Nucleosome Disruption by Human SWI/SNF Is Maintained in the Absence of Continued ATP Hydrolysis

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We have examined the requirement for ATP in human (h) SWI/SNF-mediated alteration of nucleosome structure and facilitation of transcription factor binding to nucleosomal DNA. hSWI/SNF-mediated nucleosome alteration requires hydrolysis of ATP or dATP. The alteration is stable upon removal of ATP from the reaction or upon inhibition of activity by excess ATPγS, indicating that continued ATP hydrolysis is not required to maintain the altered nucleosome structure. This stable alteration is sufficient to facilitate binding of a transcriptional activator protein; concurrent ATP hydrolysis was not required to facilitate binding. These data suggest sequential steps that can occur in the process by which transcription factors gain access to nucleosomal DNA.

In vivo, DNA is compacted via association with histones and nonhistone proteins to form chromatin, which, in general, inhibits the interaction of the transcriptional machinery with promoter sequences and is therefore refractory to gene expression. While inactive promoters are generally incorporated in nucleosomal arrays, regulatory sequences controlling the expression of genes being actively transcribed have been shown to exist in a more open conformational state, as shown by their increased sensitivity to cleavage by nucleases. Thus, there must be mechanisms utilized by the cell to disrupt chromatin and render relevant DNA sequences accessible to the transcriptional machinery.

A number of different mechanisms may exist to explain how chromatin structure is altered on promoter/enhancer sequences. Many activators, such as Sp1 (Chen et al., 1994; Li et al., 1994), synthetic derivatives of the yeast GAL4 transcriptional activator (Taylor et al., 1991; Workman and Kingston, 1992), progesterone receptor (Pham et al., 1992), glucocorticoid receptor (Perlmann and Wrangle, 1988; Pina et al., 1990; Archer et al., 1991; Li and Wrangel, 1993; Li and Wrangel, 1995), TFIIIAα (Rhodes, 1985; Lee et al., 1993), upstream stimulatory factor (Chen et al., 1994; Adams and Workman, 1995), and Max and c-Myc-Max heterodimers (Wechsler et al., 1994) have been shown to bind to nucleosome particles in vitro. In some cases, the binding of activators can destabilize the nucleosome, as shown by the observation that the binding of GAL4 derivatives to mononucleosome particles containing five GAL4 sites facilitates the displacement of histones to histone acceptors (Workman and Kingston, 1992; Chen et al., 1994; Walter et al., 1995).

In other cases, direct modification of the histone proteins comprising the nucleosome can alter the accessibility of the nucleosome to transcription factors. For example, TFIIIA binding to mononucleosomes can be facilitated by acetylation of the N-terminal tails of the core histones (Lee et al., 1993).

Other proposed mechanisms involve activities that mediate energy-dependent chromatin alteration (Cote et al., 1994; Kwon et al., 1994; Imbalzano et al., 1994a; Tsukiyama et al., 1994; Tsukiyama and Wu, 1995; Wall et al., 1995; Varga-Weisz et al., 1995; Pazin et al., 1994). Some of these activities are due to large protein complexes (e.g. SWI/SNF, NURF) that hydrolyze ATP and structurally alter nucleosome particles (Cote et al., 1994; Kwon et al., 1994; Imbalzano et al., 1994a; Tsukiyama and Wu, 1995; Tsukiyama et al., 1995). The yeast SWI/SNF complex is comprised of the products of several yeast SWI and SNF genes that function together in a large, multienzyme complex (Laurent and Carlson, 1992; Peterson and Herskowitz, 1992; Peterson et al., 1994; Cairns et al., 1994) that is required for the transcription of a large number of inducible genes and has been shown to enhance the function of many yeast, fly, and human transcriptional activators in yeast cells (Laurent and Carlson, 1992; Peterson and Herskowitz, 1992; Yoshinaga et al., 1992; reviewed in Winston and Carlson, 1992). Similarly, two human homologs of the helicase-related ATPase SNF2/SWI2 have been shown to enhance nuclear hormone receptor function in mammalian cells (Khavari et al., 1993; Muchardt and Yaniv, 1993; Chiba et al., 1994) and to purify as part of a large molecular weight complex (Khavari et al., 1993; Kwon et al., 1994).

