Methyl-CpG Binding Domain 1 (MBD1) Interacts with the Suv39h1-HP1 Heterochromatic Complex for DNA Methylation-based Transcriptional Repression*

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Cytosine methylation and posttranslational modifications of the amino termini of the core histones in the nucleosome provide epigenetic codes for genome regulation. In the nucleus, not only is the DNA methylated, but the methylated DNA is also interpreted by methyl-CpG binding domain (MBD) proteins. MBD1 possesses an MBD involved in mediating DNA methylation-dependent transcriptional repression. The MBD of MBD1 binds a symmetrically methylated CpG sequence, but the precise roles of this domain have not been investigated. In addition, little is understood about the state of histone modifications within MBD1-containing heterochromatin on methylated gene promoters. Here we show that histone H3 methylase Suv39h1 and the methyl lysine-binding protein HP1 directly interact with MBD of MBD1 in vitro and in cells. Suv39h1 was found to enhance MBD1-mediated transcriptional repression via MBD but not via the C-terminal transcriptional repression domain of MBD1. Furthermore, MBD1 links to histone deacetylases through Suv39h1, resulting in methylation and deacetylation of histones for gene inactivation. These data indicate that MBD1 may tether the Suv39h1-HP1 complex to methylated DNA regions, suggesting the presence of a pathway from DNA methylation to the modifications of histones for epigenetic gene regulation.

DNA and protein modifications create a new surface for interaction with target molecules (1–5). Cytosine methylation is involved in gene regulation and the formation of transcriptionally inactive chromatin, together with methyl-CpG binding domain proteins (MBD1 proteins) (6–7). There are at least five mammalian MBD proteins, namely MeCP2, MBD1, MBD2, and MBD3 for transcriptional repression, and MBD4 (also known as MED1) for mismatch repair as a thymine glycosylase. In addition, posttranslational modifications of amino termini of the core histones cooperatively produce epigenetic codes for genome dynamics in the nucleus. These include acetylation, phosphorylation, and methylation of the histone molecules.

Recent studies of cytosine hypomethylation mutants have revealed that both DNA methylation and histone modifications share a common pathway in chromatin organization (8). The Neurospora crassa DIM-5 gene product is a histone H3 methylase containing a SET domain, which specifically methylates lysine 9 of histone H3, abbreviated through as H3 (Lys-9). The mutant dim-5 completely abolished cytosine methylation of the genome. Furthermore, Arabidopsis thaliana DDM1, which shows amino acid similarity to a helicase of the SWI2/SNF2 family, is also required for the maintenance of genomic methylation (9), and disruption of the homolog Lah gene caused demethylation of the mouse genome (10). These suggest that DNA methylation may depend on methyl-H3 (Lys-9) and unique chromatin structure. However, interrelations between cytosine methylation, MBD proteins, and the histone-modifying system remain to be elucidated (1, 3, 11).

MBD1 is known to act as a transcriptional repressor through the cooperation of MBD, cysteine-rich CXXC domains, and a C-terminal transcriptional repression domain (TRD) (12–15). The conserved CXXC sequence was originally found in DNMT1 and the trithorax group protein ALL-1, but its precise role is unknown (12, 15). MBD1 produces an active transcriptional repression that was partially reversed by the addition of histone deacetylase inhibitors (13). During investigation of the mechanism of transcriptional repression by the TRD of MBD1, we have recently found that a transcriptional mediator, MBD1-containing chromatin associated factor (MCAF), binds the TRD of MBD1 to form the repressive complex (16). In addition, the MBD of MBD1 binds a symmetrically methylated CpG sequence (17), but transcriptional roles of this domain have not been investigated. Little is understood about the state of histone modifications within MBD1-containing heterochromatin on methylated gene promoters. In this study, we show that H3 (Lys-9) methylase Suv39h1 and the methyl lysine-binding protein HP1 interact with MBD of MBD1. Suv39h1 enhances MBD1-mediated transcriptional repression via MBD but not the C-terminal TRD of MBD1. Furthermore, MBD1 associates with histone deacetylases through Suv39h1. Our data suggest that MBD1 tethers the Suv39h1-HP1 complex to methylated DNA regions. We discuss the possible pathway from DNA...
methylation to the modifications of histones for epigenetic gene regulation.

