MBD3L1 Is a Transcriptional Repressor That Interacts with Methyl-CpG-binding Protein 2 (MBD2) and Components of the NuRD Complex

Received for publication, August 10, 2004, and in revised form, September 28, 2004
Published, JBC Papers in Press, September 28, 2004, DOI 10.1074/jbc.M409149200

Chun-Ling Jiang, Seung-Gi Jin, and Gerd P. Pfeifer‡

From the Division of Biology, Beckman Research Institute of the City of Hope, Duarte, California 91010

Methyl-CpG-binding domain proteins 2 and 3 (MBD2 and MBD3) are transcriptional repressors that contain methyl-CpG binding domains and are components of a CpG-methylated DNA binding complex named MeCP1. Methyl-CpG-binding protein 3-like 1 (MBD3L1) is a protein with substantial homology to MBD2 and MBD3, but it lacks the methyl-CpG binding domain. MBD3L1 interacts with MBD2 and MBD3 in vitro and in yeast two-hybrid assays. Gel shift experiments with a CpG-methylated DNA probe indicate that recombinant MBD3L1 can supershift an MBD2-methylated DNA complex. In vivo, MBD3L1 associates with and colocalizes with MBD2 but not with MBD3 and is recruited to 5-methylcytosine-rich pericentromeric heterochromatin in mouse cells. In glutathione S-transferase pull-down assays MBD3L1 is found associated with several known components of the MeCP1-NuRD complex, including HDAC1, HDAC2, MTA2, MBD2, RbAp46, and RbAp48, but MBD3 is not found in the MBD3L1-bound fraction. MBD3L1 enhances transcriptional repression of methylated DNA by MBD2. The data are consistent with a role of MBD3L1 as a methylated-dependent transcriptional repressor that may interchange with MBD3 as an MBD2-interacting component of the NuRD complex. MBD3L1 knockout mice were created and were found to be viable and fertile, indicating that MBD3L1 may not be essential or there is functional redundancy (through MBD3) in this pathway. Overall, this study reveals additional complexities in the mechanisms of transcriptional repression by the MBD family proteins.

Mammalian genomes encode several proteins that can bind specifically to methylated CpG sequences, providing one mechanism by which DNA methylation has the capacity to inhibit gene expression (1–3). The first methyl-CpG-binding protein discovered was MeCP2, a polypeptide that can bind selectively to a single methylated CpG site (4, 5). MeCP2 is associated with a transcriptional repressor complex containing Sin3 and histone deacetylases in vertebrate cells (6, 7). Recruitment of MeCP2 to a promoter leads to transcriptional repression that can be partially relieved by inhibitors of histone deacetylation (6, 7). Four additional genes, which encode methyl-CpG binding domain (MBD) proteins, were identified later (8). The five MBD-containing proteins (MBD1, MBD2, MBD3, MBD4, and MeCP2) are homologous within their MBD domains (1–3). MBD2 and MBD3 may be derived from a common ancestor and share >65% identity over most of the length of the smaller MBD3 protein. MBD4 is a G/T mismatch-specific DNA glycosylase functioning in DNA repair and may play a role in removing thymines derived from 5-methylcytosines by deamination (9).

MeCP2, MBD1, MBD2, and MBD3 function in gene silencing imposed by methylated DNA (6, 7, 10–16). MBD2 is highly similar to MBD3 except for the additional N-terminal glycine-arginine-rich region of 140 amino acids, which is not found in MBD3 (8). There are two translated protein products of MBD2 mRNA, MBD2a and MBD2b. MBD2b is the shorter version lacking the N-terminal 140 amino acids due to usage of a different initiation codon. The recombinant MBD2b protein has been reported to remove 5-methylcytosine residues from DNA through a DNA demethylase activity (17), although this finding has not been repeated yet by other laboratories (11, 16, 18). Transcriptional repression by MBD2 is, at least for certain promoters, sensitive to the histone deacetylase inhibitor trichostatin A (11). In cotransfection experiments, MBD2b increases transcriptional repression with methylated reporter constructs (16). Recent evidence indicates that MBD2b is the methyl CpG-binding protein of a larger complex named MeCP1, a methylated DNA binding complex identified earlier in the laboratory of Adrian Bird (11, 19, 20).

