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# The Histone Variant MacroH2A Interferes with Transcription Factor Binding and SWI/SNF Nucleosome Remodeling

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## Summary

**The unusual histone variant macroH2A (mH2A) has been associated with repression of transcription, but the molecular mechanisms by which it exerts this function are unknown. Here we have identified a mechanism by which the different domains of mH2A may be involved in the repression of transcription. Evidence is presented that the presence of mH2A in a positioned nucleosome interferes with the binding of the transcription factor NF-κB. The nonhistone region of mH2A was identified to be associated with this interference. Importantly, the presence of macroH2A was found to severely impede SWI/SNF nucleosome remodeling and movement to neighboring DNA segments. This property of mH2A was demonstrated to reside only in its H2A-like domain. A hypothesis explaining the role of histone variants in transcriptional regulation is proposed.**

## Introduction

In eukaryotic cells, DNA is packaged into chromatin. The basic unit of chromatin, the nucleosome, comprises an octamer of core histones (two of each H2A, H2B, H3, and H4) around which two ~80 bp superhelical turns of DNA are wrapped. The structure of both the histone octamer and the nucleosome was solved by X-ray crystallography (Arents et al., 1991; Luger et al., 1997). The nucleosome acts as a barrier to the access of transcription factors to their target sites. Transcription factors can be classified in two categories depending on how they gain access to their cognate sites in chromatin: (i) factors which are able to bind to their target sites in canonic nucleosomes and (ii) factors which require nucleosome remodeling before they can bind to nucleosomally organized DNA (Beato and Eisfeld, 1997). The transcription factor NF-κB, which is essential for the regulation of expression of over 150 genes (Pahl, 1999), belongs to the first category. Indeed, in vitro studies have shown that NF-κB is able to bind to its cognate sequence on a nonpositioned nucleosome reconstituted on a DNA fragment isolated from the HIV-1 enhancer (Angelov et al., 2000; Steger and Workman, 1997).

Cells use chromatin remodeling complexes to overcome the general repression of transcription associated with the organization of DNA into nucleosomes (Kornberg and Lorch, 1999; Langst and Becker, 2001; Peterson and Workman, 2000; Travers, 1999; Tsukiyama and Wu, 1997; Wu et al., 1998). Different families of such complexes have been described (Cairns et al., 1996; Ito et al., 1997; Peterson and Tamkun, 1995; Tsukiyama and Wu, 1995; Varga-Weisz et al., 1997). Each of the chromatin remodeling complexes contains an ATPase that is required for the function of the complex. The known remodeling ATPases belong to either the SWI2/SNF2 type or the ISWI type or the CHD type (Becker, 2002; Langst and Becker, 2001). All three ATPases are able to assist histone octamer sliding in an ATP-dependent manner. In addition, the SWI/SNF types of remodeling machines can strongly perturb histone-DNA interactions. SWI/SNF is a multisubunit chromatin remodeling complex which has been show to play important roles in gene expression throughout eukaryotes (Peterson and Workman, 2000; Sudarsanam and Winston, 2000). This remodeling complex is required for both activation and repression of transcription (Sudarsanam et al., 1999, 2000; Sudarsanam and Winston, 2000). Despite many efforts, neither the mechanism by which SWI/SNF acts nor the conformation of the SWI/SNF remodeled nucleosome are clear yet (Langst and Becker, 2001; Peterson and Workman, 2000).

During different periods of a cell's life, distinct subsets of genes have to be completely shut down. One way of achieving this is by building a specialized nucleosome structure which is unlikely to remodel and bind transcription factors and therefore impairs transcription. All eukaryotes have histone variant proteins which show only a variable identity to their conventional counterparts (van Holde, 1988; Tsanev et al., 1993). These histone variants could be used by the cell to build nucleosomes with a different architecture, which might interfere with the remodeling process. No such examples have, however, been reported.

The histone variant macroH2A (mH2A) is a potential candidate for such a role. Indeed, the histone macroH2A is an unusual histone variant which is nearly three times the size of H2A (Chadwick and Willard, 2001; Pehrson and Fuji, 1998; Pehrson and Fried, 1992). Its amino-terminal third (H2A-like), which shares more than 60% identity with histone H2A, is fused to a long nonhistone region (NHR) (Chadwick and Willard, 2001; Pehrson and Fried, 1992). Immunofluorescence data suggested that mH2A is enriched in different regions of the nucleus including the inactive X chromosome (Chadwick and Willard, 2001; Costanzi and Pehrson, 1998, 2001 ; Mer-

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moud et al., 1999). Recent experiments have shown that the proximity of the nonhistone region of mH2A to a promoter leads to a specific repression of transcription of this promoter (Perche et al., 2000). The large nonhistone region of mH2A may act as a partial roadblock for the RNA polymerase and therefore, may directly impede the passage of RNA polymerase through nucleosomal DNA. The molecular mechanisms by which mH2A exerts its repressive function are, however, largely unknown.

Here we analyzed the properties of nucleosomes containing macroH2A (mH2A nucleosomes). It is shown that these nucleosomes exhibit structural alterations. These alterations interfere with the binding of a transcription factor to the variant nucleosomes as well as with the variant particle remodeling by the SWI/SNF remodeling complex. Distinct domains of mH2A are responsible for these effects. Based on these data, a model is proposed for the role of mH2A and histone variants in general in the regulation of gene expression.

## Results

### MacroH2A Nucleosomes Exhibit Altered Structure in the Vicinity of the Dyad Axis

mH2A is a highly unusual histone variant (Figure 1A), and its incorporation into a histone octamer may lead to the formation of a noncanonical nucleosome. To test this hypothesis, we first expressed and purified the full-length mH2A protein (Figure 1B, lane 2). This protein, together with recombinant H2B, H3, and H4 (lanes 3–6), were used to reconstitute nucleosomes on a radioactively end-labeled 152 bp DNA fragment comprising a *Xenopus borealis* somatic 5S RNA gene. This fragment contains a strong positioning signal which allows the reconstitution of a precisely translationally and rotationally positioned nucleosome (FitzGerald and Simpson, 1985; Thiriet and Hayes, 1998). Analysis of the proteins present in the reconstituted variant (Figure 1B, lane 7) and control (Figure 1B, lane 8) nucleosomes indicates that mH2A is efficiently incorporated in the nucleosome particles, a result in agreement with the available data (Changolkar and Pehrson, 2002). High-resolution electrophoretic mobility shift assay (EMSA) of the reconstituted particles showed that, under the experimental conditions used, complete reconstitution was achieved: i.e, no or very little free DNA was observed on the gel (Figure 1C). It should be noted that on this high-resolution EMSA (5.5% polyacrylamide gel), the mH2A nucleosomes showed a lower electrophoretic mobility than the conventional ones (Figure 1C). This could be related to the higher molecular mass and/or the different conformation of the variant mH2A nucleosomes.