EXPERIMENTAL PROCEDURES

Cell Culture, Transfection, Treatment, and Luciferase Assay—HeLa, U2OS, NCI-H1299, and SBC5 cells were cultured in Dulbecco’s modified Eagle’s medium/F12 (Invitrogen) supplemented with heat-inactivated fetal bovine serum (BioWhittaker). The cells transfected by a liposome- or-DNA- mediated gene transfer were treated with 100 ng/ml of trichostatin A (TSA) or 1 mM sodium butyrate (Wako) for 12 h. The luciferase activities were determined as described (12, 16). Values are the means and standard deviations of the results from three independent experiments.

Plasmids—pEGFP-Suv39h1, pEGFP-G9a, pEGFP-MBD1v3, pEGFP-MBD1 (MBD1-NLS), pCGN-MBD1v3, pCGN-MBD1v3 ΔN, pCMV-GAL4-MBD1ΔA, pCMV-GAL4-MBD1ΔT, pCMV-GAL4-MBD1Δ111, and reporters that contain GAL4-binding elements upstream of the SNRPN and VHL promoters were as described (12, 14, 18). The cDNA for Suv39h1 was inserted into pcDNA3 (Invitrogen) and pCMV-GAL4 (GAL4 mock). The cDNA for HP1α was inserted into pcDNA3. The cDNA for MBD1v3 was ligated into pcDNA3 and pDsRed-N1 (Clontech).

Chemical Cross-linking and Chromatin Immunoprecipitation Assay (ChIP)—Cells (5 × 10⁶) were treated for 30 min on ice with dimethyl 3,3’-dithiobispropionimidate-2HCl (DTBP) (5 mM) (Pierce) in phosphate-buffered saline and then with the addition of 1% formaldehyde for 10 min. The cell lysates were sonicated to generate DNA fragments 200–1000 bp in length. Chromatin immunoprecipitation was performed with anti-FLAG, anti-HP1α, and anti-Pol II antibodies or control IgG. Specific sequences for the p16 gene promoter in the immunoprecipitates were detected by PCR amplification with the p16-W primers (14). In vitro Translocation and Glutathione S-Transferase (GST) Pull-down—Plasmid DNA (1 μg) was used in vitro transfection and translation (50 μl) according to the manufacturer (Promega). Bacterially expressed GST and GST-fused proteins (2 μg) were immobilized on glutathione-agarose beads and incubated with whole lysates from HeLa cells, in vitro translated proteins, or His-tagged proteins (2 μg) in a buffer (250 μl) containing 0.1% TritonX-100, 50 mM HEPES, pH 7.4, 50–300 mM NaCl, 5% glycerol, 2 mM dithiothreitol, and protease inhibitors for 1 h at 4 °C. The input indicates 5% of cell lysates, in vitro translated and His-tagged proteins, in the reaction mixture.

Immunoprecipitation—HeLa cell lysates were incubated with specific antibodies or control IgG (Santa Cruz Biotechnology) for 1 h at 4 °C. This was followed by incubation for 1 h after adding 30 μl of protein G/agarose beads (Oncogene) in a buffer (250 μl) containing 0.2% Nonidet P-40, 40 mM Tris-Cl, pH 7.5, 100 mM KCl, 20% glycerol, and protease inhibitors. The immunoprecipitations of endogenous methyl-H3 (lysine 9) and FLAG-MBD1 and endogenous MBD1 and HP1α in HeLa cells were performed after cross-linking with DTBP. The input indicates 5% of cell lysates.

