Roles of the Histone H2A-H2B Dimers and the (H3-H4)$_2$ Tetramer in Nucleosome Remodeling by the SWI-SNF Complex

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SWI-SNF is an ATP-dependent chromatin remodeling complex required for expression of a number of yeast genes. Previous studies have suggested that SWI-SNF action may remove or rearrange the histone H2A-H2B dimers or induce a novel alteration in the histone octamer. Here, we have directly tested these and other models by quantifying the remodeling activity of SWI-SNF on arrays of (H3-H4)$_2$ tetramers, on nucleosomal arrays reconstituted with disulfide-linked histone H3, and on arrays reconstituted with histone H3 derivatives site-specifically modified at residue 110 with the fluorescent probe acetylatedylenediamine- (1,5)-naphthol sulfonate. We find that SWI-SNF can remodel (H3-H4)$_2$ tetramers, although tetramers are poor substrates for SWI-SNF remodeling compared with nucleosomal arrays. SWI-SNF can also remodel nucleosomal arrays that harbor disulfide-linked (H3-H4)$_2$ tetramers, indicating that SWI-SNF action does not involve an obligatory disruption of the tetramer. Finally, we find that although the fluorescence emission intensity of acetylatedylenediamine- (1,5)-naphthol sulfonate-modified histone H3 is sensitive to octamer structure, SWI-SNF action does not alter fluorescence emission intensity. These data suggest that perturbation of the histone octamer is not a requirement or a consequence of ATP-dependent nucleosome remodeling by SWI-SNF.

The assembly of eukaryotic DNA into folded nucleosomal arrays has drastic consequences for many nuclear processes that require access to the DNA sequence, including RNA transcription, DNA replication, recombination, and repair. The nucleosome, which consists of 147 bp of DNA wrapped nearly twice around an octamer of histones H2A, H2B, H3, and H4, can occlude DNA sequences both in vivo and in vitro. The nucleosome is not a static structure but appears to be a dynamic and flexible assembly. For instance, moderate concentrations of NaCl can lead to several distinct changes in nucleosome conformation (1–4). In addition, nucleosomes isolated from transcriptionally active chromatin appear to be depleted of histone H2A-H2B dimers (discussed in Ref. 5) and contain histone octamers the interiors of which are more accessible to enzymatic and chemical modifications (6–8). Nucleosomes from transcriptionally active chromatin can also be visualized microscopically as extended, largely unfolded structures (9, 10). These and other studies have led to the view that regulatory factors might antagonize the repressive effects of chromatin by disrupting the structure or conformation of the histone octamer (discussed in Ref. 11).

Two types of highly conserved chromatin remodeling enzymes have been implicated as regulators of the repressive nature of chromatin structure (12, 13). Several members of the SWI2/SNF2 family of DNA-stimulated ATPases use the energy of ATP hydrolysis to disrupt nucleosome structure, which can lead to an enhanced mobility of nucleosomes (14–16, 64). The second type consists of the nuclear histone acetyltransferases that covalently modify lysine residues within the flexible N-terminal domains of the histone proteins. The Saccharomyces cerevisiae SWI-SNF complex is the prototype ATP-dependent chromatin remodeling complex. This widely conserved 2-MDa multisubunit assembly is required for the inducible expression of a number of diversely regulated yeast genes and for the full functioning of many transcriptional activators (reviewed in Refs. 17 and 18). SWI-SNF can be recruited to target genes via direct interactions with gene-specific activators (19–21), and in several cases, SWI-SNF facilitates the binding of activators to nucleosomal sites in vivo (22, 23). In vitro, the purified SWI-SNF complex is a DNA-stimulated ATPase that can use the energy of ATP hydrolysis to disrupt histone-DNA interactions. Although the mechanism by which SWI-SNF disrupts nucleosome structure is not known, this “remodeling” reaction leads to an enhanced accessibility of nucleosomal DNA to DNase I (24–26), restriction enzymes (27, 28), and sequence-specific DNA-binding proteins (24, 25, 29). Moreover, SWI-SNF has been shown to increase DNA accessibility of nucleosomal arrays in a catalytic manner that is dependent on the presence of histone N-terminal domains (27, 28).

