Effects of Rett Syndrome Mutations of the Methyl-CpG Binding Domain of the Transcriptional Repressor MeCP2 on Selectivity for Association with Methylated DNA

Esteban Ballestar,[‡,§] Timur M. Yusufzai,[§,||] and Alan P. Wolffe*[,‡]

Laboratory of Molecular Embryology, National Institute of Child Health and Human Development, National Institutes of Health, Building 18T, Room 106, Bethesda, Maryland 20892-5431, and Department of Genetics, The George Washington University, 2121 I Street NW, Washington, D.C. 20052

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ABSTRACT: We have investigated the properties of mutant forms of the methyl-CpG binding transcriptional repressor MeCP2 associated with Rett syndrome, a childhood neurodevelopmental disorder. We find that four Rett syndrome mutations at known sites within the methyl-CpG binding domain (MBD) impair binding to methylated DNA, but have little effect on nonspecific interactions with unmethylated DNA. Three of these mutations (R106W, R133C, and F155S) have their binding affinities for methylated DNA reduced more than 100-fold; this is consistent with the hypothesis that impaired selectivity for methylated DNA of mutant MeCP2 contributes to Rett syndrome. However, a fourth mutant, T158M, has its binding affinity for methylated DNA reduced only 2-fold, indicative either of additional distinct regulatory functions associated with the MBD or of an exquisite sensitivity of developing neurons to the selective association of MeCP2 with methylated DNA.

Rett syndrome (*1*) is a childhood neurodevelopmental disorder and one of the most common causes of mental retardation in females, with an incidence of 1 in 10000−15000 (*2*). Rett syndrome patients are characterized by a period of normal growth and development (6−18 months) followed by regression with loss of speech and purposeful hand use. Patients also develop seizures, autism, and ataxia. After initial regression, the condition stabilizes and patients survive into adulthood. Studies of familial cases provided evidence that Rett is caused by X-linked dominant mutations in a gene subject to X-chromosome inactivation. Recently, a number of mutations in the gene encoding the methyl-CpG binding transcriptional repressor MeCP2 have been associated with Rett syndrome (*3*, *4*).

MeCP2 is the archetypical methyl-CpG binding protein defined in vertebrates (*5*) and is representative of a family of proteins containing similar methyl-CpG binding domains (MBDs). CpG methylation, the major modification of vertebrate genomes, is associated with transcriptional repression (*6*−*8*), and it has been implicated in stable alterations of gene expression in development. Microinjection of methylated and unmethylated templates into *Xenopus* oocytes and mammalian tissue culture cells indicates that chromatin assembly is necessary for the herpes simplex virus thymidine kinase and *Xenopus* heat shock protein 70 promoters to establish selective transcriptional repression on methylated DNA under conditions where unmethylated templates remain active (*9*, *10*). Inactive chromatin assembled on methylated DNA can also confer transcriptional silencing and nuclease resistance on adjacent unmethylated promoters (*10*, *11*). These results demonstrate that chromatin structure and function are sensitive to DNA methylation. DNA methylation can repress transcription through multiple mechanisms (*6*, *8*, *10*, *12*, *13*). Pathways of repression include direct inhibition of transcription through the failure of transcription factors to associate with methylated recognition elements and indirect pathways involving either occlusion of methylated sequences by transcriptional repressors that recognize methylated DNA or the modification of chromatin structure targeted by methyl-CpG specific transcriptional repressors (*9*, *10*). All these mechanisms may operate in vivo; however, attention has been focused on chromatin modification because the C-terminal transcription domain of MeCP2 (*14*) can interact with Sin3A and recruit histone deacetylase to repress transcription (*15*, *16*).

MeCP2 contains two defined domains: a methyl-CpG binding domain (MBD) that has about 84 amino acids and binds specifically to methylated DNA and a transcriptional repression domain (TRD) of about 102 amino acids (*14*). The solution structure of the MBD has recently been determined (*17*, *18*) and consists of a wedge-shaped structure with four antiparallel β-strands constituting one face of a wedge, of which the two longer β-strands are proposed to interact with the major groove of DNA, where a methylated CpG pair would be located.

In this work, we approach the issue of the consequences of the Rett syndrome mutations within the MBD of MeCP2 on its selective binding to methylated DNA. It has been hypothesized that MBD mutations would impair specific selectivity for methylated DNA. Although our results are in agreement with the above hypothesis, surprisingly, we

* Corresponding author. Phone: (301) 402-2722. Fax: (301) 402-1323. E-mail: awlme@helix.nih.gov.
[‡] National Institutes of Health.
[§] These authors made equal contributions to this work.
[||] The George Washington University.

