

The Dermatomyositis-Specific Autoantigen Mi2 Is a Component of a Complex Containing Histone Deacetylase and Nucleosome Remodeling Activities

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Summary

Histone acetylation and deacetylation were found to be catalyzed by structurally distinct, multisubunit complexes that mediate, respectively, activation and repression of transcription. ATP-dependent nucleosome remodeling, mediated by different multisubunit complexes, was thought to be involved only in transcription activation. Here we report the isolation of a protein complex that contains both histone deacetylation and ATP-dependent nucleosome remodeling activities. The complex contains the histone deacetylases HDAC1/2, histone-binding proteins, the dermatomyositis-specific autoantigen Mi2 β , a polypeptide related to the metastasis-associated protein 1, and a novel polypeptide of 32 kDa. Patients with dermatomyositis have a high rate of malignancy. The finding that Mi2 β exists in a complex containing histone deacetylase and nucleosome remodeling activities suggests a role for chromatin reorganization in cancer metastasis.

Introduction

The eukaryotic genome is compacted with histone and other proteins to form chromatin (Van Holde, 1989), which allows for efficient storage of genetic information. However, this packaging also prevents the transcription machinery from gaining access to the DNA template (Paranjape et al., 1994). In order for the transcription machinery to access DNA, the compacted chromatin structure needs to be altered. Recent studies have revealed two mechanisms that alter chromatin structure. One mechanism, believed to function exclusively in transcriptional activation, utilizes the energy derived from ATP hydrolysis to remodel nucleosome structure (reviewed in Tsukiyama and Wu, 1997; Kadonaga, 1998; Varga-Weisz and Becker, 1998). The other mechanism involves posttranslational modification, in particular the acetylation of lysine residues at the N-terminal tails of

the core histones (reviewed in Grunstein, 1997; Wade et al., 1997; Struhl, 1998).

Extensive biochemical studies have led to the purification of several ATP-dependent nucleosome remodeling complexes from different organisms. These include the SWI/SNF and RSC complexes from yeast; the NURF, CHRAC, ACF, and Brahma complexes from *Drosophila*; the mammalian SWI/SNF complex from human (reviewed in Tsukiyama and Wu, 1997; Kadonaga, 1998; Varga-Weisz and Becker, 1998); and the recently purified RSF complex from human (G. L. et al., unpublished). Although these protein complexes have different components and different properties, all contain a related subunit that possesses a conserved SWI2/SNF2-type helicase/ATPase domain (reviewed in Eisen et al., 1995). It has been postulated that this subunit might function as a processive, ATP-driven motor to disrupt DNA–histone interactions (Pazin and Kadonaga, 1997).

The notion that acetylation of core histones affects transcription comes from the observation that hyperacetylated chromatin correlates with active genes and hypoacetylated chromatin correlates with repressed genes (Hebbes et al., 1988; Braunstein et al., 1993). This general correlation is supported by the finding that several transcriptional coactivators have histone acetyltransferase (HAT) activity, while the transcriptional corepressor Sin3 is associated with histone deacetylases (reviewed in Grunstein, 1997; Wade et al., 1997; Struhl, 1998). In addition, targeting of Sin3-HDAC/Rpd3 complexes to promoter elements results in transcriptional repression (Yang et al., 1996; Alland et al., 1997; Hassig et al., 1997; Heinzel et al., 1997; Kadosh and Struhl, 1997; Laherty et al., 1997; Nagy et al., 1997; Zhang et al., 1997), and targeting of yeast HAT complexes to nucleosomes within the vicinity of the E4 promoter results in transcriptional activation in vitro (Utley et al., 1998). Moreover, mutagenesis studies with Gcn5 and Rpd3/HDAC1 strongly suggest that the histone acetyltransferase and deacetylase activities are required for transcriptional regulation (Hassig et al., 1998; Kadosh and Struhl, 1998; Kuo et al., 1998; Wang et al., 1998). Finally, Rpd3/Sin3-dependent repression has been shown to be directly associated with the deacetylation of lysine 5 of histone H4 in the promoters of UME6-regulated genes (Rundlett et al., 1998). These studies have established the involvement of core histone acetylation/deacetylation in transcriptional regulation. However, the mechanism by which histone acetylation regulates transcription is still not clear. In addition, exceptions to the general correlation between core histone acetylation and gene activation have also been reported. For example, loss of RPD3 function in yeast and *Drosophila* results in enhanced heterochromatin silencing (De Rubertis et al., 1996; Rundlett et al., 1996; Vannier et al., 1996).

Toward a mechanistic understanding of how transcription is regulated by core histone acetylation, we have focused on purifying and characterizing histone deacetylase complexes from HeLa cell nuclear extracts. Using a combination of conventional and affinity chromatography, we previously purified an HDAC1-containing

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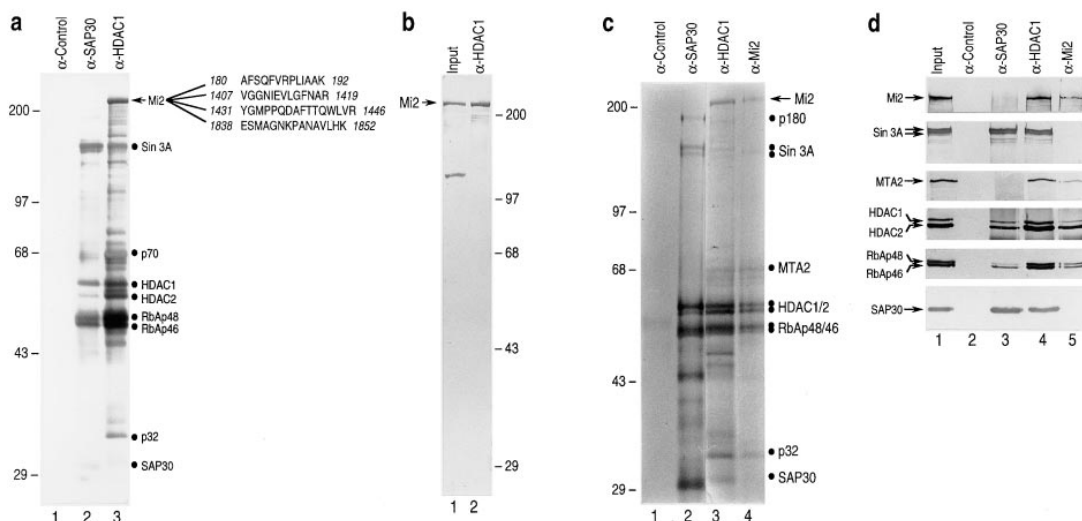


Figure 1. Affinity Purification of Histone-Deacetylase-Containing Complexes

(A) Silver staining of a polyacrylamide–SDS gel containing aliquots of samples derived from affinity columns using antibodies against SAP30 (lane 2), HDAC1 (lane 3), or GST (lane 1). Common polypeptides between the SAP30- and HDAC1-affinity-purified complexes are indicated (Sin3A, HDACs, RbAps, SAP30). Polypeptides uniquely retained in the anti-HDAC1 column (Mi2, p70, and p32) are also indicated. The amino acid sequence at the top of the panel denotes 4 of the 43 peptide sequences obtained from the 230 kDa protein that allowed us to identify it as Mi2 β .

