Brg-1 Is Required for Maximal Transcription of the Human Matrix Metalloproteinase-2 Gene*

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Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases whose aberrant expression are correlated with tumor invasion and angiogenesis. The transcription factors Sp1, Sp3, and AP-2 are required for constitutive expression of MMP-2 in tumor cells; however, the regulatory mechanisms of MMP-2 expression are not well understood. We investigated the involvement of Brg-1, the ATPase subunit of the SWI/SNF complex, in human MMP-2 gene transcription. Reconstitution of Brg-1 enhances MMP-2 transcription in Brg-1-deficient SW-13 cells. Chromatin immunoprecipitation assay demonstrates that Brg-1 is required for recruitment of Sp1, AP-2, and polymerase II to the MMP-2 promoter, whereas the binding of Sp3 to the MMP-2 promoter is decreased upon Brg-1 reconstitution. Furthermore, Sp1 interacts with Brg-1 in vitro. Restriction enzyme accessibility assays indicate that accessibility of the MMP-2 promoter region is not changed in the absence or presence of Brg-1. These results illustrate the connection between the SWI/SNF complex and optimal expression of MMP-2 and highlight the critical function of Brg-1 in regulating the recruitment of Sp1, Sp3, AP-2, and polymerase II to the MMP-2 promoter.

Matrix metalloproteinase-2 (MMP-2), also called 72-kDa type IV collagenase, belongs to the MMP family, which is composed of structurally conserved zinc-dependent endopeptidases that are involved in proteolytic modeling of the extracellular matrix. MMP-2 has been shown to play a critical role in invasion/metastasis and angiogenesis of malignant tumors (1, 2). MMP-2 is constitutively expressed by normal and transformed cells, and enhanced expression of MMP-2 is found in a variety of tumor cells that correlates with the malignancy grade of the original tumor (1, 2). We have previously shown that the transcription factors Sp1, Sp3, and AP-2 are functionally important in regulating constitutive expression of the MMP-2 gene (3). However, how the MMP-2 gene is constitutively transcribed in the generally repressive chromatin context under the regulation of Sp1, Sp3, and AP-2 is unknown.

The Sp family of transcription factors, including Sp1, Sp2, Sp3, and Sp4, play a major role in regulating a large number of eukaryotic genes, which in turn, have indispensable effects in controlling development, differentiation, tumorigenesis, and viral infection (4, 5). Sp1 and Sp3 are ubiquitously expressed in eukaryotic cells, and both can recognize and bind GC boxes present in the promoters of a variety of constitutive or inducible genes (4, 5). Although Sp1 and Sp3 share a high degree of homology in structure, they have distinct functions in transcriptional regulation. Sp1 is generally a transcriptional activator, whereas Sp3 is considered as a relatively weak activator or a transcriptional repressor depending on the context of the gene and the assay system (5–8). In addition, the ratio of Sp1/Sp3 that binds on the GC box of a specific gene is also dynamically changed in response to regulatory signals (9–11). Therefore, differential binding between Sp1 and Sp3 to a target gene is another regulatory mechanism to refine the transcriptional output. However, the mechanisms that control the ratio of Sp1/Sp3 or switching of Sp1/Sp3 are unknown.

The genomic DNA of eukaryotic cells is packaged into chromatin, which is composed of the basic structural unit of nucleosomes. Nucleosomes are formed by histone octamers wrapping around ~147 bp of DNA in eukaryotes. Chromatin is generally a repressive structure for gene transcription; therefore, a central issue of eukaryotic gene transcription is to elucidate how transcription is regulated in the context of chromatin (12–15). Two mechanisms contribute to the relaxation of chromatin structure to accommodate the binding of transcription complexes. One is by chemical modifications, such as acetylation, phosphorylation, and methylation, of the histone tails of nucleosomes (16). The other is by disruption of histone-DNA contact by ATP hydrolysis-dependent chromatin-remodeling complexes. Several ATP-dependent chromatin-remodeling complexes have been described, including the SWI/SNF complex. SWI/SNF is a protein complex of ~2 MDa containing 10–15 subunits that is conserved among eukaryotes (17). Two of the ATPase subunits of SWI/SNF, Brg-1 and Brm, have critical functions in SWI/SNF-mediated chromatin remodeling (14, 15). Homozygous inactivation of Brg-1 causes early embryonic lethality in mice (18), whereas deletion of Brm results in increased body weight in mice (19).