Although individual yeast SNF genes have been shown to function as activators when fused to DNA binding domains (Laurent et al., 1990; Laurent et al., 1991; Laurent and Carlson, 1992), the SWI/SNF complex appears not to function as an activator or bridging coactivator. Instead, many lines of evidence point to a role in chromatin disruption. First, phenotypes caused by mutations in yeast SWI and SNF genes can be suppressed by mutations in histone and nonhistone chromatin proteins and by mutations that alter histone expression levels (Kruger and Herskowitz, 1991; Peterson et al., 1991; Hirschhorn et al., 1992; Kruger et al., 1995). SWI/SNF mutations have also been shown to mediate structural changes in chromatin in vivo (Hirschhorn et al., 1992; Matallana et al., 1992). More recently, both the yeast and human SWI/SNF complexes have been purified and shown to directly alter nucleosome structure as well as to facilitate transcription factor binding to nucleosomal DNA in an ATP-dependent manner (Cote et al., 1994; Kwon et al., 1994; Imbalzano et al., 1994a).
Another due to the mechanism of SWI/SNF complex function is provided by the observation that active SWI/SNF complex is a component of the yeast RNA polymerase II holoenzyme (Wilson et al., 1996), which is thought to be the form of polymerase responsible for mRNA synthesis in vivo (reviewed in Carey, 1995; Emili and Inges, 1995; Koleske and Young, 1995). This suggests that holoenzyme, perhaps targeted to an inactive promoter by activators capable of weakly binding chromatin, carries with it an activity capable of disrupting repressive chromatin structure and allowing preinitiation complex formation.

Thus, understanding the mechanism by which SWI/SNF complexes alter chromatin structure is likely to be important in determining how transcriptionally inert genes become activated and may be applicable as well to other processes involving utilization of the DNA in chromatin, such as replication and viral integration (Dunaief et al., 1994; Kalpana et al., 1994). To further characterize the mechanism by which the human SWI/SNF (hSWI/SNF) complexes alter chromatin structure, we have investigated the ATP requirement for hSWI/SNF activity as well as the nature of the structural change induced by hSWI/SNF. We report that the change in chromatin structure induced by hSWI/SNF is stable, even in the absence of continued ATP hydrolysis, on both mononucleosome and nucleosomal plasmid templates. In addition, hSWI/SNF-mediated transcription factor binding to nucleosomal DNA requires nucleosome alteration but does not require concurrent ATP hydrolysis.

MATERIALS AND METHODS

Protein Purification—HeLa cell pellets were obtained from the National Cell Culture Center (Minneapolis, MN). hSWI/SNF “A” and “B” fractions were isolated from HeLa cell nuclear extract according to the method of Kwon et al. (1994) through the Econo Q stage. The hSWI/SNF A preparation was 150 ng/ml (determined by Bradford assay) and was estimated to be ~5% pure by comparing silver-stained SDS-polyacrylamide gels to silver-stained gels depicting hSWI/SNF preparations further purified on a Superose column (Kwon et al., 1994). The hSWI/SNF B preparation used in all experiments except that presented in Fig. 4 was 200 ng/ml and was estimated to be ~3% pure. The hSWI/SNF B preparation used in the experiment presented in Fig. 4 was 90 ng/ml and was estimated to be ~10% pure. The estimates of purity were corroborated by activity assays showing that each complex is able to fully alter nucleosome structure at an apparent stoichiometry of 1:1 when calculations are based on these estimates. GAL4-AH (i.e. GAL4-(1–147)-amphiphatic helix) was purified as described (Lin et al., 1988). HeLa core histone octamers were purified as described (Workman et al., 1991b).

