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Transcriptional Specificity of Human SWI/SNF BRG1 and BRM Chromatin Remodeling Complexes

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Summary

Mammalian SWI/SNF chromatin remodeling complexes are involved in critical aspects of cellular growth and genomic stability. Each complex contains one of two highly homologous ATPases, BRG1 and BRM, yet little is known about their specialized functions. We show that BRG1and BRM associate with different promoters during cellular proliferation and differentiation, and in response to specific signaling pathways by preferential interaction with certain classes of transcription factors. BRG1 binds to zinc finger proteins through a unique N-terminal domain that is not present in BRM. BRM interacts with two ankyrin repeat proteins that are critical components of Notch signal transduction. Thus, BRG1 and BRM complexes may direct distinct cellular processes by recruitment to specific promoters through protein-protein interactions that are unique to each ATPase.

Introduction

The regulation of gene expression through chromatin is essential for proper development, differentiation, and genomic stability. All genes are packaged into chromatin structures that can be modulated to generate transcriptionally active or repressed configurations in different cellular contexts and under changing environmental conditions. This remarkable plasticity is governed in part by multisubunit protein complexes that enzymatically regulate chromosomal structure and activity. These complexes can either chemically modify the histone tails of nucleosomes or disrupt histone-DNA contacts through ATP hydrolysis (Berger, 2002; Narlikar et al., 2002). Specific combinations of modifying and "remodeling" complexes often act in a temporal manner to modulate the nucleosomal structure and expression of individual genes (Cosma, 2002). A critical issue is how gene specificity is achieved by these diverse chromatin enzymatic machines.

One important chromatin remodeling enzyme is SWI/SNF, which is a 2 MDa multisubunit complex that was first identified in yeast and is highly conserved among eukaryotes (Peterson and Workman, 2000). Mammalian SWI/SNF interacts with a wide variety of proteins and is implicated in regulating critical cellular processes such as differentiation and cell cycle arrest (Klochendler-Yeivin et al., 2002). Mammalian SWI/SNF complexes are present in biochemically diverse forms, indicating that they may have specialized nuclear functions (Wang et al., 1996). Each of these complexes contains one of two ATPases, BRG1 or BRM, and a variable subunit composition of BRG1-associated factors (BAFs). Interestingly, BRG1 and BRM are highly homologous ATPases, yet they appear to direct very different cellular pathways. Gene knockout studies in mice have demonstrated that homozygous inactivating mutations in BRG1 or INI1/SNF5 (a core BAF subunit) are embryonic lethal whereas BRM-inactivated mice are mainly characterized as having increased body weight (Reyes et al., 1998; Bultman et al., 2000; Klochendler-Yeivin et al., 2000). BRG1 and BRM activities are partially redundant but they have different expression profiles; BRG1 protein levels are relatively constant in all cells whereas BRM protein concentrations increase during cellular differentiation. A variety of human malignancies are associated with mutations in BRG1 and INI1/SNF5 but not BRM, suggesting that loss of function of certain SWI/SNF complexes may contribute to tumorigenesis (Klochendler-Yeivin et al., 2002). Critical unresolved issues are to determine why BRG1 and BRM direct such distinct SWI/SNF activities and to identify genes which are specifically regulated by each ATPase.

We have previously examined the role of mammalian SWI/SNF in transcriptional activation of chromatin-assembled templates and demonstrated that SWI/SNF functions through selective association with certain classes of regulatory proteins (Armstrong et al., 1998; Kadam et al., 2000). For example, SWI/SNF facilitates remodeling and transcription of chromatin-assembled β-globin genes by the erythroid-restricted activators EKLF and GATA-1. This occurs through direct interaction between SWI/SNF and the zinc finger DNA binding domains (ZF DBD) of these proteins. By contrast, SWI/SNF does not interact or function with the helix-loop-helix factor TFE-3 or rel-containing NF-κB to activate HIV-1 chromatin templates. Association of specific protein domains with individual SWI/SNF subunits directs recruitment of the enzymatic complex to nucleosomal sites where stable remodeling occurs (Kadam et al., 2000). Consistent with these findings are recent studies showing that specific isoforms of SWI/SNF preferentially bind and coactivate in a factor-selective manner (Nie et al., 2000; Lemon et al., 2001). Thus, the functional diversity of mammalian SWI/SNF could result from differential gene targeting of specific complexes by transcription factor interactions with individual SWI/SNF subunits.

We investigated the basis by which BRG1- and BRM-containing human SWI/SNF complexes regulate distinct genes by identifying members of protein families that can discriminate between the two ATPases and target each one to selected promoters. We find that BRG1 but not BRM functions specifically with zinc finger proteins (ZFP). These represent the most common class of eukaryotic regulatory factors whose members control a wide range of cellular processes including differentiation, proliferation, metabolism, and apoptosis. Thus far, SWI/SNF is the only chromatin remodeling complex shown to mediate the activity of this important family of diverse proteins. We find that members of other protein

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families containing leucine zippers, rel domains, HMG boxes, tryptophan clusters, arm repeats, and ankyrin repeats either fail to interact with SWI/SNF or display high specificity for either BRG1 or BRM complexes but not both. Interestingly, two critical components of the Notch signaling pathway associate exclusively with BRM SWI/SNF and recruit this complex to natural target promoters in vivo. This supports the notion that the BRM ATPase may be specifically dedicated to modulating the chromatin structure of some genes that are regulated by this particular pathway. An examination of the recruitment of SWI/SNF to a variety of genes during cellular proliferation, differentiation, or signal transduction reveals an exclusivity of BRG1 or BRM association that occurs before transcriptional reprogramming. The final switch to activation or repression is mechanistically diverse among tissue-specific, signal-activated, cell cycle-regulated, and housekeeping genes, and can be correlated with changes in histone modification, activator binding, and relative ratios of CBP/HDAC.

Results

Zinc Finger DNA Binding Domains Generate Chromatin Accessibility to Proteins that Do Not Function with SWI/SNF

We have shown previously that a zinc finger DNA binding domain alone is sufficient to target hSWI/SNF to specific nucleosomal sites for stable remodeling. Transcription requires, in addition, an activation domain fused to the ZF DBD (Kadam et al., 2000). Based upon this observation, we examined whether ZF DBD recruitment of SWI/SNF to a chromatin-assembled promoter could generate a remodeled structure that was accessible to transcription factors which cannot normally bind to their nucleosomal sites and do not function with SWI/SNF. To address this question, we focused on the helix-loop-helix protein, TFE-3, which is a potent transcriptional activator of the HIV-1 promoter (Sheridan et al., 1995) but does not function with hSWI/SNF (Armstrong et al., 1998; Kadam et al., 2000). Plasmids containing the HIV-1 promoter were assembled into chromatin using *Drosophila* embryonic extracts (Bulger and Kadonaga, 1994) and examined by in vitro transcription and nucleosome remodeling. As shown in the transcription analysis in Figure 1A, TFE-3 functions only when prebound to HIV-1 promoter DNA prior to chromatin assembly (lane 2). If added after assembly, it cannot access its binding site at −177 or activate transcription in the presence or absence of purified hSWI/SNF (lanes 3 and 5). Addition of the ZF DBD from the erythroid factor EKLF to TFE-3 also fails to coactivate HIV-1 transcription through a −50 CACC element (lane 7). However, if hSWI/SNF is included with TFE-3 and the ZF DBD, robust transcription occurs which is dependent upon TFE-3 since the ZF DBD lacks an activation motif (lane 9). hSWI/SNF does not coactivate transcription with the ZF DBD alone (lane 8) or with TFE-3 in the absence of the ZF DBD (lane 5). To correlate these results with chromatin remodeling, DNase 1 footprinting was performed on the assembled HIV-1 templates. As shown in Figure 1B, TFE-3 alone does not bind to chromatinized HIV-1 promoters (lane 2) and SWI/SNF does not facilitate this interaction (lane 4). The ZF DBD also fails to interact with its recognition site at −50 unless catalyzed by SWI/SNF (lanes 5 and 7). However, consistent with the transcription results, TFE-3 interacts with the nucleosomal HIV-1 promoter very strongly in the presence of both SWI/SNF and the ZF DBD (lane 8) whereas the ZF DBD alone does not enable TFE-3 to bind (lane 6).

A model depicting these results is shown in Figure 1C. TFE-3 belongs to a class of proteins that does not interact with SWI/SNF and whose binding to chromatin is not directly facilitated by this remodeling complex in vitro. Therefore, no targeted chromatin restructuring of the HIV-1 promoter is catalyzed by SWI/SNF in the presence of TFE-3. However, a ZF DBD, which is insufficient for transcription, can recruit SWI/SNF through a −50 CACC site to the nucleosome-repressed promoter. This generates an accessible structure extending to at least −177 which enables TFE-3 to interact and subsequently activate transcription. These results support the notion that among the multiple proteins that regulate eukaryotic promoters and enhancers, only one may be required to initially target a remodeling complex to render a genetic element accessible to interact with other factors in a temporal or conditional manner. The widespread occurrence of ZFPs and their ability to target SWI/SNF to unique nucleosomal sites suggests that this may be a critical role for some members of this family.

