REST Repression of Neuronal Genes Requires Components of the hSWI-SNF Complex

A function of the transcription factor REST is to block the expression of neuronal phenotypic traits in non-neuronal cells. Previous studies have shown that REST-mediated repression requires histone deacetylase activity and that recruitment of deacetylases is mediated by two co-repressors, Sin3A and CoREST. In this study, we show that a repressor domain in CoREST interacts with BRG1-associated factor (BAF) 57, a component of the hSWI-SNF complex. In vivo, BAF57 occupies the neuronal sodium channel gene (Nav1.2) promoter, and targeting to this gene requires REST. In addition to BAF57, the ATPase BRG1 and BAF170, other members of the hSWI-SNF complex, are also present in the REST/CoREST repressor complex. Microinjection of specific antibodies against BRG1, BAF57, or BAF170 into Rat1 fibroblasts relieves repression of RE1 reporter genes. Together, our data suggest that ATP-dependent chromatin remodeling, as well as histone deacetylation, is needed for REST-mediated repression.

The acquisition and the maintenance of cell phenotype depend upon precisely controlled transcriptional events. For neurons, intrinsic repressor mechanisms are important for controlling multiple steps in the differentiation program, from neural induction to neural specification. In addition, an extrinsic repression mechanism, which correlates with modification of chromatin structure for at least one gene (1), blocks expression of neuronal traits outside the nervous system. The transcriptional repressor, REST (also called NRSE) (2, 3), blocks expression of neuronal-specific genes in non-neuronal cells by binding to a conserved 23-bp sequence, RE1 (repressor element 1; also called NRSE) (2–7). Because ectopic expression of REST target genes, such as voltage-dependent ion channels and synaptic proteins, is likely to have deleterious effects in non-neuronal cells, there has been much interest in determining the long term repressor mechanism underlying REST function.

REST is a modular protein that represses via two distinct repressor domains. Repression from the amino terminus is mediated by a Sin3-histone deacetylase (HDAC) complex (8–12), whereas repression from the carboxyl-terminal domain is mediated by the CoREST protein (7, 13). CoREST is in complexes with class I HDACs (1 and 2) both in non-neuronal and neuronal cell lines (7, 14, 15). Like REST, CoREST contains two repressor domains. The amino-terminal domain coincides with the REST binding site and is dependent upon HDAC activity for repression (7, 14). Trichostatin A treatment, as well as HDAC2 antibody microinjection experiments, suggest that the carboxyl-terminal repressor domain is also associated with HDAC activity (7).

It is not known why REST needs two distinct repressor domains if each recruits HDAC activity. One possibility is that CoREST recruits an additional activity specifically involved in maintaining long term repression. Indeed, it is now very well accepted that multiple covalent and non-covalent chromatin modifications are required for proper gene expression. The best studied covalent modifications occur on histones and include acetylation, methylation, phosphorylation, and ubiquitination (16, 17). In particular, hypoacetylated histones have been associated primarily with repressed or silenced genes, and many bona fide co-repressor complexes have been described that contain HDAC activity (18). Non-covalent modifications are energy-dependent chromatin modifications made by ATP-dependent chromatin-remodeling complexes, such as the SWI-SNF complex. The mammalian SWI-SNF complex is a family of 2-MDa multisubunit ATP-dependent chromatin-remodeling complexes that exists in a variety of biochemically diverse forms (19, 20). Central to the activity is either the ATPase BRG1 or BRM that serve as the catalytic components. Although ATP-dependent chromatin-remodeling activity was associated originally with transcriptional activation, it has become increasingly clear that remodeling activity is also involved in transcriptional repression. For example, microarray analysis of both yeast and human SWI-SNF mutants result in both repression and de-repression of genes (21–23). More specifically, the human homologue of yeast SWI-SNF (hSWI-SNF) has been implicated in repression of the c-fos gene (24) and is required for retinoblastoma-mediated repression during the cell cycle (25, 26). The Mi-2/NUD complex contains both HDACs and ATP-dependent chromatin-remodeling components, suggesting that these activities can be coupled to regulate transcription in vivo (27, 28). Furthermore, two mammalian co-repressors, Sin3 and NcoR, have been identified in complexes with hSWI-SNF.
components (29–31). Most recently, BAF57, a subunit of the hSWI-SNF complex, has been demonstrated to be required for the silencing of the CD4 gene (32).