Antibodies, Western Blot, and Immunofluorescence Analyses—Polyclonal antibodies against HP1α (amino acids 1–191) were generated by immunizing rats against the His-tagged HP1α. For affinity purification of the antibodies, GST-fused HP1α (1–191) was coupled to an Aff-Gel activated matrix (Bio-Rad). Anti-MBD1 antibodies were generated previously (16) or purchased from Santa Cruz Biotechnology. Antibodies utilized were anti-histone deacetylase 1 (anti-HDAC1; H-51), anti-HDAC2 (C-8), anti-RNA polymerase II (Santa Cruz Biotechnology), anti-FLAG (M5) (Sigma), anti-His (Qiagen), anti-hemagglutinin 1 (HA) (Roche Applied Science), and anti-dimethyl-histone H3 (lysine 9) antibodies (Upstate Biotechnology). Western blot and immunofluorescence analyses were carried out as described (12).

RESULTS

Localization of MBD1, Suv39h1, G9a, and HP1α in the Nucleus—To address the mechanism(s) of transcriptional repression by MBD1, we considered whether MBD1 can associate with histone methylases such as Suv39h1 and G9a by using an immunofluorescence analysis in HeLa cells (Fig. 1). Previous reports clarified that the subnuclear localization of MBD1 depends on the genome methylation (12, 14). Fluorescent protein-fused Suv39h1 and MBD1 showed a similar punctate distribution with multiple foci in the nuclei except for nucelus (Fig. 1A). In contrast, MBD1 did not preferentially colocalize with G9a (Fig. 1B), emphasizing the specific correlation between MBD1 and Suv39h1. As previous reports demonstrated that Suv39h1 associates with HP1α (19, 20), MBD1 was also found to mostly coexist with HP1α (Fig. 1C). Because Suv39h1 selectively methylates H3 (Lys-9), we further observed the existence of both methyl-H3 (Lys-9) and MBD1 in the nucleus. The methyl-H3 (Lys-9) proteins concentrated on multiple foci with intense staining of MBD1 (data not shown). Almost all the nuclei observed exhibited the patterns shown here. The observations suggested that MBD1 associates with Suv39h1-HP1 on both methyl-H3 (Lys-9) and methylated DNA-containing regions.

To investigate the localization of these proteins on chromosomal gene promoters, we chose the p16 tumor suppressor gene in which hypermethylation of the promoter-associated CpG island associates with transcriptional repression in many cancers (21, 22). NCI-H1299 cells possessed a methylated p16 promoter; meanwhile, the same DNA region was unmethylated in SBC5 cells (14, 16). After the cells expressing FLAG-tagged
FIG. 2. MBD1 interacts with the Suv39h1-HP1α complex via MBD. A, interaction between MBD1 and Suv39h1. HA-MBD1 and FLAG-Suv39h1 were co-expressed in HeLa cells and immunoprecipitated with anti-HA, anti-FLAG antibodies, and control IgG. B, Suv39h1 binds MBD of MBD1. GST and GST-fused portions of MBD1 isoform v1 (full-length (full), MBD, CXXC1, CXXC2, CXXC3, and the TRD) (2 μg) were immobilized on glutathione-agarose beads and incubated with in vitro translated FLAG-Suv39h1. C, loss of interaction of ΔN MBD1 lacking MBD
MBD1 or Suv39h1 were cross-linked with dimethyl 3,3′-dithiobispropionimidate-2HCl and formaldehyde, the coprecipitated DNAs with appropriate antibodies were subjected to PCR-amplification using a set of primers for p16 promoter sequences (Fig. 1D). MBD1 and Suv39h1 were present on the methylated but not the unmethylated p16 promoter. Using the untransformed cells, HP1α was also found in the methylated promoter. In contrast, RNA polymerase II-containing complexes associated exclusively with the unmethylated p16 promoter in SBC-5 cells. These results suggested that MBD1 coexists with the Suv39h1-HP1α heterochromatic complex and may tether it to methylated DNA regions for establishing histone methylation.