The coding sequence of MBD3 is highly similar to that of MBD2 (8). MBD3 mRNA is ubiquitously expressed in most mouse and human tissues. There are several splice variants of MBD3 including some that encode a non-functional MBD domain. Mammalian variants of MBD3 do not bind to methylated DNA in vitro (8, 18, 20, 21). However, *Xenopus* and zebrafish MBD3 proteins can bind to methylated DNA, most likely due to an amino acid difference in the MBD domain (1). NuRD/Mi-2 is a multisubunit repressor complex that contains nucleosome remodeling and histone deacetylase activities (22–25). The histone deacetylases HDAC1 and HDAC2 and the histone-binding proteins RbAp46 and RbAp48 form a core complex shared between the NuRD- and Sin3-histone deacetylase complexes. MBD3 was found to be a subunit of the NuRD complex, thus establishing another potential link between DNA methylation and transcriptional repression (20, 21, 23). The MeCP1 complex contains 10 major polypeptides including

‡ To whom correspondence should be addressed: Division of Biology, Beckman Research Institute of the City of Hope, 1500 E. Duarte Rd., Duarte, CA 91010. Tel.: 626-301-8853; Fax: 626-358-7703; E-mail: gpfeifer@coh.org.

* This work was supported by National Institutes of Health Grant CA104967. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The abbreviations used are: MBD, methyl-CpG binding domain; GST, glutathione S-transferase; DAPI, 4′,6′-diamidino-2-phenylindole; PBS, phosphate-buffered saline; CMV, cytomegalovirus; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; EGFP, epidermal growth factor.
MBD2 and all of the known NuRD components (26). Thus, NuRD may be an important mediator between DNA methylation and gene silencing. In mammals, the MBD3-containing NuRD complex can be targeted to methylated DNA by MBD2 (26).

Recently we have identified and cloned MBD3L1 and MBD3L2, two proteins with homology to MBD2 and MBD3 (27). MBD3L1 is expressed at low levels in pancreas and at higher levels in testis, specifically in round spermatids. Given their homology with MBD3/MBD2, it is likely that these two proteins may play an important role in transcriptional gene silencing or regulation of silencing and may be involved in pathways of transcriptional repression by CpG-methylated DNA. Here we have characterized the biological function of MBD3L1 using biochemical and genetic approaches.

**MATERIALS AND METHODS**

**Cell Culture and Transfection**—AD293, 293T, HeLa, NIH3T3, and COS7 cells were obtained from the American Type Culture Collection and were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% bovine serum and 5% CO2. COS7 cells were obtained from the American Type Culture Collection and were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% bovine serum and 5% CO2.

**Plasmids**—Most cDNA clones were obtained by reverse transcription-PCR and confirmed by DNA sequencing. For the yeast two-hybrid assay, full-length human MBD3L1, MBD2b, and MBD3 cDNA sequences were inserted in frame into yeast two-hybrid vectors. For expression of the recombinant proteins in *Escherichia coli*, the cDNAs of MBD3L1, MBD2, and MBD3 were subcloned into the pET28a His-tag vector (Novagen, Madison, WI). Full-length MBD3L1 was cloned into the pGEX-5X-1 GST expression vector (Amersham Biosciences). The deletion constructs of His-tagged MBD2b (1–82, 1–210, 27–82, and 201–262 amino acids) and GST-fused MBD3L1 (1–48, 1–103, 49–103, 49–146, 104–146, 141–194, and 161–194 amino acids) were created in the same vectors. For the expression of recombinant proteins in mammalian cells, full-length MBD3L1 was cloned into pEBG, a mammalian GST expression vector. The cDNAs of MBD3L1, MBD2a, and MBD3 were subcloned into pCMV-Tag1 (Stratagene, La Jolla, CA) in-frame with FLAG and Myc epitopes (FLAG-MBD3L1, FLAG-MBD2a, and Myc-MBD3) for in vitro binding assays. They were eluted from the beads with 250 mM of GST or GST-MBD3L1 protein and added to the cells and incubated in blocking buffer (PBS containing 1% bovine serum albumin and 0.2% Tween 20) for 1 h at room temperature. Rabbit anti-Myc (Santa Cruz, A-14; 2 μg/ml) was added in blocking buffer and incubated for 2 h at room temperature. Cells were washed 3 times with PBS containing 0.2% Tween 20 for 5 min each. Rhodamine-labeled goat-anti-mouse secondary antibody (Alexa Fluor 594; Molecular Probes) was added to the cells and incubated in blocking buffer for 1 h at room temperature followed by 3 washes as above. Nuclear counterstaining was performed with 0.2 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) in PBS for 5 min. The cells were washed five times with PBS, mounted with Fluoromount G (Southern Biotechnology Associates, Birmingham, AL), and visualized using a fluorescence microscope (Olympus IX81).