(B) Western blot analysis using antibodies against an internal fragment of Mi2 β . Input (lane 1) denotes the sample used in the affinity purification procedure. Lane 2 is the material that was purified using anti-HDAC1 antibodies.

(C) Silver staining of a polyacrylamide–SDS gel comparing the polypeptides present in different affinity-purified samples. Antibodies used for affinity purification are indicated on the top of the panel. Polypeptides retained in the different antibody columns are indicated on the right of the panel. MTA2 denotes a polypeptide present in the anti-Mi2 and anti-HDAC1 affinity-purified samples. MTA2 was identified by peptide sequences from p70 (see [A]).

(D) Western blot analysis of the different affinity-purified complexes, as indicated at the top of the panel. The antibodies used in the Western blot are indicated on the left side of the panel.

histone deacetylase complex (Zhang et al., 1997). This complex contains, in addition to the corepressor Sin3, the histone deacetylases HDAC1 and HDAC2 (Taunton et al., 1996; Yang et al., 1996), the retinoblastoma (Rb)-associated proteins RbAp48 and RbAp46 (Qian et al., 1993; Qian and Lee, 1995), and two novel Sin3-associated polypeptides, SAP30 and SAP18. SAP30 was recently found to be required for the establishment of repression at some promoters in yeast and mammals (Laherty et al., 1998; Zhang et al., 1998). The RbAp proteins are believed to function as molecular bridges that bring histone/nucleosome-modifying enzymes to their targets. This assumption is based on the fact that the RbAp proteins interact with the core histones H2A and H4 and exist in protein complexes that modify core histones and alter nucleosome structure (Verreault et al., 1997).

During the process of purifying the Sin3/HDAC complex, we noticed that only a portion of the HDAC1/2 polypeptides copurify with Sin3A. This led us to postulate that more than one HDAC1/2-containing complex exists in cells. To characterize the different HDAC1/2-containing complexes, we utilized an affinity chromatography approach. Using antibodies against HDAC1, we isolated different HDAC1-associated polypeptides (Zhang et al., 1998). Characterization of these HDAC1-associated polypeptides should allow us to characterize the different HDAC1-containing complexes. Here we report the identification of a protein complex containing

a subset of the polypeptides present in the HDAC1 affinity-purified complex possessing histone deacetylase and ATP-dependent nucleosome remodeling activities.

Results

Mi2 β Is a Component of a Histone Deacetylase Complex

In order to isolate different HDAC1/2-containing complexes, we attempted to identify the polypeptides associated with HDAC1 using antibodies against the C-terminal fragment of HDAC1. The results of a representative purification are shown in Figure 1A. Many polypeptides, ranging in size from 230 to 30 kDa, were retained on the anti-HDAC1 column. These polypeptides appear to be specific, since they were not retained on a control column (Figures 1A and 1C). Moreover, antibodies against SAP30, a component of the Sin3/HDAC complex, only retained a subset of the polypeptides observed in the anti-HDAC1 column (Figure 1A, compare lanes 2 and 3). Although Sin3A, HDAC1/2, RbAp48/46, and SAP30 were retained on both columns, several polypeptides were only retained on the HDAC1 column. The three most abundant HDAC1-specific polypeptides have apparent molecular weights of 230, 70, and 32 kDa (Figure 1A, compare lanes 2 and 3). The 230 kDa polypeptide was subjected to in-gel tryptic digestion, and the resulting peptides were sequenced by microcapillary HPLC ion trap mass spectrometry as described (Nash et

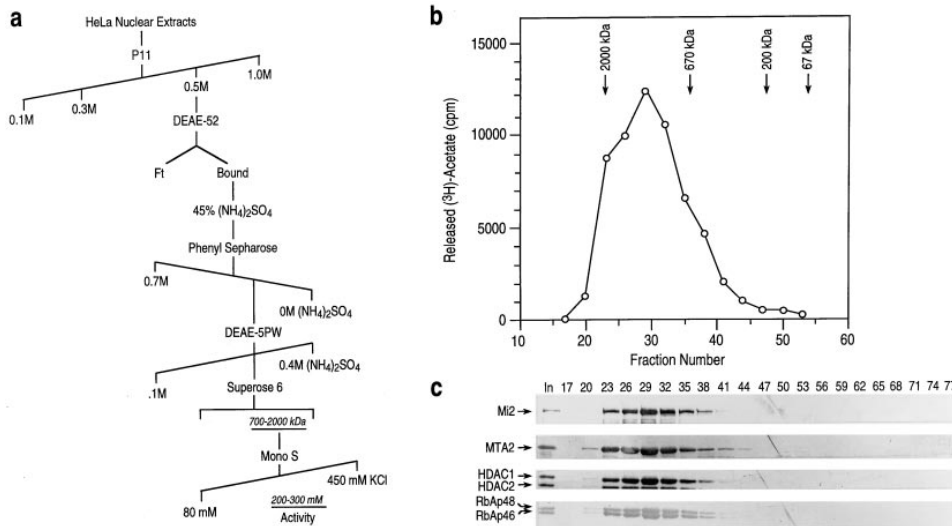


Figure 2. Conventional Purification of the NuRD Complex

(A) Schematic representation of the steps used to purify NuRD.

(B) Histone deacetylase assay of fractions derived from a Superose-6 column. An aliquot (2 μ l) of the fractions (250 μ l) derived from the Superose-6 column was assayed for histone deacetylase activity as described in Experimental Procedures. The column was calibrated using different protein markers, and their elutions from the column are indicated.

(C) Western blot analysis of the same samples described in (B). The antibodies used are indicated on the left side of the panel.

al., 1996). Forty-three peptide sequences derived from p230 were contained in a protein known as Mi2 (Seelig et al., 1995). The identity of this polypeptide was also confirmed by Western blot analysis (Figure 1B). The peptide sequences obtained from p70 (Y. Z. et al., unpublished) revealed that it is a novel protein highly related to the metastasis-associated protein MTA1 (Toh et al., 1994). Thus, we named p70 MTA2. The peptide sequences obtained from p32 by identical means revealed that it is a novel protein.