In order to study the structural consequences of the incorporation of mH2A into the reconstituted particles, we performed hydroxyl radical and DNase I footprinting. The hydroxyl radical patterns of the control and mH2A nucleosomes were identical, showing clear cleavage peaks separated by approximately 10 base pair intervals in both cases (Figure 2A, compare lanes 1 and 2, and Figure 2B, lanes 1 and 2). This demonstrated a lack of steric hindrance to this small probe and a wrapping of the DNA around the histone octamer within the reconstituted particles (Hayes et al., 1991). The DNase I cleavage

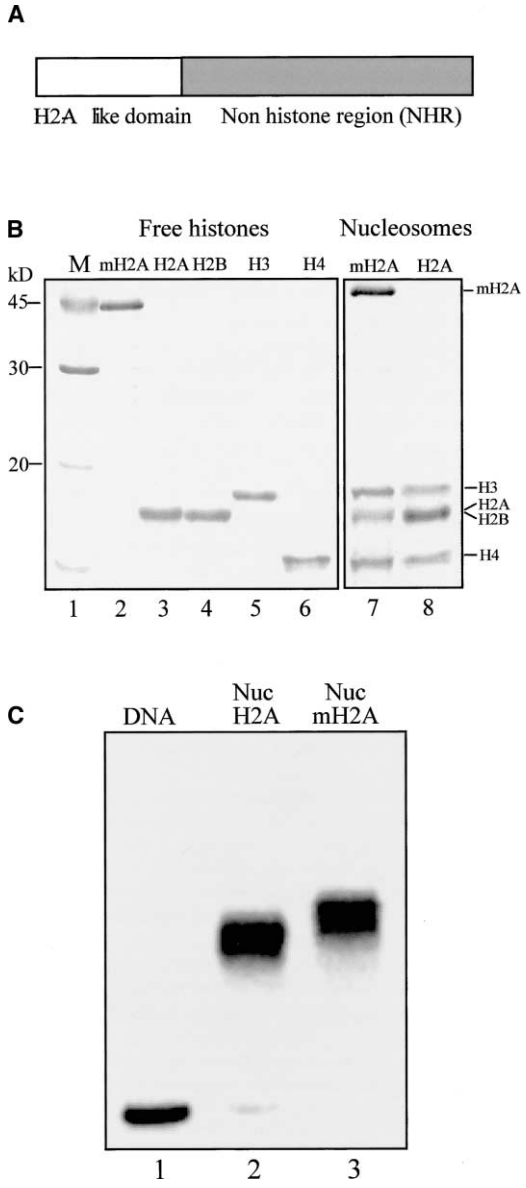


Figure 1. Macro H2A Is Able to Substitute for H2A in the Nucleosome

(A) Schematic presentation of macroH2A (mH2A) histone variant.

(B) 18% SDS-PAGE electrophoresis of the recombinant histones. *Xenopus laevis* H2A and H2B histone proteins comigrate in this type of gel. In lanes 2–6 individual proteins are analyzed. In lanes 7 and 8, proteins extracted from nucleosomes reconstituted with variant mH2A or conventional H2A are respectively analyzed.

(C) High-resolution EMSA in 5.5% polyacrylamide gel of the reconstituted conventional (lane 2) and variant (lane 3) nucleosomes. The slightly slower migration of mH2A containing nucleosomes is likely due to the much higher molecular weight of mH2A compared to conventional H2A histone, and/or to conformational changes of the reconstituted particles.

pattern, however, showed alterations in the structure of the mH2A nucleosome (Figure 2A, compare lanes 4 and 5, and Figure 2B, compare lanes 3 and 4). These alterations were observed mainly in vicinity of the nucleosome dyad axis. More precisely, the DNA regions of the top strand around +10 and of the bottom strand around

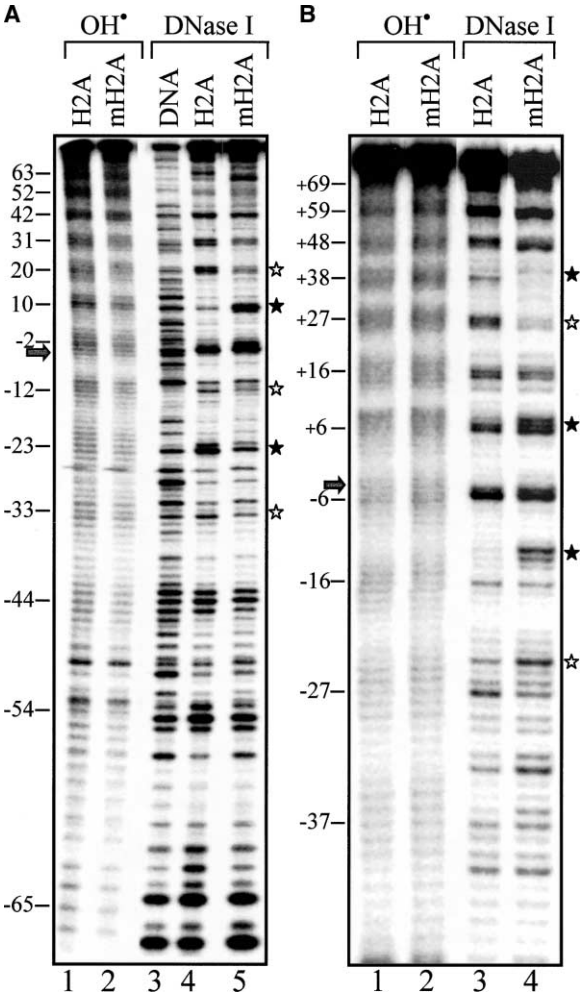


Figure 2. Hydroxyl Radical and DNase I Footprinting Analysis of Reconstituted Nucleosomal Particles

Reconstituted nucleosomes were subjected either to hydroxyl radical (OH•) or to DNase I cleavage, and the cleavage pattern was analyzed on a 8% sequencing gel. (A) Hydroxyl radical (OH•) and DNase I footprinting (0.1 U) of the reconstituted nucleosomes containing 5′ end-labeled DNA (top strand); (B) same as (A) but for 3′ end-labeled DNA (bottom strand). The arrows show the dyad axis. The major alterations in the nucleosomal structure are designated by filled stars, while the minor ones are designated by empty stars.

−11 and +6 exhibited a marked increased in accessibility to DNase I, whereas the regions of the top strand in vicinity of −23 and of the bottom strand in vicinity of +27 and +38 showed a pronounced decrease of the accessibility of the enzyme. Some other minor changes in the DNase I cleavage pattern were also noticed at few other positions as noted by the open star on the figures.