**Luciferase Assays**—We used pM1Luc (Roche Applied Science) as the luciferase reporter vector, which contains the firefly luciferase gene driven by a CMV promoter. pM1Luc was methylated in vitro using SssI DNA methylase as described by the manufacturer (New England Biolabs, Beverly, MA). 293T cells were seeded into 24-well plates at 2.2 × 10^5 per well one day before transfection. A total of 120 ng of plasmid DNA including 20 ng of reporter construct, 2.5 ng of internal control Renilla luciferase vector (pRL-CMV, Promega, Madison, WI), and different amounts of effector plasmids (from 20 to 100 ng) was used for each transfection using Lipofectamine-Plus reagent (Invitrogen) following the instructions of the manufacturer, and the firefly luciferase activities were normalized relative to Renilla luciferase expression.

**Gene Targeting**—The MBD3L1 gene-targeting vector was designed to replace exon 3 of the mouse MBD3L1 gene, which encodes the entire dithiothreitol (DTT) domain of MBD3L1. The DTT domain was made by high fidelity PCR with the proofreading-capable Pfu Turbo DNA polymerase (Stratagene) and 129/SvJmJ mouse ES cell DNA as the template. The PcG-neo cassette was flanked by 0.8 kilobases of upstream genomic MBD3L1 sequence and 5 kilobases of downstream sequence in the pKO Scranner V924 cloning vector (Lexicon Genetics). The resultant construct replaces exon 3 of MBD3L1 with the 1.8-kb PcG-neo cassette, leaving only the 7 N-terminal codons of the MBD3L1.
Characterization of MBD3L1

RESULTS

Among the family of human methyl-CpG binding domain proteins, MBD2 and MBD3 are the most closely related. We previously reported the identification of two mammalian homologues of MBD2 and MBD3, which we named MBD3L1 and MBD3L2 (27). MBD3L1 is expressed at low levels in pancreas and at higher levels in testis, specifically in post-meiotic haploid cells and has been shown to act as a transcriptional repressor (27). Both MBD3L1 and MBD3L2 lack the MBD domain but all four proteins contain a coiled-coil domain near the N-terminal glycine-arginine-rich repeat, which is absent from the MBD2a/b transcript, the primers were 5'-TCCAAGTGAGAAGGTTCAAGG-3' (upper) and 5'-CCAGGCTTCAATTGAGAAGG-3' (lower); for the MBD2b transcript, the primers were 5'-TCCCGCTCATAGAAAGGAGAC-3' (upper) and 5'-CGCCCTTGTGCAGAAAGGAGAC-3', respectively. For MBD3L1, the primers were 5'-CAGAAGAGAAGAGAGACCAGATTGTC-3' and 5'-CGTGGGTGAGAGAGACCAGATTGTC-3', and for MBD3L2, the primers were 5'-CAGAAGAGAAGAGAGACCAGATTGTC-3' and 5'-CGTGGGTGAGAGAGACCAGATTGTC-3'.

The PCR was carried out for 35 cycles at 98 °C for 45 s, 60 °C for 35 s, 72 °C for 5 min, and 72 °C for 5 min for 35 cycles (MBD3L1, MBD3L2, and MBD2) or 28 cycles (GAPDH). For MBD3, the PCR was carried out for 35 cycles at 98 °C for 45 s, 58 °C for 45 s, 72 °C for 60 s, and 72 °C for 5 min for 35 cycles (MBD3L1, MBD3L2, and MBD2) or 28 cycles (GAPDH). For MBD3, the PCR was carried out for 35 cycles at 98 °C for 45 s, 58 °C for 45 s, 72 °C for 60 s, and 72 °C for 5 min for 35 cycles (MBD3L1, MBD3L2, and MBD2) or 28 cycles (GAPDH).

