Functional Involvement of the Brahma/SWI2-related Gene 1 Protein in Cytochrome P4501A1 Transcription Mediated by the Aryl Hydrocarbon Receptor Complex*

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Chromatin remodeling is a key step in overcoming the nucleosomal repression of active transcription in eukaryotes. The mammalian SWI/SNF ATP-dependent chromatin-remodeling complexes contain multiple subunits. The ATPase activities in these complexes are attributable to either BRG-1 or the related Brahma protein. The aryl hydrocarbon receptor (AHR), after binding xenobiotic ligands such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), associates with the AHR nuclear translocator (ARNT), and the dimer so formed activates transcription of several genes, including the cytochrome P4501A1 (CYP1A1) gene. We show that BRG-1 potentiates AHR/ARNT-mediated reporter gene activity in a TCDD-dependent fashion in Hepa1c1c7 cells. Introduction of BRG-1 into the BRG-1- and hBrg-deficient SW13 and C33A human cell lines also enhances expression from a transiently transfected AHR/ARNT-dependent reporter gene. Replenishment of BRG-1 to SW13 cells also restores endogenous cytochrome P4501A1 (CYP1A1) gene expression, whereas an ATPase-deficient mutant of BRG-1 is unable to do so. Chromatin immunoprecipitation analysis demonstrated that BRG-1 associates with the enhancer region of the mouse CYP1A1 gene in vivo in a TCDD- and ARNT-dependent fashion, suggesting the specific recruitment of BRG-1 by AHR/ARNT. Finally, we demonstrate that the glutamine-rich subdomain of the transcriptional activation domain of AHR can interact with BRG-1. Together these studies reveal a functional involvement of BRG-1 in activating CYP1A1 gene transcription and implicate the importance of ATP-dependent chromatin remodeling activity on inducible gene expression mediated by AHR/ARNT.

The aryl hydrocarbon receptor (AHR) is a member of the basic helix-loop-helix/Per-ARNT-Sim family of transcription factors. It is a ligand-activated transcription factor. In the absence of the ligand, AHR resides in the cytoplasm where it is associated with heat-shock protein 90 (2), the cochaperone protein p23 (3), and the immunophilin-like AIP/ARA9/XAP2 protein (4–6). Upon binding of ligand, AHR translocates into the nucleus where it dimerizes with the AHR nuclear translocator (ARNT) (7, 8). The dimer then recognizes its cognate DNA sequence, termed the xenobiotic-responsive element (XRE), and modulates expression of a battery of genes (1).

AHR recognizes a wide spectrum of ligands. These include wide-spread environmental pollutants such as polycyclic and halogenated aromatic hydrocarbons (PAH, HAH) and aromatic amines. Many of these ligands are known to be potent carcinogens. The principal role AHR plays in PAH carcinogenesis is via induction of several cytochrome P450s by the ligand-bound AHR/ARNT. These cytochrome P450s can convert PAHs into genotoxic electrophilic derivatives. HAHs such as TCDD act as tumor promoters (non-genotoxic carcinogens) rather than tumor initiators. TCDD is resistant to metabolism and has a long half-life in the organism, and it is likely that most of its toxic and carcinogenic effects are attributable to prolonged activation of the AHR/ARNT leading to abnormalities in gene expression (1). Endogenous ligands have been postulated for AHR, suggesting an important role for the receptor in physiological settings. Indeed, AHR-null mice show defects in liver development, a compromised immune system, and impairment of retinoic acid metabolism (9–11). It is thus evident that AHR-regulated transcription plays a pivotal role in mediating the toxic effects of many environmental pollutants and perhaps also normal physiological functions.

Most of our knowledge of AHR/ARNT-regulated transcription comes from studies of the induction of the cytochrome 4501A1 (CYP1A1) gene by TCDD (12). The regulatory region of the CYP1A1 gene, particularly that of the mouse, has been well studied (1, 12). A cluster of XREs functioning as an enhancer is located ∼1 kb upstream of the transcription initiation start site. The promoter region of the gene, located within 300 bp of the transcription initiation site assumes a nucleosomal configuration (13). Following the occupation of the enhancer region by liganded AHR/ARNT, chromatin remodeling occurs over the promoter region, allowing the RNA polymerase II transcription pre-initiation complex to interact with this region and to activate transcription (14, 15). This sequence of events, which is dependent on both AHR and ARNT, requires the transcription activation domain (TAD) of AHR (14). The transcription activator protein; PAH, polycyclic aromatic hydrocarbon; Rb, retinoblastoma protein; RIP140, receptor interacting protein 140; RT, reverse transcriptase; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; XRE, xenobiotic response element; ChIP, chromatin immunoprecipitation; DTT, dithiothreitol; HAT, histone acetyltransferase.