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observe that one of the mutations does not impair substantially selectivity for methylated DNA. Since patients harboring that mutation present standard Rett syndrome, we propose that additional functions might be shared by the MBD.

MATERIALS AND METHODS

Construction of Mutations. Point mutations in *Xenopus* MeCP2 (*15*) were introduced by PCR, and the mutant MeCP2s were subsequently subcloned into the *Eco*RI site of pET21a, in frame with the C-terminal poly-His region of the vector (*15*, *19*).

Recombinant Protein Purification. Recombinant wild-type and mutant His-tagged MeCP2 were expressed in *Escherichia coli* BL21(DE3) (*19*). One liter of Luria-Bertani broth (LB) was inoculated with 5 mL of an overnight culture and incubated at 37 °C to an A_{600} of 0.7. Induction was performed by addition of isopropyl β-thiogalactosidase to a final concentration of 1 mM and incubation at 37 °C for an additional 3 h. Cells were harvested and resuspended in 10 mL of sonication buffer [20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 5 mM imidazole, 0.1% Nonidet P-40 (NP-40), and 1 mM 2-mercaptoethanol]. Purification of the soluble His-tagged protein fractions was carried out with TALON resin (Clontech) according to the manufacturer's protocol, with the elution carried out with 100 mM imidazole. Fractions were assayed for protein content by SDS−PAGE, and dialyzed against 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 1 mM 2-mercaptoethanol, and 2 mM $MgCl_2$. Quantitation of the preparation was carried out using the Bio-Rad protein assay and SDS−PAGE analysis.

Gel Mobility Shift Assays. Gel mobility shifts were performed in 10% polyacrylamide gels carried out in 0.5× TBE buffer [45 mM Tris (pH 8.0), 45 mM boric acid, and 1 mM EDTA]. GAC12, GAM1, or GAM12 double-stranded oligonucleotides were used as probes. These 42 bp oligonucleotide duplexes (see ref *20*) contain 12 CpG repeats which either are unmethylated in the case of GAC12, contain one methylated repeat in GAM1, or have all of them methylated in GAM12. One picomole of radiolabeled probe was mixed with different amounts (20−300 ng) of recombinant wild-type or mutant MeCP2 as indicated in the figure legends in 10 mM Tris-HCl (pH 8.0), 3 mM $MgCl_2$, 50 mM NaCl, 0.1 mM EDTA, 0.1% NP-40, 2 mM DTT, 5% glycerol, and 0.4 mg/mL BSA. The samples were incubated for 30 min at 37 °C. Either GAC12 or GAM12 was used as a competitor. Thirty or sixty picomoles of competitor DNA was added to the mixture containing GAM12 or GAM1, respectively. Gels were scanned on a Molecular Dynamics phosphorimager, and the percentage of bound probe was calculated. GraphPad Prism software was used to estimate dissociation constants.

Southwestern Assay. The procedure followed is based on the protocol described in ref *5*. Wild-type and mutant proteins were resolved by 10% SDS−PAGE and transferred at 20 V overnight to PVDF membranes in a Bio-Rad electroblotter. After transfer, immobilized proteins were denatured for 5 min in 6 M guanidine hydrochloride, 20 mM HEPES (pH 7.5), 3 mM $MgCl_2$, 40 mM KCl, and 10 mM 2-mercaptoethanol, followed by four successive 2-fold dilutions of the denaturing buffer with binding buffer (denaturing buffer without guanidine HCl). After two further washes with binding buffer, the filter was preblocked for 10 min with 2% nonfat dried milk in binding buffer and washed again with binding buffer. The filters were incubated for 1 h at room temperature in the presence of ^{32}P-labeled probe (GAC12, GAM1, or GAM12), in binding buffer (about 2×10^6 cpm mL^{-1}), together with 0.1% Triton X-100 and nonspecific competitor DNA (20 μg/mL native *E. coli* DNA, or 2 μg/mL denatured *E. coli* DNA). Finally, the filters were washed four times in binding buffer supplemented with 0.01% Triton X-100. After being air-dried, the filters were exposed to X-OMAT film. After Southwestern assay, filters were probed with MeCP2 antibodies.

Circular Dichroism. Spectra in the far-UV region (195−240 nm) were recorded in a J-710 JASCO spectropolarimeter. Spectra were obtained at a protein concentration of ∼0.2 mg/mL in cells with a 0.5 mm optical path. The scan speed was 10 nm/min, the resolution 0.1 nm, and the sensitivity 20 mdeg. The average of four runs was expressed in molar ellipticities in units of degrees per square centimeter per decimole of residue.

