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Repression of GR-Mediated Expression of the Tryptophan Oxygenase Gene by the SWI/SNF Complex during Liver Development

Yujin Inayoshi[1], Hidenori Kaneoka[1], Yuichi Machida[1], Masaomi Terajima[1], Takeaki Dohda[1], Katsuhide Miyake[2,*] and Shinji Iijima[1]

[1]Department of Biotechnology, Graduate School of Engineering, and [2]Ecotopia Science Institute, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603

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The chromatin remodeling complex, SWI/SNF, is known to regulate the transcription of several genes by altering the chromatin structure in an ATP-dependent manner. SWI/SNF exclusively contains BRG1 or BRM as an ATPase subunit. In the present study, we studied the role of SWI/SNF containing BRM or BRG1 in the expression of the liver-specific tryptophan oxygenase (TO) and tyrosine aminotransferase genes. Chromatin remodeling factors significantly repressed the expression of these genes induced by glucocorticoid receptor and dexamethasone. Since the repression was not reversed by trichostatin A treatment, it seemed to be independent of the well-known histone deacetylase pathway. Knock-down of BRG1 by small interfering RNA reversed the repression in primary fetal hepatocytes. These results support a model in which SWI/SNF containing BRG1 represses late stage-specific TO gene expression at an early stage of liver development.

Key words: glucocorticoid receptor, primary hepatocytes, SWI/SNF, transcriptional repression, tryptophan oxygenase.

Abbreviations: GR, glucocorticoid receptor; GRE, glucocorticoid responsive element; HDAC, histone deacetylase; MMTV, mouse mammary tumor virus; pRb, retinoblastoma protein; siRNA, small interfering RNA; TAT, tyrosine aminotransferase; TO, tryptophan oxygenase.

Eukaryotic genes are highly organized into chromatin structures that can be altered to generate transcriptionally active or repressed configurations in different cellular contexts. The structural changes in chromatin that accompany transcriptional activation often require multi-protein complexes that manipulate the nucleosomal structure (*1*, *2*). There are two general classes of complexes that disrupt the chromatin structure: those that covalently modify histones by altering the acetylation, methylation, phosphorylation and ubiquitination patterns, and those that use ATP hydrolysis to disrupt histone-DNA interactions (*3–8*).

The mammalian chromatin remodeling complex (SWI/SNF) uses the energy of ATP hydrolysis to modify the chromatin structure. The complex contains various central ATPase subunits that exhibit similarity to those of the yeast SWI2/SNF2. These ATPases can be divided into three different subfamilies, the SWI2/SNF2, Mi-2/CHD, and ISWI families, based on structure (*2*, *9*). Mammalian SWI/SNF consists of ~15 subunits and falls into two broad classes, depending on whether it contains BRM or BRG1 as the ATPase subunit (*10*). SWI/SNF interacts with a wide variety of proteins including transcription factors and nuclear receptors (*11–17*). In many cases, the chromatin remodeling complex is essential for the activation of a number of inducible genes (*12–14*, *16*, *17*). However, it was recently reported that SWI/SNF represses transcription mediated by several transcription factors (*18–22*). Since a previous report confirmed that a SWI2/SNF2-deficient mutant of yeast showed significant enhancement as well as suppression of many genes (*23*), it is no surprise that SWI/SNF represses gene expression mediated by several transcription factors.

In mammals, steroid hormones play important roles in the control of the response of the organisms to stress, whereas during development, they prepare various organs for the metabolic adaptations allowing autonomous life after birth. The nuclear hormone receptor is a ligand-activated transcriptional regulator, which makes use of coactivator complexes to modulate both the chromatin structure and the activity of the basal transcriptional machinery (*24*). The best characterized coactivators include the p160 family proteins (SRC-1-3), histone acetyltransferase CBP/P300, the ATP-dependent chromatin remodeling complex and the mediator complex (DRIP/TRAP) that bridges the basal transcriptional machinery during transactivation (*25*, *26*).

The glucocorticoid receptor (GR) regulates gene expression primarily through direct interaction with the glucocorticoid responsive element (GRE). It has been shown that GR recruits SWI/SNF to promoter-enhancer regions that contain GRE, resulting in disruption of the local nucleosomal structure (*27*). The mouse mammary tumor virus (MMTV) promoter and the tyrosine aminotransferase (TAT) gene enhancer have been used to study the processes involved in transcriptional activation by GR (*27*, *28*).

*To whom correspondence should be addressed. Tel: +81-52-789-4278, Fax: +81-52-789-3221, E-mail: miyake@proc.nubio.nagoya-u.ac.jp

In the liver, the activated GR plays central roles in expression of the tryptophan oxygenase (TO) and TAT genes in postnatal life (*28*, *29*). The induction of the TO and TAT genes is thought to be mediated by GREs located at –0.45 kb and –1.2 kb, and at –2.5 kb, respectively. Expression of the TO and TAT genes is useful for studying the mechanism of control of gene expression, since their activities are subject to developmental and organ specificity.

We show here that SWI/SNF down-regulated GR-mediated transcription of the TO and TAT genes. This repression did not depend on histone deacetylase (HDAC). Since restriction enzyme accessibility decreased in the repressed state, alteration of the chromatin structure may cause the repression.

MATERIALS AND METHODS

Plasmids—Mouse BRM cDNA was obtained by reverse transcription (RT)-PCR using mRNA prepared from cultured mouse neural precursor cells (NPCs), and a 5′ primer (5′-ATTAGAATTCAAGATGTCCACACCCACAGACCCAG-C-3′, *Eco*RI site is underlined) and a 3′ primer (5′-TTGC-GTCGACCTCGTTATCAGTTCCACTTGC-3′, *Sal*I site is underlined). Full-length cDNA of BRM was introduced into the *Eco*RI and *Xho*I sites of pcDNA4/TO/*myc*-His (Invitrogen), and an expression vector for BRM, pcDNA4-BRM, was constructed. A BRG1 expression vector, pcDNA4-BRG1, was also constructed. The BRG1 cDNA was amplified from mRNA of cultured NPCs by RT-PCR using a 5′ primer (5′-CCACAAGCTTAGTTCCAGTAAA-GATGTCTACTCC-3′, *Hin*dIII site is underlined) and a 3′ primer (5′-AAAAGCGGCCGCGTCTTCCTCACTGCCA-CTTCCTG-3′, *Not*I site is underlined), and then introduced into pcDNA4/TO/*myc*-His. These primers were designed from the reported sequence of BRG1 (*30*). The DNA sequences of these cloned fragments were confirmed. For expression of GR, GR cDNA was amplified from RNA of mouse NPCs by PCR using a 5′ primer (5′-CATG-GATCCGGAAGTTAATATTTGCCAATG-3′, *Bam*HI site is underlined) and a 3′ primer (5′-CATCTCGAGC-TTTAAGGCAGCCTTTCTTAG-3′, *Xho*I site is under-lined), and then introduced into pcDNA4/TO/*myc*-His (pcDNA4-GR).