Four different models have been proposed to explain the mechanism by which ATP-dependent remodeling by the SWI-SNF complex increases nucleosomal DNA accessibility. 1) Several studies have suggested that SWI-SNF might remove or rearrange the H2A-H2B dimers (24, 30–34). 2) SWI-SNF remodeling may induce a novel conformation of the histone octamer (Refs. 35 and 36; discussed in Ref. 11) that might involve conformational changes in the (H3-H4)$_2$ tetramer analogous to the transcription-associated transitions described above. 3) SWI-SNF may use the energy of ATP hydrolysis to translocate along DNA and destabilize histone-DNA interactions (discussed in Ref. 37). This model is similar to the octamer spoiling mechanism described by Studitsky et al. (38) for passage of polymerases through nucleosomes. 4) Finally, SWI-SNF might bind directly to nucleosomes and use the energy of ATP hydrolysis to change the path of nucleosomal DNA (25) or to peel DNA
off the surface of the histone octamer without changing octamer structure (39).

In this study, we have directly tested whether ATP-dependent chromatin remodeling by the SWI-SNF complex alters the composition or conformation of the histone octamer. We use a nucleosomal array remodeling assay (27, 28) to quantify SWI-SNF activity on arrays of histone (H3-H4)$_2$ tetramers and on nucleosomal arrays reconstituted with histone octamers containing internally cross-linked tetramers. In order to monitor more subtle or transient changes in octamer structure, we also measured the effects of SWI-SNF remodeling on the steady state fluorescence of nucleosomal and tetramer arrays harboring a histone H3 derivative site specifically modified at residue 110 with the fluorescent probe acetylatedenediamine-(1,5)-napthol sulphonate (AEDANS) (1, 2, 40). Taken together, our data are consistent with a model in which substrate recognition by SWI-SNF requires an intact histone octamer, and subsequent ATP-dependent remodeling disrupts histone-DNA contacts without a concomitant loss of histone proteins or perturbation of the histone octamer.

**EXPERIMENTAL PROCEDURES**

Reagent Preparation—Array DNA template was isolated by digestion of plasmid pCL7c with NotI, HindIII, and HhaI (New England Biolabs) followed by FPLC purification on Sephacryl-500 (Amersham Pharmacia Biotech) essentially as described (27, 41). Array DNA template was end-labeled as described (27, 41).

Chicken erythrocyte histone octamers were purified from chicken whole blood (Pel-Freez) as described previously (42). Tetramers were provided as a kind gift from Jeff Hansen and were purified by stepwise elution from hydroxylapatite columns as described (42). Tetramers were dialyzed against Buffer T (1 mM NaCl, 10 mM Tris-HCl, pH 8.0, 0.25 mM EDTA, 0.1 mM dithiothreitol) prior to array reconstitution. Disulfide-linked histone octamers were generated by first diluting histone octamers 2-fold with Buffer D1 (10 M urea, 2 M NaCl, 20 mM Tris-HCl, pH 8.0) followed by dialysis against Buffer D2 (5 M urea, 20 mM Tris-HCl, pH 8.0) for 4 days. Histone octamers were then reconstituted by dialysis against Buffer D3 (2 mM NaCl, 10 mM Tris-HCl (pH 8.0, 0.25 mM EDTA). Histone H3 oxidation efficiency was analyzed by SDS-polyacrylamide gel electrophoresis in the absence of reducing agent (see Fig. 1B, lane 3). AEDANS-modified histone H3 was generated by first diluting histone octamers 2-fold with Buffer D1, followed by addition of iodoacetethylenediamine-(1,5)-naphthol sulphonate (1.5-AEDANS) (Molecular Probes, Inc.) at a molar ratio of 100:1 (1.5-AEDANS:H3) and incubation for 2 h at room temperature or on a nutator (Adams). Reactions were quenched with an excess of β-mercaptoethanol (Sigma) and dialyzed against Buffer D2 to remove unreacted 1,5-AEDANS. Samples were then dialyzed against Buffer D3 to reconstitute histone octamers. Labeling specificity was verified by visualization of the corresponding fluorescent histone band upon illumination with long wave ultraviolet light.

SWI-SNF complex was purified from yeast strain CY396 or CY743 (sin3Δ) as described (41). The concentration of complex was determined to be approximately 300 nM by comparative Western blot and by ATPase assays (27, 41). Reconstitution and Analysis of Nucleosomal Arrays—Arrays were reconstituted onto the 208-11 S DNA template (Fig. 1A) in a Slide-a-lyzer dialysis cassette (Pierce) using the salt dialysis protocol of Hansen and Lühr (43). Octamer concentrations were determined by A$_{280}$ (44). Octamer saturation and nucleosome/tetramer positioning was determined by EcoRI or MspI digestions using approximately 20 nM array in re- modeling buffer (5 mM MgCl$_2$, 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol) as described previously (27, 28, 41, 42). Arrays were digested for 30 min at 37 °C, and the reactions were electrophoresed on 4% native polyacrylamide gels (see Fig. 1C). The gel was briefly soaked in 2 μg/ml ethidium bromide and photographed under ultraviolet illumination.