To test whether a CoREST co-repressor complex might also recruit remodeling components, in addition to HDAC, to a specific RE1 sequence, we performed a two-hybrid screen in yeast using the carboxyl-terminal repressor domain of CoREST as bait. We found that this domain interacted with a component of the mammalian SWI-SNF complex, the high mobility group (HMG) protein, BAF57 (33). Further analysis showed that CoREST, as well as REST, was in complexes with two additional hSWI-SNF components, BRG1 and BAF170. When this association was perturbed in living cells by the microinjection of specific antibodies against BRG1, BAF57, or BAF170, repression of RE1-containing reporter genes was relieved. Furthermore, BAF57 was associated with the RE1 element of the endogenous brain sodium channel gene Nav1.2 in vivo, and this association occurred only in the presence of REST. Our findings suggest that the hSWI-SNF complex plays an important role in REST-mediated repression of neuronal genes, most likely through its ATP-dependent chromatin-remodeling activity.

**EXPERIMENTAL PROCEDURES**

### Plasmid Construction

**Yeast Two-hybrid Constructs—** pbTMCoREST, a fusion between the LexA DNA binding (LexA) domain with full-length CoREST, pbTMCoREST–1–293, and pbTMKIA containing the LexA DNA binding domain, respectively, fused to amino acids 1–293 and amino acids 102–482 of CoREST, have been described previously (9, 13). A fusion of the LexA DNA binding domain to CoREST fragments encompassing amino acids 292–381, amino acids 292–354, and amino acids 373–442 were made by cloning a BamHI/SacI-blunt fragment of Gal4 CoREST (292–381, 292–354, and 373–442, respectively) into pbMT116 cut with BamHI/PolI-blunt. A fusion of the LexA DNA binding domain and CoREST fragments encompassing amino acids 187–442 and amino acids 291–442 were obtained by cloning into pbTMT116 by standard PCR cloning techniques. pGADBAF57 and pGADBAF57v were obtained by cloning full-length BAF57 and full-length GSTBAF57v into pGEX-3X by standard PCR cloning techniques. pGADBAF57 and pGADBAF57v were obtained by cloning full-length BAF57 and full-length BAF57v from pbLU/EBAF57 and pbLU/EBAF57v into the BamHI/EcoRI restriction sites in pGAD4. Fusions between the activation domain of Gal4 (Gal4AD) and different fragments of BAF57 proteins were obtained by standard PCR techniques and were named as follows: pGADBAF57 (1–280), pGADBAF57 (1–218), pGADBAF57 (105–363), pGADBAF57v (219–363), pGADBAF57 (219–280), pGADBAF57 (219–249), and pGADBAF57 (258–280).

**GST Fusion Constructs for Bacterial Expression—** Full-length GST-BAF57 and full-length GSTBAF57v were cloned into pGEX-3X by standard PCR cloning techniques.

**Mammalian Expression Constructs—** The Tet-On plasmid was purchased from Clontech, and the tetracycline-inducible pTREFLAGREST was described previously (7, 9). The pBJS-BRG1 expression vector was generously donated by G. R. Crabtree. Full-length BAF57 and full-length BAF57v were cloned in the SmaI site of pbSIIKS by reverse transcription and standard PCR techniques with primers designed in the 5′ and 3′-untranslated regions of the human BAF57 cDNA sequence using a HeLa cDNA library. pcDNAcoREST was described previously (7). All fusion protein constructs were sequenced across the junction to ensure that inserts were in-frame. All constructs generated by PCR were sequenced completely.

**Yeast Two-Hybrid Screening and Assay**

The yeast two-hybrid method used here has been described previously (13). Briefly, pbTMKIA was co-transformed into L40 yeast, together with a HeLa cell cDNA library fused to the activation domain of Gal4. An estimated 9 × 10^6 transformants were screened. Positive clones were identified by growth on selective medium lacking histidine and confirmed by assaying for ß-galactosidase activity. Specificity of interaction was tested by a mating assay between the positive L40 transformants and an AMR-70 strain expressing pbTMKIA and several LexA fusion proteins not related to CoREST. Positive clones were characterized by sequence analysis. Direct yeast interaction assays were performed using yeast transformed with either LexACoREST or smaller fragments of CoREST fused to the LexA DNA binding domain and either pGADBAF57 or pGADBAF57v or smaller fragments of these proteins fused to the activation domain of Gal4.