Human SWI/SNF Is Targeted to Chromatin through Specific Zinc Fingers of the KLF and GATA Protein Families that Preferentially Bind DNA

To gain more insight into SWI/SNF recruitment by ZFPs, we examined the nature of interaction between this remodeling complex and distinct structural motifs within different ZF DBDs. Our previous results indicated that hSWI/SNF can function with three ZFPs, Sp1 and the erythroid factors EKLF and GATA-1, to facilitate chromatin remodeling and transcriptional activation (Kadam et al., 2000). The Sp1 and KLF (Kruppel-like factors) proteins contain a C2H2 DBD consisting of three individual ZFs whereas the GATA family possesses two ZFs within a C4 structural motif (Mackay and Crossley, 1998). These domains are diagrammed in Figure 2A. Individual ZFs within both families have been shown to have distinct roles in DNA and cofactor interaction; notably, the GATA-1 C-finger and KLF fingers 1 and 3 are most critical for DNA binding (Bieker, 2001; Cantor and Orkin, 2002). We purified recombinant forms of each ZF within the C2H2 and C4 DBDs to examine their individual roles in targeted SWI/SNF remodeling and transcriptional activation.

As shown in the DNase hypersensitivity experiment in Figure 2B, C4 and C2H2 ZF DBDs each function with hSWI/SNF to direct chromatin remodeling (lanes 3–10). Interestingly, when individual C4 zinc fingers of GATA-1 were examined in this assay, only the C-finger could target SWI/SNF (lanes 11 and 12). Protein interaction studies by GST-pull-down experiments indicate that SWI/SNF exclusively associates with the GATA C-finger whereas no binding is observed to the N-finger (see Figure 3). A similar examination of SWI/SNF interaction with each of the three individual C2H2 KLF ZFs revealed that fingers 1 and 3 but not 2 interact with the recombi-

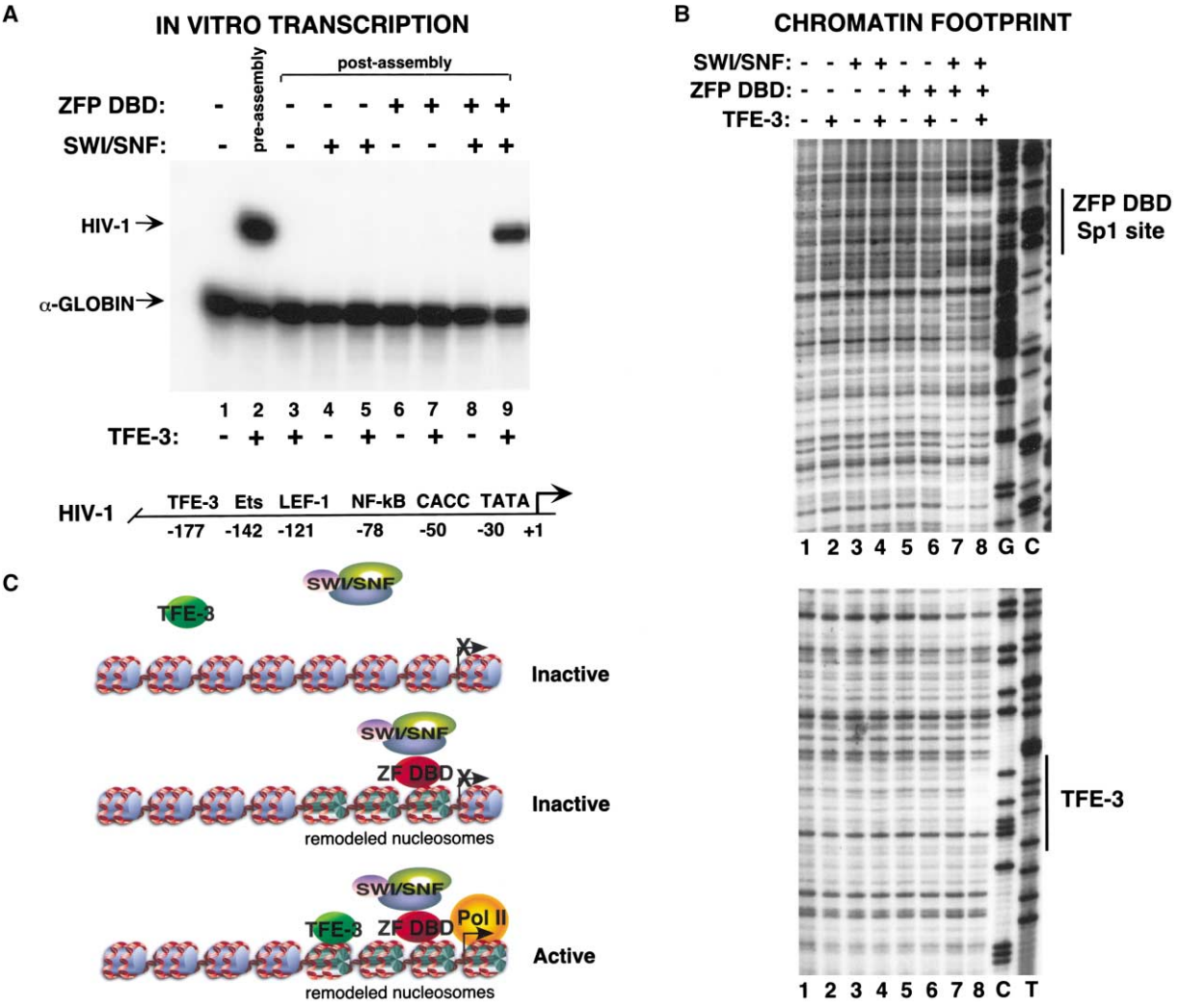

Figure 1. A Zinc Finger DNA Binding Domain Directs SWI/SNF Remodeling to Enable Interaction by Proteins that Cannot Access Their Sites in Chromatin

(A) In vitro transcription of chromatin-assembled HIV-1 promoters; effect of zinc finger DBD-targeted SWI/SNF remodeling on TFE-3 activation. One microgram of chromatin was incubated in the presence or absence of hSWI/SNF and, where indicated, 50 pmol of TFE-3 and 125 pmol of the EKLF ZF DBD. All factors were added after chromatin assembly (postassembly) except in lane 2. Reactions were split in half and analyzed separately by transcription and footprinting. α-globin plasmids were transcribed as internal controls. A diagram of the HIV-1 promoter and protein binding sites is depicted below.

(B) DNase I footprint of chromatin-assembled HIV-1 promoters; effect of zinc finger DBD-targeted SWI/SNF remodeling on TFE-3 binding. The bars indicate binding sites for ZFP DBD (CACC site) and TFE-3.

(C) A model showing how SWI/SNF-dependent structural remodeling by a zinc finger DBD facilitates binding and transcriptional activation by factors (TFE-3) that cannot access their sites in chromatin.

nant BRG1 subunit (Figure 2C). The importance of SWI/SNF interaction with specific KLF fingers was examined functionally by in vitro transcription of chromatin-assembled β-globin genes. In this case, individual fingers were tested for their ability to act as dominant negative inhibitors of EKLF-dependent activation. As shown in Figure 2D, fingers 1 or 3 individually represses transcription by intact EKLF (lanes 10, 11, 14, and 15) whereas finger 2 has no effect (lanes 12 and 13). A corresponding footprint analysis of these chromatin templates in Figure 2E reveals that EKLF interaction with the β-globin promoter is abolished by addition of fingers 1 and 3 (lanes 5, 6, 9, and 10) but not by finger 2 (lanes 7 and 8). These results indicate that SWI/SNF is targeted to chromatin by ZFPs through association with specific ZFs within C4 and C2H2 DBD motifs that are the most critical for DNA binding.

Specificity of BRG1 versus BRM SWI/SNF Complexes for Zinc Finger DNA Binding Domains

We explored further the observed selectivity of hSWI/SNF for ZF-containing proteins by examining which particular form of SWI/SNF these factors associate with. Our purified preparation of hSWI/SNF is composed of both BRG1- and BRM-containing complexes since a common subunit, INI1, is tagged (Figure 3A). In order to discriminate between the BRG1 and BRM complexes present in hSWI/SNF, we performed protein interaction