### Limited Access of NF-κB to mH2A Nucleosomes Containing the NF-κB Target Site in Vicinity to the Nucleosome Dyad Axis

In order to see if the observed alterations of mH2A nucleosome structure interfered with the binding of a transcription factor, two 152 bp 5S DNA constructs were built, each one containing a single recognition sequence of the transcription factor NF-κB located either at the vicinity of the dyad axis at positions −16 to −26 (where the major alterations of the structure of mH2A nucleosome were observed) or close to a nucleosomal DNA end at positions −53 to −63 (Figure 3A). These constructs were used to reconstitute control (H2A) and mH2A nucleosomes which were then allowed to interact with NF-κB. The binding of NF-κB was assessed by both EMSA and DNase I footprinting. The EMSA showed that the NF-κB binds quite efficiently to the control nucleosome, in agreement with the available data (Angelov et al., 2000; Steger and Workman, 1997). However, the interaction of NF-κB with the variant mH2A nucleosomes containing the NF-κB target sequence in the vicinity of the nucleosome dyad was strongly affected since even at the higher concentration of NF-κB only a very faint binding of the transcription factor was observed (Figure 3B). In order to further confirm this weak interaction of NF-κB with the variant nucleosome we performed DNase I footprinting analysis using NF-κB concentrations that allow the detection of a clear specific binding of the transcription factor to its target DNA sequence within the conventional nucleosome (Figure 3C, lanes 2–5). Under these experimental conditions, no protection was seen in the variant mH2A nucleosomes containing the NF-κB binding site close to the dyad (lanes 7–10) in contrast to the conventional ones (lanes 2–5). The situation was, however, quite different with the mH2A nucleosomes having the NF-κB binding site at positions −53 to −63, i.e., close to the end of the nucleosomal DNA. In this case, as shown by the footprinting analysis, NF-κB binds equally well to both conventional and mH2A nucleosomes (Figure 3D). It should be noted that in these experiments, the level of DNase I digestion has been adjusted to allow the detection of the footprint of NF-κB on its binding site. These conditions were slightly different (see Experimental Procedures) than those used in Figure 2B, and this is why the alteration of the pattern of digestion of the mH2A nucleosome is not as clear as in Figure 2. In conclusion, both the EMSA and the DNase I footprinting data demonstrated that the mH2A histone octamer impeded the specific binding of NF-κB to a site located in the vicinity of the dyad where the major alterations in the mH2A nucleosome structure were observed.

### SWI/SNF Is Unable to Remodel the mH2A Nucleosomes

We then asked if the ATP-dependent chromatin remodeling SWI/SNF complex was able to remodel the mH2A nucleosomes. For this purpose SWI/SNF was allowed to interact with control (H2A) and mH2A nucleosomes, and the efficiency of SWI/SNF interaction with these particles was studied by EMSA. The data clearly show that the SWI/SNF complex binds efficiently to the two types of nucleosomes (Figure 4A). In addition, the presence of mH2A resulted in no more than a 10% decrease of the SWI/SNF ATPase activity compared to that observed in the presence of conventional H2A (Figure 4B). Hence, mH2A affected the ATPase activity of SWI/SNF only very slightly. Once these control experiments were carried out, we next analyzed the remodeling capacity of SWI/SNF on these two types of nucleosome particles by DNase I footprinting. The SWI/SNF complex effi-

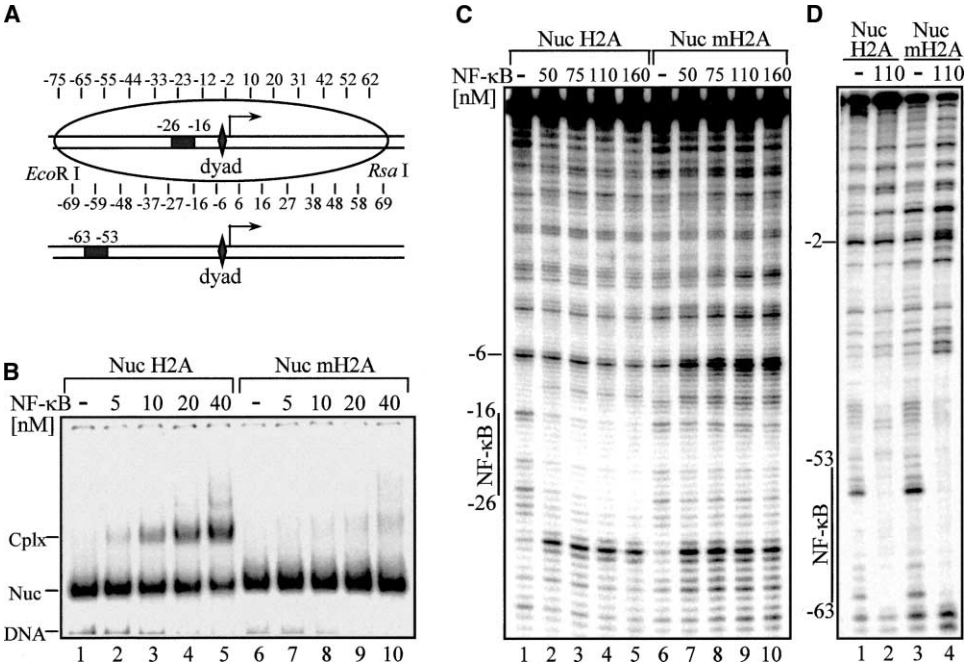


Figure 3. mH2A Interferes with the Binding of NF-κB when the NF-κB Binding Site Is Inserted in Vicinity to the Nucleosome Dyad Axis

A single NF-κB binding site was introduced either in vicinity to the dyad axis (positions at −16 to −26) or close to the end (positions −53 to −63) in the 152 bp EcoRI-RsaI fragment comprising a *Xenopus borealis* somatic 5S DNA. These DNA fragments were used to reconstitute control and mH2A nucleosomes. The nucleosomes were allowed to interact with increasing amounts of NF-κB, and the binding of NF-κB was assessed by DNase I footprinting (0.07 U) and EMSA.

(A) Schematic of the reconstituted nucleosome containing the NF-κB binding site in vicinity to the nucleosome dyad axis (−16 to −26) or close to the end (−53 to −63). The diamond indicates the dyad axis. The bold line shows the NF-κB binding sequence.

(B) EMSA of the NF-κB-nucleosome complexes analyzed on 4% polyacrylamide gels. The position of the nucleosomes (Nuc) and the NF-κB-nucleosome complexes (Cplx) and free DNA (DNA) are indicated. Note that with this low percentage of polyacrylamide, it is not possible to see the difference of migration of these two types of nucleosomes as shown in Figure 1C. The NF-κB concentrations used in this EMSA experiment were chosen to show the large difference in binding affinity of NF-κB between the conventional and mH2A nucleosomal particles.

(C) Footprint analysis of the binding of NF-κB to H2A- and mH2A nucleosomes containing the NF-κB binding site in vicinity of the nucleosome dyad axis. The result with the bottom strand is shown. The NF-κB binding site is indicated on the left of the figure. It should be noted that the amount of NF-κB used in this experiment was adjusted to get full protection of the binding site in order to get the footprint. The DNase I digestion conditions have been also modified compared to those used in Figure 2 in order for the NF-κB footprinting to be seen. In these conditions (weaker digestion), the modification of cleavage pattern of DNase I for the variant mH2A containing nucleosome is not as pronounced as in Figure 2. The major differences between normal and variant nucleosomes (−11, +6, and +38) are, however, visible (compare control lanes 1 and 6).