It has been reported that a variety of human malignancies are associated with mutations of Brg-1, thus suggesting that Brg-1 may contribute to tumor suppression (20). Indeed, Brg-1 represses c-fos gene transcription, and Brg-1 also interacts with the corepressor complex Sin3A or the tumor suppressor RB to induce cell cycle arrest (21–24). Reconstitution of Brg-1 into cells that are Brg-1-deficient can reverse the transformed...
phenotype of tumor cells (25). Because Brg-1 does not contain a sequence-specific DNA-binding domain, the selective recruitment of the SWI/SNF complex to target genes depends on protein-protein interactions through Brg-1 and other transcription factors or transcription regulators (26–28). Thus far, it has been shown that Brg-1 is required for the expression of CSF1, CD44, a subset of interferon-α-inducible genes and some interferon-γ-inducible genes, including MHC class II, CIITA, IF-ITM2, IFITM3, and GBP (28–37). Besides inducing chromatin remodeling, Brg-1 interacts with transcription factors such as Sp1, STAT-2, AP-1 family members, HP1α, BRAC1, ER, and MyoD to target active genes (28–37).

Herein, we demonstrate that Brg-1, the ATPase subunit of the SWI/SNF complex, is responsible for regulating the constitutive expression of the MMP-2 gene by recruitment of Sp1, AP-2, and pol II to the MMP-2 promoter. In addition, the binding of Sp3 to the MMP-2 promoter is diminished in the presence of Brg-1. Therefore, Brg-1 can regulate gene transcription by modifying the DNA-binding capacity of Sp1 and Sp3.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Reagents—**HeLa and SW-13 cells were maintained in Dulbecco’s modified Eagle’s medium with 2 mM l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum. Micrococal nuclease (MNaS) was purchased from Worthington Biochemicals (Lakevood, NJ). Antibodies against Sp1, Sp3, AP-2, pol II, Brg-1, and normal rabbit IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibody against actin was purchased from Sigma. Antibody against human MMP-2 was a generous gift from Dr. J. Engler (University of Alabama at Birmingham, AL).

**Plasmid DNAs—**pREP was derived from pREP7 (Invitrogen). The fragment containing the RSV promoter and hygromycin coding sequence of pREP was released by SalI/BstBI digestion, then pREP7 was generated by ligation of the blunt-ended pREP7 backbone. The promoterless luciferase expression plasmid was generated by using specific MMP-2 primer and labeled with [α-32P]dCTP. Hybridization was carried out with 50% formamide prehybridization buffer (6× SSC, 0.1% SDS) at 65°C. Blots were washed four times with 1% SDS in 2× SSC for two times, and two times in 1× SSC before exposure to the PhosphorImager (Amersham Biosciences).

**Restriction Enzyme Hypersensitivity Analysis—**Purified nuclei were digested with 10 units of Aval per μg of DNA for 20 min at 25°C. Genomic DNA was then purified and subjected to digestion by EcoRI, and Southern blotting was performed as described above.

**Chromatin Immunoprecipitation Assay—**The ChIP assay was performed as previously described (41). Cells were fixed by adding formaldehyde to a final concentration of 1% in serum-free Dulbecco’s modified Eagle’s medium for 15 min at 37°C. Cells were then washed twice in phosphate-buffered saline, and nuclei were purified as described above. Nuclei were then resuspended in Tris-EDTA buffer supplemented with protease inhibitors and sonicated until the size of DNA fragments were in the range of 500–1000 bp. Soluble chromatin was then clarified by centrifugation for 15 min at 14,000 rpm at 4°C, and ~1% of the sonicate was saved as input. The chromatin sample was adjusted to 1 μg/ml with the chromatin dilution buffer (0.5% Tween-20, 1% bovine serum albumin, 10% glycerol, 1 mM glucose in Tris-buffered saline) at 4°C overnight. Blots were washed four times in Tris-buffered saline with 0.01% Tween-20, and subsequently incubated in sheep anti-mouse peroxidase-conjugated antibody (1:3000) in antibody dilution buffer. After a 45 min incubation at room temperature, the blots were washed four times, and ECL reagents used for development.