Mi2 was originally identified as the dermatomyositis-specific autoantigen (Seelig et al., 1995). It contains two PHD (plant homeodomain)-zinc-finger domains, two chromo domains, and a SWI2/SNF2-type helicase/ATPase domain (Seelig et al., 1995; Woodage et al., 1997). Two closely related genes, *Mi2 α* and *Mi2 β* (also known as *HsCHD3* and *HsCHD4*), have been described (Seelig et al., 1996; Woodage et al., 1997). Interestingly, while the 43 peptide sequences derived from the 230 kDa polypeptide correspond to the sequence of Mi2 β , only 13 share identity with the Mi2 α sequence. Despite an exhaustive effort combining manual interpretation and correlation analysis using the Sequest algorithm (Eng et al., 1994), we were unable to find any Mi2 α -specific peptides. Therefore, we conclude that Mi2 β is the 230 kDa polypeptide in the complex.

In order to identify which of the polypeptides retained on the anti-HDAC1 column were specifically associated with Mi2, we raised antibodies against a portion (amino acids 475-970) of Mi2 β and used them in an affinity purification procedure. The polypeptides retained in the anti-Mi2 column were visualized by silver staining and were compared to those retained in the anti-HDAC1 and anti-SAP30 columns (Figure 1C). Seven predominant polypeptides were retained in the anti-Mi2 column (Figure 1C, lane 4). All were present in the HDAC1-affinity-purified sample (Figure 1C, compare lanes 3 and 4).

However, with the exception of the HDAC1/2 and the RbAp48/46 polypeptides, all other polypeptides (Mi2, MTA2, and p32) were absent in the SAP30-affinity-purified sample (Figure 1C, lane 2). Interestingly, the Mi2 complex does not contain Sin3A or SAP30 (Figure 1C). This result was confirmed by Western blot analysis (Figure 1D, lane 5). Furthermore, the SAP30 complex contains SAP30 and Sin3A but is devoid of Mi2 and MTA2 (Figure 1D, lane 3). However, the histone deacetylases HDAC1/HDAC2 and the histone-binding proteins RbAp48/46 are present in all complexes (Figures 1C and 1D). The polypeptides present in each of the affinity-purified samples are specific, as they are absent in the column containing control antibodies (Figures 1C and 1D). The above biochemical data collectively establish that Mi2 β is a component of an HDAC1/2-containing histone deacetylase complex.

The Affinity-Purified Mi2 β Complex Is a Single Histone Deacetylase Complex

The affinity purification approach described above could not distinguish whether the polypeptides associated with Mi2 β constitute one or multiple Mi2 β -containing complexes. In addition, we could not rule out the possibility that the absence of Sin3A or SAP30 in the affinity-purified Mi2 β complex was due to their displacement by the antibodies. To address these questions, we attempted to purify the Mi2 β complex from HeLa nuclear extracts using conventional chromatography (Figure 2A). Fractionation of the Mi2 β -containing complex was followed by Western blot analysis and histone deacetylase activity. We found that both Sin3A and SAP30 were separated from Mi2 β upon chromatography on Phenyl Sepharose and DEAE-5PW columns (data not shown). Therefore, the native Mi2 β complex is devoid of Sin3A and SAP30. Analysis of Superose-6 gel filtration column

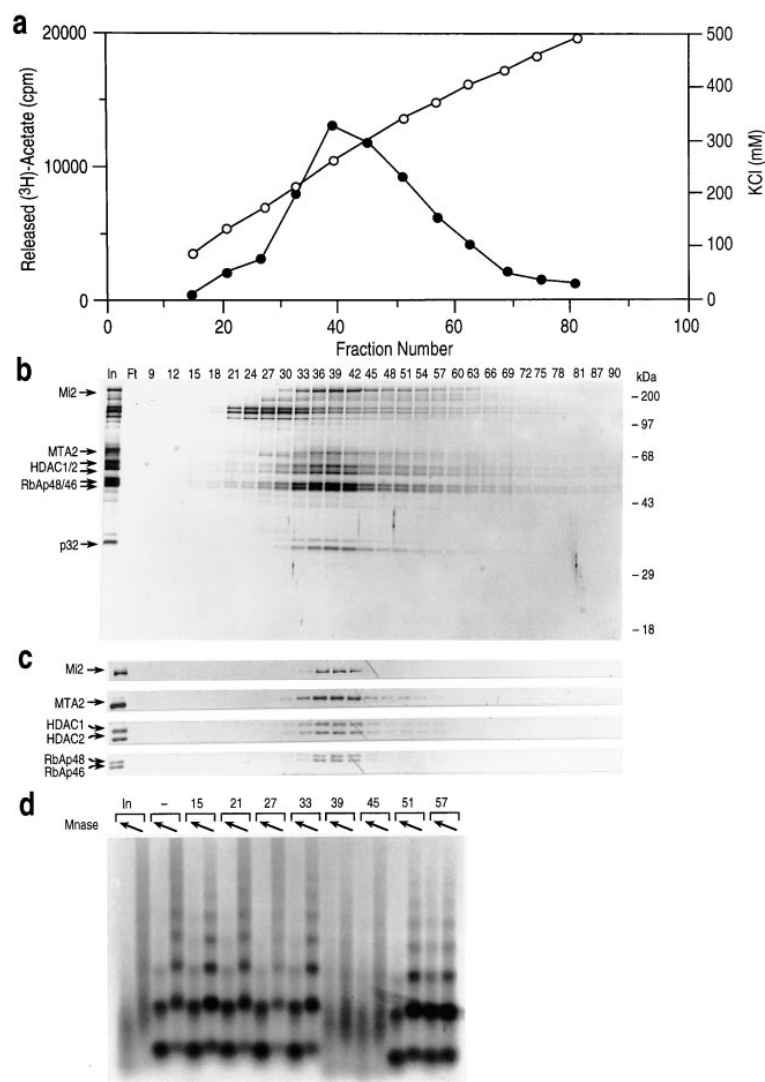


Figure 3. Elution Profile of the NuRD Complex from a Mono S Column

(A) Histone deacetylase assay. An aliquot (2 μ l) of the fractions (250 μ l) derived from the Mono S column was assayed for histone deacetylase activity as described in Figure 2B. The salt concentrations of the different fractions are indicated.

(B) Silver staining of a polyacrylamide-SDS gel containing an aliquot (10 μ l) derived from the different fractions of the Mono S column. The identities of the polypeptides are indicated on the left side of the panel.

(C) Western blot analysis of the fractions derived from the Mono S column. The antibodies used in the analysis are indicated on the left side of the panel.

(D) An aliquot (3 μ l) of the fraction derived from the Mono S column was analyzed for nucleosome remodeling activity as described in Experimental Procedures. The panel shows a Southern blot of micrococcal nuclease digestion (two different concentrations) products detected using a 32 P-labeled probe that hybridizes within the promoter (Ad-ML).

fractions for histone deacetylase activity identified a single peak of activity eluting with an apparent mass of \sim 1 MDa (Figure 2B). Moreover, Western blot analysis demonstrated that Mi2 β , MTA2, HDAC1/2, and RbAp48/46 coeluted with deacetylase activity (Figure 2C).

