Accelerated Publications

ATP-Dependent Histone Octamer Mobilization and Histone Deacetylation Mediated by the Mi-2 Chromatin Remodeling Complex

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ABSTRACT: The Mi-2 complex has been implicated in chromatin remodeling and transcriptional repression associated with histone deacetylation. Here, we use a purified Mi-2 complex containing six components, Mi-2, Mta 1-like, p66, RbAp48, RPD3, and MBD3, to investigate the capacity of this complex to destabilize histone−DNA interactions and deacetylate core histones. The Mi-2 complex has ATPase activity that is stimulated by nucleosomes but not by free histones or DNA. This nucleosomal ATPase is relatively inefficient, yet is essential to facilitate both translational movement of histone octamers relative to DNA and the efficient deacetylation of the core histones within a mononucleosome. Surprisingly, ATPase activity had no effect on deacetylation of nucleosomal arrays.

Dynamic changes in chromatin structure are associated with transcription, replication, recombination, and repair (*1*−*3*). Two major classes of chromatin remodeling occur: those that are dependent on posttranslational modification of the histones (*4*) and those that are dependent on the activity of ATP-driven motors such as the DNA and RNA polymerases (*5*, *6*) and the SWI/SNF family of enzymes (*7*, *8*). The posttranslational modifications of the histones that occur in chromatin are often coupled to the activities of ATP-driven motors. For example, acetylation of histone H4 is associated with the assembly of nascent chromatin at the replication fork (*9*, *10*), histone acetyltransferase is a component of elongating RNA polymerase II (*11*), and histone deacetylase is found associated with a member of the SWI/SNF family of ATPases known as Mi-2 (*12*).

The Mi-2 protein was initially characterized as an autoantigen in a human connective tissue disease (*13*, *14*). In *Xenopus laevis* eggs the Mi-2 protein cofractionates with five other protein components (*12*, *15*). These include Mta 1-like, a DNA binding protein (*16*) that is homologous to a tumor antigen *met*astasis-*a*ssociated protein 1 (*17*, *18*); p66, a serine- and proline-rich protein of unknown function; RbAp48, a WD-40 repeat protein that interacts specifically with the histone-fold domain of H4 (*19*, *20*); RPD3, the *Xenopus* homologue of the histone deacetylases HDAC1 and 2 (*21*); and MBD3, a *m*ethyl CpG *b*inding *d*omain protein (*12*, *15*, *16*). Since the initial characterization of the Mi-2 complex (*12*), numerous investigators have described the Mi-2 protein as a component of the nucleosome rearrangement and disruption NRD/NURD complex that has been fractionated from mammalian cells (*22*−*24*). The NRD/NURD complex contains from 10 to 40 proteins in addition to Mi-2 (reviewed in ref *25*) including HDAC 1 and 2 andhomologues of RbAp48, Mta 1-like, and MBD3 (*22*−*24*). The NRD/NURD complex has been described as either requiring ATP hydrolysis for deacetylation of nucleosomal histones (*22*, *23*) or not requiring ATP for chromatin deacetylation (*24*). These discrepancies may depend on the heterogeneity of NRD/NURD preparations, on details of experimental protocols, or on the properties of the chromatin substrates themselves. We have investigated this issue using the purified Mi-2 complex and biochemically defined chromatin substrates.

To assay chromatin disruption, we made use of nucleosome mobility assays (*26*, *27*). The ISWI nucleosomal ATPase will promote sliding of the histone octamer relative to the DNA sequence either in isolation or as a component of the NURF or CHRAC complexes (*26*, *27*). This mobilization requires ATP hydrolysis presumably to direct the substantial destabilization of histone−DNA contacts necessary to move the histone octamer relative to the double helix (*28*). To determine histone deacetylase activity, we have made use of core histones that were specifically acetylated on the amino- (N-) terminal tails of histone H3 and/or H4, either as free histones or when incorporated into chromatin. Our results establish that the purified Mi-2 complex is a nucleosomal ATPase that uses the energy of ATP hydrolysis both to mobilize histone octamers relative to DNA and to facilitate the deacetylation of nucleosomal histones in certain contexts.

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EXPERIMENTAL PROCEDURES

Mi-2 Complex Purification. The Mi-2 complex was prepared from *Xenopus* egg extract as described (*12*, *15*, *16*). Recombinant ISWI was purified as described (*27*).

Chromatin and Nucleosome Preparation. Purified chicken erythrocyte histones were acetylated in vitro with various histone acetyltransferases as described (*29*, *30*). Chromatin and histone octamers were isolated from chicken nuclei as previously described (*31*, *32*). Core particles were assembled using the salt dialysis method (*33*, *34*). Reconstitutes were digested with micrococcal nuclease, and core particles were isolated via a sucrose gradient (*35*). These core particles were used for nucleosomal ATPase assays and for histone deacetylation assays. Nuclesome arrays for these experiments were assembled onto reiterated repeats of the *Lytechinus variegatus* 5S rRNA gene (208-12) as previously described (*36*−*38*). Trypsinized chromatin was prepared by incubating trypsin beads (Sigma) with chicken chromatin fragments at a ratio of 10 mg of trypsin/1 mg of oligonucleosomes at room temperature. Aliquots were taken at times ranging from 10 to 120 min and checked on 18% SDS−PAGE. Preparative reactions followed the trypsinization trials described above; reactions were stopped by the removal of beads by centrifugation and addition of soybean trypsin inhibitor (Sigma) to a final concentration of 50 μg/mL.

For nucleosome mobility assays, a 250 bp fragment from the *Xenopus* thyroid hormone receptor βA gene (*39*) was reconstituted with histone octamers by salt dialysis as described (*33*, *34*). Nucleosomes at distinct positions on the DNA fragment were then resolved by nondenaturing gel electrophoresis. Individual populations of positioned nucleosomes were then eluted by the "crush and soak" methodology (*26*).

Nucleosome Mobility and Positioning Assays. Individual populations of positioned nucleosomes were resolved using nondenaturing polyacrylamide gel electrophoresis. Nucleosome mobilization was performed at 37 °C for 40 min in nucleosome mobilization buffer containing 10 mM HEPES, pH 7.5, 0.2 mM EDTA, 5% glycerol, and 2 mM $MgCl_2$, with 1 mM ATP or AMP-PNP added as indicated. Reactions were initiated by addition of 0.5−1 μL of the Mi-2 complex (100 ng/μL) or recombinant ISWI (100 ng/μL). Following a 40 min incubation at 37 °C, remodeled nucleosomes were resolved on polyacrylamide gels. Individual octamer positions were mapped using micrococcal nuclease as previously described (*35*).