**Chromatin Immunoprecipitation**

Chromatin immunoprecipitation assays were performed as described previously (7). Briefly, cells were cross-linked by addition of formaldehyde to 1% final concentration for 10 min at room temperature. Cells were harvested and lysed in a buffer containing 5 mM HEPES, pH 8.5, 150 mM KCl, and 0.5% Triton. Isolated nuclei were resuspended in buffer containing 50 mM Tris–Cl, pH 8, 10 mM EDTA, and 1% SDS and sonicated to obtain DNA fragments of a length between 200 and 600 bp. Sonicated chromatin was pre清除ed with protein G-agarose beads at 4 °C for 2 h. Precleared chromatin was incubated with 5 µg of each antibody (anti-REST, anti-BAF57, anti-BAF57v, and preimmune serum) and 5 µl of anti-Ac.H4 (Upstate Biotechnology, Inc.) in immunoprecipitation buffer (Upstate Biotechnology, Inc.) at 4 °C overnight. Immunocomplexes were collected on protein G-agarose beads preadsorbed with sonicated single-stranded DNA. Following washes and elution, cross-linking was reversed by heating overnight at 65 °C. Samples were purified, and the specific DNA sequences in the immunoprecipitates were detected by PCR. For real-time PCR detection of immunoprecipitated chromatin, an ABI Prism 7700 sequence detector was used to measure the incorporation of the fluorescent dye SYBR-green into PCR product. 8% of the DNA purified from the immunoprecipitation was used in the PCR reaction with primers for the rat Nav1.2 regulatory region (forward, 5'-AGGAGAAGGCTCTAATGGGTTC; reverse, 5'-CAAGCAGGTTGCAGAACCA). DNA samples were heat in 95 °C for 10 min, followed by 40 cycles of heating to 95 °C for 15 s and a combined annealing/extension at 65 °C for 1 min. Cycle thresholds for each immunoprecipitation were normalized to immunoprecipitation inputs, and comparisons were performed using the cycle threshold comparative method.

**GST Pull Down**

Expression and purification of GST fusion proteins and GST pull down assays were performed as described (13). In vitro transcription/translation was performed using a rabbit reticulocyte lysate-based TransTain system from Promega according to the manufacturer’s instructions. The in vitro-translated CoREST in pcDNA 1.1Amp was described previously (13).

**Cell Culture and Transient Transfection Analysis**

HEK293 cells were grown as described previously (13) and transfected using the LipofectAMINE 2000 transfection reagent (Invitrogen). When transfected with pTetOn and pTRE-RESTFLAG doxycycline (Sigma) was added to the medium to a final concentration of 1 µg/ml to induce the expression of FLAGREST.

**Co-immunoprecipitation and Western Blotting**

Immunoprecipitation assays were performed essentially as described (7). Antibodies used were anti-REST (2) and anti-CoREST (13). Anti-BAF57 and anti-BAF57v were generated as follows: peptides corresponding to BAF57 (amino acids 384–411, ATVEEPPTDPIPEDEK) and BAF57v (amino acids 349–363, CPRKTLTSSYDTPFD) were synthesized, injected into rabbits, and used to prepare peptide affinity columns for antibody purification from antisera by ResGen (Invitrogen). Anti-CoREST monoclonal was generated following standard hybridoma methodology (4). The antibody was raised against GST fusion protein containing amino acids 105–363 of hCoREST, and anti-BRG1 and anti-BAF170 were kindly provided by G. R. Crabtree; preimmune rabbit IgG was from Sigma. Western blot analysis was carried out using standard techniques.

**Nuclear Microinjection, Staining, and Fluorescence Microscopy**

Microinjection analysis was performed essentially as described previously (6). Prior to the injection, Rat-1 fibroblasts were rendered quiscent by incubation in serum-free medium for 24–36 h. Plasmids were injected into the nuclei of cells at a concentration of 100 µg/ml. In all cases, where no antibody was used in the experiment, preimmune rabbit or guinea pig IgG was co-injected. Injected cells were identified by co-injection of fluorescein-conjugated dextran (Molecular Probes). ß-Galactosidase activity was measured by incubation with 5-bromo-4-chloro-3-indolyl-ß-d-galactoside (X-gal). All cells showing any trace of blue staining were scored as positive for expression, to avoid any possible subjectivity in the analysis. All experiments were done at least two times, with a minimum of 250 microinjected cells in each experiment.
and probed for the presence of BRG1 epitopes by Western blot. Cells were immunoprecipitated using BAF57 and BAF57v antibodies with BRG1 in human HEK293 cells. Whole cell extracts from HEK293 (as well as transfected hemagglutinin-tagged BAF57v. empty vector body recognizes endogenous BAF57v protein (45kDa; BAF57v is shown. The translational stop codon is indicated for each protein that interacts with BRG1. Pressed in both non-neuronal (SKNBE, D283, DAOY) cell lines, d, BAF57 and BAF57v both associate with BRG1 in human HEK293 cells. Whole cell extracts from HEK293 cells were immunoprecipitated using BAF57 and BAF57v antibodies and probed for the presence of BRG1 epitopes by Western blot. Input represents 5% of the total cell extract.