**In Vivo DSP Cross-linking, Immunoprecipitation, and Immunoblot—**In vivo cross-linking and immunoprecipitation were performed as described by Liu et al. (39). 107 HeLa cells or SW-13 cells transfected with Brg-1 or Brg-2 were cross-linked with 0.6 mg/ml DSP in phosphate-buffered saline for 15 min at room temperature. After extensive washing with phosphate-buffered saline, the nuclei were purified as described below. Nuclei were lysed, and equal amounts of nuclear protein were subjected to immunoprecipitation with antibodies against Brg-1, Sp1 or normal IgG as a negative control. Protein A/G-agarose beads were washed for 2 h at 4°C, and the immunoprecipitates were washed five times with washing buffer (20 mM Tris (pH 7.5), 0.5 mM NaCl, 1 mM EDTA, 0.2% Triton X-100). Immunocomplexes were eluted from the agarose beads by boiling in 2× SDS-sample buffer with 0.2 M dithiothreitol, and subjected to 8% SDS-PAGE. Proteins were transferred to nitrocellulose, and the membrane was detected with specific antibody against Sp1 by Western blot analysis.

**Preparation of Nuclei—**Purified nuclei were resuspended in 200 μl of MNase digestion buffer (30 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, and 0.5% (w/v) Nonidet P-40. After 5 min of incubation on ice, the lysate was spun at 1500 rpm for 10 min at 4°C then the nuclei were resuspended in MNase digestion buffer.

**Preparation of Nuclei Nuclear Extractions—**Antisense RNA was generated by using specific MMP-2 primers and labeled with [α-32P]dCTP. Hybridization was carried out with 50% formamide prehybridization buffer (6× SSC, 0.1% SDS) at 65°C. Blots were washed four times with 1% SDS in 2× SSC for two times, and two times in 1× SSC before exposure to the PhosphorImager (Amersham Biosciences).

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ase K digestion and phenol-chloroform extraction and, finally, precipitated by ethanol. Approximately one-fifth of the immunoprecipitated DNA was used for semi-quantitative PCR. Immunoprecipitated DNA was amplified by a primer pair corresponding to a 247-bp fragment (H11002 to H11001) from the human MMP-2 promoter, or a primer pair corresponding to a 331-bp fragment (H11002 to H11001) of the human U6 snRNA gene (42), and subjected to semi-quantitative PCR. The PCR reaction included 26–32 PCR cycles. The PCR reaction products were resolved in 2% agarose gels in 1/10 TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.3), and the gels were stained with ethidium bromide. In experiments with transfected SW-13 cells, PCR was performed for 25–28 cycles in the presence of 2.5 μCi of [α-32P]dCTP, and the products were fractionated in 4% polyacrylamide gels. The dried gels were analyzed with the PhosphorImager. Densitometry was used to quantify the PCR results, and all results were normalized by respective input values and then normalized to the intensity of the internal control U6 snRNA.