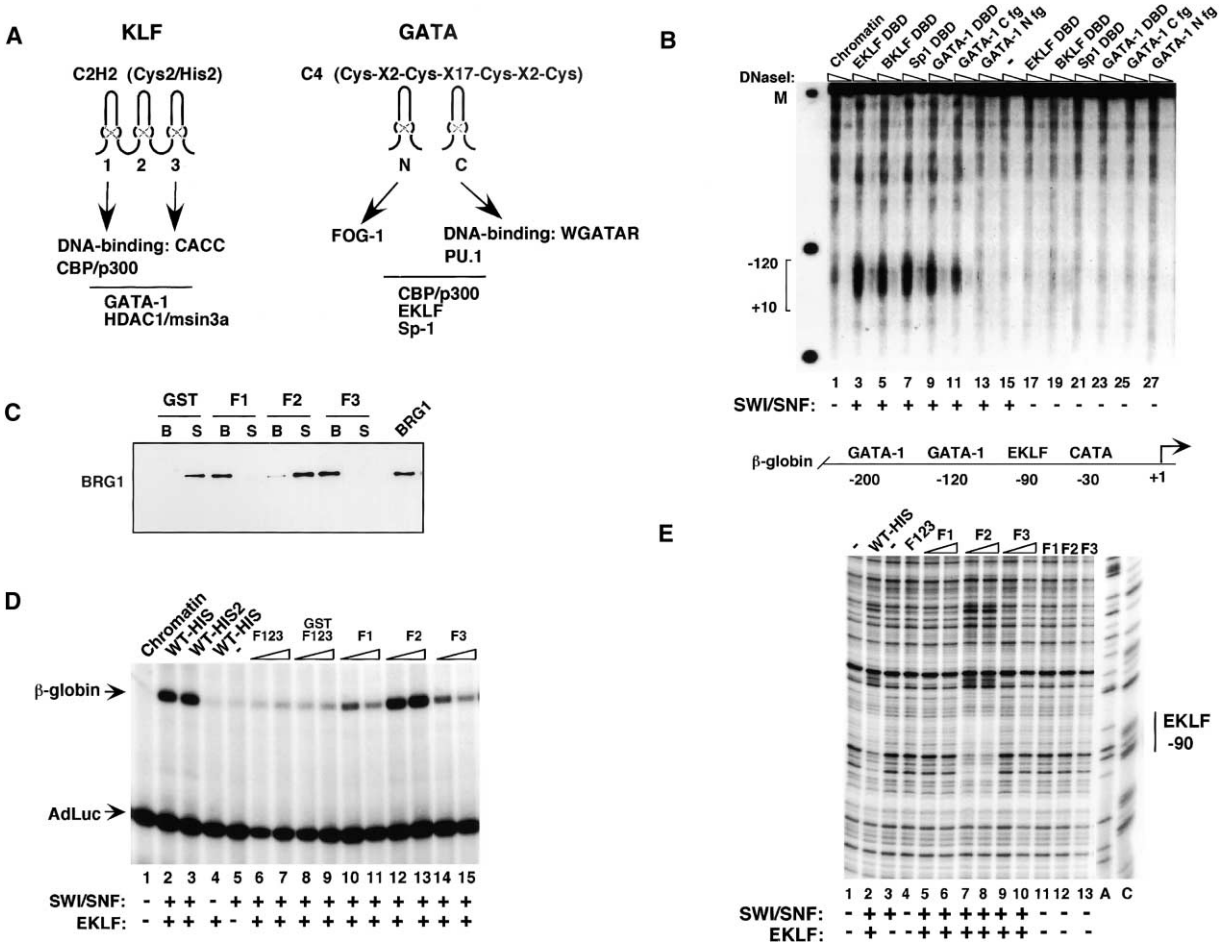

Figure 2. SWI/SNF-Dependent Remodeling and Transcription Is Targeted by Individual Zinc Fingers within the C2H2 and C4 DNA Binding Domains

(A) Schematic representation of the zinc finger DBD structures of the KLF (C2H2) and GATA (C4) protein families. Cofactors that associate with each DBD or with individual ZFs are indicated.

(B) DNase 1 hypersensitivity of chromatin-assembled β-globin promoters is generated by SWI/SNF and ZF DBDs from KLF, Sp1, and individual GATA-1 ZFs. Assembled chromatin was incubated with hSWI/SNF and 35 pmol of each protein as shown before digestion with 2 and 1 U DNase1. Brackets indicate the −120 to +10 region of the promoter. A diagram of the β-globin promoter and protein binding sites is depicted below.

(C) GST-pull-down analysis of protein interactions between 2 μg recombinant BRG1 and 1 μg individual ZFs (F1, F2, F3) within the KLF ZF DBD. B, bound; S, supernatant. BRG1 was detected by immunoblotting using BRG1 antisera.

(D) In vitro transcription of chromatin-assembled β-globin promoters; effect of individual KLF ZFs as dominant-negative inhibitors of SWI/SNF-dependent EKLF activation. Assembled chromatin was incubated with the following proteins as indicated: WT-HIS, -HIS2, two preparations of histidine-tagged EKLF (37 pmol); F123, histidine-tagged EKLF DBD; GST F123, GST-tagged EKLF DBD; F1, F2, F3, individual KLF zinc fingers (250 pmol). AdLuc transcripts are shown as internal controls. In these experiments, ZF DBD or individual ZF was incubated with EKLF and SWI/SNF for 15 min on ice before addition to assembled chromatin.

(E) DNase I footprint of chromatin-assembled β-globin promoters; effect of individual KLF ZFs as dominant-negative inhibitors of SWI/SNF-dependent EKLF binding.

experiments using hSWI/SNF and GST-tagged ZF DBDs. As shown in Figure 3B, the ZF DBDs of KLF factors, Sp1, hormone receptors (RAR, RXR), and GATA-1 each bind specifically to BRG1-containing SWI/SNF. In the case of GATA-1, SWI/SNF associates through the C-finger, consistent with the role of this finger in nucleosome remodeling (Figure 2B). We repeated this analysis using recombinant BRG1 and BRM subunits and found that interactions with various ZF DBDs occur specifically with the BRG1 ATPase (Figure 3C). Our in vitro interactions were confirmed by in vivo immunoprecipitation using mouse erythroleukemia cell extracts. As expected, EKLF interacts strongly with SWI/SNF complexes containing BRG1 (Figure 3D, lane 3) but not BRM (lane 2). Antisera to the core subunits BAF 155 (lane 4) and BAF 170 (lane 5) also coprecipitate EKLF, apparently from BRG1 complexes only. We conclude that the ZF family of transcription factors interacts exclusively with BRG1-containing SWI/SNF and that this occurs through specific ZFs and the BRG1 ATPase subunit. No binding is observed between the ZF DBDs examined and native or recombinant forms of BRM-containing SWI/SNF.

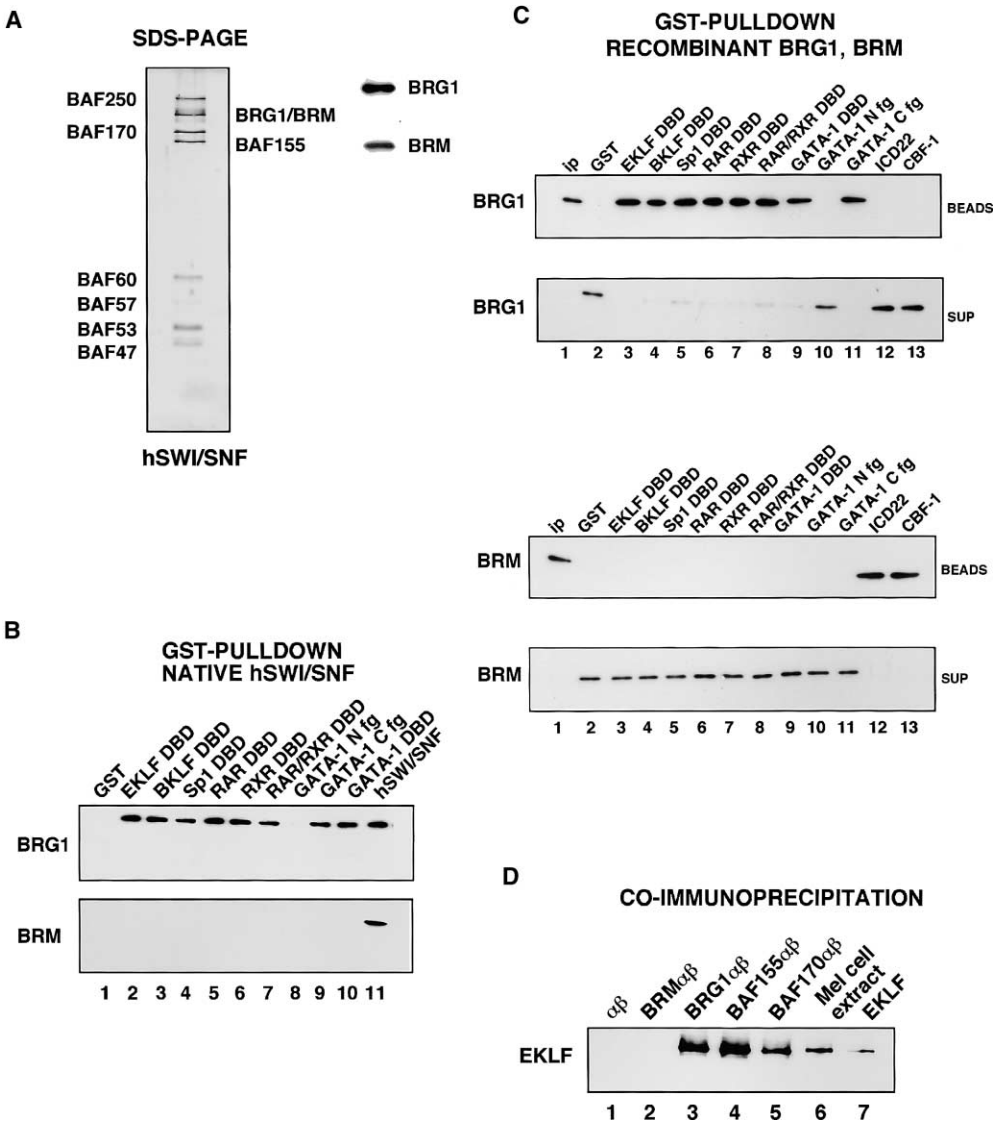

Figure 3. Zinc Finger DNA Binding Proteins Specifically Interact with BRG1-Containing SWI/SNF Complexes

(A) SDS-PAGE silver stain analysis of epitope-tagged native hSWI/SNF complexes purified from HeLa INI11 cells (left panel). Immunoblot analysis of hSWI/SNF using antisera to BRG1 and BRM subunits shows the presence of both complexes in our preparations (right panel).

