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The Core Histone N-Terminal Domains Are Required for Multiple Rounds of Catalytic Chromatin Remodeling by the SWI/SNF and RSC Complexes†

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ABSTRACT: SWI/SNF and RSC are large, distinct multi-subunit complexes that use the energy of ATP hydrolysis to disrupt nucleosome structure, facilitating the binding of transcription factors or restriction enzymes to nucleosomes [Cote, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994) *Science 265*, 53−60 (*1*); Lorch, Y., Cairns, B. R., Zhang, M., and Kornberg, R. D. (1998) *Cell 94*, 29−34 (*2*)]. Here we have used a quantitative assay to measure the activities of these ATP-dependent chromatin remodeling complexes using nucleosomal arrays reconstituted with hypoacetylated, hyperacetylated, or partially trypsinized histones. This assay is based on measuring the kinetics of restriction enzyme digestion of a site located within the central nucleosome of a positioned 11-mer array [Logie, C., and Peterson, C. L. (1997) *EMBO J. 16*, 6772−6782 (*3*)]. We find that the DNA-stimulated ATPase activities of SWI/SNF and RSC are not altered by the absence of the histone N-termini. Furthermore, ATP-dependent nucleosome remodeling is also equivalent on all three substrate arrays under reaction conditions where the concentrations of nucleosomal array and either SWI/SNF or RSC are equivalent. However, SWI/SNF and RSC cannot catalytically remodel multiple nucleosomal arrays in the absence of the histone termini, and this catalytic activity of SWI/SNF is decreased by histone hyperacetylation. These results indicate that the histone termini are important for SWI/SNF and RSC function; and, furthermore, our data defines a step in the remodeling cycle where the core histone termini exert their influence. This step appears to be after remodeling, but prior to intermolecular transfer of the remodelers to new arrays.

Nuclear processes, such as transcription, replication, recombination, and DNA repair, require that enzymes gain access to the DNA template. Eukaryotic DNA, however, is not freely accessible in vivo; instead, it is complexed with histone and non-histone proteins to form a chromatin fiber. The basic building block of chromatin is the nucleosome core particle, which contains 145−147 bp[1] of DNA wrapped about twice around an octamer of histone proteins. Nucleosome cores are further organized into long arrays which can compact into more refractory higher order structures (*4*) that are stabilized by the linker histone H1 (*5*). Not unexpectedly, both nucleosomes and nucleosomal arrays are potent repressors of transcription (for a review, see ref *6*).

The histone octamer is a tripartite assembly that is composed of a central heterotetramer of histones H3 and H4, flanked by two heterodimers of histones H2A and H2B. Each histone protein has a central tertiary structural motif, called the histone-fold, that mediates the histone−histone interactions required for octamer and nucleosome assembly (*7*, *8*). In addition to the structured domain, each core histone also contains N-terminal (21−45 residues) domains that extend from the surface of the histone octamer and are not required for assembly of nucleosomes in vitro. These domains contain sites for posttranslational modifications of histones, such as acetylation and phosphorylation. These "tail" domains are also essential for both intramolecular and intermolecular folding of nucleosomal arrays into more compact, higher order structures (reviewed in ref *9*).

In vivo studies in yeast have shown that the core histone N-terminal domains play important roles in the repression of transcription. For instance, deletions of much of the histone H4 or H3 N-terminal domains cripple or eliminate the heterochromatin-like transcriptional silencing of genes located adjacent to yeast telomeres or at the silent mating type loci (*10*−*13*). In addition, N-terminal residues directly adjacent to the structured histone-fold domains of all four core histones are required for global repression of transcription in vivo (*14*). In the case of H3 and H2B, this N-terminal repression domain (*14*) encompasses the residues that contact the two gyres of nucleosomal DNA as the "tail" protrudes from the core particle (*8*). Furthermore, many gene-specific repressor complexes appear to trigger transcriptional repression by deacetylation of specific lysine residues located within one or more of the core histone N-terminal domains (for review, see ref *15*). Thus, modulating the activities of

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[1] Abbreviations: bp, base pair(s); *r*, mol of histone octamer/mol of 208-bp DNA.

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the N-terminal domains is a specific target for alleviating chromatin-mediated transcriptional repression in vivo.

The SWI/SNF complex is a 2 MDa protein complex that appears to be conserved in all eukaryotes (for review, see ref *16*). The yeast SWI/SNF complex is composed of 11 different polypeptide subunits, and it is required in vivo for the transcriptional induction of a subset of yeast genes and for the functioning of a variety of sequence-specific transcriptional activators. Genetic studies in yeast indicate that the role of SWI/SNF complex in transcriptional regulation is to antagonize chromatin-mediated transcriptional repression. Likewise, a mammalian counterpart of SWI/SNF, the BRG1 complex, is associated with hormone-bound glucocorticoid receptor and is required for GR-dependent changes in chromatin structure in vivo (*17*). In vitro the purified yeast or mammalian SWI/SNF complex can use the energy of ATP hydrolysis to disrupt nucleosome structure, facilitating the binding of transcription factors or restriction enzymes to mononucleosomes (*1*, *18*−*20*) or to nucleosomal arrays (*3*, *21*). This ATP-dependent activity has been termed SWI/SNF-dependent "nucleosome remodeling".

In addition to the SWI/SNF complex, yeast cells have a second, highly related chromatin remodeling complex, called RSC (*22*). The RSC complex is a 1 MDa protein complex comprised of 16 polypeptide subunits. Four of these subunits are highly related in sequence to four SWI/SNF subunits (STH1 = SWI2, RSC8 = SWI3, SFH1 = SNF5, RSC6 = SWP73), and thus they may define a catalytic core for both complexes. Consistent with this view, RSC and SWI/SNF complexes have similar DNA-stimulated ATPase activities, and both complexes can use the energy of ATP hydrolysis to disrupt the accessibility of nucleosomal DNA to nuclease digestion (*22*). In the case of RSC, however, there is no genetic link between RSC function and transcriptional regulation in vivo. RSC is an essential complex in yeast, and mutations in several RSC subunit genes cause a G2/M cell division cycle arrest phenotype (*23*−*25*).

