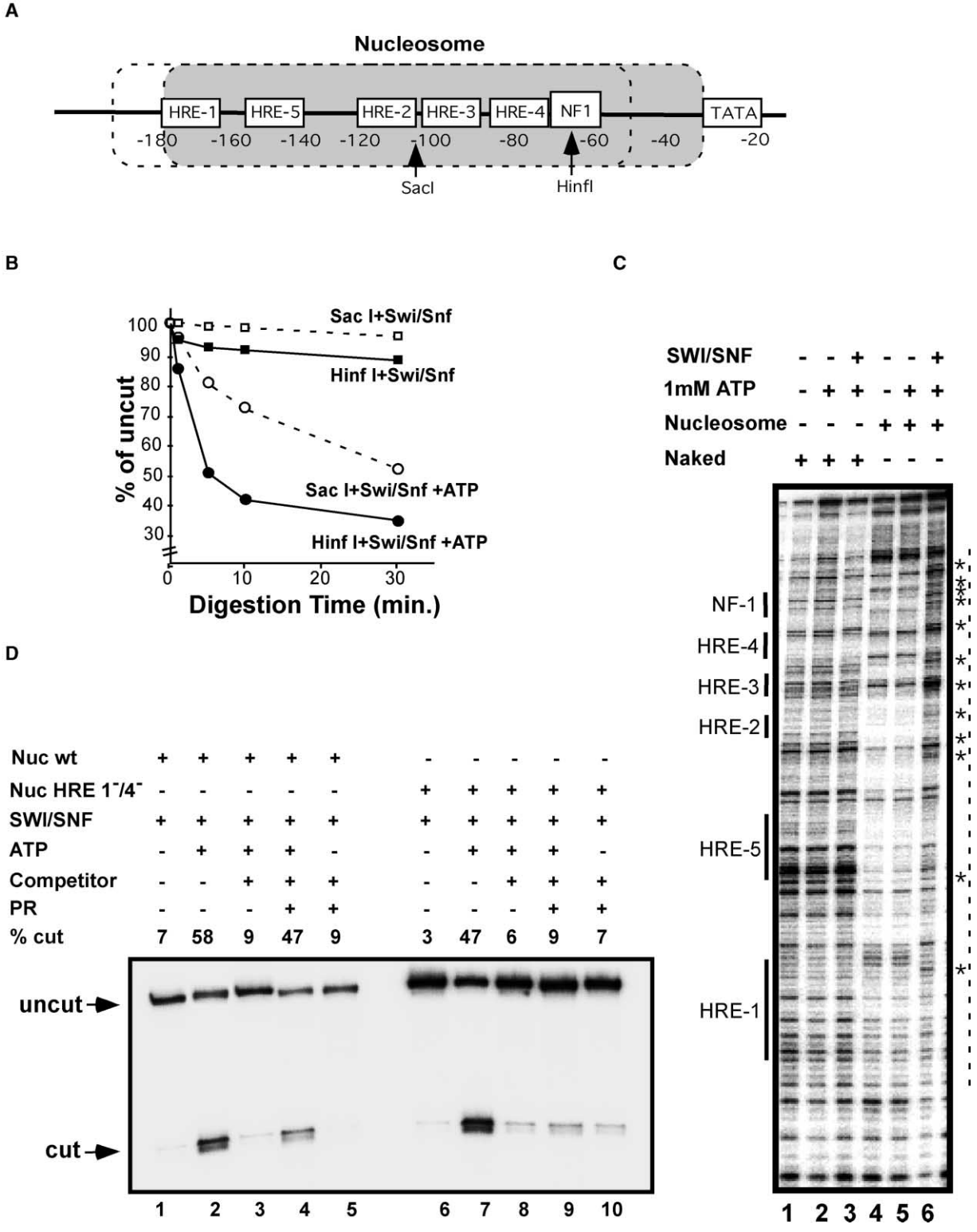


Figure 2. SWI/SNF Remodels MMTV Nucleosomes and Is Recruited by PR

(A) Scheme of the positions of the histone octamer relative to the *cis* elements of the MMTV promoter. HRE, hormone regulatory element; NF1, binding site for NF1. Restriction sites for SacI and HinfI are indicated by arrows. The two dashed rounded squares indicate the regions covered by the two nucleosome frames with the dyad axis at −107 and −127 (Spangenberg et al., 1998).

(B) Time course of digestion of labeled MMTV nucleosomes. Nucleosomes treated with SWI/SNF without ATP (squares) and with ATP (circles) were digested at 37°C with SacI and HinfI for 0, 1, 5, 10, and 30 min and electrophoresed in 8% denaturing polyacrylamide gels. The percentage of uncut DNA is plotted as a function of the incubation time.

(C) End-labeled naked DNA and reconstituted nucleosomes were treated with SWI/SNF with or without ATP as indicated and digested with

access to the hidden HREs in the presence of ATP, as demonstrated by the formation of a slower migrating complex (Figure 3A, lane 6). We conclude that the remodeled MMTV nucleosome generated by the action of purified yeast SWI/SNF is likely able to bind PR to all HREs, mimicking the in vivo situation (Truss et al., 1995). These results also show that full occupancy of the HREs is possible in the absence of covalent modifications of the core histones.

To study further the functional relevance of SWI/SNF recruitment, we analyzed the binding of recombinant PR and NF1 by DNase I footprinting. In the absence of competitor DNA, SWI/SNF made the NF1 site accessible for factor binding (Figure 3B, lane 9). However, in the presence of an excess of competitor DNA, NF1 could not bind to its cognate site even upon addition of SWI/SNF and ATP (Figure 3B, lane 10). Likewise, limiting amounts of PR did not generate a footprint under these conditions (Figure 3B, lane 13). However, in the presence of both transcription factors, the SWI/SNF complex enabled simultaneous binding of PR and NF1 (Figure 3B, lanes 11 and 12), which was not observed on free DNA (Figure 3B, left, lane 4). We conclude that PR binds to the accessible HREs 1 and 4 in a fashion unable to generate a footprint but sufficient to recruit SWI/SNF, which remodels the nucleosome and facilitates synergistic binding of NF1 and further PR molecules to the previously hidden cognate sites. Thus, the hallmark of transcriptional activation of the MMTV promoter in vivo (Truss et al., 1995) and in dynamic minichromosomes (Di Croce et al., 1999), namely the functional synergism between hormone receptors and NF1, can be reproduced in a defined system composed of recombinant nucleosomes made of unmodified core histones and a purified remodeling complex.

SWI/SNF Catalyzes Displacement of Histones H2A and H2B from MMTV Promoter Nucleosomes, but Not from rDNA Nucleosomes

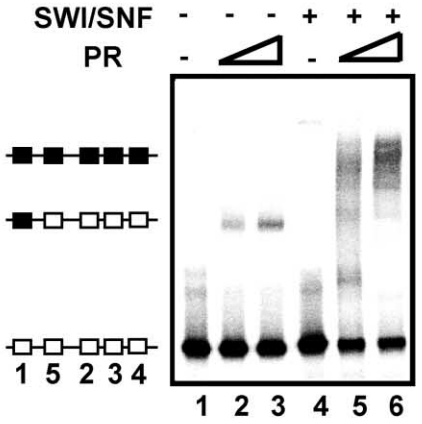
We next investigated the nature of the remodeling event leading to synergistic factor binding with mobility shift assays. In 5% (60:1) polyacrylamide gels, the MMTV promoter nucleosome migrated as a main band (Figure 4A, left, lane 1, double arrows) that represents a mixture of the two main populations with the dyad axis at −107 and −127 (Spangenberg et al., 1998). Weak faster migrating bands are also observed (black arrow), corresponding to nucleosomes with the octamer of histones located at the end of the fragment (Spangenberg et al., 1998). Incubation with low concentrations of SWI/SNF and ATP (lane 2) resulted in faster mobility of a fraction of the MMTV nucleosomes, whereas higher concentrations (lane 3) generated exclusively the fast migrating population (Figure 4A, left, gray arrow). Nucleosomes assembled on a similar size fragment of mouse ribosomal DNA (rDNA) migrated as two populations corresponding to previously reported octamer positions (Figure 4A, right, lane 1, black arrows) (Längst et al., 1999). Already low concentrations of SWI/SNF moved the two nucleosome populations to a new faster migrating position (Angelov et al., 2003) (Figure 4A, right, lanes 2 and 3, gray arrow). Using lambda exonuclease, we confirmed that SWI/SNF generated a new nucleosome position on rDNA, but the results on MMTV nucleosomes were not clear (data not shown). These data are consistent with previous studies demonstrating that yeast SWI/SNF can slide a 5S mononucleosome to the DNA ends (Jaskelioff et al., 2000) and showing that sliding is more efficient on rDNA than on MMTV DNA.

