Cell, Vol. 97, 833–842, June 25, 1999, Copyright ©1999 by Cell Press

ATP-Dependent Histone Octamer Sliding Mediated by the Chromatin Remodeling Complex NURF

Ali Hamiche, Raphael Sandaltzopoulos, David A. Gdula, and Carl Wu*
Laboratory of Molecular Cell Biology
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20892-4255

Summary

***Drosophila* NURF is an ATP-dependent chromatin remodeling complex that contains ISWI, a member of the SWI2/SNF2 family of ATPases. We demonstrate that NURF catalyzes the bidirectional redistribution of mononucleosomes reconstituted on *hsp70* promoter DNA. In the presence of NURF, nucleosomes adopt one predominant position from an ensemble of possible locations within minutes. Movements occur in *cis*, with no transfer to competing DNA. Migrating intermediates trapped by Exo III digestion reveal progressive nucleosome motion in increments of several base pairs. All four core histones are retained quantitatively during this process, indicating that the general integrity of the histone octamer is maintained. We suggest that NURF remodels nucleosomes by transiently decreasing the activation energy for short-range sliding of the histone octamer.**

Introduction

The compaction of the eukaryotic genome in nucleosomes and their subsequent assembly in higher order structures of chromatin create barriers to access by the regulatory proteins and multienzyme complexes that process genetic information. Several strategies have been shown to be employed by cells to circumvent these structural constraints. Included among these are the prominent activities of histone modifying enzymes and a growing class of multicomponent protein complexes that require ATP hydrolysis for their actions (for reviews, see Wu, 1997; Armstrong and Emerson, 1998; Cairns, 1998; Kadonaga, 1998; Pollard and Peterson, 1998; Workman and Kingston, 1998; Travers, 1999).

ATP-dependent chromatin remodeling was first biochemically demonstrated during an analysis of transcription factor–mediated disruption of an in vitro reconstituted nucleosome array (Tsukiyama et al., 1994). Subsequently, purified protein assemblies containing the SWI2/SNF2 DNA-stimulated ATPase (SWI/SNF complexes), which had previously been characterized genetically as a transcriptional regulator, were shown to alter nucleosome structure in an ATP-dependent fashion (Cote et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994; Wang et al., 1996a, 1996b; Papoulas et al., 1998). Protein purification has revealed other complexes possessing ATP-dependent chromatin perturbing activities, including *Drosophila* NURF (Tsukiyama and Wu, 1995), CHRAC (Varga-Weisz et al., 1997), and ACF (Ito et al., 1997); the yeast RSC (Cairns et al., 1996), ISW1, and ISW2 complexes (Tsukiyama et al., 1999); and the vertebrate ERC1 (Armstrong et al., 1998), RSF (LeRoy et al., 1998), and Mi-2/NRD/NURD complexes (Tong et al., 1998; Wade et al., 1998; Xue et al., 1998; Zhang et al., 1998). At the core of each protein assembly is a subunit bearing high sequence similarity to the ATPase domain of SWI2/SNF2, the activity of which is essential to function in several studied examples.

Members of the SWI2/SNF2 and ISWI subfamilies of chromatin remodeling complexes have been shown to assist transcriptional activation of reconstituted chromatin templates in vitro (Ito et al., 1997; Mizuguchi et al., 1997; Armstrong et al., 1998; LeRoy et al., 1998; Okada and Hirose, 1998). Despite this similarity, the compositions and biochemical modes of action of the two types of complexes are distinct. Yeast SWI/SNF and its close relative RSC are very large ~2 MDa assemblies composed of 10–15 polypeptides (Cairns et al., 1994; Cote et al., 1994; Peterson et al., 1994). In contrast, complexes containing ISWI or its close relatives are smaller (~0.5 MDa), with 2–5 components (Tsukiyama and Wu, 1995; Tsukiyama et al., 1995, 1999; Ito et al., 1997; Varga-Weisz et al., 1997; LeRoy et al., 1998). The SWI2/SNF2 protein and SWI/SNF complexes exhibit DNA-stimulated ATPase activity (Laurent et al., 1993; Cairns et al., 1994; Cote et al., 1994; Kwon et al., 1994), while the ATPase activity of ISWI alone or in complex is only stimulated by nucleosomes (Tsukiyama and Wu, 1995; Varga-Weisz et al., 1997; Corona et al., 1999). Moreover, although SWI/SNF complexes alter broadly the DNase I accessibility of mononucleosomes (Cote et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994), NURF, an ISWI-containing complex, induces more subtle changes (Tsukiyama and Wu, 1995). ISWI-containing assemblies either impose (CHRAC, ACF, and RSF) or degrade (NURF) the regularity of spacing of nucleosome arrays.

To investigate mechanisms of ATP-dependent chromatin remodeling, our laboratory has undertaken a comprehensive study of the structure and function of *Drosophila* nucleosome remodeling factor (NURF) (Wu et al., 1998). In this report, we examine the remodeling action of NURF on a histone octamer complexed with DNA. We have found that NURF is able to reposition a histone octamer to vicinal positions on the same DNA fragment, while retaining the full complement of core histones. Nucleosome movement on DNA occurs within minutes, and migrating intermediates can be captured at increments of a few base pairs. Our results suggest a mechanism of remodeling in which NURF lowers the activation energy for short-range sliding of a histone octamer under physiological conditions.

Results

Nucleosomes Can Be Reconstituted at Several Positions on the *hsp70* Promoter

In an earlier study, we noted that the ability of NURF to rearrange nucleosomes was substantially greater on an

*To whom correspondence should be addressed (e-mail: carlwu@helix.nih.gov).

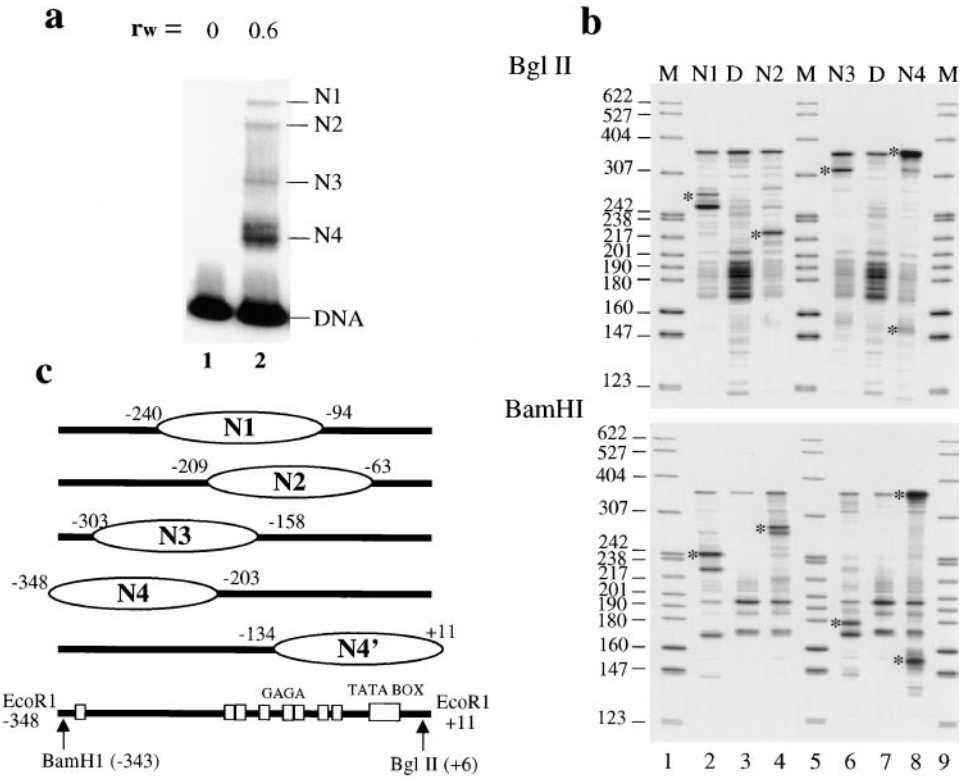

Figure 1. Reconstituted Nucleosomes Occupy Several Positions on the *hsp70* Promoter

(a) Native polyacrylamide gel electrophoresis of mononucleosomes reconstituted with a histone:DNA weight ratio (r_w) of 0.6 on a radiolabeled 359 bp EcoRI fragment carrying the *hsp70* promoter. The four major nucleosome species are indicated as N1, N2, N3, and N4.