ATPase Assays. The Mi-2 complex or recombinant ISWI was incubated with [γ-^{32}P]ATP and purified chicken erythrocyte mononucleosomes, salmon sperm DNA, or purified chicken erythrocyte core histones for 30 min at room temperature. Reactions were spotted on PEI−cellulose thin-layer chromatography plates and developed in 1 M formic acid and 0.5 M LiCl. ATP hydrolysis was quantitated using a phosphorimager with Image Quant Software.

Histone Deacetylase Assays. Histone deacetylase activity was measured by incubating the indicated amounts of the Mi-2 complex with core histones acetylated in vitro with tritiated acetyl-coenzyme A and the recombinant acetyltransferases indicated as described (*29*). We made use of recombinant yeast Hat1p that acetylates lysines 5 and 12 of histone H4 (*40*), recombinant PCAF (*41*) that predominantly acetylates lysine 14 of H3 (*30*), and recombinant p300 (*41*) that predominantly acetylates lysines 14 and 18 of H3 and lysines 5 and 8 of H4 (*30*). Reactions proceeded for 30 min at 30 °C, were terminated by acid addition (hydrochloric plus acetic acid), extracted with ethyl acetate, and counted in a liquid scintillation counter. Released acetate is indicated in the figures. As indicated, acetylated recombinant histones were reconstituted into nucleosome cores and nucleosomal arrays.

RESULTS

The Mi-2 Complex Is an Inefficient Nucleosomal ATPase. We purified the Mi-2 complex from *Xenopus* eggs as previously described (Experimental Procedures). The peak of nucleosomal ATPase and histone deacetylase activity corresponded to fraction 5 on a sucrose gradient (Figure 1A,B). The Mi-2 complex in fraction 5 contained six polypeptides, including two splice variants of MBD3 (Figure 1B). We examined the ATPase activity of the Mi-2 complex and found that it was stimulated by nucleosomes but not by free DNA or by free histones (Figure 1C). To gain additional insight into the features of chromatin substrates that contribute to stimulation of the ATPase activity of the Mi-2 complex, we examined ATPase activity in the presence of mononucleosomes and nucleosomal arrays with or without removal of the histone amino termini by trypsin. We observed that nucleosomal arrays (>2000 bp), like mononucleosomes, stimulated the ATPase activity of the Mi-2 complex (Figure 1D). Further, this stimulation was insensitive to the presence of the core histone amino termini. Thus, core histone tails do not play a significant role in stimulating the nucleosomal ATPase activity of the Mi-2 complex. The N-termini of the core histones have been found to be necessary for the ATPase activity of NURF, a nucleosomal ATPase (*42*), but not for the ATPase activities of the yeast SWI/SNF and RSC complexes (*43*). As the SWI/SNF and RSC complexes exhibit stimulation of ATPase activity by free DNA, the Mi-2 complex thus constitutes a third class of chromatin ATPase—one stimulated by nucleosomes but not free DNA, which does not require histone tails. Considerable evidence exists for the rearrangement of histone−DNA contacts upon the assembly of nucleosomal arrays (*44*−*49*). In particular, the positions of the core histone amino- (N-) and carboxyl- (C-) termini rearrange during the folding of nucleosomal arrays into higher order structures (*47*, *50*, *51*). We observed a roughly 2-fold difference in ATPase activity of the Mi-2 complex in the presence of nucleosomal arrays as compared to mononucleosomes (Figure 1D). This difference likely reflects a preference of the Mi-2 complex to associate with higher order chromatin structures. A comparable preference of the yeast histone acetyltransferase GCN5 for nucleosomal arrays relative to mononucleosomes has been demonstrated (*52*).

We next undertook a kinetic analysis of ATPase activity in the presence of nucleosomal arrays (Figure 1E). We observed a linear increase in ATP hydrolysis over the time course of this experiment (30 min). The enzyme was saturated with respect to chromatin at approximately 12 ng (DNA) per reaction—an approximate nucleosome concentration of 10 nM. The slopes of the individual curves indicated increasing reaction velocity with increasing chromatin concentration. We determined the apparent maximal velocity