RESULTS

The REST Co-repressor, CoREST, Interacts in Yeast Two-hybrid Assay with BAF57v, an Alternatively Spliced Form of BAF57—A fragment of the human CoREST cDNA encoding amino acids 102–482 was fused to LexA and used as bait in a yeast two-hybrid screen with a HeLa cell cDNA library. A screen of $9 \times 10^6$ transformants yielded several positive clones that failed to interact with unrelated LexA fusion proteins (data not shown). One of the positive cDNA clones encoded an open reading frame of 255 amino acids in-frame with the fused Gal4 activation domain. The first 235 amino acids were identical to a portion of BAF57, a known component of the hSWI/SNF ATP-dependent chromatin-remodeling complex, and corresponded to the amino acids coded by the exons 4 through 10 of the BAF57 gene (Fig. 1a). The carboxyl terminus of the clone was distinct from the BAF57 sequence published previously. The clone corresponded to an alternatively spliced form containing a unique exon, exon 11 (shaded in Fig. 1a). Exon 11 encodes 20 amino acids followed by a stop codon and the 3’-untranslated region. The alternatively spliced variant was BAF57v (BAF57 variant), and full-length cDNA was cloned by reverse transcriptase PCR using primers specifying the 5’- and 3’-untranslated regions of the human BAF57 cDNA. PCR amplification using these primers yielded two products corresponding to the expected cDNA sizes of both BAF57 and BAF57v. BAF57v cDNA encodes a putative protein of 363 amino acids of which the first 343 amino acids are identical to BAF57.

Antibodies specific for BAF57 and BAF57v were raised against peptides from the unique carboxyl termini of each variant. The anti-BAF57v antibody recognized the recombinant hemagglutinin-tagged BAF57v from transfected HEK293 nuclear extracts, as well as the endogenous protein (45 kDa; Fig. 1b). The presence of the respective immunizing peptides blocked immunoreactivity in the Western blots (data not shown). Like BAF57, BAF57v is expressed in several human cell lines of distinct cellular origin, both non-neuronal and neuronal (Fig. 1c). Co-immunoprecipitation assays using whole cell extracts of HEK293 cells revealed that the ATPase BRG1 was present in both anti-BAF57 and anti-BAF57v immunocomplexes suggesting that BAF57v, as well as BAF57, is associated with the hSWI/SNF complex (Fig. 1d). Using an antibody raised against an epitope shared by both the splice variants, we found that the two proteins were expressed to similar levels (data not shown).

The Coiled-coil Domain in BAF57 Proteins Is Sufficient for Interaction with the Carboxyl Repressor Domain of CoREST—To define the minimal domains required for the interaction between CoREST and the BAF57 proteins, we performed a yeast two-hybrid assay. A family of deletion mutants of LexACoREST was generated and assayed for the ability to interact with Gal4ADBAF57 fusion protein in yeast (Fig. 2a). Full-length CoREST interacted strongly with full-length BAF57v in this assay. Deletional analysis showed that BAF57v interacted specifically with the carboxyl-terminal repressor domain of CoREST located between amino acids 292 and 442 (shaded in Fig. 2a) (7). A family of deleted BAF57v proteins fused to the Gal4AD was also generated and assessed for interaction with CoREST (Fig. 2b). The minimal fragment of BAF57v able to interact with CoREST contained amino acids 219 to 280, spanning the region in the molecule containing the coiled-coil domain.

Full-length BAF57v did not interact with CoREST in two-hybrid assay (data not shown) although BAF57 and BAF57v share identical coiled-coil domains. To test whether this result was because of a peculiarity of the assay, we investigated the interaction between CoREST and both BAF57 and BAF57v in an in vitro assay independent of transcription. GST fusion proteins containing full-length BAF57 or full-length BAF57v were expressed and purified from bacteria and immobilized on glutathione-agarose beads. Fig. 3a shows that in vitro-translated CoREST proteins labeled with [35S]methionine interacted with both GSTBAF57 and GSTBAF57v fusion proteins but not GST alone (Fig. 3a). These results indicate that, in vitro, both BAF57 and BAF57v are able to interact with CoREST and that the CoREST carboxyl repressor domain interacts with the coiled-coil domain of the BAF57 proteins.

BAF57 Targets Components of the hSWI/SNF Complex to the REST-CoREST Repressor Complex—To verify the relevance of the in vitro interaction between CoREST and the BAF57 proteins, we tested whether BAF57 and/or BAF57v were part of a CoREST complex in vivo. CoREST immunocomplexes from HEK293 whole cell extracts were analyzed by Western blotting for the presence of BAF57 and BAF57v. Fig. 3b shows that BAF57 is part of the CoREST complex; we were unable to detect BAF57v epitopes in the CoREST immunoprecipitates (data not shown). Therefore, we focused our attention on the in vivo interaction between CoREST and BAF57. Our experiments showed that BAF57 and REST were in a complex together (Fig. 4a), indicating that BAF57 is relevant for REST-mediated repression. Because BAF57 is part of the hSWI/SNF complex, we asked subsequently whether BRG1, the catalytic subunit of the hSWI/SNF complex, and BAF170, an additional hSWI/SNF component, were also associated with REST (Fig. 4, b–d). FLAG-tagged REST epitopes were present in anti-BRG1 and anti-BAF170 immunocomplexes isolated from whole cell extracts of HEK293 cells overexpressing FLAGREST (Fig. 4, b and c). Additionally, anti-REST immunoprecipitates from HEK293 cells over-expressing BRG1 contained BRG1 epitopes.
CoREST is also associated with BRG1 and BAF170 in immunocomplexes (Fig. 4, e and f). Thus, REST and CoREST are associated with components of the hSWI-SNF complex. However, because two hSWI-SNF complexes have been shown to contain Sin3 (29–31), we cannot exclude the possibility that BRG1 could also be associated with REST through a Sin3 interaction.