Assay Conditions—Coupled array remodeling-restriction reactions were performed in a final concentration of 5 mM MgCl$_2$, 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 1 mM ATP, and 500 units/ml Hin$^c$I (New England Biolabs) as described previously (27, 28, 41).

Fluorescence spectroscopic studies were carried out using a PTI QM1S.E.9010S.E.910Q fluorescence spectrophotometer. Samples were excited at 344 nm (slit width, 2.5 nm), and emission intensities were recorded from 450 to 500 nm (slit width, 4 nm). Samples (80 μl in quartz microcuvettes) contained 4 nM AEDANS-labeled octamer or tetramer array in remodeling buffer (described above) at room temperature. In experiments assessing effects of monovalent salt, emission intensities were recorded initially at 15 min and subsequently every 0.5, 15, 30, and 50 min after subsequent adjustment to 50, 75, 150, and 300 mM NaCl and 0.5, 60, 120, 240, 480, and 960 min after subsequent adjustment to 600 mM NaCl. In experiments assessing effects of SWI-SNF, emission intensities were recorded initially at 5 min after addition of a final concentration of 2, 10, or 20 nM purified SWI-SNF and 5, 10, and 60 min after subsequent addition of a 1 mM final concentration of ATP. For samples in which SWI-SNF, ATP, or salt was added to the arrays, data were corrected for a buffer addition control. All data were corrected for dilution.

**RESULTS**

**SWI-SNF Remodels Arrays of Histone (H3-H4)$_2$ Tetramers—** It has been proposed that SWI-SNF increases the accessibility of nucleosomal DNA by depletion or rearrangement of the histone H2A-H2B dimers (Refs. 24, 30, 31, 32, and 34; discussed in Ref. 33). In order to investigate whether loss of the dimers is equivalent to the SWI-SNF remodeled state, we used purified chicken erythrocyte histone octamers or (H3-H4)$_2$ tetramers (Fig. 1B, lanes 1 and 4) to reconstitute nucleosomal or tetramer arrays. The DNA template used for these reconstitutions is composed of 11 head-to-tail repeats of a nucleosome positioning sequence from the *Lytechinus variegatus* 5 S rRNA gene, each of which is flanked by EcoRI restriction sites. Nucleosomes that are reconstituted onto each of these 5 S repeats assume a major translational position that is present in at least 50% of the population (45, 46). Minor translational positions also exist and differ from the major frame by multiples of 10 bp. The central repeat of our array template also bears a unique *Sal*I/Hin$^c$I restriction site close to the dyad axis of symmetry of a nucleosome positioned at the major frame (27, 47). Array reconstitutions were analyzed for extent of DNA repeat saturation and for correct positioning by restriction enzyme cleavage (Fig. 1C; see also under “Experimental Procedures”) (27, 41, 42). EcoRI digestion of nucleosomal or tetramer arrays releases primarily mononucleosome-sized particles and few of the high molecular weight partial digestion products that would be indicative of alternative positioning or oversaturation (Fig. 1C). Digestion of nucleosomal or tetramer arrays with *Msp*I (the site of which is located ~30 bp from the predicted dyad axis of a nucleosome positioned at the major frame) demonstrates that these sites are fully protected by nucleosomal arrays but accessible in tetramer arrays (Fig. 1C). These data are consistent with the fact that an (H3-H4)$_2$ tetramer assumes the same translational positions as an intact octamer (48, 49) but that the tetramer assembles less DNA (34).

We then exploited a sensitive nucleosomal array remodeling assay in which SWI-SNF remodeling activity is coupled to restriction enzyme activity (27, 28). Previously we used this assay to determine the kinetic parameters of ATP-dependent nucleosomal array remodeling by the SWI-SNF and RSC (remodels the structure of chromatin) complexes (27, 28, 41). To quantify the remodeling capability of SWI-SNF complex, 3 nM of nucleosomal or tetramer array was exposed to 500 units/ml Hin$^c$I, either in the presence or absence of 3 nM SWI-SNF complex and 1 mM ATP (Fig. 2A). As described previously, Hin$^c$I digestion of nucleosomal arrays is biphasic (27); the first, rapid phase of the reaction represents digestion of arrays having Hin$^c$I sites positioned between nucleosomes, and the second, slow phase represents digestion of the nucleosomal Hin$^c$I sites. By limiting our analysis to the second phase of Hin$^c$I digestion, the first order rate of Hin$^c$I cleavage yields a quantitative measurement of nucleosomal DNA accessibility (27, 28, 47).