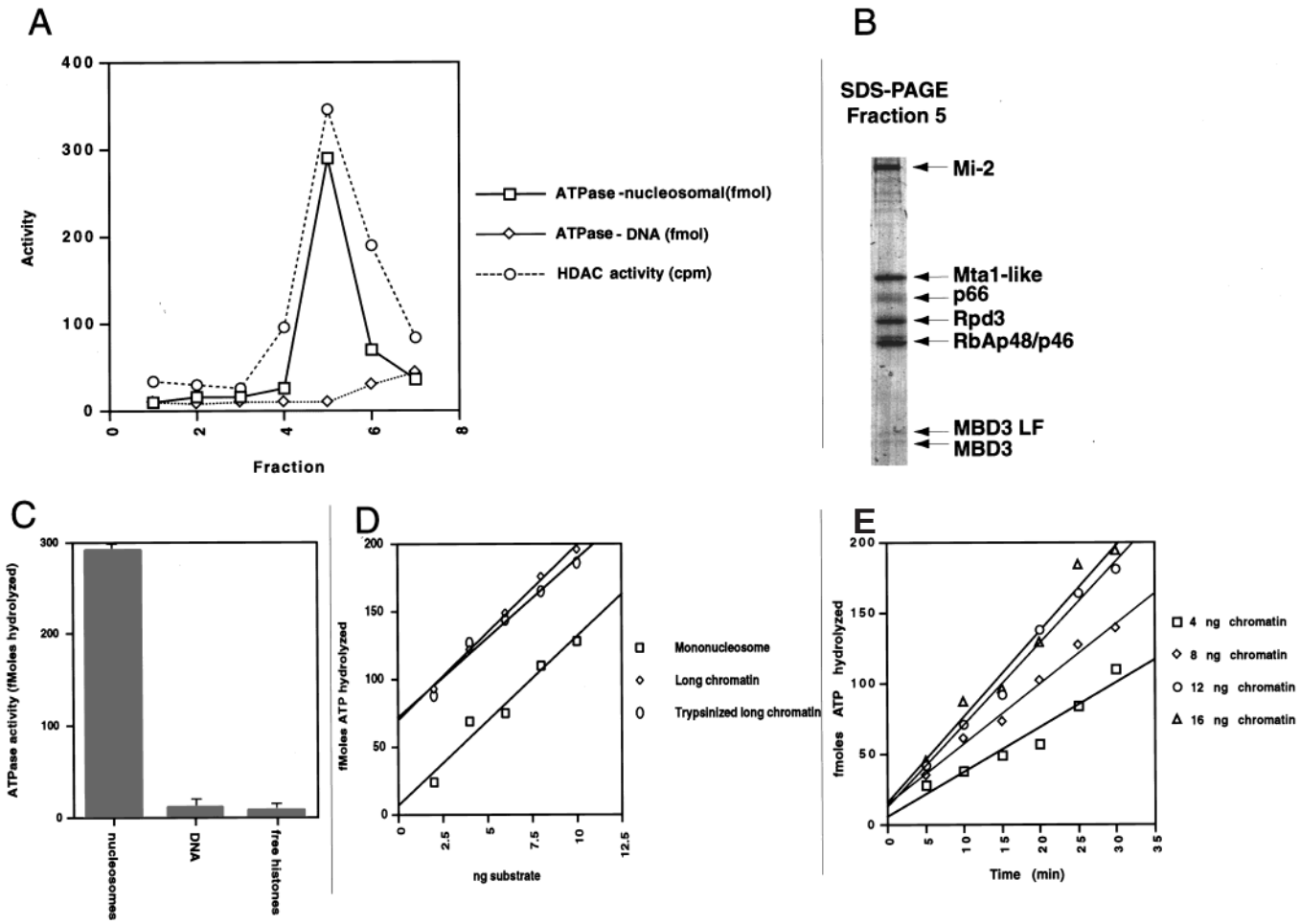

FIGURE 1: The Mi-2 complex is a nucleosome-stimulated ATPase. (A) Cosedimentation of the ATPase and deacetylase activities of the Mi-2 complex across a sucrose gradient. Aliquots of each fraction were assayed for ATPase and HDAC activity as described in Experimental Procedures. (B) Polypeptide composition of the *Xenopus* Mi-2 complex. The Coomassie-stained gel depicts the components of the purified *Xenopus* Mi-2 complex. Subunit identification by chemical and immunologic means has been described elsewhere (*12*, *16*). (C) The ATPase activity of sucrose gradient fraction 5 was determined in the presence of nucleosomes (100 ng of DNA), free DNA (100 ng), or purified core histones (100 ng). (D) Substrate specificity of Mi-2 ATPase. Equivalent masses of mononucleosomes, long chicken chromatin (depleted of linker histones), and trypsinized long chromatin were titrated against a constant amount of the Mi-2 complex (100 ng). (E) Kinetic analysis of Mi-2 ATPase activity. Long chicken chromatin (linker histone depleted) was used to stimulate ATPase activity of the Mi-2 complex (100 ng) in the amounts indicated in the graph. Reactions were stopped at the indicated points by spotting on PEI−cellulose plates.

(V_{max}) and Michaelis constant (K_M) using the double-reciprocal plot method (not shown). Under our experimental conditions, these values indicated a fairly slow reaction rate (V_{max} = 8.5 fmol of ATP hydrolyzed per minute) but quite high-affinity interaction between the enzyme and chromatin substrate (K_M = 7 nM nucleosome). Further, calculation of the enzymatic turnover number (K_{cat} = 0.1 per minute) confirmed that even under optimal conditions the rate of ATP hydrolysis is less than one molecule per Mi-2 complex per minute. This is less than one-tenth of the value obtained for the yeast ISWI complexes (*53*) and more than 100-fold less than the values obtained with yeast SWI/SNF or RSC complexes (*43*).

We concluded that the *Xenopus* Mi-2 complex is a relatively inefficient ATPase under these reaction conditions. This is consistent with the excess of Mi-2 complex over nucleosomes required for optimal ATPase activation (Figure 1E). Earlier chromatin remodeling experiments using the SWI/SNF and RSC complexes have demonstrated the requirement for equimolar amounts of complex to nucleosomes for optimal remodeling (*7*, *43*).

The Mi-2 Complex Directs ATP-Dependent Translational Movement of Histone Octamers. The requirement for ATP to facilitate chromatin remodeling by the mammalian chromatin remodeling complexes NRD and NURD is controversial (*22*−*24*). Since the Mi-2 complex is a nucleosomal ATPase, we wished to explore the functional consequence of this albeit inefficient ATP hydrolysis. An attractive assay for the transient disruption of the histone−DNA interaction is to monitor changes in histone octamer position or mobility on a defined DNA fragment (*35*, *54*, *55*). The mobility of histone octamers occurs spontaneously at 37 °C but can be facilitated by the action of an RNA polymerase (*56*) or through the activity of the ISWI nucleosomal ATPase (*26*, *27*). We examined whether the Mi-2 complex would direct ATP-dependent repositioning of histone octamers.

We reconstituted a single histone octamer onto a 250 bp DNA fragment encoding the 5′UTR of the *Xenopus* thyroid hormone receptor TRβA gene (*39*). We used nondenaturing gel electrophoresis to resolve mononucleosomes with the histone octamer in four major translational positions (Figure 2A). Micrococcal nuclease was used to map the translational

