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mSin3A/Histone Deacetylase 2- and PRMT5-Containing Brg1 Complex Is Involved in Transcriptional Repression of the Myc Target Gene *cad*

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The role of hSWI/SNF complexes in transcriptional activation is well characterized; however, little is known about their function in transcriptional repression. We have previously shown that subunits of the mSin3A/histone deacetylase 2 (HDAC2) corepressor complex copurify with hSWI/SNF complexes. Here we show that the type II arginine-specific methyltransferase PRMT5, which is involved in *cyclin E* repression, can be found in association with Brg1 and hBrm-based hSWI/SNF complexes. We also show that hSWI/SNF-associated PRMT5 can methylate hypoacetylated histones H3 and H4 more efficiently than hyperacetylated histones H3 and H4. Protein-protein interaction studies indicate that PRMT5 and mSin3A interact with the same hSWI/SNF subunits as those targeted by c-Myc. These observations prompted us to examine the expression profile of the c-Myc target genes, *carbamoyl-phosphate synthase-aspartate carbamoyltransferase-dihydroorotase* (*cad*) and *nucleolin* (*nuc*). We found that *cad* repression is altered in cells that express inactive Brg1 and in cells treated with the HDAC inhibitor depsipeptide. Using chromatin immunoprecipitation assays, we found that Brg1, mSin3A, HDAC2, and PRMT5 are directly recruited to the *cad* promoter. These results suggest that hSWI/SNF complexes, through their ability to interact with activator and repressor proteins, control expression of genes involved in cell growth and proliferation.

During cell growth and differentiation several genes become either repressed or activated. These variations in expression often correlate with changes in chromatin structure and occur in the context of the cell cycle. Recruitment of the highly related Brg1 and hBrm chromatin remodeling complexes, which can disrupt nucleosome structure and increase accessibility to DNA, has been implicated in transcriptional activation of many inducible genes (21, 41). However, in view of recent findings, which show that subunits of mSin3/histone deacetylase (HDAC) corepressor complexes can be found in association with Brg1 and hBrm chromatin remodelers and that HDACs 1 and 2 are integral components of the NuRD complex, it appears that ATP-dependent chromatin remodeling might also be involved in transcriptional repression (32, 51, 56, 63, 65). Consistent with this notion, mutation of yeast SWI2/SNF2 can lead to gene derepression (28, 35, 53). Furthermore, Brg1, hBrm, and BAF45/Ini1 have been shown to be involved in transcriptional repression of cell cycle-regulated genes (39, 57, 64, 67). Nevertheless, the molecular mechanisms underlying the regulation of repression by hSWI/SNF complexes are not clearly understood.

Histone deacetylation by mSin3A/HDAC corepressor complexes has been linked to transcriptional silencing of genes regulated by the retinoid and thyroid hormone receptors, Ikaros, E2F, and Myc/Max/Mad proteins, but it is not known whether mSin3A/HDAC complexes can efficiently modify nucleosomal histones (1, 27, 31, 33, 40, 44). Previous work has shown that members of the Myc family of oncoproteins control cell proliferation by modulating transcription of genes that are important for G_1-to-S phase transition (13, 16). Transcriptionally active c-Myc is always in complex with Max and can bind to the E-box-related sequence CACGTG (4). Myc-Max heterodimers activate transcription of target genes by recruiting distinct activities that can phosphorylate the RNA polymerase II carboxy-terminal domain and acetylate histone H4 (5, 12, 37). In addition, c-Myc has been shown to interact with BAF45/Ini1 and to activate transcription from a reporter gene in a Brg1-dependent manner (10). Unlike c-Myc, Max can also heterodimerize with Mad proteins (Mad1, Mxi1, Mad3, and Mad4), which are known to repress transcription by recruiting mSin3A/HDAC complexes (2, 3, 48). Both c-Myc and Mad protein levels fluctuate as cells either proliferate or withdraw from the cell cycle, whereas Max levels remain constant (25, 26). Therefore, regulation of c-Myc target genes appears to be governed by the balance between Myc-Max and Mad-Max complexes and the histone-modifying enzymes with which they interact. Currently, there is limited information on the requirement for chromatin remodeling complexes and the mechanisms used to repress Myc/Max/Mad target genes.

Histone methylation by SET (Suvar3-9, Enhancer of Zeste, and Trithorax) proteins, which can methylate histone lysine residues, and protein arginine methyltransferase (PRMT) proteins has been implicated in transcriptional activation and repression, depending on the histone residue(s) being targeted (29, 52, 66). For example, histone methylation by PRMT4/CARM1, a type I PRMT that targets arginine residues in the N- and C-terminal regions of histone H3, can enhance transcriptional activation by nuclear receptors, while histone meth-

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ylation by PRMT5, a type II PRMT, leads to transcriptional repression of *cyclin E1* (6, 9, 15, 49). PRMT5 was first identified in *Schizosaccharomyces pombe* as a Shk1 kinase binding protein 1 (Skb1) and was shown to positively regulate Shk1 protein-activated kinase, which is a component of the Ras1/Cdc42 signaling module in *S. pombe* that is involved in the control of cell morphology (23). Subsequently, PRMT5 was shown to inhibit mitosis by interacting with Shk1 and the Cdc2 complex (24). Furthermore, human PRMT5 was able to substitute for Skb1 in *S. pombe*, suggesting that both proteins are functionally conserved (24). More recently, PRMT5 has been shown to methylate free histones as well as Sm proteins, which are involved in biogenesis of the snRNP components of the pre-mRNA splicing machinery (19, 20, 43). Despite its numerous functions, the mechanism by which PRMT5 is recruited to regulate transcription is not known.

To further understand how hSWI/SNF complexes regulate chromatin structure, we have analyzed their subunits by mass spectrometry and determined that the previously unidentified 66-kDa subunit is identical to PRMT5. We demonstrate that immunopurified hSWI/SNF-associated PRMT5 can efficiently methylate hypoacetylated histones H3 and H4. Furthermore, we provide evidence linking PRMT5, mSin3A/HDAC2, and the Brg1-based hSWI/SNF complex to *cad* transcriptional repression. These findings indicate that chromatin remodeling in combination with histone modification play an essential role in *cad* gene regulation.

MATERIALS AND METHODS

Plasmid constructions. Plasmids for in vitro transcription and mammalian cell expression of full-length mSin3A and hSWI/SNF cDNAs have been described previously (50, 51, 59). Plasmids pFastBac1/Fl-PRMT5 and pBS(KS+)/Fl-PRMT5 were generated by inserting a 2-kbp *Bam*HI-*Eco*RI DNA fragment, which encodes full-length PRMT5, into *Bam*HI-*Eco*RI-linearized pFastBac1 or pBS(KS+). Full-length PRMT5 was PCR amplified from a human peripheral blood cDNA library by use of a 5′ primer (5′-CGCGGATCCGTGATTGGCT ACTAGTATCAAGGAATCCCGGCGTGGACA-3′), which introduced a *Bam*HI site, and a 3′ primer (5′-CCGGAATTCTTACTATTTGTCATCGTCG TCCTTGTAGTCGAGGCCAAT-3′), which introduced a Flag tag before the stop codon, followed by an *Eco*RI site. To generate pFastBac1 vector for expression of catalytically inactive Fl-PRMT5, glycine 367 and arginine 368 were mutated to alanines with the Quick Change site-directed mutagenesis kit (Stratagene, Inc.). Plasmid pGEX-2TK/Fl-PRMT5 was constructed by inserting a 1.9-kbp *Bam*HI-*Eco*RI DNA fragment, which encodes PRMT5 (amino acids [aa] 4 to 637), into *Bam*HI-*Eco*RI-linearized pGEX-2TK. Plasmids for bacterial expression of glutathione *S*-transferase (GST)-paired amphipathic helix (PAH) fusion proteins were constructed by subcloning PCR-amplified DNA fragments that spanned each PAH domain into pGEX-2TK. All 5′ primers included a *Bam*HI site sequence, while 3′ primers included an *Eco*RI site, except for the primer for the PAH3 domain of mSin3B, in which a *Sma*I site was included due to the presence of an internal *Eco*RI site. PCR-amplified DNA sequences used to generate each construct encoded PAH1A (aa 119 to 210), PAH2A (aa 300 to 404), PAH3A (aa 454 to 544), PAH4A (aa 939 to 1034), PAH1B (aa 20 to 105), PAH2B (aa 140 to 235), PAH3B (aa 281 to 365), and PAH4B (aa 759 to 868).

Purification of recombinant Fl-PRMT5 and Fl-hSWI/SNF complexes and protein identification. Wild-type and mutant Fl-PRMT5 as well as Fl-hSWI/SNF complexes were purified as described previously (42, 50, 51). Glycerol gradient fractions were concentrated by trichloroacetic acid (TCA) precipitation, and samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie blue staining. Gel-resolved subunits were digested with trypsin and partially fractionated, and the resulting peptide mixtures were analyzed by matrix-assisted laser desorption ionization–reflectron time of flight mass spectrometry (MS) (Reflex III; BRUKER Daltonics, Bremen, Germany) as described previously (14) and also by use of an electrospray ionization (ESI) triple quadrupole MS/MS instrument (API300; ABI/MDS SCIEX, Thornhill, Canada) modified with an ultra-fine ionization source (22). Selected masses from the matrix-assisted laser desorption ionization–reflectron time of flight spectra were used to search the human segment of a protein nonredundant database. MS/MS spectra were inspected for "y" ion series to compare with the computer-generated fragment ion series of the predicted tryptic peptides.