We used micrococcal nuclease (MN) digestion to map the positioning of the various nucleosome populations. As previously reported (Vicent et al., 2002), MN digestion of MMTV nucleosomes yielded a 146 bp fragment (Figure 4B, left, lane 1), which corresponds to the nucleosome core particle DNA. Restriction mapping revealed two translational frames with the dyad axis at −107 (marked with an asterisk) and −127 (marked with an open circle) as well as octamers located at the end of the DNA fragment (Figure 4C, left, lanes 1, 4, and 7, top figure on the scheme). MN digestion of MMTV nucleosomes treated with low concentrations of SWI/SNF and ATP generated the 146 bp DNA fragment and an additional 96 bp fragment (Figure 4B, left, lane 2). Restriction nuclease mapping revealed new octamer positions and a higher proportion of particles located at either the 5′ end (marked with a plus sign) or at the 3′ end (marked with a closed circle) of the DNA fragment (Figure 4C, left, lanes 2, 3, 5, 6, 8, and 9, middle figure on the scheme). MMTV nucleosomes treated with higher SWI/SNF concentrations yielded almost exclusively an MN digestion product of 96 bp (Figure 4B, left, lane 3). We obtained a similar MN product with MMTV promoter DNA assembled on tetramers of histones H3 and H4 (data not shown). Restriction mapping of the 96 bp MN product revealed a majority of modified core particles located at the 5′ end of the DNA fragment (Figure 4C, right, bottom figure on the scheme). We conclude that low concentrations of SWI/SNF induced octamer sliding toward the ends of the DNA with a mixed population of MN products, the 146 bp DNA fragment expected from core particle, and a smaller 96 bp DNA fragment. High SWI/SNF concentrations generated particles located at the end of the DNA fragment, protecting only 96 bp from MN digestion, compatible with the particles containing H3/H4 tetramers.

Treatment with MN of rDNA nucleosomes generated exclusively the 146 bp DNA product after preincubation with low as well as with high concentrations of SWI/SNF (Figure 4B, right, lanes 4–6). Restriction mapping confirmed the exonuclease data, showing a change of translational octamer positioning to the fragment ends,

DNase I, and the DNA fragments were analyzed on 6.5% sequencing gels. Untreated naked DNA and nucleosomes are shown in lanes 1 and 4, respectively. The SWI/SNF-dependent bands are marked with an asterisk. The dashed line indicates the region covered by nucleosome B. (D) Nucleosomes assembled on wild-type MMTV promoter DNA and on MMTV promoter mutated in HREs 1 and 4 (HRE, $1^{-}/4^{-}$) were treated with SWI/SNF in the absence or in the presence of ATP, competitor DNA, and PR as indicated. After incubation at room temperature for 20 min, the nucleosomes were digested with SacI. The DNA fragments were electrophoresed in 8% denaturing polyacrylamide gels. The percentage of cut DNA is shown on top of each lane.

A



B

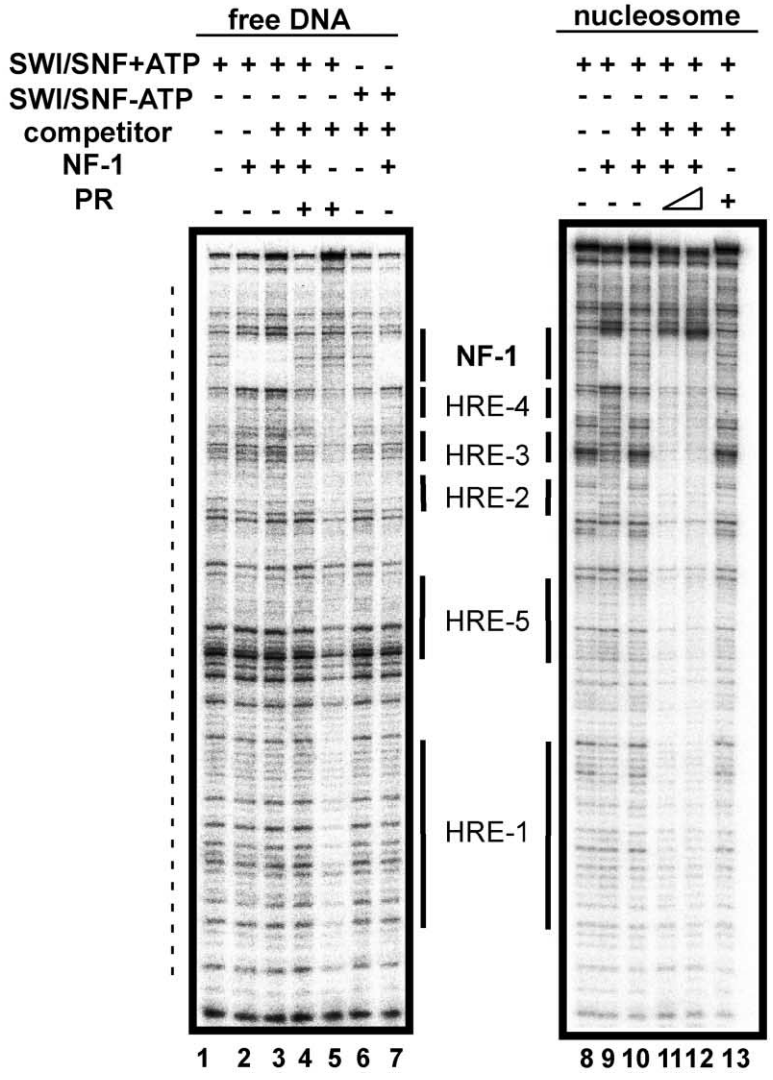


Figure 3. SWI/SNF Facilitates Synergistic Binding of PR and NF1 to MMTV Nucleosomes

(A) Control and SWI/SNF-treated MMTV nucleosomes were incubated at room temperature for 20 min with increasing amounts of PR and analyzed by electrophoresis on acrylamide/agarose gels. The schemes show the MMTV HREs with the occupied sites marked in black.

(B) Naked DNA or nucleosomes were preincubated at 30°C for 30 min in the presence of SWI/SNF and ATP followed by incubation in the additional presence of buffer, NF1, PR, and competitor DNA as indicated. The samples were then digested with DNase I, and the extracted DNA was electrophoresed on 6.5% sequencing gels. The positions of the five HREs and the NF1 binding site are indicated in the scheme between the two panels. The broken lines indicate the region covered by the promoter nucleosome.

already at low concentrations of SWI/SNF (Figure 4D). No 96 bp MN product could be identified. Thus, on rDNA, SWI/SNF promotes nucleosome sliding but does not change the size of DNA protected from MN digestion.