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vation domain of ARNT plays a less prominent role (14, 16). AHR has a potent and complex transcription activation domain. The TAD of human AHR consists of three subdomains: an acidic domain, a glutamine-rich (Q-rich) domain, and a proline-serine/threonine-rich domain (17). The Q-rich domain has been shown to be capable of interacting with the coactivator/corepressor protein RIP140 (18). The coactivator proteins CBP/p300 (19), SRC-1 (20),^2 NCoA-2, and p/CIP^2 have also been implicated in AHR/ARNT-mediated transcription. All these coactivators can enhance AHR/ARNT-mediated transcription in reporter gene assays.

As mentioned above, transcription activation of the CYP1A1 gene involves alleviation of nucleosomal repression over the promoter region. At least two general types of chromatin remodeling factors/complexes have been shown to be recruited to promoters by transcription activators (21, 22). One type possesses histone acetyltransferase (HAT) activity, and includes yeast GCN5 (23), and the aforementioned mammalian coactivators, SRC-1, NCoA-2, p/CIP, and CBP/p300 (24–26). These coactivators acetylate histones on their N-terminal tails thereby reducing histone-DNA affinity and nucleosome-nucleosome interactions (27). Another type consists of multisubunit protein complexes containing an ATPase subunit and utilizes ATP to disrupt histone-DNA interactions and drive histone octamer transfer (28, 29). The ATPase complexes are further divided into three groups, based upon whether the ATPase subunit is more related to the yeast SWI2/SNF2 protein (yeast SWI/SNF, yeast RSC, human SWI/SNF, Brahma complexes), Drosophila ISWI (ISWI, ISW2, human RSC, Drosophila NURF, Drosophila ACF complexes), and human Mi-2 (human NURD and xMi-2 complexes) (30). The ATPase activities of the mammalian SWI/SNF multisubunit complexes are conferred either by the BRG-1 or Brm proteins, which are related to the yeast SWI2/SNF2 protein. Both BRG-1 and Brm can interact with certain steroid hormone receptors, such as GR (31), ER (32), and enhance their transcriptional potential. Furthermore, mammalian SWI/SNF-like complexes have been shown to associate with a number of transcription factors, including c-Myc (33), EKLF (34), and C/EBPβ (35), and transcription of a number of mammalian genes have been shown to be dependent on the action of SWI/SNF complexes, such as mammalian heat-shock protein 70 (36), β-globin (34), myeloid genes (35), and interferon β (37). Genome-wide studies of mutants deleted for genes encoding certain components of the SWI/SNF complex in yeast reveals that the yeast SWI/SNF complex controls only a small fraction of genes and acts at the individual gene level rather than over extended chromosomal domains. (38) BRG-1 and other subunits of mammalian SWI/SNF complexes are also found associated with Rb and histone deacetylases and exert transcriptional repression (39). BRG-1 null mice die very early in development (40). Although viable, heterozygous BRG-1 mice are prone to exencephaly and tumors (40). Together, these observations indicate that BRG-1 or Brm containing SWI/SNF complexes actively engage in a wide range of cellular activities during development and proliferation.

In this work, we examined the possible involvement of mammalian SWI/SNF complexes in AHR/ARNT-mediated CYP1A1 gene expression. This is the first reported investigation on the role of an ATPase containing chromatin-remodeling factor in transcription activation by AHR/ARNT and has important implications for chemical carcinogenesis and other cellular responses mediated by AHR/ARNT.