Immunoprecipitation and GST pull-down assays. Approximately 2 mg of HeLa nuclear extracts was incubated with either preimmune or immune anti-PRMT5 antibodies in a 250-μl reaction containing IP buffer (40 mM Tris-HCl [pH 8.0], 100 mM NaCl, 0.5% NP-40, 1% aprotinin). When partially purified hSWI/SNF complexes were incubated with either preimmune or immune anti-Brg1 and anti-hBrm antibodies, a total of 600 to 800 μg of proteins was used in each reaction. After incubation on ice for 1 h, 30 μl of protein A-agarose beads was added, and samples were incubated at 4°C overnight. Beads were collected and washed six times with 500 μl of IP buffer containing 250 mM NaCl, and bound protein complexes were analyzed by SDS-PAGE followed by Western blotting. Expression of GST fusion proteins was carried out as described previously (17). To immobilize GST fusion proteins, approximately 500 to 800 μg of bacterial extracts was incubated with 30 to 40 μl of GST beads on ice for 30 min. Bound proteins were washed with buffer STE-100 (20 mM Tris-HCl [pH 7.6], 5 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA) supplemented with 0.5% NP-40 and 1% bovine serum albumin (BSA). Washed beads were then blocked in 250 μl of buffer STE-100 containing 1 mg of uninduced bacterial extract per ml, 1% BSA, 0.5% Carnation milk, and 100 μg of ethidium bromide per ml. Approximately 8×10^4 cpm of in vitro-translated and ^{35}S-labeled hSWI/SNF subunits was added to immobilized GST fusion proteins and incubated at 4°C for 12 to 16 h. Beads were washed three times with 300 μl of STE-100 containing 1% BSA and 0.5% Carnation milk, followed by two washes with STE-150, which contains 150 mM NaCl, and the retained proteins were analyzed by SDS-PAGE. For immunoprecipitation of in vitro-cotranslated proteins, 5×10^4 cpm was incubated with antibodies as described previously (51). Beads were then washed three times with 300 μl of washing buffer (20 mM Tris-HCl [pH 7.6], 5 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 0.05% NP-40, 1% aprotinin) before the retained proteins were analyzed by SDS-PAGE and detected by autoradiography.

Histone methylation and deacetylation assays. Histone methylation was performed with 2 μg of H1-depleted HeLa core histones. Each reaction contained 15 mM HEPES (pH 7.9), 5 mM $MgCl_2$, 20% glycerol, 1 mM ETDA, 0.25 mM dithiothreitol, and 2.75 μCi of [^{3}H]*S*-adenosyl methionine (Amersham Pharmacia Biotech., Inc.) in a total volume of 25 μl. Samples were incubated at 30°C for 1.5 h and separated on an SDS–18% polyacrylamide gel, and histones were visualized by Coomassie blue staining. Gels were treated with 1 M salicylic acid (pH 6.0), and methylated histones were detected by autoradiography. To measure the HDAC activity of immunopurified Brg1 and hBrm complexes, histone peptides were labeled as specified by the manufacturer (Upstate Biotechnology, Inc.). Equal amounts of each purified histone peptide were incubated with 0.25 to 0.5 μg of immunopurified hSWI/SNF complexes at 37°C for 2 h in a reaction containing 20 mM HEPES (pH 7.9), 20% glycerol, 100 mM KCl, 2 mM EDTA, and 0.5 mM phenylmethylsulfonyl fluoride. The released [^{3}H]acetate was extracted according to the manufacturer's instructions (Upstate Biotechnology, Inc.) and quantitated by scintillation counting.

Northern blot analysis. Total RNA was extracted from cells by the guanidium thiocyanate-phenol-chloroform protocol as previously described (62). To measure the mRNA levels, approximately 30 μg of total RNA was loaded in a 1% formaldehyde–agarose gel and transferred onto a Hybond-XL nylon membrane (Amersham Pharmacia Biotech, Inc.) as described previously (47). Membranes were blocked and hybridized using ExpressHyb solution (Clontech, Inc.) according to the manufacturer's protocol. Probes used to detect *cad* (forward primer, 5′-CTTAGTGCTCACTCCTCTGATCG-3′, and reverse primer, 5′-GGGATG AAGGTTCTGTTCCATTCTG-3′), *nuc* (5′-GAAAGCAGCTGTCACTCCAG GCAAA-3′ and 5′-TCATCGTCCTCATCCTCTGAGGCAG-3′), and *GAPDH* (5′-CTCAACTACATGGTTTACATGTTC-3′ and 5′-CCTTCCACGATACCA AAGTGGTCATG-3′) were synthesized by reverse transcription-PCR using the indicated primer pairs and 10 μg of total RNA from HeLa cells. Each probe was ^{32}P labeled and used at 1×10^6 to 2×10^6 cpm/ml.

Chromatin immunoprecipitation (ChIP) assay. Cross-linked chromatin was prepared as described previously (18). Briefly, cells were cross-linked with 1% formaldehyde and harvested in 1 ml of lysis buffer (50 mM Tris-HCl [pH 8.1], 100 mM NaCl, 5 mM EDTA, 0.5% SDS, protease inhibitors). Cells were collected by centrifugation and resuspended in 250 μl of IP buffer (100 mM Tris-HCl [pH 8.6], 5 mM EDTA, 0.3% SDS, 1.7% Triton X-100, protease inhibitors). Chromatin was solubilized to a bulk size of 0.25 to 2 kbp by sonication with a microtip Branson sonifier 450 and was immunoprecipitated by specific antibodies in the presence of 40 μl of preblocked protein A-Sepharose beads overnight at 4°C. Bound nucleoprotein complexes were washed once successively with 300 μl of

mixed micelle buffer (20 mM Tris-HCl [pH 8.1], 150 mM NaCl, 5 mM EDTA, 5% [wt/vol] sucrose, 0.2% SDS, 0.2% Triton X-100), buffer-250 (50 mM HEPES [pH 7.5], 250 mM NaCl, 1 mM EDTA, 0.1% deoxycholine, 0.2% Triton X-100), LiCl detergent buffer (10 mM Tris-HCl [pH 8.0], 250 mM LiCl, 1 mM EDTA, 0.5% deoxycholine, 0.25% NP-40), and Tris-EDTA (pH 7.5). Eluted DNA was PCR amplified with 100 pmol of *cad*-specific primers (forward, 5′-AGTCTCTG CTGCTGCCGCCAA-3′, and reverse, 5′-GAGAGGCGCATCACAGAGTGG GATAA-3′) or *odc*-specific primers (forward, 5′-GCTTTGTCAGTCCCTCCT GTAGCCG-3′, and reverse, 5′-ATCACCCTTATCCAGCCGCGGGAGAA-3′) in a 50-μl reaction containing 2 μCi of [α-P^{32}]dCTP for 35 cycles.

Antibodies and Western blot analysis. Proteins were electrophoresed on an SDS–8 to 10% polyacrylamide gel, transferred onto a nitrocellulose membrane, and detected by ECL according to the manufacturer's recommendations (Amersham Pharmacia Biotech, Inc.). Antibodies to hSWI/SNF subunits have been described previously (50, 51). Anti-PRMT5 rabbit polyclonal antibodies were raised against GST-PRMT5 (aa 4 to 637) by Covance, Inc. Anti-Myc, anti-Mad1, anti-mSin3A, and anti-Flag M2 antibodies were purchased from Santa Cruz, and anti-HDAC2 antibodies were purchased from Zymed.

RESULTS

Immunopurified Fl-Brg1 and Fl-hBrm complexes contain the type II protein arginine methyltransferase PRMT5. To enhance our understanding of how Brg1- and hBrm-based hSWI/SNF complexes regulate chromatin structure and affect transcription, we have conducted a thorough analysis of their subunits. Using stable cell lines that express Flag-tagged versions of Brg1 and hBrm, we immunopurified hSWI/SNF complexes (Fig. 1A). Affinity-purified hSWI/SNF peak fractions were concentrated by TCA precipitation, and the Brg1- and hBrm-associated subunits were visualized by Coomassie blue staining (Fig. 1B). Prominent bands were excised from the gels and subjected to in-gel tryptic digestion, and the resulting peptides were analyzed by MS (60).

All previously identified hSWI/SNF subunits were present in the Brg1 and hBrm fractions purified by this method. In addition, a previously unidentified 66-kDa polypeptide was found to be identical to the type II arginine methyltransferase protein PRMT5 (Fig. 1B), which can mono- or dimethylate arginine residues (6). Using polyclonal antibodies raised against full-length PRMT5 (predicted molecular mass, ~72 kDa), we detected a single band of the correct size in the immunopurified Brg1 and hBrm fractions, further confirming that PRMT5 and the Brg1- and hBrm-associated 66-kDa subunit are identical (Fig. 1C). PRMT5 was not detected when fractions affinity purified from HeLa nuclear extracts were used, indicating that its association with hSWI/SNF complexes is specific (Fig. 1C, lane 1). To further characterize the interaction between PRMT5 and Brg1 and hBrm complexes, we immunoprecipitated PRMT5-containing complexes from HeLa nuclear extracts by using either preimmune or immune anti-PRMT5 antibodies (Fig. 1D). After extensive washing, the retained proteins were analyzed by Western blotting. Both Brg1 and hBrm were immunoprecipitated only in the presence of anti-PRMT5 antibodies, indicating that PRMT5 is associated with endogenous hSWI/SNF complexes.