Since the mechanism of ATP-dependent nucleosome remodeling is not known for either SWI/SNF or RSC, and given the importance of the core histone N-terminal domains in chromatin-mediated transcriptional repression, we have investigated potential roles for the histone tails in RSC- and SWI/SNF-mediated nucleosome remodeling. To quantitate the activity of these two remodeling complexes, we exploited a sensitive nucleosomal array assay where the activity of a nucleosome remodeling complex is coupled to restriction enzyme activity (*3*). Previously we used this assay to determine the kinetic parameters of ATP-dependent nucleosome remodeling by the SWI/SNF complex (*3*). In this study, a single SWI/SNF complex was found to remodel multiple nucleosomal arrays at a rate of about one nucleosomal array per 50 min (*3*). Here we use this quantitative assay to compare the remodeling activity of SWI/SNF and RSC complexes on nucleosomal arrays that have been reconstituted with either hypoacetylated, hyperacetylated, or trypsinized histone octamers. We find that the nucleosome remodeling activity per se of these complexes is not sensitive to the presence or hyperacetylation of the N-terminal tail domains. However, the histone termini are required for SWI/SNF or RSC to carry out multiple rounds of nucleosomal array remodeling. Template competition studies suggest that the core histone N-terminal tails may control the rate of dissociation of SWI/SNF and RSC from nucleosomal arrays. We propose a model whereby the core histone N-terminal tail domains are allosteric effectors of SWI/SNF and RSC remodeling complexes.

EXPERIMENTAL PROCEDURES

Protein Purification and General Reagents. Chemicals were purchased from Sigma or from Boehringer Mannheim, and restriction enzymes and BSA from New England Biolabs. The yeast SWI/SNF complex was purified through four chromatographic steps as previously described (*26*, *27*). Purified RSC complex (50 μL of a 200 nM solution) was a kind gift of Dr. B. Cairns (University of Utah) and corresponded to the peak monoS fraction of the published purification scheme (*22*). Chicken erythrocyte histone octamers were purified as described previously (*28*). Partial trypsin digestion of nucleosomal histones was carried out using trypsin immobilized on glass beads as described (*29*, *30*). The intact and trypsinized core histone preparations have been analyzed previously by SDS−PAGE and Coomassie staining (see Figure 1, ref *3*; see Figure 1, ref *30*). Hypoacetylated and hyperacetylated HeLa cell histone octamers were kind gifts of Dr. T. Imbalzano (UMMC) and were isolated from HeLa cells grown in the absence or presence of sodium butyrate, respectively (*31*, *32*). Hyperacetylated histones contained primarily tetraacetylated histone H4 and have an average of about 12 acetyl groups per octamer.

Analytical Ultracentrifugation. Sedimentation velocity analyses were performed using a Beckman XL-A analytical ultracentrifuge equipped with absorption optics as described (*33*). Samples were loaded in a final volume of ~420 μL and sedimented in 12 mm double sector cells. The final A_{260} of the samples was between 0.6 and 0.8. The integral distribution of sedimentation coefficients was determined by the method of van Holde and Weischet using Ultrascan data analysis software version 2.95.

Enzyme Assay Conditions. ATPase reactions were performed at 37 °C using 100 μM ATP and 0.2 μCi of [γ-^{32}P]-ATP (Amersham) in 0.1% Tween, 20 mM Tris, pH 8.0, 5% glycerol, 0.2 mM DTT, 5 mM $MgCl_2$, 10 mM sodium butyrate, 1 mM PMSF, and 0.1 mg/mL BSA (*1*). Released phosphate was resolved from ATP on plastic plates coated with PEI cellulose (EM Science) with 0.75 M KPO_4 (pH 3.5) as solvent (*27*); quantitation was by phosphorimager analysis.

Array DNA template was generated from plasmid pCL7c and labeled by Klenow fill in reaction (*3*). Nucleosome arrays were reconstituted by stepwise salt dialysis as described previously (*3*, *27*). 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 DTT, 0.1 mg/mL BSA, 10 mM sodium butyrate, 1 mM PMSF, and 1 mM ATP unless otherwise indicated. *Hin*cII was added to reactions at a final concentration of 500 units/mL. Time points were taken by vigorously mixing aliquots of the reactions, ~5000 cpm, with 50 μL of 1:1 phenol/chloroform plus 20 μL of 15 mM EDTA for 10 s. The DNA fragments were separated by electrophoresis on 1% agarose gels, and *Hin*cII cleavage was quantified by phosphorimager analysis (*3*, *27*).