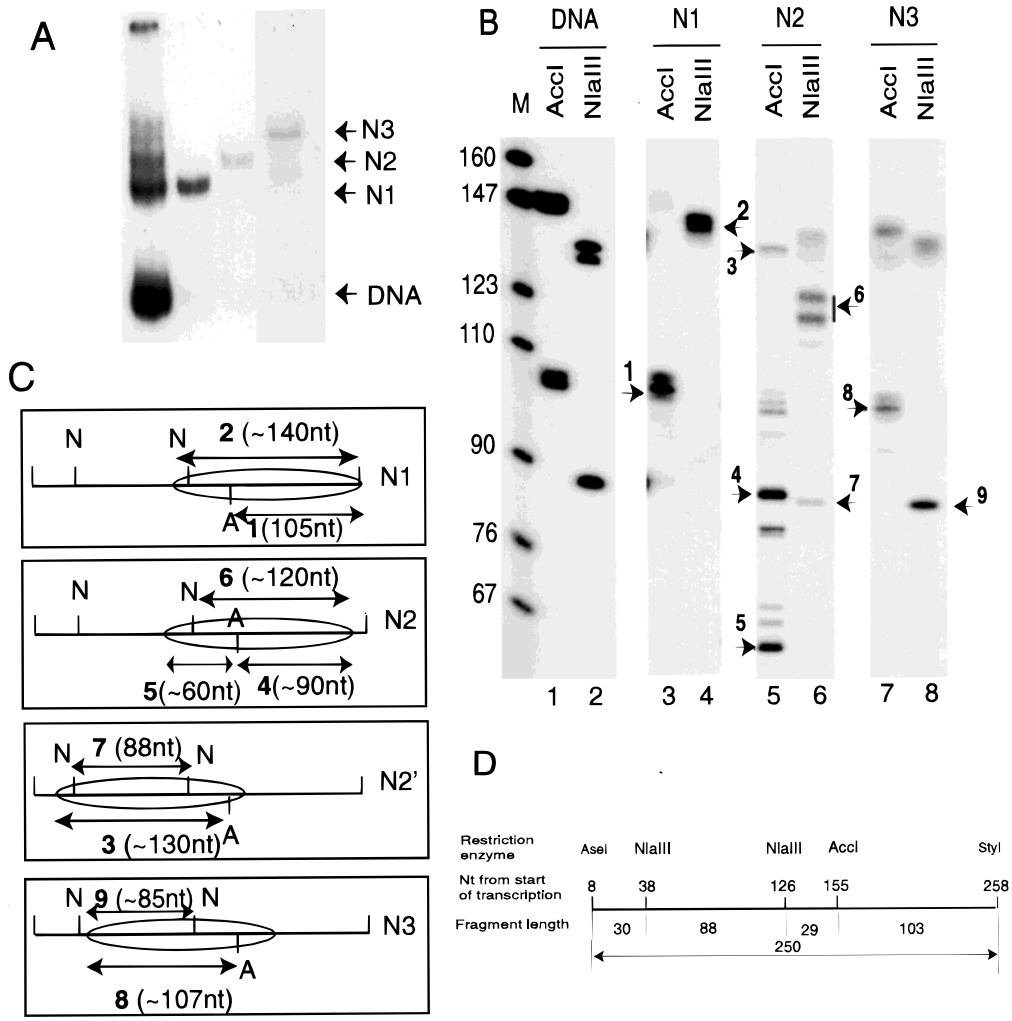

FIGURE 2: Mapping of histone octamer positions on the TRβA fragment. (A) Isolation of major mononucleosome species. Three uniquely positioned mononucleosomes were purified by preparative gel electrophoresis and reanalyzed on an analytical gel (as in Figure 1A). (B) Micrococcal nuclease mapping of histone octamer positions on reconstituted mononucleosome complexes. Nucleosome core particles for each species were isolated by nondenaturing gel electrophoresis. DNA was recovered and digested with restriction enzymes *Acc*I or *Nla*III to determine the positions of the octamer boundaries. DNA fragments generated by the restriction enzymes were analyzed on a sequencing gel (6% polyacrylamide, 7 M urea, 1× TBE). A ^{32}P-labeled *Msp*I digest of pBR322 was used as size markers. (C) Restriction maps of histone octamer positions on the TRβA fragment. Predominant octamer positions are indicated by solid ovals. (D) Map of relative sizes (in nucleotides) of restriction fragments of the TRβA 5′UTR.

positions of the histone octamer relative to the DNA sequence for three of the distinct nucleosome positions as follows (*35*). First, native polyacrylamide gel electrophoresis was used to resolve the positional isomers (see, for example, Figure 2A). Each unique nucleosome was then eluted (*26*) and subjected to digestion with micrococcal nuclease. Following digestion, DNA was deproteinized and resolved on polyacrylamide gels (*35*). The 147 bp product from each parent nucleosome was then eluted and digested with the restriction endonucleases *Acc*I and *Nla*III, and the resulting fragments were resolved on a sequencing gel (see Figure 2B). The lengths of these restriction fragments allow deduction of the limits of micrococcal digestion, and thus the translational boundaries of the histone octamer, relative to the DNA sequence. These deduced positions are represented diagrammatically in Figure 2C. The position labeled as N2 is a mixture of two unique positions (termed N2 and N2′) symmetrically located relative to the center of the fragment. As expected, nucleosomes positioned near the edge of the DNA fragment (i.e., N1) migrate faster in the native polyacrylamide gel than those positioned near the middle of the fragment (i.e., N3). The fourth uniquely positioned nucleosome is unstable during the micrococcal mapping protocol, and we have thus far been unable to map its position by this technique.

To test the spontaneous mobility of the histone octamer relative to the DNA sequence in these nucleoprotein complexes, we first resolved the conformational isoforms on a native gel (Figure 3A). The gel lane was excised and incubated at 37 °C for 1 h before resolution in a second dimension (Figure 3B). The movement of a significant fraction of the sample away from the diagonal indicates that a substantial equilibrium exists between different translational positions of the histone octamer on this DNA sequence. Importantly, the accumulation of nucleoprotein complexes in equivalent amounts above and below the diagonal indicates that octamer mobility is random.

We next asked whether the Mi-2 complex would influence this equilibrium. We reconstituted nucleosome cores and established that they would stimulate the ATPase activity of the Mi-2 complex (Figure 4A). Recombinant ISWI was used as control for this assay (*27*). We excised the nucleosome complex labeled as "N2" that reflects the histone

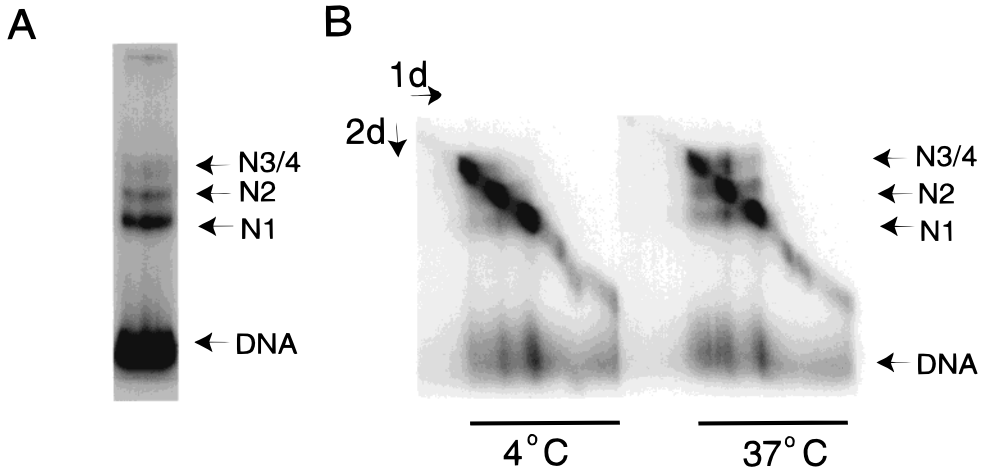

FIGURE 3: Temperature-dependent octamer mobilization on *Xenopus* TRβA 5′UTR. (A) Native polyacrylamide gel electrophoresis (5% acrylamide, 0.5× TBE) of mononucleosomes reconstituted on the 250 bp TRβA fragment. Major mononucleosome species are indicated. (B) Redistribution of histone octamers on the 5′UTR of the TRβA gene is induced by elevated temperature. Mononucleosomes were resolved on a nondenaturing 5% polyacrylamide gel before excision of the gel and incubation at 4 or 37 °C for 1 h as indicated, followed by electrophoresis in a second dimension.