We and others have previously shown that hSWI/SNF complexes contain components of the mSin3A/HDAC corepressor complex (32, 51). Therefore, we tested whether anti-PRMT5 immune complexes contain mSin3A (Fig. 1D). Like Brg1 and hBrm, mSin3A was detected only in the presence of immune anti-PRMT5 antibodies, indicating that PRMT5-containing hSWI/SNF complexes also include mSin3A. To unambiguously demonstrate whether mSin3A and PRMT5 can interact with Brg1 and hBrm complexes, we immunoprecipitated partially purified hSWI/SNF complexes by using either preimmune, anti-Brg1, or anti-hBrm antibodies and tested for the presence of mSin3A and PRMT5 by Western blotting (Fig. 1E). Both mSin3A and PRMT5 were detected only in the presence of immune anti-Brg1 and anti-hBrm antibodies. Furthermore, purification of Flag-tagged hSWI/SNF complexes by glycerol gradient sedimentation revealed that Brg1 can be found in two distinct complexes, while hBrm cosediments with hSWI/SNF subunits in one major complex (51). To determine whether mSin3A and PRMT5 comigrate with hSWI/SNF complexes, we tested affinity- and glycerol gradient-purified Brg1 and hBrm complexes by Western blotting (Fig. 1F). mSin3A and PRMT5 were present in Brg1 complex I, as well as in the hBrm complex, demonstrating that both proteins coexist in the same Brg1- and hBrm-based hSWI/SNF complexes.

PRMT5 can directly interact with components of the mSin3A-containing Brg1 and hBrm complexes. To identify the subunits that mediate the interaction with PRMT5, we conducted GST pull-down experiments with full-length PRMT5. Both catalytic subunits, Brg1 and hBrm, were able to interact with GST-PRMT5 (Fig. 2A). Among the other hSWI/SNF subunits tested, only BAF57 and BAF45/Ini1 were able to interact with PRMT5. We also found that mSin3A can form a complex with GST-PRMT5. To confirm these results, we cotranslated PRMT5 with the interacting subunits and performed immunoprecipitation assays by using specific anti-hSWI/SNF and anti-mSin3A antibodies (Fig. 2B). PRMT5 coimmunoprecipitated with Brg1, hBrm, mSin3A, BAF57, and BAF45/Ini1, but not with BAF155. To show that the antibodies used were specific, we conducted immunoprecipitation experiments using in vitro-translated and ^{35}S-labeled PRMT5. We found that PRMT5 was immunoprecipitated only in the presence of anti-PRMT5 antibodies (Fig. 2C). These results demonstrate that PRMT5 can associate with mSin3A-containing Brg1 and hBrm complexes by establishing multiple contacts with specific hSWI/SNF subunits and mSin3A.

Recombinant and hSWI/SNF-associated PRMT5 methylate histones H3 and H4. Since PRMT5 contains a highly conserved region that is involved in *S*-adenosyl-L-methionine (SAM) binding and catalysis, we tested whether GST-PRMT5 can methylate free histones. Our results indicated that bacterially expressed GST-PRMT5 is inactive (data not shown). We have observed by Western blot analysis that immunopurified Brg1- and hBrm-associated PRMT5 does not migrate as a distinct band, but rather as a mixture of posttranslationally modified isoforms (Fig. 1C). Therefore, we generated a baculovirus for Sf9 expression of Flag-tagged PRMT5 and tested its ability to methylate HeLa core histones (Fig. 3A). When affinity-purified Fl-PRMT5 was incubated with the four core histones, it was able to methylate histones H3 and H4, suggesting that PRMT5 requires posttranslational modification in order to be active. Methylation of H3 and H4 was lost when catalytically inactive Fl-PRMT5 (G367A/R368A) was added to the reaction. When either immunopurified Fl-Brg1 or Fl-hBrm complex was incubated with all four core histones, only H3 and H4 were methylated. The addition of increasing amounts of wild-type Fl-PRMT5 to immunopurified Fl-Brg1 or Fl-hBrm complex enhanced H3 and H4 methylation. In stark contrast,

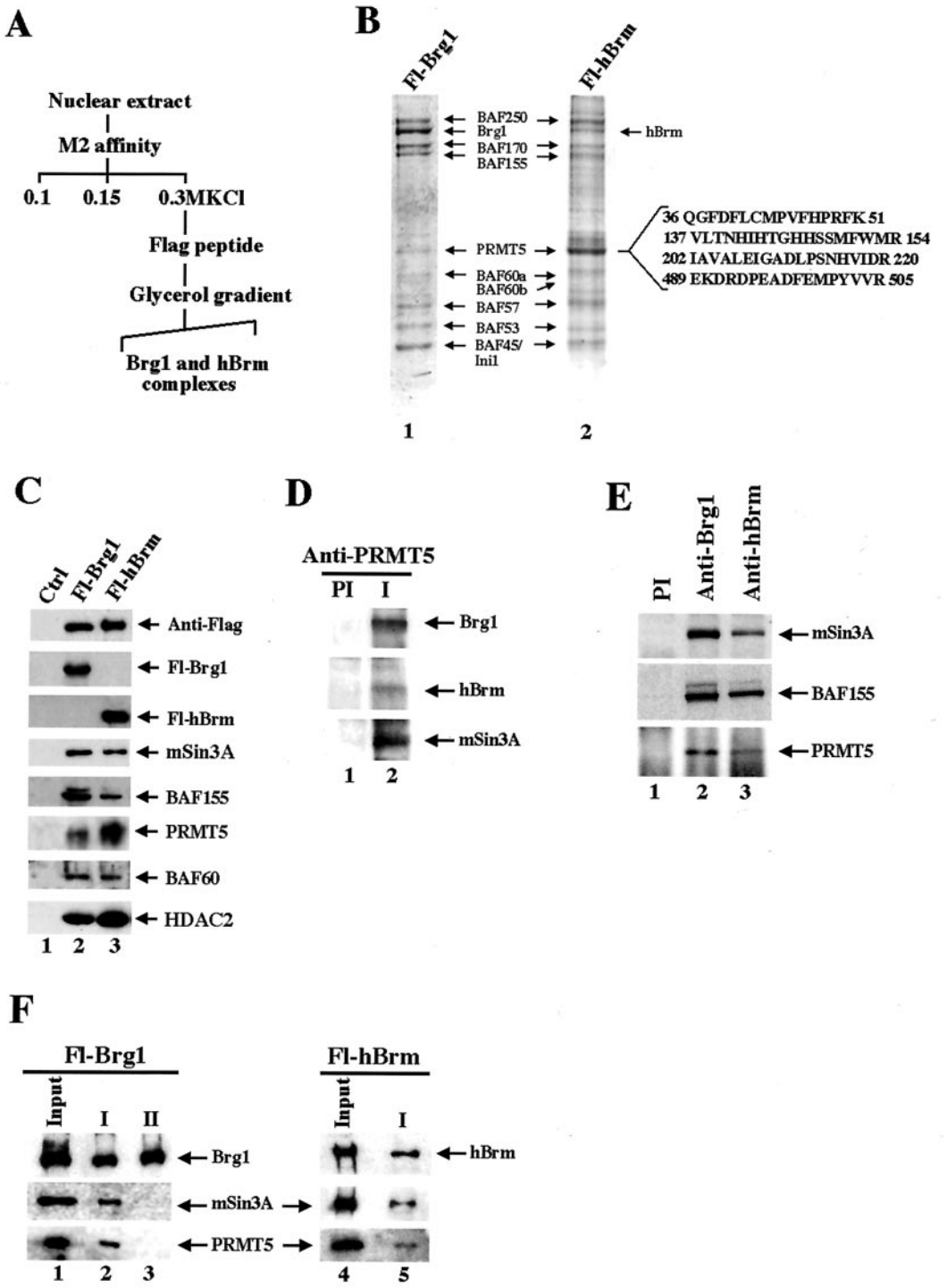

FIG. 1. Flag-tagged Brg1 and hBrm complexes contain PRMT5. (A) Scheme for purification of Flag-tagged Brg1 and hBrm complexes. (B) Coomassie blue-stained gels showing affinity-purified hSWI/SNF complexes. Arrows indicate bands that were excised and identified by MS. Amino acid sequences of 4 of the 13 peptides analyzed, which identify the 66-kDa polypeptide as PRMT5, are shown. (C) Antibodies raised against cloned PRMT5 recognize the hSWI/SNF-associated 66-kDa polypeptide. Approximately 250 ng of affinity-purified Flag-tagged Brg1 and hBrm complexes was analyzed by Western blotting using the indicated antibodies. (D) PRMT5 can specifically interact with mSin3A-containing hSWI/SNF complexes. HeLa nuclear extracts were incubated with either preimmune (PI) or immune (I) anti-PRMT5 antibodies, and the retained proteins were analyzed by Western blotting using anti-Brg1, anti-hBrm, or anti-mSin3A antibodies. (E) PRMT5 and mSin3A coexist in the same Brg1 and hBrm complexes. Partially purified hSWI/SNF complexes were incubated with either preimmune, anti-Brg1, or anti-hBrm antibodies, and protein complexes were analyzed by Western blotting using the indicated antibodies. (F) Affinity- and glycerol gradient-purified Brg1 and hBrm complexes were analyzed by Western blotting using 20 μl of either Brg1 complex I or II and hBrm complex. Input lanes 1 and 4 show affinity-purified Fl-Brg1 and Fl-hBrm complexes.