(b) The bands corresponding to each nucleosome species (DNA radiolabeled either at Bgl II or BamHI positions) were excised from the gel, and nucleosomes were eluted and digested with 400 U/ml of Exo III for 2 min at 37°C. DNAs were analyzed by electrophoresis on a sequencing gel. The lanes represent N1–N4 nucleosomes; D, free DNA; M, pBR322 Hpa II digestion markers; asterisk, nucleosome boundaries.

(c) Nucleosome positions on the 359 bp EcoRI fragment (bars) are represented as ovals; positions of nucleosome boundaries are given ± 2 bp. The GAGA and TATA promoter elements are indicated.

array of nucleosomes than on a 161 bp mononucleosome (Tsukiyama and Wu, 1995). To resolve this paradox, and to investigate the mechanism of nucleosome remodeling, we developed a remodeling assay that utilizes a mononucleosome reconstituted on a longer, 359 bp, fragment of the *Drosophila hsp70* promoter. This 359 bp, loosely defined "nucleosome" provides DNA arms of sufficient length extending from the ~146 bp nucleosome core particle (Kornberg, 1977; Van Holde, 1989) to accept nucleosomal histones after remodeling.

As observed previously using other DNAs, mononucleosome reconstitution by salt gradient dialysis on the 359 bp fragment generates a mixed nucleosome population distributed over several translational positions, whose locations are dependent on the underlying DNA sequence. These nucleosomes can be fractionated by native polyacrylamide gel electrophoresis on the basis of differences in length and spatial orientation of the linker DNA (Figure 1a: N1, N2, N3, and N4). Such position-dependent electrophoretic mobility (Linxweiler and Horz, 1984; Duband-Goulet et al., 1992; Meersseman et al., 1992) has been described for DNA-bending proteins such as CAP (Wu and Crothers, 1984). The native gel mobility is minimal for DNA molecules with centrally positioned nucleosomes and increases linearly as the nucleosome location approaches the end of the fragment.

We mapped the nucleosome positions precisely by gel elution of each of the N1-N4 species, followed by Exonuclease III (Exo III) digestion (Figure 1b, top and bottom). The pause positions of Exo III define nucleosome boundaries, which are displayed relative to the *hsp70* promoter elements (Figure 1c). Thus, nucleosomes N1 and N2 overlap and are located over GAGA elements of the *hsp70* promoter. The N3 position is located further upstream from, and partially overlaps, N1 and N2. As is generally observed when nucleosomes are reconstituted on linear fragments, there appears to be a greater preference for nucleosome location at either fragment end (N4 and N4′) (e.g., Hamiche et al., 1996).

NURF Promotes Nucleosome Redistribution to One Favored Position

Native gel electrophoresis revealed that when NURF is added to the mixed population of N1-N4 nucleosomes in the presence of ATP, there was a clear increase in the N3 species and a corresponding loss of the N1 and N2 nucleosome positions (Figure 2, lanes 5 and 6). A subtle change in the electrophoretic mobility of the N4 species could also be observed. To illustrate the effects of NURF on a single positioned species, we purified the N1 nucleosome by native gel electrophoresis and eluted and incubated it with NURF and ATP. As shown in Figure

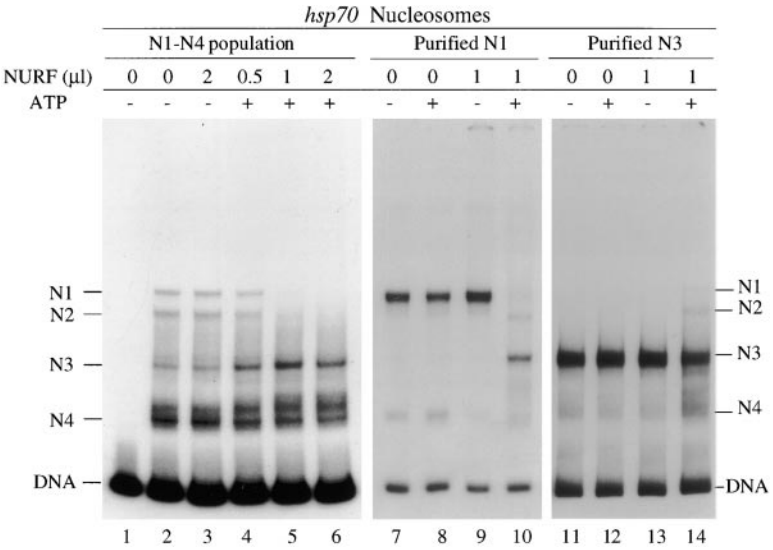

Figure 2. NURF Facilitates Nucleosome Mobility on the *hsp70* Promoter

Native polyacrylamide gel electrophoresis of mononucleosomes reconstituted on the radiolabeled 359 bp *hsp70* promoter fragment results in four major nucleosome species, indicated as N1, N2, N3, and N4 (lane 2). The mixed nucleosome population was incubated with NURF (3 ng/μl) in the absence or presence of ATP as indicated for 30 min at 26°C (lanes 3–6). Lane 1 shows free DNA only. Lanes 7–14: nucleosome species N1 and N3 were eluted from the gel slice in buffer containing 50 μg/ml sheared salmon sperm DNA and incubated as above with NURF and ATP as indicated, before electrophoresis on a second native gel. The gel purification and elution procedure results in some redistribution of the purified N1 and N3 nucleosomes to the N4 position and in the release of naked DNA fragment.

2 (lane 10), nucleosomes at the N1 position moved primarily to the N3 position, but a small, clearly defined signal was also observed at N2, and between the N3 and N4 positions.

The results demonstrate that nucleosome redistribution mediated by NURF is bidirectional, repositioning nucleosomes from N1 to either upstream (N3) or downstream (N2) positions on the same DNA fragment. This can be further illustrated by incubation of gel-purified N3 nucleosomes with NURF and ATP. A fraction of the N3 species was relocated to the positions corresponding to N1, N2, N4, and between N3 and N4 nucleosomes (Figure 2, lane 14). However, most of the nucleosomes remained at N3, confirming this location as the favored one in the ensemble of equilibrium positions.

Remodeling mediated by NURF does not proceed by the complete dissociation of the histone octamer from DNA. As the remodeling reactions were performed in a vast excess of carrier DNA, nucleosome transfer to an unlinked DNA fragment would readily have been detected by the production of free labeled DNA (O'Donohue et al., 1994; Lorch et al., 1999). No increase in the free 359 bp DNA was observed after remodeling by NURF. Furthermore, we argue that NURF acts catalytically; remodeling reactions were performed at a ratio of 1 NURF:50–100 nucleosomes.

Histone Octamer Retains Integrity after Remodeling

To investigate the fate of core histone proteins in the remodeling reaction, we analyzed the protein composition of the N1 nucleosome species after NURF-catalyzed redistribution to N3. As shown by two-dimensional electrophoresis and silver staining for the core histones H3, H2B, H2A, and H4, the histone composition of the N1 nucleosome after NURF repositioning to N3 remained the same, with no significant change in histone stoichiometry (Figure 3). Thus, chromatin remodeling by NURF does not entail the overt loss of, for example, H2A-H2B dimers whose interactions with the H3-H4 tetramer are known to be relatively weak (Baxevanis et al., 1991). It remains possible that NURF may induce a transient and subtle disorganization of the interface between the H2A-H2B dimer and the H3-H4 tetramer, or a change in some other aspect of histone octamer or DNA structure to faciliate nucleosome repositioning.