RESULTS

Among the family of human methyl-CpG binding domain proteins, MBD2 and MBD3 are the most closely related. We previously reported the identification of two mammalian homologues of MBD2 and MBD3, which we named MBD3L1 and MBD3L2 (27). MBD3L1 is expressed at low levels in pancreas and at higher levels in testis, specifically in post-meiotic haploid cells and has been shown to act as a transcriptional repressor (27). Both MBD3L1 and MBD3L2 lack the MBD domain but all four proteins contain a coiled-coil domain near the C terminus (Fig. 1). The longer form of MBD2 contains an N-terminal glycine-arginine-rich repeat, which is absent from

Characterization of MBD3L1

RESULTS

Among the family of human methyl-CpG binding domain proteins, MBD2 and MBD3 are the most closely related. We previously reported the identification of two mammalian homologues of MBD2 and MBD3, which we named MBD3L1 and MBD3L2 (27). MBD3L1 is expressed at low levels in pancreas and at higher levels in testis, specifically in post-meiotic haploid cells and has been shown to act as a transcriptional repressor (27). Both MBD3L1 and MBD3L2 lack the MBD domain but all four proteins contain a coiled-coil domain near the C terminus (Fig. 1). The longer form of MBD2 contains an N-terminal glycine-arginine-rich repeat, which is absent from

Characterization of MBD3L1

RESULTS

Among the family of human methyl-CpG binding domain proteins, MBD2 and MBD3 are the most closely related. We previously reported the identification of two mammalian homologues of MBD2 and MBD3, which we named MBD3L1 and MBD3L2 (27). MBD3L1 is expressed at low levels in pancreas and at higher levels in testis, specifically in post-meiotic haploid cells and has been shown to act as a transcriptional repressor (27). Both MBD3L1 and MBD3L2 lack the MBD domain but all four proteins contain a coiled-coil domain near the C terminus (Fig. 1). The longer form of MBD2 contains an N-terminal glycine-arginine-rich repeat, which is absent from
the shorter form, MBD2b. A truncated form of MBD2, termed MBD2t, is expressed in testis. However, it is not clear if this isoform has a biological function.

The presence of coiled-coil domains in MBD2, MBD3, and MBD3L1 suggested that these proteins may have the potential to heterodimerize through this type of motif, which is often involved in protein-protein interactions. To test interactions between MBD3L1 and MBD2 and MBD3, we carried out a yeast two-hybrid analysis (Fig. 2A). The sequences were cloned into the GAL4 activation domain and GAL4 binding domain vectors, respectively. For MBD2, we used MBD2b as well as the testis-specific variant, MBD2t. Two-hybrid interactions were observed between MBD2b and MBD3, which confirms a previous report (29). Binding between MBD2 and MBD3 may form the basis for the recruitment of a larger transcriptional repressor complex containing MBD3 to methylated DNA, since mammalian MBD3 alone cannot bind to CpG-methylated DNA (1, 8, 21). When MBD3L1 was present in the activation domain vector, it interacted with MBD2b and MBD3 and with itself. However, when MBD3L1 was present in the DNA binding domain vector, a significant interaction was observed only with MBD3 and with itself (Fig. 2A). No interaction was seen between MBD3L1 and the testis-specific variant MBD2t.

We then tested if MBD3L1 can interact with MBD2 or...
MBD3L1 was expressed in E. coli. After binding of the protein to glutathione-Sepharose beads, the beads were incubated with recombinant His-tagged MBD3 or MBD2b. A significant interaction of MBD3L1 was observed with both proteins. To identify the domains in MBD3L1 that are required for interaction with MBD2, we incubated His-tagged MBD2b with GST-fused deletion constructs of MBD3L1 (Fig. 3). We found that two domains of MBD3L1 had the capacity to interact with MBD2b. These two domains included amino acids spanning the N-terminal part of the protein (amino acids 1–48 and amino acids 49–103). The data indicate that the coiled-coil domain present in MBD3L1 was not required for the interaction.