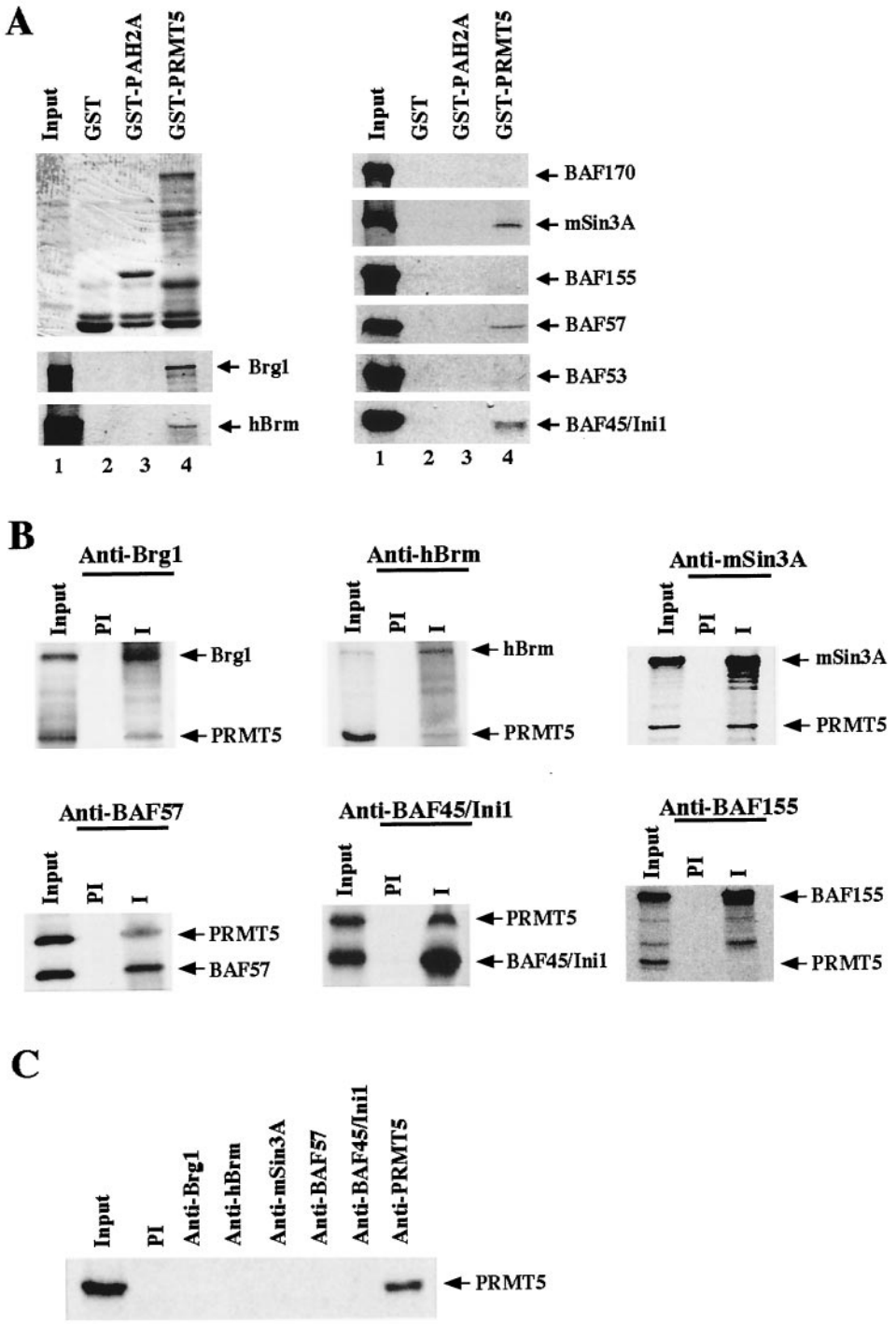

FIG. 2. PRMT5 can specifically interact with hSWI/SNF subunits and mSin3A. (A) In vitro-translated hSWI/SNF subunits and mSin3A were synthesized in the presence of [^{35}S]methionine-cysteine using the Promega TNT-coupled reticulocyte lysate as described previously (51) and were incubated with either GST (lane 2), GST-PAH2A (lane 3), or GST-PRMT5 (lane 4). For reference, 25% of the input is shown in lane 1. (B) PRMT5 cDNA was cotranslated with either Brg1, hBrm, mSin3A, BAF57, BAF45/Ini1, or BAF155 cDNA in the presence of [^{35}S]methionine-cysteine, and the protein mixtures were subjected to immunoprecipitation using preimmune (PI) and immune (I) antibodies as indicated. (C) Anti-hSWI/SNF and anti-mSin3A antibodies do not cross-react with PRMT5. In vitro-translated and ^{35}S-labeled PRMT5 was incubated with the indicated antibodies and the reactions were treated as for panel B. As a control, anti-PRMT5 immunoprecipitation is shown.

the addition of inactive Fl-PRMT5 did not change the levels of H3 and H4 methylation. Taken together, these results show that cloned PRMT5 and Brg1- and hBrm-associated PRMT5 target the same histones.

PRMT5-containing Brg1 and hBrm complexes also include mSin3A and HDAC2 (Fig. 1C to F). Since histone acetylation and deacetylation can influence histone methylation and vice versa, we compared the ability of hSWI/SNF-associated