(D) Same as (C), but for control and mH2A nucleosomes containing the NF-κB binding sequence located close to the end of nucleosomal DNA (positions −53 to −63). Note that in these mH2A nucleosomes, the binding of NF-κB is not affected by the presence of mH2A.

ciently remodels the control H2A nucleosomes in the presence of ATP. The DNase I 10 bp specific nucleosomal cleavage pattern was dramatically perturbed in presence of increasing amount of SWI/SNF (Figure 5, lanes 1–4), a result in complete agreement with previously published data (Côté et al., 1998). This is in contrast with the complete absence of remodeling activity of SWI/SNF on the mH2A nucleosomes (Figure 5, lanes 6–9). Therefore, the presence of the histone variant mH2A within the nucleosome impeded severely the remodeling of the particle by SWI/SNF.

This conclusion was further strengthened by single mH2A nucleosome mobilization experiments (Langst et al., 1999). Conventional H2A- and variant mH2A nucleosomes positioned at the center of a 248 bp rDNA fragment were incubated with increasing amounts of SWI/SNF in the presence of ATP. The changes in their positions were analyzed by EMSA on native polyacrylamide gels (Figure 6). A clear, energy-dependent, SWI/SNF-induced sliding of the conventional histone octamers was detected, a result in full agreement with previously published data (Hamiche et al., 1999; Langst et al., 1999). In contrast, no sliding of the centrally positioned mH2A variant histone octamer was observed (Figure 6). Therefore, SWI/SNF was unable to generate movement of mH2A variant nucleosomes.

### Distinct Effects of the Nonhistone Region and the H2A-like Domain of MacroH2A on NF-κB Binding and SWI/SNF Nucleosome Remodeling

The presence of mH2A affects the SWI/SNF nucleosome remodeling and the binding of NF-κB to its target site when it is inserted in vicinity to the nucleosome dyad. Since mH2A is an unusual histone variant which consists of a H2A-like domain and a large nonhistone region, the question arises whether these both parts of mH2A are important for the interference with both nucleosome remodeling and NF-κB binding. To answer this question,

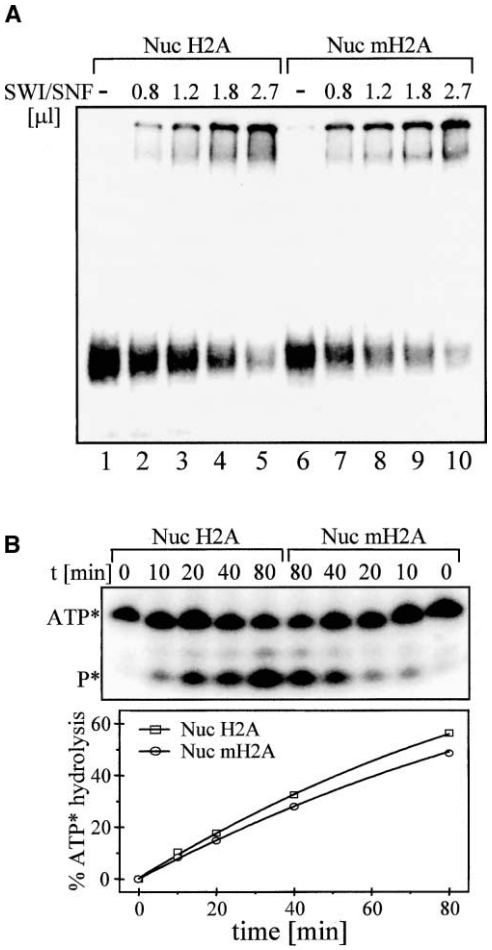


Figure 4. The Presence of mH2A Does Not Interfere with the Binding of SWI/SNF to the Nucleosome and with the SWI/SNF ATPase Activity

(A) Binding of SWI/SNF to conventional H2A- and variant mH2A nucleosomes. Conventional H2A and variant mH2A nucleosomes were allowed to interact with increasing amount of SWI/NF in the presence of 1 mM ATP and 3.5 mM $MgCl_2$, and the SWI/SNF-nucleosome complexes was visualized by EMSA on a 4% polyacrylamide gel.

(B) mH2A slightly affects the ATPase activity of SWI/SNF. The kinetics of SWI/SNF-induced ATP hydrolysis were analyzed in the presence of conventional H2A nucleosomes or variant mH2A particles.

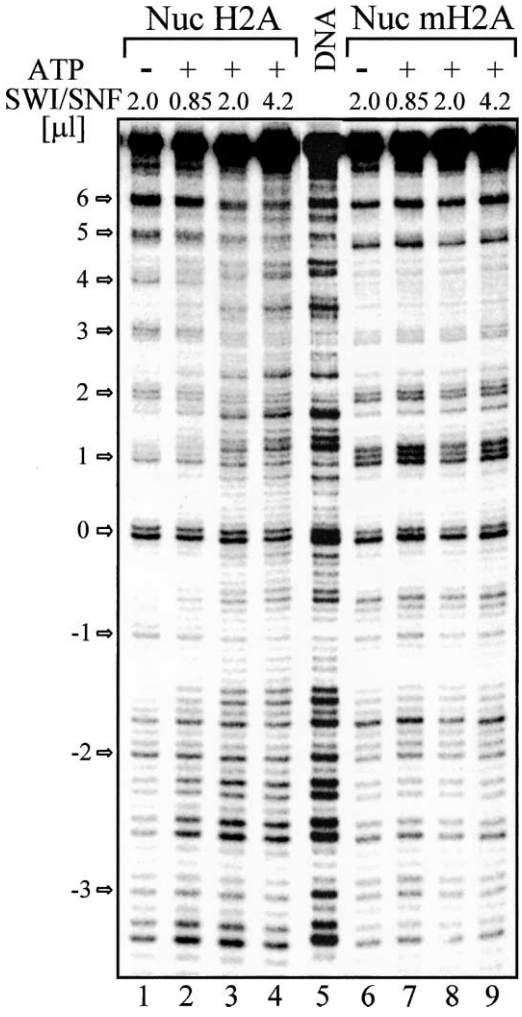


Figure 5. The Histone Variant mH2A Interferes with the Remodeling of the Nucleosome by SWI/SNF

Different amounts of SWI/SNF were added to a solution of either conventional (Nuc H2A) or variant (Nuc mH2A) nucleosomes, and the remodeling reaction was carried out in the presence (+) or the absence (−) of ATP. The 3′ end DNA (bottom strand) was labeled, and nucleosome remodeling was assessed by DNase I footprinting (0.15 U) and subjected to an 8% sequencing gel. Digestion of the free DNA (lane 5) was performed with 0.015 U of DNase I. The position of the DNase I cleavage repeat is indicated on the left of the figure.

we have cloned, expressed, and purified the H2A-like domain of mH2A (Figure 7A). Then the H2A-like protein was used to reconstitute variant nucleosomes on both the 152 bp DNA fragment comprising the *Xenopus borealis* 5S RNA gene which contained the NF-κB binding site in vicinity to the dyad and the 248 bp rDNA fragment. These reconstituted nucleosomes were used to study the NF-κB binding and SWI/SNF induced histone octamer sliding, respectively. As shown, the H2A-like protein was efficiently incorporated into the histone octamer, and H2A-like nucleosome particles were formed (Figures 7B–7D). These particles, however, behaved differently than the parental mH2A nucleosomes relative to the interaction of NF-κB. Indeed, NF-κB binds equally well to its target sequence located in vicinity to the dyad in control and H2A-like nucleosomes: in both cases, a very clear NF-κB-dependent footprint is observed (Figure 7C). Remarkably, the H2A-like histone octamer preserves the property of the parental mH2A octamer to interfere with the nucleosome mobilization induced by SWI/SNF. As shown, no H2A-like nucleosome sliding is observed in the presence of SWI/SNF (Figure 7D). Therefore, we conclude that the macroH2A nonhistone region is responsible for the NF-κB binding impediment, while the H2A-like domain interferes with nucleosome remodeling by SWI/SNF.