We also mapped the domains on MBD2b, which are required for binding to MBD3L1 and MBD3, respectively (Fig. 4). Since MBD2a is identical to MBD2b except for an N-terminal extension, the MBD3L1-binding domain(s) in MBD2a are expected to be identical to those in MBD2b. These two domains included amino acids spanning the N-terminal part of the protein (amino acids 1–48 and amino acids 49–103). The data indicate that the coiled-coil domain present in MBD3L1 was not required for the interaction.

We also mapped the domains on MBD2b, which are required for binding to MBD3L1 and MBD3, respectively (Fig. 4). Since MBD2a is identical to MBD2b except for an N-terminal extension, the MBD3L1-binding domain(s) in MBD2a are expected to be identical to those in MBD2b. The C-terminal region of MBD2b (amino acids 201–262, which forms the coiled-coil domain) was capable of binding to MBD3L1 in vitro (Fig. 4A). The same region of MBD2b was involved in binding to MBD3 (Fig. 4B). However, the fragment 1–210 (but not 1–82) also bound to MBD3. Thus, MBD3L1 and MBD3 bind to overlapping regions near the C terminus of MBD2 via its coiled-coil domain.

To obtain further evidence that MBD3L1 can interact with MBD2b, we conducted gel mobility shift assays with a CpG-methylated DNA probe (Fig. 5). MBD3L1 alone did not bind to methylated DNA. His-tagged MBD2b produced a gel-retarded complex with the methylated probe but not with the unmethylated probe. Upon the addition of recombinant MBD3L1 to the MBD2b reaction, several supershifted bands appeared in a concentration-dependent manner. The supershifted complexes contained MBD2 as shown by the addition of an anti-MBD2 antibody (Fig. 6).
These data show that MBD3L1 and MBD2 can interact and form a larger methylated-DNA binding complex. To further corroborate the observed interactions between MBD3L1 and MBD2, we analyzed the intracellular localization and co-localization of the EGFP- or Ds-RED-tagged proteins by immunofluorescence microscopy in NIH3T3 cells (Fig. 6). In mouse cells ~50% of all 5-methylcytosine is concentrated in major satellite DNA, which is organized in foci of constitutive heterochromatin and stains brightly with Hoechst 33258 or DAPI (30). These sequences have been shown to attract several of the methyl-CpG-binding proteins in transfected cells (8). The MBD2a protein (and also MBD2b, data not shown) displayed a speckled nuclear localization pattern and co-localized with DAPI-bright areas. On the other hand, MBD3L1 showed a mostly diffuse nuclear localization pattern when transfected alone (Fig. 6). However, when MBD3L1 and MBD2a were co-transfected, MBD3L1 formed intense bright spots that co-localized with MBD2a-DsRED and with DAPI-bright areas. This localization pattern was dependent on the level of EGFP-MBD3L1 expression in individual cells. At low levels of expression, there was only weak co-localization of MBD3L1 with MBD2, but at higher expression levels, the two proteins co-localized to DAPI-bright spots. The data indicate that MBD3L1 can adopt the nuclear localization pattern of MBD2 when the two proteins are co-expressed.

**FIG. 8. Enhancement of MBD2-dependent transcriptional repression by MBD3L1. A, unmethylated promoter. B, methylated promoter.** The CMV-promoter luciferase plasmid was methylated at all CpG sites using SssI DNA methylase. This vector was transfected into AD293 cells along with expression vectors encoding MBD2a (20 ng) and different amounts of MBD3L1 or its N-terminal domain (MBD3L1-N). Co-expression of full-length MBD3L1 leads to an enhancement of MBD2a-mediated repression.
The MBD2 protein has been shown to be a component of a larger complex, termed MeCP1, which consists of the Mi2-NuRD complex and MBD2. NuRD is involved in nucleosome remodeling and transcriptional repression and contains histone deacetylases and histone-binding proteins (18, 20, 23, 24). To determine whether MBD3L1 associates with components of this complex in vivo, we transfected a GST-tagged MBD3L1 construct into AD293 cells and carried out GST pull-down experiments. Western blot analysis was used to determine the possible association of several endogenous proteins with GST-MBD3L1. We found that MBD3, p66, and Sin3 were not associated with MBD3L1, and only weak binding was seen for Mi2 (Fig. 7). The proteins specifically retained by GST-MBD3L1 construct into AD293 cells and carried out GST pull-down experiments. Western blot analysis was used to determine the possible association of several endogenous proteins with GST-MBD3L1. We found that MBD3, p66, and Sin3 were not associated with MBD3L1, and only weak binding was seen for Mi2 (Fig. 7). The proteins specifically retained by GST-MBD3L1 were MTA2 (with weak binding of MTA1), HDAC1, HDAC2, RbAp46, RbAp48, and MBD2. The data confirm that MBD3L1 and MBD2 interact in vivo. They also suggest that the in vitro and yeast two-hybrid interactions seen for MBD3L1 and MBD3 (Fig. 2) are not relevant in vivo since MBD3 was undetectable in the MBD3L1 pulled-down fraction. The interaction of MBD3L1 with HDAC1, HDAC2, RbAp46, RbAp48, MTA2, and MBD2 indicates that MBD3L1 is associated with a form of the MeCP1 complex.