(B) GST-pull-down analysis of protein interactions between 3 μg native hSWI/SNF and 1 μg recombinant ZF DBDs of EKLF, BKLF, Sp1, retinoic acid receptors (RAR and RXR), GATA-1, and individual GATA-1 C-and N-ZFs. Interactions with BRG1 or BRM were detected by immunoblotting using appropriate antisera.

(C) GST-pull-down analysis of protein interactions between 500 ng recombinant BRG1 (upper panel) or BRM (lower panel) and 500 ng recombinant ZF DBDs, individual ZFs, ICD22, and CBF-1. One hundred percent of bound proteins (beads), 50% of unbound proteins (sup), and 50% of input BRG1 and BRM were analyzed on 10% SDS-PAGE gels and immunoblotted with antibodies against BRG1 or BRM.

(D) In vivo interaction of EKLF with SWI/SNF. Coimmunoprecipitation of EKLF with antisera to BRG1, BRM, BAF155, and BAF170 subunits of SWI/SNF using 100 μg mouse erythroid (MEL) cell nuclear extract. Immune complexes were separated by 10% SDS-PAGE and immunoblotted with antibodies against EKLF. Recombinant EKLF (25 ng) was used as a positive control (lane 7).

Native and Recombinant BRG1 but Not BRM SWI/SNF Complexes Function with Zinc Finger Proteins to Activate Transcription through Chromatin

To confirm the functional significance of the interaction specificity between BRG1-containing SWI/SNF and ZFPs, we compared the ability of immunopurified native BRG1 and BRM SWI/SNF as well as recombinant BRG1 and BRM complexes to coactivate transcription by EKLF. We have shown previously that a minimal recombinant SWI/SNF complex composed of BRG1 and BAF 155 is sufficient to facilitate EKLF-dependent nucleosome remodeling and transcription (Kadam et al., 2000). As shown in Figure 4A, native BRG1 SWI/SNF immunodepleted of BRM complexes (lane 21) and recombinant BRG1/BAF155 (lanes 7, 9, and 19) can each activate β-globin transcription by EKLF. However, no EKLF-dependent transcription is observed with native BRM SWI/SNF complexes (lane 25) or with recombinant BRM/BAF155 (lanes 13 and 23). A mononucleosome disruption assay was performed with recombinant BRM to

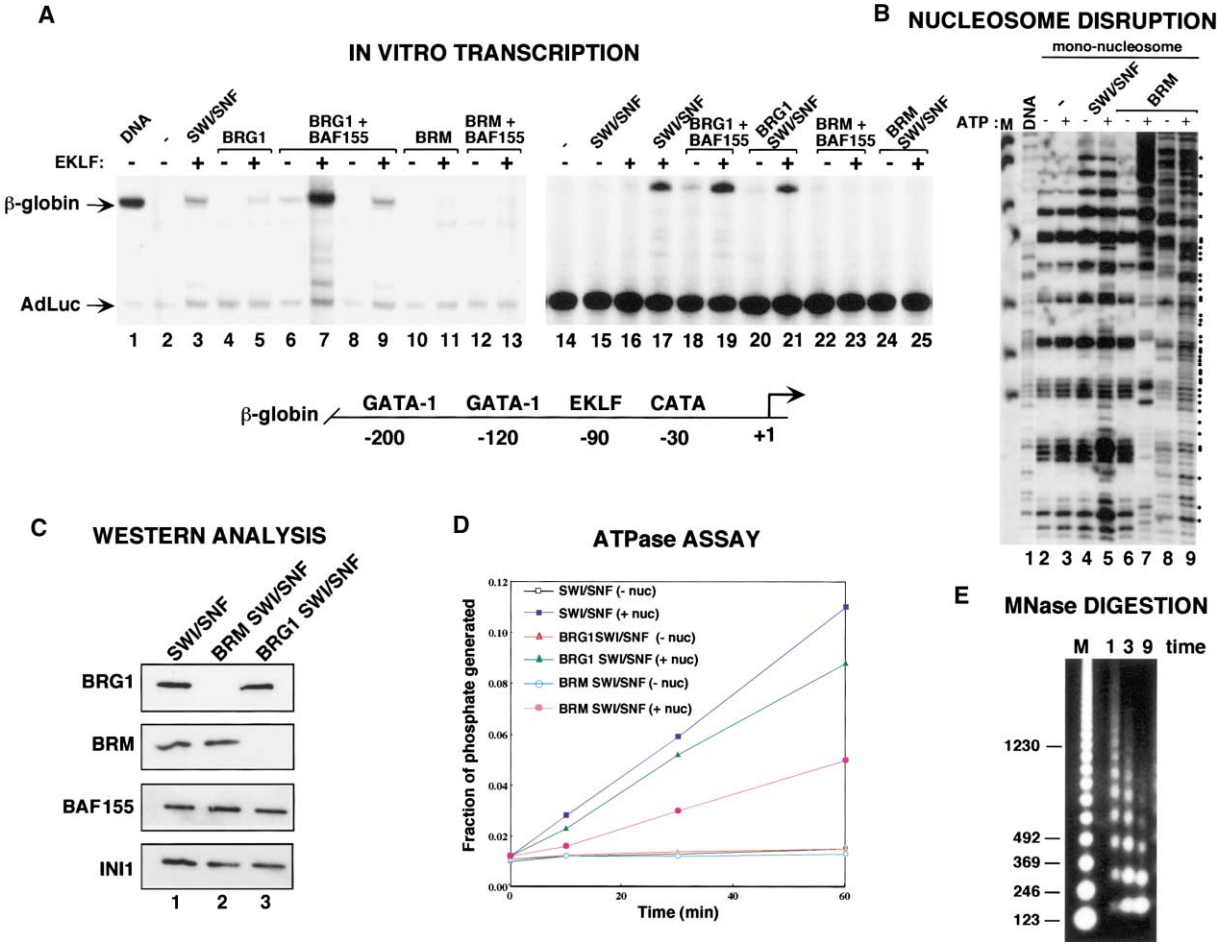

Figure 4. Native and Recombinant BRG1 but Not BRM SWI/SNF Complexes Coactivate Transcription by Zinc Finger Proteins

(A) EKLF-dependent transcription from chromatin-assembled β-globin promoters with native INI-1 tagged SWI/SNF (containing both BRG1 and BRM), immunopurified native BRG1 or BRM SWI/SNF, and recombinant BRG1 or BRM complexes. One hundred nanograms of chromatin was incubated with 3.7 pmol EKLF, 20 ng F-BRG1, 100 ng F-BRM, 100 ng of F-BAF155, 58 ng INI-1 tagged SWI/SNF, 60 or 120 ng immunopurified BRG1- or BRM-containing SWI/SNF as indicated. Two separate experiments are represented in the right and left panels. AdLuc transcripts are shown as internal controls. A diagram of the β-globin promoter is depicted below.

(B) Nucleosome disruption analysis showing that the recombinant BRM ATPase possesses classical chromatin remodeling activity. Three hundred nanograms of SWI/SNF (lanes 4 and 5), 100 ng (lanes 6 and 7), and 500 ng (lanes 8 and 9) of hBRM were incubated with reconstituted 5S mononucleosomes followed by DNase 1 digestion. M, MspI digested pBR322 as a molecular weight marker.

(C) Equal amounts (100 ng) of native INI-1 tagged SWI/SNF and immunopurified BRG1 or BRM SWI/SNF complexes were analyzed by Western blotting using antisera to BRG1, BRM, BAF 155, and INI-1.

(D) ATPase activity of SWI/SNF complexes. Approximately 55 ng of native SWI/SNF, immunopurified BRG1 and BRM SWI/SNF complexes was incubated in the presence or absence of 150 nM nucleosomes and (γ-^{32}P) ATP for the indicated times. The phosphate present at time zero is due to the presence of (γ-^{32}P) phosphate in the (γ-^{32}P) ATP stock. The ratio of inorganic phosphate to ATP was quantitated for each time point using a Molecular Dynamics PhosphoImager.

(E) Chromatin structural analysis of in vitro assembled β-globin plasmids by micrococcal nuclease digestion.

confirm that this protein preparation is active (Figure 4B, lanes 6–9). Native BRG1 and BRM SWI/SNF were examined by Western blotting to evaluate the efficiency of separation of these complexes after immunodepletion (Figure 4C). These complexes were analyzed for their catalytic activity by measuring ATP hydrolysis (Figure 4D). BRM SWI/SNF has lower ATPase activity than BRG1 complexes, consistent with previous reports (Sif et al., 2001), and comparable catalytic activities of all SWI/SNF complexes were analyzed in our in vitro transcription experiments (Figure 4A). A micrococcal nuclease digest was performed on nucleosomal β-globin plasmids to assess the quality of chromatin assembly (Figure 4E).Taken together, these results demonstrate that EKLF can functionally discriminate between native and recombinant BRG1 and BRM SWI/SNF complexes through interaction specificity (Figure 3) and that the BRM catalytic subunit cannot replace BRG1 in ZFP-dependent transcription.