To characterize the Mi2 β -containing histone deacetylase complex further, the active fractions from the Superose-6 column were pooled and fractionated on a Mono S column. The Mono S-derived fractions were analyzed by assaying histone deacetylase activity (Figure 3A), silver staining (Figure 3B), and Western blots (Figure 3C). Silver staining revealed that only seven polypeptides copurified with histone deacetylase activity. Western blot analysis confirmed that the seven polypeptides included Mi2 β , MTA2, HDAC1/2, and RbAp48/46. This result is in perfect agreement with the result obtained using the affinity purification approach (Figure 1C). Therefore, we conclude that the seven-subunit Mi2 β -containing histone deacetylase complex purified by both conventional and affinity approaches is a bona fide histone deacetylase complex.

The Mi2 β -Containing Histone Deacetylase Complex Contains Nucleosome Remodeling Activity

Mi2 contains a SWI2/SNF2-type helicase/ATPase domain. Many proteins containing this domain have been found to exist in protein complexes that possess nucleosome remodeling activity. This observation prompted us to analyze whether the Mi2-HDAC1/2 complex contains nucleosome remodeling activity. Data presented in Figure 3D demonstrate that the Mi2 complex possesses nucleosome remodeling activity, as indicated by the loss of periodic nucleosome spacing between fractions 36 and 45, which correlates with the elution profile of the Mi2-containing histone deacetylase complex (Figure 3).

All nucleosome remodeling protein complexes characterized thus far require ATP hydrolysis for function. Moreover, most of the known nucleosome remodeling complexes remodel nucleosomes in an activator (DNA-binding domain)-dependent manner. To examine whether the Mi2 complex has similar properties, we characterized the nucleosome remodeling activity further. We found that the activity was dependent on ATP and a

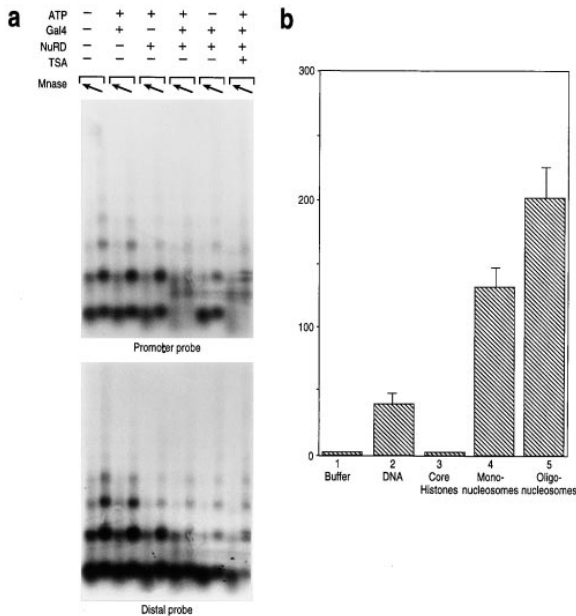


Figure 4. Characterization of the Nucleosome Remodeling Activity Present in the NuRD Complex

(A) Requirements for nucleosome remodeling activity by the NuRD complex. An aliquot (3 μ l) of the Mono S-derived fraction was assayed for activity as described in Figure 3D and in Experimental Procedures. Additions were as indicated on the top of the panel (TSA was added to a final concentration of 400 ng/ml). The upper panel shows a Southern blot hybridized with a 32 P-labeled probe hybridizing to sequences within the promoter. The bottom panel shows a Southern blot hybridized with a 32 P-labeled probe hybridizing approximately 1000 base pairs away from the promoter.

(B) An aliquot (2 μ l) of the Mono S pool was analyzed for ATPase activity as described in Experimental Procedures. Reactions contained DNA, core histones, mononucleosomes, or oligonucleosomes, as indicated at the bottom of the panel. Products of the reaction were separated by TLC, and the amount of 32 Pi-released was quantitated and expressed as fold released over buffer after deduction of background without addition of the NuRD complex.

DNA-binding domain. Therefore, the remodeling activity was only observed in the vicinity of the promoter, whereas distal nucleosomes were not affected (Figure 4A). However, at high concentrations of the complex, the nucleosomes were remodeled in a transcription factor-independent manner (data not shown, see below). To analyze whether the histone deacetylase activity of this complex affects the nucleosome remodeling activity, we performed nucleosome remodeling assays in the presence of the deacetylase inhibitor TSA. Results presented in Figure 4A demonstrate that the remodeling activity is not affected by TSA, indicating that the histone deacetylase activity is not required for nucleosome remodeling. Collectively, the above results allow us to conclude that the Mi2 β -containing histone deacetylase complex has ATP-dependent nucleosome remodeling activity that is independent of the deacetylase activity. Thus, we have named this complex NuRD (*n*ucleosome remodeling histone *d*eacetylase complex).

To further characterize the ATP requirement for the nucleosome remodeling activity of the NuRD complex,

we analyzed the ability of the complex to hydrolyze ATP in the presence of equal amounts of either sonicated calf thymus DNA (200–600 bp), mononucleosomes, or oligonucleosomes purified from HeLa cells (Figure 4B). This analysis uncovered that the NuRD complex possesses a DNA-dependent ATPase activity that was further stimulated by nucleosomes (approximately 3-fold for mononucleosome and 6-fold for oligonucleosome when compared to DNA). The activity was not stimulated by core histones.

The NuRD Complex Can Deacetylate Nucleosomal Histones

Promoter-proximal remodeling by the NuRD complex suggested that nucleosomal histone deacetylation by NuRD may require targeting of the complex to a promoter. An ideal system for testing targeted nucleosomal histone deacetylation requires the assembly of a properly spaced nucleosome array with acetylated histones and a plasmid DNA containing a transcription factor-binding site. The presence of histones as well as histone deacetylase activity in the *Drosophila* S190 extracts prevented us from using this assembly system to obtain highly acetylated nucleosomal templates. In addition, the alternative salt dialysis method for nucleosome assembly does not generate a properly spaced nucleosome array. Thus, we used properly spaced, highly acetylated nucleosomes isolated from sodium butyrate-treated HeLa cells as a substrate. The limitation of using these substrates is that the NuRD complex cannot be targeted.

The physiological substrate of the NuRD complex is most likely nucleosome arrays. However, for comparison, acetylated core histone octamers and mononucleosomes (Figure 5A) were also purified and used as substrates for histone deacetylase assays. In agreement with the promoter proximal remodeling property of the NuRD complex, we could not detect deacetylation of nucleosomes when using catalytic amounts of the NuRD complex. However, the NuRD complex was active in deacetylating core histone octamers under the same conditions (data not shown, see below). This result suggests that nucleosomal histone deacetylation may require targeted remodeling. Since high concentrations of the NuRD complex could remodel nucleosomes in a transcription factor (DNA-binding domain)-independent manner (data not shown), we predicted that a higher concentration of the NuRD complex should be able to deacetylate nucleosomal histones. Results shown in Figure 5B, using a triton-acid-urea (TAU) gel, confirmed this prediction. However, the histone deacetylase activity was not dramatically affected by ATP (Figure 5B).