**MBD1 Interacts with Suv39h1-HP1α Complex via MBD**—To address the molecular interaction between MBD1 and the Suv39h1-HP1α complex, we performed an immunoprecipitation analysis in HeLa cells (Fig. 2A). FLAG-Suv39h1 was detected in the immunoprecipitates with HA-fused MBD1. Likewise, MBD1 was present in the Suv39h1-immunoprecipitates. To identify the direct interaction between MBD1 and Suv39h1, in vitro pull-down analysis was carried out (Fig. 2B). GST and GST-fused portions of MBD1 were immobilized on glutathione-agarose beads and incubated with in vitro translated FLAG-Suv39h1. Full-length MBD1 bound Suv39h1. Interestingly, Suv39h1 was found to selectively bind the MBD but not the cysteine-rich CXXC domains or the C-terminal TRD of the MBD1 protein. In agreement with this data, an immunoprecipitation analysis showed that the HA-ΔN MBD1 mutant, which was deleted for N-terminal MBD (amino acids 1–61), lost the binding ability to FLAG-Suv39h1 (Fig. 2C).

To map the region of Suv39h1 that interacts with MBD1, GST-fused portions of Suv39h1 were utilized for a pull-down analysis of in vitro translated MBD1 (Fig. 2D). MBD1 associated with a middle part of Suv39h1 (Δ2), termed the PreSET region, which is located between the chromo domain (Δ1) and the C-terminal SET catalytic domain (Δ3). This result indicated the presence of an MBD1-interacting domain in Suv39h1. Next, we investigated whether HP1α participates in the MBD1-containing complex, using an immunoprecipitation analysis (Fig. 2E). Endogenous HP1α was found in the immunoprecipitates with FLAG-MBD1, and MBD1 was present in the HP1α-immunoprecipitates. To evaluate the direct interaction of MBD1 with HP1α, bacterially expressed proteins were used for in vitro pull-down analysis (Fig. 2F). GST-fused Suv39h1 or parts of MBD1 on glutathione-agarose beads were incubated with His-tagged HP1α. Both full-length Suv39h1 and MBD1 complexed with HP1α. Significantly, the MBD of MBD1, but not the CXXC domains or TRD, bound HP1α. Thus, the data in Fig. 2, B, C, and F evidently suggested that MBD is an interacting surface to Suv39h1-HP1α. We further examined the association of these endogenous proteins in HeLa cells without any overexpression (Fig. 2G). The band that reacted to anti-HP1α antibodies was concentrated in the MBD1 immunoprecipitates. Endogenous MBD1 and HP1α were also colocalized in the nucleus, indicating the existence of complexes containing both proteins in vivo. Because HP1 tethers Suv39h1 to methyl-H3 (Lys-9), we tested whether MBD1 associates with methyl-H3 (Lys-9) by using immunoprecipitation. FLAG-MBD1 was detected in the immune complexes with endogenous methyl-H3 (Lys-9), and methyl-H3 (Lys-9) was also present in the MBD1-immunoprecipitates (data not shown).

To examine whether the MBD in MBD1 tethers the Suv39h1-HP1 complex to methylated DNA regions in the nucleus, immunofluorescence analyses of fluorescent protein-fused Suv39h1, HP1α, and MBD1 (MBD1-NLS), which includes only the MBD and nuclear localization signal (NLS) of MBD1, as well as FLAG-ΔN MBD1 were performed in HeLa cells (Fig. 2H). As described above, the punctate distribution of MBD1 with multiple foci depends on the presence of N-terminal MBD in proportion to genome methylation (12, 14). The MBD1 (MBD1-NLS) showed multiple foci formation in the interphase nuclei and colocalized with both Suv39h1 and HP1α. On the other hand, ΔN MBD1 was found to be present throughout the nuclei, and both Suv39h1 and HP1α localized into differently characteristic foci in the nuclei. Ectopic expression of MBD1 (MBD1-NLS) altered the localization of Suv39h1 and HP1α, suggesting that MBD of MBD1 is likely to recruit Suv39h1-HP1 complex to methylated DNA sites. However, our data do not exclude the possibility that Suv39h1-HP1α recruits MBD1 to H3 (Lys-9)-methylated chromatin.