EXPERIMENTAL PROCEDURES

Plasmid Constructs and Vectors—pB5-J-4-BRG-1 and pB5-J-4-BRG-1 (K785R) were kind gifts of Dr. Myles Brown. pCMX-SRC-1 and pCMX-CBP were kind gifts from Dr. Michael G. Rosenfeld. p-tAHRR was made by cloning human AHR cDNA into pTARGET vector (Promega). Reporter gene pGL-CYP1A1 contains 2.6 kb of the regulatory sequence of the rat CYP1A1 gene, including the enhancer and promoter region and the transcription initiation site. The minimal XRE-promoter luciferase reporter construct (pGL-XRE) was a kind gift of Dr. Dianne Duncan. To construct glutathione S-transferase (GST)-tAHRR and GST-ARNT TAD deletion mutants, individual domains were amplified by PCR and inserted into the EcoRI and XhoI restriction sites of the pGEX-SX-2 vector (Amersham Biosciences, Inc.).

Cell Culture and Transfections—The SW-13 adrenal carcinoma cells and the C33A cervical carcinoma cells were grown in Dulbecco’s modified Eagle medium (Invitrogen) with 10% fetal calf serum (Omega), l-glutamine (Invitrogen), fungizone, and penicillin-streptomycin (Invitrogen) at 37 °C and 5% CO2. The Hepa1c1c7 strain and the c4 mutant derived from Hepa1c1c7 were grown in minimal essential media under the same conditions as above. Transfections of expression plasmids were performed using SuperFect reagent (Qiagen). For Hepa1c1c7 cells, a dual-luciferase system (Promega) was used. DNA mixtures of the indicated amount of pB5-J-BRG1 were cotransfected with 100 ng of Renilla luciferase reporter (pRL-TK) driven by the herpes simplex virus thymidine kinase promoter and 500 ng of pGL-CYP1A1 firefly luciferase reporter plasmid into six-well plates of Hepa1c1c7 cells. The final DNA concentrations were adjusted using empty expression vector to ensure equal amounts of DNA were used in each plate. After 16 h, transfected cells were treated with 1 μg of TCDD or vehicle (MeSO4) for 24 h. Cells were then harvested and lysed in Passive lysis buffer (Promega). Luciferase activities were measured using luciferase assay reagent (Promega). The Renilla luciferase activity was used to normalize the firefly luciferase activity of each sample. For SW13 and C33A cells, DNA mixtures of the indicated amount of pB5-J-BRG1, pB5-J-BRG1-K785R, and pCMX-SRC-1 were cotransfected with pRL-TK reporter, and 500 ng of either of pGL-CYP1A1 luciferase or pGL-XRE luciferase reporter plasmids into six-well plates of SW-13 cells or C33A cells. The final DNA concentrations were adjusted using empty expression vector to ensure equal amount of DNA was used in each plate. After 16 h, transfected cells were treated with either TCDD or MeSO4 for 24 h. Cells were then harvested and lysed in reporter lysis buffer (Promega). Luciferase activities were measured using luciferase assay reagent (Promega). The β-galactosidase activity was used to normalize the luciferase activity of each sample. All the transfection experiments were performed in triplicate.

Semiquantitative RT-PCR—SW-13 and C33A cells were transfected by SuperFect (Qiagen) with plasmids (2 μg each) containing pTHAHR, pB5-J-BRG1, pB5-J-BRG1-K785R, and pCMX-SRC-1 were cotransfected with pGL-CYP1A1 reporter, and 500 ng of either of pGL-CYP1A1 luciferase or pGL-XRE luciferase reporter plasmids into six-well plates of SW-13 cells or C33A cells. The total DNA concentrations were adjusted using empty expression vector to ensure equal amount of DNA was used in each plate. After 16 h, transfected cells were treated with either TCDD or MeSO4 for 24 h. Total RNA was prepared from cells transfected with expression plasmids using TRIzol (Invitrogen). 1 μg of total RNA was reverse-transcribed using a Thermoscript Reverse Transcriptase kit (Invitrogen). PCR was performed with 4% of the RT reaction using Taq polymerase (Promega). For controls, similar procedures were followed except that in no reverse transcriptase was added and the reaction mixture was digested with RNaseH before the PCR amplification. The primer sets for RT-PCR are as follows: 5′-CCCTGATCCTGTCAAGGAC-3′ and 5′-ACGAAAGGAGG-AGTGCAGG-3′ for the human CYP1A1 gene; 5′-GGTTGTCTCTGAGGA-TAAATCCATC-3′ and 5′-ACCAAGATCTATGCAGTGG-3′ for the human AHR; 5′-TCCATCTCCATGCTCTTGG-3′ and 5′-TCAATCCCGCGGAT-CCTG-3′ for the ribosomal protein L23 (RPL23).