Migrating Intermediates Trapped at Increments of a Few Base Pairs

We characterized the kinetics of nucleosome mobilization by mapping of the shift in the boundary of nucleosome N1 after reaction with NURF and ATP. In these experiments, gel-purified N1 nucleosomes were incubated with NURF and ATP for varying time periods at 26°C, followed by digestion with Exo III for 1 min at 37°C (incubation time was registered after the Exo III digestion step). As shown in Figure 4, a near quantitative shift from the N1 to N3 position is evident after 5 or 10 min incubation. Interestingly, after 1 and 2 min in the presence of NURF, a continuous series of Exo III–resistant fragments extending at increments of a few base pairs between the N1 and N3 positions (the limit of resolution of the gel) can be observed. These sites probably represent migration intermediates trapped by the exonucleolytic action of Exo III, in which destruction of one strand from both DNA arms extending from the core particle would freeze further motion of the histone octamer. While underlying DNA sequence may influence the action of the Exo III enzymatic probe, the incremental nature of the Exo III stops suggests that the migration of the histone octamer is not entirely smooth, occurring in small processive steps, rather than large jumps on DNA.

Little Effect of NURF on *5S* Nucleosomes

NURF facilitates nucleosome redistribution to one preferred location from several major positions on the 359 bp *hsp70* promoter fragment. To explore the action of NURF on other nucleosomes, we analyzed its impact on a mixed nucleosome population reconstituted on a 256 bp Nci I fragment containing the sea urchin *5S* RNA gene (Simpson et al., 1985). As reported previously, histone octamers occupy several positions on this fragment, leading to at least three electrophoretically separable species of mononucleosomes (N1-N3; Hamiche et

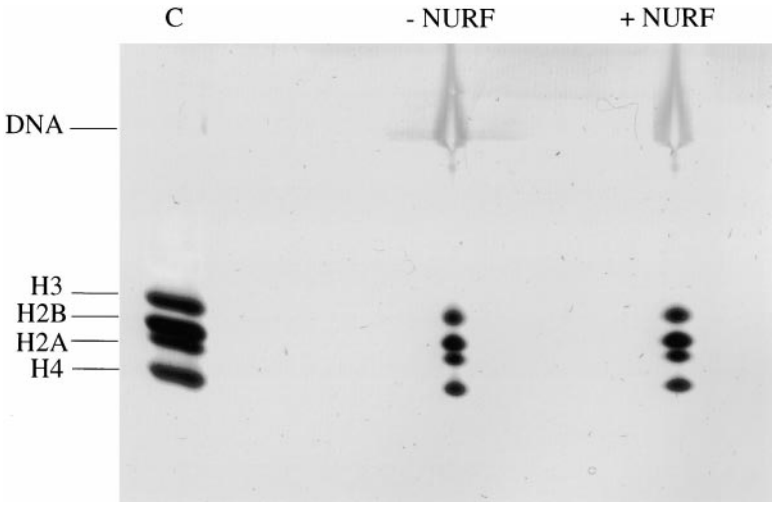

Figure 3. Two-Dimensional Gel Electrophoresis of the N1 Nucleosome before and after Remodeling by NURF

The first dimension was performed as in Figure 2 to isolate the N1 and N3 nucleosomes. The corresponding gel band was then excised and analyzed by SDS–PAGE. Lane C represents histone octamer control showing *Drosophila* histones H3, H2B, H2A, and H4.

al., 1996) (Figure 5, lane 1). Incubation of this mixed *5S* nucleosome population with NURF and ATP under conditions in which redistribution of *hsp70* nucleosomes was readily apparent revealed little or no net change in the *5S* nucleosome positions (Figure 5, lane 4).

We further analyzed the effects of NURF and ATP on gel-purified *5S* N1 and N3 nucleosomes. A small fraction of the purified *5S* N1 species was found to be relocated to the N2 and N3 positions (Figure 5, lane 8), and a small fraction of the purified *5S* N3 species was relocated to N1 (Figure 5, lane 12; a trace of N2 can also be seen in the original autoradiogram). However, most of the purified *5S* N1 and N3 nucleosomes remained at their original positions, respectively, confirming retention of site preferences upon remodeling by NURF. Moreover, the redistribution of purified *5S* nucleosomes was found to be similar after 15, 30, and 45 min of incubation with NURF and ATP, indicating that remodeling had reached equilibrium (data not shown). As *5S* gene sequences are known to organize stable nucleosomes (Shrader and Crothers, 1989), the results suggest that, for any given gene sequence, the intrinsic mechanical properties of nucleosome core particle and linker DNAs may substantially restrict the extent of nucleosome redistribution by NURF.

Remodeling Efficiencies of NURF and ISWI

NURF is a four-subunit assembly consisting of NURF-215, the largest NURF component; NURF-38, an inorganic pyrophosphatase (Gdula et al., 1998); NURF-55, a WD/β-transducin repeat protein shared with other complexes involved in histone metabolism (Martinez-Balbas et al., 1998); and NURF-140, the ISWI ATPase (Elfring et al., 1994; Tsukiyama et al., 1995). To compare the activities of NURF and the ISWI ATPase subunit, we have purified FLAG-tagged ISWI protein from Sf9 cells

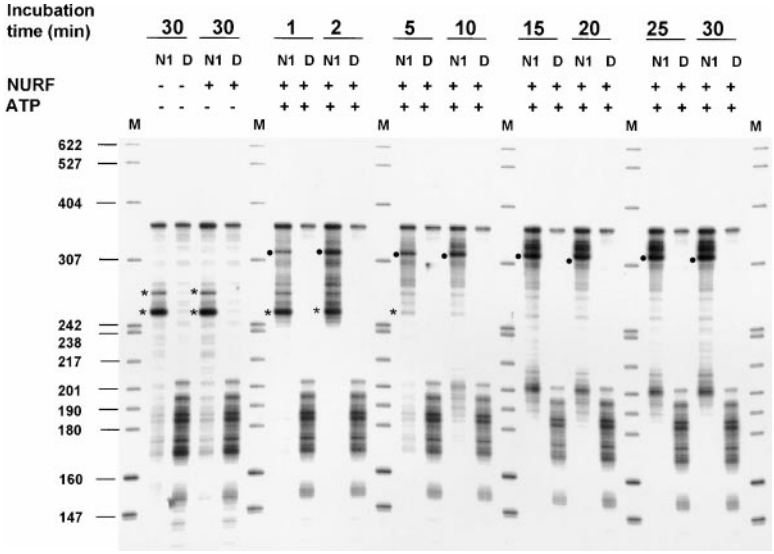

Figure 4. Exo III Digestion and DNA Sequencing Gel Electrophoresis Showing Shifts in the N1 Nucleosome Boundary upon Reaction with NURF

Gel-purified N1 nucleosome from the mixed nucleosome population reconstituted on the 359 bp *hsp70* EcoRI fragment labeled at the Bgl II position was incubated in the absence or presence of 6 ng NURF and 1 mM ATP, for the indicated min in 25 μl volume. At (t − 1) min, 800 U/ml of Exo III was introduced and digestion was allowed to occur for 1 min at 37°C. The lanes marked D correspond to Exo III digestions of naked DNA as for samples containing nucleosomes. Lanes M indicate the marker HpaII fragments of pBR322. Asterisks, Exo III pause corresponding to the N1 nucleosome boundary. Dots, Exo III pause corresponding to N3 nucleosome boundary. (The Exo III pauses near the 201 bp marker and above the N3 boundary observed at 10 min and longer incubation times are likely to reflect the fractional redistribution of N1 nucleosomes to positions at or near N2, and between N3 and N4, respectively, consistent with data from native gel electrophoresis in Figure 2, lane 10).