BRG1 Specificity for Zinc Finger Proteins Occurs through N-terminal Protein Interaction Domains which Are Not Present in BRM

The ability of EKLF to activate transcription with only SWI/SNF complexes containing the BRG1 ATPase is particularly striking since BRG1 and BRM share signifi-

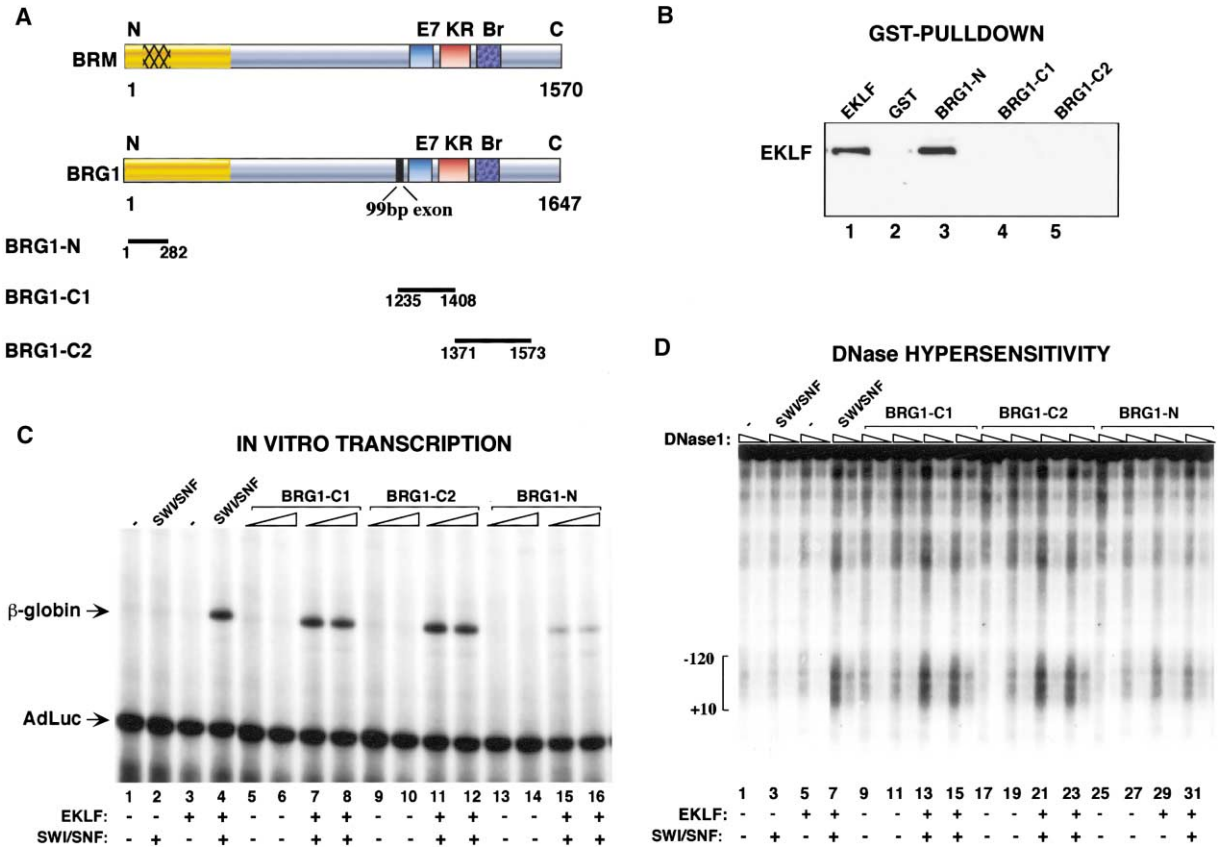

Figure 5. BRG1 Interacts with EKLF through Unique N-Terminal Sequences which Are Nonhomologous in BRM to Target Remodeling and Activate Transcription

(A) Diagram of human BRM and BRG1 proteins comparing common (HPV E7, KR, Bromodomain) and unique (99 bp exon, N terminus) motifs between the ATPases. The hatched area near the BRM N terminus designates a region that is nonhomologous with BRG1. Cloned subdomains of BRG1 containing common and unique sequences with BRM are indicated below.

(B) GST-pull-down analysis of protein interactions between 350 ng of His-tagged EKLF and 500 ng of each BRG1 subdomain. Interactions were detected by immunoblotting using EKLF antisera. Purified EKLF was used as a positive control (lane 1).

(C) In vitro transcription of chromatin-assembled β-globin promoters; effect of recombinant BRG1 subdomains as dominant-negative inhibitors of SWI/SNF-dependent EKLF activation. Assembled chromatin templates were incubated with EKLF (37 pmol per 1 μg of chromatin in a 100 μl reaction volume), SWI/SNF, and 300 pmol of each BRG1 subdomain. Reactions were divided in half and used for transcription or DNase hypersensitivity. AdLuc transcripts are shown as internal controls.

(D) DNase I footprint of chromatin-assembled β-globin promoters; effect of recombinant BRG1 subdomains as dominant-negative inhibitors of SWI/SNF-dependent EKLF binding. Each chromatin reaction (see above) was again divided into two tubes of 150 ng and digested with 1 and 2 U DNase1.

cant sequence homology (approximately 75% identical). Moreover, both ATPases are coexpressed in many cells, yet it is poorly understood why two mammalian homologs of SWI2/SNF2 exist. A comparison of protein domains within the BRG1 and BRM subunits is diagrammed in Figure 5A. BRG1-N spans the N-terminal residues 1–282 and shows high sequence divergence from the corresponding region of BRM. BRG1-C1 contains a 99 base pair exon that is unique to BRG1, the conserved E7 sequence, and a portion of the lysine-arginine (KR) region. BRG1-C2 spans the KR region and a bromodomain, both of which are conserved in BRM. We were interested in determining whether any of these regions within BRG1 were responsible for the observed functional specificity with zinc finger proteins. To address this, we expressed and purified recombinant proteins containing each of the three distinct BRG1 domains and performed GST-pull-down experiments to determine whether EKLF interaction occurred through one of these sequences.

Our analysis revealed that EKLF interacts specifically with the N-terminal region of BRG1 (BRG1-N) which shares little sequence homology with BRM (Figure 5B). The importance of this interaction was confirmed functionally by in vitro transcription and chromatin remodeling analyses by testing each of the three BRG1 domains as competitive inhibitors. As shown in Figure 5C, the BRG1-N protein is a potent inhibitor of SWI/SNF-dependent EKLF activation of chromatin-assembled β-globin genes, presumably by interfering with the ability of EKLF to associate with this region of BRG1 within the native complex (lanes 15 and 16). The BRG1-N protein similarly interferes with EKLF-targeted chromatin remodeling by SWI/SNF (Figure 5D, lanes 29–32). Two other subdomains of BRG1 that do not interact with EKLF (BRG1-C1, -C2) have no effect on either transcription (Figure

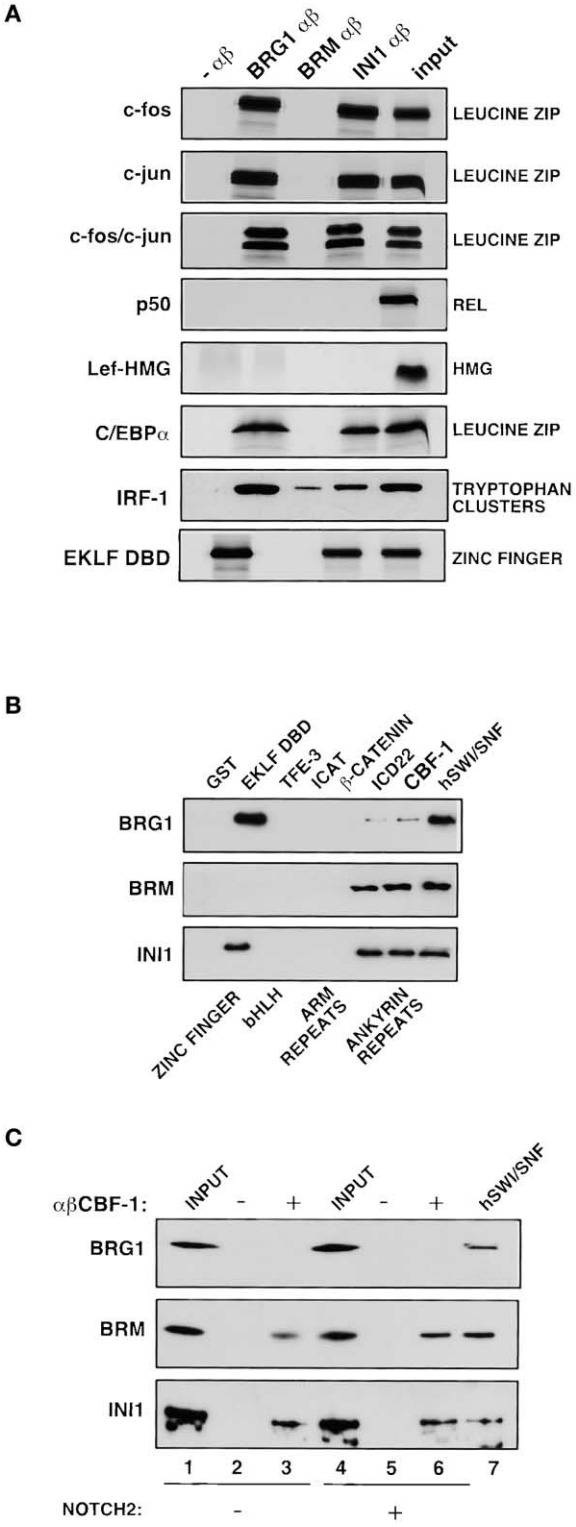

Figure 6. BRG1 and BRM Possess Distinct Interaction Specificities toward Different Transcription Factors

(A) In vitro binding studies between 500 ng native hSWI/SNF and 200 ng of the following in vitro translated proteins: c-*fos*, c-*jun*, heterodimer of c-*fos*/c-jun, NF-κB p50 DBD subunit, LEF HMG DBD, C/EBPα, IRF-1, and EKLF DBD. Immunoprecipitation was carried out using antisera to BRG1, BRM, and INI1 subunits of the hSWI/SNF complex and analyzed by SDS-PAGE. Ten percent of the in vitro translated protein was loaded as an input.