Chromatin Immunoprecipitation Assay—The procedure was adopted from that described by Brown and coworkers (32) with some modifications. Hepa1c1c7 or c4 cells were treated with TCDD to a final concentration of 10−8 M for 40 min. Cross-linking was achieved by adding formaldehyde to a final concentration of 1% at room temperature for 10 min. Cells were washed twice with ice-cold phosphate-buffered saline and resuspended in 1 ml of ice-cold phosphate-buffered saline. Cells were pelleted at 700 × g at 4 °C and resuspended in 0.3 ml of cell lysis buffer (50 mM Tris-HCl (pH 8.1), 10 mM EDTA, 1% SDS, Roche Complete protease inhibitor mixture) and incubated on ice for 10 min. Cell lysates were sonicated to give a DNA size range from 200 to 900 bp. Samples were centrifuged for 10 min at 4 °C. Supernatants were adjusted to give a final solution of 15 mM Tris-HCl (pH 8.1), 1% Triton X-100, 1 mM EDTA, 150 mM NaCl, 0.1% SDS, and Complete protease inhibitor

^2 T. Beischlag and O. Hankinson, unpublished results.
mixture. The solutions were pre-cleared with 50 µl of a 50% slurry of protein A-Sepharose containing 2.5 µg of sheared salmon sperm DNA for 2 h at 4 °C, and then treated with antibodies against either BRG-1 (a kind gift of Dr. Weidong Wang) or AHR overnight at 4 °C. 50 µl of protein A-Sepharose containing 2.5 µg of salmon sperm DNA and 2 mg/ml bovine serum albumin solution added to the solution for 1 h. The beads were washed and resuspended in the following buffers: buffer A (20 mM Tris-HCl (pH 7.8), 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% Triton X-100); buffer B (20 mM Tris-HCl (pH 8.1), 500 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% Triton X-100), and LiCl-detergent buffer (10 mM Tris-HCl (pH 8.1), 0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA), and TE buffer (twice). Immunoprecipitates were extracted from the beads with 1% SDS-0.1 M NaHCO3. Cross-linking was reversed by heating the elutes at 65 °C overnight. The elutes were then digested with proteinase K at 45 °C for 1 h. The solutions were extracted with phenol-chloroform-isomyl alcohol. DNA was purified by ethanol precipitation, and 20 mM Tris (pH 7.9), 150 mM NaCl, 2 mM EDTA, 0.5 mM DTT, and 5% glycerol. A small amount of individual hAHR TAD subdomain GST fusion protein was eluted off the beads in SDS sample buffer and subjected to SDS-PAGE. The protein concentrations were determined by comparing to Coomassie Blue-stained bovine serum albumin.}[50x389]Nonidet P-40, 10% glycerol, and 1 mM DTT. The cell lysate was then subjected to 6% SDS-PAGE and Western blot using the BRG-1 antibody. BRG-1 Is a Coactivator of AHR/ARNT—Enhancer-promoter communication and chromatin remodeling is a hallmark of CYP1A1 gene transcriptional activation. To test the potential involvement of ATP-dependent mammalian SWI/SNF chromatin-remodeling complexes in this process, we focused on the AP45 element subunit BRG-1. Using a reporter gene pG-L-CYP1A1, in which the luciferase gene was driven by a fragment extending from the transcriptional initiation site to 2.6 kb upstream of the rat CYP1A1 gene, we found that in the Hepa1c1c7 cell line, which has endogenous BRG-1 expression, BRG-1 co-activated a reporter gene in a BRG-1-dependent manner (Fig. 1A). In two cell lines that are defective in BRG-1 and Brm expression: the SW13 bovine adrenal carcinoma cell line and the C33A human cervical carcinoma cell line (41), BRG-1 also enhanced the AHR/ARNT-dependent reporter gene activity in a dose-dependent fashion, with an approximate 5-fold increase when the greatest amount of DNA was used (Fig. 1, B and D). This enhancement was AHR-dependent, because it could not be observed when exogenous AHR was not provided to SW13 cells (Fig. 1C). SW13 and C33A cells express ARNT (data not shown) but not AHR, see Fig. 3). Furthermore, enhancement was much less with a BRG-1 mutant defective in AP45 activity (Fig. 1, B and D). We also employed a different reporter gene system (pGL-XRE) in which the luciferase gene is driven by five XREs and a minimal promoter. As shown in Fig. 1E, BRG-1 was also able to augment the AHR-mediated transcription of this reporter gene in SW13 cells. This effectively addresses the concern that the enhancement may result from an effect of BRG-1 on other constitutive transcription factors whose binding sites are present in the regulatory region of the CYP1A1 gene.