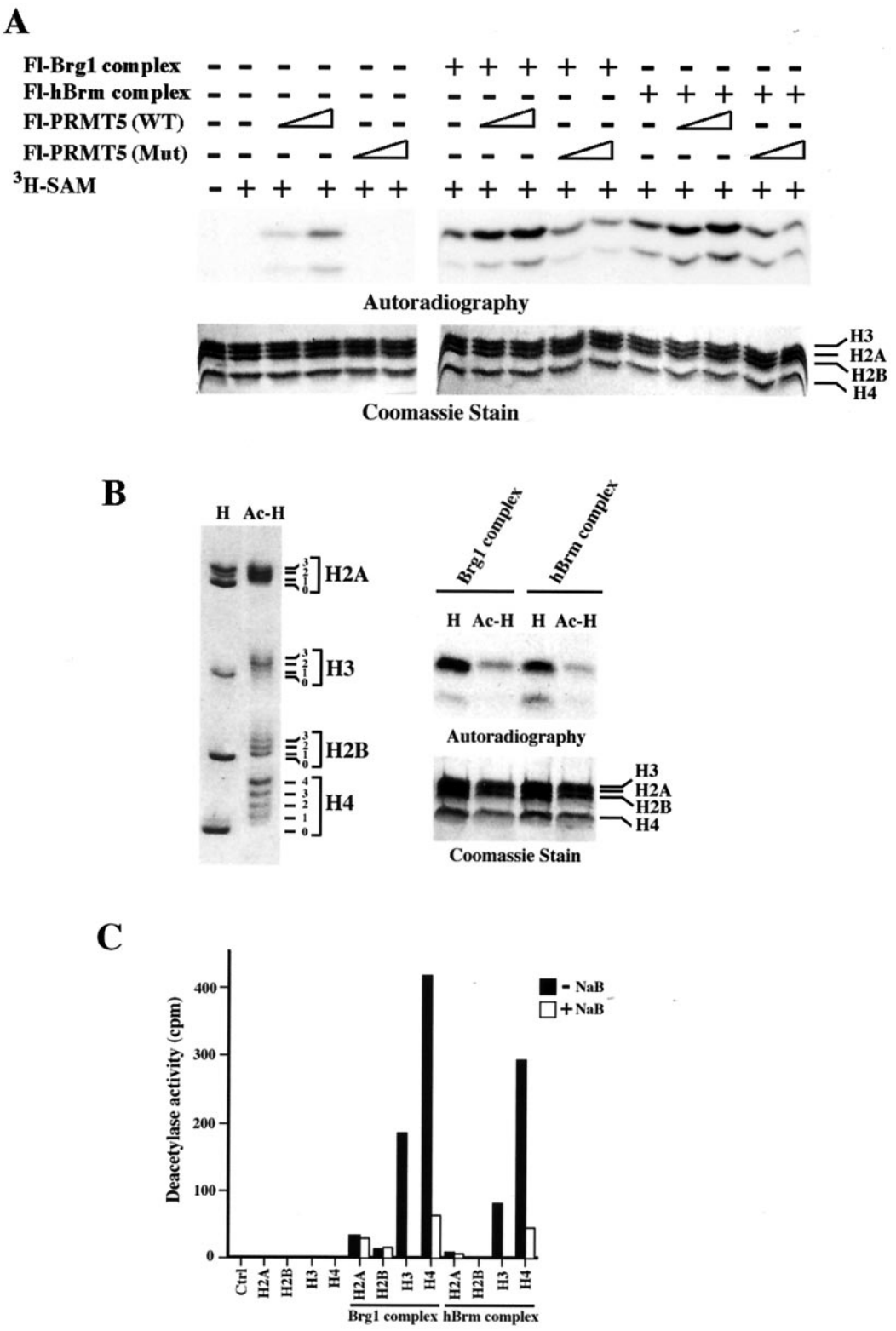

FIG. 3. Recombinant and hSWI/SNF-associated PRMT5 can methylate histones H3 and H4. (A) Histone methylation was performed by incubating HeLa core histones with increasing amounts (75 and 150 ng) of individually expressed and affinity-purified wild-type (WT) Fl-PRMT5 or mutant Fl-PRMT5 (G367A/R368A) as indicated. Similar amounts of either wild-type or mutant Fl-PRMT5 were added to reactions containing affinity-purified Brg1 (500 ng) or hBrm (250 ng) complex. (B) Brg1- and hBrm-associated PRMT5 can efficiently methylate hypoacetylated H3 and H4. The left panel shows separation of the different isoforms of HeLa core histones isolated from either asynchronous cells or cells treated with 10 mM sodium butyrate on a Triton-acetic acid-urea gel. The right panel shows methylation of hypoacetylated (H) and hyperacetylated (Ac-H) HeLa core histones using affinity-purified Brg1- and hBrm-associated PRMT5. (C) mSin3A/HDAC2- and PRMT5-containing Brg1 and hBrm complexes can deacetylate H3 and H4 peptides. Equal amounts of acetylated H2A, H2B, H3, or H4 peptide were incubated with or without affinity-purified Brg1 (500 ng) and hBrm (250 ng) complexes in the presence or absence of 20 mM sodium butyrate as indicated. Ctrl, reaction without acetylated peptides.

PRMT5 to methylate acetylated and nonacetylated histones (Fig. 3B). Both PRMT5-containing hSWI/SNF complexes methylated hypoacetylated H3 and H4 more efficiently than the hyperacetylated forms, indicating that histone deacetylation is required for efficient histone methylation. Consistent with these results, incubation of mSin3A/HDAC2- and PRMT5-containing Brg1 and hBrm complexes with equal amounts of labeled and acetylated histone N-terminal tails revealed that both hSWI/SNF complexes can specifically deacetylate H3 and H4 (Fig. 3C). These results show that the mSin3A/HDAC2- and PRMT5-containing Brg1 and hBrm complexes preferentially methylate deacetylated histones H3 and H4.

mSin3A and -B can directly interact with hSWI/SNF subunits. We have previously shown that individually expressed mSin3A can interact with Brg1 and hBrm ATPases (51). Both mSin3A and its homolog, mSin3B, contain four highly conserved PAH domains, which have been shown to mediate interactions with a wide variety of specific transcription factors (30). To determine whether any of the four PAH domains of mSin3A and mSin3B could directly interact with hSWI/SNF subunits, GST pull-down assays were used. When GST fusion proteins, which included individual PAH domains, were incubated with in vitro-translated and ^{35}S-labeled hSWI/SNF subunits, specific interactions were detected (Fig. 4A). Both Brg1 and hBrm were able to interact with the PAH3 and -4 domains of mSin3A and -B, whereas BAF57 interacted only with PAH4. Quantitation of these results indicated that the interaction of BAF57 with PAH4 was threefold stronger than that of Brg1 and hBrm with PAH4. In contrast, BAF170, BAF155, BAF53, and BAF45/Ini1 were unable to interact with any of the GST-PAH fusion proteins tested, although a weak interaction was observed between PAH4 and BAF45/Ini1, suggesting that the observed interactions are specific. These results and previous work, which showed that mSin3A can directly interact with HDACs 1 and 2 (33), suggest that the association of mSin3A/HDAC2 with hSWI/SNF complexes is mediated through direct interactions between PAH3 and PAH4 and specific hSWI/SNF subunits.

***cad* expression is altered in the presence of either catalytically inactive Brg1 or the HDAC inhibitor depsipeptide.** We have found that PRMT5 and mSin3A/HDAC2 can form a complex with hSWI/SNF chromatin remodelers by directly interacting with Brg1, hBrm, BAF57, and BAF45/Ini1 (Fig. 1, 2, and 4A). To understand the relevance of these interactions, we sought to determine whether Brg1 and hBrm complexes could be recruited to genes known to be regulated by the mSin3A/HDAC corepressor complex. Based on previous work which showed that Mad-Max heterodimers can repress transcription by recruiting mSin3A/HDAC (2, 3), we hypothesized that Brg1 and hBrm complexes might play a role in repression of Myc/Max/Mad target genes. We reasoned that if chromatin remodeling were required for the establishment of a repressed state, hSWI/SNF complexes would be recruited to the promoter region; however, in cells that express inactive Brg1 or hBrm, repression would be lost or decreased.

For Myc target genes, we chose to analyze *cad* and *nuc*, which are repressed in the absence of serum and are induced when c-Myc levels increase (8). Both wild-type HeLa cells and HeLa cells that express catalytically inactive Fl-Brg1 (K798R) were synchronized by serum starvation, and total RNA was examined by Northern blot analysis (Fig. 4B). Experiments using catalytically inactive hBrm were unsuccessful, because serum starvation reduced mutant Fl-hBrm levels severalfold (A. Datta and S. Sif, unpublished results). Fluorescence-activated cell sorting analysis revealed that 85 to 88% of wild-type and mutant cells were blocked in G_1 by serum starvation (data not shown). When we examined the expression profile of *cad* in wild-type HeLa cells, we found that there was a three- to fourfold increase in *cad* mRNA after serum stimulation (Fig. 4B and C). The levels of *cad* mRNA decreased to basal levels by 14 to 16 h, consistent with results obtained with other cell lines, including NIH 3T3 and Rat1 fibroblasts (8, 34). In the presence of inactive Brg1, *cad* was derepressed threefold under conditions that should have induced silencing (Fig. 4B and C, compare HeLa and mutant Brg1 *cad* mRNA levels at 0, 14, and 16 h). To test whether *cad* repression was dependent on histone deacetylation, wild-type HeLa cells were synchronized by serum starvation and then subjected to depsipeptide (a cyclic tetrapeptide HDAC inhibitor) treatment. Quantitation of *cad* mRNA after depsipeptide treatment revealed that there was a six- to eightfold derepression at all times (Fig. 4B and C). Taken together, these results show that chromatin remodeling and histone deacetylation are involved in *cad* transcriptional repression.

To determine whether expression of mutant Brg1 had a global effect on other Myc/Max/Mad target genes, we examined *nuc* mRNA levels with the same blot used to detect *cad*. Despite the delayed kinetics of induction in the presence of mutant Brg1, *nuc* repression was unaffected both at the onset of G_1 arrest and after cells had traversed S phase (Fig. 4B and C, compare HeLa and mutant Brg1 *nuc* mRNA levels at 0 and 16 h). In marked contrast, depsipeptide treatment resulted in a three- to fourfold derepression, suggesting that histone deacetylation is required for *nuc* repression. Expression of mutant Brg1 and depsipeptide treatment had no noticeable effects on *GAPDH* expression (Fig. 4B and C). These results show that not all Myc/Max/Mad target genes require Brg1 complexes for their repression.

c-Myc can interact with hSWI/SNF complexes by targeting Brg1, hBrm, BAF57, and BAF45/Ini1. Having found that *cad* induction was deficient in cells expressing mutant Brg1 (Fig. 4C, compare fold induction between HeLa and mutant Brg1 at 0 and 6 h), we tested whether the Brg1-based hSWI/SNF complex could directly interact with c-Myc. Previous work has shown that c-Myc can interact with BAF45/Ini1 and transactivate E-box-containing reporter genes in a Brg1-dependent manner (10). To verify these results and to determine whether Brg1- and hBrm-based hSWI/SNF complexes can interact with c-Myc, we incubated GST-Myc with HeLa nuclear extracts and tested for the presence of hSWI/SNF subunits by Western blotting (Fig. 5A, lanes 1 to 4). Components of the Brg1 and hBrm complexes were detected only in the presence of GST-Myc, but not with GST alone or GST-PAH2A, which includes a domain that is known to mediate interaction of mSin3A with various transcription factors (30). Similarly, when anti-BAF45/Ini1, anti-BAF57, or anti-BAF155 antibodies were used to immunoprecipitate ^{35}S-labeled HeLa nuclear extracts, c-Myc was detected only in the presence of immune, not preimmune, antibodies (S. Pal and S. Sif, unpublished results).