(B) GST-pull-down analysis of interactions between 1 μg hSWI/SNF and 500 ng of the following GST-fused proteins: EKLF DBD, TFE-3, ICAT, β-catenin, ICD22, and CBF-1. Interactions were detected by immunoblotting using antibodies against SWI/SNF BRG1, BRM, and INI1 subunits. Purified hSWI/SNF was used as a positive control.

(C) In vivo interaction of CBF-1 with BRM-containing SWI/SNF. Coimmunoprecipitation of hSWI/SNF with antibodies to CBF-1 using whole-cell extracts from Notch2-expressing mouse myoblasts (C2C12) and control cells. Immune complexes were separated by SDS-PAGE and analyzed by Western blots with antisera against BRG1, BRM, and INI1 subunits of SWI/SNF. Native hSWI/SNF was used as a positive control (lane 7).

5C, lanes 7, 8, 11, and 12) or chromatin remodeling (Figure 5D, lanes 13–16 and 21–24). These results demonstrate that the functional specificity between ZFPs and BRG1-containing SWI/SNF complexes is due to protein-protein interactions that occur through individual ZF within ZF DBD motifs and the N-terminal sequences of BRG1 which are nonhomologous with BRM.

BRG1 and BRM SWI/SNF Complexes Possess Unique Interaction Specificities with Different Protein Domains

We have shown previously that mammalian SWI/SNF preferentially associates with certain DNA binding proteins and that this may enable it to be targeted to specific promoters (Armstrong et al., 1998; Kadam et al., 2000). To further understand the basis of factor selectivity, we screened representative classes of different transcription factors for their ability to preferentially interact with BRG1- or BRM-containing native hSWI/SNF complexes. We synthesized some factors by in vitro translation (Figure 6A) and expressed others as GST-fusion proteins (Figure 6B). GST-pull-down and immunoblotting experiments revealed that some proteins fail to interact with hSWI/SNF whereas others associate preferentially or exclusively with BRG1- or BRM-containing complexes. In no case did we observe proteins interacting with equal affinity to both BRG1 and BRM SWI/SNF.

Among the factors that we examined, those that failed to interact with hSWI/SNF are: TFE-3 (bHLH); components of the Wnt signaling pathway, ICAT and β-catenin (ARM repeats); LEF-1 HMG DBD; and NF-κB p50 (rel DBD). Factors that associate preferentially or exclusively with BRG1 SWI/SNF complexes include: c-*fos*, c-*jun*, and C/EBPα (LEU zipper), IRF-1 (TRYP cluster), and, as expected, the EKLF ZF DBD. Interestingly, two proteins of the ankyrin repeat family showed a strong interaction preference for BRM rather than BRG1 hSWI/SNF (also see Figure 3C). These proteins, ICD22 and CBF-1, are critical regulators of the Notch signaling pathway and function with the cofactor Mastermind to activate transcription of chromatin-assembled templates (Fryer et al., 2002). To confirm our in vitro observations, coimmunoprecipitation experiments were carried out using protein extracts from control and Notch2-expressing mouse myoblast cells using antibodies to CBF-1. As shown in Figure 6C, CBF-1 interacts specifically with BRM-containing SWI/SNF complexes in both Notch2-expressing and -nonexpressing cells. No association between CBF-1 and BRG1 SWI/SNF was detected.

Thus, out of eight distinct protein domains examined

for interaction specificity between BRG1 and BRM, four do not associate with SWI/SNF (bHLH, ARM repeats, HMG, rel), three bind to BRG1 complexes (LEU zip, TRYP cluster, ZF), and one shows marked preference for BRM complexes (ANK repeats). All proteins that interact with either BRG1 or BRM SWI/SNF also coprecipitate with INI1 since it is a core subunit of both remodeling complexes. Taken together, these results demonstrate that BRG1 and BRM-containing SWI/SNF have distinct interaction preferences for regulatory proteins which can be classified on the basis of protein domain identity. The ability of mammalian SWI/SNF to discriminate among proteins in this manner provides a possible mechanism by which BRG1 and BRM complexes are targeted to specific genes that control distinct cellular functions or pathways.

Promoter Localization of BRG1 and BRM SWI/SNF Complexes and Chromatin Modifiers during Cellular Proliferation, Differentiation, and Signaling

The existence of two broad classes of SWI/SNF remodeling complexes and their clear interaction specificities for distinct transcription factors suggest that BRG1 and BRM may each regulate different genes. However, it is unclear which genes or biological processes require the activity of either SWI/SNF complex. To address these issues and to gain further insight into promoter targeting by BRG1- or BRM-containing SWI/SNF, we examined protein occupancies of a variety of tissue-specific and cell cycle regulatory genes during erythroid proliferation and differentiation in mouse erythroleukemia cells. We chose the β-globin gene, which is activated after chemically induced differentiation; the Cdk inhibitors p16, p21, and p27, which are also upregulated; cyclin A and the housekeeping gene, DHFR, which are downregulated after differentiation; and the T cell receptorα (TCRα) gene, which is permanently inactive in erythroid cells. In addition to analyzing these promoters for BRG1 and BRM interaction, we examined the occupancy of other chromatin enzymatic complexes, the histone acetyltransferase CBP, and the histone deacetylase HDAC1. The histone modification status within the proximal promoter of each gene was also determined.

Chromatin immunoprecipitation (ChIP) analyses were conducted using antibodies to a variety of proteins including the transcriptional activators EKLF, in the case of the β-globin promoter, or Sp1, as a ubiquitous factor found on many promoters. As shown in Figure 7A, during erythroid proliferation the β-globin, p16, and p21 promoters are remodeled and poised for transcription by the binding of EKLF or Sp1 (lane 2) and BRG1 (lane 3) plus INI1 (lane 11) but not BRM (lane 4). The nucleosomes assembled on these promoters are acetylated on lysines 4 and 9 of histones H3 and H4 (lanes 7 and 8) and dimethylated on H3 lysine 4 (lane 9). These modifications are characteristic of active genes. Interestingly, among the proteins we examined, the major change that occurs on these promoters upon upregulation after differentiation is the loss of HDAC1 (lane 17) and a decrease in bound BRG1 (lane 15), presumably because the chromatin is already stably remodeled. In the p27 promoter, Sp1, BRG1, and CBP are not bound in proliferating cells when expression is downregulated (lanes 2, 3, and 6) but are recruited after differentiation (lanes 14, 15, and 18). This is correlated with a dramatic increase in H4 acetylation (lane 20) and H3 Lys4 dimethylation (lane 21). DHFR is an example of a promoter that is apparently not regulated by SWI/SNF since neither BRG1, BRM, nor INI1 are bound (lanes 3, 4, and 11). Instead, when DHFR is upregulated in proliferating cells, Sp1 and CBP are recruited (lanes 2 and 6), H3 and H4 are acetylated, and H3 is methylated (lanes 7–9). Upon differentiation, DHFR downregulation is associated with the loss of Sp1 and CBP (lanes 14 and 18), a decrease in histone acetylation and Lys4 dimethylation (lanes 19–21), and the appearance of HDAC1 (lane 17). The cyclin A promoter is the only one in our erythroid cell survey that interacts with BRM. Interestingly, there is a switch in BRG1 and BRM occupancy of this promoter since BRG1 is predominantly bound during proliferation (lanes 3 and 4) but only BRM remains after differentiation (lane 16). This is consistent with previous studies which showed that Rb-dependent repression of the cyclin A promoter is correlated with the loss of BRG1 and the gain of BRM (Dahiya et al., 2001). The TCRα gene is permanently inactive in erythroid cells and is not associated with SWI/SNF subunits, CBP, acetylated or methylated histones, or even the corepressor HDAC1.