Mononucleosome Assembly—Mononucleosome particles were assembled by salt dilution as described (Imbalzano et al., 1994a) using PH MLT, PH MLT (+3), or PH GAL4, 150 bp, gel-purified restriction fragments (Imbalzano et al., 1994a; Kwon et al., 1994). The GAL4 site in PH GAL4 is the synthetic consensus site GGAGAACCTCCTCCG defined by Ginner et al. (1985). Assembly reactions contained 0.45 μg of 32P-labeled DNA fragment (end-labeled by Klenow fill-in with ['γ-32P]ATP (DuPont NEN) at the EcoRI end or with ['γ-32P]ATP (DuPont NEN) at the BamHI end), 5 μg of Hae III-digested pUC18 DNA, and 8.6 μg of core histone octamers purified from HeLa cell nuclei (Workman et al., 1991b). The concentration of core histone octamers was determined by Bradford assay using BSA as a standard. Mononucleosomes were separated from unincorporated DNA on 5-ml 5–30% glycerol gradients by centrifugation in a Beckman SW55.1 rotor spun at 35,000 rpm at 4°C for 4 h.

Reactions contained nucleosomal template (1–2 ng of DNA), 1 unit of topoisomerase I (Promega), 2.5 μl of 30% glycerol gradient buffer, 4 mM MgCl2, and, where indicated, hSWI/SNF, 0.4 mM ATP, 7 mM ATP5S/MgCl2, and/or 2.5 units of apyrase, and were brought to a final volume of 12.5 μl with Buffer A (20 mM HEPES, pH 7.9, 10 mM KCl, 1 mM dithiotreitol, 1 mM EDTA, 0.1 mg of BSA per ml at a concentration of 1 unit/μl). Reactions were incubated at 30°C for the times indicated in each figure legend. Following incubation, reactions were treated with 0.01 unit (for reactions containing nucleosomal DNA) or 0.1 unit (for reactions containing naked DNA) of RQ1 DNase I (Promega) for 2 min at room temperature. Digestion was stopped by addition of 2 μl of 0.5 M EDTA. Samples were prepared for electrophoresis on 8% polyacrylamide sequencing gels as described (Imbalzano et al., 1994b).

Assembly of Nucleosomal Plasmid Templates and Supercoiling Assay—pG5HC2AT (Workman et al., 1992a), a 3.5-kilobase plasmid, was linearized with BamHI, labeled with ['γ-32P]ATP (DuPont NEN) and T4 polynucleotide kinase (New England Biolabs), extracted with phenol:chloroform (1:1), purified through a Sephadex G-50 spin column (Pharmacia), equilibrated in TE (TE, Tris/EDTA), and re-ligated at a concentration of 1 μg/ml. The closed circular, internally labeled plasmid was reconstituted as described with purified HeLa core histone octamers in a XbaI-linearized heat-treated empty vector that is competent for nucleosome assembly (Workman et al., 1991b), in the presence of wheat germ topoisomerase I (Promega). The reconstituted plasmids were purified on 5-10–40% glycerol gradients in a Beckman SW55.1 rotor spun at 35,000 rpm at 4°C for 4 h.

Results

Assessment of Nucleosome Stability—Identical reactions containing naked or nucleosomal PH MLT or PH MLT + 3 DNA were set up and incubated at 30°C under the reaction conditions described above, in the absence or presence of 4 mM ATP. At the times indicated, reactions were removed from the 30°C bath and loaded directly onto adjacent lanes of native 5%, 0.5× TBE polyacrylamide gel that had been pre-run at 4°C for 1 h. Electrophoresis was performed at 6 V/cm. Gels were then dried and exposed to x-ray film (and to x-ray film screen(s)). The proportion of free DNA present in the nucleosomal lanes was quantified by the calculation: volume free DNA band (volume nucleosomal DNA band + volume free DNA band).