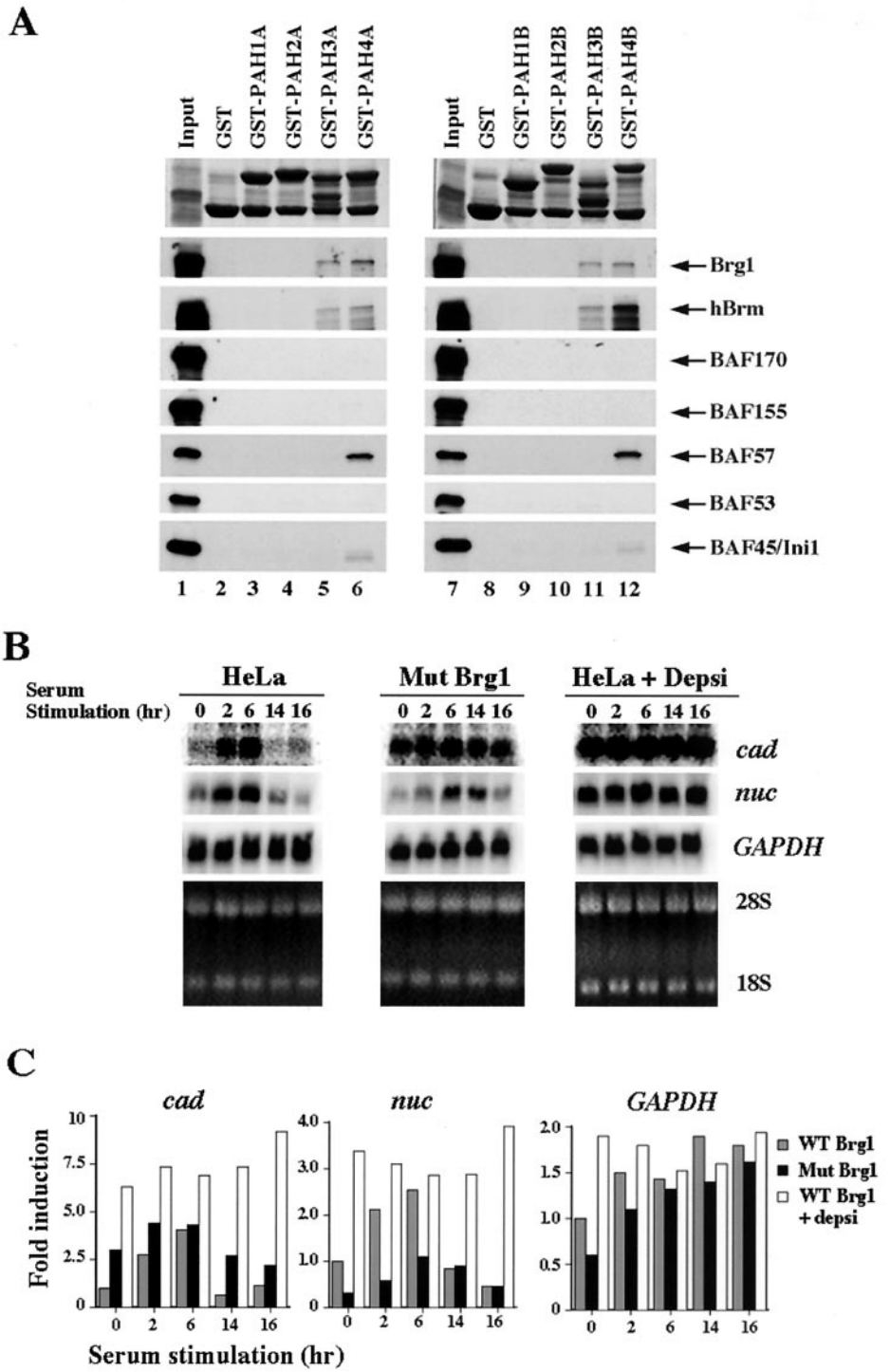

FIG. 4. Brg1 chromatin remodeling and histone deacetylation are essential for efficient repression of the Myc target gene *cad*. (A) Both isoforms of mSin3 can interact with hSWI/SNF subunits. Approximately 1 to 2 μg of either GST (lanes 2 and 8), GST-mSin3A PAH1 through -4 (lanes 3 to 6), or GST-mSin3B PAH1 through -4 (lanes 9 to 12) was immobilized on GST beads and tested for the ability to interact with the indicated ^{35}S-labeled hSWI/SNF subunits. The upper panels show Coomassie blue-stained gels of the GST and GST-PAH fusion proteins. (B) Cells were serum starved as described in Materials and Methods, and total RNA was isolated from either wild-type HeLa cells, HeLa cells that express catalytically inactive Brg1 (Mut Brg1), or HeLa cells treated with 60 nM depsipeptide (depsi) at the indicated times. Northern blot analysis was performed using equal amounts of total RNA, and mRNA was detected by use of ^{32}P-labeled cDNA probes. (C) Bar graphs show quantitation of the bands shown in panel B. Fold induction for each gene is reported as the ratio of the total number of counts for each sample to that for the wild-type uninduced sample (0 h). Quantitation of mRNA was performed with a Molecular Dynamics PhosphorImager. To control for sample loading, 18S and 28S rRNAs were visualized by ethidium bromide staining.

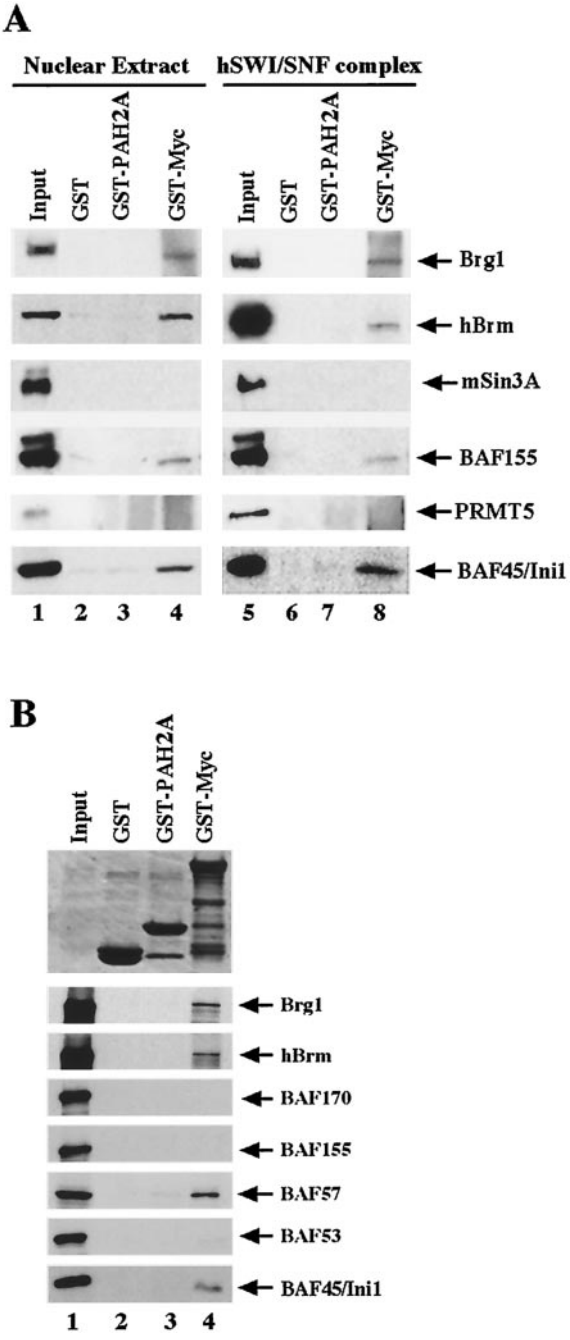

FIG. 5. c-Myc can directly interact with Brg1 and hBrm complexes. (A) Approximately 1 to 2 μg of either GST, GST-PAH2A, or GST-Myc was incubated with 2 mg of HeLa nuclear extracts (lanes 2 to 4) or 500 ng of immunopurified PRMT5- and mSin3A-containing hSWI/SNF complexes (lanes 6 to 8), and the indicated subunits were detected by Western blotting. Input lanes 1 and 5 show 1 and 50%, respectively, of the total amount of proteins used for each experiment. (B) c-Myc can associate with hSWI/SNF complexes by directly interacting with Brg1, hBrm, BAF57, and BAF45/Ini1. In vitro ^{35}S-labeled hSWI/SNF subunits were incubated with either GST (lane 2), GST-PAH2A (lane 3), or GST-Myc (lane 4), and bound proteins were detected by autoradiography. The input lanes represent 10% of the total amount of protein used in each reaction.

Because c-Myc is known to interact with histone-modifying activities via TRRAP (36, 37), we examined whether immunopurified hSWI/SNF complexes, which lack TRRAP, can still interact with GST-Myc (Fig. 5A, lanes 5 to 8, and data not shown). All hSWI/SNF subunits detected by use of crude nuclear extracts were present in the reaction containing GST-Myc (lane 8). PRMT5 and mSin3A were not detected in GST-Myc pull-down assays when either HeLa nuclear extract or affinity-purified hSWI/SNF complexes were used (Fig. 5A). These results demonstrate that c-Myc can directly interact with hSWI/SNF complexes that lack PRMT5 and mSin3A. To identify the hSWI/SNF subunits that can interact with c-Myc, individually translated and ^{35}S-labeled hSWI/SNF subunits were incubated with immobilized GST-Myc and analyzed by SDS-PAGE. Brg1, hBrm, BAF57, and BAF45/Ini1 were all able to specifically interact with GST-Myc, but not with GST or GST-PAH2 (Fig. 5B). BAF170, BAF155, and BAF53 did not interact with any of the GST fusion proteins tested. Also note that Brg1, hBrm, BAF57, and BAF45/Ini1 failed to interact with other GST fusion proteins, including GST-ERK2 and GST-MEK1 (data not shown). Collectively, these results suggest that c-Myc can associate with Brg1 and hBrm complexes by physically interacting with the same hSWI/SNF subunits targeted by mSin3A and PRMT5.