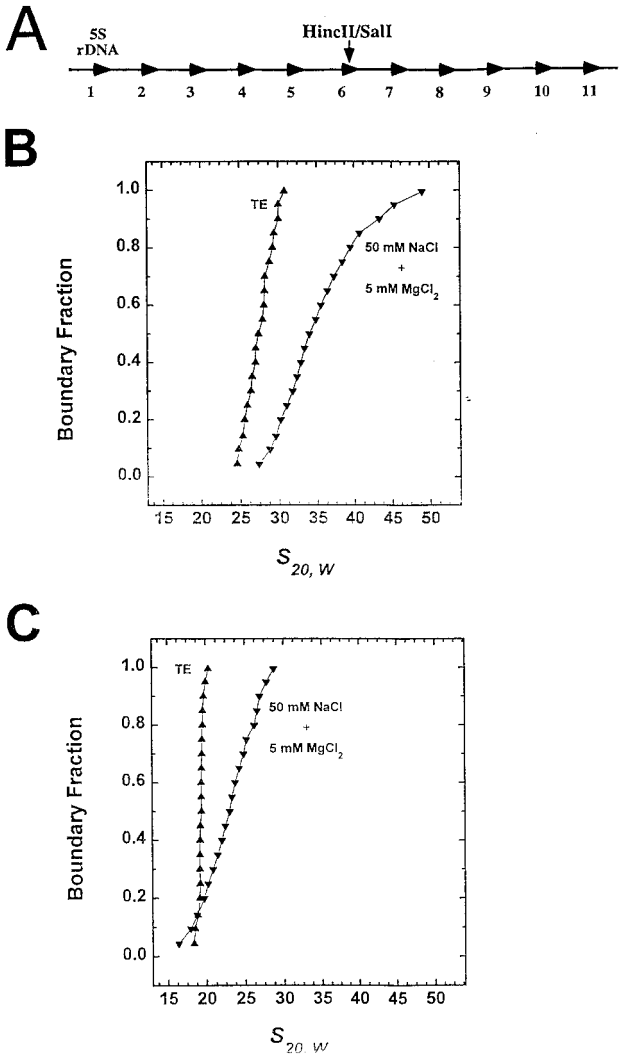

FIGURE 1: Characterization of reconstituted nucleosomal arrays. (A) Schematic representation of the *L. variegatus* 5S rDNA concatemer used to reconstitute 11mer nucleosomal arrays. A unique *Hinc*II/*Sal*I site is located close to the predicted dyad axis of the sixth nucleosome. This restriction site is used to assess nucleosomal DNA accessibility. (B) Sedimentation velocity analysis of intact, hypoacetylated, nucleosomal arrays in either TE (▲) or TE + 50 mM NaCl + 5 mM $MgCl_2$ (▼). Samples were equilibrated at 21 °C and sedimented at 18 000 rpm. Shown are the sedimentation coefficient distributions obtained after analysis of the boundaries by the method of van Holde and Weischet (*40*). (C) Sedimentation velocity analysis of trypsinized nucleosomal arrays in either TE (▲) or TE + 50 mM NaCl + 5 mM $MgCl_2$ (-▼). Experiments were carried out as described in panel B.

RESULTS

Assembly and Salt-Dependent Folding of Defined Nucleosomal Arrays. The DNA template that we have used for array reconstitution is composed of 11 head to tail repeats of a *L. variegatus* 5S rRNA gene (208-11; Figure 1A). Each repeat can rotationally and translationally position a nucleosome after in vitro reconstitution with purified histone octamers, yielding a homogeneous array of positioned nucleosomes (*28*, *34*, *35*). The sixth repeat of this DNA template contains a *L. variegatus* 5S sequence engineered by Polach and Widom (*36*) that bears a unique *Sal*I/*Hinc*II restriction site close to the dyad axis of symmetry of the reconstituted nucleosome, thereby allowing for a sensitive kinetic assay for nucleosome remodeling within nucleosomal arrays. Three different types of nucleosomal arrays were reconstituted onto this DNA template by using either hypoacetylated histone octamers, hyperacetylated histone octamers, or partially trypsinized histone octamers that lack most of their N- and C-terminal tail domains (*30*, *37*, *38*).

To compare SWI/SNF and RSC activities with different types of nucleosomal arrays, it was necessary to ensure that each nucleosomal array had a similar number of nucleosomes per DNA template (saturation extent). To meaure the saturation of templates with nucleosomes, we digested reconstituted nucleosomal arrays with *Eco*RI restriction endonuclease. Each 5S positioning sequence is flanked by two *Eco*RI sites; thus, digestion of the nucleosomal arrays will release either naked, 195 bp DNA fragments (232 bp in the case of the repeat that contains the *Sal*I/*Hinc*II site), or mononucleosomes which migrate on a native acrylamide gel with an apparent mobility of 400 bp (*3*, *28*). When this assay was performed using arrays that were reconstituted at histone octamer to 5S repeat ratios of 1.0, <10% of the 5S repeats migrated as naked DNA, indicating that all of the different nucleosomal arrays were near saturation (data not shown; see ref *4*, *30*). To confirm the extent of template saturation by an alternative method, intact and trypsinized nucleosomal arrays were subjected to sedimentation velocity in the analytical ultracentrifuge (*30*, *39*). The integral distribution of sedimentation coefficients of either intact or trypsinized nucleosomal arrays obtained from the analysis of a typical sedimentation velocity experiment by the method of van Holde and Weischet (*40*) is shown in Figure 1, panels B and C. In low-salt TE buffer, intact nucleosomal arrays exhibited an $S_{midpoint}$ of ~28 S which is consistent with >80% of the nucleosomal arrays being saturated with 11 nucleosomes (Figure 1B; ref *3*). Likewise, trypsinized nucleosomal arrays exhibited an $S_{midpoint}$ of ~20 S indicative of a saturated 11-mer trypsinized nucleosomal array (Figure 1C; ref *30*). Thus, the intact and trypsinized nucleosomal arrays used in our experiments were comprised of nearly homogeneous populations of equal saturation extent.

Nucleosomal arrays undergo reversible folding equilibria under the ionic conditions of our restriction enzyme assay. Previous studies have shown that the folding of model nucleosomal arrays requires intact histone termini (*30*, *37*, *38*), and that folding is destabilized by high levels of histone acetylation (*4*, *41*). Thus, sedimentation velocity experiments were conducted to determine the extent of folding of the reconstituted nucleosomal arrays in our remodeling reaction buffer (50 mM NaCl, 5 mM $MgCl_2$; Figure 1, panels B and C). Consistent with previous studies, intact nucleosomal arrays were capable of higher order folding as indicated by the dramatic increase in $s_{20,w}$ through the entire distribution (Figure 1B; ref *4*, *30*, *42*). In contrast, the trypsinized nucleosomal arrays were incapable of folding and were only able to re-form the same structures as the intact nucleosomal arrays in low salt (Figure 1C). Essentially identical results have been observed for hyperacetylated nucleosomal arrays incubated in buffers containing monovalent and divalent cations (data not shown; see ref *4*). Thus, although our different nucleosomal array substrates have similar numbers of nucleosomes per template, the intact, hypoacetylated nucleosomal arrays were highly folded (Figure 1B), whereas the trypsinized (Figure 1C) and hyperacetylated nucleosomal