To explore further the mechanisms of remodeling, we performed immunoprecipitation experiments of formaldehyde crosslinked MMTV nucleosomes with antibodies to core histones (Angelov et al., 2000). MMTV nucleosomes incubated in the absence of SWI/SNF or ATP prior to crosslinking were efficiently immunoprecipitated with αH4, αH2A, or αH2B antibodies (Figure 5A, lanes 2, 4, and 7 and data not shown). In contrast, after incubation with SWI/SNF and ATP followed by crosslinking, the amount of MMTV nucleosome precipitated with αH4 was slightly enhanced (Figure 5A, lane 5), whereas precipitation with αH2A or αH2B was markedly reduced (Figure 5A, lanes 3 and 8). A quantitative PCR analysis of the immunoprecipitated DNA from two different experiments revealed that, in average, 70% of histone H2A was displaced from MMTV nucleosomes treated with SWI/SNF and ATP at a nucleosome to SWI/SNF molar ratio of 13:1 (see Supplemental Figure S1 at http://www.molecule.org/cgi/content/full/16/3/439/DC1/). Under these conditions, not all nucleosomes could have been converted to the remodeled state that generates a 96 bp MN product (Figure 4B). This result indicates that even at this low concentration of SWI/SNF, both H2A/H2B dimers are displaced from DNA in a large proportion of the nucleosomes. Because formaldehyde is known to crosslink H2A to H2B and H2B to H4 (Jackson, 1978), SWI/SNF seems to weaken the interaction between H2A/H2B dimers and H3/H4 tetramers, leading to selective displacement of both H2A/H2B dimers. Under our crosslinking conditions, the majority of core histones were indeed crosslinked to each other in untreated nucleosomes and formed larger complexes in SDS polyacrylamide gels (data not shown). Importantly, under similar incubation conditions SWI/SNF displaced neither H2A nor H2B from rDNA nucleosomes (Figure 5B and data not shown). Because the only difference with the MMTV nucleosomes resides in the DNA sequence, these results indicate that the nucleotide sequence of the DNA influences the outcome of the remodeling process by purified SWI/SNF.

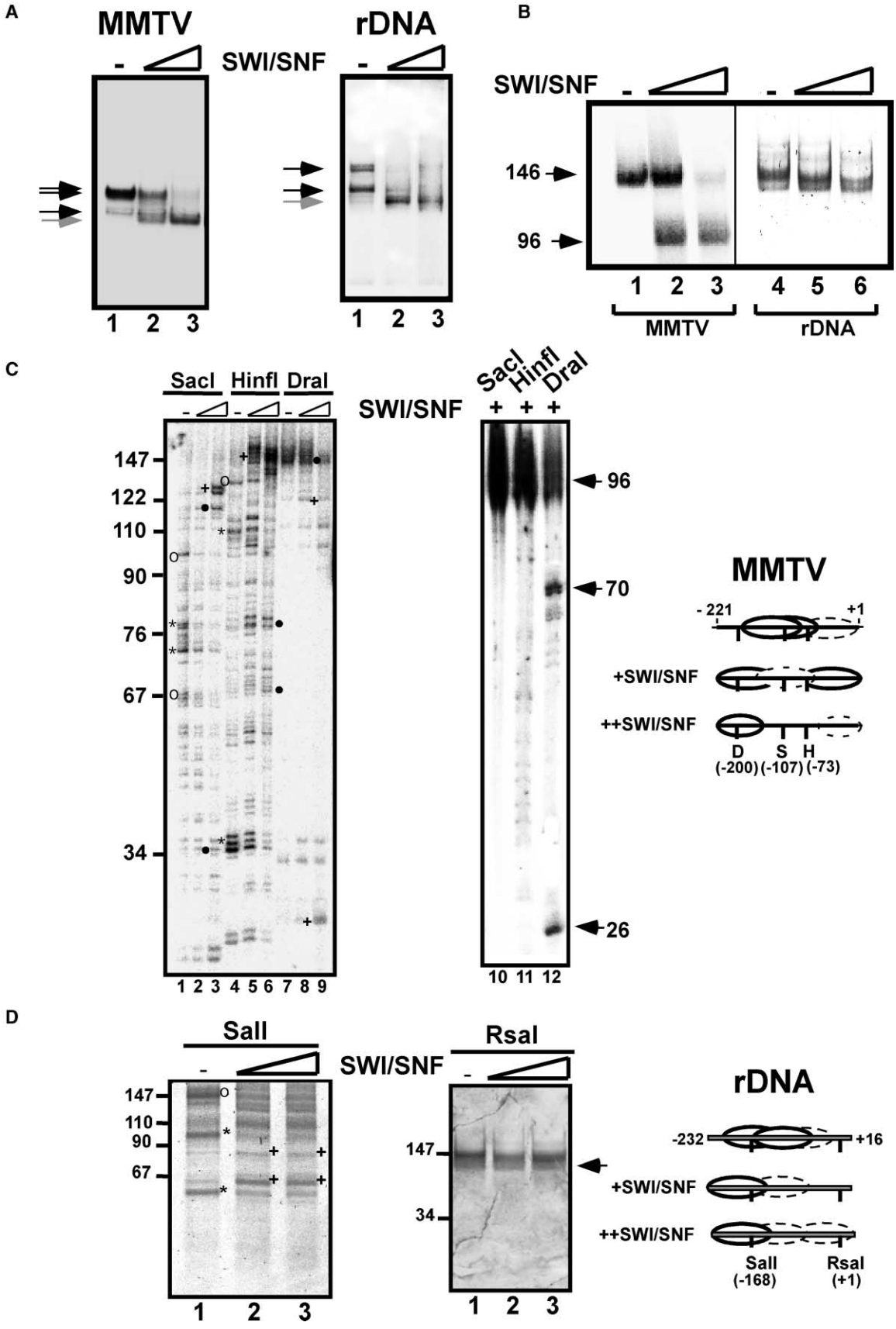
Under conditions of PR-mediated SWI/SNF recruitment in the presence of an excess of competitor DNA, there was a significant ATP-dependent displacement of histone H2A from MMTV nucleosome B, as demonstrated by quantitative RT-PCR (Supplemental Figure S1). As shown above, this promotes synergistic binding of PR and NF1 (Figure 3B). Thus, the SWI/SNF recruited by PR to the MMTV promoter nucleosome is able to promote H2A/H2B displacement in the presence of DNA bound receptors.

To confirm that histones H2A and H2B are physically displaced from MMTV nucleosomes treated with SWI/SNF in the presence of ATP, we performed incubations with large amounts of nucleosomes and SWI/SNF and analyzed the products of remodeling by two-dimensional gel electrophoresis and Coomassie blue staining. Whereas no changes in core histones stoichiometry were observed with rDNA nucleosomes treated with SWI/SNF and ATP (Figure 5C, right), the amount of H2A/H2B relative to H3 in treated MMTV nucleosomes decreased by 30%, as calculated from a densitometric scan of the autoradiogram (Figure 5C, left). The fact that less H2A/H2B dimers are displaced under these conditions than in ChIP experiments is likely due to the 3-fold higher nucleosome to SWI/SNF ratio used in the staining experiments. At this nucleosome to SWI/SNF molar ratio, only 35% of the H2A/H2B dimers were displaced in ChIP experiments, as determined by quantitative PCR (Supplemental Figure S1). Thus, we conclude that H2A/H2B are physically displaced from the H3/H4 tetramer particle containing MMTV promoter DNA.

To provide direct proof of H2A/H2B displacement under the conditions used for ChIP assays, we performed additional experiments with an H2B* chimera with its N-terminal tail replaced by the N-terminal tail of histone H3, which has been shown not to alter the properties of reconstituted nucleosomes (Angelov et al., 2004). The chimeric H2B* was phosphorylated in vitro with purified Aurora kinase a $\gamma[^{32}P]$-ATP and used to assemble nucleosomes with the other three unlabeled core histones. As a control, nucleosomes were assembled with H3 labeled with $[^{32}P]$ in a similar way and the other three unlabeled core histones. The two types of nucleosomes were incubated with SWI/SNF in the absence or presence of ATP, and the products were separated in an agarose/acrylamide gel (Figure 5D). In these gels, nonremodeled MMTV and rDNA nucleosomes migrate as doublets corresponding to the nucleosome populations described previously. Upon remodeling, a single faster band is observed corresponding to the particles located at the end of the fragments. This demonstrates that both MMTV and rDNA nucleosomes were remodeled in an ATP-dependent process. Measurement of the radioactivity of rDNA nucleosomes showed no significant changes upon incubation with SWI/SNF and ATP (Figure 5D, lanes 5–8), confirming that neither H3 nor H2B were displaced from these nucleosomes upon remodeling. In contrast, whereas there was no significant changes in MMTV nucleosomes labeled with H3 (Figure 5D, lanes 3 and 4), the amount of radioactivity in H2B*-labeled MMTV nucleosomes decreased by 74% upon remodeling with SWI/SNF and ATP (Figure 5D, compare lanes 1 and 2). Thus, in a high proportion of MMTV promoter nucleosomes, there is displacement of both H2A/H2B dimers, confirming that the results obtained in ChIP experiments are not due to epitope masking during remodeling. We have not followed the fate of the displaced H2A/H2B dimers, but they could be associated with the excess of competitor DNA present in the reactions.