The majority of promoters in our ChIP analyses interact with BRG1 rather than BRM. This correlates with protein-protein interaction experiments which showed that most transcription factors in our survey, which may recruit SWI/SNF to different promoters, associate with BRG1-containing complexes (Figure 6). BRG1 interacts with an erythroid-specific gene, β-globin, and cell cycle regulatory genes, p16, p21, and p27 whereas both BRG1 and BRM bind to cyclin A. The preponderance of promoter-associated BRG1 complexes in our studies may reflect a more widespread role of BRG1 in cellular function as indicated by the severity of phenotype in BRG1 knockout mice (Bultman et al., 2000). This raises the issue of identifying which genes BRM SWI/SNF complexes regulate. In this regard, one intriguing observation is that BRM preferentially associates with proteins that regulate the Notch signaling pathway, CBF-1 and ICD22 (Figures 6B and 6C). Upon signaling, the transmembrane Notch receptor is proteolytically cleaved to release the ICD (Notch intracellular domain) which translocates to the nucleus and forms a complex with CBF-1. This complex then activates Notch target genes which regulate a variety of cell fate decisions (Artavanis-Tsakonas et al., 1999; Anderson et al., 2001).

We examined the physiological relevance of these protein interactions by determining whether BRM is indeed recruited by CBF-1 to natural Notch target genes. As shown in the ChIP analysis in Figure 7B, CBF-1 is bound to two Notch-regulated promoters, Hes1 and Hes5, in mouse C2C12 myoblasts which express the Notch2 ICD as well as in control myoblasts (lanes 3 and 14). Significantly, BRM but not BRG1 is predominantly associated with these promoters (lanes 6 and 17) in Notch2 expressing and nonexpressing cells. This suggests that CBF-1 recruits SWI/SNF BRM complexes to Hes1 and Hes5 promoters through preferential interaction with BRM rather than BRG1 and that SWI/SNF-dependent chromatin remodeling occurs before Notch signaling. INI1 is also bound to each promoter because

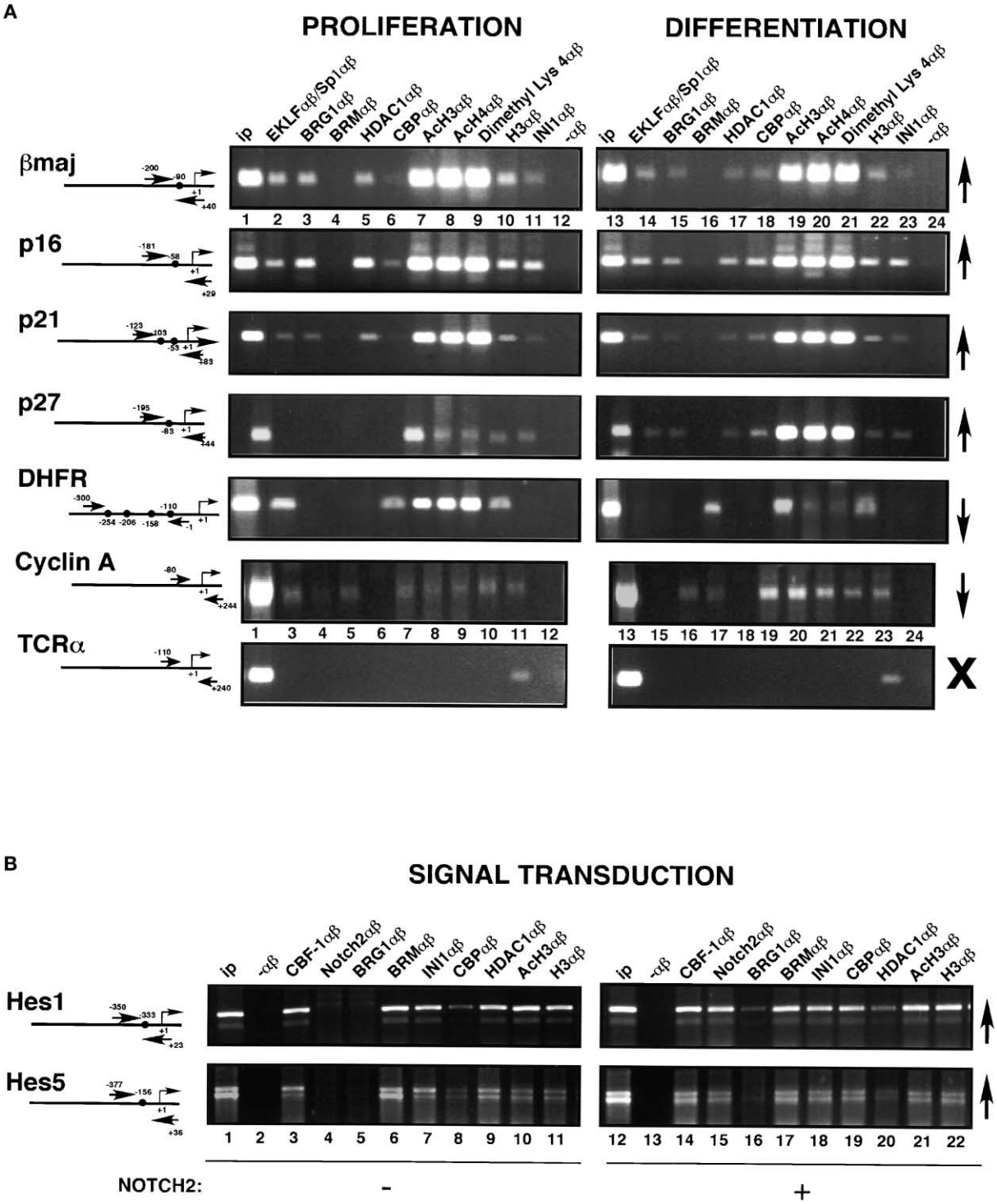

Figure 7. Promoter-Specific Recruitment of BRG1 or BRM SWI/SNF and Chromatin Modifiers during Proliferation, Differentiation, and Signal Transduction

Chromatin immunoprecipitation analyses of SWI/SNF remodeling complexes (BRG1, BRM, core subunit INI1), transcriptional activators (EKLF/Sp1, CBF-1, Notch2), chromatin coactivators/corepressors (HDAC1, CBP), and histone modifications (AcH3, AcH4, dimethyl Lys4 H3, core H3) were performed using a variety of promoters (left side) which are up- or downregulated or permanently inactivated (right side, indicated by arrows or by an "X") in the cell types examined. In brief, formaldehyde crosslinked DNA was immunoprecipitated, using the antibodies indicated above each lane, and the recovered DNA was employed as a template in PCR amplification using primers directed against different promoters.

(A) ChIP analyses of individual promoters during DMSO-induced mouse erythroid cell differentiation (βmaj, adult β-globin; DHFR, dihydrofolate reductase; TCRα, T cell receptor α chain). Amplification products were in the range of 250 to 350 bp and contain the promoter sequence elements indicated on the left of the panel. Input DNA (5%) and minus antibody controls were also included.

(B) ChIP analyses of Hes1 (hairy enhancer of split) and Hes5 promoters during Notch signaling in mouse myoblasts. Amplification products were in the range of ~400 bp and contain the CBF-1 sequence elements indicated on the left of the panel. Input DNA (5%) and minus antibody controls were also included.

it is a core subunit of all SWI/SNF complexes (lanes 7 and 18). The Hes1 and Hes5 promoters appear to be poised for transcription by CBF-1 binding, BRM recruitment, and histone acetylation (lane 10). Upon signaling, Notch2 associates with Hes promoters (lane 15) and provides an activation domain for CBF-1, which then stimulates transcription. Interestingly, another pronounced change after signaling is a switch in the relative amounts of promoter-bound CBP and HDAC1 which correlates with the induction of Hes gene expression (lanes 8, 9, 19, and 20).

Taken together, these results provide insight into the specificity of promoter recruitment of BRG1 or BRM SWI/SNF complexes during proliferation, differentiation, and cell signaling. SWI/SNF interaction normally precedes transcription suggesting that nucleosome remodeling poises target promoters for eventual activation. With the exception of cyclin A, either BRG1 or BRM, but not both, preferentially associates with individual promoters. BRG1 binds to a tissue-specific gene and several cell cycle regulators whereas BRM interacts with cyclin A in combination with BRG1 and exclusively with two genes that are induced by Notch signaling. Our experiments also reveal consistent patterns of SWI/SNF occupancy, nucleosome modifications, and relative CBP/HDAC levels among some promoters and marked differences in others. This reflects the distinct levels at which genes are regulated in response to changes in cellular function.