## Discussion

Eukaryote cells have developed different ways to modulate nucleosome structure and to use these modified

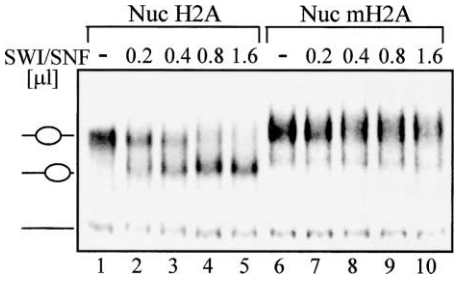


Figure 6. mH2A-Nucleosome Mobilization by SWI/SNF

Control (Nuc H2A) and variant mH2A (Nuc mH2A) nucleosomes positioned at the center of the 248 bp rDNA fragment were incubated with increasing amount of SWI/SNF in the presence of ATP. The nucleosome positions were then analyzed on native 5.5% polyacrylamide gels. The migration of the end- and the center-positioned nucleosomes and free DNA are indicated.

structures for the regulation of transcription. Covalent modifications of the histone tails and the nucleosome remodeling by high molecular mass protein complexes are two well-studied mechanisms. Here we report the study of a third largely unknown mechanism: the modulation of nucleosome structure by histone variants and its potential physiological consequences. By using recombinant histone proteins, positioned variant nucleosomes containing a mH2A histone octamer or a histone octamer containing only the H2A-like domain of mH2A were reconstituted. The particles containing the variant mH2A histone exhibited structural alterations, the strongest being found around the dyad axis at the vicinity of sites where the C terminus of histone H2A is believed to be localized in canonical H2A nucleosomes (Luger et al., 1997). The presence of mH2A interfered with the binding of NF-κB when the target sequence of the latter was inserted in vicinity to the dyad, but not with the NF-κB binding to mH2A nucleosomes containing a NF-κB target sequence close to a DNA nucleosomal end. Since the H2A-like domain of mH2A exhibited a high similarity to conventional H2A, the H2A-like C terminus should be positioned similarly to that of conventional H2A. Hence, the C-terminal nonhistone portion of mH2A should be located close to the dyad and could participate in the impediment of the binding of NF-κB. And indeed, we have found this to be the case since it was demonstrated that NF-κB binds equally well to control and to H2A-like nucleosomes, i.e., nucleosomes lacking the mH2A nonhistone region. The other perturbations observed in the mH2A nucleosome structure might be related to some other altered interactions of the mH2A variant octamer with nucleosomal DNA.

The presence of mH2A impeded the SWI/SNF remodeling and the mobilization of the variant nucleosomes. It could be noted that histone H1, which is known to contact H2A, was also able to partially inhibit the SWI/SNF nucleosome remodeling (Hill and Imbalzano, 2000). However, neither the binding of SWI/SNF to mH2A nucleosomes nor its ATPase activity were significantly affected by mH2A. The 10% lower ATPase activity of SWI/SNF observed in presence of mH2A containing particles might be the consequence of a slightly lower binding efficiency of SWI/SNF to the variant nucleosomes which is difficult to detect by the gel shift assay used

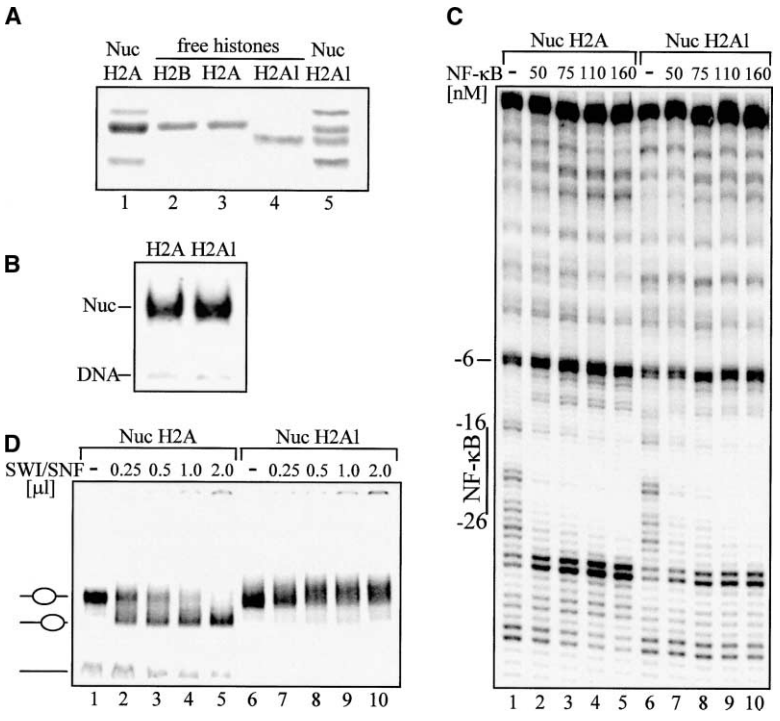


Figure 7. The Nonhistone Region of mH2A Interferes with the Binding of NF-κB while the H2A-like Domain Is Involved in the Impairment of mH2A Nucleosome Remodeling by SWI/SNF

(A) 18% SDS-PAGE electrophoresis of the recombinant histones H2B (lane 2), H2A (lane 3), and H2A-like (H2Al, lane 4) used for nucleosome reconstitution. Proteins extracted from nucleosomes reconstituted with either conventional H2A or H2A-like (H2Al) are shown in lanes 1 and 5, respectively.

(B) EMSA of nucleosomes reconstituted with conventional H2A (H2A) and H2A-like (H2Al) histones. The analysis was carried out on a 4% polyacrylamide gel.

(C) Footprinting analysis of the NF-κB binding to conventional H2A and variant H2A-like nucleosomes. Conventional (Nuc H2A) or H2A-like (Nuc H2Al) nucleosomes containing the NF-κB binding site in vicinity to the dyad were allowed to interact with increasing amounts of NF-κB. The binding of NF-κB was visualized by DNase I footprinting. The data of the bottom strand are shown only. The open arrow designates the cleavage site at −6 nt. The vertical bold line indicates the binding site of NF-κB. Note that NF-κB binds efficiently to both types of nucleosomes.