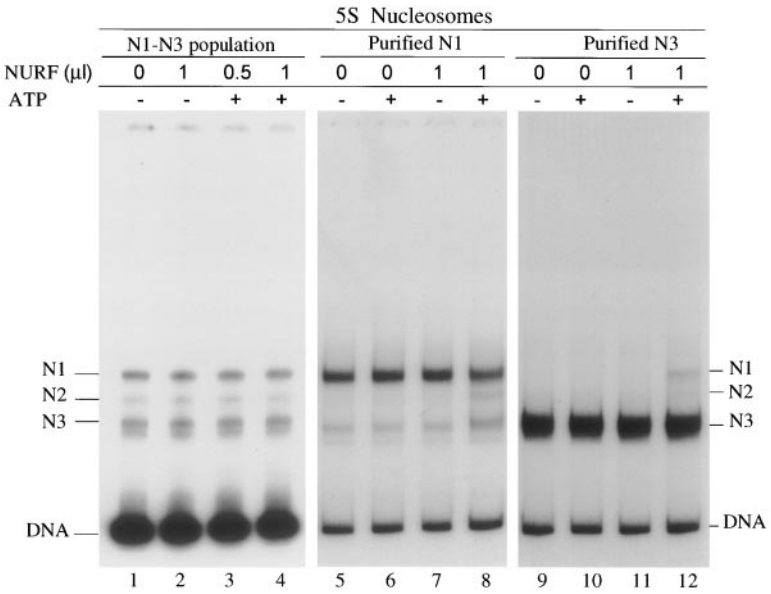

Figure 5. Nucleosomes Assembled on the Sea Urchin *5S* Fragment Are Relatively Resistant to NURF Action

Mononucleosomes reconstituted on a 256 bp Nci I fragment containing the sea urchin *5S* RNA gene migrate as three major bands on a native gel (lane 1). The mixed population of *5S* nucleosomes (lanes 2–4) was incubated at 26°C for 30 min with NURF (3 ng) in the absence or presence of ATP in a reaction volume of 10 μl. Lanes 5–12: *5S* nucleosome species N1 and N3 were eluted from the gel slice in buffer containing 50 μg/ml sheared salmon sperm DNA and incubated at 26°C with NURF and ATP as indicated for 45 min (lanes 5–8) or for 30 min (lanes 9–12), before electrophoresis on a second native gel. The gel purification and elution procedure result in the release of naked DNA fragment and in some redistribution of the purified *5S* N1 nucleosome to the N3 position.

under control of the baculovirus expression vector. Like NURF, the purified ISWI protein was found to exhibit a nucleosome-stimulated ATPase activity, consistent with a recent study using bacterially expressed ISWI (Corona et al., 1999) (Figure 6a). However, the ATPase activity of ISWI was substantially weaker than that of NURF, requiring a 50-fold molar excess to give equivalent hydrolysis of ATP in a standard reaction (Figure 6a, compare lanes 1 and 2 with lanes 5 and 6).

We also compared NURF with the ISWI subunit using the nucleosome mobilization assay. When used at amounts equimolar to levels of NURF that gave strong nucleosome mobility (2.8 fmol), ISWI was unable to mobilize nucleosomes in the mixed nucleosome population reconstituted on the 359 bp *hsp70* fragment (Figure 6b, compare lanes 3 and 7). A 10-fold higher level of ISWI (28 fmol; lane 8) was also ineffective. However, at 50- and 100-fold higher levels of ISWI (140 and 280 fmol), we observed decreased signal intensities and a smeared pattern for nucleosomes N1, N2, as well as N3, while the signal increased slightly for nucleosome N4 (Figure 6b, lanes 11 and 13). These ATP-dependent effects on nucleosome positions by the recombinant ISWI subunit stand in contrast to those displayed by NURF (i.e., movement of N1 and N2 to the N3 position) (Figure 6b, lanes 3 and 4).

Discussion

In this report we show that, in an ATP-dependent manner, the purified NURF complex can catalyze the redistribution of a histone octamer reconstituted on *hsp70* promoter DNA. Movement occurs bidirectionally and results in redistribution to one preferred location from an ensemble of positions. Mobilization does not entail a loss of core histone proteins, indicating that the histone octamer does not undergo a radical change in composition during this process. Movement of the histone octamer occurs in moderate concentrations of monovalent and divalent salt (50 mM NaCl, 3 mM $MgCl_2$) and in a time scale of minutes or less. Migration intermediates trapped by Exo III digestion reveal progressive movement of the histone octamer every few base pairs. Taken together, these results suggest a remodeling mechanism in which the ATP-driven action of NURF lowers the activation energy for short-range sliding of the histone octamer under physiological conditions.

Nucleosome Sliding

In principle, a histone octamer could be translocated in several ways (Beard, 1978). The octamer could be made to jump by complete detachment from one position and reassociation at a new location. The octamer could undergo displacement transfer in which a stretch of free DNA could displace another stretch of nucleosomal DNA from its interaction with the histone octamer. The nucleosome could also be relocated by a rolling or sliding of the histone octamer relative to DNA without dissociation. We favor sliding as a means of nucleosome mobilization by NURF because it would require only the transient alteration of individual weak interactions between histones and DNA, whereas jumping entails the energetically unfavorable disruption of all histone octamer–DNA contacts simultaneously. The crystal structure of the histone octamer complexed with DNA (Luger et al., 1997) indicates sites of interaction between the core histones and DNA approximately every ten base pairs over the entire 146 bp length of nucleosomal DNA, with the notable insertion of an arginine residue in the minor groove at every site. To maintain critical minor groove contacts during translational sliding, a rotation of the double helix as in the advance of a screw in its screwthread would also be required.

The experimental data we have obtained suggest that NURF promotes nucleosome sliding. First, we observed migration of the core histone octamer on the same DNA fragment without displacement in *trans*, even in the presence of a 3000-fold excess of exogenous carrier DNA (50 μg/ml). This concentration of competing DNA is 3-fold greater than that which we (A. H.) found effective

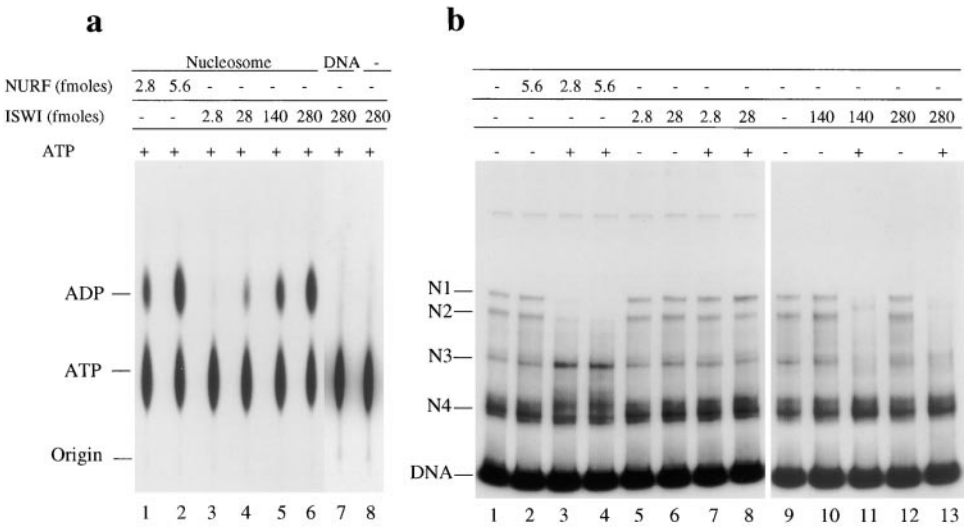

Figure 6. Comparison between NURF and the ISWI Subunit

(a) Nucleosome-stimulated ATPase activity of the indicated femtomoles of NURF and recombinant ISWI, as measured by hydrolysis of [α-P^{32}]ATP to [α-P^{32}]ADP, followed by TLC separation.