The regulatory regions of the TAT and TO genes were amplified from rat genomic DNA. The TAT enhancer-promoter region (2.5 kb) was amplified using a 5′ primer (5′-CATCTCGAGGCAAGTTAGCTCCAGAGCGC-3′, *Xho*I site is underlined) and a 3′ primer (5′-CATAAGCTT-GAGCTCAGGTGCAGTCCTGAC-3′, *Hin*dIII site is under-lined). The TO regulatory region (1.2 kb) was amplified using a 5′ primer (5′-CTACTCGAGACGCCAATGAATTG-GATCA-3′, *Xho*I site is underlined) and a 3′ primer (5′-CTAGTCGACCCAGATATTTTCGTGCTTGC-3′, *Sal*I site is underlined). The amplified DNA fragments were introduced into pGL3-basic (Promega) and pREP4 (Novagen), and the resulting modified luciferase reporter vectors were designated as pGL3-TO/TAT and pREP4-TO/TAT. Two deletion mutants of the TO regulatory region were also constructed from the TO reporter plasmid. For deletion of the proximal and distal GREs, a plasmid without the proximal or distal GRE was directly amplified from pGL3-TO using primers (Δproximal GRE, 5′-ATCCTGCAGGTTGGAAGACTACCGCACCG-3′ and 5′-TTCCTGCAGCCACACCCAGCCTGTATT-3′; Δdistal GRE, 5′-TTCCTGCAGCGATGTGATGGCCTATCTCA-3′ and 5′-TTCCTGCAGACTGATCCAATTCATTGGCG-3′, *Pst*I sites are underlined) and then self-ligated, and these primers were also used for the deletion of both GREs (ΔGREs).

We constructed an ATPase mutant of BRG1 using a PCR-based method. For this purpose, two primers proximal to the ATP-binding motif (5′-CATAAGCTTCCCAGCCCCAT-CTCAT-3′, 5′-CTAAAGCTTCATCCAGACCATCGCGCTC-3′, *Hin*dIII sites are underlined) were designed. The 5′- and 3′-fragments of BRG1 were amplified using these primers and the BRG1 5′- and 3′-primers, and then ligated to produce a BRG1 ATPase mutant (BRG1ΔATP). This mutation changed the KT sequence (785–786 aa) in the BRG1 ATP-binding motif to SF.

Antibodies and Western Blot Analysis—Antibodies to BRM and BRG1 were prepared as described previously (*31*). Antibodies to GR and actin, and normal rabbit antibodies were purchased from Santa Cruz Biotechnology. Western blot analysis was performed by the standard procedure.

Cell Culture—Hepatocytes were isolated from fetal (15, 17 and 19 days) and adult Sprague-Dawley rats as described previously (*32*). Fetal hepatocytes at the 15 and 17-day stages were cultured in William's medium E (WE) (Invitrogen) containing 0.1 μM $CuSO_4$, 25 nM Na_2SO_4, 0.1 μM insulin (Sigma), 48 μg/ml of gentamicin sulfate (Sigma), and 100 μg/ml of chloramphenicol (Wako). Adult hepatocytes were cultured in WE medium without serum. Dexamethasone (Wako) was added (1 μM) when necessary. C33A and SW-13 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum.

Immunoprecipitation Assays—For immunoprecipitation assays, 3×10^6 C33A cells were seeded into 100-mm dishes the day before transfection and then co-transfected with 2.5–5 μg of BRM or BRG1, and GR expression vectors by the calcium phosphate method (*33*). Transfected C33A cells and hepatocytes (1×10^7 cells) were used for immunoprecipitation assays.

RT-PCR Analysis—Total RNA was isolated using EASY-Prep RNA isolation reagent (Takara). Five micrograms of RNA was subjected to a RT reaction. A reverse transcriptase, ReverTra Ace (Toyobo), was used to generate cDNAs in a total volume of 20 μl according to the manufacturer's instructions. One microliter of the cDNAs was mixed with 10 pmol of primers specific for TO cDNA (5′-AAACTCCCC-GTAGAAGGCAG-3′ and 5′-TGCAACTCTGGAAGCCTG-3′), for TAT cDNA (5′-CAATCCGAACAAGACCGTGA-3′ and 5′-TTCCCAAGACTTCTCAGGCAG-3′), and for BRG1 cDNA (5′-GATGTCGACCCGTGCTGGGGGGCT-3′ and 5′-CATTCTAGAGTCTTCCTCACTGCCACTTCC-3′), and then amplified by PCR. As a control, PCR using primers specific to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was carried out using the same samples.

Chromatin Immunoprecipitation (ChIP) Assay—The ChIP assay was performed using salmon sperm DNA/protein A agarose beads (Upstate Cell Signaling Solutions) according to the manufacturer's instructions with some modifications. Fetal (17 days) and adult hepatocytes were treated with 1% formaldehyde for 10 min at room temperature. Crosslinking was stopped by adding glycine

to 0.125 M. The immunoprecipitated DNA and input DNA were analyzed by PCR using primers for the distal GRE region of TO (direct primer, 5′-GGGAGATGGGGGAAGGAGAA-3′; reverse primer, 5′-CATGGGGAAGGGTAGTG-3′), and for the proximal GRE region of TO (direct primer, 5′-GAAAGGTCATTGTACGGCTGCCATCTT-3′; reverse primer, 5′-TGATTTCTCCCGGTGCGGTAGTCTT-3′).

Luciferase Reporter Gene Assays—For reporter assays, C33A or SW-13 cells seeded into 24-well plates were transfected using LipofectAMINE 2000 reagent (Life Technologies, Inc.) according to the manufacturer's instructions. Typically, 0.05 μg each of the luciferase reporter plasmids and the *Renilla* luciferase plasmid containing the promoter region of the elongation factor Iα gene were transfected with or without 0.1 μg of GR expression vector and 0.5 μg of BRG1 or BRM expression vector. Dexamethasone (1 μM) was added 24 h post-transfection when necessary. The amount of total DNA was kept constant by adding empty expression vector. Reporter assays were performed with a dual luciferase assay kit (Promega) according to the manufacturer's instructions. Luciferase activity was measured with a luminometer (Atto JNR2) and normalized as to *Renilla* luciferase activity. Data shown are for representative experiments carried out three times.

Restriction Enzyme Accessibility Assay—Nuclei were isolated from SW-13 cells transfected with the pREP4-TO luciferase reporter plasmid, and GR and BRG1 or BRM expression vectors. The nuclei from 5×10^6 cells were digested with *Fok*I (5 U) by incubation for 10 min at 37°C. Digestion was stopped by adding a stop solution containing proteinase K. After purification by phenol-chloroform extraction and ethanol precipitation, 10 μg of DNA was digested with *Hin*cII, separated on a 0.6% agarose gel, and then analyzed by Southern blotting followed by densitometric analysis (*34*).