The TAU gel assay shown in Figure 5B revealed an interesting feature about the histone deacetylase activity of the NuRD complex. Although the NuRD complex could deacetylate all four core histones, the extent of deacetylation on histone H4 varied depending on whether H4 was present in an octamer, mononucleosome, or oligonucleosome. When the substrate was an octamer, histone H4 was completely deacetylated (Figure 5B, lanes 2 and 3), indicating that all four lysines were targeted for deacetylation by NuRD. However, when the

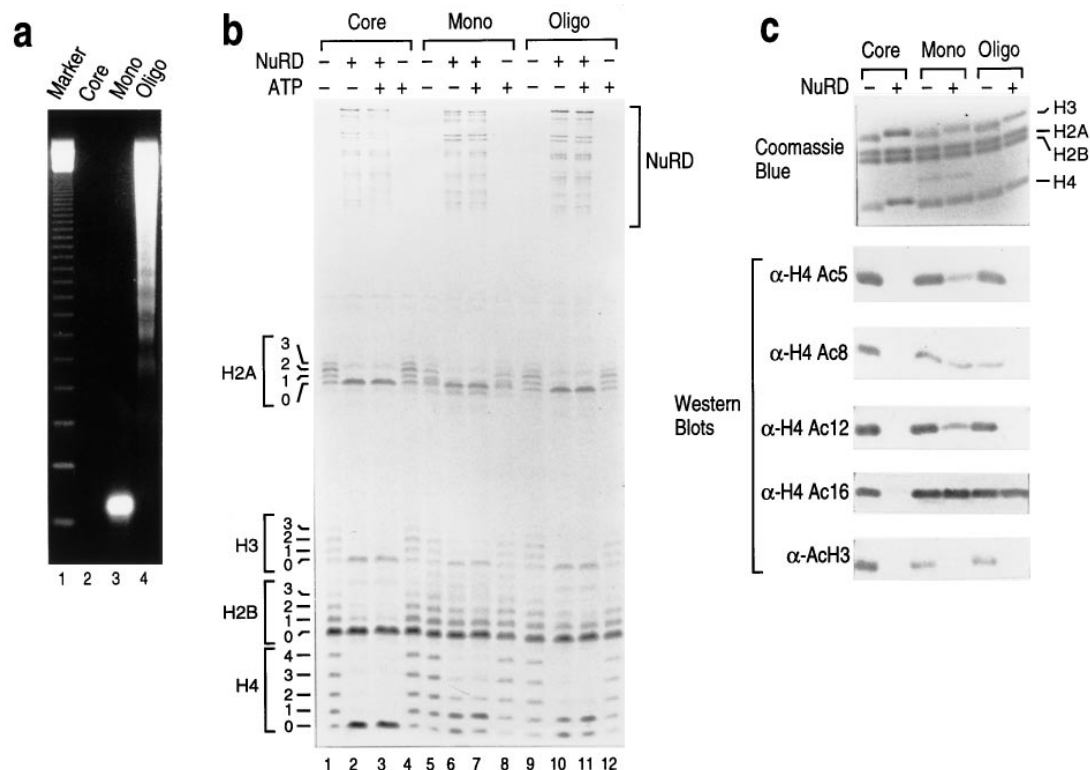


Figure 5. Characterization of the Histone Deacetylase Activity of the NuRD Complex

(A) An ethidium bromide-stained agarose gel containing mononucleosomes, oligonucleosomes, and core histones, as indicated on the panel, used in the experiments.

(B) Substrate specificity of the NuRD complex assayed on a TAU gel. A histone deacetylase assay was performed using an aliquot (10 μ l) of NuRD complex derived from the Mono S column supplemented with core histones, mononucleosomes, or oligonucleosomes, as indicated on the panel. The migration of the different histone isoforms is indicated on the left side of the panel. The bands at the top of the gel represent polypeptides present in the NuRD complex.

(C) Determination of the lysine residues deacetylated by the NuRD complex. The panel shows a Western blot analysis of the reactions using core histones, mononucleosomes, or oligonucleosomes as substrates. The reactions were performed as in (B). The reactions were divided and loaded onto polyacrylamide-SDS gels for five blots. The top is a Coomassie blue staining gel containing aliquots of the deacetylation reaction. The bottom panels are Western blots that were probed with antibodies recognizing acetylated histone H3 or histone H4 acetylated at lysine 5, 8, 12, or 16, as indicated on the panel.

substrate was a mononucleosome or oligonucleosome, H4 was only partially deacetylated (Figure 5B, compare lanes 6, 7, 10, and 11 with lanes 2 and 3). Moreover, histone H2B is also not as efficiently deacetylated when assembled into nucleosomes (Figure 5B, compare lanes 6, 7, 10, and 11 with lanes 2 and 3). This suggests that some lysine residues are not accessible to the NuRD complex when DNA is wrapped around the octamer.

To determine which lysine residues of histone H4 were targeted for deacetylation by the NuRD complex, we examined the acetylation state of H4 using acetylation site-specific antibodies (Figure 5C). Equal amounts of core histones in the form of octamers, mononucleosomes, and oligonucleosomes were incubated with the NuRD complex before subjecting the samples to SDS-PAGE followed by Western blot analysis. Identical blots were probed with antibodies recognizing acetylated histone H3 or histone H4 acetylated at lysine 5, 8, 12, or 16. Results shown in Figure 5C confirmed the TAU gel assay, demonstrating that histone H3 is deacetylated in all three substrate forms. In addition, this assay also confirmed that all four sites of H4 were deacetylated

when octamers were used as a substrate (Figure 5C, lane 2). Interestingly, lysine 16 could not be deacetylated when H4 was assembled into nucleosome (Figure 5C, compare lanes 3 and 4 and lanes 5 and 6). Moreover, lysine 8 of histone H4 could be deacetylated in the oligonucleosome but not in the mononucleosome substrate. These results are in good agreement with the TAU gel assay, demonstrating that histone H4 is more efficiently deacetylated in oligonucleosomes than in mononucleosomes. These results also generally agree with the *in vivo* results obtained in yeast (Rundlett et al., 1996, 1998).