octamer at an intermediary position between the "N1" and "N3" conformations (Figures 2 and 3). As expected, the process of dilution, electroelution, and addition of the Mi-2 reaction buffer leads to some dissociation of the histone octamer from DNA (*57*). However, the octamer position remains substantially unchanged, indicating that nucleosome mobility does not provide a major contribution to this dissociation (Figure 4B). In the absence of ATP, the Mi-2 complex does not change the position of the histone octamer (Figure 4B, lane 1). In the presence of ATP, however, the octamer was displaced toward the center of the DNA fragment, leading to a more substantial retardation relative to naked DNA in the nondenaturing gel (Figure 4B, lane 2). Note in particular that histone octamers including those wrapped at positions N3 and N4 accumulate. This nucleosome mobilization does not result in dissociation of the histone−DNA complex, because the amount of free DNA in the sample remains constant.

In earlier work it has been shown that recombinant ISWI protein will also promote nucleosome mobility (*26*, *27*). We compared the nucleosome mobilization activity of rISWI to that of the Mi-2 complex in our assay system (Figure 4C). We find that r-ISWI will induce nucleosome mobility, but in the opposite direction to that of the Mi-2 complex. As previously reported for nucleosomes assembled on the *Drosophila* hsp 70 promoter (*26*) and the *Drosophila* hsp 26 promoter (*27*), nucleosome mobilization by r-ISWI on the TRβA DNA fragment moves the histone octamer to the end of the fragment (Figure 4C, lane 3). In contrast, nucleosome mobilization by Mi-2 moves the octamer to the center of the fragment (Figure 4D, lanes 6 and 7). This result is similar to that reported for the chromatin remodeling complex CHRAC using the *Drosophila* hsp 26 promoter (*27*) but distinct from that observed with NURF using the hsp 70 promoter (*26*). ATP hydrolysis is necessary for mobilization since the nonhydrolyzable ATP analogue AMP-PNP (Figure 4D, lanes 4 and 8) did not promote mobility (data not shown).

We next examined whether the efficiency of chromatin remodeling could be influenced by increasing the concentration of Mi-2. An increase in the relative concentration of the Mi-2 complex to nucleosomes from equimolar to a 10-fold excess promoted more efficient nucleosome movement (data not shown). At a 10-fold excess of Mi-2 complex over nucleosomes, mobilization occurred progressively over a 20 min period at 37 °C (Figure 4D), consistent with the continual hydrolysis of ATP previously observed (Figure 1E). Note that in the absence of the Mi-2 complex no detectable mobilization of the histone octamer over this time period occurred (Figure 4C, lane 1).

The Mi-2 Complex Directs the Efficient Deacetylation of Acetylated H3 and Acetylated H4 in Nucleosomes. We wished to determine whether the ATP-dependent destabilization of histone−DNA contacts observed with the Mi-2 complex might impact on the efficiency of deacetylation of nucleosomal histones. Histone deacetylase complexes will deacetylate free histones in the absence of ATP (*58*, *59*) but generally have their activity constrained on nucleosomal substrates (*22*, *23*, *60*). The human NURD/NRD complex has been reported to deacetylate histones incorporated into oligonucleosomes (*22*−*24*). However, the effect of ATP hydrolysis on HDAC activity has been controversial, with some reporting a modest stimulation (*22*, *23*) and others reporting no effect (*24*). In addition, Zhang et al. (*24*) reported a requirement for noncatalytic ratios of NURD to nucleosomes and stipulated that the NURD complex requires targeting for efficient deacetylation of chromatin substrates. All of these groups made use of oligonucleosomes isolated from cultured cells (*22*−*24*), and two utilized enzyme preparations immobilized on beads (*22*, *23*).

We wished to explore the ATP requirements for deacetylation of chromatin templates in a biochemically defined system. Thus, we prepared purified histones which are expected to be acetylated at lysines 5 and 12 of histone H4 (using recombinant yeast HAT1 (Figure 5A, lane 1)), at lysine 14 of H3 [using recombinant PCAF (Figure 5A, lane 2)], and at lysines 14 and 18 of H3 and lysines 5 and 8 of histone H4 [with recombinant p300 (Figure 5A, lane 3)] as chemically verified by others (*30*, *40*). We found that the Mi-2 complex efficiently deacetylated free histones independent of the site of modification (Figure 5B and data not shown). ATP hydrolysis had no effect on deacetylation of the free histone substrates (Figure 5B and data not shown).