Small Interfering RNA (siRNA) Knock-Down—Following culture at 37°C for 18 h, the transfection of siRNAs into fetal or adult hepatocytes at 50% confluency in 24-well plates was performed with Oligofectamine Reagent (Invitrogen). The siRNAs for rat BRG1 (sense: 5′-UUUGAACUUCUUGAUAGAAdTdT-3′, antisense: 5′-UUCUAUCAAGAAGUUCAAAdTdT-3′) were purchased from Darmacon Research Inc. Hepatocytes were harvested at 48 or 72 h post-transfection, and the expression of BRG1 and TO was analyzed by RT-PCR.

RESULTS

Expression Patterns of the TO and TAT Genes during Liver Development—To confirm the expression of the TO and TAT genes during the development of the liver, RT-PCR and luciferase reporter gene assays were performed using primary hepatocytes. These genes are known to be regulated by GR and its ligand through GREs that exist in their regulatory regions (*28*, *29*, *35*). While the expression of GR was almost constant during the development, that of the TO and TAT genes only was enhanced in adult hepatocytes, as reported previously (*28*, *29*, *36*, *37*) (Fig. 1A). To investigate the transcriptional regulation of the TO gene in hepatocytes, a pGL3 luciferase reporter plasmid containing the regulatory region of the TO gene was introduced into fetal and adult hepatocytes, and then luciferase activity was measured. As shown in Fig. 1B, luciferase activity

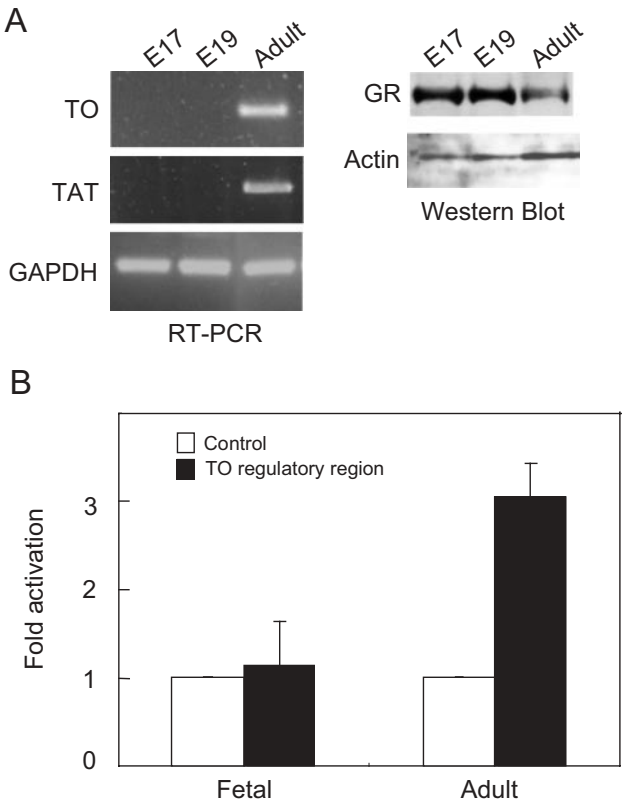

Fig. 1. **Developmental changes in the expression levels of the TO and TAT genes.** (A) Expression of TO, TAT and GR during liver differentiation. Hepatocytes were isolated from E17, E19, and adult rats. Equal amounts of hepatocyte proteins were subjected to SDS-PAGE for Western blotting and equal amounts of RNAs were used for RT-PCR. As controls, anti-actin antibodies were used for Western blotting and GAPDH cDNA was amplified for RT-PCR analysis. (B) Transcriptional activity of the regulatory region of the TO gene in primary hepatocytes. A pGL3 luciferase reporter plasmid containing the regulatory region was transfected into fetal and adult hepatocytes. As a negative control, a pGL3-basic plasmid was also transfected. The reporter activity of the pGL3-basic plasmid is expressed as 1. Error bars represent the standard error of the mean of three independent experiments.

was not stimulated in fetal hepatocytes but was significantly enhanced in adult hepatocytes, indicating that the TO regulatory region on the reporter plasmid is transcriptionally repressed at an early stage of liver development, and induced in adult hepatocytes.

GR-Mediated Expression of the TO and TAT Genes is Regulated by Chromatin Remodeling Factors—GR-mediated transcription of the MMTV promoter is known to be enhanced by SWI/SNF containing BRG1 (*13*). Therefore, it is likely that SWI/SNF containing either BRG1 or BRM also regulates the GR-mediated transcription of the TO and TAT genes in liver. To study this possibility, we performed luciferase reporter assays in the presence and absence of these chromatin remodeling complexes. As a host cell line, SW-13 cells, which are deficient in BRG1 and BRM but express other subunits of SWI/SNF (BRG1 associated factors, BAFs), were used. As shown in Fig. 2A, GR stimulated transcription from the regulatory region (1.2 kb) of the TO gene upon the addition of dexamethasone.