(D) Mobilization of H2A-like nucleosomes by SWI/SNF. Control (H2A) and H2A-like (H2Al) nucleosomes positioned at the center of the 248 bp fragment of rDNA were incubated with increasing amounts of SWI/SNF. Then the alterations in the positions of both nucleosomes were analyzed on native 5.5% polyacrylamide gel. The migrations of the centered- and end-position nucleosomes as well as of free DNA are indicated on the left part of the figure. Note the absence of sliding of the H2A-like histone octamer.

in this study. This cannot, however, explain the complete absence of remodeling of the mH2A containing nucleosomes. Importantly, the H2A-like domain was able alone to inhibit nucleosome remodeling, suggesting that this property reflected some peculiarities of the structure of the H2A-like histone octamer. Hence, the failure of SWI/SNF to remodel the variant mH2A nucleosomes as well as the impairment of NF-κB binding is likely related with the noncanonical structure of these particles. Inserting the variant mH2A histone into the histone octamer thus creates a nucleosome with novel properties.

How might the cell use this noncanonical mH2A nucleosome to regulate transcription? At different times during the cell life span, distinct groups of genes have to be completely transcriptionally locked up. To achieve this, the cell could specifically position a mH2A octamer on a selected sequence. The chromatin containing the mH2A variant histone would lead to a significant repression of transcription since: (i) the variant mH2A particle cannot be efficiently remodeled, and (ii) an initiator transcription factor, which is able to invade a conventional nucleosome, would be unable to bind to the mH2A nucleosome and initiate the process of transcriptional set up. To secure a complete inhibition of transcription of the desired subset of genes, additional mechanisms could be used by the cell, this time using only the nonhistone domain of mH2A. Indeed, previous data have shown that the presence of this mH2A C-terminal region at the proximity of a promoter can efficiently hinder its transcriptional activity (Perche et al., 2000). The nonhistone domain of mH2A may therefore act as a signal for the recruitment and the assembly of specific protein complexes, which would in turn create transcriptionally inactive regions. In addition, the mH2A nonhistone region may act as a roadblock for the passage of RNA polymerases.

It should be noted that the suggested scenario of transcriptional regulation through a histone variant differs considerably from the "histone code" hypothesis (Strahl and Allis, 2000). Indeed, the latter hypothesis suggests that the cell uses a histone "language" created by different combinations of histone tail modifications. These modifications of the histone tails as well as the tails themselves have, however, negligible effect on the nucleosome structure (Hans and Dimitrov, 2001; Luger and Richmond, 1998; Mutskov et al., 1998). Thus, the "histone code" hypothesis would not imply changes in the nucleosome structure, while the histone variant transcriptional regulation model is built on the alterations of the nucleosome structure due to the insertion of a variant histone in the histone octamer. Since the histone tail modifications can be quickly reversed, they could be used for rapid changes of the transcriptional status in a given promoter, while the presence of some variant histones as mH2A may be viewed as a long-term approach used by the cells to control transcription.

The data presented show that incorporation of the variant histone mH2A in a nucleosome prevents the binding of a specific transcription factor and to an inhibition of remodeling by the SWI/SNF machinery, thus suggesting that mH2A might be associated with the establishment of silent chromatin structure. Such a type of chromatin structure might be characteristic of the inactive X chromosome or heterochromatin: gene expression might be silenced by inhibition of both transcription site exposure and nucleosome mobility (Ahmad and Henikoff, 2002a). This could also have some epigenetic consequences (Ahmad and Henikoff, 2002a). On the other hand, two other histone variants, H3.3 (Ahmad and Henikoff, 2002b) and H2A.Z (Abbott et al., 2001; Santisteban et al., 2000; Suto et al., 2000), seem to be important for transcriptional activation. Interestingly, H2A.Z has also been found to be associated with gene silencing (Dhillon and Kamakaka, 2000). Therefore, the picture is quite complex since the same histone variant may be involved in both positive and negative regulation of transcription. This recalls the situation with histone tails where the same modifications participate both in the activation of transcription and in mitotic events (Hans and Dimitrov, 2001; Strahl and Allis, 2000). Although, the histone tail modifications have been the subject of numerous studies, the role of the histone variants in the control of gene expression has just begun to be explored. Several in vivo and in vitro approaches will have to be applied in order to solve this fascinating problem.

## Experimental Procedures

### DNA Fragments

The 152 bp EcoRI-RsaI fragment containing a *Xenopus borealis* somatic 5S gene was derived from plasmid pXP10 (Lee and Hayes, 1998). The recognition sequence of NF-κB (GGGGATTCCCC) was introduced at positions −16 to −26 or at positions −53 to −63 by site-directed mutagenesis using the QuickChange site-directed mutagenesis kit (Stratagene). The fragment was $^{32}$P-labeled at 5′ or 3′ ends at the EcoRI site by using either polynucleotide kinase and [γ-$^{32}$P]ATP or klenow fragment and [α-$^{32}$P]ATP.

A 248 bp fragment representing sequences between −238 and +16 relative to the mouse rDNA transcription site (+1) was produced by PCR using the plasmid PMR974 as template and $^{32}$P-labeled primer (Langst et al., 1999). All labeled DNA fragments were purified on a native 6% polyacrylamide gel.

### Protein Expression and Purification

Recombinant *Xenopus laevis* full-length histone proteins were produced in bacteria and purified as described (Luger et al., 1999). Bacterially expressed human NF-κB (i.e., p50 subunit) was prepared according to the protocol of Adams and Workman (1995). Yeast SWI/SNF complex was purified as described previously (Côté et al., 1994).

Human macroH2A1.2 cDNA, encoding amino acids 1–372, was amplified by PCR. The primers used were as follows: 5′-CCGGAATTCATGTCGAGCCGCGGTGGGAAGAAGAAG (forward, including an EcoRI restriction site (underlined) for in-frame cloning) and a 5′-GGAAGATCTTTAGTTGGCGTCCAGCTTGGCCATTTCCTG (reverse, including a BglII site, underlined). The resulting fragment was subcloned in PGEX-6P-1 vector. A GST-mH2A fusion protein was produced in *Escherichia coli* BL21 cells. The GST moiety was removed by digestion with the protease precision, and the nontagged mH2A histone was purified as described by Changolkar and Pehrson (2002).

For the production of the H2A-like domain of macroH2A, the cDNA encoding full-length macroH2A was subcloned in the pet 15B vector (Novagen) using the BamHI restriction site. Then, the H2A-like cDNA encoding amino acids 1–120 of the human macroH2A protein was obtained using the oligonucleotides H2AI− (5′CTTCCCACCGCGGCTCGACATGGTATATCTCCTTCTTAAAGTTAAAC) and H2AI + (5′GTTTAACTTTAAGAAGGAGATATACCATGTCGAGCCGCGGTGGGAAG) and the QuickChange site-directed mutagenesis kit (Stratagene). In the resulting pet 15B plasmid, the histidine tag was deleted; therefore, the N-terminal end of the protein corresponds exactly to that of wild-type protein (MSSR). The recombinant H2A-like protein produced from this plasmid was purified like the conventional histones

(Luger et al., 1999). All plasmids used in this study were checked by DNA sequencing.