In the absence of SWI-SNF, the first order rate of Hin$^c$I digestion...
cleavage was $1.3 \times 10^{-5}$ for tetramer arrays and $2.4 \times 10^{-6}$ for nucleosomal arrays (Fig. 2; see also Table I). The 5-fold higher rate for the tetramer arrays is comparable to the rate observed previously for nucleosomal arrays reconstituted with histones that lack their trypsin-sensitive N-terminal domains (28) and is consistent with nucleosomal DNA being more accessible in the absence of the histone H2A-H2B dimers (31, 48, 50, 51). In the presence of SWI-SNF, the first order rate of Hin cII cleavage was $6.4 \times 10^{-5}$ for tetramer arrays and $9.2 \times 10^{-5}$ for nucleosomal arrays (Fig. 2; Table I). The fact that the rate of cleavage of nucleosomal arrays in the presence of SWI-SNF significantly exceeded the rate of cleavage of tetramer arrays in the absence of SWI-SNF (see Table I) indicates that remodeling is not equivalent simply to loss of histone H2A-H2B dimers. The fact that the rate of cleavage of tetramer arrays in the presence of SWI-SNF was 5-fold higher than that of nucleosomal arrays in the presence of SWI-SNF (Fig. 2C; see also Table I).

SWI-SNF Activity Is Not Catalytic on Arrays of (H3-H4)2 Tetramers—Previously, we showed that SWI-SNF is able to catalytically remodel multiple nucleosomal arrays and that histone N-terminal domains are required for this reaction (27, 28). As remodeling of tetramer arrays appears to be quantitatively less effective than remodeling of nucleosomal arrays (see above), we investigated whether SWI-SNF was able to function catalytically, or only stoichiometrically, on tetramer arrays. Remodeling assays were carried out in which there was a 10-fold molar excess of array to remodeling complex (12 nM array to 1.2 nM SWI-SNF). Consistent with previous data, SWI-SNF was able to stimulate Hin cII cleavage of nucleosomal arrays throughout the 150-min time course (Fig. 3A, left panel; see also Refs. 27 and 28). However, there was only minor stimulation of Hin cII cleavage by SWI-SNF on tetramer arrays (Fig. 3A, right panel). In fact, the rate of Hin cII cleavage on tetramer arrays in the presence of SWI-SNF approximated the rate determined in the absence of remodeling complex (Fig. 2A, right panel; and Table I). Fig. 3B shows the quantification of
the amount of SWI-SNF-dependent array cleavage during the 150-min time course. As expected, SWI-SNF was able to perform approximately 2.5 rounds (3 nM array cleaved by 1.2 nM SWI-SNF) of nucleosomal array remodeling in 150 min (±50 min per round; see also Ref. 27). In contrast, SWI-SNF was unable to complete even one round of remodeling on tetramer arrays (0.3 nM array cleaved due to 1.2 nM SWI-SNF) during the time course. Therefore, SWI-SNF is unable to catalytically remodel multiple arrays of (H3-H4)2 tetramers.

One possible explanation for the lack of catalytic remodeling of tetramer arrays may be that SWI-SNF has a higher affinity for tetramer arrays and is thus defective for product release (analogous to the explanation for the lack of catalytic remodeling of trypsinized nucleosomal arrays (28)). In order to investigate this possibility, SWI-SNF remodeling reactions were assembled that contained labeled nucleosomal array and a 3- or 12-fold molar excess of free DNA, nucleosomal, trypsinized nucleosomal, or tetramer competitor array. Under these conditions, tetramer arrays competed for SWI-SNF activity to the same extent as nucleosomal arrays or free DNA. In contrast, trypsinized nucleosomal arrays were markedly more potent in competing for SWI-SNF activity (data not shown; see also Ref. 28). This suggests that the defect in catalytic remodeling of tetramer arrays, unlike the defect in catalytic remodeling of trypsinized nucleosomes, is not due to a higher affinity of SWI-SNF for these arrays.

SWI-SNF Remodeling Does Not Require Disruption of the (H3-H4)2 Tetramer—Chicken histone octamers contain only two cysteine residues (Cys-110 of each of the two copies of histone H3), and these cysteine residues are in close apposition within the interior of the histone octamer near the dyad axis of symmetry (34, 52). The cysteine residues normally are inaccessible to chemical modification, although denatured histone H3 can be disulfide-linked or chemically modified in vitro with minimal perturbation to the subsequently reconstituted histone octamer (52-54). In contrast, these cysteine residues appear to be more exposed to solvent in nucleosomes isolated from transcriptionally active chromatin (6–8) or when nucleosomes are exposed to higher salt concentrations (1, 2).