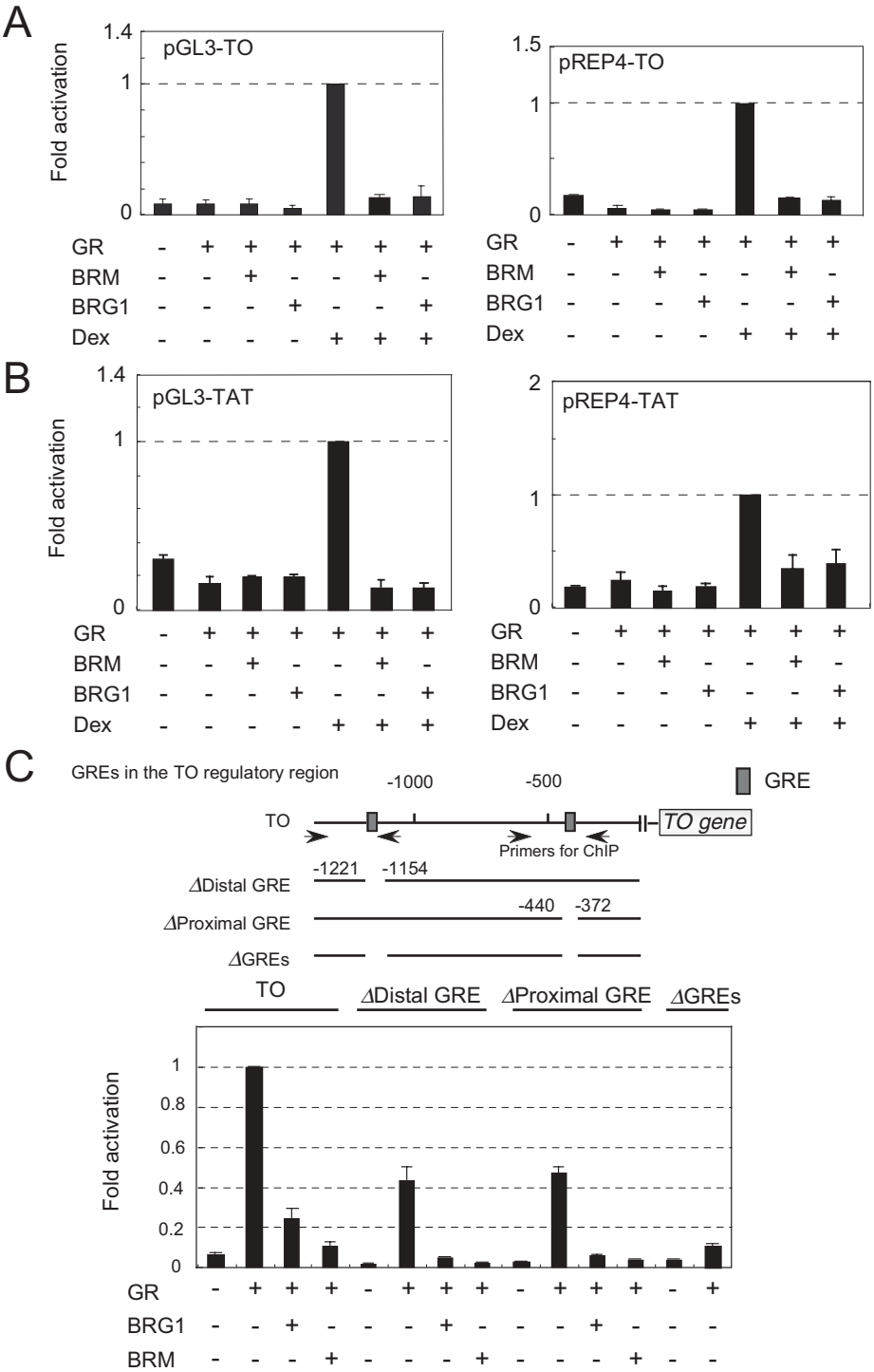

Fig. 2. **BRM and BRG1 significantly repress GR-mediated expression of the TO and TAT genes.** (A) pGL3 and pREP4 luciferase reporter plasmids containing the TO regulatory region were transfected into SW-13 cells with expression vectors for GR and BRG1 or BRM. (B) pGL3 and pREP4 luciferase reporter plasmids containing the TAT enhancer-promoter region were transfected into SW-13 cells with expression vectors for GR and BRG1 or BRM. Dexamethasone (Dex) was added at 1 μM, if necessary. (C) pGL3 reporter plasmids containing a mutated TO regulatory region were transfected into SW-13 cells and the effect of deletion in GREs was examined. The structures of these mutants are also shown. Dex (1 μM) was added 24 h post-transfection. Reporter activation with GR is expressed as 1. Error bars represent the standard error of the mean of three independent experiments.

However, when the BRG1 or BRM expression vector was transfected into SW-13 cells together with the GR expression vector, both BRG1 and BRM significantly repressed the expression of this gene. We performed these assays using two different reporter plasmids, pREP4 and pGL3. pREP4 is thought to replicate as an episomal vector and to form chromatin structures in transfected cells (*38*), and in several cases, SWI/SNF could enhance transcription of the gene only on pREP4 (*39*). Repression by BRG1 and BRM was observed in both pREP4 and pGL3 (Fig. 2A), and the level of repression was almost the same for these luciferase vectors. Similar results were obtained for the enhancer-promoter region of the TAT gene (Fig. 2B).

Since two GREs reside in the regulatory region of the TO gene, we then examined the roles of the respective GREs in the GR-mediated expression, and in the repression of the GR-mediated transactivation by SWI/SNF. A luciferase assay was performed using reporter plasmids with a deletion in the respective GREs. As shown in Fig. 2C, the reporter plasmid with either the distal or proximal

GRE deleted exhibited weaker transactivation by GR, compared to that containing an intact regulatory region; they still retained about 50% of the activity exhibited by the intact regulatory region. This finding shows that the effect of the GREs is additive, as reported previously (*29*). The extent of repression by SWI/SNF was similar for both deletions. Moreover, the luciferase activity of the reporter plasmid with deletion of both GREs was drastically reduced (Fig. 2C). These results suggest that both the GREs contribute to GR-mediated transactivation and that the repression is not dependent on either the proximal or distal GRE alone.

BRG1 and BRM Interact with GR and Exist in the Regulatory Region of the TO Gene in Primary Hepatocytes—It has been reported that GR interacted with SWI/SNF *in vivo*, when BRG1 and BRM were overexpressed (*13*, *40*). To determine whether or not SWI/SNF interacts with GR and exists in the regulatory region of the TO gene in hepatocytes, we performed a co-immunoprecipitation assay and ChIP analysis. Since BRG1 and BRM were preferentially expressed in fetal and adult hepatocytes, respectively (Inayoshi *et al*., unpublished results), the interaction of GR with SWI/SNF (BRG1) for fetal and with SWI/SNF (BRM) for adult hepatocytes was examined (Fig. 3A). SWI/SNF containing either BRG1 or BRM interacted with GR in fetal and adult hepatocytes, respectively. We then performed a ChIP assay to determine whether or not BRG1 and BRM are recruited to the GREs of the TO gene. In fetal hepatocytes, in which TO was not expressed, both GREs were at least partly occupied by GR as in adult hepatocytes. This finding suggests that binding of GR to GRE alone may not cause the transactivation, and additional events may be essential for the initiation of transactivation.

Anti-BRG1 antibodies precipitated the distal GRE region of the TO gene mainly in fetal hepatocytes, and anti-BRM antibodies precipitated this region mainly in adult hepatocytes (Fig. 3B). A similar finding was made for the proximal GRE region. However, in adult hepatocytes, anti-BRG1 antibodies also precipitated the proximal GRE region, but the amount of precipitated DNA was less than that with anti-BRM antibodies. A similar but reciprocal finding was made for fetal hepatocytes. These results indicate that either or both of the ATPase subunits exist in the regulatory region containing GREs of the TO gene depending on their expression levels in hepatocytes.