RESULTS AND DISCUSSION

Rett Syndrome and Mutations in MeCP2. Our experimental approach has consisted of preparing point mutations of MeCP2, according to those recently described as being associated with Rett syndrome (*3*, *4*). These mutations are found in both the methyl-CpG binding domain (MBD) and transcription repression domain (TRD) of MeCP2 (Figure 1A; *14*, *20*). To analyze the in vitro ability of recombinant mutant proteins to bind methylated DNA, we have initially focused on point mutations within the MBD, in particular R106W, R133C, F155S, and T158M (see ref *3*). All of the mutated residues are conserved from human to *Xenopus* MeCP2 (Figure 1B), although only three of these residues (R106, R133, and F155) are conserved in other MBD-containing proteins (*21*, *22*). T158 is found in MeCP2, but is not present in human MBD1, human MBD2, or *Xenopus* MBD3 (*21*, *22*).

The different Rett mutations we have analyzed are all found at potentially key sites within the MBD, either for interaction with DNA or for the integrity of the domain (see Figure 5). R106W, where the basic amino acid arginine is replaced with tryptophan, is located in the second β-sheet of the MBD, which is proposed to run along the major groove of the DNA near the methylated CpG (*18*). In R133C, a cysteine is substituted for an arginine located between the fourth β-sheet and the α-helix domain, which are close to the phosphate backbone of the DNA. The last two mutations (F155S and T158M) are substitutions of hydrophobic amino acids with polar amino acids and are placed in the hairpin loop (*17*). It has been proposed that these hydrophobic residues play a role in stabilizing the orientation of the hairpin relative to the rest of the molecule (*17*).

Qualitative Analysis of Selectivity for Methylated DNA of Rett Syndrome Mutations of MeCP2. We subcloned cDNAs encoding the different MBD mutants of MeCP2 into a pET21a vector, induced protein expression, and purified recombinant proteins from *E. coli*. We first compared the binding properties of these four MBD mutants to that of the wild-type protein by Southwestern analysis. To qualitatively determine the ability of the Rett syndrome MBD mutations