To investigate whether SWI-SNF action might require a structural transition of the (H3-H4)2 tetramer, we reconstituted nucleosomal arrays with histone octamers that contain disulfide-linked histone H3 (Fig. 1B, lane 3; see under “Experimental Procedures” for details). As described above, 3 nM of nucleosomal or disulfide-linked nucleosomal array was incubated in the presence or absence of SWI-SNF and 1 mM ATP. In the absence of SWI-SNF, the first order rate of cleavage by HinII was 3.8 × 10^{-6} for the disulfide-linked nucleosomal array compared with 2.4 × 10^{-6} for nucleosomal arrays (Fig. 4A; Table I). This is consistent with previous observations that disulfide-linked histone octamers can be reconstituted into nucleosomes that are not grossly different from canonical nucleosomes (Ref. 52–54; see also Fig. 1C). We then quantified the ability of SWI-SNF to increase the accessibility of nucleosomal DNA to restriction enzyme cleavage on the disulfide-linked and nucleosomal arrays. In the presence of SWI-SNF complex, HinII cleavage was stimulated 25-fold on the disulfide-linked arrays and 37-fold on the control nucleosomal arrays (Fig. 4A; Table I). Furthermore, the first order rate of HinII cleavage in the presence of SWI-SNF was nearly identical for the disulfide-linked and control nucleosomal arrays (Fig. 4B; Table I). These data strongly suggest that structural perturbation of the hist-
FIG. 3. SWI-SNF action does not catalyze on arrays of (H3-H4)$_2$ tetramers. A, representative time course for HincII digestion of 12 nM nucleosomal (left panel) or (H3-H4)$_2$ tetramer (right panel) arrays in the presence ($\bullet$) or absence ($\oplus$) of 1.2 nM SWI-SNF complex. Time point 0 reflects a 30-min preincubation with HincII in the absence of SWI-SNF. B, quantification of the data shown in A. Data are presented as the remodeller-dependent HincII cleavage of either nucleosomal ($\bullet$) or tetramer ($\oplus$) arrays versus time. Remodeller-dependent HincII cleavage events were obtained by subtracting the fraction of cleaved arrays in the absence of SWI-SNF (A, $\oplus$) from the fraction of cleaved arrays in the presence of SWI-SNF (A, $\bullet$). Data are presented as nanomolar remodeled nucleosomal arrays versus time.

Consistent with previous results (1, 2), a biphasic decrease in fluorescence emission intensity was observed, with a fast component on the second time scale (complete within 30 s) and a slow component on the hour time scale (complete within 4 h). These salt-dependent decreases in fluorescence emission intensity were observed for both nucleosomal and tetramer arrays, indicating that the observed decreases are not due to disruption of the interface between the (H3-H4)$_2$ tetramer and the H2A-H2B dimers. However, we note that AEDANS-modified tetramer arrays exhibit a ~3-fold lower fluorescence emission intensity than AEDANS-modified nucleosomal arrays (see Fig. 5B, left panels), suggesting that the presence of the H2A-H2B dimers decreases solvent accessibility of the (H3-H4)$_2$ tetramer.

We next measured the effect of SWI-SNF remodeling activity on the steady state fluorescence of AEDANS-modified nucleosomal or tetramer arrays. SWI-SNF (2, 10, 20 nM) was added to arrays (4 nM) equilibrated in remodeling buffer (see under “Experimental Procedures” for details) in the absence of ATP, and emission intensities were recorded (Fig. 5B, right panel, and data not shown). Subsequently, ATP was added to a final concentration of 1 mM, and emission intensities were recorded 5, 10, and 60 min after its addition (Fig. 5B; right panel). In striking contrast to NaCl titration (Fig. 5B, left panels), neither the binding of SWI-SNF (which occurs in the absence of ATP) nor ATP-dependent remodeling altered the fluorescence emission intensities of arrays (Fig. 5B, right panels). Addition of HincII directly to the reaction cuvettes confirmed that SWI-SNF and ATP stimulated HincII cleavage of the arrays and thus was active under these conditions (data not shown). Thus, these data indicate that SWI-SNF remodeling is not accompanied by the loss of the H2A-H2B dimers or by disruption or changes in the internal accessibility of the (H3-H4)$_2$ tetramer.