It has been shown that repression mediated by the carboxyl-terminal repressor domain of CoREST is trichostatin A-sensitive and can be relieved by microinjection of HDAC2 antibody (7). The interaction between CoREST and BAF57 occurs through this repressor domain. Therefore, to determine whether components of the hSWI-SNF complex could target HDACs to a repressor complex containing the carboxyl repressor domain of CoREST, whole cell extracts were immunoprecipitated with antibodies to anti-BAF57, anti-BRG1, and anti-BAF170, and anti-CoREST. These immunocomplexes all contained epitopes for HDACs 1 and 2. Input represents 5% of total cell extracts.

**Fig. 2. Identification of the CoREST-BAF57v interaction domains by yeast two-hybrid assay.** a, the carboxyl repressor domain of CoREST, containing the SANT II domain, is required for BAF57v interaction. A family of deleted CoREST molecules fused to the LexA DNA binding domain (LexA) was tested for interaction with full-length BAF57v fused to Gal4AD. The activity of β-galactosidase (β gal) is shown as + or −. Black rectangles correspond to SANT motifs. The shaded region indicates the minimal interaction fragment on CoREST. b, the coiled-coil region of BAF57v is required for its interaction with CoREST. Gal4AD fusion proteins containing portions of BAF57v were interacted with LexA full-length CoREST. The HMG and coiled-coil domains are indicated, and the minimal interaction domain is shaded.

**Fig. 3. CoREST interacts with BAF57 in vitro and in vivo.** a, CoREST-BAF57 interactions by GST pull-down assay. CoREST was transcribed and translated in vitro to yield 35S-labeled products that were incubated with immobilized GST, GSTBAF57, or GSTBAF57v. Input corresponds to 10% of the total protein. b, Western blot indicating that whole cell immunoprecipitates from HEK293 cells using a monoclonal anti-CoREST antibody, but not preimmune IgG (PI IgG), contain BAF57 epitopes. Input corresponds to the 5% of the total cell extract.

**Fig. 4. REST and CoREST are in complexes with components of the hSWI-SNF complex and HDACs.** Whole cell immunocomplexes from HEK293 cells overexpressing REST using antibodies to (a) BAF57, (b) BRG1, and (c) BAF170, but not preimmune IgG (PI IgG), were resolved by SDS-PAGE, and the Western blot was probed with antibodies to REST. Conversely, in d, REST whole cell immunocomplexes from HEK293 cells overexpressing BRG1 were probed with antibody to BRG1. Immunocomplexes formed using antibodies to (e) BRG1 and (f) BAF170 contain CoREST epitopes. g, immunoprecipitates using antibodies to CoREST, BAF57, BRG1, BAF170, or PI IgG were resolved on SDS-PAGE and probed by Western blotting with antibodies specific for HDACs 1 and 2. Input represents 5% of total cell extracts.

The Presence of BAF57 on the Neuronal Nav1.2 Sodium Channel RE1 Sequence in Vivo Is Dependent upon REST—We performed chromatin immunoprecipitation analysis of the Nav1.2 RE1 to identify proteins associated with this sequence. Antibodies directed against REST, CoREST, and BAF57 were used to immunoprecipitate chromatin from L6 muscle cells that do not express the Nav1.2 gene. Associated DNA fragments were amplified using primers that flanked the Nav1.2
RE1 sequence (304 bp; see Fig. 5a) (7). All three proteins occupied the RE1 sequence; no amplification products were observed using preimmune IgG or in the absence of any antibodies. The presence of BAF57 on the RE1 DNA sequence further supports the involvement of hSWI/SNF components in REST-mediated repression.