SWI/SNF Selectively Displaces H2A/H2B from Nucleosome B and Not from the Adjacent Nucleosome in an MMTV Nucleosome Array

We next asked whether the selective loss of histone H2A/H2B dimers from nucleosome B within the MMTV LTR observed in intact cells was due to PR binding or was an intrinsic property of nucleosome B that could be reproduced in vitro in the absence of PR. To answer this question, we generated a recombinant linear array of MMTV nucleosomes containing nucleosomes A, B, C, and D, as present in the cellular construct (Figure 6A, top scheme). When digested with MN, a nucleosome ladder was observed indicative of a correct nucleosome


A
MMTV
rDNA
SWI/SNF
1 2 3
1 2 3
B
SWI/SNF
146
96
1 2 3 4 5 6
MMTV
rDNA
C
SacI
HinfI
DraI
SWI/SNF
147
122
110
90
76
67
34
1 2 3 4 5 6 7 8 9
96
70
26
10 11 12
MMTV
- 221
+1
+SWI/SNF
++SWI/SNF
D S H
(-200) (-107) (-73)
D
SalI
RsaI
SWI/SNF
147
110
90
67
1 2 3
147
34
1 2 3
rDNA
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SalI
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RsaI
(+1)

spacing (Figure 6A). The array was incubated with or without SWI/SNF and ATP and analyzed for remodeling by digestion with the restriction enzymes SacI and RsaI that cut over nucleosomes B and C, respectively (Figure 6B, left). Both nucleosomes were remodeled by SWI/SNF, as judged by increased accessibility to the restriction enzymes (Figure 6B, right). The array was digested with MN to yield mainly mono- and some dinucleosome-sized fragments to test for H2A and H2B displacement. After formaldehyde crosslinking, ChIP assays were performed with antibodies to H2A, H2B, and H4, and the products were analyzed with primers selective for nucleosomes B and C. Whereas remodeling did not significantly change the content of H4, H2A, or H2B in nucleosome C (Figure 6C, bottom), there was a significant decrease in the content of H2A and H2B, but not H4, in nucleosome B (Figure 6C, top). Thus, the selective decrease in H2A and H2B reactivity of nucleosome B could be reproduced with recombinant histones and purified SWI/SNF complex. These data do not support epitope masking as an explanation for our results, because one would have to explain why the epitopes are masked only in nucleosome B and not in nucleosome C, which is also remodeled. Moreover, the results demonstrate that within a continuous array of nucleosomes and in the absence of sequence-specific transcription factors, SWI/SNF generates different remodeled products from adjacent nucleosomes. Therefore, it must be the underlying DNA sequence that determines the outcome of the remodeling process.

Conclusions

Taken together, our data indicate that PR bound to the accessible HREs recruits SWI/SNF to the promoter, which remodels the nucleosome in an ATP-dependent fashion, leading to displacement of H2A and H2B. Since MMTV promoter sequences assembled on H3/H4 tetramers are able to bind NF1 rather efficiently (Spangenberg et al., 1998) and can allow binding of PR to all HREs (Supplemental Figure S2), we assumed that the product of remodeling is an MMTV H3/H4 tetramer able to bind NF1 and PR simultaneously. The synergistic binding of both factors is likely mediated by the stabilizing effect of NF1 binding on the remodeled nucleosome state (Di Croce et al., 1999). In this way, NF1 facilitates access of additional PR molecules to the central HREs and further recruitment of SWI/SNF, leading to full remodeling of nucleosome B. Additional kinetics experiments will be required to establish the precise order of events leading to the final remodeled particle with bound PR and NF1.

Originally remodeling by SWI/SNF was claimed to occur by changing the core histone interaction with DNA but without a loss of histones (Bazett-Jones et al., 1999; Côte et al., 1994; Schnitzler et al., 1998). Recently, the potential of ATP-dependent remodeling complexes to remove one dimer of H2A/H2B and exchange it with an exogenous dimer has been associated to their ability to move the octamer particle into positions in which DNA is unraveled from one end (Bruno et al., 2003). However, this reaction was much less efficient and required higher relative concentrations of SWI/SNF and the presence of exogenous histone dimers, which are absent in our remodeling assays. It will be interesting to test whether this type of exchange reaction at high concentrations of SWI/SNF take place independently of nucleosomal DNA sequence. Although we do not have any indication, it is conceivable that the observed displacement of H2A/H2B dimers on the MMTV promoter represents the first step in a process leading to replacement by modified or variant H2A/H2B dimers. The remodeling on MMTV nucleosome B is also distinct from the transient displacement of a single H2A/H2B dimer catalyzed by the transcription elongation factor FACT (Kireeva et al., 2002), which generates a hexamer particle (Belotserkovskaya et al., 2003). We do not have evidence for a hexamer particle as a remodeling product of MMTV promoter nucleosomes.

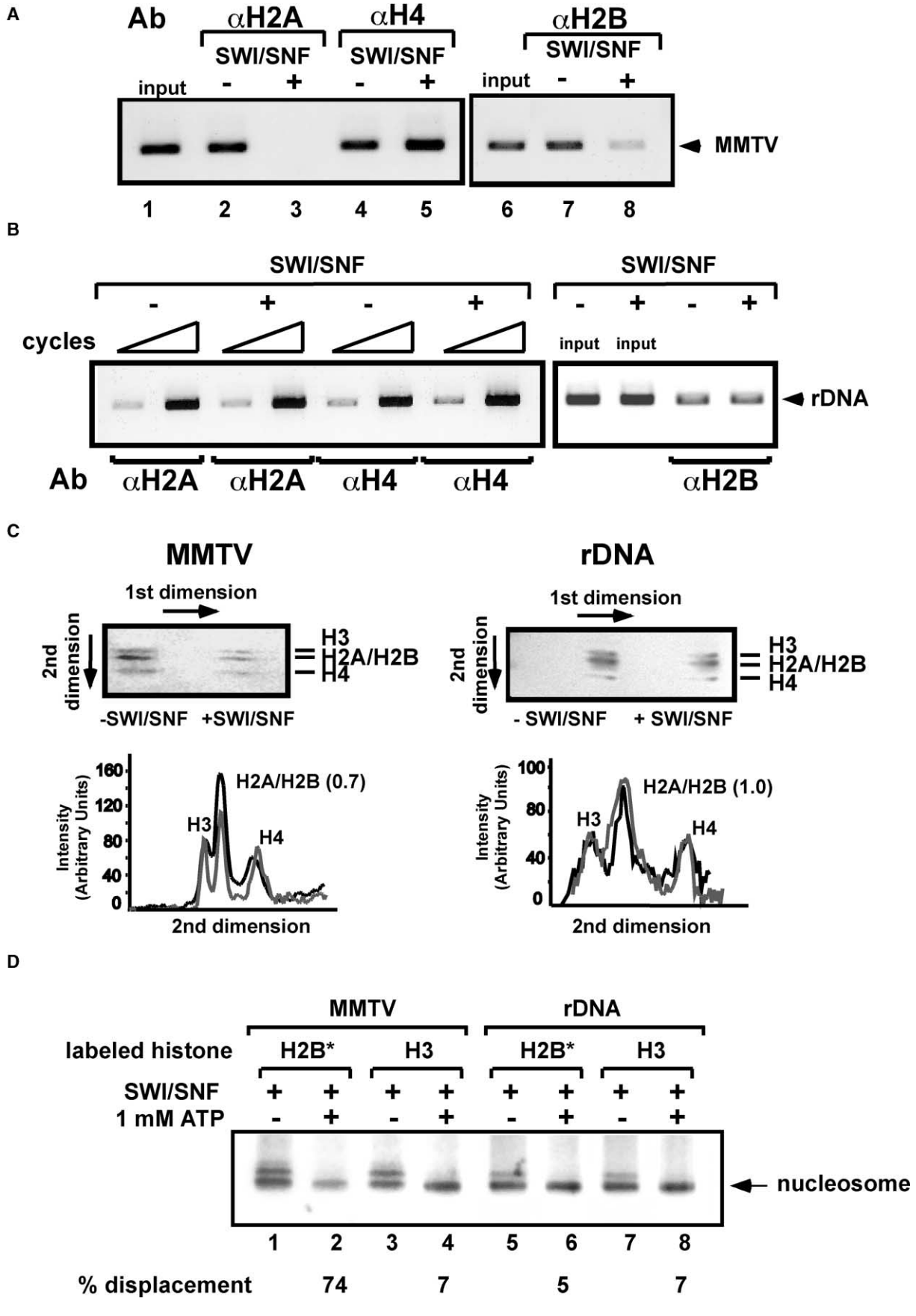
Figure 4. SWI/SNF Catalyzes Different Nucleosome Rearrangements on MMTV and rDNA Sequences