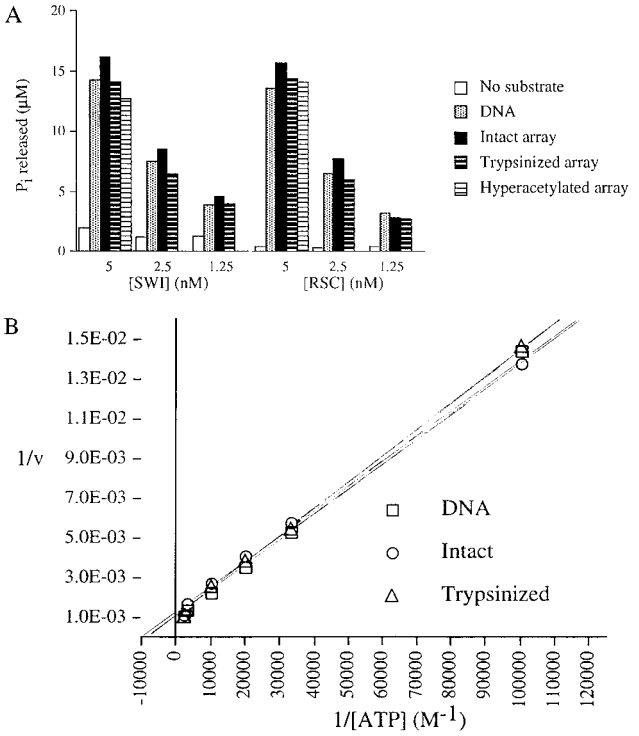

FIGURE 2: Comparison of SWI/SNF and RSC ATPase activities with different nucleic acid substrates. (A) ATPase activities of RSC and SWI/SNF were measured in standard assay conditions in the presence of a saturating concentration of substrate (12 nM DNA, intact, trypsinized, or hyperacetylated nucleosomal arrays) at three different concentrations of remodeling complex. (B) Lineweaver−Burk plot of ATPase activity of SWI/SNF. Velocity (v) is plotted as moles of ATP hydrolyzed per minute per mole of SWI/SNF complex. Similar results were obtained in two additional, independent experiments.

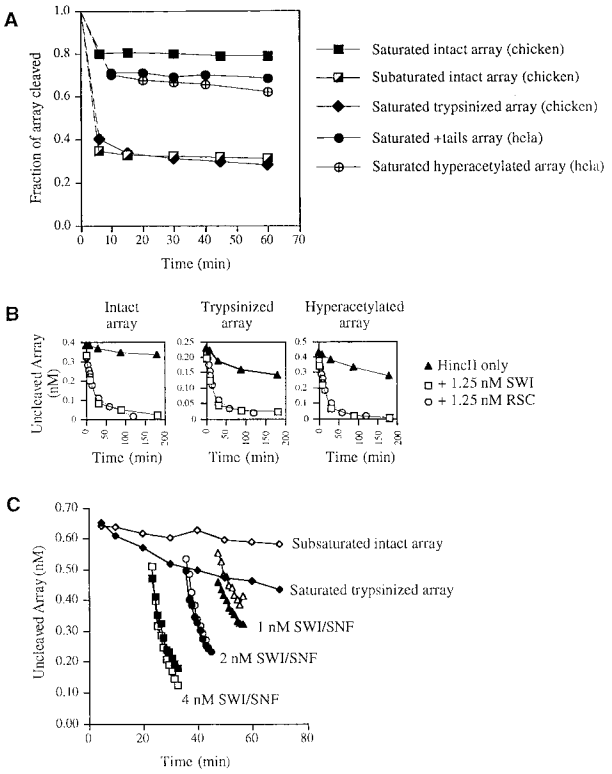

FIGURE 3: SWI/SNF and RSC can remodel intact, trypsinized, and hyperacetylated arrays. (A) Biphasic kinetics of *Hinc*II digestion of the three types of arrays in the absence of remodeler. Note that the saturated, trypsinized and subsaturated, intact nucleosomal arrays display an increased extent of digestion during the first phase. Subsaturated intact arrays were generated by reconstituting the DNA template with a ratio of 0.5 mol of histone octamers per mole of 5S repeat. (B) Remodeling assays. Remodeling reactions were performed using 1 nM saturated, nucleosomal arrays with a small excess of remodeler (1.25 nM). The arrays were preincubated with *Hinc*II (500 units/mL) for 20 min to eliminate nucleosome-free *Hinc*II/*Sal*I sites prior to addition of remodeling complex. Note that the reactions proceeded to completion. (C) Apparent initial SWI/SNF remodeling velocities were measured at three concentrations of SWI/SNF (4 nM, 2 nM, and 1 nM) on 3 nM intact (subsaturated) or trypsinized (saturated) nucleosomal arrays. Time courses denoted by the open and closed diamonds denote reactions that lacked SWI/SNF. SWI/SNF was added at 20, 31, or 42 min to initiate a 10 min remodeling reaction. Time points were taken every minute. Similar results were obtained in two additional, independent experiments.

arrays (*4*) had a more extended conformation under our reaction conditions.

Kinetics of HincII Digestion of Reconstituted Nucleosomal Arrays. Previously, we showed that *Sal*I digestion of nucleosomal arrays reconstituted with intact histone octamers results in a biphasic kinetic profile (*3*). In the first phase (0−15 min), 30−50% of the template DNA is digested at a rate similar to that of naked DNA. This is followed by a second phase where the template DNA is cleaved 300 times more slowly (*3*). This biphasic profile is attributable to the adoption of alternative positions of the histone octamers that leave the *Hinc*II/*Sal*I site in internucleosomal linker DNA (*3*; see ref *35*, *39*). Figure 3A shows the biphasic kinetic profiles for *Hinc*II digestion of the three different nucleosomal array substrates. The fraction of nucleosomal arrays that are cleaved in the first, rapid phase of the reaction (dashed lines) is comparable for the hypoacetylated and hyperacetylated arrays (20−30%). In contrast, over 60% of the trypsinized arrays were digested in the first phase of the reaction. This level of digestion in the first phase of the reaction is comparable to a nucleosomal array that contains only about half the density of intact histone octamers (subsaturated intact array; $r = 0.5$). Additional restriction enzyme mapping suggests that this increase in the first phase of the reaction is due to the trypsinized octamers adopting an alternative, major translational position that leaves the *Sal*I/*Hinc*II site in the linker between two positioned nucleosomes (unpublished results). This alternative, translational positioning is not observed when trypsinized histone octamers are reconstituted onto a single 5S rRNA repeat (*35*, *43*).