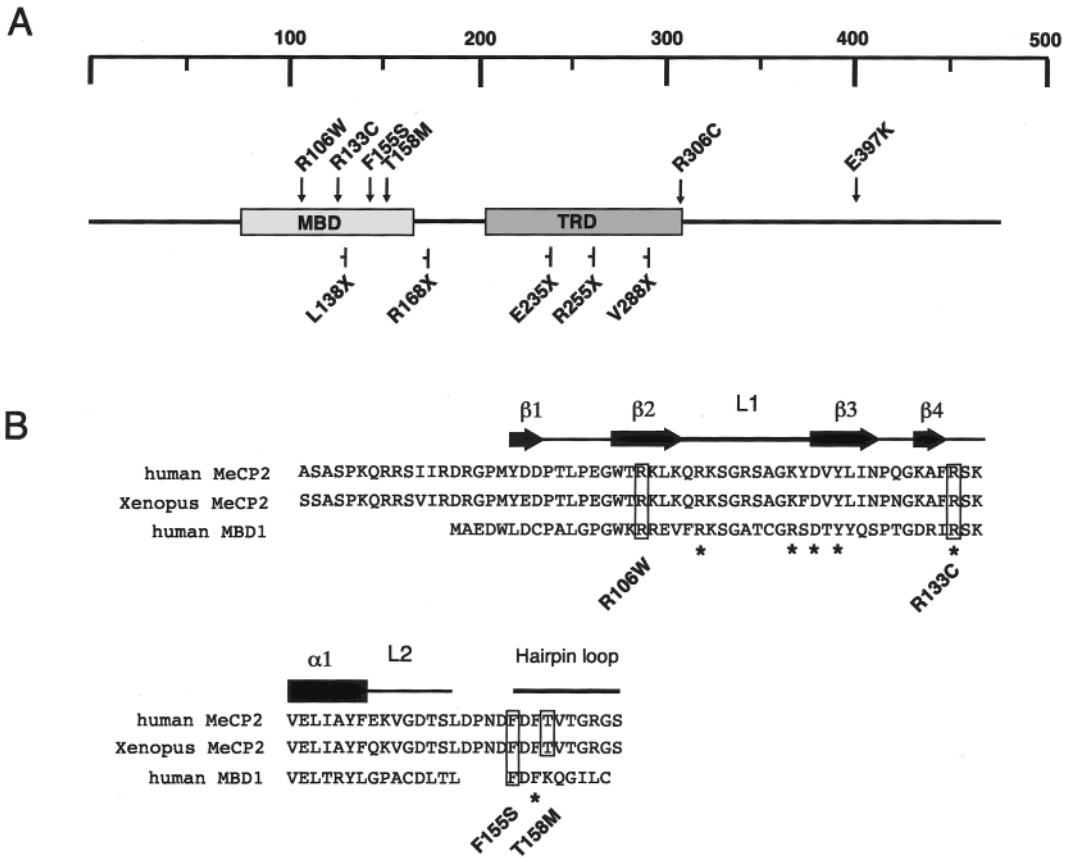

FIGURE 1: (A) Representation of MeCP2 with all the Rett mutations described to date (refs *3* and *4*). MBD stands for methyl binding domain, whereas TRD stands for transcription repression domain. Point mutations are indicated by an arrow. Deletions are indicated by a segment. (B) Sequence alignment of the MBD of human and *Xenopus* MeCP2 and MBD1 (adapted from ref *17*). The secondary structure is indicated at the top. Arrows represent β-sheet segments, and an α-helix is represented as a rectangle. Loops appear as thick lines. Sequence comparison of the methyl-CpG binding domain of human and *Xenopus* MeCP2 and MBD1. The four Rett mutations analyzed in this work are indicated by an open rectangle. Point mutations within the MBD of MBD1 (see ref *17*) that result in loss of specificity for methylated DNA are indicated with an asterisk.

to bind methylated DNA, we electroblotted equal amounts of protein to PVDF membranes and incubated them with radiolabeled oligonucleotides (Southwestern assay). Probes were 42 bp in length and had 12 CpG repeats that were either nonmethylated (GAC12), methylated at one CpG (GAM1), or methylated at all 12 CpGs (GAM12).

It has previously been shown that MBD is able to bind one single methylated CpG pair (*19*, *20*). The issue of the effects of DNA methylation density on MeCP2 DNA binding is important, because if it is possible to alter the efficiency of Rett syndrome mutant forms of MeCP2 to bind to DNA by altering the number of methylated CpGs in the genome then this provides a potential approach to therapy. Our Southwestern analysis (Figure 2) reveals a dramatic reduction in the ability to bind methylated DNA for Rett syndrome mutants R106W, R133C, and F155S compared to that of the wild type. The different behavior between wild-type MeCP2 and the Rett syndrome mutants is enhanced as more methyl-CpGs are present in the probe (compare GAM12 and GAM1). These results are quantitated in Figure 2B. All four of the MBD mutant forms of MeCP2 have a very low level of association with unmethylated DNA as seen with the wild-type protein (Figure 2A, GAC12). Remarkably, T158M exhibits an even lower ability to bind unmethylated DNA. Nan et al. (*20*) have shown that the MBD flanking regions are involved in nonspecific binding to DNA. T158 is very close to the C-terminal side of the MBD. A change in the structure of this region could also affect nonspecific interactions with DNA, explaining the decrease in the level of binding to unmethylated DNA.

Also in marked contrast to the other Rett syndrome mutants, T158M retains the capacity to bind selectively to methylated DNA, although with a lower apparent affinity than that of wild-type MeCP2 (Figure 2 and see Figures 4 and 5). Interestingly, T158 is a residue that is not conserved through the MBD family. In fact, MBD1 (Figure 1B) and other proteins such as MBD2 or MBD3 have a basic residue at this site within the MBD instead. Our results indicate that T158 is not essential for conferring selectivity for association with methylated DNA.

Circular Dichroism Spectra of Wild-Type and Rett Syndrome Mutant Forms of MeCP2. To compare the degree of structural change between the wild-type and the Rett syndrome mutant forms of MeCP2, CD spectra were recorded. The analysis of the far-UV (Figure 3) and near-UV regions (not shown) indicated that no major changes occur. However, slight changes in secondary structure were observed for all four mutants. R106W, R133C, and F155S exhibited a similar decrease in the percentage of organized secondary structure. Changes in T158M were more subtle; however, changes in structure in T158M may well account for the 2−3-fold decrease in the affinity for methylated DNA.

Quantitative Analysis of DNA Binding Properties of Rett Syndrome Mutations of MeCP2. We next determined the