RESULTS

To investigate the requirements for and to assess changes in nucleosome structure due to hSWI/SNF activity, rotationally phased nucleosome particles were assembled in vitro from 32P-end-labeled, gel-purified 150-bp DNA fragments and purified HeLa cell histone octamers. Mononucleosome particles were separated from un assembled DNA by glycerol gradient centrifugation. The purified mononucleosome particles showed decreased mobility relative to naked DNA on native polyacrylamide gels (see below), were resistant to micrococcal nuclease digestion (Imbalzano et al., 1994a), and exhibited a 10-bp cleavage ladder upon digestion with DNase I (Fig. 1), which is typical of a mononucleosome population that is rotationally phased.

Nucleotide Requirements for hSWI/SNF Function—Previous work has demonstrated that hSWI/SNF-mediated nucleosome disruption requires ATP and is not promoted by nonhydrolyzable ATP analogs (Kwon et al., 1994). To further investigate the nucleotide requirements for nucleosome disruption, ATP was replaced by different nucleoside di- or triphosphates, and concentrations ranging from 20 μM to 2 mM of each nucleotide were tested (Fig. 1). hSWI/SNF activity resulted in a decrease in the
intensity of the cleavage products comprising the 10-bp repeat pattern and the appearance of novel cleavages throughout the length of the template. 20 μM ATP was almost as effective as 2 mM ATP at eliciting maximal activity (lanes 4–6; similar results were seen at lower amounts of hSWI/SNF, data not shown). This change in accessibility to DNase I indicates that hSWI/SNF mediates an ATP-dependent alteration in the structure of the nucleosomal DNA (compare lanes 3 and 4). Lanes 22–27 confirm that ATP hydrolysis is required for hSWI/SNF function as neither ATPγS nor AMP-PNP, both nonhydrolyzable ATP analogs, supported nucleosome disruption. Of the analogs tested, only dATP could substitute for ATP, although approximately 10-fold more dATP than ATP was required to see disruption (lanes 7–9). UTP, GTP, CTP, and ADP did not promote disruption (lanes 10–21).

Prior work has demonstrated that two chromatographically separable fractions that contain hSWI/SNF nucleosome disruption activity can be obtained from fractionation of HeLa cell nuclear extract. These fractions were termed A and B (Kwon et al., 1994). Both contained immunoreactivity to BRG1, a SWI2/SNF2 homolog (Khavari et al., 1993), and they showed no functional differences in all previous studies (Kwon et al., 1994; Imbalzano et al., 1994a). Fig. 2A demonstrates that the alteration in the DNase I digestion pattern of mononucleosomes mixed with either hSWI/SNF A or hSWI/SNF B is the same. Both hSWI/SNF A and hSWI/SNF B were used separately in all of the experiments presented in this paper, and essentially identical results were obtained for each experiment (data not shown). The fact that all results are observed with both SWI/SNF complexes argues that they are unlikely to be caused by a fortuitously co-purifying activity because the hSWI/SNF A and B fractions are both highly enriched and they chromatograph differently on both phosphocellulose and single strand DNA-cellulose columns.
ATP Is Not Required to Maintain Disruption of Mononucleosomes—To characterize the alteration of nucleosome structure by hSWI/SNF, we sought to determine whether ATP was continuously required in order to maintain a disrupted pattern in the presence of hSWI/SNF. If ATP were continuously required for activity, that is, if the structural change induced by hSWI/SNF and ATP were transient, and the nucleosome reverted to its original form after ATP mediated disruption, then removal of ATP from the reaction prior to DNase I cleavage should generate the same 10-bp ladder of cleavage products seen when untreated nucleosomes are digested with DNase I. Alternatively, if the change in structure induced by hSWI/SNF is stable, then the altered DNase I digestion pattern should be maintained, even after ATP is removed from the reaction. To facilitate this experiment, the ATP concentration in the reaction was decreased to 20 μM, which is sufficient for disruption (Fig. 1, lane 4).