The hSWI/SNF complex can be present on a genomic locus prior to the binding of a specific transcription factor or it can be targeted specifically to DNA through interactions with a transcription factor (35). To determine whether the targeting of
BAF57 to the RE1 sequence depends upon REST, we took advantage of a stable neuronal PC12 cell line, PC12tet REST, in which REST expression was under control of doxycycline (dox) and in which REST was shown to repress the endogenous Nav1.2 gene. We analyzed the Nav1.2 RE1 element for occupancy by BAF57, in the absence (Fig. 5b) and presence (Fig. 5c) of REST expression. Neither CoREST nor BAF57 were present on the RE1 element in PC12tet cells despite the expression of both CoREST and BAF57. Chromatin immunoprecipitation with an anti-acetyl histone H4 antibody was used as control for chromatin quality (Fig. 5b).

Upon induction of REST expression in the PC12tet REST cell line, REST, CoREST, and BAF57 were all found to occupy the RE1 site (Fig. 5c). These data suggest that BAF57 is recruited to the RE1 sequence only upon REST expression. Furthermore, to determine whether the induction of REST modified the chromatin of an endogenous gene in vivo, we compared the acetylation state of histone H4 proximal to the RE1 element in different cellular contexts using both semiquantitative and quantitative chromatin immunoprecipitation analysis (Fig. 5a and b). The amount of H4 acetylated histone in PC12 cells was similar to that of the PC12tet line. In contrast, Rat1 fibroblast expressing endogenous REST, or PC12tet REST treated with dox showed a 50% reduction of H4 acetylation. The uninduced PC12tet REST cells expressed a low level of REST protein (data not shown), and this likely accounts for the lower level of Ac. H4 when compared with PC12 and PC12tet cells.

Components of the hSWI/SNF Complex Are Required for REST Repression in Transient Assays—The association of components of the hSWI/SNF complex with the REST/CoREST complex raises the question of the functional significance of this interaction. We tested the ability of REST to repress an RE1 tk-lacZ reporter gene in the presence of neutralizing antibodies. Under normal conditions in Rat1 fibroblasts, the reporter gene is repressed by the presence of the endogenous REST repressor complex. It has already been shown that the same reporter gene is de-repressed by the microinjection of both anti-REST and anti-CoREST antibodies (7). Antibodies against BAF57, BRG1, or BAF170 relieved repression of the RE1 reporter gene (Fig. 6). The amount of de-repression was quantified for each microinjection experiment and was expressed as the percentage of cells that stained positively for β-galactosidase in the total number of injected cells (fluorescein-positive) within a field. Microinjection of control antibody did not relieve repression. Furthermore, to exclude the possibility that microinjection of antibodies against the hSWI/SNF complex components could interfere with the general transcription machinery, we used as a control a reporter gene identical to the RE1 tk-lacZ except the upstream activating sequences replaces the RE1 sequence (6). This reporter was active in fibroblast, and we showed that the microinjection of BRG1, BAF57, or BAF170 did not affect basal activity (Fig. 6b). Together these results indicate that REST repression of RE1 reporter gene requires the presence of components of the hSWI/SNF complex.

**DISCUSSION**

It has been suggested previously that REST might utilize the Sin3 and CoREST complexes differentially depending upon their relative availabilities during development (9). In this paper, we suggest an additional possibility. We have identified an interaction of CoREST with BAF57, a component of the hSWI/SNF ATP-dependent chromatin-remodeling complex. Further analysis revealed that REST was in complexes with the ATPase BRG1, as well as with BAF170, both intrinsic components of the hSWI/SNF complex. Microinjection experiments using specific antibodies against the ATPase BRG1, as well as against BAF57 and BAF170, relieved repression from a RE1-reporter gene, indicating that these components were required for repression in this assay. Thus, it is possible that the
presence of two repressor domains in CoREST reflects a requirement for remodeling activity recruited by the carboxy-terminal repressor domain.

Our results suggest that BAF57 targets the hSWI-SNF complex to the Nav1.2 RE1 DNA element by binding to CoREST via its coiled-coil domain. This role for BAF57 is consistent with the idea that specific subunits of the hSWI-SNF complex might either modulate the activity of the core ATPase (36) or be involved in the targeting of the complex to specific sites in chromatin (19, 37). Our findings with BAF57 are similar to the findings for two other hSWI-SNF subunits, hSNF5/Ini1/BAF47 (38) and BAF250 (39). Interaction of hSNF5/Ini1/BAF47 with the co-activator EBNAA2 leads to the recruitment of BRG1 to a specific set of target genes controlled by the DNA-binding proteins RBP-JK and PV.1 (38). Similarly, BAF250 is involved in the targeting of the remodeling complex to genes regulated by the glucocorticoid receptor, by directly interacting with the glucocorticoid receptor (39). In addition to containing a coiled-coil motif, BAF57 is an HMG protein. Its ability to bind to cruciform DNA structures (33) suggests that it might also contribute to stabilization of the interaction of the hSWI-SNF complex with the DNA. Another HMG protein, BRAF35 (BRACA-2-associated factor 35), has recently been shown to be a component of a CoREST-HDAC core-complex purified from HeLa cells (40). Like BAF57, BRAF35 also contains a coiled-coil domain. Whether BRAF35 interacts directly with CoREST is not known, but it could subserve a similar “bridge” function in another CoREST complex.