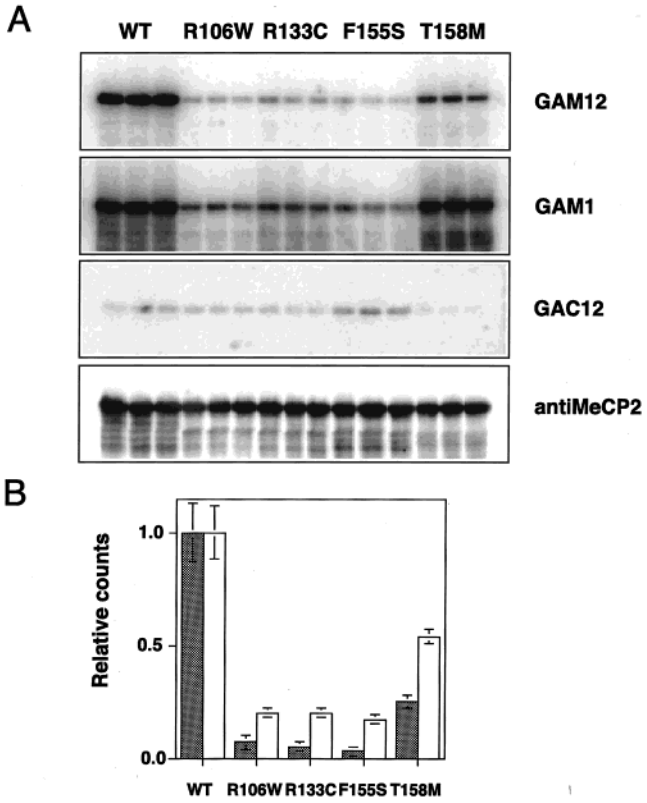

FIGURE 2: Rett mutants of MeCP2 lose partially or totally the ability to bind selectively methylated DNA. (A) The top three panels depict Southwestern assays performed with recombinant *Xenopus laevis* wild-type MeCP2 and different Rett syndrome point mutations: (top) probed with GAM12, (middle) probed with GAM1, and (bottom) probed with unmethylated DNA (GAC12). Filters were probed with 2×10^6 cpm/mL (approximately 5 pmol/mL) of probe (see Materials and Methods). After Southwestern assays, the filters were also probed with antiMeCP2 antibodies (antiMeCP2). (B) Quantitation of the results depicted in panel A. The ratio of the counts per minute in each lane for the Southwestern assays to the signal from the Western assay is represented (gray bars, GAM12; white bars, GAM1). Every column corresponds to the average of three lanes.

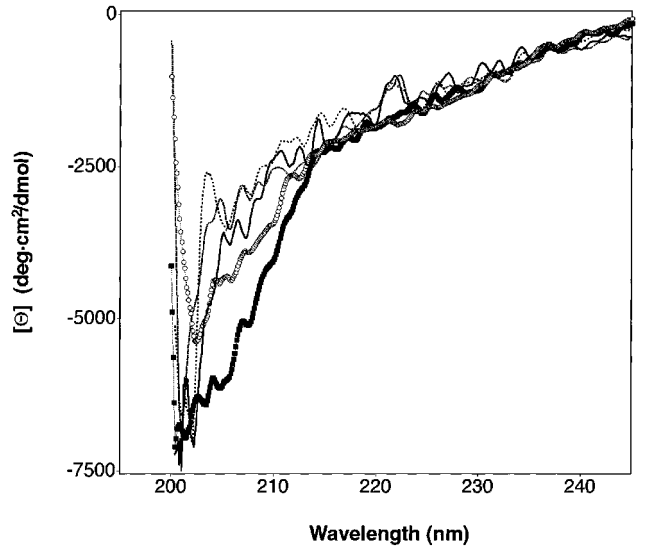

FIGURE 3: Far-UV circular dichroism spectra of recombinant *X. laevis* wild-type MeCP2 (■), R106W (−), R133C (•••), F155S (- - -), and T158M (○).

DNA binding properties of Rett syndrome mutations of MeCP2 using a more quantitative analysis. Wild-type MeCP2