(b) The mixed population of mononucleosomes assembled on the 359 bp *hsp70* EcoRI fragment radiolabeled at the 5′ end (lanes 1–8) or internally labeled at the Bgl II site to circumvent a terminal phosphatase activity evident at high ISWI concentration (lanes 9–13) was incubated in a standard mobilization assay (26°C) with increasing fmol of recombinant ISWI protein or NURF, in the absence or presence of ATP.

for capturing histone octamers displaced from the *5S* RNA gene by the SP6 RNA polymerase (O'Donohue et al., 1994). Second, at early times in the reaction with NURF, we observed migration intermediates trapped by Exo III digestion at increments of a few base pairs. The presence of these migration intermediates is not consistent with a jumping or displacement transfer process, except over a distance of a few base pairs.

Sliding has long been known to be an intrinsic property of nucleosomes (Beard, 1978; Spadafora et al., 1979; Glotov et al., 1982; Van Holde and Yager, 1985; Meersseman et al., 1992; Ura et al., 1997; Flaus and Richmond, 1998). However, this passive and spontaneous sliding of the histone octamer, which occurs over a period of hours, is readily distinguished from the rapid sliding that we show here to be facilitated by NURF. Moreover, while spontaneous sliding is enhanced by elevated temperatures and ionic strengths and is suppressed by 2 mM $MgCl_2$ (Pennings et al., 1994), sliding faciliated by NURF can occur at room temperature and in moderate concentrations of both mono- and divalent cations.

We note that an independent study of the *Drosophila* CHRAC complex has also found ATP-driven movement of a nucleosome without displacement of the histone octamer from DNA (Längst et al., 1999 [this issue of *Cell*]). It is intriguing that although both NURF and CHRAC facilitate sliding of the histone octamer, NURF promotes the bidirectional movement of a centrally located nucleosome, with less impact on end nucleosomes, while CHRAC moves a nucleosome from a fragment end toward the middle. If these distinct sliding properties can be separated from the effects of the different underlying DNA sequences, they may provide a basis for the different biochemical actions of NURF and CHRAC on nucleosome arrays (Tsukiyama and Wu, 1995; Varga-Weisz et al., 1997). It will be of interest to compare directly the sliding of histone octamers for the three known ISWI-containing complexes (ACF, CHRAC, and NURF).

NURF (and CHRAC)-promoted sliding of the histone octamer is in contrast to the recently described remodeling mechanism of the SWI/SNF and RSC complexes, in which significant unwrapping of nucleosomal DNA is observed (Logie and Peterson, 1997; Cote et al., 1998; Lorch et al., 1998, 1999; Schnitzler et al., 1998; Bazett-Jones et al., 1999). Most strikingly, remodeling by RSC leads to transfer of the entire histone octamer from a nucleosome core particle to naked DNA (Lorch et al., 1999). Thus, at least two separate modes of ATP-dependent chromatin remodeling can now be discerned for the SWI2/SNF2 and ISWI subfamilies of complexes, namely histone octamer transfer and histone octamer sliding, respectively (Figure 7).

How Could NURF Assist Histone Octamer Sliding?

NURF may promote movement of the histone octamer by several mechanisms. In one model, NURF could promote histone octamer sliding by progressive capture, advance, and release of spontaneous unwrapping fluctuations of DNA (Widom, 1998), initiating at one end of the core particle, which is particularly susceptible to reversible dissociation (Weischet et al., 1978; Simpson, 1979). It may be envisaged that such propagation of a "DNA bulge" would lead to the eventual transfer of the nucleosome to a new location without direct perturbation of histone–DNA interactions. NURF could also utilize the energy of ATP hydrolysis to alter the conformation (e.g., the twist) of nucleosomal DNA (Van Holde and Yager, 1985), as does occur, for example, during the action of Sp6 RNA polymerase (O'Donohue et al., 1994; Studitsky et al., 1994). To date, we have not observed a stable complex of NURF with DNA or nucleosomes,

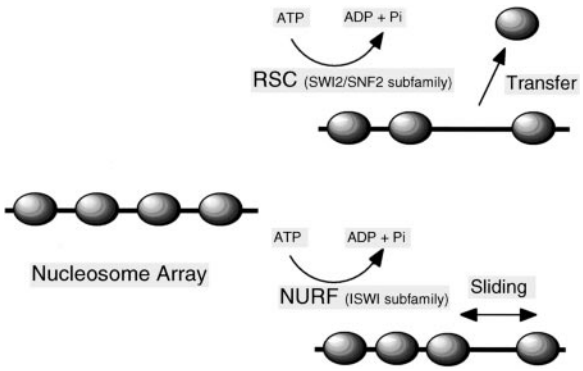

Figure 7. Two Models for Nucleosome Remodeling

nor any evidence of ATP-driven, processive movements of NURF on DNA.

In an alternative model, NURF could directly alter histone–DNA interactions by a "hit and run" mechanism. The histone elements that organize the wrapping of nucleosomal DNA over the histone octamer include the histone fold, structured elements beyond the histone fold, as well as elements of the histone tails (Arents et al., 1991; Luger et al., 1997). A transient perturbation of certain histone octamer–DNA interactions by NURF could lower the activation energy for spontaneous sliding of the histone octamer. In this case, sliding could occur as a random walk process along the DNA until the octamer is captured by a DNA sequence with favorable anisotropic bendability (Travers and Drew, 1998) or is blocked from sliding further by a particularly rigid DNA linker. Our observation of a bidirectional redistribution of the purified *hsp70* N1 nucleosome species to the preferred N3 location, as well as to N2 and N4 positions, and the redistribution of purified *hsp70* N3, and *5S* N1 and N3 nucleosomes is consistent with a random walk hypothesis.

Two observations suggest that the ISWI subunit and perhaps other components of NURF make direct contact with nucleosomes to initiate sliding. First, the ATPase activity of NURF or recombinant ISWI is stimulated by nucleosomes but not by free DNA or histones (Tsukiyama and Wu, 1995; Corona et al., 1999; this study). Second, the nucleosome-stimulated ATPase activity of NURF or recombinant ISWI is abrogated by trypsinization of the histone tails and inhibited by competition with GST–histone tail fusions (Georgel et al., 1997; Corona et al., 1999). Thus, we suggest, as part of the mechanism to facilitate histone octamer sliding, that recombinant ISWI in the context of, or in concert with, other subunits of the NURF complex, utilizes the energy of ATP hydrolysis to alter transiently the conformation of the histone tails.

Restrictions on Nucleosome Sliding

The position of the histone octamer with respect to the DNA sequence is a function of the sequence-dependent flexibility of DNA and its bendability over the histone octamer (Luger et al., 1997; Travers and Drew, 1998). In our study of nucleosome movements facilitated by NURF, the migration of nucleosomes to one favored location on the *hsp70* promoter and the preferred retention of *5S* nucleosomes at several major locations provide examples of free energy minima where the anisotropic flexibility of core particle DNA, or the rigidity of an adjacent linker, restrict free sliding of histone octamers. For the *hsp70* promoter, octamer migration from N1 to N3 positions unveils (though not completely) the GAGA factor–binding sites. It will be of interest to analyze the fate of the N3 nucleosome when GAGA factor is included in a nucleosome sliding experiment on the *hsp70* promoter.

More generally, it would be important to define how, on any given promoter or enhancer sequence, histone octamer sliding mediated by NURF (or by remodeling complexes of the ISWI subfamily) aligns the nucleosomal array relative to the underlying DNA sequence, how nucleosome alignment or spacing is established during S phase and modulated subsequently, and how alignment is perturbed (or not) upon binding of sequence-specific transcription factors. We have demonstrated that there are sequence-dependent restrictions on NURF-assisted octamer sliding. It is conceivable that on promoters which contain relatively immobile nucleosomes, octamer transfer or eviction by one of the RSC- or SWI/SNF-like complexes might be necessary. In this view, the functional requirement for one or another type of chromatin remodeling complex would depend not only on the constellation of relevant DNA-binding factors and the coactivators they recruit, but also on the mechanical properties of genomic DNA.