When apyrase, which cleaves ATP, was added to a reaction containing nucleosomes and ATP before the addition of hSWI/SNF, ATP-dependent nucleosome disruption was inhibited (Fig. 2b, compare lanes 4 and 5). In the reactions presented in lanes 6–10, identical samples containing nucleosomes, ATP, and hSWI/SNF were incubated for 10 min, were subsequently exposed to apyrase, and then were digested with DNase I at times ranging from 2 to 30 min following apyrase addition to determine whether the altered nucleosome would revert to its original structure. Lanes 6–10 of Fig. 2b indicate that the altered DNase I digestion pattern was maintained for up to 30 min past the addition of apyrase. Other experiments indicate that the altered digestion pattern was maintained for up to 2.5 h after apyrase addition (data not shown). This result indicates that the alteration in nucleosome structure induced by hSWI/SNF is stable, even in the absence of ATP.

To confirm that hSWI/SNF could stably alter nucleosome structure, the nonhydrolyzable ATP analog, ATPγS, was used to competitively inhibit ATP hydrolysis by hSWI/SNF. Fig. 3 shows that concurrent addition of ATP and a 200-fold excess of ATPγS before addition of hSWI/SNF prevented nucleosome disruption for up to 60 min, presumably because ATPγS acts as a competitive inhibitor (lanes 6 and 13). When a 200-fold excess of ATPγS was added subsequent to hSWI/SNF addition, the altered DNase I digestion pattern was maintained (lanes 7–11), confirming that hSWI/SNF induced a stable change in nucleosome structure that was maintained in the absence of further hydrolysis. Addition of a 200-fold excess of AMP-PNP to the reaction did not inhibit hSWI/SNF activity (data not shown), probably reflecting a lower affinity of this analog for the ATP binding site.

ATP Is Not Required to Maintain Altered Supercoiling of Plasmid Templates—Previously, we demonstrated that the hSWI/SNF fractions can reduce the linking number of closed circular DNA that was assembled into a nucleosomal template (Kwon et al., 1994). This result is consistent with the observation that hSWI/SNF fractions can increase the accessibility of mononucleosomes to nucleases such as DNase I and indicates that hSWI/SNF can alter chromatin structure on nucleosomal plasmid templates as well as on mononucleosomes particles. In the experiment presented in Fig. 4A, 32P-labeled, closed circular DNA was reconstituted into nucleosomes using octamers purified from HeLa cells and a Xenopus egg heat-treated extract that is competent for nucleosome assembly (Workman et al., 1991b). The reconstituted template was purified by glycerol gradient centrifugation and was mixed with hSWI/SNF, ATP, and topoisomerase I. Following a 90-min incubation, the DNA was purified, and topoisomers were resolved by agarose gel electrophoresis. In an ATP-dependent manner, hSWI/SNF caused the appearance of a number of DNA topoisomers that have reduced mobility in the gel, indicating a loss of superhelical density (compare lanes 2, 5, and 7). Addition of an 18-fold excess of ATPγS prior to the addition of hSWI/SNF prevented alteration of the template (lane 6). When an 18-fold excess of ATPγS was added to the reaction subsequent to addition of hSWI/SNF and ATP, DNA species with reduced superhelical density were still present (lanes 9 and 10), indicating that the structural alteration in the nucleosomal plasmid template caused by hSWI/SNF was stable, even upon inhibition by excess ATPγS. Similarly, addition of apyrase at the start of the reaction but prior to the addition of hSWI/SNF prevented alteration of the template (lane 13). When apyrase was added after the addition of hSWI/SNF and ATP, topoisomers with reduced superhelical density were present (lanes 14–15), indicating that removal of ATP from the reaction did not reverse the alteration in nucleosomal DNA structure. We therefore conclude that the alteration in structure of a nucleosomal plasmid template by hSWI/SNF is also maintained in the absence of ATP.
of ATP hydrolysis.