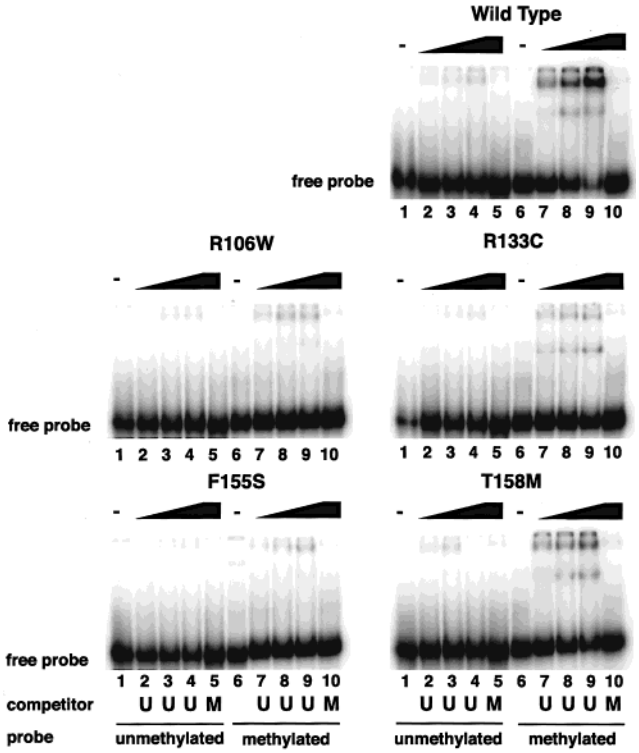

FIGURE 4: Wild-type and Rett mutants of MeCP2 (see ref *3*) were examined for the ability to bind to methylated DNA probes (GAM12 or GAC12). Binding reactions were performed as described in Materials and Methods. Lanes 1−5 of each gel contained radiolabeled GAC12 as a probe, and lanes 6−10 contained radiolabeled GAM12 as a probe. For each gel, lanes 1 and 6 contained only the probe without any added MeCP2. Lanes 2 and 7 contained 50 ng of MeCP2; lanes 3 and 8 contained 75 ng of MeCP2, and lanes 4, 5, 9, and 10 contained 150 ng of MeCP2. Binding was carried out with either GAC12 or GAM12 as the competitor (U, unmethylated GAC12; M, methylated GAM12).

and Rett mutant forms of MeCP2 were incubated with unmethylated and methylated probes, and the complexes were resolved on nondenaturing polyacrylamide gels using GAM1 (data not shown) and GAM12 (Figure 4). In our binding experiments, samples contain a certain amount of unlabeled competitor DNA (GAC12) for competition in nonspecific interactions between MeCP2 and the labeled probe. Since the affinity of the MBDs for GAM12 is higher than that for GAM1, we found that we could also use a larger amount of competitor DNA with the GAM12 probe than the amount used for the GAM1 probe. Under these conditions, the component of nonspecific interactions is minimized for the GAM12 samples.

All MBD mutants, including T158M, exhibit a reduced affinity for methylated DNA. In fact, with the exception of the wild-type MeCP2 and T158M, selectivity for methylated DNA was so greatly reduced in the Rett syndrome mutants that it was not possible to saturate the free DNA probe under our experimental conditions (see below). Under these conditions, significant nonspecific binding to DNA also occurs. It has been suggested (*20*) that regions of MeCP2 flanking the MBD are primarily responsible for that nonspecific binding. Mutations in the MBD do not alter that component, and therefore, the mutants would be expected to retain significant nonspecific binding to unmethylated probes (see Figure 2A). The gel shift assay also reveals this to be true for T158M (Figure 4).

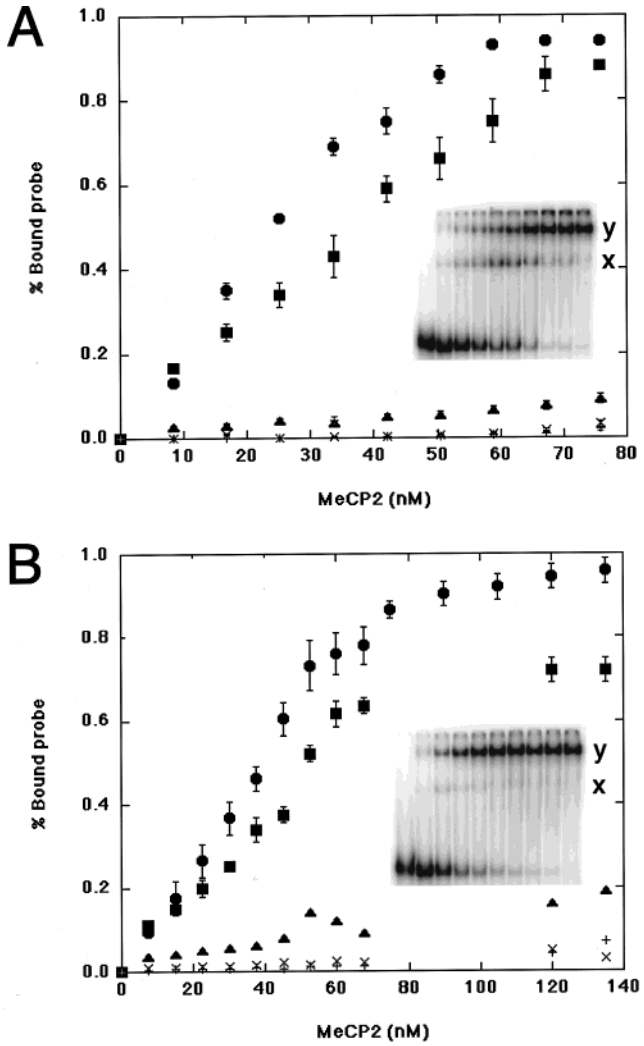

FIGURE 5: Saturation plots for GAM12 (A) and GAM1 (B). A saturation curve is plotted, where the percentage of bound probe is plotted vs MeCP2 concentration. Titrations were performed for wild-type and Rett mutants of MeCP2. The right bottom inset in each graph shows a gel as an example where the probe is titrated with 3.3, 6.6, 9.9, 13.2, 16.5, 19.8, 23.1, 26.4, and 29.7 ng of wild-type MeCP2 (A) and 6.6, 13.2, 19.8, 26.4, 33, 39.6, 46.2, and 52.8 and 3.3, 6.6, 9.9, 13.2, 16.5, 19.8, 23.1, 26.4, and 29.7 ng of wild-type MeCP2 (B): wild-type MeCP2 (●), T158M (■), R133C (▲), F155S (+), and R106W (×).