Comparing NURF and Recombinant ISWI

In a recent study, bacterially expressed ISWI protein was shown to possess nucleosome-stimulated ATPase activity as well as ATP-dependent nucleosome spacing and remodeling activities (Corona et al., 1999). In partial agreement with these findings, we have found that recombinant ISWI protein purified from a baculovirus expression system also displays nucleosome-stimulated ATPase activity. However, a 50-fold molar excess of recombinant ISWI (a 1:1 ratio of ISWI to nucleosomes) was required to generate nucleosome-stimulated ATPase activity equivalent to the activity shown by NURF. Similarly, recombinant ISWI had no effect on nucleosome sliding when compared with NURF on an equimolar basis—only a stoichiometric level of ISWI could display effects on nucleosome positioning. Moreover, stoichiometric ISWI affected nucleosomes in a qualitatively different manner than did catalytic amounts of NURF. Bacterially expressed ISWI also showed qualitative and quantitative differences from CHRAC in affecting nucleosome movement (Längst et al., 1999). It is possible that only a fraction of recombinant ISWI is properly folded and active. Alternatively, another subunit(s) of NURF participates with the ISWI engine in the overall process of nucleosome remodeling. Indeed, the ISWI-containing complex ACF requires both ISWI and a large subunit, Acf1, to achieve efficient nucleosome assembly (Ito et al., 1999). It will be of interest to examine this issue by comparing the activities of recombinant ISWI, alone and in various combinations with NURF-215, NURF-55, or NURF-38, using histone octamer sliding and other assays to elucidate the function of this chromatin remodeling complex.

Experimental Procedures

DNA

The 359 bp *hsp70 (87A)* fragment spans the promoter from positions −348 to +11. It was amplified by the polymerase chain reaction from pdhspXX3.2 (Tsukiyama et al., 1994) using the following primers, which contain an EcoRI site: primer 1, 5′-GCGGAATTCGGATCC ACGATAAGCATAACCAA-3′ and primer 2, 5′-GCGGAATTCAGATCT GAATTGACGCTCCGTCG-3′. The resulting DNA fragment was digested with EcoRI, gel purified, and cloned in the vector pBSK (Stratagene) as a tandem repeat (construct pBSK359.3m). pBSK359.3m was digested with either BglII or BamHI, dephosphorylated, and labeled at the 5′ end with T4 kinase. After circularization using T4 ligase, the circle was gel-purified and then relinearized by EcoRI cleavage.

The 256 bp fragment containing a sea urchin *5S* RNA gene (Simpson and Stafford, 1983) was gel-purified from a Nci I digest of plasmid pLV405-10 (Simpson et al., 1985).

Histones

Drosophila melanogaster nuclei were prepared according to Kamakaka et al. (1991). Long soluble chromatin obtained from micrococcal nuclease digestion of nuclei was adsorbed onto a hydroxyapatite column (1–2 mg DNA/ml packed hydroxyapatite) as described in Simon and Felsenfeld (1979). The column was first washed with elution buffer (0.1 M potassium phosphate [pH 6.8], 0.25 mM phenylmethylsulfonyl fluoride, and 1 mM β-mercaptoethanol) containing 0.65 M NaCl. Core histones were then eluted stepwise in wash buffer containing 2 M NaCl. Aliquots of histone preparations (1–2 mg/ml) were stored at −80°C until use.

Nucleosome Reconstitution

Mononucleosomes were assembled on linear DNA according to the "salt jump" method (Stein, 1979). Carrier DNA (supercoiled plasmid DNA from which the fragment originated) was mixed with the ^{32}P-labeled DNA (20,000–100,000 cpm, final DNA concentration, 200 μg/ml). The suitable amount of histones (histone:DNA weight ratio [r_w] = 0.6) in 2 M NaCl, 10 mM Tris-HCl (pH 7.5), and 100 mg/ml bovine serum albumin were added. The mixture was first incubated for 10 min at 37°C, diluted to 50 μg/ml DNA and 0.5 M NaCl, incubated at the same temperature for 30 min, and finally dialyzed at 4°C against 10 mM Tris-HCl (pH 7.5), 1 mM EDTA for at least 2 hr. We found that a histone:DNA weight ratio of 0.6 gave optimal reconstitution of mononucleosomes, with little formation of dinucleosomes, as judged by sucrose gradient centrifugation. For experiments involving analysis of histone composition (two-dimensional gel electrophoresis, see below), nonradioactive, 359 bp DNA fragment was substituted for the carrier plasmid DNA.

Gel Electrophoresis

Mononucleosomes were electrophoresed at room temperature in 4% polyacrylamide (acrylamide/bisacrylamide, 29:1, w/w) slab gels (0.15 cm × 17 cm × 18 cm) in TE buffer (Tris-HCl 10 mM, EDTA 1 mM [pH 7.6]). Gels were preelectrophoresed for 1 hr at 200–250 V and electrophoresed at the same voltage for 3 to 4 hr with extensive buffer recirculation. Gels were dried and autoradiographed at −80°C.

For two-dimensional gel analysis, the mixed population of nucleosomes was first fractionated on a native gel as above, and the N1 species was gel eluted, incubated with or without NURF, and reelectrophoresed on a native gel in TE buffer. The nucleosome bands N1 and N3 were excised and loaded directly onto the second dimension 15% SDS-polycarylamide gel. DNA and histones were visualized by silver staining (Wray et al., 1981).

DNA was electrophoresed at room temperature in 4% polyacrylamide (acrylamide/bisacrylamide, 20:1, w/w) slab minigels (0.15 × 10 × 8 cm) for 2 hr at 100 V in 20 mM sodium acetate, 2 mM EDTA, 40 mM Tris acetate (pH 7.8). Sequencing gels (0.02 × 30 × 40 cm) contained 7 M urea and 6% polyacrylamide (acrylamide to bisacrylamide = 19:1, w/w) in TBE buffer (90 mM Tris base, 90 mM boric acid, 2 mM EDTA [pH 8.5]). Gels were electrophoresed at 1200 V for 2 hr.

Mononucleosome Purification

Gel-fractionated nucleosome species were eluted after excision of gel slices by crushing them with a plastic pestle (Polylabo, France) in 1.5 ml microcentrifuge tubes (Eppendorf). The slurry was suspended and incubated for 30 min on ice in 100–150 μl of 10 mM Tris-Cl (pH 7.6), 0.2 mM EDTA, 1 mM $MgCl_2$, 5% glycerol, 0.02% bovine serum albumin, containing 50 μg/ml of sheared sperm salmon DNA. The eluate was recovered from the supernatant after centrifugation at 4°C.

Purification of NURF

NURF was purified to the final, glycerol gradient step from 200 g of 0–12 hr *Drosophila* embryos as described by Tsukiyama and Wu (1995) and Sandaltzopoulos et al. (1999). The concentration of NURF was estimated to be 0.8 ng/μl ISWI equivalents (3 ng NURF/μl, assuming ISWI comprises 26% of NURF), using recombinant ISWI (see below) as a calibration standard for Western blotting.