The presence of multiple copies of the same structural motif in the REST-CoREST repressor complex is not restricted to the HMG and coiled-coil domains of BAF57 and BRAF35. Another domain present in more than one copy in the complex is the SANT domain, a domain common to several other transcriptional activators and repressors that may have evolved from the myb DNA binding domain. The SANT domain can mediate DNA binding and protein-protein interactions (41). CoREST itself contains two SANT motifs that are required for repression. SANT domain I is required for interaction with REST and is involved in recruiting HDAC activity (7, 14). We show here that SANT domain II is involved in the interaction with BAF57, in addition to recruiting HDAC activity. At least four additional components of the REST-CoREST complex contain SANT or SANT-like domains: KIAA0601 (a protein with homology to polyamine oxidases) (14, 15, 40), BAF170 (42), and HDACs 1 and 2 (Fig. 7). Interestingly, MeCP2, another protein involved in repression, also contains a SANT-like motif. Thus, it is likely that these conserved domains provide "hot spots" for building distinct complexes with similar components.

How might ATP-dependent chromatin-remodeling activity affect REST-mediated repression? ATP-dependent chromatin remodeling has been postulated to be involved at different levels in gene regulation. It can facilitate the binding of a transcription factor to its cognate DNA binding site by relocating nucleosomes and exposing the DNA target. Alternatively, it can be targeted by a transcription factor to a specific genomic site and regulate recruitment and/or the enzymatic activity of other factors in situ (37). In the case of the Mi-2-NURD complex, which contains both HDACs and ATP-dependent chromatin-remodeling components, enhanced deacetylation activity is dependent upon ATP, a result that suggests that nucleosome remodeling improves the access or the affinity of HDACs to chromatin (43). On the other hand, genetic and biochemical evidence suggests that the activity of the Drosophila Imitation Switch (ISWI) ATPase is affected by the acetylation state of histone H4 (44). In our study, we show that the recruitment of BAF57 to the RE1 sequence occurs only upon expression of the DNA-binding protein, REST. Therefore, the hSWI-SNF complex might modulate the enzymatic activity of other components in the chromatin-remodeling complex, for example the HDACs associated with the REST complex. The need to recruit complexes with distinct activities for the repression of specific genes has been shown for other transcription factors (37). It has been suggested that coordination among different chromatin-remodeling activities might involve a "histone code" that reflects specific combinations of covalent and non-covalent histone modifications for each promoter (45, 46). The modular structure of REST, and its association both with factors that covalently modify histones and factors that are involved in ATP-dependent remodeling of chromatin, presents an opportunity to characterize the histone code (46) responsible for REST-mediated, long-term neuronal gene repression.