To test the possible interplay between MBD2 and MBD3L1 in transcriptional repression, we carried out transcriptional repression assays. We conducted repression assays with CpG-methylated and unmethylated luciferase reporter genes (Fig. 8). We used the firefly luciferase gene under control of the CMV promoter. When this construct was methylated at CpG sites by using SssI DNA methylase, the expression of the reporter gene was strongly (~11-fold) reduced 48 h after transfection (data not shown). When an MBD2a expression vector was co-transfected, this activity was further reduced (Fig. 8B), indicating that MBD2a can mediate repression from a CpG-methylated promoter. No effect of MBD2a co-expression was observed with the unmethylated promoter (Fig. 8A). Notably, the repression by MBD2a on the methylated promoter was efficiently in-
creased when MBD3L1 was co-transfected ($p = 0.038$; Wilcoxon signed rank test; two-tailed) (Fig. 8B). An MBD3L1 deletion construct containing only the N-terminal 103 amino acids, shown to promote interaction with MBD2 (Fig. 3), enhanced MBD2a repression only marginally ($p = 0.31$; Wilcoxon signed rank test), suggesting that the C-terminal part of MBD3L1 is also required for the repression effect. The data suggest that MBD3L1 can enhance MBD2-mediated repression at CpG-methylated promoters.

As an important tool to study the function of MBD3L1, we generated MBD3L1 knockout mice. Almost the entire exon 3, which presents 100% of the MBD3L1-coding sequence, was replaced with a neo cassette leaving only the seven N-terminal codons of the MBD3L1 gene intact (Fig. 9). The targeted ES cell clones were injected into blastocysts. After germ line transmission, heterozygous mice were bred, and homozygous mice were obtained at the expected Mendelian ratio. In Fig. 9C, the lack of expression of MBD3L1 in testis of homozygous mutant mice was confirmed, but levels of MBD2, MBD2d, and MBD3 mRNAs were unaffected. Homozygous MBD3L1 knockout mice were viable and fertile and have shown no adverse health effects after 8 months of observation.

**DISCUSSION**

We have characterized the MBD3L1 protein, a homologue of MBD2 and MBD3, as a transcriptional repressor. We show that MBD3L1 is a transcriptional repressor and is associated with specific components of the NuRD complex. This complex contains histone deacetylase activity. MBD3L1 repression was sensitive to inhibitors of histone deacetylases, but this effect was promoter-dependent (data not shown). The interaction between MBD3L1 and MBD2 was confirmed by yeast two-hybrid assays, by *in vitro* binding assays, by *in vivo* pull-down assays, and by co-localization studies. The expression of MBD3L1 is tissue-specific, and MBD3L1 mRNA levels were detectable by Northern blot analysis only in pancreas and testis (27). The major form of MBD2 mRNA present in testis tissue is an alternative splice variant, named MBD2a, which lacks the C-terminal part of MBD2a/b (8). This C-terminal domain of MBD2 is required to interact with MBD3L1 (Fig. 4). However, the full size MBD2 mRNA, which is present in most somatic tissues, is also present in testis of mice and humans at about one-third the level of MBD2b mRNA. Given the restricted expression of MBD3L1, *in vivo* interactions of MBD3L1 and MBD2 are likely to be highly tissue-selective.