It should be noted that luciferase activity in the absence of TCDD was nearly as great as that in the presence of TCDD in the experiments performed in SW13 and C33A cells. A similarly reduced effect of exogenous ligand has been observed by ourselves and other investigators in analogous experiments using AHR/ARNT-dependent reporter genes (42, 43), and overexpression of AHR and ARNT in CV-1 cells has been shown to trigger nuclear translocation and activation of CYP1A1 tran-
FIG. 1. BRG-1 enhances AHR/ARNT-mediated reporter gene transcription. A, Hepa1c1c7 cells were transfected with a CYP1A1 enhancer-promoter driven firefly luciferase reporter (pGL-CYP1A1) and 3–5 μg of BRG-1 expression construct. Their luciferase activities were measured and normalized with Renilla luciferase activity expressed from a cotransfected pRL-TK plasmid. B, SW-13 cells were transfected with a CYP1A1 enhancer-promoter-driven luciferase reporter (pGL-CYP1A1), 200 ng of pT-HAHR, and 3–6 μg of BRG-1 or BRG-1 ATPase mutant expression constructs. Luciferase activities were measured and normalized with β-galactosidase activity. C, SW-13 cells were transfected with a CYP1A1 enhancer-promoter-driven luciferase reporter (pGL-CYP1A1) and 3–6 μg of BRG-1 expression construct in the absence of HAHR expression construct. Luciferase activities were measured and normalized with β-galactosidase activity. D, similar experiments described in B were repeated with C33A cells. E, SW-13 cells were transfected with an XRE-minimal promoter-driven luciferase reporter (pGL-XRE), 200 ng of pT-HAHR, and 3–6 μg of BRG-1 expression constructs.
with the histone acetyltransferase, Gcn5, in controlling expression of certain inducible genes in yeast (47). We wished to investigate whether coactivators possessing HAT activity such as SRC-1 and p300/CBP serve redundant or partially redundant roles with respect to the BRG-1-containing SWI/SNF complexes in AHR/ARNT-mediated transcription. As shown in Fig. 4, when either SRC-1 or CBP was overexpressed with AHR, no CYP1A1 mRNA was detectable. Only when BRG-1 was provided was a marked increase in CYP1A1 mRNA levels observed. The results strongly suggest that HAT activity cannot substitute for BRG-1 with regard to AHR/ARNT-dependent transcription.

**BRG-1 Is Specifically Targeted to the Murine CYP1A1 Enhancer Region by AHR/ARNT**

—Direct recruitment of SWI/SNF complexes and HATs by activators to specific genes is the prevailing model for precise targeting of chromatin remodeling activities. To test whether BRG-1 can be recruited by AHR/ARNT to the regulatory region of the CYP1A1 gene, we used a chromatin immunoprecipitation (ChIP) assay. We chose to use the CYP1A1 gene from the mouse, because its regulatory region has been well studied, and in addition, mouse hepatoma mutant lines lacking either AHR or ARNT are available. In the ChIP assay, Hepa1c1c7 cells were exposed in culture briefly to TCDD or the vehicle Me2SO. Chromatin-associated proteins and DNA were cross-linked with formaldehyde, and the chromatin was subjected to sonication to generate fragments containing DNA between 200 and 900 bp. Antibodies against either AHR or BRG-1 were then added to precipitate protein-bound DNA fragments. After reversal of cross-linking, DNA fragments were purified and amplified by PCR using primers specific for human CYP1A1 or human ribosomal protein S14. PCR amplifications failed to produce any bands representative of HAHR when previous RT reactions were carried out in the absence of reverse transcriptase and digested with RNaseH, indicating bands produced by PCR amplification for HAHR were result of mRNA from the transfected plasmid, not the plasmid DNA itself.