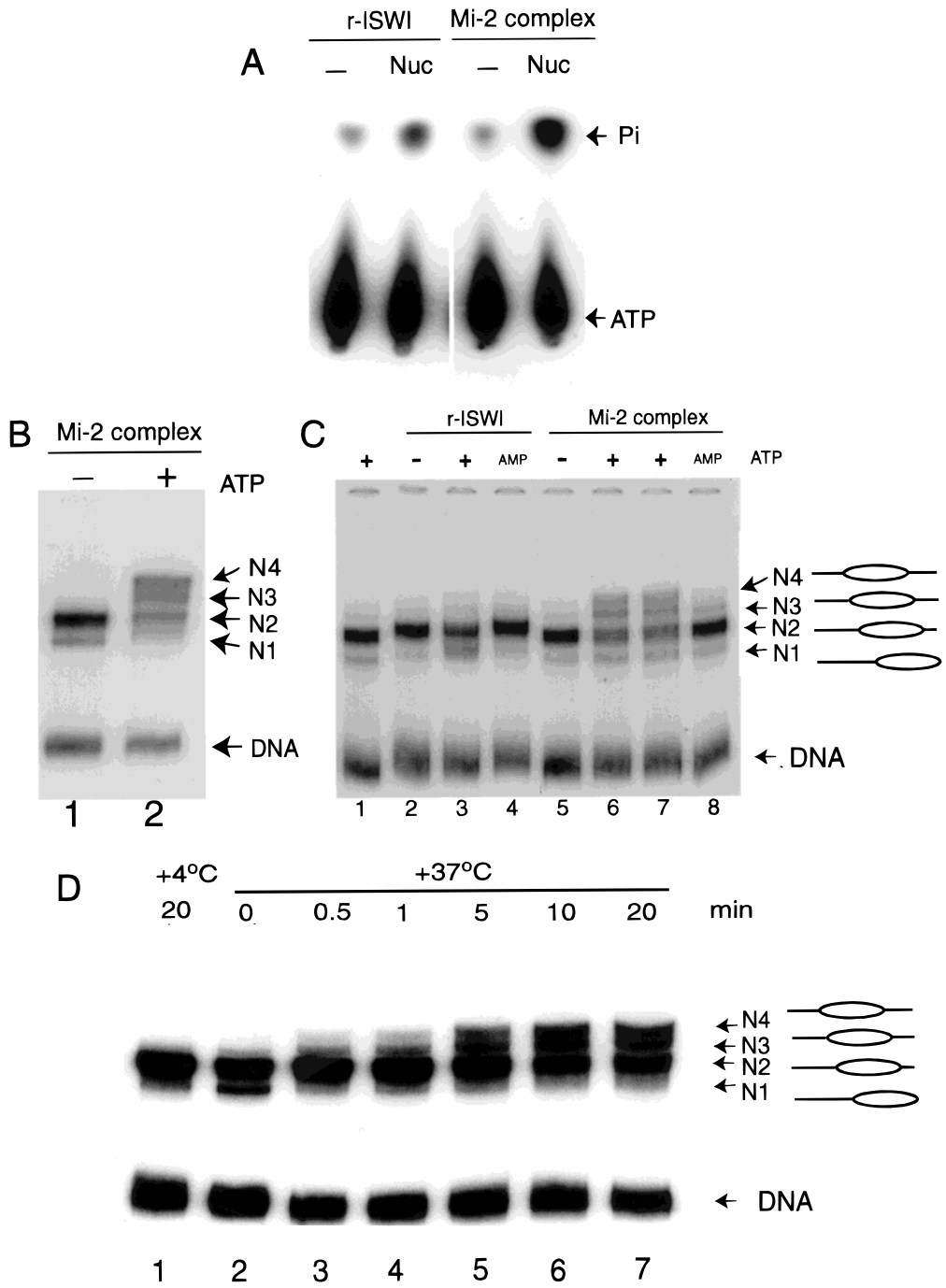

FIGURE 4: The Mi-2 complex is an ATP-dependent nucleosome remodeling enzyme. (A) Nucleosome-inducible ATPase activity of recombinant ISWI and the Mi-2 complex. ATPase assays were performed as in Figure 1E. (B) The Mi-2 complex induces redistribution of histone octamers from the edges of the DNA fragment (N2) to the center (N3). The mobilization assays were performed as described in Experimental Procedures. (C) Histone octamer mobilization by the Mi-2 complex requires ATP hydrolysis. The uniquely positioned N2 nucleosome (lane 1) was incubated in the presence of recombinant ISWI or the Mi-2 complex and nucleotides as shown in the figure. Mobilization assays were performed as described in Experimental Procedures. (D) Time course of octamer mobilization by the Mi-2 complex. N2-positioned nucleosomes (lane 1) were incubated with 80 ng of the Mi-2 complex for the indicated times (lanes 2−7) as described in Experimental Procedures.

For mononucleosome substrates, the efficiency of deacetylation was significantly reduced in the absence of ATP (Figure 5B); this result is partially consistent with previous reports (*22*, *23*), although these authors used oligonucleosomal substrates. The presence of ATP stimulated deacetylation severalfold (Figure 5B) as observed by others on oligonucleosomes (*22*, *23*). We extended these observations by examining the ability of the Mi-2 complex to deacetylate oligonucleosomal substrates acetylated at unique positions. We made use of the ability of the *L. variegatus* 5S rRNA gene to position nucleosomes to assemble a spaced nucleosomal array using the salt dialysis method (*36*−*38*). This method has the virtue of using purified core histones and purified unique sequence DNA—resulting in a biochemically defined chromatin substrate with histones acetylated at defined positions. We observed that the Mi-2 complex was able to efficiently catalyze the deacetylation reaction on these substrates, releasing in excess of 90% of the input counts