By limiting our analysis to the second phase of the reactions, we determined the conformational equilibrium constants (K_{conf}) for the central nucleosome in each of our different nucleosomal array substrates. The value K_{conf} provides a quantitative measurement of the accessibility of DNA within a reconstituted nucleosome (*36*). Nucleosomal arrays reconstituted with intact, hyperacetylated, or trypsinized histone octamers have *Hinc*II conformational equilibrium constants of 1.2×10^{-3}, 5.8×10^{-3}, and 8.2×10^{-3}, respectively (Figure 3A). Thus, trypsinization of the histone octamer leads to about a 7-fold increase in the nucleosomal conformational constant, whereas histone hyperacetylation enhances K_{conf} 4-fold in this assay. These increases in nucleosomal DNA accessibility correlate with the decreased folding of the hyperacetylated (*4*, *41*) and trypsinized arrays (Figure 1), suggesting that restriction enzyme−nucleosomal

array interactions are sensitive to the degree of array compaction.

SWI/SNF and RSC ATPase Activities Are Stimulated Equally by DNA and Different Nucleosomal Array Substrates. The SWI/SNF and RSC complexes are potent ATPases when in the presence of a nucleic acid substrate. Previous studies have shown that SWI/SNF and RSC ATPase activities are stimulated equally well by double-stranded plasmid DNA and the DNA component of intact nucleosomes, i.e., nucleosomal DNA (*1*, *22*). In contrast, the ATPase activity of the *Drosophila* NURF remodeling complex is stimulated only by nucleosomal DNA (*44*). Furthermore, the ATPase activity of NURF is dependent on the core histone N-terminal domains (*45*). To determine if the SWI/SNF or RSC ATPase activities might be sensitive to the presence, absence, or hyperacetylation of the histone N-termini, we compared the ATPase activity of SWI/SNF and RSC complexes after incubation with 208-11 DNA template, hypoacetylated nucleosomal arrays, hyperacetylated nucleosomal arrays, or trypsinized nucleosomal arrays (Figure 2).

SWI/SNF and RSC complexes displayed nearly identical levels of ATPase activity regardless of the type of nucleosomal array substrate (Figure 2A). Furthermore, titration of ATP yielded an estimated K_M of 100 ± 50 μM ATP and a V_{max} of 1000 ± 200 ATP hydrolysis events per minute per SWI/SNF complex, in the presence of DNA, intact nucleosomal arrays, or trypsinized nucleosomal arrays (Figure 2B and data not shown). We conclude that the substrate-stimulated ATPase activity of both RSC and SWI/SNF is primarily stimulated by the DNA component of the arrays rather than by the core histone N-terminal domains. These data also indicate that the extent of nucleosomal array folding (Figure 1B) does not affect the capacity of arrays to stimulate SWI/SNF and RSC ATPase activities.

SWI/SNF and RSC Remodeling Activities Do Not Require Intact Core Histone Termini. To assess the capacity of SWI/SNF and RSC to remodel the three different types of array substrates, 1 nM of each array was incubated with 1.25 nM SWI/SNF or RSC complex (array:remodeler complex ratio = 0.8), and the rate of *Hinc*II digestion was measured. At this ratio of nucleosomal array to remodeling complex, the enhancement of *Hinc*II digestion by both SWI/SNF and RSC proceeded with very similar rates on all three types of nucleosomal arrays, and the reactions proceeded to completion (Figure 3B). We also measured the initial rates of *Hinc*II digestion on the intact and trypsinized nucleosomal arrays at three different SWI/SNF concentrations (Figure 3C). In this case as well, SWI/SNF enhanced the cleavage of both types of nucleosomal arrays with very similar kinetics. Thus, the nucleosome remodeling activities of SWI/SNF and RSC appear to be insensitive to the status of the core histone tail domains, even though the absence or hyperacetylation of the core histone N-termini eliminates the folding of nucleosomal arrays and enhances the accessibility of nucleosomal DNA to restriction enzymes.

The Histone Tail Domains Are Required for Catalytic Remodeling of Multiple Nucleosomal Arrays. Previously we showed that SWI/SNF is able to catalytically remodel multiple nucleosomal arrays at a rate of one array per 50 min (3). To investigate whether this catalytic activity of SWI/SNF or RSC might require the core histone N-terminal

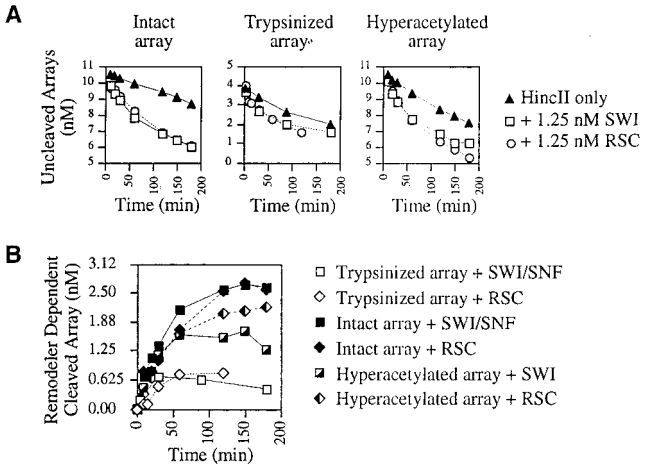

FIGURE 4: SWI/SNF and RSC complexes require histone termini for catalytic remodeling of multiple arrays. (A) Time courses of array remodeling were performed using 15 nM aliquots of the three different types of saturated nucleosomal arrays and 1.25 nM remodeling complex. The arrays were preincubated with *Hinc*II (500 units/mL) for 20 min to eliminate nucleosome-free *Hinc*II/*Sal*I sites prior to addition of remodeling complex. (B) Remodeler-dependent *Hinc*II cleavage events were obtained by subtracting the fraction of uncleaved arrays (filled triangles in panel A) in the absence of remodeler from the fraction of cleaved arrays in the presence of remodeling complexes (open symbols of panel A). Data are presented as nanomolar remodeled nucleosomal arrays versus time. Similar results were obtained in three separate experiments using independent nucleosomal array reconstitutions and two different SWI/SNF preparations.