The PHD-Zinc Fingers of Mi2 β Are Required for Mi2 β -HDAC1 Interaction

Previous studies have suggested that Sin3 functions as a scaffold for the formation of HDAC1/2-containing complexes (Alland et al., 1997; Hassig et al., 1997; Heinzel et al., 1997; Kadosh and Struhl, 1997; Laherty et al., 1997; Nagy et al., 1997; Zhang et al., 1997). The finding that the NuRD complex is devoid of Sin3 prompted us to analyze how the histone deacetylases HDAC1/2 were

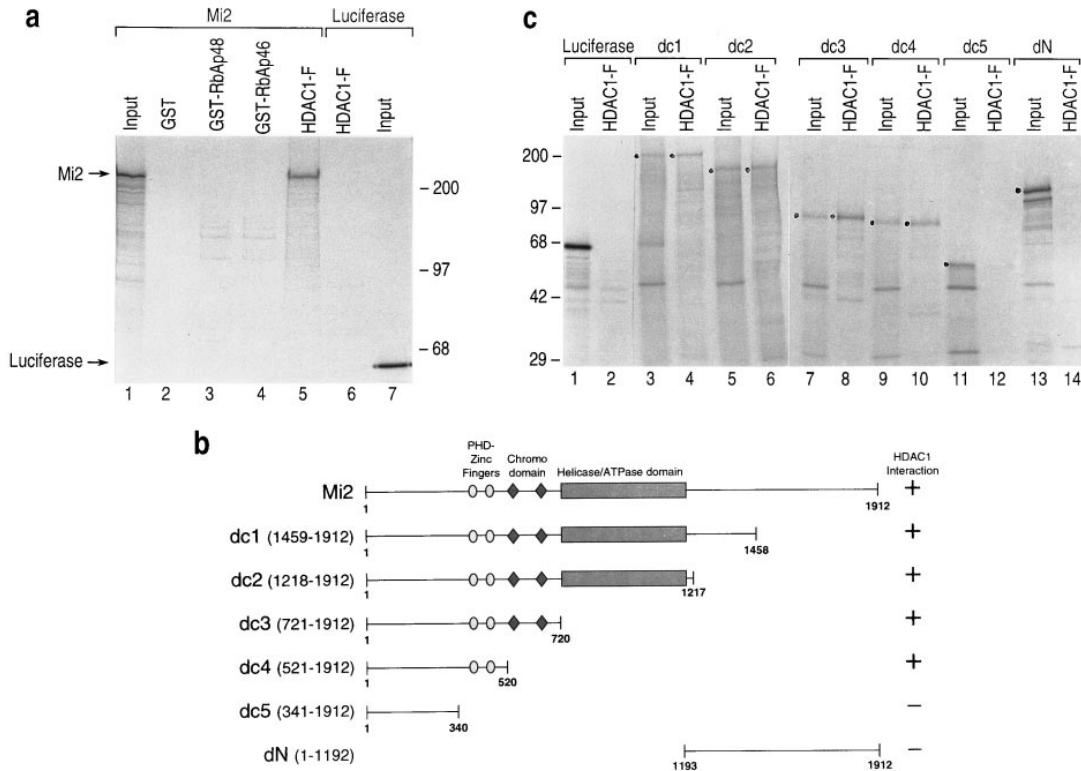


Figure 6. The PHD-Zinc Fingers Are Required for Mi2 β -HDAC1 Interaction

(A) Autoradiogram of a pull-down assay. Different proteins, as indicated on the panel, were attached to beads and incubated with in vitro-translated Mi2 β or luciferase. The beads were then washed three times with a buffer containing 400 mM KCl and 0.05% NP40 before elution with SDS loading buffer.

(B) Schematic representation of the Mi2 β polypeptide and different truncated proteins that were used in the analysis described in (C). The + symbol at the right of the panel denotes the protein that interacts with HDAC1.

(C) Different truncated Mi2 β expression vectors, described in (B), or a luciferase expression vector was transcribed/translated in vitro and incubated with HDAC1 as indicated at the top of the panel. The interaction between the different truncated Mi2 β proteins and HDAC1 was analyzed by a pull-down assay as described in (A). HDAC1-Flag was attached to beads coated with monoclonal antibodies against the Flag epitope.

incorporated into the NuRD complex. Therefore, we examined whether Mi2 β interacts with HDAC1 and the histone-binding proteins RbAp48/46. HDAC1 was purified from baculovirus-infected SF9 cells as an HDAC1-FLAG fusion protein, while RbAp48/46 were purified from *E. coli* as GST-fusion proteins. These proteins were bound to beads and were incubated with in vitro-translated Mi2 β protein. After extensive washing, the proteins remaining bound to the beads were resolved by SDS-PAGE and visualized by fluorography. We found that Mi2 β interacts with HDAC1 (Figure 6A, lane 5). This interaction appears to be specific, as GST and GST-RbAp48/46 failed to pull down Mi2 β (Figure 6A, lanes 2-4). Furthermore, HDAC1 failed to pull down in vitro-translated luciferase (Figure 6A, lane 6).

Mi2 β contains several interesting domains (Figure 6B). To determine whether these domains are involved in the Mi2/HDAC1 interaction, different Mi2 truncations were analyzed for their ability to interact with HDAC1. A schematic representation of these truncations is shown in Figure 6B. The results from pull-down experiments, shown in Figure 6C, demonstrate that amino acids (521-1912) at the C-terminal of the PHD-zinc fingers of Mi2

are dispensable for the interaction between Mi2 and HDAC1 (Figures 6B and 6C). However, deletion of the PHD-zinc fingers abrogated the interaction (Figure 6C, lane 12). To analyze whether the PHD-zinc fingers were sufficient for the interaction, polypeptides containing one or both fingers were produced in bacteria and analyzed for their ability to interact with HDAC1. This analysis failed to demonstrate an interaction (data not shown). Therefore, we conclude that the PHD-zinc fingers are required but not sufficient for the interaction. While the function of PHD-zinc fingers is not known, they have been implicated in the regulation of chromatin-mediated transcription (Aasland et al., 1995). The functional significance of PHD-zinc-finger domains has been highlighted by the recent finding that mutations within the PHD-zinc finger of the transcriptional regulator ATRX are associated with the ATRX syndrome (Gibbons et al., 1997).

Discussion

Using conventional chromatography and immuno-affinity purification approaches, we have purified a novel protein complex from human cells containing both

nucleosome remodeling and histone deacetylase activities. The complex, which we named NuRD, contains the two histone deacetylases HDAC1/2, the two histone-binding proteins RbAp48/46, the helicase/ATPase domain-containing protein Mi2 β , an MTA1-related protein, and a novel protein of 32 kDa. We show that the NuRD complex has DNA-dependent nucleosome-stimulated ATPase activity. In addition, we demonstrate that this complex remodels nucleosomes in an ATP-dependent manner. Furthermore, the NuRD complex is able to deacetylate nucleosomal histones.