RESULTS

Reconstitution of Brg-1 Potentiates Transcription of the Human MMP-2 Gene in SW-13 Cells—It has been shown that the SWI/SNF complex enhances the expression of 80 genes in FIG. 1. Reconstitution of Brg-1 potentiates transcription of the human MMP-2 gene in SW-13 cells. A, SW-13 cells were transfected with BJ-5 (empty vector), Brg-1 or Brg-1(K798R) for 36 h, then the supernatants were collected and concentrated. The concentrated supernatants were subjected to zymography and immunoblotting for analysis of MMP-2 protein. B, expression of wild-type and mutant Brg-1 protein was examined by Western blotting using total cell lysates from BJ-5-, Brg-1-, and Brg-1(K798R)-transfected SW-13 cells. Expression of actin was used as an internal control. C, SW-13 cells were transfected with BJ-5, Brg-1, or Brg-1(K798R) for 24 h. Total RNA was purified, and the expression level of human MMP-2 mRNA was evaluated by RPA. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels were used as an internal control. Quantification of the above experiment and three others is shown by setting the normalized MMP-2 mRNA level (against glyceraldehyde-3-phosphate dehydrogenase mRNA level) in BJ-5-transfected cells as 1.0. Mean ± S.D. of four experiments is shown. D, constitutive expression of MMP-2 protein was detected in supernatants from HeLa cells by zymography and immunoblotting. Expression of MMP-2 mRNA was detected by RPA in HeLa cells. Brg-1 protein expression was detected by immunoblotting of total cell lysates from HeLa cells. Representative of three experiments.
SW-13 cells, which lack expression of both the Brg-1 and Brm subunits of SWI/SNF, or lack a functional SWI/SNF chromatin remodeling complex (29). We initiated our study on the function of Brg-1 in MMP-2 gene transcription by reconstitution of Brg-1 or mutant Brg-1 (K798R), which lacks ATPase function, into SW-13 cells, and then evaluated the expression of MMP-2. As shown in Fig. 1A, SW-13 cells transfected with the empty vector (BJ-5) express very low levels of MMP-2, however, reconstitution of Brg-1 enhanced expression of the MMP-2 protein as evaluated by both zymography and immunoblotting. Reconstitution of Brg-1 (K798R) had only a modest effect on MMP-2 protein expression compared with transfection with the BJ-5 vector. Equivalent levels of Brg-1 and Brg-1 (K798R) were expressed upon transfection into SW-13 cells (Fig. 1B). We also examined MMP-2 mRNA levels expressed in Brg-1 and Brg-1 (K798R)-transfected SW-13 cells. Reconstitution of Brg-1 enhanced MMP-2 mRNA expression ~5.2-fold compared with the control vector BJ-5, whereas Brg-1 (K798R) transfection had a minimal influence on MMP-2 mRNA expression (~1.5-fold) (Fig. 1C). Similar to the data on MMP-2 protein, expression of MMP-2 mRNA is potentiated by Brg-1, but not by the mutant Brg-1 protein. HeLa cells were used as a positive control for these experiments, because they express high levels of Brg-1 and express MMP-2 mRNA and protein constitutively (Fig. 1D). Thus, our data suggest that Brg-1 is required for maximal expression of the MMP-2 gene.

Proper Chromatin Context Is Required for Brg-1 to Potentiate MMP-2 Promoter Activity—To study the influence of Brg-1 on MMP-2 promoter activity, we cloned the 1.7-kb promoter of the human MMP-2 gene into both pGL3 and pREP vectors. This construct contains the Sp1/Sp3 site at −91 and AP-2 site at −61 that are critical for constitutive MMP-2 promoter activity (3). The pREP vector is an episomal reporter vector that can form a proper chromatin structure when transfected into host cells (29, 31, 43). Both pGL3 and pREP-based MMP-2 luciferase vectors were transiently transfected into SW-13 cells with increasing amounts of BJ-5, Brg-1, or Brg-1 (K798R), then luciferase activity was determined 24 h later. Our results indicate that the MMP-2 promoter in the pREP episomal vector was enhanced by Brg-1 (~5-fold), whereas Brg-1 (K798R) did not affect MMP-2 promoter activity (Fig. 2A). In contrast, Brg-1 did not enhance MMP-2 promoter activity in the pGL3-based vector (Fig. 2B), which can not form a regular-phased nucleosome structure in transfected cells. Thus, our data suggest that proper chromatin structure is required for Brg-1 to enhance MMP-2 promoter activity.