Recombinant ISWI

A BamHI-HindIII fragment from pBS-ISWI, constructed from partial ISWI cDNAs by T. Tsukiyama (unpublished data) and Corona et al. (1999), was inserted into the corresponding cloning sites of pBlueBac4 (Invitrogen). Recombinant baculovirus carrying the FLAG-tagged ISWI was used to infect 1 l of High Five cells (1.0–1.5 × 10^6 cells per ml; MOI = 5), and cells were harvested after 48 hr at 29°C. All further manipulations were conducted at 4°C. Cells were washed in PBS, resuspended in 10 ml HEGN-0.2M KCl (HEGN: 25 mM HEPES-KOH [pH 7.6], 1 mM EDTA, 10% glycerol, 0.02% NP40), supplemented with 1 mM DTT, 0.5 mM sodium metabisulfite, and 1× complete protease inhibitors (Boehringer Mannheim). Cells were homogenized with ten strokes of a homogenizer (B. Braun) at 700–800 rpm and centrifuged for 30 min at 40,000 rpm (Beckman 50-Ti rotor). The supernatant was incubated with a 0.5 ml volume of anti-FLAG beads (Sigma) equilibrated in HEGN-0.2M KCl and rotated gently for 2 hr. Beads were washed thrice, each with 5 ml of HEGN-0.5 M KCl (with supplements) for 5 min. (Between washes, beads were pelleted briefly in a Beckman JS-5.2 rotor; centrifuge was stopped upon reaching 1000 rpm.) Beads were then washed twice, each wash for 5 min with 5 ml of HEGN-0.1 M KCl (with supplements), followed by elution for 30 min by addition of 0.5 ml of the same buffer containing 2 mg/ml FLAG peptide. Beads were pelleted by microcentrifugation at 5000 rpm (Eppendorf), and the supernatant containing purified ISWI protein and FLAG peptide was collected. Aliquots were frozen in liquid nitrogen and stored at −80°C. The purity (~95%) and concentration of the ISWI preparation (40 ng/μl) were judged by SDS–PAGE and Coomassie blue staining using BSA as a standard.

Nucleosome Mobilization Assay

Nucleosome mobilization was performed at 26°C or 37°C for 30 min in nucleosome sliding buffer: 10 mM Tris-HCl (pH 7.6), 50 mM NaCl, 3 mM $MgCl_2$, 1 mM β-mercaptoethanol, 1 mM ATP at a nucleosome concentration of ~10 μg DNA equivalent/ml, in the presence of 0.5–2 μl of NURF. The standard reaction volume was 10 μl, except for Figure 4 (25 μl). The amount of competing carrier DNA is given in the figure legends.

ATPase Assays

ATPase assays were performed using α-P^{32}-labeled ATP as described by Gdula et al. (1998). The standard reaction (5 μl vol) contained 10 mM HEPES-KOH (pH 7.6), 50 mM KCl, 0.1 mM EDTA, 2 mM $MgCl_2$, 0.5 mM DTT, 7.5% glycerol, 0.01% NP40, 30 μM ATP, 5 μCi [α-P^{32}]ATP (300 Ci/mmol, Amersham), 25 ng mononucleosomes (DNA weight equivalent), or 25 ng sheared salmon sperm DNA as control, and ISWI (0.4–40 ng). Reactions were incubated at 26°C for 30 min. The labeled ADP produced by the ATPase reaction (1.0 μl) was separated by TLC (polyethyleneimine cellulose on polyester, Sigma) using 0.75 M KH_2PO_4.

Nucleosome Digestion with Exo III

Nucleosomes were fractionated by native gel electrophoresis, and the bands corresponding to N1-N4 nucleosomes were excised and nucleosomes were eluted as described above. The supernatant containing eluted nucleosomes was made up to 1× nucleosome sliding

buffer and incubated at 26°C with NURF for 0 min and increasing times, followed by digestion with 800 U/ml of Exo III for 1 min at 37°C. The reaction was immediately stopped with 10 mM EDTA, and DNA was extracted with phenol according to standard procedures.

Acknowledgments

We thank Paul Badenhorst, Gary Felsenfeld, and members of our laboratory for helpful comments and discussion, Helene Richard-Foy for useful reagents, Toshi Tsukiyama and Davide Corona for constructing full-length ISWI cDNA, Toshi Tsukiyama for a NURF sample, Gernot Längst and Peter Becker for an exchange of manuscripts and comments, and the reviewers for helpful criticisms. A. H. and R. S. were supported by fellowships from the Human Frontiers of Science Program, and D. A. G. was supported by the Leukemia Society of America. This work was supported by the Intramural Research Program of the National Cancer Institute.

Received April 9, 1999; revised May 14, 1999.

References

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

Armstrong, J.A., and Emerson, B.M. (1998). Transcription of chromatin: these are complex times. Curr. Opin. Genet. Dev. *8*, 165–172.

Armstrong, J.A., Bieker, J.J., and Emerson, B.M. (1998). A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF in vitro. Cell *95*, 93–104.

Baxevanis, A.D., Godfrey, J.E., and Moudrianakis, E.N. (1991). Associative behavior of the histone (H3-H4)2 tetramer: dependence on ionic environment. Biochemistry *30*, 8817–8823.

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

Beard, P. (1978). Mobility of histones on the chromosome of simian virus 40. Cell *15*, 955–967.

Cairns, B.R. (1998). Chromatin remodeling machines: similar motors, ulterior motives. Trends Biochem. Sci. *23*, 20–25.

Cairns, B.R., Kim, Y.J., Sayre, M.H., Laurent, B.C., and Kornberg, R.D. (1994). A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. Proc. Natl. Acad. Sci. USA *91*, 1950–1954.

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

Corona, D.F., Längst, G., Clapier, C.R., Bonte, E.J., Ferrari, S., Tamkun, J.W., and Becker, P.B. (1999). ISWI is an ATP-dependent nucleosome remodeling factor. Mol. Cell *3*, 239–245.

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

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

Duband-Goulet, I., Carot, V., Ulyanov, A.V., Douc-Rasy, S., and Prunell, A. (1992). Chromatin reconstitution on small DNA rings. IV. DNA supercoiling and nucleosome sequence preference. J. Mol. Biol. *224*, 981–1001.

Elfring, L.K., Deuring, R., McCallum, C.M., Peterson, C.L., and Tamkun, J.W. (1994). Identification and characterization of Drosophila relatives of the yeast transcriptional activator SNF2/SWI2. Mol. Cell. Biol. *14*, 2225–2234.

Flaus, A., and Richmond, T.J. (1998). Positioning and stability of nucleosomes on MMTV 3′LTR sequences. J. Mol. Biol. *275*, 427–441.

Gdula, D.A., Sandaltzopoulos, R., Tsukiyama, T., Ossipow, V., and Wu, C. (1998). Inorganic pyrophosphatase is a component of the Drosophila nucleosome remodeling factor complex. Genes Dev. *12*, 3206–3216.

Georgel, P.T., Tsukiyama, T., and Wu, C. (1997). Role of histone tails in nucleosome remodeling by Drosophila NURF. EMBO J. *16*, 4717–4726.

Glotov, B.O., Rudin, A.V., and Severin, E.S. (1982). Conditions for sliding of nucleosomes along DNA: SV 40 minichromosomes. Biochim. Biophys. Acta *696*, 275–284.

Hamiche, A., Schultz, P., Ramakrishnan, V., Oudet, P., and Prunell, A. (1996). Linker histone-dependent DNA structure in linear mononucleosomes. J. Mol. Biol. *257*, 30–42.

Imbalzano, A.N., Kwon, H., Green, M.R., and Kingston, R.E. (1994). Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature *370*, 481–485.

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

Ito, T., Levenstein, M.E., Fyodorov, D.V., Kutach, A.K., Kobayashi, R., and Kadonaga, J.T. (1999). ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. Genes Dev., in press.

Kadonaga, J.T. (1998). Eukaryotic transcription: an interlaced network of transcription factors and chromatin-modifying machines. Cell *92*, 307–313.

Kamakaka, R.T., Tyree, C.M., and Kadonaga, J.T. (1991). Accurate and efficient RNA polymerase II transcription with a soluble nuclear fraction derived from Drosophila embryos. Proc. Natl. Acad. Sci. USA *88*, 1024–1028.

Kornberg, R.D. (1977). Structure of chromatin. Annu. Rev. Biochem. *46*, 931–954.