The NuRD Complex Couples Histone Deacetylation with Nucleosome Remodeling

As discussed above, two types of activities involving ATP hydrolysis and covalent modification of histone tails, respectively, have been discovered. However, a protein complex possessing both activities has not been reported. Here we demonstrate that the NuRD complex has both nucleosome remodeling and histone deacetylation activities. Similar to NURF, the NuRD complex remodels nucleosomes in an ATP-dependent and transcription factor-dependent manner when catalytic amounts of the factor are used. At higher concentrations, the NuRD complex loses transcription factor dependence. Unlike other nucleosome remodeling factors such as ACF, CHRAC, and RSF, NuRD does not possess ATP-dependent nucleosome spacing activity. With regard to its deacetylase activity, the NuRD complex is able to deacetylate all four core histones (Figure 5B). However, the NuRD complex is not able to deacetylate lysine 16 of histone H4 when H4 is incorporated into a nucleosome, although this lysine can be efficiently deacetylated when histone octamers are used as a substrate (Figure 5C). Interestingly, lysine 8 of histone H4 also becomes resistant to deacetylation when present in a mononucleosome, although it is efficiently deacetylated in oligonucleosomes (Figure 5C). These results indicate that in addition to DNA, the nucleosome structure also influences the accessibility of the NuRD complex to its target.

The finding that both nucleosome remodeling and histone deacetylation activities exist in the same complex seems to be a paradox, since ATP-dependent nucleosome remodeling was thought to be involved in transcriptional activation, while histone deacetylation has been linked to transcriptional repression. However, we believe that the coupling of a nucleosome remodeling activity to HDAC or HAT activity may provide an efficient way for the cell to achieve transcriptional regulation. It has been previously demonstrated that RbAp48 and RbAp46 cannot gain access to nucleosomal histones, although they efficiently bind to core histones (Verreault et al., 1997; Zhang et al., 1998). This is likely because helix 1 of histone H4, which is involved in RbAp binding (Verreault et al., 1997), also interacts with DNA when packaged into a nucleosome (Luger et al., 1997). Therefore, in order for the RbAp proteins to access histones, the nucleosome structure needs to be altered. If nucleosomal histones were freely accessible to the NuRD or other complexes, one would expect that histone deacetylases would work on a genome-wide basis. In this

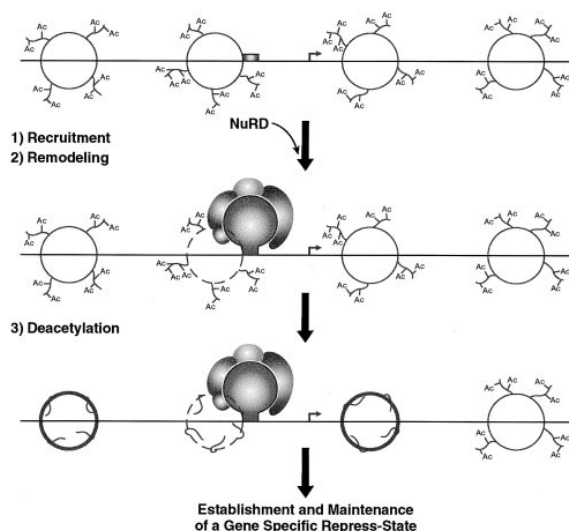


Figure 7. A Speculative Model for the Function of the NuRD Complex

The NuRD complex is tethered to specific sites in promoters by a sequence-specific DNA-binding protein interacting, directly or indirectly, with a component of the NuRD complex. Alternatively, one of the NuRD subunits may bind DNA directly. The ATP-dependent nucleosome remodeling activity of NuRD remodels the nucleosome(s) and stabilizes the protein-DNA interaction. The remodeling activity also permits the RbAp polypeptides to gain access to the core histones. The histone deacetylases in the NuRD complex then deacetylate (or maintain in a deacetylated state) the histone tails such that the neighboring nucleosomes are tightly packed and do not allow formation of transcription-competent complex. This model permits the establishment and maintenance of a gene-specific repressed state.

case, the loss of histone deacetylase activity should cause a general increase in histone acetylation, resulting in a global increase in gene expression. However, disruption of RPD3 in yeast or treatment of human cells with histone deacetylase inhibitors does not cause global gene activation. In fact, only a limited number of genes are affected, and some genes are repressed rather than activated (Vidal and Gaber, 1991; Van Lint et al., 1996). One possible explanation is outlined in Figure 7. A remodeling histone deacetylase complex, such as NuRD, is recruited to the promoter region of a subset of genes through the interaction with a DNA-binding protein. Alternatively, recruitment may be achieved by the complex itself recognizing a specific DNA element. Upon recruitment, the complex uses energy from ATP hydrolysis to remodel adjacent nucleosomes, allowing the histone tails to become accessible for deacetylation. Deacetylation of the core histone tails results in the formation of a more compacted nucleosomal structure, leading to transcriptional repression. The discovery that nucleosome remodeling and histone deacetylase activities coexist in the same complex supports this model. We observed that the NuRD complex deacetylates nucleosomal histones in the absence of ATP. This result does not seem to be compatible with this model. However, this only occurs in the presence of large amounts of the NuRD complex. When catalytic amounts of the NuRD complex were used, no nucleosomal histone deacetylation was observed, although core

histones were efficiently deacetylated. It is likely that nucleosomal histone deacetylation by the NuRD complex requires targeting of this complex to the vicinity of a specific nucleosome. We believe that it will be possible to obtain ATP-dependent nucleosomal histone deacetylation if the NuRD complex is targeted to a specific site in a reconstituted nucleosome array.

The Connection between Histone Deacetylase, Chromatin Structure, Dermatomyositis, and Cancer

Chromatin is the *in vivo* substrate for all biological processes involving DNA. Thus, interfering with chromatin structure should influence many fundamental biological processes, likely resulting in the development of specific diseases. Several lines of evidence indicate that factors that alter chromatin structure could lead to malignancy. First, histone deacetylase inhibitors, such as TSA, traxopin, and depudecin, are able to cause cell cycle arrest and morphological changes in a number of tumor cell lines (Yoshida et al., 1995; Kwon et al., 1998). In addition, histone deacetylases may affect cell cycle regulation through their association with the retinoblastoma tumor suppressor protein (Brehm et al., 1998; Luo et al., 1998; Magnaghi-Jaulin et al., 1998). Moreover, a chimeric mutant of the retinoic acid receptor, which causes acute promyelocytic leukemia, was found to be associated with the histone deacetylase HDAC1 (Grignani et al., 1998; Lin et al., 1998). Finally, mutations in hSNF5, a component of the human SWI/SNF nucleosome remodeling factor, have recently been linked to the development of malignant rhabdoid tumors (Versteeg et al., 1998). The finding that the NuRD complex contains a component that is highly related to the metastasis-associated factor MTA1 reinforces the idea that changes in chromatin structure, either by nucleosome remodeling factors or histone deacetylases, could affect the cell cycle.