**BRG-1 Is a Coactivator of AHR/ARNT**

A. total RNAs were extracted from SW-13 cells and analyzed by RT-PCR with primers for human AHR or ribosomal protein S14. B. SW-13 cells were transfected with either pT-hAHR or pT-hAHR and pBJ5-BRG-1. After 24 h, transfected cells were treated with either TCDD or Me2SO for 12 h. Total RNAs were then extracted and analyzed by RT-PCR using primers specific for human CYP1A1, human AHR, or human ribosomal protein S14. C. SW-13 cells were transfected with either pBJ5-BRG-1 alone or pT-hAHR and pBJ5-BRG-1 (K785R). 24 h post-transfection, cells were treated with either TCDD or Me2SO for 12 h. Total RNAs were then extracted and analyzed by RT-PCR using primers specific for human CYP1A1 or human ribosomal protein S14. PCR amplifications failed to produce any bands representative of HAHR when previous RT reactions were carried out in the absence of reverse transcriptase and digested with RNaseH, indicating bands produced by PCR amplification for HAHR were result of mRNA from the transfected plasmid, not the plasmid DNA itself.
precipitated by either AHR or BRG-1 antibodies (data not shown). When similar experiments were conducted with the ARNT-deficient mutant of Hepa1c1c7 cells, c4, neither AHR nor BRG-1 antibodies were capable of precipitating the enhancer region of CYP1A1 (Fig. 5B). These experiments demonstrate that BRG-1 is targeted to the murine CYP1A1 enhancer by AHR/ARNT in a ligand-dependent fashion.

The Glutamine-rich (Q-rich) Region in the Human AHR Transcriptional Activation Domain Interacts with BRG-1—We next investigated whether BRG-1 interacts with AHR’s TAD. AHR was studied in detail, because its transcription activation domain is dominant over that of ARNT during the activation of CYP1A1 gene transcription (14, 16). We made glutathione S-transferase (GST) fusion proteins of individual subdomain of AHR’s TAD as well as the TAD of ARNT and performed GST pull-down experiments using cell lysate from HK293T cells overexpressing BRG-1 (Fig. 6). The glutamine-rich (Q-rich) domain of AHR was capable of pulling down BRG-1, whereas GST alone, the acidic domain (AAD), the proline-serine/threonine-rich domain (PST), and the transcription activation domain of ARNT were unable to do so. A fusion of the Q-rich and the acidic domain of AHR pulled down a similar amount of BRG-1 as the Q-rich domain on its own.

**DISCUSSION**

CYP1A1 gene transcription is frequently used as a model for studying in transcriptional activation by AHR/ARNT. This system provides a means for elucidating mechanistic details of AHR/ARNT-mediated transcription and also of ligand-activated mammalian gene expression in general. Studies on transcriptional regulation of the CYP1A1 gene should also contribute to an understanding of carcinogenesis and other toxicological effects of PAHs and HAHs. PAHs, which constitute a major class of carcinogens found in tobacco smoke, are AHR ligands and activate CYP1A1 expression. CYP1A1 is a major player in converting these compounds into genotoxic derivatives. Gene regulation by AHR/ARNT is also very probably involved in mediating many of the carcinogenic and toxic effects of TCDD and other HAHs. Detailed understanding of the transcriptional regulation of CYP1A1 may also provide insight into differential susceptibility to the deleterious effects of PAHs and HAHs between individuals and may thus impact risk assessment.

It is known that there are positioned nucleosomes over the promoter region of the mouse CYP1A1 gene that pose a hindrance to active transcription (14). TCDD treatment induces nucleosomal loss over this region. TCDD-inducible DNase I hypersensitivity and increased micrococcal nuclease and restriction enzyme cleavage over the promoter region provide compelling evidence for the occurrence of chromatin remodeling (13–15). At least two classes of chromatin remodeling moieties can actively catalyze chromatin reconfiguration: factors possessing histone acetyltransferase (HAT) activity and ATP-dependent remodeling complexes. We show here that BRG-1, a component of some SWI/SNF ATP-dependent remodeling complexes, can specifically enhance AHR/ARNT-mediated transcription from a transiently transfected template. More importantly, addition of BRG-1 restores AHR/ARNT-dependent endogenous CYP1A1 expression to such cells. Although much reduced, some enhancement of transcription from the transiently transfected reporter plasmid was elicited by an ATPase-abolished mutant of BRG-1, which is unable to carry out chromatin remodeling. A similar observation was previously made for the r1 transcriptional activation domain of the glucocorticoid receptor (48). This could result from aberrant nucleosomal packaging of the transiently transfected reporter and the possibility that BRG-1 affects gene transcription by additional mechanisms besides chromatin remodeling that are less dependent on ATPase activity, such as facilitating interactions between AHR and other coactivators, or stabilizing interactions with the RNA polymerase II holoenzyme. Importantly, we did not observe any enhancement of transcription of the endogenous CYP1A1 gene using the ATPase-abolished mutant, indicating that BRG-1’s chromatin remodeling activity is necessary for coactivation of AHR/ARNT-mediated CYP1A1 gene expression in vivo.