In contrast to MBD3L1, MBD2L2, the closest homologue of MBD3L1, interacted preferentially with MBD3 rather than with MBD2. Thus, a picture emerges in which several hetero-oligomeric (and possibly homo-oligomeric) complexes can form between the four related proteins, MBD2, MBD3, MBD3L1, and MBD3L2. The functional significance of each of these interactions will need to be investigated further. All four proteins operate as transcriptional repressors in assays where they are recruited to promoters through a heterologous (GAL4) DNA binding domain. Here these proteins are recruited *in vivo* to promoters has not yet been determined, except for MBD2, for which its association with CpG methylated DNA has been shown to result in transcriptional repression (11, 14, 16). MBD3, as a component of the NuRD complex, also is apparently involved in repression of methylated DNA. However, this can only be one of the functions of MBD3, since MBD2 and MBD3 appear to have different characteristics. The two proteins are localized to different areas of the nucleus where MBD2 is attracted to 5-methylcytosine-rich heterochromatin, but MBD3 is not. The phenotype of knockout mice for the two proteins is very different. Mbd3<sup>−/−</sup> mice die during early embryogenesis, whereas Mbd2<sup>−/−</sup> mice are viable and fertile (15).

It is also of note that we did not find evidence for specific binding of the Mi2 and p66 proteins in the MBD3L1-pulled down fractions (Fig. 7). It is becoming recognized that there are different sub-forms of the NuRD complex (31). Every subunit of this complex exhibits heterogeneity at the protein or gene level. For example, the reported complexes differ in their composition with respect to whether they contain MTA1 or MTA2 (20, 22, 32). Thus, specific NuRD complexes may differ also with respect to whether they contain MBD3L1 or not.

We have shown that MBD3L1 can increase repression mediated by MBD2a acting on a CpG-methylated promoter (Fig. 8). Both MBD3L1 and MBD3 and can bind to the C-terminal portion of the MBD2 protein (Fig. 4). This data would suggest a model in which MBD2, through its C-terminal domain, may interact either with MBD3 or with MBD3L1 (Fig. 10). Both complexes may be involved in methylation-mediated repression. The two complexes may be functionally redundant as suggested by the lack of an obvious phenotype of MBD3L1 knockout mice (due to the presence of MBD3). However, the situation is likely to be more complex since MBD2 knockout mice, in which the binding partner of MBD3L1 has been deleted, are also viable and fertile (15). In future work, the gene targets for MBD3L1-induced transcriptional silencing as well as the mechanisms of this pathway will need to be determined. Our data are consistent with a role of MBD3L1 as a methylation-dependent transcriptional repressor that can interact with MBD2 and can interchange with MBD3 as a component of the NuRD complex.

**Acknowledgments**—We thank Piroska E. Szabó, Ahmad Besaratinia, and Tibor Rauch for discussions.

**REFERENCES**

1. Wade, P. A. (2001) *Oncogene* **20**, 3166–3173
2. Bird, A. P., and Wolffe, A. P. (1999) *Cell* **99**, 451–454
3. Hendrich, B., and Bird, A. (2000) *Curr. Top. Microbiol. Immunol.* **249**, 55–74
4. Lewis, J. D., Meehan, R. R., Henzel, W. J., Maurer-Fogy, I., Jeppesen, P., Klein, F., and Bird, A. (1992) *Cell* **69**, 905–914
5. Nan, X., Meehan, R. R., and Bird, A. (1993) *Nucleic Acids Res.* **21**, 4886–4892
6. Nan, X., Ng, H. H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N., and Bird, A. (1998) *Nature* **393**, 386–389

![Model of MBD3L1 function.](Image 347x532 to 533x737)

FIG. 10. Model of MBD3L1 function. The data suggest that MBD3L1 is a component of the MeCP1 CpG-methylated DNA binding complex. Because MBD3L1 interacts with MBD2 but not MBD3 in *in vivo* and MBD3L1 and MBD3 bind to overlapping regions on the MBD2 protein, we suggest that MBD3L1 can take the place of MBD3 and may comprise a special form of the MeCP1 complex.  