Constitutive Accessibility of the Genomic Locus of the Human MMP-2 Promoter Region—The results above indicate that chromatin structure is important for Brg-1 to potentiate MMP-2 gene expression, thus, we next studied the chromatin structure and chromatin remodeling of the MMP-2 promoter in vivo. As we had previously shown that the ~200-bp proximal promoter region of the MMP-2 gene, which contains the Sp1/Sp3 and AP-2 binding sites, is necessary and sufficient to maintain constitutive MMP-2 gene expression (3), we focused our study on chromatin structure and changes of accessibility within this region. As shown in Fig. 3A, there is an ApaL1 site located ~206 bp upstream of the transcription start site. We used this ApaL1 restriction site, combined with limited MNase digestion, to elucidate the chromatin structure of the MMP-2 promoter. Nuclei purified from HeLa cells were digested with increasing amounts of MNase, then genomic DNA was purified and subsequently digested with ApaL1. A probe located downstream of ApaL1 (Fig. 3A) was used to map the double-stranded cleavage sites induced by MNase digestion, which correspond to the linker regions of nucleosomes. At the same time, purified genomic DNA from HeLa cells was also subjected to MNase and ApaL1 digestion, and subsequent Southern blotting, with the same probe. Limited digestion of nuclei from HeLa cells and indirect end-labeling resulted in a series of bands ~180 bp apart, which correlates with the position of nucleosomes spanning the MMP-2 gene promoter (Fig. 3B). However, limited digestion of deproteinized genomic DNA (G) with MNase did not produce a similar pattern (Fig. 3B), thus confirming that the origin of these bands is solely a result of digestion of nucleosomes at the linker regions. To evaluate the effects of Brg-1 on the global chromatin structure of the MMP-2 promoter, SW-13 cells were transfected with BJ-5 or Brg-1, then nuclei from transfected cells were subjected to limited MNase digestion and indirect end-labeling analysis. Brg-1 did not induce a global change of chromatin structure in SW-13 cells, such as total disruption of transcription of nucleosomes, in the MMP-2 promoter (Fig. 3C). We used the restriction enzyme accessibility assay to examine changes in chromatin structure of the MMP-2 promoter in the absence or presence of Brg-1. As shown in Fig. 3D, within the 2.9-kb EcoRI fragment spanning the MMP-2 promoter, there are several AvaI sites in this region. One of them is located in the ~200-bp proximal promoter region of the MMP-2 gene, which contains the Sp1/Sp3 and AP-2 binding sites (Fig. 3, A and C). Therefore, we
used AvaI accessibility as a marker of local chromatin remodeling, because the remodeling process results in the relaxation of nucleosomes and enhances the exposure of the AvaI cutting site, which in turn produces a 0.94-kb band that is detected by Southern blotting. SW-13 cells were transfected with either BJ-5 or Brg-1 for 36 h, and then purified nuclei were digested with AvaI. Purified genomic DNA was further restricted by EcoRI and indirectly end-labeled with the probe as shown in Fig. 3D. Reconstitution of Brg-1 did not enhance the sensitivity of AvaI digestion on the promoter region of MMP-2 gene, because the 0.94-kb band caused by AvaI in vivo digestion was detected in both BJ-5- and Brg-1-transfected SW-13 cells, and

**FIG. 3.** Brg-1 does not change the accessibility of chromatin on the human MMP-2 promoter. A, schematic diagram of the proximal region of the human MMP-2 promoter; the position of the restriction site for ApaL1 is indicated. The horizontal bar shows the position of the probe used for Southern blotting. B, nuclei from HeLa cells were digested with increasing amounts of MNase (0–500 units/ml). The purified DNA and genomic DNA directly digested by MNase were subsequently digested by ApaL1. DNA fragments were analyzed by Southern blotting with the corresponding probe shown above. EtBr-stained agarose gels of the respective digested genomic DNA are also shown. C, SW-13 cells were transfected with BJ-5 or Brg-1 for 36 h. Purified nuclei were digested with increasing amounts of MNase. Genomic DNA was purified and restricted with ApaL1. Nucleosome positions were determined by Southern blotting. EtBr-stained agarose gels of the respective digested genomic DNA are also shown. D, schematic diagram of the genomic structure of the human MMP-2 gene. There is a 2.9-kb fragment covering the MMP-2 promoter. The position of AvaI sites are shown, and one of them covering the Sp1/Sp3 element is ~0.94 kb downstream of the upstream EcoRI site. The horizontal bar shows the position of the probe used for Southern blotting. E, nuclei purified from SW-13 cells transfected with BJ-5 or Brg-1 were digested with AvaI. The purified DNA was then subsequently digested by EcoRI. DNA fragments were analyzed by Southern blotting with the probe shown above. Accessibility was defined as the ratio (%) of in vivo digestion (the 0.94-kb AvaI band) normalized against the sums of in vivo (AvaI) and in vitro (EcoRI) digestion. Accessibility of BJ-5-transfected SW-13 cells was set as 1.0. Mean ± S.D. of three experiments are shown.
the quantified accessibility was not changed in the absence or presence of Brg-1 (Fig. 3E). Therefore, Brg-1 does not affect accessibility of the chromatin structure in the MMP-2 promoter.