MTA1 was initially identified by differential screening using the rat mammary adenocarcinoma metastatic system (Toh et al., 1994). It was found that the expression level of MTA1 correlates with the metastatic potential of several human cancer cell lines and tissues (Toh et al., 1994, 1997). In agreement with a role for MTA2 in cell proliferation, we found that MTA2 is highly expressed in rapidly dividing cells (Y. Z. and D. R., unpublished data). Another important component of the NuRD complex is Mi2 β , an autoantigen for the autoimmune disease dermatomyositis. While the relationship between the normal function of Mi2 β and the development of dermatomyositis is not clear, it has been reported that patients with dermatomyositis also face an increased risk of malignancy (Airio et al., 1995; Shorr et al., 1997). About 15% to 30% of patients with dermatomyositis develop cancer (Airio et al., 1995). The finding that the dermatomyositis-associated autoantigen Mi2 β and the candidate metastasis-associated protein MTA2 exist in the same protein complex may provide a molecular explanation to the above observation.

Experimental Procedures

Purification of the NuRD Complex

Affinity purification of the HDAC1, SAP30, and NuRD complexes was based on a previously published procedure (Zhang et al., 1998)

using HeLa nuclear extracts fractionated on phosphocellulose and DEAE-52 columns. The affinity columns were prepared using purified antibodies against the C-terminal domain of HDAC1, against the full-length SAP30, or against a fragment of Mi2 β (amino acids 475–970) coupled to 1 ml of protein A–agarose beads (Repligen) as described (Harlow and Lane, 1988).

The procedure for conventional purification of the NuRD complex is outlined in Figure 2A. Approximately 6 g of HeLa nuclear extracts was fractionated on phosphocellulose and DEAE-52 columns. The DEAE-52-bound proteins were eluted with buffer C (20 mM Tris-HCl [pH 7.9], 0.2 mM EDTA, 10 mM β -ME, 0.2 mM PMSF, and 10% glycerol) containing 350 mM KCl (BC350) and proteins were precipitated with saturated ammonium sulfate (final concentration of 45%). Following centrifugation at 35,000 rpm for 1 hr, the pellets were resuspended in buffer D (40 mM HEPES [pH 7.9], 0.2 mM EDTA, 1 mM DTT, 0.2 mM PMSF, and 10% glycerol), and the ammonium sulfate concentration was adjusted to 700 mM. The sample (approximately 340 mg) was then loaded onto a 55 ml FPLC Phenyl Sepharose column (Pharmacia). The column was then washed with 4 column volumes (cv) of BD700. Proteins were eluted with a linear gradient (20 cv) of ammonium sulfate from 700 to 0 mM in buffer D. The fractions containing the NuRD complex (150 to 400 mM ammonium sulfate) were pooled and dialyzed against BD100. The sample (75 mg) was then loaded onto an HPLC-DEAE-5PW column (TosoHaas, 45 ml) equilibrated with BD100. Proteins bound to the column were eluted with a 10 cv linear gradient from 100 to 400 mM ammonium sulfate in buffer D. Fractions containing the NuRD complex (190 to 230 mM ammonium sulfate) were pooled (5 mg) and precipitated with saturated ammonium sulfate to a final concentration of 65%. After centrifugation at 35,000 rpm for 1 hr, the pellets were resuspended in BC500 and proteins were separated by chromatography on a Superose-6 column (Pharmacia). The NuRD complex eluted with an apparent mass of 1 MDa. The NuRD complex pool (approximately 0.5 mg) was dialyzed against BC80 and loaded onto a 1 ml Mono S column (Pharmacia) equilibrated in BC80. The proteins were then eluted with 20 cv linear gradient of buffer C from 80 to 450 mM KCl. The NuRD complex eluted between 200 to 300 mM KCl.

Mass Spectrometric Peptide Sequencing

Affinity-purified samples from anti-HDAC1 column was concentrated 10-fold using a centricon concentrator and was resolved on an 8% SDS-PAGE. After Coomassie staining, the 230 kDa band was subjected to in-gel reduction, S-carboxyamidomethylation, and tryptic digestion (Promega), and a 10% aliquot of the resultant mixture was analyzed as described (Nash et al., 1996). Interpretation of the resulting MS/MS spectra of the peptides was facilitated by searching the NCBI nr and dbest databases with the algorithm Sequest (Eng et al., 1994), followed by manual inspection. Forty-three MS/MS spectra were individually confirmed to correlate with sequences from Mi2.

Nucleosomes and Core Histones Purification, Histone Deacetylase Assays, and TAU Gel

Acetylated nucleosomes and core histones were purified from HeLa cells as described (Zhang et al., 1998). Procedures for labeling, purification of core histones, and electrophoresis on TAU gels were as described (Zhang et al., 1998). Histone deacetylase assay conditions were modified from our previously published procedure (Zhang et al., 1998) to facilitate nucleosome remodeling. Briefly, about 10 μ g of 3 H-labeled core histone octamers (Figures 2B and 3A) or 4 μ g of acetylated core histones and nucleosomes (Figure 5) were incubated with column fractions or purified NuRD complex at 30°C for 1 hr in the presence or absence of 4 mM ATP in buffer containing 10 mM HEPES (pH 7.5), 5 mM MgCl₂, 50 mM KCl, 2 mM DTT, and 0.5 mM EDTA. The released [3 H]acetate was extracted with ethyl acetate and quantified by liquid scintillation counting (Figures 2B and 3A). Alternatively, deacetylation was monitored by resolving histones on a TAU gel followed by Coomassie staining (Figure 5B) or by resolving on an SDS-PAGE followed by Coomassie staining and Western blot (Figure 5C).

Nucleosome Assembly, Nucleosome Remodeling, and ATPase Assays

Chromatin was assembled and purified as described (Bulger and Kadonaga, 1994; Orphanides et al., 1998). The nucleosome remodeling assay was performed using a modification of a published procedure (Tsukiyama and Wu, 1995). The ATPase activity of the NuRD complex was analyzed using a modification of a published procedure (Tsukiyama and Wu, 1995). Briefly, 2 μ l of purified NuRD complex (Mono S fraction) was incubated with 500 ng of sonicated calf thymus DNA (200–600 bps), core histones, mononucleosomes, or oligonucleosomes (purified from HeLa cells, see above section) in the presence of 2 mM ATP, 8 mM MgCl₂, 50 mM KCl, and 0.5 μ l of [γ -³²P]ATP (3000 Ci/mmol, 5 mCi/ml, NEN Dupont) in a 10 μ l volume at 28°C for 30 min. The reactions were stopped by adding 5 μ l of 0.5 M EDTA. Products were separated using a TLC glass chamber presaturated with 1 M formic acid and LiCl. The TLC plates were dried, exposed to X-ray film, and quantitated with an Image Master Densitometer (Pharmacia).

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