To increase the resolution of the topoisomers showing a hSWI/SNF-induced reduction in supercoiling, similar reactions were resolved on agarose gels containing chloroquine (Fig. 4B). The change in superhelical density in the presence of hSWI/SNF and ATP is shown in lane 9 (compare to lane 1). Addition of apyrase after the addition of hSWI/SNF did not change the distribution of topoisomers, again indicating that removal of ATP from the reaction did not reverse the alteration in nucleosomal DNA structure (lanes 7–8). The increase in resolution provided by the presence of chloroquine also indicated that hSWI/SNF has a small effect on nucleosome structure in the absence of ATP (lanes 3, 5, and 6). ATP-independent effects of yeast SWI/SNF on DNA topology have previously been noted (Quinn et al., 1996).

Estimations of the size of the hSWI/SNF complex (1 MD by gel filtration (Kwon et al., 1994)) and of the purity of the fractions used (3–10%, as estimated by visual examination of silver-stained SDS-polyacrylamide gels, see "Materials and Methods") allowed a crude estimate of stoichiometry to be made in these experiments. We calculate that hSWI/SNF is approximately equimolar with mononucleosome particles at concentrations of hSWI/SNF where nucleosome disruption is maximal. For nucleosomal plasmid templates, we calculated that a ~20-fold excess of hSWI/SNF to template was sufficient to see maximal changes in supercoiling. This template contains 16 nucleosomes (on average), thereby resulting in an approximate equimolar ratio between hSWI/SNF and nucleosomes.

Fig. 5. Nucleosome disruption by hSWI/SNF requires the simultaneous presence of nucleosomes and ATP. Reactions were as described in Fig. 1. PH MLT nucleosomes were labeled at the EcoRI end. Where indicated, reactions contained 600 ng of hSWI/SNF B fraction and 0.02 mM ATP. In the control reactions (lanes 2–4), ATP-dependent nucleosome disruption is seen upon DNase I digestion 25 min after the start of the reactions (lane 3). For the reactions shown in lanes 5–11, reactions were initiated in the absence of nucleosomes. 1 unit of apyrase was added to the reaction prior to the addition of hSWI/SNF (lane 9) or 25 min after addition of hSWI/SNF (lane 10). PH MLT nucleosomes were added to each reaction (lanes 5–11) 35 min after the start. Nucleosome disruption was analyzed by DNase I digestion 60 min after the start of the reactions. No disruption was observed when apyrase was added after hSWI/SNF but before the nucleosomes (lane 10), indicating a concurrent requirement of ATP and nucleosomes for hSWI/SNF-mediated nucleosome disruption. N represents naked DNA.
Fig. 6. Facilitated binding of GAL4-AH to PH GAL4, mononucleosomes requires prior disruption by hSWI/SNF but does not require concurrent hSWI/SNF activity. Reaction conditions were as described for Fig. 1. Where indicated, reactions contained 600 ng of hSWI/SNF B fraction, 4 mM ATP, and no GAL4-AH (lanes 3, 7, 11, 15, 19), 1 × 10^{-7}M GAL4-AH dimer (lanes 4, 8, 12, 16, 20), 1 × 10^{-6} M GAL4-AH dimer (lanes 5, 9, 13, 17, 21), or 1 × 10^{-5} M GAL4-AH dimer (lanes 6, 10, 14, 18, 22). In lanes 3–10 reactions were treated with hSWI/SNF in the absence (lanes 3–6) or in the presence (lanes 7–10) of ATP for 30 min, and then GAL4-AH was added for another 30 min, and samples were assayed by DNase I digestion. If 1 unit of apyrase was added prior to hSWI/SNF addition (lanes 11–14), no facilitated GAL4-AH binding was subsequently observed. 1 unit of apyrase was added to the reactions presented in lanes 15–18 30 min after addition of hSWI/SNF; 10 min later, GAL4-AH was added for 30 min, followed by DNase I digestion. Reactions in lanes 19–22 were identical to those in lanes 15–18, except that apyrase was added after the 30 min exposure to GAL4-AH, followed by DNase I digestion 10 min later. For comparison, naked PH GAL4, DNA (lanes 1–2) was incubated for 30 min at 30°C, followed by an additional 30 min incubation in the absence (lane 1) or presence (lane 2) of 2 × 10^{-8}M GAL4-AH. Samples were then digested with DNase I. Bars span the sequences protected by GAL4-AH on naked and nucleosomal DNA.