---

*a* C.-L. Jiang and G. P. Pfeifer, unpublished results.  
*b* S.-G. Jin and G. P. Pfeifer, unpublished results.
Characterization of MBD3L1

7. Jones, P. L., Veenstra, G. J., Wade, P. A., Vermaak, D., Kass, S. U., Landesberger, N., Strousoulis, J., and Wolfe, A. P. (1998) Nat. Genet. 19, 187–191
8. Hendrich, B., and Bird, A. (1998) Mol. Cell. Biol. 18, 6538–6547
9. Hendrich, B., Hardeland, U., Ng, H. H., Jiricny, J., and Bird, A. (1999) Nature 401, 301–304
10. Nan, X., Campoy, F. J., and Bird, A. (1997) Cell 88, 471–481
11. Ng, H.-H., Zhang, Y., Hendrich, B., Johnson, C. A., Turner, B. M., Erdjument-Bromage, H., Tempst, P., Reinberg, D., and Bird, A. (1999) Nat. Genet. 23, 58–61
12. Fujita, N., Takebayashi, S., Okumura, K., Kudo, S., Chiba, T., Suya, H., and Nakao, M. (1999) Mol. Cell. Biol. 19, 6415–6426
13. Ng, H. H., Jeppesen, P., and Bird, A. (2000) Mol. Cell. Biol. 20, 1394–1406
14. Yu, F., Thiesen, J., and Stratling, W. H. (2000a) Nucleic Acids Res. 28, 2201–2206
15. Hendrich, B., Guy, J., Ramsahoye, B., Wilson, V. A., and Bird, A. (2001) Genes Dev. 15, 710–723
16. Boeke, J., Ammerpohl, O., Kegel, S., Moehren, U., and Renkawitz, R. (2000) J. Biol. Chem. 275, 34963–34967
17. Bhatnacharya, S. K., Ramchandani, S., Cervoni, N., and Szyf, M. (1999) Nature 397, 579–583
18. Wade, P. A., Gegonne, A., Jones, P. L., Ballestar, E., Aubry, F., and Wolfe, A. P. (1999) Nat. Genet. 23, 62–66
19. Meehan, R. R., Lewis, J. D., McKay, S., Kleiner, E. L., and Bird, A. P. (1989) Cell 58, 499–507
20. Zhang, Y., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Bird, A., and Reinberg, D. (1999) Genes Dev. 13, 1924–1935
21. Saito, M., and Ishikawa, F. (2002) J. Biol. Chem. 277, 35434–35439
22. Xue, Y., Wong, J., Moreno, G. T., Yeung, M. K., Cote, J., and Wang, W. (1998) Mol. Cell 2, 851–861
23. Wade, P. A., Jones, P. L., Vermaak, D., and Wolfe, A. P. (1998) Curr. Biol. 8, 843–846
24. Zhang, Y., LeRoy, G., Seelig, H. P., Lane, W. S., and Reinberg, D. (1998) Cell 95, 279–289
25. Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998) Nature 395, 917–921
26. Feng, Q., and Zhang, Y. (2001) Genes Dev. 15, 827–832
27. Jiang, C. L., Jin, S. G., Lee, D. H., Lan, Z. J., Xu, X., O’Connor, T. R., Szabo, P. E., Mann, J. R., Cooney, A. J., and Pfeifer, G. P. (2002) Genomics 80, 621–629
28. Kiefer, S. M., McDill, B. W., Yang, J., and Rauchman, M. (2002) J. Biol. Chem. 277, 14869–14876
29. Tatematsu, K. I., Yamazaki, T., and Ishikawa, F. (2000) Genes Cells 5, 677–688
30. Miller, O. L., Schnedl, W., Allen, J., and Erlanger, B. F. (1974) Nature 251, 636–637
31. Bowen, N. J., Fujita, N., Kajita, M., and Wade, P. A. (2004) Biochim. Biophys. Acta 1677, 52–57
32. Yao, Y. L., and Yang, W. M. (2003) J. Biol. Chem. 278, 42560–42568
MBD3L1 Is a Transcriptional Repressor That Interacts with Methyl-CpG-binding Protein 2 (MBD2) and Components of the NuRD Complex
Chun-Ling Jiang, Seung-Gi Jin and Gerd P. Pfeifer

J. Biol. Chem. 2004, 279:52456-52464.
doi: 10.1074/jbc.M409149200 originally published online September 28, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M409149200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 32 references, 10 of which can be accessed free at http://www.jbc.org/content/279/50/52456.full.html#ref-list-1