Brg-1 Enhances Binding of Sp1, AP-2, and pol II to the MMP-2 Promoter in Vivo but Decreases the Association of Sp3—We have previously shown that the Sp1, Sp3, and AP-2 transcription factors have critical functions in constitutive transcription of the human MMP-2 gene (3). Because Brg-1 is required for transcription of the MMP-2 gene, we further investigated the effect of Brg-1 on recruitment of transcription factors and other transcription regulators on the MMP-2 promoter in vivo by ChIP assay. The recruitment of transcription factors and pol II was examined in both Brg-1-positive cells (HeLa cells), Brg-1-negative cells (SW-13 cells), and in SW-13 cells transfected with BJ-5, Brg-1, or Brg-1 (K798R). Cells were fixed by formaldehyde, and soluble chromatin was purified by sonication. DNA was purified from the chromatin input. The amount of the MMP-2 genomic fragment was determined by semi-quantitative PCR with serially diluted chromatin input. The amount of the MMP-2 sequence present in the immunopurified chromatin was determined by PCR. The pol III transcribed U6 snRNA gene was used as an internal control. The amount of U6 snRNA genomic sequence was utilized to normalize the levels of MMP-2. The data are representative of three experiments. The fold-induction shown is the mean of three experiments.

FIG. 4. Brg-1 enhances recruitment of Sp1, AP-2, and pol II to the MMP-2 promoter in vivo, but decreases the association of Sp3.

A. schematic diagram shows the position of PCR primers used to amplify the human MMP-2 promoter from immunoprecipitated chromatin. B. SW-13 and HeLa cells were cross-linked with formaldehyde, and chromatin was prepared from purified nuclei by sonication. DNA was purified from the chromatin input. The amount of the MMP-2 genomic fragment was determined by semi-quantitative PCR with serially diluted chromatin input. C. SW-13 cells were transfected with BJ-5, Brg-1, or Brg-1 (K798R) for 24 h. Chromatin samples prepared as described above were subjected to immunoprecipitation by antibodies against IgG, Sp1, Sp3, AP-2, Brg-1, and pol II. The amount of MMP-2 sequence present in the immunopurified chromatin was determined by PCR. The pol III transcribed U6 snRNA gene was used as an internal control. The amount of U6 snRNA genomic sequence was utilized to normalize the levels of MMP-2. The data are representative of three experiments. The fold-induction shown is the mean of three experiments. D. SW-13 cells were transfected with BJ-5 or Brg-1, and nuclear extracts were analyzed by Western blotting for Sp1 and Sp3 protein expression.
these samples (Fig. 4B). As a negative control, immunoprecipitation by IgG resulted in very faint bands in the two cell lines (Fig. 4B), likely due to nonspecific interactions between IgG and the chromatin complexes. In SW-13 cells, binding of Sp1 is undetectable, and AP-2 and pol II binding on the MMP-2 promoter is modest. The Brg-1 antibody generated a signal comparable to that of IgG alone, in agreement that SW-13 cells are deficient in Brg-1. Interestingly, there was a very strong association of Sp3 with the MMP-2 promoter in SW-13 cells (Fig. 4B). However, in Brg-1-positive HeLa cells, the recruitment of Sp1, AP-2, pol II, and Brg-1 on the MMP-2 promoter was stronger than that in SW-13 cells, and the association of Sp3 on the MMP-2 promoter was weaker (Fig. 4B). Thus, our results suggest that Brg-1 enhances the recruitment of Sp1, AP-2, and pol II to the MMP-2 promoter in vivo, but decreases the binding of Sp3 at the same time. To substantiate these findings, SW-13 cells were transfected with BJ-5, Brg-1, or Brg-1 (K798R), and ChIP assays performed with equal amounts of input from these transfected cells (Fig. 4C). Reconstitution of Brg-1 in SW-13 cells enhanced the binding of Sp1, AP-2, and pol II to the MMP-2 promoter, concomitant with increased association of the Brg-1 protein on the MMP-2 promoter in vivo (Fig. 4C). Of note, the binding of Sp3 on the MMP-2 promoter was decreased upon reconstitution of Brg-1. SW-13 cells transfected with the BJ-5 control vector (Fig. 4C) showed a similar pattern as wild-type SW-13 cells (Fig. 4B). In addition, SW-13 cells transfected with Brg-1 (K798R) mutant showed a similar pattern of transcription factor binding compared with SW-13 cells transfected with BJ-5, although a modest enhancement of AP-2 and pol II binding was observed (Fig. 4C). To exclude the possibility that Brg-1 may modify protein levels of Sp1 or Sp3, we examined Sp1 and Sp3 levels in BJ-5- or Brg-1-transfected SW-13 cells. The results indicate that Brg-1 does not change the expression levels of Sp1 or Sp3 (Fig. 4D). In summary, using the ChIP assay to examine the in vivo binding of transcription factors and pol II to the MMP-2 promoter, we demonstrate that Brg-1 is required for recruitment of Sp1, AP-2, and pol II to the MMP-2 promoter, and at the same time, Brg-1 also decreases the association of Sp3 to the MMP-2 promoter. The ATPase activity of Brg-1 is required for this response as demonstrated using the Brg-1 (K798R) mutant.