stimulated by the presence of DNA (Kwon et al., 1994), this experiment was repeated with 3–30 ng of free plasmid DNA present in the preincubation. As before, no alteration in the DNase I cleavage ladder was observed (data not shown). These results suggest that the hydrolysis of ATP is required for altering the structure of the nucleosome and not for modifying the hSWI/SNF. This experiment does not exclude the possibility that ATP modifies hSWI/SNF structure, but it indicates that even if such a modification occurs, it is not sufficient for nucleosome disruption.

Facilitation of GAL4 Binding Does Not Require Continued ATP Hydrolysis—Previous work has shown that if the nucleosome contains a transcription factor binding site, treatment of the nucleosome with hSWI/SNF and ATP facilitates the binding of transcription factors to it (e.g. GAL4 derivatives, TATA binding protein (Kwon et al., 1994; Imbalzano et al., 1994a)). Addition of hSWI/SNF in the absence of ATP to nucleosomes containing a single GAL4 binding site at the dyad axis of symmetry results in no change in the DNase I digestion pattern, and, upon addition of increasing amounts of GAL4 fusion protein, GAL4-AH, no binding to the GAL4 site was observed at the GAL4-AH concentrations utilized (Fig. 6, lanes 3–6). In contrast, when hSWI/SNF was added in the presence of ATP, a change in the DNase I digestion pattern was observed, and subsequent addition of increasing amounts of GAL4-AH resulted in specific occupancy of the GAL4 site (lanes 7–10), confirming previous observations. To determine whether the increased ability of a transcription factor to bind to an altered nucleosome requires continued ATP hydrolysis, nucleosomes were mixed with hSWI/SNF and ATP, and apyrase was added either before hSWI/SNF addition (lanes 11–14), after hSWI/SNF addition (lanes 15–18), or after the subsequent addition of GAL4-AH (lanes 19–22). Whereas addition of apyrase prior to the reaction prevented an increase in the ability of GAL4-AH to bind (lanes 11–14), addition of apyrase after addition of hSWI/SNF and ATP did not (lanes 15–18). Thus, prior alteration of nucleosome structure by hSWI/SNF is sufficient to allow an increase in GAL4-AH binding to nucleosomal DNA; concurrent ATP hydrolysis was not required.

hSWI/SNF Activity Is Not Due to Spontaneous Nucleosome Disruption—In the experiments presented here and in previous work, mononucleosome particles were present at a final concentration of 1.2 × 10^{-9} M, or approximately 0.12 ng/μl. It has recently been suggested that absolute concentration of nucleosomes may affect nucleosomal stability under certain reaction conditions; for example, when mononucleosomes are assembled from DNA containing Xenopus 5 S RNA sequences, they are not stable at 3 ng/μl under some conditions (Goddard and Wolffe, 1995), while under other conditions they are stable at 0.3–0.6 ng/μl (Lee et al., 1993). We therefore wanted to demonstrate that the nucleosomes were stable under the conditions used here.