Although some studies in yeast suggest that HAT factors and SWI/SNF complexes have somewhat overlapping roles in controlling transcription of certain genes, recent evidence in both yeast and mammalian systems indicates that they may fulfill different roles to relieve nucleosomal repression. Previous studies have implicated the participation of the HAT coactivators SRC-1, NCoA-2, p300/CBP, and p300/CBP in AHR/ARNT-dependent transcription. BRG-1 possesses a bromodomain, which is known to bind acetyl-lysine with high affinity (49). This suggests the possibility that HAT activity may create a preformed target for SWI/SNF complexes. However, the exact roles of the two types of chromatin remodeling factors/complexes may vary for different promoters. Although transcriptional ac-
tivation of the yeast PHO8 gene requires both yeast SWI/SNF and HAT-containing GCN5, the absence of SWI/SNF causes complete loss of chromatin remodeling, whereas deletion of GCN5 permits partial and local chromatin perturbation (50). For the yeast HO gene, association of the HAT complex seems to require preceding recruitment of the yeast SWI/SNF complex (51), whereas for the mammalian interferon γ promoter, in contrast, recruitment of the SWI/SNF complex appears to require prior association of the HAT complex (37). Here we provide evidence that neither of the HAT factors SRC-1 nor CBP can substitute for BRG-1 with regard to AHR/ARNT-dependent transcription but that SRC-1 and BRG-1 cooperatively enhance AHR/ARNT-mediated transcription. These observations suggest that these two types of remodeling factors/complexes may act sequentially and synergistically during transcriptional activation by AHR/ARNT.

Our ChIP assays demonstrate the direct targeting of BRG-1 to the CYP1A1 gene enhancer region. The enhancer region of CYP1A1 harbors several XREs. This effectively increases the local concentrations of the activator as well as coactivators, which may be advantageous for capturing coactivator complexes cross-linked to DNA in our assay. The inability of BRG-1 to associate with the enhancer in ARNT-deficient cells demonstrates that an active AHR/ARNT complex is needed for this targeting. Previous studies have shown that the BRG-1 or Brahma subunits of mammalian SWI/SNF complexes can interact with transcription activation domains of other mammalian transcription factors, including C/EBPβ and the glucocorticoid receptor (31, 35, 48). Using GST pull-down assays, we showed an interaction between BRG-1 and the glutamine-rich domain of human AHR. Our experiments cannot distinguish between a direct or indirect interaction between these two proteins. Previously, deletion of the Q-rich domain has been shown to abolish transactivation by human AHR. The Q-rich domain may comprise a major docking surface for coactivator complex assembly. Assembly may well be temporally and spatially regulated to allow for efficient transactivation. The functions of the other subdomains of human AHR’s TAD remain elusive, but they may be important in different tissue and promoter contexts. Interestingly BRG-1 did not interact with ARNT’s TAD, consistent with previous observations indicating that AHR’s TAD is dominant over ARNT’s TAD during transcriptional activation (at least in the case of the CYP1A1 gene).

In conclusion, our observations indicate that BRG-1, the ATPase subunit of certain mammalian SWI/SNF complexes, is a bona fide coactivator for AHR/ARNT-dependent transcription of the CYP1A1 gene. These studies thus provide a functional link between chromatin remodeling of the CYP1A1 gene regulatory region and the mammalian SWI/SNF complexes. It is an interesting question as to whether BRG-1 is required for expressions of other genes controlled by AHR/ARNT, given the different nucleosomal architecture for some of these genes (52). This relates to an important issue with regard to complex combinatorial regulation of inducible mammalian gene expression.

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