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REFERENCES

1. Vandenbergh, D. J., Waenschell, C. W., Mori, N., and Anderson, D. J. (1989) Neuron 3, 507–518
2. Chong, J. A., Tapia-Ramirez, J., Kim, S., Toledos-Alar, J. J., Zheng, Y., Boutros, M. C., Alshuller, Y. M., Frohwirt, M. A., Kramer, S. D., and Mandel, G. (1995) Cell 80, 949–957
3. Schoencker, C. J., and Anderson, D. J. (1995) Science 267, 1360–1363
4. Chen, Z. F., Paquette, A. J., and Anderson, D. J. (1998) Nat. Genet. 20, 136–142
5. Jones, P. S., and Meech, R. (1999) Bioessays 21, 372–376
6. Paquette, A. J., Perez, S. E., and Anderson, D. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12128–12133
7. Ballas, N., Battaglioli, E., Atouf, P., Andres, M. E., Chenoweth, J., Anderson, M. E., Burger, C., Moniwa, M., Davie, J. B., Bowers, W. J., Oredjo, F. J., Rose, D. W., Rosenfeld, M. G., Brehm, P., and Mandel, G. (2001) Neuron 31, 352–365
8. Tapia-Ramirez, J., Eggen, B. J., Peral-Rubio, M. J., Toledo-Aral, J. J., and Mandel, G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1177–1182
9. Grimes, J. A., Nielsen, S. J., Battaglioli, E., Miska, E. A., Speh, J. C., Berry, D. L., Atouf, F., Holdener, B. C., Mandel, G., and Kouzarides, T. (2000) J. Biol. Chem. 275, 9461–9467
10. Huang, Y., Myers, S. B., and Dingledine, R. (1999) Nat. Neurosci. 2, 867–872
11. Roopra, A., Sharling, L., Wood, I. C., Briggs, T., Bachfischer, U., Paquette, A. J., and Buckle, N. J. (2000) Mol. Cell. Biol. 20, 2147–2157
12. Naruse, Y., Aoki, T., Kojima, T., and Mori, N. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13691–13696
13. Alles, M. E., Burger, C., Peral-Rubio, M. J., Battaglioli, E., Anderson, M. E., Grimes, J., Jallman, J., Ballas, N., and Mandel, G. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 9873–9878
14. You, A., Tong, K. J., Grozinger, C. M., and Schreiber, S. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 14558–14563
15. Humphrey, G. W., Wang, Y., Russanova, Y. R., Hirai, T., Qin, J., Nakatani, Y., and Howard, B. B. (2001) J. Biol. Chem. 276, 6817–6824
16. Cheung, P., Allis, C. D., and Sassone-Corsi, P. (2000) Cell 103, 263–271
17. Wu, J., and Grunstein, M. (2000) Trends Biochem. Sci. 25, 619–625
18. Khochbin, S., Verdel, A., Lemerrier, C., and Seigneurin-Berny, D. (2001) Curr. Opin. Genet. Dev. 11, 162–166
19. Kingston, R. E., and Narlikar, G. J. (1999) Genes Dev. 13, 2339–2352
20. Vignali, M., Hasson, A. H., Neely, K. E., and Workman, J. L. (2000) Mol. Cell. Biol. 20, 1899–1910
21. Holstege, F. C., Jennings, E. G., Wyrick, J. L., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S., and Young, R. A. (1998) Cell 95, 717–728
22. Sudarsanam, P., Iyer, V. R., Brown, P. O., and Winston, F. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1364–1368
23. Liu, R., Liu, H., Chen, X., Kirby, M., Brown, P. O., and Zhao, K. (2001) Cell 106, 309–318
24. Murphy, D. J., Hardy, S., and Engel, D. A. (1999) Mol. Cell. Biol. 19, 2724–2733
25. Zhang, H. S., Gavin, M., Dahiya, A., Postigo, A. A., Ma, D., Luo, R. X., Harbour, M., and Dean, D. C. (2000) Mol. Cell 5, 3364–3369
26. Zhang, Y., LeRoy, G., Seelig, H. P., Lane, W. S., and Reinberg, D. (1998) Cell 95, 279–289
27. Kuzmichev, A., Zhang, Y., Endrulf-Bromage, H., Tempst, P., and Reinberg, D. (2002) Mol. Cell. Biol. 22, 835–848
28. Sif, S., Saurin, A. J., Imbalzano, A. N., and Kingston, R. E. (2001) Genes Dev. 15, 603–618
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31. Underhill, C., Qutob, M. S., Yee, S. P., and Torchia, J. (2000) J. Biol. Chem. 275, 40463–40470
32. Chi, T. H., Wan, M., Zhao, K., Taniuchi, I., Chen, L., Littman, D. R., and Crabtree, G. R. (2002) Nature 418, 195–199
33. Wang, W., Chi, T., Xue, Y., Zhou, S., Kuo, A., and Crabtree, G. R. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 492–498
34. Trimmer, J. S., Trowbridge, I. S., and Vacquier, V. D. (1985) Cell 40, 697–703
35. Peterson, C. L., and Workman, J. L. (2000) Curr. Opin. Genet. Dev. 10, 187–192
36. Phelan, M. L., Sif, S., Narlikar, G. J., and Kingston, R. E. (1999) Mol. Cell 3, 247–253
37. Narlikar, G. J., Fan, H. Y., and Kingston, R. E. (2002) Cell 108, 517–519
38. Wu, D. Y., Kalpana, G. V., Goff, S. P., and Schubach, W. H. (1996) J. Virol. 70, 6020–6028
39. Nie, Z., Xue, Y., Yang, D., Zhou, S., Deroo, B. J., Archer, T. K., and Wang, W. (2000) Mol. Cell. Biol. 20, 8879–8888
40. Hakimi, M. A., Bochar, D. A., Chenoweth, J., Lane, W. S., Mandel, G., and Shiekhattar, R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 7420–7425
41. Aasland, R., Stewart, A. P., and Gibson, T. (1996) Trends Biochem. Sci 21, 87–88
42. Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B. R., and Crabtree, G. R. (1996) Genes Dev. 10, 2117–2130
43. Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998) Nature 395, 917–921
44. Corona, D. F., Clapier, C. R., Becker, P. B., and Tamkun, J. W. (2002) EMBO Rep. 3, 242–247
45. Strahl, B. D., and Allis, C. D. (2000) Nature 403, 41–45
46. Jenuwein, T., and Allis, C. D. (2001) Science 293, 1074–1080