We have previously reported DNase and micrococcal nuclease studies that demonstrate that nucleosomes assembled under the conditions reported here are stable (Imbalzano et al., 1994a). To further support this conclusion, we performed a time course where free DNA or mononucleosome particles assembled from PH MLT DNA or PH MLT(+3) DNA, which has previously been shown to bind to yeast TBP and TFIIA when altered by hSWI/SNF, were incubated at the concentration and under reaction conditions used here and in previous work (1.2 × 10^{-9} M: 0.12 ng/μl), in the presence or absence of ATP (Fig. 7). At times ranging from 3 min to 2.5 h, identical reactions were loaded onto native 5%, 0.5× TBE polyacrylamide gels and were subjected to electrophoresis at 6 V/cm. Since the nucleosome structure in the experiments presented was assayed at 10–70 min after the start of any given reaction, this experiment evaluates the state of the mononucleosome at times equal to and beyond the point where these and previous experiments were assayed. At times up to 90 min, no significant dissociation of nucleosomal DNA occurred, and the proportion of naked DNA present in any of the samples did not exceed 10%. A minor increase in the amount of free DNA did occur between 90 and 150 min, such that the percentage of free DNA after 2.5 h was between 10 and 15%. In no case was there a rapid or significant spontaneous nucleosome disruption, and there was no effect of ATP on stability. We conclude that the mononucleosome particles are stable throughout and beyond the time parameters used in our experiments and are not subject to “spontaneous” nucleosome disruption. The alterations in nucleosome structure and the resulting increase in the ability of transcription factors to bind to nucleosomal DNA that we have observed are therefore due to the ATP-dependent changes caused by hSWI/SNF and the presence of the purified transcription factors.

**DISCUSSION**

At least two distinct nucleosome remodeling activities in eukaryotes, NURF and SWI/SNF, require ATP in order to alter nucleosome structure (Tsukiyama and Wu, 1995; Kwon et al., 1994; Imbalzano et al., 1994a; Cote et al., 1994). The data presented here distinguish between possible mechanisms of
action by hSWI/SNF and indicate that ATP hydrolysis is not required for an activity that continually weakens histone-DNA contacts on the surface of a nucleosome. Instead, hSWI/SNF is able to alter nucleosome structure in a manner that remains completely stable after removal of ATP or after competitive inhibition of ATP hydrolysis. We do not know whether hSWI/SNF presence is required to maintain the disrupted structure. It is possible that hSWI/SNF has an ATP-dependent activity that is necessary to alter nucleosome structure and a separate activity (not ATP-dependent) that is necessary to maintain the altered structure. Recent work has demonstrated that the yeast SWI/SNF complex both binds to and can alter the topology of naked DNA in an ATP-independent manner (Quinn et al., 1996). The relevance of these observations to nucleosome disruption and whether hSWI/SNF can maintain 4 mM ATP. At the indicated times, one naked DNA and one nucleosomal DNA reaction were removed from the 30°C bath and loaded directly onto adjacent lanes of a native 5%, 0.5 × TBE polyacrylamide gel. A second gel was used to assay nucleosomes at 90–150°C after the start of the reactions. Electrophoresis was performed at 6 V/cm. Gels were then dried and exposed to film or a phosphorimager screen (Molecular Dynamics). This experiment was performed with nucleosome particles that had been assembled 24 days previously. Thus, storage of the nucleosome particles for 24 days at 4°C had no effect on nucleosome stability, and, in fact, we have observed that assembled nucleosomes are stable for up to 6 weeks post-assembly (data not shown). These observations are in agreement with the findings of Li and Wrange (Li and Wrange, 1993; Li and Wrange, 1995).

The spectrum of nucleotides that will function with hSWI/SNF in disruption is essentially identical to that of Drosophila NURF (Tsukiyama and Wu, 1995), which also disrupts nucleosome structure in an ATP-dependent manner. It is believed that the Brg1 and/or hBrm protein encodes the ATPase of hSWI/SNF (Khavari et al., 1993; Muchardt and Yaniv, 1993; Chiba et al., 1994) and that hSWI/SNF, a Drosophila protein with similarities to SWI2/SNF2 (Elfring et al., 1994), encodes the ATPase domain of NURF (Tsukiyama et al., 1995). These genes all display extensive homology in their ATPase domains, consistent with their similar nucleotide requirements.

We have used three separate protocols to assess the effect of ATP depletion on nucleosome disruption: alteration of DNaseI digestion of a mononucleosome, alteration of supercoiling in a circular plasmid, and facilitation of GAL4 binding to nucleosomal DNA. In all three cases the effects of hSWI/SNF were fully maintained in the absence of ATP. These data are consistent with the hypothesis that all three effects are a consequence of the same underlying change in nucleosome structure induced by hSWI/SNF.

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