Brg1, HDAC2, and PRMT5 are recruited to the *cad* promoter. Northern blot analysis suggests that chromatin remodeling and histone deacetylation are required for efficient *cad* repression. These results do not rule out the possibility that this could be an indirect effect. Using ChIP assays, we tested whether components of the Brg1-based hSWI/SNF and mSin3A/HDAC2 complexes could be directly recruited to the *cad* promoter (Fig. 6). Previous work has shown that *cad* induction is abolished in c-*myc* null cells (8). Therefore, we wanted to determine exactly when c-Myc and Mad1 interacted with the *cad* promoter (Fig. 6A). Using specific antibodies, we found that c-Myc associated with the promoter region maximally between 2 and 6 h after serum stimulation, while optimal binding by Mad1 was restricted to 0 and 16 h, although some binding was detected at 14 h. Based on the Northern blotting results, binding of c-Myc and Mad1 correlated with *cad* induction and repression, respectively (compare Fig. 4B and 6A).

When we analyzed recruitment of the Brg1-based hSWI/SNF complex in wild-type HeLa cells or HeLa cells expressing mutant Brg1, we found that wild-type and mutant Brg1 associated with the *cad* promoter with similar kinetics. When cells were serum starved and *cad* was repressed (Fig. 4B, 0 h), both wild-type and mutant Brg1 were found at the promoter region (Fig. 6A, 0 h). Recruitment of both ATPases increased after serum stimulation; however, wild-type and mutant Brg1 remained associated with the *cad* promoter even after *cad* transcription had been reduced to basal levels (compare Fig. 4B and 6A, 14 and 16 h), consistent with the possibility that the Brg1-based hSWI/SNF complex is involved in *cad* transcriptional repression. When we tested another Myc/Max/Mad target gene, we found that although c-Myc associated with the *ornithine decarboxylase* (*odc*) promoter 14 to 16 h after serum induction, there was a lack of Brg1 recruitment (Fig. 6B). Thus, we conclude that the Brg1-based hSWI/SNF complex is recruited specifically to the *cad* promoter.

Association of mSin3A and HDAC2 with the *cad* promoter

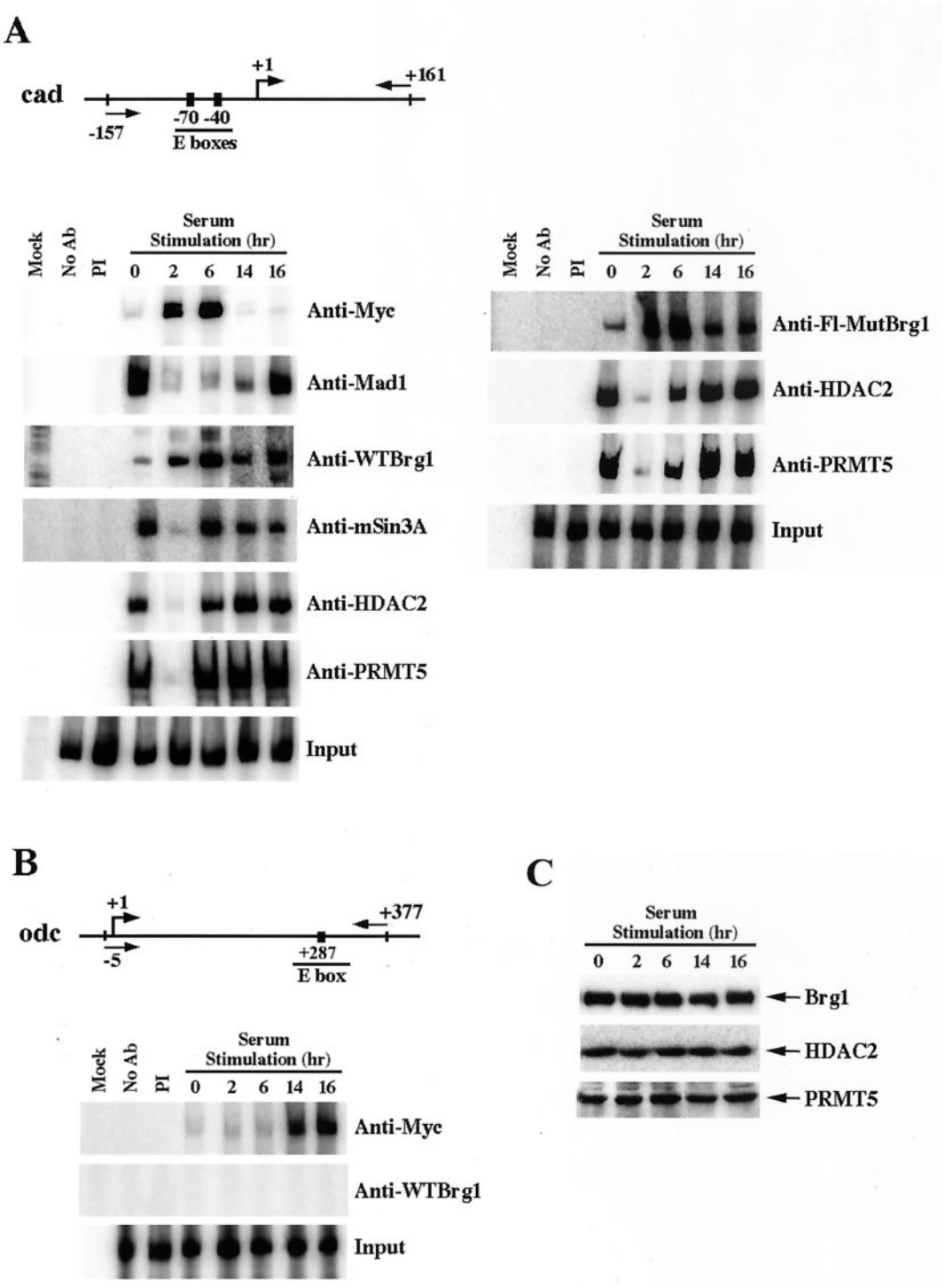

FIG. 6. Cell cycle-dependent recruitment of wild-type and mutant Brg1, mSin3A/HDAC2, and PRMT5 to the *cad* promoter. (A) Schematic representation of the *cad* promoter region showing the positions of the primer pair used to PCR amplify the sequences from −157 to +161. Black boxes (E boxes) depict Myc-Max and Mad-Max DNA binding sites. ChIP assays were performed using chromatin from either HeLa cells (anti-Myc, anti-Mad1, anti-WTBrg1, anti-mSin3A, anti-HDAC2, and anti-PRMT5) or HeLa cells that express Flag-tagged mutant Brg1 (anti-Fl-Mut Brg1, anti-HDAC2, and anti-PRMT5). Chromatin was prepared either after 48 h of serum starvation (0 h) or after serum stimulation for the indicated times. Input lanes represent 1% of the total chromatin used in each reaction. As controls, mock, which represents samples without chromatin, no antibody (no Ab), and preimmune (PI) reactions are shown. The total DNA used in the input reactions was diluted 300-fold, and because there was no variation in input lanes only two representative gels are shown. (B) Schematic representation of the *odc* promoter region showing the primers used to PCR amplify the sequences from −5 to +377. ChIP assays were performed using the indicated antibodies. (C) Nuclear extracts were prepared from either cells blocked in G_1 (0 h) or cells blocked and then released for the indicated times. Approximately 20 μg of nuclear extracts was analyzed by SDS-PAGE, and proteins were detected by using anti-Brg1, anti-HDAC2, and anti-PRMT5 antibodies.

was detected at 0 h but was lost when cells were stimulated to enter S phase (Fig. 6A, 2 h). Recruitment of mSin3A and HDAC2 to the promoter region was not detected until 6 h after serum stimulation, a time point when Brg1 was already associated with the *cad* promoter. Both Brg1 and mSin3A/HDAC2 remained associated with the promoter region 16 h after serum stimulation. Similar results were observed for cells that express catalytically inactive Brg1 (Fig. 6A, right panel). These results and our findings, which show that mSin3A can directly interact with Brg1 and BAF57 (Fig. 1 and 4A), suggest that mSin3A/HDAC2 is recruited to the *cad* promoter through direct interaction with the Brg1-based hSWI/SNF complex.