### Nucleosome Reconstitution

Stochiometric amounts of H2A or macroH2A proteins and the three remaining core histones were dialyzed overnight at 4°C against histone folding buffer (20 mM Tris-HCl, pH 7.6, 1 mM EDTA, 5 mM β-mercaptoethanol, 2 M NaCl). The next morning the histones were mixed at a 0.8:1 w/w ratio with 100 ng of the $^{32}$P-labeled DNA fragments and 400 ng of nonlabeled nucleosome-length random sequence DNA, in a final 150 μl volume of histone folding buffer containing 100 μg/ml BSA. The nucleosome reconstitution was performed by dialysis against decreasing salt concentrations as previously described (Mutskov et al., 1998). In the cases when some free DNA was present after the reconstitution, the reconstituted nucleosomes were additionally purified on a 5%–30% sucrose gradient (Mutskov et al., 1998). The sucrose gradient-purified nucleosomes behaved identically to those not passed through the gradient.

For the mobilization experiments, nucleosomes were reconstituted on a 248 bp rDNA fragment as described above. After reconstitution, the centrally located nucleosomes were purified by preparative gel electrophoresis (5.5% polyacrylamide gel) as described in Langst et al. (1999).

### Electrophoretic Mobility Shift Assay, DNase I, and Hydroxyl Radical Footprinting

The EMSA was carried out in a 4% acrylamide-bis acrylamide (29:1) (for data shown in Figure 3B and 4A) in 0.5× TBE (Tris-Borate-EDTA). For high-resolution EMSA, 5.5% polyacrylamide-bis acrylamide (60:1) gels were used. The nucleosome samples were run at 4°C at 3V/cm–6V/cm. Upon completion of the electrophoresis, the gel was dried and exposed on a Phosphor-Imager screen.

For the DNase I footprinting, 10–15 fmol of reconstituted labeled nucleosomes were digested in 10 μl with 0.07–0.15 U of DNase I (as indicated in the figure legend) for 2 min at room temperature in 10 mM Tris-HCl, pH 7.6, 3.5 mM $MgCl_2$. The reaction was stopped by adding 100 μl of stop solution (10 mM EDTA, 0.2% SDS, 50 ng/μl proteinase K) followed by 30 min incubation at 50°C. The samples were then phenol chloroform extracted, ethanol precipitated, and separated on an 8% polyacrylamide denaturing sequencing gel (8 M urea). The dried gel was exposed on a Phosphor-Imager screen.

The hydroxyl radical footprinting was performed according to the protocol of Hayes and Lee (1997).

### Transcription Factor Binding

The NF-κB transcription factor binding reactions were performed in a binding buffer (10 mM HEPES, pH 7.8, 50 mM KCl, 5 mM DTT, 0.5 mM PMSF, 200 μg/ml BSA, 5% glycerol) at 30°C for 20 min. In order to find the optimal conditions of NF-κB binding, increasing amounts of NF-κB solution were added to a fixed amount of conventional (H2A) nucleosomes. A typical reaction of 10 μl contained 10–15 fmol of reconstituted end-labeled nucleosomes. The efficiency of the binding was checked by both EMSA and DNase I footprinting. Once the optimal binding conditions (the optimal molar ratio NF-κB: nucleosomes) were found for the conventional nucleosomes, the same ratio was used for the mH2A reconstituted particles.

### SWI/SNF Experiments

The measurements of the ATPase activity of SWI/SNF in the presence of nucleosome particles was carried out as previously described (Bochar et al., 2000). Briefly, 100 ng of reconstituted nucleosomes were mixed with 1 μl of SWI/SNF and 1 μCi of [γ-$^{32}$P]ATP in a final volume of 10 μl (10 mM HEPES, pH 7.8, 50 mM KCl, 5 mM DTT, 0.5 mM PMSF, 200 μg/ml BSA, 5% glycerol, 3,5 mM $MgCl_2$). Aliquots of 1 μl were taken at the time points indicated, and the reaction was stopped with 10 μl of gel loading buffer containing 90% formamide, 0.2% SDS, 10 mM EDTA, and dyes. ATP hydrolysis was analyzed on 15% denaturing polyacrylamide gels. Gels were dried and exposed with phosphoimager screens, and quantified using the ImageQuant software (Molecular dynamic). SWI/SNF remodeling reactions were performed in 10 μl aliquots in the binding buffer described above supplemented with 1 mM ATP. Either the indicated amount of a purified SWI/SNF complex (25–50 fmole/μl of SWI/SNF) or the equivalent buffer (for the samples not receiving this protein) was added. After incubation for 60 min at 30°C, 2 μl aliquots were taken for EMSA analysis, and the remaining 10 μl were supplemented with a 50-fold excess of native nucleosomes and 1 U of apyrase. After incubation for 15 min, the samples were submitted to DNase I digestion as described above, and using 0.15 U of DNase I.

The nucleosome mobility assay was performed using the 248 bp reconstituted nucleosome as described above, and the mobility was analyzed on a 5.5% polyacrylamide gel (60:1) according to the procedure described in Langst et al. (1999).

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### References

Abbott, D.W., Ivanova, V.S., Wang, X., Bonner, W.M., and Ausio, J. (2001). Characterization of the stability and folding of H2A.Z chromatin particles. Implications for transcriptional activation. J. Biol. Chem. *276*, 41945–41949.

Adams, C.C., and Workman, J.L. (1995). The binding of disparate transcription factors to nucleosomal DNA is inherently cooperative. Mol. Cell. Biol. *15*, 1405–1421.

Ahmad, K., and Henikoff, S. (2002a). Epigenetic consequences of nucleosome dynamics. Cell *111*, 281–284.

Ahmad, K., and Henikoff, S. (2002b). The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. Mol. Cell *9*, 1191–1200.

Angelov, D., Charra, M., Seve, M., Côté, 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.

Arents, G., Burlingame, R.W., Wang, B.-C., Love, W.E., and Moudrianakis, E.N. (1991). The nucleosomal core histone octamer at 3.1 Å resolution: a tripartite protein assembly and a left-handed superhelix. Proc. Natl. Acad. Sci. USA *88*, 10148–10152.

Beato, M., and Eisfeld, K. (1997). Transcription factor access to chromatin. Nucleic Acids Res. *25*, 3559–3563.

Becker, P.B. (2002). Nucleosome sliding: facts and fiction. EMBO J. *21*, 4749–4753.

Bochar, D.A., Savard, J., Wang, W., Lafleur, D.W., Moore, P., Cote, J., and Shiekhattar, R. (2000). A family of chromatin remodeling factors related to Williams syndrome transcription factor. Proc. Natl. Acad. Sci. USA *97*, 1038–1043.

Cairns, B.R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R.D. (1996). RSC, an essential, abundant chromatin-remodeling complex. Cell *87*, 1249–1260.

Chadwick, B.P., and Willard, H.F. (2001). Histone H2A variants and the inactive X chromosome: identification of a second macroH2A variant. Hum. Mol. Genet. *10*, 1101–1113.

Changolkar, L.N., and Pehrson, J.R. (2002). Reconstitution of nucleosomes with histone macroH2A1.2. Biochemistry *41*, 179–184.

Costanzi, C., and Pehrson, J.R. (1998). Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. Nature *393*, 599–601.