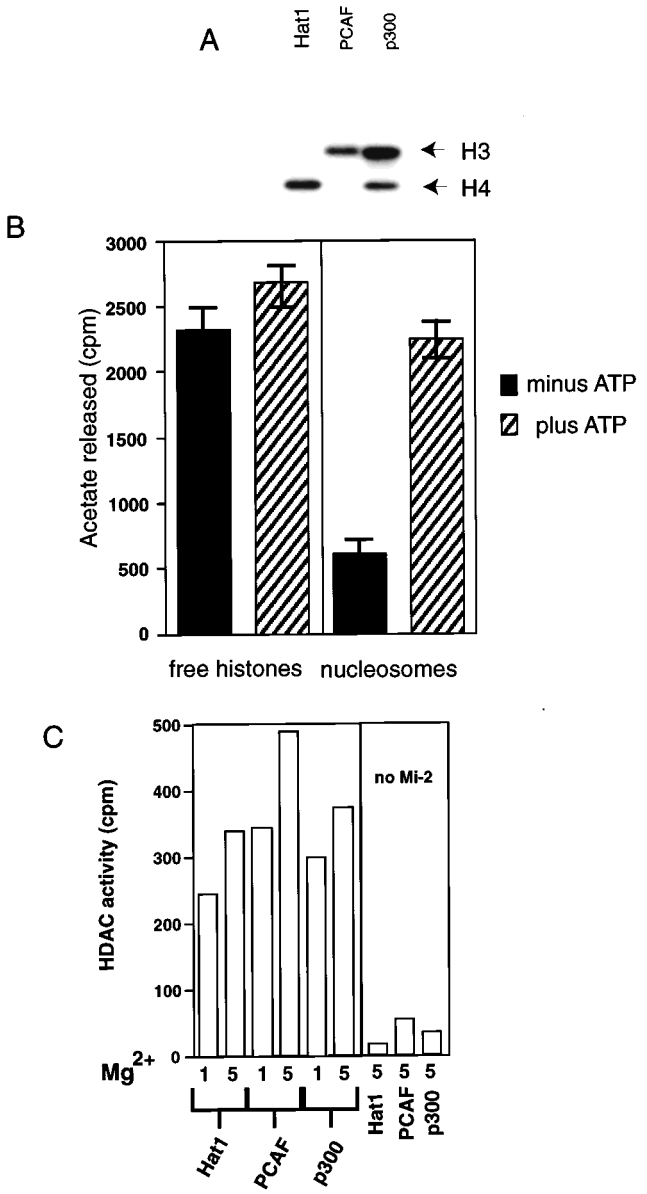

FIGURE 5: Deacetylase activity of the Mi-2 complex on chromatin substrates. (A) Characterization of histone acetylation by recombinant HATs. The fluorogram depicts incorporation of tritiated acetyl groups into core histones by the indicated recombinant histone acetyltransferases. Equivalent quantities of histones were loaded in each lane. (B) ATP stimulates the HDAC activity of the Mi-2 complex on mononucleosomal substrates. Nucleosome core particles (1 μg) prepared with histones acetylated by HAT1 as described in Experimental Procedures were used as substrates for HDAC assays in the presence or absence of ATP (1 mM). The bar graph depicts the mean of five duplicate reactions; the error bars reflect standard deviations. (C) The Mi-2 complex exhibits broad HDAC specificity on nucleosomal arrays. Nucleosomal arrays (100 ng) reconstituted with the histones shown in panel A were used as substrates for the Mi-2 complex as described in Experimental Procedures.

for each substrate examined (Figure 5C and data not shown) even at a 100-fold excess of nucleosome to complex. In contrast to a previous report (*24*), this catalysis was independent of the individual position modified (Figure 5C and data not shown). Finally, we examined the effect of ATP hydrolysis on the deacetylation of these nucleosomal arrays, failing to detect any significant stimulation of HDAC activity (data not shown) in contrast to other reports using immobilized enzymes (*22*, *23*).

DISCUSSION

We establish that the Mi-2 complex is an inefficient nucleosomal ATPase that favors interaction with oligonucleosome templates. Its ATPase activity is clearly stimulated by chromatin but not significantly by free DNA. The large class of chromatin remodeling complexes contains examples of two different types of ATPase subunit. The yeast SWI/SNF and RSC complexes represent the first class—those whose activity is stimulated by either free or nucleosomal DNA. The ISWI-containing complexes represent a second class—those which are stimulated preferentially by nucleosomes and require intact histone N-termini. The Mi-2 complex represents a third novel class of these enzymes—one whose activity is preferentially stimulated by nucleosomal substrates independent of histone N-termini.

The Mi-2 complex actively destabilizes the interaction of the histone octamer with DNA, causing the octamer to move toward the center of a DNA fragment. This destabilization of histone−DNA interactions facilitates histone deacetylation in certain contexts. Interestingly, we observe stimulation of deacetylation by ATP hydrolysis only on mononucleosomes, not on nucleosomal arrays. This is consistent with a model whereby the Mi-2 complex perturbs canonical mononucleosome structure to allow access of the RbAp48 component to the histone-fold domain of H4 (*19*, *20*), which normally lies sequestered inside the coils of nucleosomal DNA. RbAp48 interacts with histone deacetylase directly (*19*, *59*) and can enhance the efficiency of histone modification by tethering the enzyme next to the lysines being modified (*20*). As predicted by Stillman and colleagues (*20*), this nucleosome remodeling could also facilitate histone deacetylation at low nucleosome density, a situation which occurs in vivo immediately following DNA replication. In a fully assembled, mature chromatin fiber, the histone N-termini rearrange (*47*, *50*, *51*), rendering them more accessible to the deacetylase. We propose that the coupling of a nucleosomal ATPase to a histone deacetylase may serve different purposes at different times in the cell cycle. During S phase, ATPase activity likely facilitates the deacetylation of newly deposited histones by the Mi-2 complex during the process of chromatin maturation—a process known to be time dependent (*61*). At other times in the cell cycle, ATPase activity may contribute to the appropriate assembly and/or maintenance of specialized, repressive chromatin structures as suggested for Mi-2 in *Drosophila* (*62*) or perform other, as yet unknown, roles in regulation of chromatin structure and function.

REFERENCES

1. Wu, C. (1997) *J. Biol. Chem. 272*, 28171−28174.
2. Smerdon, M. J., and Conconi, A. (1999) *Prog. Nucleic Acid Res. Mol. Biol. 62*, 227−255.
3. Schlissel, M. S. (2000) *Science 287*, 438−440.
4. Strahl, B. D., and Allis, C. D. (2000) *Nature 403*, 41−45.
5. Sogo, J. M., Stahl, H., Koller, T., and Knippers, R. (1986) *J. Mol. Biol. 189*, 189−204.
6. Studitsky, V. M., Kassavetis, G. A., Geiduschek, E. P., and Felsenfeld, G. (1997) *Science 278*, 1960−1963.
7. Cote, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994) *Science 265*, 53−60.
8. Fryer, C. J., and Archer, T. K. (1998) *Nature 393*, 88−91.
9. Sobel, S. G., and Snyder, M. (1995) *J. Cell Biol. 131*, 1775−1788.