domains or be sensitive to hyperacetylation, we carried out remodeling assays in which a large excess of nucleosomal array (15 nM) was incubated with the remodeling complexes (1.25 nM; array:remodeler complex ratio = 12) (Figure 4A). In the case of the intact hypoacetylated nucleosomal arrays, both remodeling complexes were able to enhance *Hinc*II digestion throughout the 200 min time course. Less enhancement was observed for the hyperacetylated arrays, and enhanced cleavage of the trypsinized nucleosomal array did not occur. To show more clearly how many rounds of nucleosomal array remodeling were carried out on each array substrate, we quantitated the moles of nucleosomal array that were remodeled during each of the time courses (Figure 4B). On the intact hypoacetylated nucleosomal array substrate, 1.25 nM RSC or SWI/SNF was able to remodel about 2.5 nM nucleosomal array in 120 min ("2 rounds" of array remodeling). In contrast, on the trypsinized nucleosomal arrays, SWI/SNF or RSC was only able to remodel about 0.6 nM array (<"1 round" of array remodeling) even after 200 min (Figure 4B). Likewise, the apparent rate of catalytic nucleosomal array remodeling of the hyperacetylated arrays was reproducibly slower relative to the intact arrays after the first round (1.25 nM) had been completed. This apparent defect in multiple rounds of nucleosomal array remodeling for the hyperacetylated array was more pronounced for SWI/SNF, as 1.25 nM of this complex was only able to remodel 1.5 nM hyperacetylated array even after 200 min (Figure 4B). Thus, remodeling of multiple nucleosomal arrays by SWI/SNF and RSC complexes requires intact histone termini, and the rate of this reaction appeared to be slower when the histone termini were hyperacetylated. Since the rates of nucleosome remodeling per se are very similar on all three nucleosomal array substrates (Figure 3), these results indicate

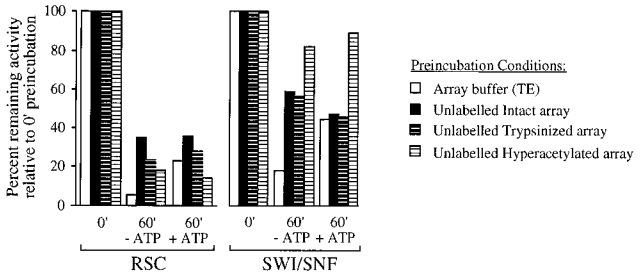

FIGURE 5: Stability of SWI/SNF and RSC remodeling activities. SWI/SNF or RSC (5 nM) was preincubated for 1 h in reaction buffer (+500 units/mL *Hinc*II) in the absence or presence of unlabeled nucleosomal array (intact, trypsinized, or hyperacetylated) (6 nM) and in the absence or presence of ATP. The remaining remodeling activity was assayed by subsequently adding 1 nM labeled, intact nucleosomal array (precleaved with *Hinc*II for 20 min) and incubating for an additional 10 min. The remaining remodeling activity is shown as a percentage of the activity present when the remodeler is added to a reaction that contains both unlabeled and labeled nucleosomal arrays (0 min preincubation). For reactions containing either buffer only, unlabeled intact array, or unlabeled hyperacetylated array, 100% activity corresponds to 21−23% cleavage of the labeled intact nucleosomal array. In reactions that contained the unlabeled trypsinized array, 100% activity corrresponds to 5% (SWI/SNF) or 8% (RSC) cleavage of the labeled, intact nucleosomal array. Similar results were obtained in several independent time courses where the time of preincubation varied from 20 to 120 min.

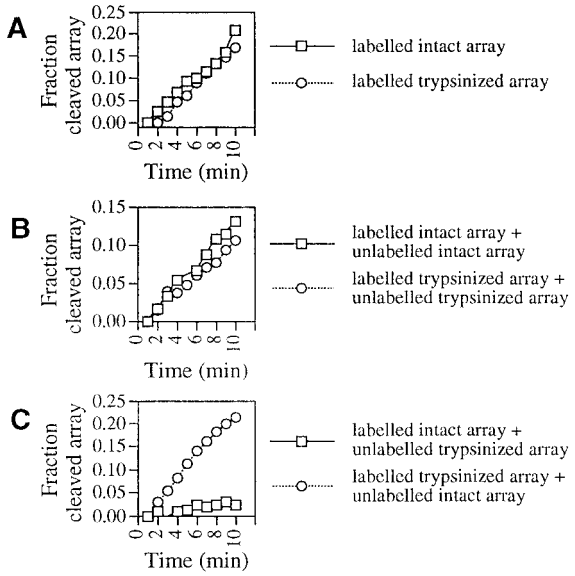

FIGURE 6: SWI/SNF has a high apparent affinity for trypsinized nucleosomal arrays. (A) Labeled intact or trypsinized nucleosomal arrays (3 nM) were exposed to *Hinc*II for 20 min, and apparent initial remodeling rates were measured upon addition of 1.25 nM SWI/SNF to the reactions. (B) Same as (A) but reactions contained an additional 3 nM unlabeled competitor array of the same type. (C) Same as (B), but reactions contained 3 nM unlabeled competitor array of opposite type. Similar results were obtained in six additional, independent experiments.

that the core histone termini influence a step of the remodeling reaction that occurs after nucleosome remodeling but prior to intermolecular transfer of the remodeling complexes to new nucleosomal arrays.