BRG1 Represses the TO Gene in the Genome of SW-13 Cells and Primary Hepatocytes—To determine whether or not the repression of GR-mediated transactivation by SWI/SNF can take place in a genomic environment, we studied GR-mediated expression of the TO gene from the SW-13 genome by RT-PCR. Since SW-13 is a non-hepatic cell line, the TO gene is likely repressed or silenced in a cell type-specific manner such as methylation of DNA. However, as shown in Fig. 4A, GR could partly trigger the expression of

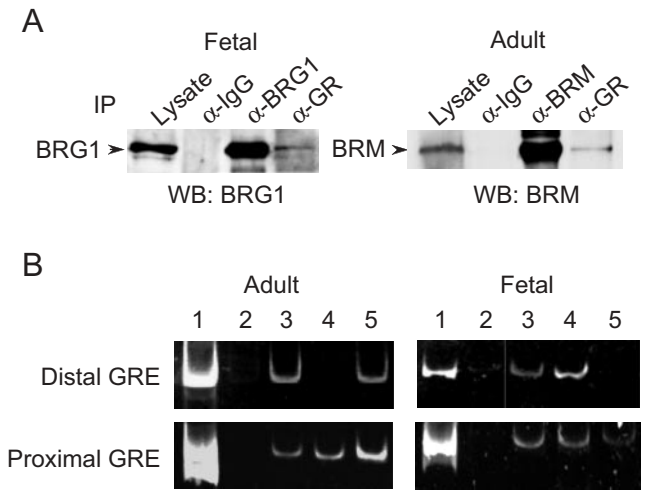

Fig. 3. **BRG1 and BRM associate with GR in the TO regulatory region in primary hepatocytes.** (A) Co-immunoprecipitation of BRG1 or BRM with GR in primary hepatocytes. Crude extracts were prepared from fetal and adult hepatocytes, and immunoprecipitated with anti-BRG1, anti-BRM, and anti-GR antibodies. Western blotting was performed using anti-BRG1 or anti-BRM antibodies. (B) ChIP analysis of the TO regulatory region with anti-GR (lane 3), anti-BRG1 (lane 4), and anti-BRM (lane 5) antibodies. Fetal and adult rat hepatocytes were isolated and cultured for one day before use. The primers used for PCR are shown in Fig. 2C. The DNA was amplified by PCR (35 cycles) and analyzed by Tris-borate-EDTA polyacrylamide gel electrophoresis. As negative controls, samples precipitated with anti-OVA antibodies (lane 2) were used. Input (10%) was used as a positive control (lane 1). Representative data of one of three independent experiments are shown.

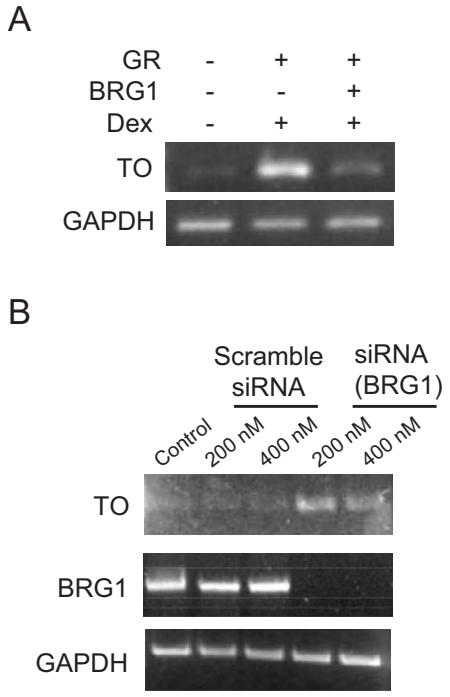

Fig. 4. **BRG1 represses expression of the TO gene in SW-13 cells and primary hepatocytes.** (A) The BRM or BRG1 expression vector (0.5 μg) was transiently transfected into SW-13 cells together with the GR expression vector. The expression of the TO gene from the genome of SW-13 cells was determined by RT-PCR. As a control, cDNA of GAPDH was amplified. (B) BRG1 siRNA was transfected into primary fetal hepatocytes isolated from rat E15 embryos. The expression of BRG1 and TO was detected by RT-PCR. A sample without siRNA was electrophoresed in the control lane. GAPDH expression was analyzed as a control. Representative data of one of three independent experiments are shown.

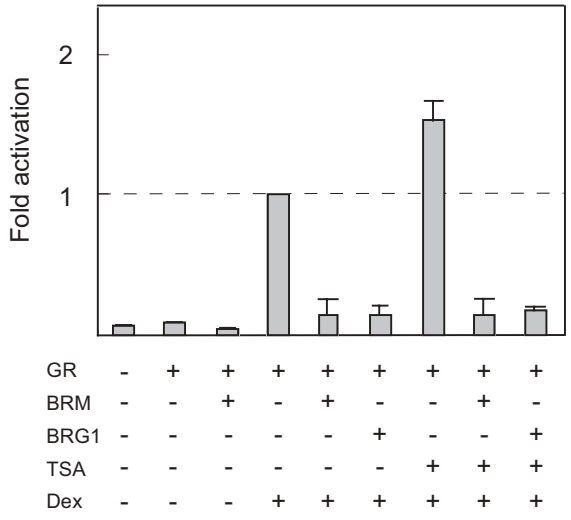

Fig. 5. **Repression of GR-mediated transcription of the TO gene by SWI/SNF is independent of HDAC.** SW-13 cells were transiently transfected with the pREP4-TO luciferase reporter plasmid, and GR and BRG1 or BRM expression vectors in the presence or absence of TSA (200 nM). Error bars represent the standard error of the mean of three independent experiments.

the gene and the GR-mediated transactivation of the TO gene was clearly repressed by BRG1, this being consistent with the results of luciferase reporter assays. This finding indicates that the repression could occur in a genomic environment. To study the repression in primary fetal hepatocytes, in which no expression of TO was observed, we then performed siRNA experiments for the knock-down of BRG1. As shown in Fig. 4B, BRG1 siRNA could function in primary fetal hepatocytes and successfully reduced the expression of the protein, and caused enhancement of the expression of the TO gene (Fig. 4B). This finding indicates that BRG1 represses GR-mediated expression of the TO gene to a certain extent in fetal hepatocytes.

BRG1 Requires Its ATPase Activity but Does Not Cooperate with HDAC to Suppress GR-Mediated Transactivation in Hepatocytes—Transcriptional repression by SWI/SNF was previously reported for the *c-fos* gene (*18*). This repression is known to be mediated by the retinoblastoma protein (pRb)-HDAC pathway and to be sensitive to a HDAC inhibitor, trichostatin A (TSA) (*41*). To determine whether or not the repression of the GR-mediated transactivation by SWI/SNF is dependent on the pRb-HDAC pathway, luciferase reporter assays were performed in the presence of TSA (200 nM). As shown in Fig. 5, TSA did not have any effect on the repression by SWI/SNF.