**Suv39h1 Enhances MBD1-dependent Transcriptional Repression**—Throughout our study in vivo, we chose the use of the splice isoform v3 of MBD1 (shown in Fig. 3A), because this isoform most clearly exhibits DNA methylation-dependent gene silencing (12, 14). The protein structure of isoforms MBD1v1 and v3 is identical except for the presence of CXXC3 in MBD1v1 (Fig. 2B), and the CXXC3 itself seems to have some DNA binding activities. We investigated whether Suv39h1 is involved in MBD1-mediated transcriptional silencing. As reported previously (12), the MBD1 moderately reduced methylated promoter activities, either by promoter occupation through the contact between MBD and methylated DNA or by unidentified mechanisms. To avoid the possibility of the promoter occupation, we utilized GAL4-fused portions of MBD1v3 (MBD1Δ7 (MBD, amino acids 1–61), MBD1Δ4 (CXXC1 and 2, amino acids 62–327), and MBD1Δ11 (TRD, amino acids 460–523)) and examined their effects on a Photinus pyralis luciferase reporter that contains five GAL4 binding elements (5×GAL4) just upstream of the human SNRPN gene promoter in HeLa cells (Fig. 3A). A Western blot analysis showed that GAL4-fused MBD1 proteins were equally expressed in a dose-dependent manner (data not shown). MBD1Δ11 strongly repressed transcription (Fig. 3A, hatched bars), whereas MBD1Δ7 moderately suppressed promoter activities (Fig. 3A, gray bars). In contrast, MBD1Δ4 did not repress transcription (Fig. 3A, black bars). Very similar results were obtained with another CpG island-associated promoter from the VHL gene (data not shown). Next, Suv39h1 is known to function as a transcriptional corepressor (23, 24). To confirm whether Suv39h1 affects transcription, we expressed GAL4-fused Suv39h1 in HeLa cells to test the effect of this protein on 5× GAL4-containing the SNRPN and VHL promoters (Fig. 3B). Suv39h1 inhibited both promoter activities in a dose-dependent manner. The efficiency of repression by Suv39h1 seemed to be similar to that of MBD1 by MBD. The GAL4-Suv39h1 was with Suv39h1. HA-ΔN MBD1 and FLAG-Suv39h1 proteins were co-expressed in HeLa cells and immunoprecipitated with anti-FLAG and control IgG. D, MBD1 binds the PreSET region between the chromo and SET domains of Suv39h1. Immobilized GST and GST-fused portions of Suv39h1 (2 μg) were incubated with in vitro translated FLAG-MBD1. E, association of MBD1 with HP1α. Endogenous HP1α and FLAG-MBD1 were immunoprecipitated from HeLa cells. F, HP1α directly binds MBD of MBD1. Immobilized GST, GST-Suv39h1, and GST-MBD1 (full, MBD, CXXC1, CXXC2, CXXC3, and TRD) (2 μg) were incubated with His-tagged HP1α. A Western blot analysis was performed with anti-His antibodies. G, coexistence of MBD1 and HP1α in vivo. Endogenous MBD1 and HP1α were co-immunoprecipitated from HeLa cells. The input indicates 5% of the HeLa cell lysates. H, the MBD of MBD1 tethers the Suv39h1-HP1α complex in the nucleus. An immunofluorescence analysis of ΔSuv39h1-BD1 (MBD1-NLS) containing only the N-terminal MBD and nuclear localization signal, FLAG-ΔN MBD1, EGFP-Suv39h1, and EGFP-HP1α was performed in HeLa cells.
also expressed in proportion to the amount of transfected plasmid (data not shown). Furthermore, we examined whether Suv39h1 can affect transcriptional repression by MBD1 (Fig. 3C). FLAG-Suv39h1 and GAL4-fused portions of MBD1 were expressed in HeLa cells together with the 5× GAL4-containing promoter-driven luciferase vectors. The luciferase activity, in combination with FLAG mock, was normalized to 100 (Fig. 3C, leftmost bar), and Suv39h1 alone mildly repressed transcription from both promoters. GAL-fused MBD1Δ7 and MBD1Δ11 were appropriately expressed to repress transcription to 60–80%. Importantly, Suv39h1 synergistically enhanced the repression by MBD1Δ7, but not by MBD1Δ11, in a dose-dependent manner. No additive effect of Suv39h1 was found in the expression of GAL4 mock and MBD1Δ4. In addition, the FLAG-Suv39h1 active mutant (H320R) (25) enhanced repression by GAL4-MBD1Δ7 more than was the case with the wild-type Suv39h1 (data not shown). These data supported the idea that Suv39h1 contributes to transcriptional repression by MBD1.