Interaction of Sp1 and Brg-1 in Vivo—Previous studies have shown that Brg-1 interacts with Sp1 (26, 27, 39). We observed that Brg-1 influenced the binding of Sp1 and Sp3 on the MMP-2 promoter; a possible mechanism is that Brg-1 can specifically interact with Sp1 and recruit Sp1 to the MMP-2 promoter. To test this hypothesis, communoprecipitation experiments were performed to detect the in vivo interaction between Sp1 and Brg-1. We used in vivo cross-linking to perform communoprecipitation in HeLa cells. HeLa cells were cross-linked with DSP, and then nuclear extracts were subject to immunoprecipitation by IgG, anti-Sp1, or anti-Sp3 antibodies, and subsequent blotting with antibody against Sp1. As shown in Fig. 5A, communoprecipitation with Brg-1 antibody and blotting with anti-Sp1 demonstrated that the anti-Brg-1 antibody can precipitate the Sp1 protein, indicating complex formation between these proteins in HeLa cells (Fig. 5A, lane 2). Furthermore, SW-13 cells were transfected with Brg-1 or BJ-5 and cross-linked with DSP, and then nuclear extracts were subject to
immunoprecipitation by IgG, anti-Brg-1, or anti-Sp1 antibodies, and subsequent blotting with antibody against Sp1. The data demonstrate that transfected Brg-1 forms a complex with Sp1 in vivo (Fig. 5B, lane 2). As a negative control, no complex formation between Brg-1 and Sp1 was detected in SW-13 cells transfected with B.J.5 (Fig. 5C, lane 2).

**DISCUSSION**

We used SW-13 cells, which lack Brg-1, as a model system to study transcription of the human MMP-2 gene. We demonstrate that expression of the MMP-2 gene in SW-13 cells is decreased compared with a Brg-1-positive cell line such as HeLa cells. Reconstitution of Brg-1, but not the ATPase-deficient Brg-1 mutant (K798R), enhanced transcription of the MMP-2 gene. Further analysis of chromatin structure and chromatin remodeling demonstrated that Brg-1 is not required for chromatin remodeling in the MMP-2 genomic locus. We further investigated the binding of transcription factors and pol II to the MMP-2 promoter in the absence or presence of the Brg-1 protein. Interestingly, we found that Brg-1 regulated Sp1 and Sp3 binding to the MMP-2 promoter by increasing the association of Sp1, decreasing the association of Sp3, and enhancing the recruitment of AP-2 and pol II. Coimmunoprecipitation by in vivo cross-linking indicated that Brg-1 is associated with Sp1. Thus, Brg-1 has important functions in regulating MMP-2 gene expression, because Brg-1 regulates MMP-2 gene transcription by promoting the binding of Sp1 and the dissociation of Sp3. We have previously shown that Sp1 is a strong activator of MMP-2 expression, whereas Sp3 has much weaker activity for MMP-2 expression (3). Based on our finding that Brg-1 interacts with Sp1 in vivo, this suggests that the recruitment of Sp1 on the MMP-2 promoter may be dependent on its association with the SWI/SNF complex and ultimately results in optimal MMP-2 gene transcription.