**DISCUSSION**

The *S. cerevisiae* SWI-SNF complex provides a paradigm for a family of eukaryotic protein assemblies that function in an ATP-dependent manner to alter chromatin structure. Although it is evident that chromatin remodeling by SWI-SNF and other related complexes results in enhanced accessibility of nucleosomal DNA and an increased mobility of nucleosomes, the mechanism by which this reaction occurs remains controversial. This study was designed to directly test simple predictions.
for several models that have been proposed for ATP-dependent chromatin remodeling.

The “Dimer Disruption” Model—In the extreme case, the dimer disruption model predicts that ATP-dependent remodeling by SWI-SNF will generate a (H3-H4)2 tetramer. Although assembly of a (H3-H4)2 tetramer is sufficient to position at least 90 bp of DNA (34), several studies have shown that DNA-tetramer particles are more accessible to DNA-binding proteins (31, 50, 51), and removal of the histone H2A-H2B dimers facilitates transcription in vitro (58). A simple prediction of such a model is that the accessibility of DNA within (H3-H4)2 tetramer arrays should be equivalent to that of nucleosomal DNA after SWI-SNF remodeling. However, we find that DNA wrapped around a (H3-H4)2 tetramer is only about 5-fold more accessible to HinclI digestion compared with DNA assembled onto a complete histone octamer. SWI-SNF action, on the other hand, enhances nucleosomal DNA accessibility by ~35-fold (see Table I). Thus, the remodeled state of a nucleosome is not equivalent to a (H3-H4)2 tetramer. The dimer disruption model also predicts that SWI-SNF will not be able to enhance the accessibility of (H3-H4)2 tetramers. We found, however, that SWI-SNF can remodel arrays of (H3-H4)2 tetramers, which leads to an additional 5-fold enhancement of HinclI cleavage rates. Finally, we show that fluorescence emission intensity of AEDANS-modified tetramer arrays is significantly lower than that of AEDANS-modified nucleosomal arrays (presumably due to increased solvent accessibility of the internal structure of the tetramer) (Fig. 5B). The fact that SWI-SNF remodeling of AEDANS-modified nucleosomal arrays does not decrease fluorescence emission intensity from the level characteristic of nucleosomal arrays to the level of tetramer arrays (Fig. 5B, right panels) also indicates that remodeling does not involve dimer disruption. Thus, SWI-SNF action does not convert a nucleosome into a tetramer.

The “Nucleosome Spooling” Model—The nucleosome spooling model for SWI-SNF action is based on the mechanism for passage of some RNA polymerases through a nucleosome (38). This model proposes that the energy of ATP hydrolysis might be used to translocate SWI-SNF along DNA and around a nucleosome in a “wave-like” fashion (discussed in Ref. 37). Such ATP-driven translocation of SWI-SNF along the DNA would disrupt histone-DNA contacts and may also lead to movement of the histone octamer. Such a reaction mechanism might also result in transfer of intact histone octamers onto an acceptor DNA, which has been observed during nucleosome remodeling by the yeast RSC complex (59). The nucleosome spooling model predicts that SWI-SNF will not discriminate between a tetramer and nucleosomal substrate. In fact, one might predict that the absence of H2A-H2B dimers might facilitate the ability of SWI-SNF to translocate through the residual histone-DNA interactions of the (H3-H4)2 tetramer. Although SWI-SNF does remodel the (H3-H4)2 tetramer arrays, the apparent rate of remodeling was approximately 30% slower than the rate for nucleosomal array remodeling. Furthermore, SWI-SNF was inactive on the (H3-H4)2 tetramers in remodeling reactions in which the concentration of tetramer array was in excess over SWI-SNF. These results demonstrate that arrays of (H3-H4)2 tetramers are poor substrates for ATP-dependent remodeling by SWI-SNF, a result that is not predicted by the nucleosome spooling model. Furthermore, SWI-SNF does not show ATP-dependent tracking activity in a DNA supercoiling assay (60), nor do other SWI2/SNF2 family members (e.g. Mot1p) demonstrate DNA tracking activity (61). Together, these data suggest that ATP-dependent remodeling by SWI-SNF does not involve DNA tracking, nor is it equivalent to the loss of the H2A-H2B dimers. Our data do indicate that efficient remodeling activity requires a canonical histone octamer that contains both an (H3-H4)2 tetramer and one or more H2A-H2B dimers.