We then performed a reporter assay using a BRG1 mutant that had a mutation in the ATP-binding motif (BRG1ΔATP) to clarify whether the repression by BRG1 requires its ATPase activity. This mutant lost all repressive activity on GR-mediated transactivation (Fig. 6A). We confirmed that the ΔATP mutant still binds to GR and BAF155 in a co-immunoprecipitation assay (data not shown). This finding indicates that the repression of GR activity by BRG1 requires ATPase activity, which may result in a conformational change of chromatin. We therefore performed a restriction enzyme accessibility assay to determine

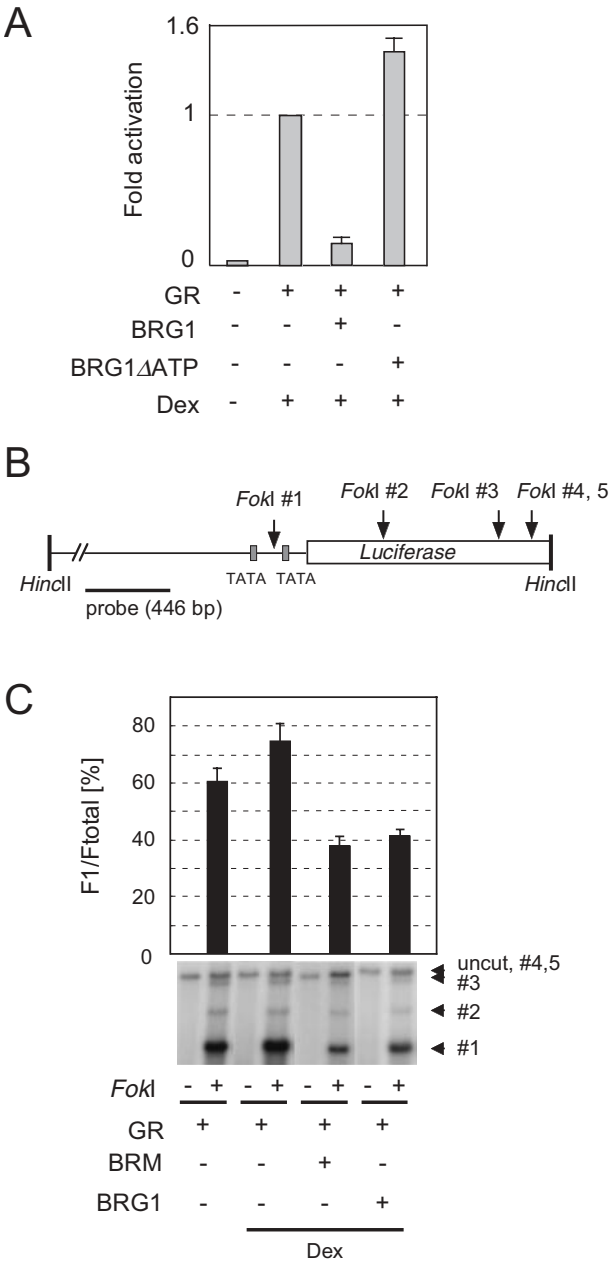

Fig. 6. **Requirement of chromatin remodeling for repression of GR-mediated transcription of the TO gene by BRG1.** (A) SW-13 cells were transiently transfected with the pREP4-TO luciferase reporter plasmid, and GR and BRG1 or BRG1ΔATP expression vectors. (B) Physical map of the TO promoter region. The locations of the five *Fok*I sites in the TO promoter luciferase reporter plasmid (#1–#5) and the position of the probe are shown. *Fok*I site #1 resides in the TO promoter region and the other sites (#2–#5) are located in the luciferase gene. (C) Restriction enzyme accessibility of the TO promoter region. Nuclei from SW-13 cells transfected with the pREP4-TO luciferase reporter plasmid, and GR and BRG1 or BRM expression vectors were digested with *Fok*I. Ten micrograms of DNA per lane was separated on a 0.6% agarose gel and hybridized with the indicated probe. Bands produced on *Fok*I digestion are indicated by arrowheads. The signal from each band was quantitated using a scanner. The relative cutting ratio at site 1 was calculated by dividing the signal from band 1 by the sum of the signals from all the bands in the same lane (*34*). Error bars represent the standard error of the mean of three independent experiments.

whether or not SWI/SNF alters the chromatin structure of the TO promoter in the reporter plasmid. Figure 6B shows the restriction sites used in the assay. SWI/SNF caused a reduction in sensitivity to the restriction enzyme *Fok*I, whose site exists in the promoter region of the TO gene (Fig. 6C). This observation suggests that SWI/SNF alters the conformation of chromatin possibly from an open state to a closed state in the TO promoter region. This is consistent with the fact that SWI/SNF requires ATPase activity for repression of the TO gene.

DISCUSSION

It has been speculated that SWI/SNF plays important roles in the induction or maintenance of tissue-specific gene expression and cell differentiation (*16*, *17*, *42–45*). In this study, we selected the TO and TAT genes, which are regulated by GR, to examine the role of SWI/SNF in liver-specific gene expression. It has been reported that SWI/SNF is required for GR-mediated transcriptional activation of the MMTV promoter and synthetic DNA-containing GRE sequences (*12*, *13*). The TO and TAT genes are also activated by GR in a hormone-dependent manner (*28*, *29*, *36*), as is the case with the MMTV promoter. However, in contrast to the MMTV promoter, SWI/SNF containing either BRG1 or BRM significantly repressed the transactivation of the TO and TAT regulatory regions by GR and dexamethasone in the luciferase assay. The repression by BRG1 or BRM may be considered problematic in that these proteins are usually credited with a coactivator function. However, this phenomenon seems to have some physiological importance, since GR-mediated partial activation of the endogenous TO gene of SW-13 cells was repressed by the expression of BRG1, and a reduction of BRG1 expression by siRNA in fetal hepatocytes induced GR-mediated TO expression in part.

The reason why SWI/SNF enhances GR-mediated transactivation of the MMTV promoter, but represses that of the TO and TAT enhancer and promoter has not been elucidated. It was recently reported that GR-mediated expression of the MMTV promoter was repressed by SWI/SNF in the presence of overexpressed nuclear factor-κB in SW-13 cells (*46*). Thus, it seems likely that the opposite functions of SWI/SNF depending on promoters are partly due to the difference in structure of the promoter and/or enhancer regions of these genes and recruited transcription factors that form preinitiation complexes (PIC). In fact, the glucocorticoid responsive unit of the TAT enhancer contains CCAAT/enhancer-binding protein and hepatocyte nuclear factor 3 binding sites in addition to GRE (*47*, *48*), and a nuclear factor 1 binding site was identified in the MMTV promoter (*49*). So far, precise analysis of transcription factors that require full activation of TO expression has not been reported except for that of GR. Therefore, further analyses are essential to clarify the mechanism of repression of the TO gene by SWI/SNF. In addition, ordered binding of transcription factors is prerequisite for the active PIC formation. It has been reported that GR binding leads to the recruitment of SWI/SNF and nucleosome remodeling, and that GR is displaced from the chromatin template during the remodeling process in the MMTV promoter (*50*). Therefore, the dynamics of PIC formation as well as the combinations of transcription factors involved may cause the opposite effects of SWI/SNF on GR-mediated transcription.