Hyperacetylated Arrays Protect SWI/SNF, but Not RSC, Against Time-Dependent Loss of Activity. A possible explanation for the results described in Figure 4 is that SWI/SNF and RSC activities are inactivated more quickly when they are incubated for prolonged periods of time with trypsinized or hyperacetylated nucleosomal array substrates. Such differential stabilization would lead to different levels of apparent activity. To test this possibility, we incubated SWI/SNF or RSC with reaction buffer or with unlabeled intact, trypsinized, or hyperacetylated nucleosomal arrays. At either time zero or after 60 min of preinubation, labeled, intact nucleosomal array was added to these reactions, and the rate of *Hinc*II digestion of this labeled array was determined (Figure 5). To normalize for differences in the apparent affinities of remodelers for different types of arrays (see below), the data are presented as the percent remodeling activity that remains after the 60 min incubation relative to time zero. This experiment yielded four key results. First, ATP had a 2−4-fold stabilizing effect for both SWI/SNF and RSC complexes when these enzymes were incubated in the absence of nucleosomal array substrate (buffer only). Second, RSC complex was less stable than SWI/SNF after a 60 min incubation in reaction buffer (20% vs 40% activity remaining in the presence of ATP). Third, a 60 min preincubation of SWI/SNF or RSC with trypsinized or intact nucleosomal arrays led to a similar loss of activity (40% for SWI/SNF; 60% for RSC). Thus, the striking defect in remodeling of multiple trypsinized nucleosomal array substrates (Figure 4) is not due to differential stability of SWI/SNF or RSC activities. Since SWI/SNF does lose activity during incubation with intact arrays, these results also indicate that our previous estimate of the turnover rate (50 min/array) is likely to be an under-estimate. Fourth, SWI/SNF activity, but not RSC, was dramatically stabilized by preincubation with the hyperacetylated array ($t_{1/2}$ > 2 h). Even after a 2 h incubation, over 60% of the SWI/SNF remodeling activity remained, whereas only 30% of the activity remained after preincubation with the intact, nucleosomal array substrate (data not shown). Therefore, even though SWI/SNF retains more activity when incubated with a hyperacetylated substrate, SWI/SNF is less proficient at remodeling multiple hyperacetylated nucleosomal arrays (Figure 4). In contrast, RSC complex was less stable when incubated with the hyperacetylated substrate (Figure 5), indicating that the small defect in catalytic remodeling by RSC (Figure 4) is due to instability of the RSC complex, and not to hyperacetylation. Thus, catalytic remodeling of multiple nucleosomal arrays by SWI/SNF is diminished by hyperacetylation, whereas catalytic remodeling by RSC appears to be insensitive to hyperacetylation.

SWI/SNF Has a High Apparent Affinity for Trypsinized Nucleosomal Arrays. How do the core histone termini influence the ability of SWI/SNF or RSC to remodel multiple nucleosomal arrays? One possibility is that the core histone termini modulate the dissociation of these remodeling complexes from nucleosomal arrays. If this was the case, SWI/SNF or RSC would exhibit a higher apparent affinity for a typsinized nucleosomal array due to the slower rate of dissociation. To test this possibility, we carried out a set of template competition assays (Figure 6). In these assays, the initial rates of SWI/SNF remodeling were determined on a labeled nucleosomal array in the presence of an unlabeled competitor nucleosomal array. In the control reaction, SWI/SNF (1.25 nM) was mixed with 3 nM of either an intact nucleosomal array or a trypsinized nucleosomal array (top panel). As shown previously in a similar experiment in Figure 3, the apparent initial rates of remodeling were almost

identical in intact and trypsinized nucleosomal arrays (top panel). When the concentration of nucleosomal array was doubled by the addition of 3 nM competitor nucleosomal array of the same type, the initial rates of remodeling were slowed to a similar extent on both types of nucleosomal arrays (~1.8-fold decrease, middle panel). Thus, the same nucleosomal array can compete for itself. However, when 3 nM intact competitor nucleosomal array was added to 3 nM labeled, trypsinized nucleosomal array, there was little change in the rate of trypsinized nucleosomal array remodeling. Thus, an intact nucleosomal array is a poor competitor for remodeling of a trypsinized nucleosomal array. On the other hand, addition of 3 nM trypsinized, competitor nucleosomal array to 3 nM labeled, intact nucleosomal array virtually abolished remodeling of the intact nucleosomal array (lower panel). Therefore, a trypsinized nucleosomal array is an excellent competitor for remodeling of an intact array. These results strongly suggest that an equilibrium state is rapidly established in the presence of different nucleosomal arrays, and that the SWI/SNF and RSC complexes have a higher apparent affinity for nucleosomal arrays that lack core histone termini.

DISCUSSION

One goal of our work was to simultaneously quantitate the activities of SWI/SNF and RSC complexes in the same assays. Both enzymes have nearly identical ATPase and nucleosome remodeling activities. We found that both remodeling enzymes could function with hypoacetylated, hyperacetylated, and trypsinized nucleosomal arrays when assays were restricted to a single cycle of array remodeling. In contrast, we showed that SWI/SNF and RSC require the core histone termini to transfer from one array to another ("catalytic" remodeling; Figure 4). In addition, a trypsinized array is able to sequester SWI/SNF activity when the enzyme is given a choice between trypsinized and intact array substrates (Figure 5). The latter result suggests that SWI/SNF complex may have a higher apparent affinity for nucleosomal arrays that lack most of their histone termini. This in turn suggests that SWI/SNF and RSC have a much slower rate of dissociation from the trypsinized arrays. In this model (Figure 7), the histone tails control a late step in the remodeling cycle after ATP-dependent remodeling, but prior to recycling of the enzyme from one array to another. Specifically, we also propose that one or more histone tails directly interact with the SWI/SNF or RSC complex, and that this interaction in some way induces the release of the remodeler from the nucleosomal array.