SWI-SNF Action Does Not Perturb Octamer Structure—Several groups have recently suggested the alternative possibility that SWI-SNF activity might induce a novel conformation of the nucleosome that may involve rearrangement of the histone octamer without loss of histone proteins (32, 35, 36). This model is consistent with recent electron microscopy studies that indicated that ATP-dependent remodeling by SWI-SNF does not change the protein mass of a nucleosome and that remodeling is relatively insensitive to addition of an external cross-linking reagent, dimethyl suberimidate to mononucleosomes or nucleosomal arrays (39). What is this alternate conformation? Lee et al. (32) proposed that human SWI-SNF might use the energy of ATP hydrolysis to rearrange one or both H2A-H2B dimers such that only the flexible N-terminal domain contacts DNA close to the nucleosomal dyad. This novel octamer conformation might then have a propensity to form the dinucleosome-like particle.
that was previously observed (35, 36). Alternatively, SWI-SNF action might lead to a conformational change in the (H3-H4)$_2$ tetramer that might mimic the “split nucleosome” or “lexosome” structure that has been proposed for the structure of transcriptionally active chromatin (6, 9, 10).

To test these possibilities, we took advantage of the single cysteine residue found within each copy of chicken histone H3. These two cysteines are buried within the histone octamer and are located very close to each other at the nucleosomal dyad axis (34, 52). To test for gross changes in the structure of the (H3-H4)$_2$ tetramer, we monitored the apparent rates of remodeling of nucleosomal arrays that contain disulfide-linked (H3-H4)$_2$ tetramers. We found that SWI-SNF remodeled these substrates with rates equivalent to nucleosomal arrays. These data indicate that SWI-SNF action does not require a significant rearrangement of the tetramer.

To probe for more subtle changes in the structure of the histone octamer, we also monitored the effects of ATP-dependent remodeling on the steady state fluorescence of AEDANS-modified nucleosomal and tetramer arrays. Steady state fluorescence of AEDANS-H3 has been used to detect at least three distinct conformational states of the nucleosome as a function of salt concentration (1, 2). Furthermore, the solvent accessibility of the AEDANS group, and thus its fluorescence emission intensity, is predicted from previous fluorescence studies to be highly dependent on the presence of one or both histone H2A-H2B dimers (Ref. 57; see also Fig. 5B, left panels).

We found that in contrast to increased salt concentrations, which have large effects on fluorescence emission intensities of nucleosomal or tetramer arrays (Refs. 1 and 2; see also Fig. 5B, left panels), addition of SWI-SNF (one SWI-SNF per two nucleosomes), with or without ATP, had no measurable effect on fluorescence emission intensities (Fig. 5B, right panels). These data suggest that SWI-SNF does not change the overall structure or the solvent accessibility of the histone octamer or tetramer.

What Is ATP-dependent Chromatin Remodeling?—Our data are consistent with previous suggestions that ATP-dependent nucleosome remodeling by SWI-SNF disrupts histone-DNA contacts without a structural change in the histone octamer (Ref. 25; discussed in Ref. 62). How does SWI-SNF accomplish this feat? The remodeling reaction randomizes the rotational setting of both wraps of nucleosomal DNA without removing most of the DNA from the octamer surface (25). Furthermore, SWI-SNF action does not enhance the reactivity of nucleosomal DNA to potassium permanganate, indicating...
that remodeling does not involve an unwinding of the DNA double helix (25). We favor models in which the energy of ATP hydrolysis is used to rotate the DNA helix along its long axis relative to the histone octamer. In one such model, SWI-SNF would remain at a fixed position relative to the histone octamer, and both SWI-SNF and the octamer would remain in a fixed translational position relative to DNA. In this model, SWI-SNF would rotate the DNA helix back and forth with each round of ATP hydrolysis, changing rotational phasing and disrupting histone-DNA contacts throughout both wraps of nucleosomal DNA. In another, similar model, SWI-SNF would remain in a fixed position relative to the octamer, but SWI-SNF and the octamer would not remain in a fixed translational position relative to the DNA. In this model ATP-driven DNA helix rotation would “screw” the octamer along the DNA helix, changing the translational position of the octamer (analogous to a model proposed in Ref. 63). Either of these models would be consistent with previous data (see above and Ref. 25), as well as with recent studies indicating that several members of the SWI-SNF family of chromatin remodeling complexes can enhance nucleosome mobility (14–16, 64). Studies designed to test and distinguish between these models are in progress.