Recently, PRMT5 has been implicated in transcriptional repression of *cyclin E1* (15). Because we have found PRMT5 in association with the mSin3A/HDAC2-containing Brg1 complex (Fig. 1) and because PRMT5 is able to methylate hypoacetylated histones H3 and H4 (Fig. 3B), we wanted to assess its involvement in *cad* regulation. We found that in both wild-type and mutant Brg1 cell lines, PRMT5 was recruited to the *cad* promoter concomitantly with mSin3A/HDAC2 (Fig. 6A). PRMT5 remained associated with the *cad* promoter 16 h after release from the G_1 block, indicating that it is involved in *cad* repression. To exclude the possibility that differential recruitment of Brg1, HDAC2, and PRMT5 to the *cad* promoter is not due to variations in protein expression, we examined their levels at different time points (Fig. 6C). All three chromatin-modifying enzymes were expressed equally at all times. These results and the protein-protein interaction studies, which show that mSin3A and PRMT5 can interact with each other and form a complex with specific hSWI/SNF subunits, suggest that the Brg1-based hSWI/SNF complex is able to recruit mSin3A/HDAC2 and PRMT5 to promote *cad* repression.

DISCUSSION

In this study, we show that Brg1- and hBrm-based hSWI/SNF complexes contain the type II arginine methyltransferase PRMT5 and that both recombinant and hSWI/SNF-associated PRMT5 proteins are able to methylate hypoacetylated histones H3 and H4. We also provide evidence that links the Brg1-based hSWI/SNF complex, PRMT5, and mSin3A/HDAC2 to transcriptional repression of the Myc target gene *cad*. Our findings suggest that one mechanism by which PRMT5 and mSin3A/HDAC2 repress *cad* is by directly interacting with the same hSWI/SNF subunits targeted by c-Myc. Therefore, when c-Myc levels increase either upon mitogenic stimulation or, as is the case for many cancers, by aberrant expression of c-*myc*, interaction of hSWI/SNF complexes with repressor activities such as PRMT5 and mSin3A/HDAC would be minimized. As a result, c-Myc would be able to recruit repressor-free hSWI/SNF complexes and induce transcription of its target genes. The purification of hSWI/SNF complexes that lack c-Myc and the absence of PRMT5 and mSin3A in GST-Myc pull-down assays using either HeLa nuclear extract or affinity-purified hSWI/SNF complexes, which contain mSin3A and PRMT5, support this notion and suggest that the interactions of c-Myc and mSin3A/HDAC2-PRMT5 with hSWI/SNF complexes are mutually exclusive (Fig. 5A). Moreover, the ChIP results which show decreased association of PRMT5 and mSin3A/HDAC2 with the *cad* promoter 2 h after serum stimulation and increased recruitment of Brg1 in the presence of c-Myc further substantiate this theory (Fig. 6A). After 6 h of serum stimulation, it appears that mSin3A/HDAC2 and PRMT5 colocalize with c-Myc and Brg1 on the *cad* promoter. This time point is reflective of the dynamic changes that occur at the promoter prior to the onset of *cad* repression. As c-Myc levels decrease, association of the Brg1-based hSWI/SNF complex with mSin3A/HDAC2 and PRMT5 is restored, thereby bringing back the repressed state, which becomes established at 14 to 16 h post-serum stimulation.

Recent studies have shown that histone methylation can be influenced by other modifications, such as acetylation, phosphorylation, and ubiquitination (29). In addition, there appears to be a certain level of cross talk between histone tails. For instance, ubiquitination of H2BK123 by the ubiquitin-conjugating enzyme Rad6 (Ubc2) has been shown to promote methylation of H3K4 by SET1 and to induce silencing (55). Currently, it is not known whether arginine methylation by PRMTs can be affected by other histone modifications. We have shown that the mSin3A/HDAC2 and PRMT5-containing Brg1 and hBrm complexes are able to deacetylate the N-terminal tails of histones H3 and H4 but not H2A and H2B (Fig. 3C). Furthermore, we have found that the hSWI/SNF-associated PRMT5 is able to efficiently methylate hypoacetylated histones H3 and H4 (Fig. 3B), suggesting that histone lysine deacetylation enhances H3 and H4 arginine methylation. Although the histone arginine residues targeted by PRMT5 are still not known, we have been able to show that PRMT5 and HDAC2 are concomitantly recruited to the *cad* promoter and that HDAC2 inhibition abolishes *cad* repression in vivo (Fig. 4B), indicating that histone deacetylation plays an important role in *cad* transcriptional repression. In vivo experiments aimed at addressing the role played by PRMT5 in *cad* repression were unsuccessful, because the addition of high concentrations (700 μM) of the methyltransferase inhibitor 5′-methylthioadenosine failed to inactivate the Brg1- and hBrm-associated PRMT5 (Pal and Sif, unpublished results). Currently, we are establishing a PRMT5 antisense cell line to rigorously address the role played by this arginine methyltransferase in *cad* regulation.

The biochemical association of PRMT5 and mSin3A/HDAC2 with Brg1- and hBrm-based hSWI/SNF complexes suggests that these histone-modifying and chromatin-remodeling activities are functionally related. We have observed that association of Brg1 with the *cad* promoter fluctuated as cells were either arrested in G_1 or stimulated to enter S phase. Although recruitment of Brg1 was reduced upon serum starvation, we were still able to detect its association with the *cad* promoter, but not with the *odc* promoter (Fig. 6B). Consistent with this result, Brg1 remained associated with the promoter region even when *cad* mRNA induction was reduced to basal levels (Fig. 4B and 6A, 14 and 16 h), indicating that the Brg1-based hSWI/SNF complex is involved in *cad* transcriptional repression. It is well established that c-Myc can recruit the histone acetyltransferase hGCN5 via TRRAP (36, 37). It is also known that c-Myc can induce *cad* expression by increasing acetylation of nucleosomal histone H4 at the promoter region (18). Using primers that flank the same region examined by Frank et al. (18), we have found that upon serum stimulation

there was an increase in c-Myc binding which was accompanied by a complete loss of PRMT5 and mSin3A/HDAC2 (Fig. 6A). Recruitment of PRMT5 and mSin3A/HDAC2 was not detected until 6 h poststimulation, a time point when Brg1 is associated with the *cad* promoter. Therefore, it is conceivable that the Brg1-based hSWI/SNF complex plays a dual role during *cad* transcription. Through its ability to interact with c-Myc, the Brg1 complex is able to promote *cad* transcriptional activation. However, as cells are either blocked in G_1 or traverse S phase and c-Myc levels decrease, PRMT5 and mSin3A/HDAC2 are recruited by directly interacting with the same hSWI/SNF subunits targeted by c-Myc to promote *cad* transcriptional repression.

Inactivation of Brg1, hBrm, BAF57, and BAF45/Ini1 has been linked to a wide variety of cancers, including leukemias, lymphomas, breast and lung cancers, and rhabdoid sarcomas (11, 45, 58, 61). Protein-protein interaction studies indicate that Brg1, hBrm, BAF57, and BAF45/Ini1 can directly interact with mSin3A and PRMT5 (Fig. 2 and 4A). In addition, affinity purification of Brg1 and hBrm complexes containing PRMT5 and mSin3A/HDAC2 strongly suggests that Brg1, hBrm, BAF57, and BAF45/Ini1 play a major role in tethering these corepressor activities to the promoter region of target genes. Thus, it is possible that interaction with PRMT5 and mSin3A/HDAC2 corepressor complexes is essential for the tumor suppressor function of Brg1- and hBrm-based hSWI/SNF complexes.

Brg1 and hBrm complexes are highly related to each other and can disrupt nucleosome structure and increase accessibility to nucleosomal DNA and histones. Although both complexes contain ATPases that are related and associate with similar corepressor activities, they do show some differences. For example, Brg1 and hBrm complexes purified from asynchronous cells remodel nucleosomes with different specific activities (51). Moreover, both complexes are regulated differently by phosphorylation (38, 50). Gene knockout experiments have shown that Brg1 and hBrm have different effects on cell growth and proliferation. Brg1 cannot be deleted unless it is expressed from an ectopic source (7, 54), whereas hBrm is dispensable for cell growth since deletion of both alleles has no effect on viability (46). Thus, it appears that although there is some redundancy between Brg1 and hBrm complexes, each complex might perform distinct functions that contribute to normal cell growth and proliferation.

We have shown that Brg1- and hBrm-based hSWI/SNF complexes can interact with mSin3A and PRMT5 (Fig. 1, 2, and 4A). We have also shown that *cad* repression is altered in the presence of catalytically inactive Brg1 (Fig. 4B) and that this is due to the direct recruitment of mutant Brg1 to the *cad* promoter region (Fig. 6). Using cell lines that express mutant hBrm, we have been unable to determine whether hBrm has any effect on *cad* gene expression because serum starvation decreased hBrm protein levels (Datta and Sif, unpublished results). The lists of direct target genes regulated by Brg1 and hBrm complexes remain unknown. However, recent studies have shown that hSWI/SNF complexes and their associated histone-modifying enzymes are involved in transcriptional repression of cell cycle regulators such as cyclins A, D1, and E (15, 64, 67). Based on these studies and the findings which show that mutation of hSWI/SNF subunits are associated with cancer, it is going to be important to identify more Brg1 and hBrm direct target genes, elucidate the mechanism by which Brg1- and hBrm-based hSWI/SNF complexes contribute to their regulation, and study how changes in their expression might influence cell growth and proliferation.

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