(A) Mononucleosomes reconstituted on radiolabeled MMTV (left) or rDNA (right) were incubated with increasing amount of SWI/SNF in the presence of ATP. The nucleosome positions were then analyzed on native 5% (60:1) polyacrylamide gels. The migration of the three main populations of MMTV nucleosomes (left) and the two main populations of rDNA nucleosomes (right) are indicated with black arrows. The gray arrows indicate the new population of nucleosomes generated after SWI/SNF remodeling.

(B) Control and SWI/SNF-treated MMTV and rDNA nucleosomes (left and right, respectively) were digested for 5 min at 22°C with 5 Units/reaction of MNase. The digestion products were 5′ end labeled, analyzed by electrophoresis on native 6% polyacrylamide gels, and autoradiographed. The positions of the 146 bp and 96 bp digestion products are indicated by arrows.

(C) Control and SWI/SNF-treated MMTV nucleosomes were digested with MNase as described in (B). Left: the resistant 146 bp fragment labeled at both ends was cleaved with restriction enzymes as indicated. The asterisks and open circles (lanes 1 and 4) indicate the bands corresponding to the populations of nucleosomes with the dyad axis at −107 and −127, respectively. After remodeling (lanes 2, 3, 5, and 6), two populations of nucleosomes either located at the 5′ end (indicated with $^{+}$) or at the 3′ end (indicated with closed circles) become more evident. Location of the remodeled particle mostly at the 5′ end was confirmed by DraI digestion (lanes 8 and 9). pBR322 DNA digested with HpaII was used as size marker (numbers of nucleotides indicated on the right). Right: mapping of the 96 bp MNase digestion product with Sac I, HinfI, and DraI (numbers on the right indicate the size in nucleotides). The scheme on the right summarizes the positions of the histone particles in the absence of SWI/SNF (top) and after remodeling by two different concentrations of SWI/SNF. Dotted lines represent minor populations. The numbers in parentheses indicate the sites of cleavage of the restriction enzymes (D, DraI; S, SacI; H, HinfI) relative to the transcription start site.

(D) Control and SWI/SNF-treated rDNA nucleosomes were digested with MNase as described in (B). The resistant 146 bp were cleaved with restriction enzymes as indicated, and the resulting DNA fragments were stained with SYBR Green I (Längst et al., 1999). Open circles and asterisks indicate the bands corresponding to the slow and fast migrating populations of nucleosomes, respectively (see Figure 4A, right, lane 1). After remodeling (lanes 2 and 3), new bands appear (indicated with $^{+}$ and a black arrow). pBR322 DNA digested with HpaII was used as size marker (numbers of nucleotides indicated on the left). The scheme on the right summarizes the positions of the histone particles (dotted lines represent minor populations) in the absence of SWI/SNF (top) and after remodeling by two different concentrations of SWI/SNF. The numbers in parentheses indicate the site of cleavage by the restriction enzymes relative to the transcription start site.


A
Ab
αH2A
αH4
αH2B
SWI/SNF
SWI/SNF
SWI/SNF
input
- + - +
input - +
MMTV
1 2 3 4 5 6 7 8
B
SWI/SNF
SWI/SNF
- + - +
- + - +
cycles
input input
rDNA
Ab
αH2A
αH2A
αH4
αH4
αH2B
C
MMTV
rDNA
1st dimension
1st dimension
2nd dimension
2nd dimension
H3
H2A/H2B
H4
-SWI/SNF +SWI/SNF
- SWI/SNF + SWI/SNF
Intensity (Arbitrary Units)
160
120
80
40
0
H2A/H2B (0.7)
H3
H4
2nd dimension
100
80
40
0
H2A/H2B (1.0)
H3
H4
2nd dimension
D
MMTV
rDNA
labeled histone
H2B*
H3
H2B*
H3
SWI/SNF
+ + + + + + + +
1 mM ATP
- + - + - + - +
nucleosome
1 2 3 4 5 6 7 8
% displacement
74
7
5
7

After the completion of this work, a report appeared claiming that remodeling of in vitro-assembled MMTV promoter chromatin by SWI/SNF after glucocorticoid receptor (GR) binding does not involve displacement of H2A nor H2B (Nagaich et al., 2004). We do not see a contradiction with our results, because this study used an array containing six nucleosomes from the MMTV promoter of which only nucleosome B was remodeled in response to GR binding. The depletion of histones was measured from all six nucleosomes together, and, therefore, even if H2A/H2B dimers were depleted from nucleosome B, no more than 16% decrease in dimer content will be expected. Moreover, no NF1 was used in this study. Because NF1 is essential to obtain a high proportion of remodeled promoters (see also Di Croce et al., 1999), we would not expect a quantitative remodeling of nucleosome B in the absence of NF1. Thus, the depletion of H2A/H2B from nucleosome B will be undetectable under the conditions and with the resolution used by Nagaich and coworkers.

Our studies show that the mechanism by which the SWI/SNF complex makes nucleosomal DNA more accessible is influenced by the nucleotide sequence of the DNA. On mouse rDNA, SWI/SNF catalyses nucleosome sliding and does not displace H2A/H2B dimers. On MMTV promoter nucleosome B, it also contributes to sliding of the histone octamers, but, in addition, it promotes displacement of both H2A/H2B dimers. Displacement of H2A/H2B from nucleosome B is also observed within a MMTV nucleosome array in which the adjacent nucleosome C is remodeled by SWI/SNF without loosing H2A/H2B. Therefore, sliding to the end of a DNA fragment is not a necessary requirement for H2A/H2B displacement. The determining role of nucleotide sequence for nucleosome remodeling is supported by recent findings with the PHO5 promoter in yeast, showing that induction is accompanied by total displacement of all core histones only from two of the four remodeled nucleosomes (Boeger et al., 2003; Reinke and Horz, 2003). Therefore, ATP-dependent remodeling facilitates access of transcription factors to nucleosomal DNA by processes resulting in different remodeled stages: changes in the position of the histone octamer (Jaskelioff et al., 2000; Whitehouse et al., 1999), displacement of a single H2A/H2B dimer or both dimers, and displacement of all core histones (Lorch et al., 1999). It will be interesting to know whether these various final states of the remodeled nucleosome are the product of similar or different changes in the interactions between core histones and DNA and how they are affected by post-translational histone modifications. Finally, it remains to be established what features of the nucleotide sequence determine the outcome of nucleosome remodeling.