Costanzi, C., and Pehrson, J.R. (2001). MacroH2A2, a new member of the macroH2A core histone family. J. Biol. Chem. *276*, 21776–21784.

Côté, J., Quinn, J., Workman, J.L., and Peterson, C.L. (1994). Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science *265*, 53–60.

Côté, J., Peterson, C.L., and Workman, J.L. (1998). Perturbation of nucleosome core structure by the SWI/SNF complex persists after its detachement, enhancing subsequent transcription factor binding. Proc. Natl. Acad. Sci. USA *95*, 4947–4952.

Dhillon, N., and Kamakaka, R.T. (2000). A histone variant, Htz1p, and a Sir1p-like protein, Esc2p, mediate silencing at HMR. Mol. Cell *6*, 769–780.

FitzGerald, P.C., and Simpson, R.T. (1985). Effects of sequence alterations in a DNA fragment containing the 5S RNA gene from Lytechinus variegatus on positioning of a nucleosome core particle in vitro. J. Biol. Chem. *260*, 15318–15324.

Hamiche, A., Sandaltzopoulos, R., Gdula, D.A., and Wu, C. (1999). ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. Cell *97*, 833–842.

Hans, F., and Dimitrov, S. (2001). Histone H3 phosphorylation and cell division. Oncogene *20*, 3021–3027.

Hayes, J.J., and Lee, K.M. (1997). In vitro reconstitution and analysis of mononucleosomes containing defined DNAs and proteins. Methods *12*, 2–9.

Hayes, J.J., Clark, D.J., and Wolffe, A.P. (1991). Histone contributions to the structure of DNA in the nucleosome. Proc. Natl. Acad. Sci. USA *88*, 6829–6833.

Hill, D.A., and Imbalzano, A.N. (2000). Human SWI/SNF nucleosome remodeling activity is partially inhibited by linker histone H1. Biochemistry *39*, 11649–11656.

Ito, T., Bulger, M., Pazin, M.J., Kobayashi, R., and Kadonaga, J.T. (1997). ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. Cell *90*, 145–155.

Kornberg, R.D., and Lorch, Y. (1999). Chromatin-modifying and -remodeling complexes. Curr. Opin. Genet. Dev. *9*, 148–151.

Langst, G., and Becker, P.B. (2001). Nucleosome mobilization and positioning by ISWI-containing chromatin-remodeling factors. J. Cell Sci. *114*, 2561–2568.

Langst, 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.

Lee, K.-M., and Hayes, J.J. (1998). Linker DNA and H1-dependent reorganization of histone-DNA interactions within the nucleosome. Biochemistry *37*, 8862–8628.

Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature *389*, 251–260.

Luger, K., Rechsteiner, T.J., and Richmond, T.J. (1999). Expression and purification of recombinant histones and nucleosome reconstitution. Methods Mol. Biol. *119*, 1–16.

Luger, K., and Richmond, T.J. (1998). The histone tails of the nucleosome. Curr. Opin. Genet. Dev. *8*, 140–146.

Mermoud, J.E., Costanzi, C., Pehrson, J.R., and Brockdorff, N. (1999). Histone macroH2A1.2 relocates to the inactive X chromosome after initiation and propagation of X-inactivation. J. Cell Biol. *147*, 1399–1408.

Mutskov, V., Gerber, D., Angelov, D., Ausio, J., Workman, J., and Dimitrov, S. (1998). Persistent interactions of core histone tails with nucleosomal DNA following acetylation and transcription factor binding. Mol. Cell. Biol. *18*, 6293–6304.

Pahl, H.L. (1999). Activators and target genes of Rel/NF-kB transcription factors. Oncogene *18*, 6853–6866.

Pehrson, J.R., and Fried, V.A. (1992). MacroH2A, a core histone containing a large nonhistone region. Science *257*, 1398–1400.

Pehrson, J., and Fuji, R.N. (1998). Evolutionary conservation of histone macroH2A subtypes and domains. Nucleic Acids Res. *26*, 2837–2842.

Perche, P., Vourch, C., Souchier, C., Robert-Nicoud, M., Dimitrov, S., and Khochbin, C. (2000). Higher concentrations of histone macroH2A in the Barr body are correlated with higher nucleosome density. Curr. Biol. *10*, 1531–1534.

Peterson, C.L., and Tamkun, J.W. (1995). The SWI-SNF complex: a chromatin remodeling machine? Trends Biochem. Sci. *20*, 143–146.

Peterson, C.L., and Workman, J.L. (2000). Promoter targeting and chromatin remodeling by the SWI/SNF complex. Curr. Opin. Genet. Dev. *10*, 187–192.

Santisteban, M.S., Kalashnikova, T., and Smith, M.M. (2000). Histone H2A.Z regulates transcription and is partially redundant with nucleosome remodeling complexes. Cell *103*, 411–422.

Steger, D.J., and Workman, J.L. (1997). Stable co-occupancy of transcription factors and histones at the HIV-1 enhancer. EMBO J. *16*, 2463–2472.

Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41–45.

Sudarsanam, P., and Winston, F. (2000). The Swi/Snf family nucleosome-remodeling complexes and transcriptional control. Trends Genet. *16*, 345–351.

Sudarsanam, P., Cao, Y., Wu, L., Laurent, B.C., and Winston, F. (1999). The nucleosome remodeling complex, Snf/Swi, is required for the maintenance of transcription in vivo and is partially redundant with the histone acetyltransferase, Gcn5. EMBO J. *18*, 3101–3106.

Sudarsanam, P., Iyer, V.R., Brown, P.O., and Winston, F. (2000). Whole-genome expression analysis of snf/swi mutants of Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA *97*, 3364–3369.

Suto, R.K., Clarkson, M.J., Tremethick, D.J., and Luger, K. (2000). Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. Nat. Struct. Biol. *7*, 1121–1124.

Thiriet, C., and Hayes, J.J. (1998). Functionally relevant histone-DNA interactions extend beyond the classically defined nucleosome core region. J. Biol. Chem. *273*, 21352–21358.

Travers, A. (1999). An engine for nucleosome remodeling. Cell *96*, 311–314.

Tsanev, R., Russev, G., Pashev, I., and Zlatanova, J. (1993). Replication and Transcription of Chromatin (Boca Raton, FL: CRC Press).

Tsukiyama, T., and Wu, C. (1995). Purification and properties of an ATP-dependent nucleosome remodeling factor. Cell *83*, 1011–1020.

Tsukiyama, T., and Wu, C. (1997). Chromatin remodeling and transcription. Curr. Opin. Genet. Dev. *10*, 182–191.

van Holde, K. (1988). Chromatin (Berlin: Springer-Verlag KG).

Varga-Weisz, P.D., Wilm, M., Bonte, E., Dumas, K., Mann, M., and Becker, P.B. (1997). Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. Nature *388*, 598–602.

Wu, C., Tsukiyama, T., Gdula, D., Georgel, P., Martinez-Balbas, M., Mizuguchi, G., Ossipow, V., Sandaltzopoulos, R., and Wang, H.M. (1998). ATP-dependent remodeling of chromatin. Cold Spring Harb. Symp. Quant. Biol. *63*, 525–534.