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REFERENCES

1. Dieterich, A. E., Axel, R., and Cantor, C. R. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 199–206
2. Dieterich, A. E., Axel, R., and Cantor, C. R. (1979) J. Mol. Biol. 129, 587–602
3. Yager, T. D., and van Holde, K. E. (1984) J. Biol. Chem. 259, 4212–4222
4. Czarzota G. J., and Ottensmeyer, F. P. (1996) J. Biol. Chem. 271, 3677–3683
5. Hansen, J. C., and Ausi, J. (1992) Trends Biochem. Sci. 17, 187–191
6. Prior, C. P., Cantor, C. R., Johnson, E. M., Littau, V. C., and Allfrey, V. G. (1983) Cell 34, 1033–1042
7. Allegre, P., Sternier, R., Clayton, D. F., and Allfrey, V. G. (1987) J. Mol. Biol. 196, 379–388
8. Chen, T. a., Smith M. M., Le, S. Y., Sternglanz, R., and Allfrey, V. G. (1991) J. Biol. Chem. 266, 6489–6498
9. Bazett-Jones, D. P., Mendez, E., Czarzota G. J., Ottensmeyer, F. P., and Allfrey, V. G. (1996) Nucleic Acids Res. 24, 321–329
10. Czarzota G. J., Bazett-Jones, D. P., Mendez, E., Allfrey, V. G., and Ottensmeyer, F. P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1033–1042
11. Lorch, Y., Caires, B. R., Zhang, M., and Kornberg, R. D. (1998) Cell 94, 29–34
12. Schnitzler, G., Sif, S., and Kingston, R. E. (1998) Biochemistry 37, 12642–12649
13. Xu, K. M., Sif, S., Kingston, R. E., and Hayes, J. J. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9129–9134
14. Stein, A. (1979) J. Mol. Biol. 130, 103–134
15. Dong, F., and van Holde, K. E. (1991) J. Biol. Chem. 266, 8409–8418
16. Outten, K., and Carlson, M. (1992) Trends Genet. 8, 387–391
17. Lorch, Y., and Peterson, C. L. (1997) Biochim. Biophys. Acta 1350, 159–168
18. Bahr, B. M., Zhou, H., and Workman, J. L. (1999) Trends Genet. 15, 371–382
19. Wu, C., and Whitehouse, I. (1999) Nature 378, 44–47
20. He, M., and Zhang, M. (1999) Biochemistry 38, 6929–6933
21. Yudkovsky, N., Logie, C., Hahn, S., and Peterson, C. L. (1999) Genes Dev. 15, 2369–2374
22. Lorch, Y., and Peterson, C. L. (1997) Mol. Cell. Biol. 17, 4811–4819
23. Cosma, M. P., Tanaka, T., and Nasmyth, K. (1999) Cell 97, 299–311
24. Cote, J., Quinton, J., Workman, J. L., and Peterson, C. L. (1994) Science 265, 53–60
25. Cote, J., Peterson, C. L., and Workman, J. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 4947–4952
26. Owen-Hughes, T. A., Utley, R. T., Cote, J., Peterson, C. L., and Workman, J. L. (1996) Science 273, 513–516
27. Logie, C., and Peterson, C. L. (1997) EMBO J. 16, 6772–6782
28. Logie, C., Tse, C., Hansen, J., and Peterson, C. L. (1999) Biochemistry 38, 2514–2522
29. Utley, R. T., Cote, J., Owen-Hughes, T., and Workman, J. L. (1997) J. Biol. Chem. 272, 12462–12469
30. Hirschhorn, J. N., Brown, S. A., Clark, C. D., and Winston, F. (1992) Genes Dev. 6, 2288–2298
31. Prior, C. P., Cantor, C. R., Johnson, E. M., Littau, V. C., and Allfrey, V. G. (1983) Cell 34, 1033–1042
32. Allegre, P., Sternier, R., Clayton, D. F., and Allfrey, V. G. (1987) J. Mol. Biol. 196, 379–388
33. Allegra, P., Le, S. Y., Sternglanz, R., and Allfrey, V. G. (1991) J. Biol. Chem. 266, 6489–6498
34. Bazett-Jones, D. P., Mendez, E., Czarzota G. J., Ottensmeyer, F. P., and Allfrey, V. G. (1996) Nucleic Acids Res. 24, 321–329
35. Czarzota G. J., Bazett-Jones, D. P., Mendez, E., Allfrey, V. G., and Ottensmeyer, F. P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1033–1042
36. Logie, C., Tse, C., Hansen, J., and Peterson, C. L. (1999) Biochemistry 38, 2514–2522
37. Logie, C., Tse, C., Hansen, J., and Peterson, C. L. (1999) Biochemistry 38, 2514–2522
38. Logie, C., Tse, C., Hansen, J., and Peterson, C. L. (1999) Biochemistry 38, 2514–2522
39. Logie, C., Tse, C., Hansen, J., and Peterson, C. L. (1999) Biochemistry 38, 2514–2522
Roles of the Histone H2A-H2B Dimers and the (H3-H4)$_2$Tetramer in Nucleosome Remodeling by the SWI-SNF Complex
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