Experimental Procedures

Chromatin Reconstitution and Mononucleosome Purification

The 232 bp EcoRI-BamH1 fragment containing either the wild-type MMTV promoter sequence from −221 to +1 or the MMTV HRE $1^-/4^-$ mutant were derived from the plasmids pGAW222 and pGAW222HRE$1^-/4^-$, respectively. Both fragments included additional 5 bp on each end, resulting from filling the cleavage sites of EcoRI and BamHI. The fragments were radiolabeled at the 3′ ends with the Klenow fragment of DNA polymerase and α[^{32}P]-ATP. The mouse rDNA region from −232 to +16 relative to the transcription start site was synthesized by PCR with the plasmid pMR974 (Längst et al., 1999), as template and radiolabeled at the 5′ end with polynucleotide kinase and [γ-^{32}P] ATP. All labeled fragments were purified on a native 5% polyacrylamide gel. The histones used for reconstitution experiments were recombinant *X. laevis* histones expressed in *E. coli*, and nucleosomes were reconstituted by the salt dialysis technique as described (Vicent et al., 2002).

Nucleosome Remodeling Assays

The yeast SWI/SNF was prepared as described (Logie and Peterson, 1999). Reactions (20 μl) were done in 10 mM HEPES (pH 7.9), 60 mM KCl, 6 mM $MgCl_2$, 60 μM EDTA, 2 mM DTT, 13% glycerol containing 20 nM of MMTV or rDNA nucleosomes, and 1.5 or 6 nM SWI/SNF in the presence or absence of 1 mM ATP. Nucleosomes were incubated for 30 min at 30°C followed by additional 30 min incubation with 30 ng/μl poly-dIdC as competitor in order to remove SWI/SNF from the nucleosomes. The different mononucleosome populations were separated by electrophoresis on 5% polyacrylamide gels (acrylamyde:bisacrylamide, 60:1) in 0.2× TBE (Vicent et al., 2002).

Electrophoretic Mobility Shift Assay

Recombinant human PR, isoform B, was expressed in baculovirus and purified as previously described (Di Croce et al., 1999). Intact or remodeled nucleosomes were incubated with different amounts of PR (15–75 ng) in TGA-buffer (10 mM Tris-HCl [pH 8.0], 0.5 mM EDTA, 5% glycerol, 0.5 mM 2-mercaptoethanol, 90 mM NaCl, 1 μg poly-dI-dC, 100 ng of calf thymus DNA, and 60 μg bovine serum albumin) in a 20 μl final volume. Binding reactions were incubated for 20 min at room temperature and analyzed by electrophoresis at 4°C in a 3.5% acrylamide/20% glycerol/0.5% agarose/0.3× TBE gel (Vicent et al., 2002).

Figure 5. SWI/SNF Mediates Histone H2A/H2B Displacement on MMTV Nucleosomes, but Not on rDNA Nucleosomes

(A) Chromatin immunoprecipitation (ChIP) on MMTV nucleosomes. Control and SWI/SNF-treated MMTV nucleosomes were crosslinked in 0.25% formaldehyde and immunoprecipitated with antibodies against the histones H2A, H2B, or H4. The precipitated DNA fragments were subjected to PCR analysis (30 cycles) with oligonucleotides corresponding to the MMTV promoter nucleosome B. 1% of the input DNA was used in lanes 1 and 6.

(B) Chromatin immunoprecipitation on rDNA nucleosomes. Control and SWI/SNF-treated rDNA nucleosomes were subjected to the ChIP protocol as described in (A). The results of 27 and 30 cycles of amplification with oligonucleotides corresponding to the rDNA nucleosome are shown.

(C) Scaled up remodeling reactions. MMTV (left) or rDNA nucleosomes (right) (1 μM) were incubated at 30°C for 30 min with SWI/SNF (20 nM) or control buffer and ATP (1 mM). The nucleosomes were resolved on 5% acrylamide gel (1st dimension), and the nucleosome-corresponding gel slices were excised and run into 18% SDS-PAGE gel (2nd dimension). The gels were stained with Coomassie blue. Densitometric scans of the SDS-PAGE gels are shown at the bottom. Black and gray lines indicate nucleosomes treated with control buffer or with SWI/SNF, respectively. The numbers in parenthesis indicate the fraction of H2A/H2B remaining after SWI/SNF treatment.

(D) Remodeling of nucleosomes with radioactive-labeled histones. Nucleosomes were assembled on either MMTV (lanes 1–4) or rDNA (lanes 5–8) with [^{32}P]-labeled chimeric H2B* (lanes 1, 2, 5, and 6) or H3 (lanes 3,4, 7, and 8) and incubated with SWI/SNF with or without ATP as indicated. The remodeling products were electrophoresed on agarose/acrylamide gels and revealed by autoradiography. The percentage of displaced histones was calculated by measuring the radioactivity on a PhosphorImager (Fuji FLA 5000), quantified with Image Gauge software, and is indicated below the corresponding lanes.

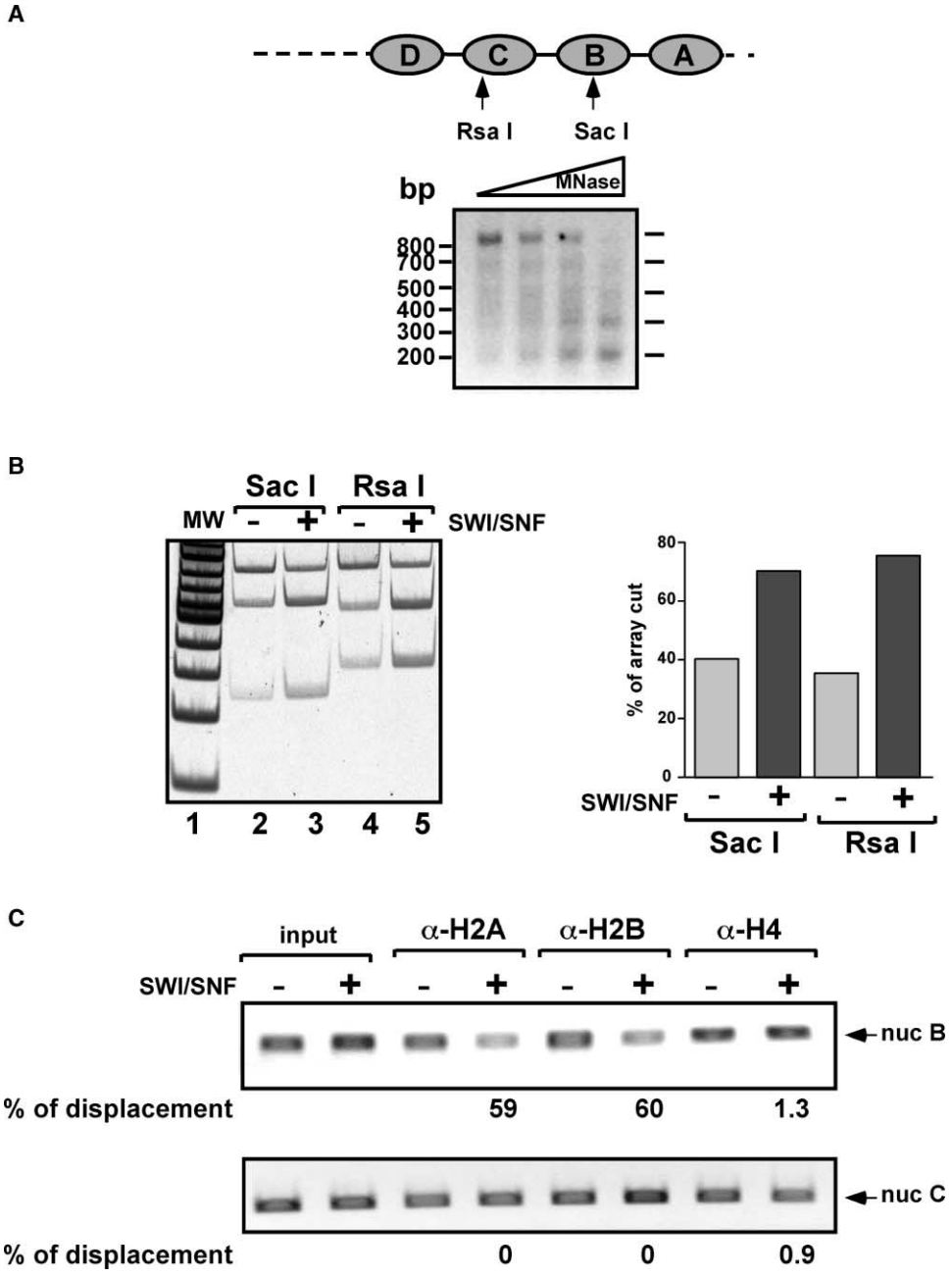


Figure 6. SWI/SNF Selectively Displaces H2A/H2B from Nucleosome B within an MMTV Nucleosome Array

(A) Schematic representation of the MMTV nucleosome array containing nucleosomes A, B, C, and D. SacI and RsaI restriction sites are indicated. MMTV arrays (5 nM) were digested with 0.05 U/reaction of MNase for 30 s, 1, 2, and 5 min at 22°C, and the resulting DNA fragments were analyzed on a 1% agarose gel. The numbers on the left indicate the size of the markers in bp. The lines on the right indicate the approximate position of the nucleosomes.