To estimate the dissociation constants for the different mutants, a titration with increasing amounts of protein was carried out. Figure 5 shows the saturation plots for wild-type MeCP2 and the different MBD mutants binding to the GAM12 and GAM1 probes. These plots were the average of three independent experiments with each of the protein samples. Only wild-type MeCP2 and T158M were able to achieve saturation of the probe. Again, the affinity was higher for GAM12 than for GAM1. Gels were scanned on a Molecular Dynamics phosphorimager, and the percentage of bound probe was used to estimate dissociation constants with GraphPad Prism software. Under our conditions, wild-type MeCP2 has a dissociation constant of approximately 2×10^{-8} M for GAM12 and of about 4×10^{-8} M for GAM1. T158M also has a dissociation constant in the same range, although the binding affinities have been reduced approximately by 2-fold compared to that of the wild type in the case of GAM12, and about 1.5-fold in the case of GAM1.

The patterns of nucleoprotein complexes resolved for GAM1, with only one symmetrically methylated CpG and GAM12, with 12 methylated CpG repeats were the same. In both cases, two discrete complexes appeared. Since MeCP2 is able to bind a single methylated CpG, one should expect to observe more bands as the number of methylated CpG dinucleotides in the oligonucleotide probes increases. Thus, the GAM12 probe would be expected to bind more MeCP2 molecules than the GAM1 probe. This is not observed either because the binding of one MeCP2 molecule to DNA occludes access to other sites (*19, 20*) or because incorporation into the complex of a second molecule of MeCP2 is driven primarily by protein−protein interactions. Further analysis will resolve the nature of these complexes.

Structural Implications of Rett Syndrome Mutations of MeCP2. The recent description of the solution structure of the MBD allows the location of the Rett syndrome mutations in their structural context. These data come from two independent reports (*17*, *18*) within which NMR data of the MBD and the results of chemical shift perturbation with DNA lead to similar models of the structure of this domain and its interaction with methylated DNA. Ohki et al. (*17*) determined the structure of the MBD of the human methylation-dependent repressor MBD1, while Wakefield et al. (*18*) determined the structure of the MBD of MeCP2. Both reports propose a wedge-shaped structure where an α/β-sandwich, with four antiparallel β-sheets, constitutes a face of the wedge, while the other face is formed by a C-terminal α-helix (Figure 5). Ohki et al. (*17*) have also identified a C-terminal hairpin loop, similar to a β-hairpin. Although the sequences from MBD1 and MeCP2 exhibit only a moderate degree of homology, sequences can easily be aligned with a number of conserved residues throughout the MBD (see Figure 1B). The current model proposes that the interaction between MBD and methylated DNA takes place along the major groove of a standard B-form DNA. The two longer β-sheet strands (β2 and β3), as well as the loop between them (L1), would interact with the major groove of the DNA. Also, the residues between β4 and α1 seem to establish contacts with the phosphate backbone (see Figure 6 for a model).

We find that R106W and F155S have lost almost completely any selective affinity for methylated DNA. The side chains of R106 and F155 (*18*) are located in the hydrophobic core of MBD and are predicted to be essential for the integrity of the structure and selectivity of interactions with methylated DNA. The guanidyl moiety of R106 is exposed to the solvent on the helical face, where it may contact the phosphodiester backbone and contribute to nonspecific protein−DNA interactions. Our data show that substitution of that Arg for a Trp results in a dramatic decrease in the level of specific binding to methylated DNA. Ohki et al. (*17*) and Wakefield et al. (*18*) proposed that the two central β-sheets of the MBD run along the major groove of the DNA. R106 is located in the middle of the second β-sheet. Ohki et al. (*17*) have performed a mutational analysis of the MBD (Figure 1B) and established that substitutions along the third β-sheet result in total loss of selectivity for association with methylated DNA. Our results indicate that the R106W mutation may lead to similar consequences. A slight change in secondary structure may be responsible for the loss of affinity for methylated DNA.