**MBD1 Links to Histone Deacetylases via Suv39h1—** HDAC inhibitors such as TSA partially relieved the transcriptional repression by MBD1 (13). In addition, the N terminus of Suv39h1 was found to physically interact with HDAC1 and HDAC2, leading to formation of transcriptionally inactive chromatin (26). Repression by GAL4-Suv39h1 was partly reversed by the HDAC inhibitors TSA and sodium n-butyrate (Fig. 4A, hatched and gray bars, respectively) in comparison with the solvent alone (Fig. 4A, black bars). To examine the interaction of MBD1 and Suv39h1 with HDAC1/HDAC2, in vitro pull-down and subsequent Western blot analyses were carried out with the indicated antibodies. Suv39h1, MBD, and TRD of MBD1 in a GST fusion were immobilized on glutathione-agarose beads and incubated with the cell lysates from HeLa cells. Suv39h1 and MBD of MBD1 bound both endogenous HDAC1 and HDAC2 (Fig. 4B, top two panels). The association between MBD1 and HDAC1/HDAC2 appeared to be indirect, because Suv39h1, but not MBD, bound in vitro translated HA-tagged HDAC1/HDAC2 (Fig. 4B, bottom two panels). Immunoprecipitation analyses further showed that HDAC1/HDAC2 were present in both FLAG-tagged Suv39h1 and MBD1 immunoprecipitates (Fig. 4C). In contrast, the use of ΔN MBD1 and control IgG failed to immunoprecipitate the HDAC1/HDAC2. Finally, we studied the effect of HDAC inhibitors on transcriptional repression by MBD1 and TRD of MBD1 (Fig. 4D). The repression by MBD1 was partially reversed by both TSA (Fig. 4D, hatched bars) and sodium n-butyrate (Fig. 4D, gray bars). In agreement with a recent study conducted under the same experimental conditions (16), these inhibitors appeared to have less effect on transcriptional inhibition of MBD1 by TRD. Collectively, these data suggest that MBD1 promotes both methylation and deacetylation of histones on methylated genomic regions through interaction with Suv39h1-HP1 and HDACs.

**FIG. 3.** Suv39h1 enhances MBD1-dependent transcriptional repression. A, effect of GAL4-fused MBD, CXXC1 and 2, and the TRD on a reporter that contains GAL4-binding elements upstream of the human SNRPN gene promoter. Gray bars, MBD (Δ7); black bars, CXXC1 and 2 (Δ4); and hatched bars, TRD (Δ11). The luciferase activity in combination with the mock was normalized to 100. pCMV-PRL (0.02 μg) was used for a transfection control. We chose the use of the splice isoform v3 of MBD1, which most clearly exhibits DNA methylation-dependent gene silencing (12, 14). B, effect of GAL4-fused Suv39h1 on SNRPN and VHL promoter-driven luciferase vectors containing GAL4-binding sites. C, Suv39h1 represses transcription via MBD of MBD1. FLAG-Suv39h1 and GAL4-MBD1 (Δ7, Δ4, and Δ11) were cotransfected into HeLa cells, together with GAL4 binding motif-containing a luciferase vector. MBD1Δ7 (0.5 μg) and MBD1Δ11 (0.01 μg) were transfected to compare the effect of FLAG-Suv39h1 (0.2 and 0.4 μg). The luciferase activity in combination with FLAG mock was normalized to 100.
DISCUSSION