Repression by BRG1 has been reported for the promoter of the *c-fos* gene (*18*, *41*). In this case, the repression was mediated by the pRb-HDAC pathway, because it was very sensitive to TSA. Since TSA did not have any effect on the repression of GR-mediated transactivation by BRG1, even at high concentrations, the down-regulation observed in this study seems not to be related to the pRb-HDAC pathway. Recently, a ligand-dependent nuclear receptor corepressor, LCoR, was reported (*51*). This repressor uses either the HDAC-dependent or HDAC-independent pathway for repression of nuclear receptor-mediated transcription, but the repression of GR-mediated transcription by the corepressor is only TSA-sensitive. Therefore, the repression of the TO and TAT genes by SWI/SNF probably is not related to this corepressor. Alternatively, chromatin remodeling itself seems to be required for the repression, since the repression studied here required the ATPase activity of BRG1, and the restriction enzyme accessibility assay suggested a change in the chromatin conformation of the TO promoter region on the plasmid. In fact, it was recently reported that SWI/SNF itself inhibits the expression of several genes possibly by remodeling the nearby chromatin structure of activation sequences (*21*, *22*, *52*).

In this study, we showed that a BRM complex as well as a BRG1 complex repressed the TO and TAT genes. Our previous report showed that the expression of BRG1 decreases and that of BRM increases during neural development (*31*). A similar phenomenon was confirmed during liver development. The repression by BRG1 in fetal hepatocytes is thus physiologically relevant since the TO gene is not expressed at an early stage of hepatocyte differentiation. However, the repression by the BRM complex acts against the physiological event observed in adult hepatocytes. At present, the mechanism that causes release from the repression by the BRM complex in adult hepatocytes is unclear. However, it is possible that the combination of differentiation-specific transcription factors involved in the expression of the TO gene in adult hepatocytes modulates the repressive effect of BRM. Alternatively, a DNase I hypersensitive site in the upstream enhancer region of the TO gene that is essential for developmental regulation of the gene may modulate the repressive effects of BRM (*53*).

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REFERENCES

1. Urnov, F.D. and Wolffe, A.P. (2001) Chromatin remodeling and transcriptional activation: the cast (in order of appearance). *Oncogene* **20**, 2991–3006
2. Narlikar, G.J., Fan, H.Y., and Kingston, R.E. (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**, 475–487
3. Berger, S.L. (2002) Histone modifications in transcriptional regulation. *Curr. Opin. Genet. Dev.* **12**, 142–148
4. Hayes, J.J. and Hansen, J.C. (2001) Nucleosomes and the chromatin fiber. *Curr. Opin. Genet. Dev.* **11**, 124–129

5. Neely, K.E. and Workman, J.L. (2002) Histone acetylation and chromatin remodeling: which comes first? *Mol. Genet. Metab.* **76**, 1–5
6. Kingston, R.E. and Narlikar, G.J. (1999) ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. *Genes Dev.* **13**, 2339–2352
7. Zhang, Y. (2003) Transcriptional regulation by histone ubiquitination and deubiquitination. *Genes Dev.* **17**, 2733–2740
8. Muchardt, C. and Yaniv, M. (1999) ATP-dependent chromatin remodelling: SWI/SNF and Co. are on the job. *J. Mol. Biol.* **293**, 187–198
9. Wang, W., Cote, J., Xue, Y., Zhou, S., Khavari, P.A., Biggar, S.R., Muchardt, C., Kalpana, G.V., Goff, S.P., Yaniv, M., Workman, J.L., and Crabtree, G.R. (1996) Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. *EMBO J.* **15**, 5370–5382
10. Martens, J.A. and Winston, F. (2003) Recent advances in understanding chromatin remodeling by Swi/Snf complexes. *Curr. Opin. Genet. Dev.* **13**, 136–142
11. Yoshinaga, S.K., Peterson, C.L., Herskowitz, I., and Yamamoto, K.R. (1992) Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science* **258**, 1598–1604
12. Muchardt, C. and Yaniv, M. (1993) A human homologue of *Saccharomyces cerevisiae* SNF2/SWI2 and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. *EMBO J.* **12**, 4279–4290
13. Fryer, C.J. and Archer, T.K. (1998) Chromatin remodelling by the glucocorticoid receptor requires the BRG1 complex. *Nature* **393**, 88–91
14. Chiba, H., Muramatsu, M., Nomoto, A., and Kato, H. (1994) Two human homologues of *Saccharomyces cerevisiae SWI2/SNF2* and *Drosophila brahma* are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. *Nucleic Acids Res.* **22**, 1815–1820
15. Ichinose, H., Garnier, J.M., Chambon, P., and Losson, R. (1997) Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. *Gene* **188**, 95–100
16. Armstrong, J.A., Bieker, J.J., and Emerson, B.M. (1998) A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF in vitro. *Cell* **95**, 93–104
17. Kowenz-Leutz, E. and Leutz, A. (1999) A C/EBPβ isoform recruits the SWI/SNF complex to activate myeloid genes. *Mol. Cell* **4**, 735–743
18. Murphy, D.J., Hardy, S., and Engel, D.A. (1999) Human SWI-SNF component BRG1 represses transcription of the *c-fos* gene. *Mol. Cell. Biol.* **19**, 2724–2733
19. Zhang, H.S., Gavin, M., Dahiya, A., Postigo, A.A., Ma, D., Luo, R.X., Harbour, J.W., and Dean, D.C. (2000) Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and Rb-hSWI/SNF. *Cell* **101**, 79–89
20. Baker, K.M., Wei, G., Schaffner A.E., and Ostrowski, M.C. (2003) Ets-2 and components of mammalian SWI/SNF form a repressor complex that negatively regulates the *BRCA1* promoter. *J. Biol. Chem.* **278**, 17876–17884
21. Ishida, M., Tanaka, S., Ohki, M., and Ohta, T. (2004) Transcriptional co-activator activity of SYT is negatively regulated by BRM and Brg1. *Genes to Cells* **9**, 419–428
22. Siddiqui, H., Solomon, D.A., Gunawardena, R.W., Wang, Y., and Knudsen, E.S. (2003) Histone deacetylation of RB-responsive promoters: Requisite for specific gene repression but dispensable for cell cycle inhibition. *Mol. Cell. Biol.* **23**, 7719–7731
23. Sudarsanam, P., Lyer, V.R., Brown, P.O., and Winston, F. (2000) Whole-genome expression analysis of *snf/swi* mutants of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **97**, 3364–3369
24. Dilworth, F.J. and Chambon, P. (2001) Nuclear receptors coordinate the activities of chromatin remodeling complexes and coactivatiors to facilitate initiation of transcription. *Oncogene* **20**, 3047–3054
25. Westin, S., Rosenfeld, M.G., and Glass, C.K. (2000) Nuclear receptor coactivators. *Adv. Pharmacol.* **47**, 89–112
26. Aranda, A. and Pascual, A. (2001) Nuclear hormone receptors and gene expression. *Physiol. Rev.* **81**, 1269–1304
27. Hebbar, P.B. and Archer, T.K. (2003) Chromatin remodeling by nuclear receptors. *Chromosoma* **111**, 495–504
28. Grange, T., Cappabianca, L., Flavin, M., Sassi, H., and Thomassin, H. (2001) *In vivo* analysis of the model tyrosine aminotransferase gene reveals multiple sequential steps in glucocorticoid receptor action. *Oncogene* **20**, 3028–3038
29. Danesch, U., Gloss, B., Schmid, W., Schütz, G., Schüle, R., and Renkawitz, R. (1987) Glucocorticoid induction of the rat tryptophan oxygenase gene is mediated by two widely separated glucocorticoid-responsive elements. *EMBO J.* **6**, 625–630
30. Sumi-Ichinose, C., Ichinose, H., Metzger, D., and Chambon, P. (1997) SNF2β-BRG1 is essential for the viability of F9 murine embryonal carcinoma cells. *Mol. Cell. Biol.* **17**, 5976–5986
31. Machida, Y., Murai, K., Miyake, K., and Iijima, S. (2001) Expression of chromatin remodeling factors during neural differentiation. *J. Biochem.* **129**, 43–49
32. Hamamoto, R., Yamada, K., Kamihira, M., and Iijima, S. (1998) Differentiation and proliferation of primary rat hepatocytes cultured as spheroids. *J. Biochem.* **124**, 972–979
33. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G., and Struhl, K. (1987) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, NY
34. Ishii, H., Sen, R., and Pazin, M.J. (2004) Combinatorial control of DNase I-hypersensitive site formation and erasure by immunoglobulin heavy chain enhancer-binding proteins. *J. Biol. Chem.* **279**, 7331–7338
35. Thomassin, H., Flavin, M., Espinás, M.L., and Grange, T. (2001) Glucocorticoid-induced DNA demethylation and gene memory during development. *EMBO J.* **20**, 1974–1983
36. Roper, M.D. and Franz, J.M. (1977) Glucocorticoid control of the development of tryptophan oxygenase in the young rat. *J. Biol. Chem.* **252**, 4354–4360
37. Sassi, H., Pictet, R., and Grange, T. (1998) Glucocorticoids are insufficient for neonatal gene induction in the liver. *Proc. Natl. Acad. Sci. USA* **95**, 5621–5625
38. van der Vlag, J., den Blaauwen, J.L., Sewalt, R.G.A.B., van Driel, R., and Otte, A.P. (2000) Transcriptional repression mediated by polycomb group proteins and other chromatin-associated repressors is selectively blocked by insulators. *J. Biol. Chem.* **275**, 697–704
39. Liu, R., Liu, H., Chen, X., Kirby, M., Brown, P.O., and Zhao, K. (2001) Regulation of CSF1 promoter by the SWI/SNF-like BAF complex. *Cell* **106**, 309–318
40. Wallberg, A.E., Neely, K.E., Hassan, A.H., Gustafsson, J.A., Workman, J.L., and Wright, A.P.H. (2000) Recruitment of the SWI-SNF chromatin remodeling complex as a mechanism of gene activation by the glucocorticoid receptor τ1 activation domain. *Mol. Cell. Biol.* **20**, 2004–2013
41. Lee, D., Lim, C., Seo, T., Kwon, H., Min, H., and Choe, J. (2002) The viral oncogene human papillomavirus E7 deregulates transcriptional silencing by Brm-related gene 1 via molecular interactions. *J. Biol. Chem.* **277**, 48842–48848
42. Pedersen, T.A., Kowenz-Leutz, E., Leutz, A., and Nerlov, C. (2001) Cooperation between C/EBPα TBP/TFIIB and SWI/SNF recruiting domains is required for adipocyte differentiation. *Genes Dev.* **15**, 3208–3216
43. Lee, C.H., Murphy, M.R., Lee, J.S., and Chung, J.H. (1999) Targeting a SWI/SNF-related chromatin remodeling complex