Regulation of the Remodeling Cycle. The remodeling cycle proposed in Figure 7 predicts several steps where the length of the cycle might be regulated by either intrinsic or extrinsic signals. For instance, when SWI/SNF binds to nucleosomes within the array (step 1), interactions with the histone tails that lead to dissociation must be avoided until after ATP-dependent remodeling (step 2). Perhaps one or more subunits of the remodeler control when the histone tails interact with the complex. Two ideal candidates for such subunits are the SWP61 and SWP59 subunits of SWI/SNF which we have recently shown to be members of the actin-related protein (ARP) family (*46*). Many ARP family members use the energy of ATP hydrolysis to govern conformational transitions (discussed in ref *47*). For instance, in the case of

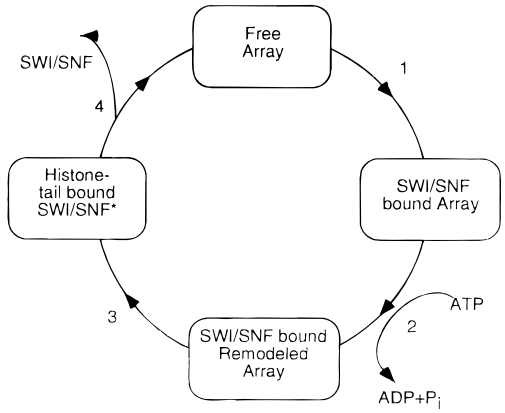

FIGURE 7: Model for the catalytic cycle of SWI/SNF and RSC chromatin remodeling complexes. The proposed remodeling cycle involves 4 steps: (1) binding of the remodeler to a nucleosomal array; (2) ATP-dependent nucleosomal array remodeling that results in enhanced accessibility of nucleosomal DNA; (3) interuption of the remodeling cycle leading to a conformation permissive for substrate release (indicated by an asterisk); and (4) substrate release. We propose that the conformation generated by step 3 requires the presence of one or more core histone N-terminal domains.

HSP70/DnaK, ATP hydrolysis results in a conformational change that controls substrate binding specificity (*48*). By analogy, perhaps ATP hydrolysis by SWP59 and SWP61 controls the ability of the histone N-termini to promote dissociation of SWI/SNF from the nucleosomal array. Alternatively, the interaction of SWI/SNF with the histone N-terminal domains may not lead to the immediate, obligatory dissociation of the remodeler. In this case, the histone N-termini may promote the release of the remodeled array due to an increased electrostatic contribution to substrate or product binding affinity.

Since the core histone N-terminal domains contain sites for histone acetylation, it is possible that posttranslational modification of the histone tails might control the remodeling cycle. In support of this model, we find that SWI/SNF activity is stabilized by hyperacetylated arrays and that the rate of multiple-round remodeling by SWI/SNF is reproducibly slower on arrays that contain hyperacetylated histones (Figure 4). In contrast, RSC does not appear to sense the acetylation status of the N-termini. The effect of histone hyperacetylation on remodeling kinetics, however, is quite weak in comparison to the elimination of multiple round remodeling on the trypsinized array substrate. If acetylation does play an important role in controlling the remodeling cycle, perhaps a particular pattern of acetylated lysines is required. In vivo SWI/SNF and the GCN5 histone acetyltransferase control expression of a similar subset of yeast genes (*49*), and in vitro GCN5 preferentially acetylates lysines 5 and 14 of histone H3 (*50*). Perhaps the in vivo functional interactions are due to regulation of the remodeling cycle by a GCN5-dependent pattern of histone acetylation.

Remodeling of Nucleosomal Arrays Requires Continuous ATP Hydrolysis. Previously we showed that the SWI/SNF-dependent remodeling of nucleosomal arrays is not stable, but is rapidly reversed after removal of ATP (*3*). In the course of these studies, we have found that remodeling of nucleosomal arrays by RSC complex also requires continuous hydrolysis of ATP (unpublished results). In contrast, several groups have shown that RSC- and SWI/SNF-dependent remodeling of mononucleosome substrates does not require

continuous ATP hydrolysis and results in a persistently disrupted structure that is stable for at least several hours (*2*, *51*−*53*). One possibility is that the rate at which remodeled nucleosomes revert back to an inaccessible state may be influenced by the locations of the histone tails which are known to undergo major rearrangements when arrays are converted to mononucleosomes (reviewed in ref *9*). Alternatively, reversal may require an interaction between nucleosome particles located on the same DNA segment or require that the linker DNA between nucleosomes be constrained. The former possibility is not likely, as SWI/SNF-dependent remodeling is still rapidly reversible on trypsinized and hyperacetylated nucleosomal arrays (our unpublished results).

SWI/SNF, RSC, and the Higher Order Folding of Arrays. One advantage of model 5S nucleosomal arrays is that they possess all of the fundamental properties required to acheive a chromosomal level of DNA compaction (reviewed in ref *9*). In our buffer conditions (50 mM NaCl, 5 mM $MgCl_2$), an intact 208-11 nucleosomal array is not an extended molecule, but assumes a highly compact state (Figure 1B; ref *42*). Previously we predicted that the folding of 208-11 nucleosomal arrays might contribute to the occlusion of nucleosomal DNA that is detected in our restriction enzyme assay (*3*). Consistent with this view, trypsinized and hyperacetylated nucleosomal arrays are more extended in our assay buffer, and array unfolding leads to a 7-fold and 4-fold increase in the accessibility of nucleosomal DNA to restriction enzymes, respectively. Surprisingly, however, the activities of SWI/SNF and RSC were equivalent on highly folded (hypoacetylated) and unfolded (trypsinized or hyperacetylated) nucleosomal array substrates during a "single round" of array remodeling. These results suggest that SWI/SNF and RSC may have evolved to interact and function on DNA that initially is encompassed in compacted chromatin fibers. In this respect, direct interactions between these remodeling complexes and the core histone N-terminal tails may interfere with tail-dependent fiber condensation (by sequestering the tail domains essential for folding), thereby leading to localized disruption of a folded chromatin domain (*54*). In this way SWI/SNF and RSC would simultaneously lead to global remodeling of chromatin fibers and specific remodeling of individual nucleosomes. Targeting the remodeling of higher order structure is an intriguing possibility in light of the fact that higher order folding is severely repressive to transcription (*4*, *55*, *56*). Since linker histones (e.g., H1) are known to stabilize higher order chromatin folding, it will be interesting to investigate how linker histones influence the remodeling cycles of SWI/SNF and RSC complexes.

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