In this study, we report that Suv39h1 and HP1α interact with MBD1. Suv39h1 enhanced MBD1-mediated transcriptional repression via MBD but not via the C-terminal TRD of MBD1. Furthermore, MBD1 associated with histone deacetylases probably through Suv39h1 and MBD1 with endogenous HDAC1/HDAC2. HDACs were coprecipitated by FLAG-MBD1, but not by ΔN MBD1, from HeLa cells. D, effect of HDAC inhibitors on repression by MBD of MBD1. GAL4-fused MBD (Δ7) and TRD (Δ11) of MBD1 were expressed in HeLa cells in combination with the reporter containing the SNRPN gene promoter. Transfected cells were treated with HDAC inhibitors. The luciferase activity in combination with GAL4 mock was normalized to 100.

Several transcription repression complexes include the deacetylases and methylases of histone molecules (1–5, 7, 11). Hypermethylated DNA usually tends to coexist with hypoacetylated histones and methyl-H3 (Lys-9) on the heterochromatic regions. In fact, MeCP2 and MBD2 interact with a co-repressor complex Sin3 containing HDACs. MBD2-MBD3 heterodimer recruits another multifunctional complex, Mi2-NuRD, which possesses both HDAC and chromatin-remodeling activities. This combination of Mi2-NuRD and MBD2 may be synonymous with the originally designated MeCP1 complex. Nevertheless, trichostatin A has been found to partially relieve transcriptional repression by MeCP2 and MBD2. Furthermore, the retinoblastoma protein (Rb) represses E2F-mediated transcription through interaction not only with histone deacetylases but also with Suv39h1 (27–29). Recent reports have shown that MeCP2 blocks transcription both by recruiting HDACs or an unidentified histone methylase at the promoter (30). Our data demonstrated that MBD1 represses transcription together with Suv39h1-HP1α complexes. In agreement with this, both MBD1 and Suv39h1-HP1α are found in the pericentromeric heterochromatin, which includes H3 (Lys-9) methylation on the methylated DNA region (1, 14). Although H3 (Lys-9) is known to be methylated by Suv39h and G9a, certain histone methylases may be able to methylate the lysine 9. In fact, double knockouts of both Suv39h1 and h2 in mouse did not reduce the level of overall H3 (Lys-9) methylation (25). We do not exclude the possibility that MBD1 can cooperate with other histone methylases and chromatin regulators.

We have characterized the solution structure of MBD of MBD1 that binds a symmetrically methylated CpG dinucleotide, resulting in identification of the amino acid residues important for recognizing a methyl-CpG pair (12, 17). The lines of evidence suggest that MBD itself is actively involved in the interaction with target proteins.
for transcriptional repression. Furthermore, MBD1 is likely to establish and/or maintain the repressive state by recruiting HDAC1/HDAC2 as well as the Suv39h1-HP1α heterochromatin complex, suggesting that both methylation and deacetylation of histones coexist in the MBD1-containing chromatin on methylated DNAs. MBD1 would provide the chance for methylating core histones in the methylated DNA regions, as some MBD proteins connect cytosine methylation to histone deacetylation (7, 32). In other words, all cytosine methylation may not be simply downstream of histone methylation. The localization of Suv39h1, HP1α, and HDACs onto MBD1-containing methylated DNA sites may exemplify the possible presence of a repression pathway from DNA methylation to these histone modifications in heterochromatic

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