10. Chang, L., Loranger, S. S., Mizzen, C., Ernst, S. G., Allis, C. D., and Annunziato, A. T. (1997) *Biochemistry 36*, 469−480.
11. Wittschieben, B. O., Otero, G., de Bizemont, T., Fellows, J., Erdjument-Bromage, H., Ohba, R., Li, Y., Allis, C. D., Tempst, P., and Svejstrup, J. Q. (1999) *Mol. Cell 4*, 123−128.
12. Wade, P. A., Jones, P. L., Vermaak, D., and Wolffe, A. P. (1998) *Curr. Biol. 8*, 843−846.
13. Nilasena, D. S., Trieu, E. P., and Targoff, I. N. (1995) *Arthritis Rheum. 38*, 123−128.
14. Seelig, H. P., Moosbrugger, I., Ehrfeld, H., Fink, T., Renz, M., and Genth, E. (1995) *Arthritis Rheum. 38*, 1389−1399.
15. Wade, P. A., Jones, P. L., Vermaak, D., Veenstra, G. J., Imhof, A., Sera, T., Tse, C., Ge, H., Shi, Y. B., Hansen, J. C., and Wolffe, A. P. (1998) *Cold Spring Harbor Symp. Quant. Biol. 63*, 435−445.
16. Wade, P. A., Gegonne, A., Jones, P. L., Ballestar, E., Aubry, F., and Wolffe, A. P. (1999) *Nat. Genet. 23*, 62−66.
17. Futamura, M., Nishimori, H., Shiratsuchi, T., Saji, S., Nakamura, Y., and Tokino, T. (1999) *J. Hum. Genet. 44*, 52−56.
18. Toh, Y., Pencil, S. D., and Nicolson, G. L. (1994) *J. Biol. Chem. 269*, 22958−22963.
19. Vermaak, D., Wade, P. A., Jones, P. L., Shi, Y. B., and Wolffe, A. P. (1999) *Mol. Cell. Biol. 19*, 5847−5860.
20. Verreault, A., Kaufman, P. D., Kobayashi, R., and Stillman, B. (1998) *Curr. Biol. 8*, 96−108.
21. Wong, J., Patterton, D., Imhof, A., Guschin, D., Shi, Y. B., and Wolffe, A. P. (1998) *EMBO J. 17*, 520−534.
22. Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998) *Nature 395*, 917−921.
23. Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Cote, J., and Wang, W. (1998) *Mol. Cell 2*, 851−861.
24. Zhang, Y., LeRoy, G., Seelig, H. P., Lane, W. S., and Reinberg, D. (1998) *Cell 95*, 279−289.
25. Kornberg, R. D., and Lorch, Y. (1999) *Curr. Opin. Genet. Dev. 9*, 148−151.
26. Hamiche, A., Sandaltzopoulos, R., Gdula, D. A., and Wu, C. (1999) *Cell 97*, 833−842.
27. Langst, G., Bonte, E. J., Corona, D. F. V., and Becker, P. B. (1999) *Cell 97*, 843−852.
28. Guschin, D., and Wolffe, A. P. (1999) *Curr. Biol. 9*, R742−R746.
29. Wade, P. A., Jones, P. L., Vermaak, D., and Wolffe, A. P. (1999) *Methods Enzymol. 304*, 715−725.
30. Schiltz, R. L., Mizzen, C. A., Vassilev, A., Cook, R. G., Allis, C. D., and Nakatani, Y. (1999) *J. Biol. Chem. 274*, 1189−1192.
31. Guschin, D., Chandler, S., and Wolffe, A. P. (1998) *Biochemistry 37*, 8629−8636.
32. Simon, R. H., Camerini-Otero, R. D., and Felsenfeld, G. (1978) *Nucleic Acids Res. 5*, 4805−4818.
33. Camerini-Otero, R. D., Sollner-Webb, B., and Felsenfeld, G. (1976) *Cell 8*, 333−347.
34. Chandler, S. P., Guschin, D., Landsberger, N., and Wolffe, A. P. (1999) *Biochemistry 38*, 7008−7018.
35. Ura, K., Hayes, J. J., and Wolffe, A. P. (1995) *EMBO J. 14*, 3752−3765.
36. Simpson, R. T., Thoma, F., and Brubaker, J. M. (1985) *Cell 42*, 799−808.
37. Hansen, J. C., and Wolffe, A. P. (1992) *Biochemistry 31*, 7977−7988.
38. Logie, C., and Peterson, C. L. (1997) *EMBO J. 16*, 6772−6782.
39. Ranjan, M., Wong, J., and Shi, Y. B. (1994) *J. Biol. Chem. 269*, 24699−24705.
40. Parthun, M. R., Widom, J., and Gottschling, D. E. (1996) *Cell 87*, 85−94.
41. Imhof, A., Yang, X. J., Ogryzko, V. V., Nakatani, Y., Wolffe, A. P., and Ge, H. (1997) *Curr. Biol. 7*, 689−692.
42. Georgel, P. T., Tsukiyama, T., and Wu, C. (1997) *EMBO J. 16*, 4717−4726.
43. Logie, C., Tse, C., Hansen, J. C., and Peterson, C. L. (1999) *Biochemistry 38*, 2514−2522.
44. Lee, K. M., and Hayes, J. J. (1998) *Biochemistry 37*, 8622−8628.
45. Usachenko, S. I., Bavykin, S. G., Gavin, I. M., and Bradbury, E. M. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 6845−6849.
46. Usachenko, S. I., Gavin, I. M., and Bavykin, S. G. (1996) *J. Biol. Chem. 271*, 3831−3836.
47. Gavin, I. M., Usachenko, S. I., and Bavykin, S. G. (1998) *J. Biol. Chem. 273*, 2429−2434.
48. Fletcher, T. M., and Hansen, J. C. (1996) *Crit. Rev. Eukaryotic Gene Expression 6*, 149−188.
49. Hansen, J. C., Tse, C., and Wolffe, A. P. (1998) *Biochemistry 37*, 17637−17641.
50. Fletcher, T. M., and Hansen, J. C. (1995) *J. Biol. Chem. 270*, 25359−25362.
51. Tse, C., and Hansen, J. C. (1997) *Biochemistry 36*, 11381−11388.
52. Tse, C., Georgieva, E. I., Ruiz-Garcia, A. B., Sendra, R., and Hansen, J. C. (1998) *J. Biol. Chem. 273*, 32388−32392.
53. Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J., and Wu, C. (1999) *Genes Dev. 13*, 686−697.
54. Meersseman, G., Pennings, S., and Bradbury, E. M. (1992) *EMBO J. 11*, 2951−2959.
55. Pennings, S., Meersseman, G., and Bradbury, E. M. (1991) *J. Mol. Biol. 220*, 101−110.
56. Studitsky, V. M., Clark, D. J., and Felsenfeld, G. (1994) *Cell 76*, 371−382.
57. Godde, J. S., Nakatani, Y., and Wolffe, A. P. (1995) *Nucleic Acids Res. 23*, 4557−4564.
58. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L., and Ayer, D. E. (1997) *Cell 89*, 341−347.
59. Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996) *Science 272*, 408−411.
60. Hassig, C. A., Tong, J. K., Fleischer, T. C., Owa, T., Grable, P. G., Ayer, D. E., and Schreiber, S. L. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 3519−3524.
61. Dimitrov, S., and Wolffe, A. P. (1995) *Biochim. Biophys. Acta 1260*, 1−13.
62. Kehle, J., Beuchle, D., Treuheit, S., Christen, B., Kennison, J. A., Bienz, M., and Muller, J. (1998) *Science 282*, 1897−1900.

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