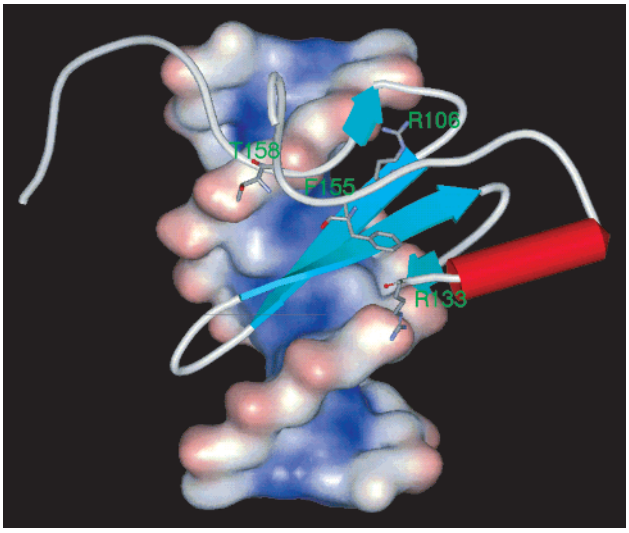

FIGURE 6: Model for the interaction of MBD of MeCP2 and methylated DNA. The four Rett mutations analyzed in this study are shown. To construct this model, the coordinates for the MBD of MeCP2 and the CpG helix were used (PDB accession numbers 1qk9 and 329G, respectively).

A partial disruption of the structure of the MBD is the most likely explanation for the loss of selective binding to methylated DNA in the case of F155. This residue is located in the C-terminal hairpin loop of the MBD. This C-terminal hairpin loop contains structurally essential hydrophobic residues. Ohki et al. (*17*) investigated the binding properties of a mutant of MBD1, F64A, which is equivalent to a F157A substitution in MeCP2. Unlike the other mutants in the MBD they prepared (see Figure 1), F64A yielded an aberrant structure and exhibited a total loss of specific binding to methylated DNA. We propose that a similar behavior may occur when F155 of MeCP2 is mutated, since this residue is also located in the same hydrophobic core and selectivity for association with methylated DNA is eliminated with mutation F64A of MBD1. In fact, F155S shows also changes in structure (see Figure 3). The F155 and F157 amino acids of MeCP2 are conserved at comparable positions among all the other members of the MBD family (*15*, *21*, *22*).

R133C of MeCP2 still retains very low selectivity for methylated DNA. This residue of MeCP2 is equivalent in position to R44 of MBD1. A substitution of this residue to an alanine, also studied by Ohki et al. (*17*), resulted in a reduction of the properties of binding of MBD1 to methylated DNA. We have also observed a slight change in secondary structure for this mutation.

The most unanticipated result from our analysis is that the replacement of threonine 158 by methionine allows the mutant MBD to retain selectivity for methylated DNA, although the mutation reduces the overall affinity 2-fold. Amir et al. (*3*) predicted that changes in both F155 and T158 might disrupt the structure of the MBD. This is the case for mutation F155S; however, the T158M mutant retains selectivity for binding methylated DNA that is very similar to that of wild-type MeCP2. We have also observed that the change in secondary structure is less important than the one observed for the other three mutant forms of MeCP2 (see Figure 3). Unlike the other residues, T158 is not conserved among members of the MBD family (see, for instance, MBD1 in Figure 1). The properties of the T158M mutant form of MeCP2 provide some insight into the MBD itself and potentially Rett syndrome. None of the other proteins from the MBD family possess a threonine at the mutant position. The fact that T158 is only present within the MBD of MeCP2 and is not essential for conferring selectivity for methylated DNA, together with its essential role for MeCP2 function, is especially interesting. T158 is in fact one of the most common mutations of MeCP2 found in Rett syndrome patients (*3*, *4*). It could be that the reduction in the affinity of T158M that we have observed in our analysis is enough to prevent or diminish physiological binding of MeCP2 to methylated DNA in vivo. A 2-fold reduction in absolute binding affinities makes this prospect unlikely (Figure 5A,B). Although the T158 residue is located within the MBD of MeCP2, it might be involved in other roles related to the specific function of MeCP2. One possibility might be the involvement of this residue in transcriptional repression through the recruitment of corepressor complexes or through contact with the basal transcriptional machinery. A precedent exists for this with the nuclear hormone receptors where the DNA binding domain can contribute directly to corepressor recruitment through contacts with histone deacetylases (*23*). Other mutations involved in a Rett syndrome phenotype have recently been described (*4*), some of which occur solely in the TRD. These observations also implicate the recruitment of corepressors as a key aspect of MeCP2 function. Future experiments will explore the interaction of the Rett syndrome MBD mutant T158 with the SIN3 corepressor complex (*15*, *16*) as well as the DNA binding and transcriptional repression properties of the Rett syndrome TRD mutants. The binding properties of the mutant forms in the context of chromatin (*19*) comprise another interesting issue to study in estimating the effect in a more physiological context.

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