(B) Restriction enzyme assay of remodeling. Control and SWI/SNF-treated MMTV arrays (5 nM) were digested with 500 U/ml of the diagnostic restriction enzymes SacI and RsaI at 37°C for 40 min. After phenol extraction, DNA fragments were analyzed on a 5% acrylamide gel and stained with SYBR Green I (Molecular probes, Oregon) (left). The percentage of DNA cleaved was determined by PhosphorImager quantification (right).

(C) Chromatin immunoprecipitation on MMTV arrays. Control and SWI/SNF-treated MMTV arrays digested with MN to mainly mononucleosome size were subjected to the ChIP protocol with specific antibodies against histones H2A, H2B, and H4. The results of 27 cycles of amplification with oligonucleotides corresponding to the nucleosomes B and C are shown. The percent displacement is indicated underneath the relevant lanes.

Cleavage by Nucleases

DNase I and Lambda Exonuclease digestion of control and remodeled nucleosomes were performed as described (Spangenberg et al. [1998] and Negri et al. [2001], respectively). For DNase I footprints, nucleosomes were incubated in remodeling buffer in the presence of SWI/SNF, 1 mM ATP, PR, and NF1 for 30 min at 30°C as indicated followed by the addition of DNase I (9.4 ng/μl) and further incubation for 1 min at room temperature. The reactions were stopped with

5 mM EDTA and analyzed on 6.5% sequencing gels. The naked DNA digestions were performed with 0.9 ng/μl of DNase I.

Restriction Endonuclease Accessibility and Nucleosome Mapping

Intact and remodeled MMTV mononucleosomes were digested at 37°C with 500 U/ml of SacI or HinfI in a total volume of 160 μl. At the indicated time points, aliquots of 19 μl were removed and added to 181 μl of stop buffer to give a final concentration of 12.5 mM EDTA and 0.5% SDS. The samples were then extracted with phenol, phenol/chloroform, and chloroform/isoamylic alcohol and precipitated with three volumes of ethanol. After washing with 80% ethanol and drying, the samples were analyzed on 8% denaturing polyacrylamide gels. The intensity of the bands was determined with a PhosphorImager (Fuji FLA 3000G) and quantification software (Image Gauge v 3.1).

The reconstituted particles were subjected to MNase digestion as described (Vicent et al., 2002). The digested DNA was 5′ end-labeled with $\gamma[^{32}P]$-ATP and T4 polynucleotide kinase and purified on native 6% polyacrylamide gels. DNA bands of defined lengths were eluted from the gels and digested with diagnostic restriction enzymes: SacI, HinfI, and DraI for MMTV and RsaI and SalI for rDNA. The lengths of the resulting DNA fragments were determined by electrophoresis on polyacrylamide sequencing gels.

Two Dimensional Gel-Shift/SDS-PAGE

Scale-up SWI/SNF-remodeling reactions were loaded onto preparative 1 mm thick 5% polyacrylamide gels. After electrophoresis, each lane was cut out and laid over the stacking gel of an 18% SDS-PAGE gel poured with 2 mm spacers. After electrophoresis, the gel was stained with Coomassie.

Chromatin Immunoprecipitation (ChIP) Assays

ChIP assays were performed as described (Strutt and Paro, 1999) with the T47D-MTVL cell line (Truss et al., 1995) and the following antibodies: anti-Progesterone receptor (H-190, Santa Cruz), anti-Brg-1 (gift from Dr Weidong Wang), anti SRC-1 (clone 1135, Upstate Biotechnologies), anti H2A, H2B, and H4 (Angelov et al., 2000). For each experiment, PCRs were performed with a different number of cycles and dilution series of input DNA to determine the linear range of the amplification; all results shown fall within this range. Primers sequences are available on request.

Quantification of chromatin immunoprecipitation was performed by real-time PCR with Roche Lightcycler (Roche). Each PCR reaction generated only the expected specific amplicon, as shown by the melting temperature profiles of final products. Calculation of the amount of immunoprecipitated DNA was performed with the Light Cycler Software with different input DNA concentrations for standard curve generation.

Assembly of Nucleosomes with Radioactive-Labeled Histones

Wild-type H3 and H2B*, a swapped tail H3-H2B mutant, histones were used. The N-terminal tail of histone H2B was replaced with the N terminus of histone H3 within the H2B* mutant protein by standard molecular biology approaches (Angelov et al., 2004). This allows the mutant H2B* to be radioactively labeled at serine 10 by the Aurora A kinase. For labeling, 10 μg of either H2B* or H3 histones were incubated for 1 hr at 30°C with 5 μl of 0.25 μg/μl Aurora A kinase and 30 μCi $\gamma[^{32}P]$-ATP in 300 μl final volume of 50 mM Tris-HCl (pH 7.4), 7.5 mM $MgCl_2$, and 1 mM DTT. The reaction was stopped by adding 65 μl of 1M HCl and incubating for 15 min on ice. After centrifugation for 15 min at 4°C, the supernatant was TCA precipitated and washed twice with acetone. The pellets were resuspended in 10 mM HCl. Labeled H2B* or H3 histones were mixed with equimolar amounts of the remaining three histones in 10 mM HCl and stepwise dialyzed overnight against histone folding buffer. Nucleosomes were reconstituted as previously described (Dyer et al., 2004). The remodeling experiments were performed as described above.

Nucleosome Arrays

A 0.8 kb Hind III/Bgl II fragment of MMTV LTR (−640, +126) was derived from the pMCBB (Di Croce et al., 1999). The reconstitution of nucleosome arrays were performed as previously described (Cirillo and Zaret, 2004). The reconstituted particles were subjected to MNase and restriction enzyme digestion as described (Vicent et al., 2002). For ChIP experiments, reconstituted material (5 nM) was digested with 0.5 U/reaction of micrococcal nuclease for 30 s at 22°C to generate mainly mononucleosome-sized fragments as source of chromatin and subjected to the ChIP protocol with specific antibodies against histones H2A, H2B, and H4 as described (Koop et al., 2003).

Acknowledgments

We thank Bernhard Gross, Institut für Molekularbiologie und Tumorforschung, for the preparation of recombinant PR; Karolin Luger and Tim Richmond, Zürich, for the *E. coli* expression plasmids encoding *Xenopus laevis* full-length histones; and Weidong Wang, National Institutes of Health, for Brg-1 antibody. G.P.V. was a postdoctoral fellow of FOMEC-Argentina. The experimental work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 397), the European Union, the Fonds der Chemischen Industrie, and the Spanish Ministry of Science and Technology (MCYT, PM 99-0030, and BMC2003-02902).

Received: June 10, 2004
Revised: October 7, 2004
Accepted: October 18, 2004
Published: November 4, 2004

References

Angelov, D., Charra, M., Seve, M., Cote, J., Khochbin, S., and Dimitrov, S. (2000). Differential remodeling of the HIV-1 nucleosome upon transcription activators and SWI/SNF complex binding. J. Mol. Biol. *302*, 315–326.

Angelov, D., Molla, A., Perche, P.Y., Hans, F., Cote, J., Khochbin, S., Bouvet, P., and Dimitrov, S. (2003). The histone variant macroH2A interferes with transcription factor binding and SWI/SNF nucleosome remodeling. Mol. Cell *11*, 1033–1041.