Chromatin remodeling is defined as persistent changes in the structure or position of nucleosomes on DNA. SWI/SNF complexes can disrupt histone-DNA contacts in an ATP-dependent manner (13, 14). It is proposed that recruitment of SWI/SNF complexes to target promoters requires the interaction of SWI/SNF with transcription factors (28). In our study, we found that Brg-1 does not change the position of nucleosomes on the MMP-2 promoter, nor does it enhance the accessibility of nucleosomes to restriction enzymes. In the absence of Brg-1, Sp3 is the major transcription factor binding on the Sp1/Sp3 element, whereas in the presence of Brg-1, Sp1 is associated with the Sp1/Sp3 element. Thus, these data suggest that the Sp1/Sp3 element is constitutively accessible for Sp1 or Sp3 binding. As we show in vivo interaction of Brg-1 and Sp1, and by ChIP assay the concurrent recruitment of Sp1 and Brg-1 on the MMP-2 promoter in vivo, a possible mechanism is that Sp1 binds to Brg-1, and the Sp1 and Brg-1 complex is targeted to the MMP-2 promoter by Sp1 binding to the Sp1/Sp3 element in the proximal region of the MMP-2 promoter. In addition, Liu et al. (29) have shown that Brg-1 facilitates Z-DNA formation in the nucleosomal template and stabilizes the open chromatin structure on the constitutively expressed human colony-stimulating factor 1 gene. Therefore, although Brg-1 does not promote chromatin remodeling events on the MMP-2 promoter, it may stabilize the open chromatin configuration of the MMP-2 promoter, and allow Sp1 to bind on the MMP-2 promoter. Whether Z-DNA formation plays an important role in regulation of human MMP-2 gene transcription warrants further investigation.

How can Brg-1 modulate the binding of Sp1 and Sp3? It has been reported that activation of the HPV-16 promoter during epithelial differentiation and transformation is determined by the ratio of Sp1/Sp3 binding (9). Furthermore, Sp3 has been shown to antagonize target gene transcription induced by Sp1, thus Sp3 is generally regarded as a repressor or mild activator depending on the context of the target gene (6–8, 10, 11, 44, 45). Thus, the switching of Sp1 and Sp3 binding by Brg-1 may be a mechanism to refine the regulation of MMP-2 gene transcription. Recently, Yu et al. reported that the Sp3-DNA complex is more stable than the Sp1-DNA complex (46). In addition, Sp3 can compete with Sp1 for promoter binding and block Sp1-mediated synergistic transcription activation (46). On the other hand, Utley et al. (47) have shown that the SWI/SNF complex can interact with recombiant Sp1 and promote its binding on target gene promoters. Furthermore, it has been found that the binding affinity of Sp1 for nucleosomal DNA increases >10-fold after adding Sp1 and the SWI/SNF complex together (47, 48). In our study, the ChIP data clearly show that, in the absence of Brg-1, the MMP-2 promoter is predominantly occupied by Sp3, however, reconstitution of Brg-1 can release Sp3 and enhance the occupancy of Sp1 on the MMP-2 promoter. Taken together, we propose that Brg-1 regulates Sp1/Sp3 binding by altering the affinity of Sp1 to nucleosomal DNA on the MMP-2 promoter, as association of Sp1 and Brg-1 enhances the affinity of Sp1 to chromatin, and at the same time, competitively releases Sp3 from the MMP-2 promoter (Fig. 6).

Brg-1 has been intensely studied in its relationship with cell transformation and tumor invasion. Brg-1 has been shown to interact with the RB protein, and induce cell cycle arrest (22, 24, 44). In addition, Brg-1 is also involved in regulating p53- and BRCA-1-induced genes (36, 37). Wong et al. (25) also reported that Brg-1 is deleted in prostate and lung tumor cells, and reintroduction of Brg-1 is sufficient to reverse the transformed phenotype. It has been reported that loss of Brg1 is correlated with poor prognosis in primary lung cancer (49). Therefore, these studies suggest that Brg-1 acts as a tumor suppressor. However, it has also been shown that increased expression of Brg-1 is associated with the advanced stage of human gastric carcinomas (50). CD44, a tumor invasion-related gene, also requires Brg-1 for optimal transcription (35). Here, our results demonstrate that transcription of the MMP-2 gene requires the function of Brg-1. In summary, Brg-1 has a more complicated role in cell transformation and tumor invasion; it can induce cell cycle arrest and inhibit cell transformation, but also enhances expression of tumor invasion and angiogenesis-related genes in the advanced stages of malignant tumors.

**Acknowledgments**—We thank G. R. Crabtree (Stanford University) for the Brg-1(K798R) vector and K. Zhao (National Institutes of Health) for the Brg-1 expression vector and helpful advice.

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J. Biol. Chem. 2004, 279:46326-46334.
doi: 10.1074/jbc.M405438200 originally published online August 17, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M405438200

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