Discussion

Our studies reveal that human SWI/SNF complexes containing either BRG1 or BRM as the catalytic subunit interact specifically with different classes of regulatory proteins. This property enables these enzymes to be selectively targeted to distinct sets of promoters to facilitate chromatin remodeling and transcription. This provides a mechanistic basis for members of these two broad categories of SWI/SNF to regulate different programs of gene expression. We find that representatives of the zinc finger family of proteins interact exclusively with BRG1-containing SWI/SNF and recruit these complexes to specific chromatin sites. The ability of SWI/SNF to coactivate ZFP was first shown with nuclear hormone receptors (Yoshinaga et al., 1992). The ZFP family is the largest class of transcription factors, and many members have diverse roles in critical cellular processes. The ZF domain is a ubiquitous structural element that exists in several major motifs. For example, the C2H2 motif is the most abundant eukaryotic DNA binding element which is estimated to be present in 600–700 proteins. We determined that SWI/SNF interacts with ZFPs through the ZF DBD and the BRG1 ATPase. The basis for the observed specificity between ZFPs and BRG1 complexes is that interaction occurs within a domain of BRG1 that is nonhomologous with BRM. We then examined the role of individual ZFs within two structural motifs, C2H2 and C4, in mediating BRG1 SWI/SNF function. Using the erythroid factors EKLF and GATA-1 as representative proteins that contain C2H2 and C4 domains, respectively, our studies demonstrate that BRG1 binds to individual ZFs which are the most critical for DNA binding. This may seem paradoxical; however, ZF DBDs have been shown to associate with both RNA and protein. The EKLF and GATA-1 DBDs interact with a variety of cofactors, often through specific ZFs (Cantor and Orkin, 2002). The significance of such critical protein-protein interactions, including that of SWI/SNF, occurring through domains that must also bind DNA has yet to be elucidated. The functional relationship between BRG1-containing SWI/SNF and the ZF DBDs of EKLF or GATA-1 may pertain to other members of these transcription factor families which contain conserved DBDs but highly divergent activation domains which contribute to their specialized functions in gene regulation.

The involvement of mammalian SWI/SNF in cell proliferation and differentiation has been shown in several systems. SWI/SNF cooperates with C/EBPα to control genes that specify either myeloid or adipocyte lineages (Kowenz-Leutz and Leutz, 1999; Pedersen et al., 2001) and MyoD to regulate muscle cell determination (de la Serna et al., 2001). The role of SWI/SNF in mediating Rb-dependent cell cycle progression is well documented (Harbour and Dean, 2000). Additionally, recent studies demonstrate that SWI/SNF influences thymic development by CD4 silencing and CD8 activation through separate contributions of BRG1 and BAF57 (Chi et al., 2002). Our analysis of SWI/SNF promoter localization in differentiating erythroid cells shows that these complexes bind to a variety of tissue-specific and cell cycle regulatory genes. SWI/SNF recruitment generally occurs during proliferation indicating that the promoters we surveyed are already poised for changes in transcriptional activity which will be induced by differentiation. It is apparent that different strategies are used to regulate gene activity during this specific window of transition. Some promoters have dramatic changes in histone modifications that correlate with altered gene expression whereas others show a clear gain or loss of activators, coactivators, and corepressors or a combination of both processes. The fascinating diversity of transcriptional mechanisms and the temporal order of specific regulatory events have been demonstrated in several elegant studies (Cosma, 2002). In our analysis, BRG1 or BRM, but not both, exclusively interacts with most SWI/SNF-responsive promoters. This correlates well with our protein-protein interaction experiments which reveal that among the factors we examined the majority associate preferentially with BRG1 or BRM or have no affinity for either form of SWI/SNF. In no case do we observe equivalent binding to both BRG1 and BRM. It is possible that the recombinant and in vitro translated forms of these proteins fail to interact without other cofactors or modifications. However, the clear binding preferences observed for a number of factors strongly support the notion that gene-targeted SWI/SNF activity can be regulated by highly specific protein interactions which discriminate between the two ATPases.

The BRM ATPase is expressed at high levels in differentiating cells, yet the functional role of this protein and the identity of the genes it regulates are poorly understood. In this regard, our observation that two components of the Notch signaling pathway, CBF-1 and ICD22, strongly associate with BRM but not BRG1 is especially intriguing. This pathway controls cell fate commitment in a broad range of developmental processes. We find

that CBF-1 recruits BRM to two natural target genes, Hes1 and Hes5, in myoblasts before Notch induction. This indicates that these promoters are already in a remodeled configuration and accessible to bind the activator, Notch2, upon signaling. Interestingly, our studies show that components of the Wnt signaling pathway, β-catenin and ICAT, fail to interact with either BRG1- or BRM-containing SWI/SNF. This suggests that different requirements may exist for specific remodeling complexes to mediate the effects of distinct types of extracellular signals. In support of this, recent reports show that BRG1-containing SWI/SNF is involved in regulating genes induced by cytokine pathways through interferon-α or -γ (Liu et al., 2002; Pattenden et al., 2002). In addition to Hes1 and Hes5, the only other reported BRM target genes are cyclin A which is repressed in an Rb-dependent manner during the cell cycle (Dahiya et al., 2001) and α_1-antitrypsin (α_1-AT) which is activated upon enterocyte differentiation (Soutoglou and Talianidis, 2002). Thus, a common feature of these BRM target genes is that they promote cellular differentiation and may have evolved to respond to BRM because its levels are more highly regulated in the cell than BRG1.

It will be interesting in the future not only to define the target genes of BRG1 and BRM and the proteins that selectively recruit them but to understand the physiological basis for this specificity.

Experimental Procedures

Cell Lines and Plasmid Constructions

Mouse erythroleukemia (MEL) cells were grown in α MEM, 10% calf serum, and 1X pen-strep-L-glutamine, and induced using 2% DMSO for 5 days. C2C12 mouse myoblasts expressing Notch2 ICD were maintained in DMEM containing 20% FBS in the presence of 400 μg G418. Control C2C12 cells were maintained only in DMEM plus FBS and split every 2 days. Cell confluency did not exceed 70%. β-CAT, HIV-1-Luc, EKLF, GATA-1, NF-κB, TFE-3, and Sp1 plasmids were constructed as described in Kadam et al., 2000. BRG1-N, -C1 and -C2 were cloned into the EcoRI and BamHI sites of pGEX-KG expression vector by introducing amino acids 1–282, 1235–1408, and 1371–1573 of BRG1 downstream of GST.

Protein Purification

Histidine-tagged or GST-fusion wild-type and mutant proteins were expressed in *E. coli* BL21(DE3)pLysS or BL21(DE3) cells (Kadam et al., 2000). Some proteins were in vitro translated with the Invitrogen trancription and translation system using ^{35}S (Amersham) as per the manufacturer's instructions. Human Flag-tagged SWI/SNF, F-BRG1, F-hBRM, F-BAF155, and F-BAF170 were purified as described (Phelan et al., 1999). HeLa FL-INI1-11 cells were grown by the National Cell Culture Center. BRG1 and BRM SWI/SNF complexes were immunopurified from 5 μg native hSWI/SNF using antisera to either BRG1 or BRM as described (Yie et al., 1999). The specific complexes were separated using Sepharose Protein A beads, and purified forms of SWI/SNF in the supernatants were assayed for activity. To measure the ATPase activity of SWI/SNF complexes, approximately 55 ng of purified complexes was incubated with or without 150 nM of nucleosomes. Reactions were initiated with 10 μM ATP, 7 mM $MgCl_2$ and a trace quantity of (γ-^{32}P) ATP in a final volume of 10 μl. At specific times after initiation of the reaction, 2 μl aliquots were quenched in 5 μl of stop solution containing 3% SDS, 100 mM EDTA, and 50 mM Tris (pH 7.5). Each time point was spotted onto PEI-Cellulose TLC plates (EM Science). Inorganic phosphate was separated from unreacted ATP by running the TLC plates in 0.5 M LiCl, 1 M formic acid. The ratio of inorganic phosphate to ATP at each time point was quantitated using a Molecular Dynamics PhosphoImager.

Chromatin Assembly and In Vitro Transcription

Chromatin was reconstituted using *Drosophila* embryonic extracts as described (Bulger and Kadonaga, 1994). Following assembly, the chromatin template (1 μg in 100 μl) was incubated with wild-type or mutant proteins, as indicated in the figure legends, and SWI/SNF for 30 min at 27°C. The reactions were then split in half and used for either transcription or structural analyses as described (Kadam et al., 2000).

Chromatin Structural Analyses

DNase 1 hypersensitivity reactions were performed as described in Kadam et al. (2000). Micrococcal nuclease digestions: 15 U enzyme was added to 500 μg of chromatin, and 150 μg aliquots were processed at timed intervals and analyzed on 1.5% agarose gels. DNase I footprinting: After incubation of chromatin templates with specific factors, samples were digested with 33 U/ml of DNase I (Boehringer Mannheim) for 80 s at 27°C. Purified DNA fragments were analyzed by primer extension. Mononucleosome disruption: Sequence-positioned mononucleosomes were reconstituted on 5S DNA by salt dialysis and subjected to sucrose gradient sedimentation as described (Armstrong et al., 1998).

Protein-Protein Interactions

Pull-down assays with GST or histidine-fusion proteins were carried out as described (Kadam et al., 2000). Immunoprecipitation of 0.2–1 μg in vitro translated proteins and 0.5–3 μg SWI/SNF was performed using 0.5 μg of primary antibody against BRG1, hBRM, or INI1 followed by binding to Protein A- and G-agarose beads.

Chromatin Immunoprecipitation Assays

Chromatin immunoprecipitation assays were performed essentially as described (Liu et al., 2001). Five micrograms of antibodies to the proteins H3, AcH3, AcH4, dimethyl H3 Lys4, CBP, HDAC1 (Upstate), EKLF, Sp1, hBRM (Santa Cruz), BRG1, and INI1 (gift from Dr. Weidong Wang) was used according to the manufacturer's recommendations with some minor modifications. Immunoprecipitated promoter fragments were detected by PCR amplification.

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