Angelov, D., Verdel, A., An, W., Bondarenko, V., Hans, F., Doyen, M.C., Studitsky, V.M., Hamiche, A., Roeder, R.G., Bouvet, P., and Dimitrov, S. (2004). SWI/SNF remodeling and p300-dependent transcription of histone variant H2ABbd nucleosomal arrays. EMBO J. *19*, 3815–3824.

Bazett-Jones, D.P., Cote, J., Landel, C.C., Peterson, C.L., and Workman, J.L. (1999). The SWI/SNF complex creates loop domains in DNA and polynucleosome arrays and can disrupt DNA-histone contacts within these domains. Mol. Cell. Biol. *19*, 1470–1478.

Beato, M., Herrlich, P., and Schütz, G. (1995). Steroid hormone receptors: many actors in search of a plot. Cell *83*, 851–857.

Belotserkovskaya, R., Oh, S., Bondarenko, V.A., Orphanides, G., Studitsky, V.M., and Reinberg, D. (2003). FACT facilitates transcription-dependent nucleosome alteration. Science *301*, 1090–1093.

Boeger, H., Griesenbeck, J., Strattan, J.S., and Kornberg, R.D. (2003). Nucleosomes unfold completely at a transcriptionally active promoter. Mol. Cell *11*, 1587–1598.

Bruno, M., Flaus, A., Stockdale, C., Rencurel, C., Ferreira, H., and Owen-Hughes, T. (2003). Histone H2A/H2B dimer exchange by ATP-dependent chromatin remodeling activities. Mol. Cell *12*, 1599–1606.

Cirillo, L.A., and Zaret, K.S. (2004). Preparation of defined mononucleosomes, dinucleosomes, and nucleosome arrays in vitro and analysis of transcription factor binding. Methods Enzymol. *375*, 131–158.

Côte, J., Quinn, J., Workmann, J.L., and Peterson, C.L. (1994). Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science *265*, 53–59.

Di Croce, L., Koop, R., Venditti, P., Westphal, H.M., Nightingale, K., Becker, P., and Beato, M. (1999). Two-steps synegism between progesterone receptor and the DNA binding domain of NF1 on MMTV minichromosomes. Mol. Cell *4*, 45–54.

Dyer, P.N., Edayathumangalam, R.S., White, C.L., Bao, Y., Chakravarthy, S., Muthurayan, U.M., and Luger, K. (2004). Reconstitution

of nucleosome core particles from recombinant histones and DNA. Methods Enzymol. *375*, 23–44.

Fazzio, T.G., and Tsukiyama, T. (2003). Chromatin remodeling in vivo: evidence for a nucleosome sliding mechanism. Mol. Cell *12*, 1333–1340.

Gavin, I., Horn, P.J., and Peterson, C.L. (2001). SWI/SNF chromatin remodeling requires changes in DNA topology. Mol. Cell *7*, 97–104.

Ichinose, H., Garnier, J.M., Chambon, P., and Losson, R. (1997). Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. Gene *188*, 95–100.

Jackson, V. (1978). Studies on histone organization in the nucleosome using formaldehyde as a reversible cross-linking agent. Cell *15*, 945–954.

Jaskelioff, M., Gavin, I.M., Peterson, C.L., and Logie, C. (2000). SWI-SNF-mediated nucleosome remodeling: role of histone octamer mobility in the persistence of the remodeled state. Mol. Cell. Biol. *20*, 3058–3068.

Kassabov, S.R., Henry, N.M., Zofall, M., Tsukiyama, T., and Bartholomew, B. (2002). High-resolution mapping of changes in histone-DNA contacts of nucleosomes remodeled by ISW2. Mol. Cell. Biol. *22*, 7524–7534.

Kassabov, S.R., Zhang, B., Persinger, J., and Bartholomew, B. (2003). SWI/SNF unwraps, slides, and rewraps the nucleosome. Mol. Cell *11*, 391–403.

Kireeva, M.L., Walter, W., Tchernajenko, V., Bondarenko, V., Kashlev, M., and Studitsky, V.M. (2002). Nucleosome remodeling induced by RNA polymerase II: loss of the H2A/H2B dimer during transcription. Mol. Cell *9*, 541–552.

Ko, M.S.H., Nakauchi, H., and Takahashi, N. (1990). The dose dependence of glucocorticoid-inducible gene expression results from changes in the number of transcriptionally active templates. EMBO J. *9*, 2835–2842.

Koop, R., Di Croce, L., and Beato, M. (2003). Histone H1 enhances synergistic activation of the MMTV promoter in chromatin. EMBO J. *22*, 588–599.

Längst, G., Bonte, E.J., Corona, D.F., and Becker, P.B. (1999). Nucleosome movement by CHRAC and ISWI without disruption or trans-displacement of the histone octamer. Cell *97*, 843–852.

Logie, C., and Peterson, C.L. (1999). Purification and biochemical properties of yeast SWI/SNF complex. Methods Enzymol. *304*, 726–741.

Lorch, Y., Zhang, M., and Kornberg, R.D. (1999). Histone octamer transfer by a chromatin-remodeling complex. Cell *96*, 389–392.

Muchardt, C., and Yaniv, M. (1993). A human homologue of Saccharomyces cerevisiae SNF2/SWI2 and Drosophila brm genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J. *12*, 4279–4290.

Nagaich, A.K., Walker, D.A., Wolford, R., and Hager, G.L. (2004). Rapid periodic binding and displacement of the glucocorticoid receptor during chromatin remodeling. Mol. Cell *14*, 163–174.

Negri, R., Buttinelli, M., Panetta, G., De Arcangelis, V., Di Mauro, E., and Travers, A. (2001). Sequence dependence of translational positioning of core nucleosomes. J. Mol. Biol. *307*, 987–999.

Nie, Z., Xue, Y., Yang, D., Zhou, S., Deroo, B.J., Archer, T.K., and Wang, W. (2000). A specificity and targeting subunit of a human SWI/SNF family-related chromatin-remodeling complex. Mol. Cell. Biol. *20*, 8879–8888.

Reinke, H., and Horz, W. (2003). Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. Mol. Cell *11*, 1599–1607.

Schnitzler, G., Sif, S., and Kingston, R.E. (1998). Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. Cell *94*, 17–27.

Spangenberg, C., Eisfeld, K., Stünkel, W., Luger, K., Flaus, A., Richmonds, T.J., Truss, M., and Beato, M. (1998). The MMTV promoter positioned on a tetramer of histones H3 and H4 binds nuclear factor 1 and OTF1. J. Mol. Biol. *278*, 725–739.

Strutt, H., and Paro, R. (1999). Mapping DNA target sites of chromatin proteins in vivo by formaldehyde crosslinking. Methods Mol. Biol. *119*, 455–467.

Truss, M., Bartsch, J., Schelbert, A., Haché, R.J.G., and Beato, M. (1995). Hormone induces binding of receptors and transcription factors to a rearranged nucleosome on the MMTV promoter in vivo. EMBO J. *14*, 1737–1751.

Vicent, G.P., Meliá, M.J., and Beato, M. (2002). Asymmetric binding of histone H1 stabilizes MMTV nucleosomes and the interaction of progesterone receptor with the exposed HRE. J. Mol. Biol. *324*, 501–517.

Wallberg, A.E., Neely, K.E., Hassan, A.H., Gustafsson, J.A., Workman, J.L., and Wright, A.P. (2000). Recruitment of the SWI-SNF chromatin remodeling complex as a mechanism of gene activation by the glucocorticoid receptor tau1 activation domain. Mol. Cell. Biol. *20*, 2004–2013.

Whitehouse, I., Flaus, A., Cairns, B.R., White, M.F., Workman, J.L., and Owen-Hughes, T. (1999). Nucleosome mobilization catalysed by the yeast SWI/SNF complex. Nature *400*, 784–787.

Yoshinaga, S.K., Peterson, C.L., Herskowitz, I., and Yamamoto, K.R. (1992). Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. Science *258*, 1598–1604.

Yudkovsky, N., Logie, C., Hahn, S., and Peterson, C.L. (1999). Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. Genes Dev. *13*, 2369–2374.