to the β-globin promoter in erythroid cells. *Proc. Natl. Acad. Sci. USA* **96**, 12311–12315
44. O'Neill, D., Yang, J., Erdjument-Bromage, H., Bornschlegel, K., Tempst, P., and Bank, A. (1999) Tissue-specific and developmental stage-specific DNA binding by a mammalian SWI/SNF complex associated with human fetal-to-adult globin gene switching. *Proc. Natl. Acad. Sci. USA* **96**, 349–354
45. de la Serna, I.L., Carlson, K.A., and Imbalzano, A.N. (2001) Mammalian SWI/SNF complexes promote MyoD-mediated muscle differentiation. *Nat. Genetics* **27**, 187–190
46. Burkhart, B.A., Hebbar, P.B., Trotter, K.W., and Archer, T.K. (2005) Chromatin-dependent E1A activity modulates NF-κB RelA-mediated repression of glucocorticoid receptor-dependent transcription. *J. Biol. Chem.* **280**, 6349–6358
47. Grange, T., Roux, J., Rigaud, G., and Pictet, R. (1991) Cell-type specific activity of two glucocorticoid responsive units of rat tyrosine aminotransferase gene is associated with multiple binding sites for C/EBP and a novel liver-specific nuclear factor. *Nucleic Acids Res.* **19**, 131–139
48. Roux, J., Pictet, R., and Grange, T. (1995) Hepatocyte nuclear factor 3 determines the amplitude of the glucocorticoid response of the rat tyrosine aminotransferase gene. *DNA Cell Biol.* **14**, 385–396
49. Hebbar, P.B. and Archer, T.K. (2003) Nuclear factor 1 is required for both hormone-dependent chromatin remodeling and transcriptional activation of the mouse mammary tumor virus promoter. *Mol. Cell. Biol.* **23**, 887–898
50. Fletcher, T.M., Xiao, N., Mautino, G., Baumann, C.T., Wolford, R., Warren, B.S., and Hager, G.L. (2002) ATP-dependent mobilization of the glucocorticoid receptor during chromatin remodeling. *Mol. Cell. Biol.* **22**, 3255–3263
51. Fernandes, I., Bastien, Y., Wai, T., Nygard, K., Lin, R., Cormier, O., Lee, H.S., Eng, F., Bertos, N.R., Pelletier, N., Mader, S., Han, V.K.M., Yang, X.J., and White, J.H. (2003) Ligand-dependent nuclear receptor corepressor LCoR functions by histone deacetylase-dependent and -independent mechanisms. *Mol. Cell* **11**, 139–150
52. Coisy, M., Roure, V., Ribot, M., Philips, A., Muchardt, C., Blanchard, J.M., and Dantonel, J.C. (2004) *Cyclin A* repression in quiescent cells is associated with chromatin remodeling of its promoter and requires Brahma/SNF2α. *Mol. Cell* **15**, 43–56
53. Kaltschmidt, C., Muller, M., Brem, G., and Renkawitz, R. (1994) DNase I hypersensitive sites far upstream of the rat tryptophan oxygenase gene direct developmentally regulated transcription in livers of transgenic mice. *Mech. Dev.* **45**, 203–210