Kwon, H., Imbalzano, A.N., Khavari, P.A., Kingston, R.E., and Green, M.R. (1994). Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature *370*, 477–481.

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

Laurent, B.C., Treich, I., and Carlson, M. (1993). The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. Genes Dev. *7*, 583–591.

LeRoy, G., Orphanides, G., Lane, W.S., and Reinberg, D. (1998). Requirement of RSF and FACT for transcription of chromatin templates in vitro. Science *282*, 1900–1904.

Linxweiler, W., and Horz, W. (1984). Reconstitution of mononucleosomes: characterization of distinct particles that differ in the position of the histone core. Nucleic Acids Res. *12*, 9395–9413.

Logie, C., and Peterson, C.L. (1997). Catalytic activity of the yeast SWI/SNF complex on reconstituted nucleosome arrays. EMBO J. *16*, 6772–6782.

Lorch, Y., Cairns, B.R., Zhang, M., and Kornberg, R.D. (1998). Activated RSC-nucleosome complex and persistently altered form of the nucleosome. Cell *94*, 29–34.

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

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

Martinez-Balbas, M.A., Tsukiyama, T., Gdula, D., and Wu, C. (1998). Drosophila NURF-55, a WD repeat protein involved in histone metabolism. Proc. Natl. Acad. Sci. USA *95*, 132–137.

Meersseman, G., Pennings, S., and Bradbury, E.M. (1992). Mobile nucleosomes—a general behavior. EMBO J. *11*, 2951–2959.

Mizuguchi, G., Tsukiyama, T., Wisniewski, J., and Wu, C. (1997). Role of nucleosome remodeling factor NURF in transcriptional activation of chromatin. Mol. Cell *1*, 141–150.

O'Donohue, M.F., Duband-Goulet, I., Hamiche, A., and Prunell, A. (1994). Octamer displacement and redistribution in transcription of single nucleosomes. Nucleic Acids Res. *22*, 937–945.

Okada, M., and Hirose, S. (1998). Chromatin remodeling mediated by Drosophila GAGA factor and ISWI activates fushi tarazu gene transcription in vitro. Mol. Cell. Biol. *18*, 2455–2461.

Papoulas, O., Beek, S.J., Moseley, S.L., McCallum, C.M., Sarte, M., Shearn, A., and Tamkun, J.W. (1998). The Drosophila trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. Development *125*, 3955–3966.

Pennings, S., Meersseman, G., and Bradbury, E.M. (1994). Linker histones H1 and H5 prevent the mobility of positioned nucleosomes. Proc. Natl. Acad. Sci. USA *91*, 10275–10279.

Peterson, C.L., Dingwall, A., and Scott, M.P. (1994). Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA *91*, 2905–2908.

Pollard, K.J., and Peterson, C.L. (1998). Chromatin remodeling: a marriage between two families? Bioessays *20*, 771–780.

Sandaltzopoulos, R., Ossipow, V., Gdula, D.A., Tsukiyama, T., and Wu, C. (1999). Purification of Drosophila nucleosome remodeling factor. Methods Enzymol. *304*, 757–765.

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

Shrader, T.E., and Crothers, D.M. (1989). Artificial nucleosome positioning sequences. Proc. Natl. Acad. Sci. USA *86*, 7418–7422.

Simon, R.H., and Felsenfeld, G. (1979). A new procedure for purifying histone pairs H2A + H2B and H3 + H4 from chromatin using hydroxylapatite. Nucleic Acids Res. *6*, 689–696.

Simpson, R.T. (1979). Mechanism of a reversible, thermally induced conformational change in chromatin core particles. J. Biol. Chem. *254*, 10123–10127.

Simpson, R.T., and Stafford, D.W. (1983). Structural features of a phased nucleosome core particle. Proc. Natl. Acad. Sci. USA *80*, 51–55.

Simpson, R.T., Thoma, F., and Brubaker, J.M. (1985). Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: a model system for study of higher order structure. Cell *42*, 799–808.

Spadafora, C., Oudet, P., and Chambon, P. (1979). Rearrangement of chromatin structure induced by increasing ionic strength and temperature. Eur. J. Biochem. *100*, 225–235.

Stein, A. (1979). DNA folding by histones: the kinetics of chromatin core particle reassembly and the interaction of nucleosomes with histones. J. Mol. Biol. *130*, 103–134.

Studitsky, V.M., Clark, D.J., and Felsenfeld, G. (1994). A histone octamer can step around a transcribing polymerase without leaving the template. Cell *76*, 371–382.

Tong, J.K., Hassig, C.A., Schnitzler, G.R., Kingston, R.E., and Schreiber, S.L. (1998). Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. Nature *395*, 917–921.

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

Travers, A., and Drew, H. (1998). DNA recognition and nucleosome organization. Biopolymers *44*, 423–433.

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

Tsukiyama, T., Becker, P.B., and Wu, C. (1994). ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. Nature *367*, 525–532.

Tsukiyama, T., Daniel, C., Tamkun, J., and Wu, C. (1995). ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell *83*, 1021–1026.

Tsukiyama, T., Palmer, J., Landel, C.C., Shiloach, J., and Wu, C. (1999). Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in Saccharomyces cerevisiae. Genes Dev. *13*, 686–697.

Ura, K., Kurumizaka, H., Dimitrov, S., Almouzni, G., and Wolffe, A.P. (1997). Histone acetylation: influence on transcription, nucleosome mobility and positioning, and linker histone-dependent transcriptional repression. EMBO J. *16*, 2096–2107.

Van Holde, K.E. (1989). Chromatin, A. Rich, ed. (New York: Springer-Verlag).

Van Holde, K.E., and Yager, T.D. (1985). Nucleosome motion: evidence and models. In Structure and Function of the Genetic Apparatus, C. Nicolini and P.O.P. Ts'o, eds. (New York and London: Plenum Press).

Varga-Weisz, P.D., Wilm, M., Bonte, E., Dumas, K., Mann, M., and Becker, P.B. (1997). Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. Nature *388*, 598–602. Erratum: Nature *389*(6654), 1997.

Wade, P.A., Jones, P.L., Vermaak, D., and Wolffe, A.P. (1998). A multiple subunit Mi-2 histone deacetylase from Xenopus laevis cofractionates with an associated Snf2 superfamily ATPase. Curr. Biol. *8*, 843–846.

Wang, W., Cote, J., Xue, Y., Zhou, S., Khavari, P.A., Biggar, S.R., Muchardt, C., Kalpana, G.V., Goff, S.P., Yaniv, M., Workman, J.L., and Crabtree, G.R. (1996a). Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. EMBO J. *15*, 5370–5382.

Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B.R., and Crabtree, G.R. (1996b). Diversity and specialization of mammalian SWI/SNF complexes. Genes Dev. *10*, 2117–2130.

Weischet, W.O., Tatchell, K., Van Holde, K.E., and Klump, H. (1978). Thermal denaturation of nucleosomal core particles. Nucleic Acids Res. *5*, 139–160.

Widom, J. (1998). Structure, dynamics, and function of chromatin in vitro. Annu. Rev. Biophys. Biomol. Struct. *27*, 285–327.

Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem. *67*, 545–579.

Wray, W., Boulikas, T., Wray, V.P., and Hancock, R. (1981). Silver staining of proteins in polyacrylamide gels. Anal. Biochem. *118*, 197–203.

Wu, C. (1997). Chromatin remodeling and the control of gene expression. J. Biol. Chem. *272*, 28171–28174.

Wu, H.M., and Crothers, D.M. (1984). The locus of sequence-directed and protein-induced DNA bending. Nature *308*, 509–513.

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

Xue, Y., Wong, J., Moreno, G.T., Young, M.K., Cote, J., and Wang, W. (1998). NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol. Cell *2*, 851–861.

Zhang, Y., LeRoy, G., Seelig, H.P., Lane, W.S., and Reinberg, D. (1998). The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. Cell *95*, 279–289.