determined by mechanisms dependent on intracortical distance, center and surround interactions, and cortical feedback.

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Derepression of BDNF Transcription Involves Calcium-Dependent Phosphorylation of MeCP2

Wen G. Chen,[1,2] Qiang Chang,[3] Yingxi Lin,[1] Alexander Meissner,[3,4] Anne E. West,[1] Eric C. Griffith,[1] Rudolf Jaenisch,[3,4] Michael E. Greenberg[1,2]*

Mutations in *MeCP2*, which encodes a protein that has been proposed to function as a global transcriptional repressor, are the cause of Rett syndrome (RTT), an X-linked progressive neurological disorder. Although the selective inactivation of MeCP2 in neurons is sufficient to confer a Rett-like phenotype in mice, the specific functions of MeCP2 in postmitotic neurons are not known. We find that MeCP2 binds selectively to *BDNF* promoter III and functions to repress expression of the *BDNF* gene. Membrane depolarization triggers the calcium-dependent phosphorylation and release of MeCP2 from *BDNF* promoter III, thereby facilitating transcription. These studies indicate that MeCP2 plays a key role in the control of neuronal activity–dependent gene regulation and suggest that the deregulation of this process may underlie the pathology of RTT.

Methylation of DNA in vertebrates occurs preferentially on cytosine residues that occur in dinucleotides in which the cytosine residues are followed by guanine residues (CpGs). Methylated CpGs bind a variety of proteins (*1*). One of these proteins, the methyl-CpG binding protein 2 (MeCP2), has been implicated in long-term silencing of gene expression (*2–5*). Inactivating mutations in the *MeCP2* gene cause the majority of cases of Rett syndrome (RTT) (*6–8*), a human X-linked disorder characterized by arrested neurological development and subsequent cognitive decline (*9–11*). MeCP2 is abundantly expressed within the central nervous system, where its expression is highly enriched in postmitotic neurons (*12–14*). Although biochemical evidence is consistent with MeCP2 functioning as a global silencer of gene transcription (*3*), microarray analyses have failed to detect significant gene derepression in brains of *Mecp2* mutant mice (*15*). This finding, as well as the strong expression of MeCP2 in mature neurons and the neuronal phenotype of RTT patients, raised the possibility that MeCP2 may function as a selective regulator of neuronal gene expression.

Activity-dependent transcription underlies the ability of the nervous system to convert the effects of transient stimuli into long-term changes in brain function. Two classes of genes are regulated by neuronal activity–dependent calcium influx (*16*): immediate-early gene (IEG)–encoded transcription factors that mediate cellular responses to extracellular stimuli and genes selectively expressed in the nervous system that directly regulate neuronal development and synaptic plasticity. Of the neural-selective activity-dependent genes, that encoding brain-derived neurotrophic factor (BDNF) is among the most extensively studied. BDNF plays important roles in neuronal survival (*17*), development (*18*), and plasticity (*19*). *BDNF* is highly expressed in neurons, and its transcription is up-regulated dramatically by membrane depolarization in vitro (*18*, *20*, *21*) and by neuronal activity during kindling, induction of long-term potentiation (LTP), and associative learning (*22–24*). BDNF is encoded by a complex gene with four well-characterized promoters that give rise to at least eight different mRNAs (*25*) (Fig. 1A). Calcium influx through L-type voltage-sensitive calcium channels (L-VSCCs) activates *BDNF* promoter III, an effect that requires the action of at least three transcriptional activators [calcium-response factor, CaRF; upstream stimulatory factors, USFs; and calcium/cyclic adenosine monophosphate (cAMP)–responsive element binding protein, CREB] that bind to enhancer elements in promoter III (*21*, *26–28*). However, activation of CREB precedes the onset of transcription from *BDNF* promoter III (*21*), raising the possibility that a repressive mechanism may need to be relieved before the activation of transcription. The mechanisms by which *BDNF* transcription is repressed and then activated upon membrane depolarization are not yet clear. In this study, we report that, in the absence of neuronal activity, MeCP2 binds specifically to *BDNF* promoter III and functions as a negative regulator of *BDNF* expression. In response to neuronal activity–dependent calcium influx into neurons, MeCP2 becomes phosphorylated and is released from the *BDNF* pro-

[1]Division of Neuroscience, Children's Hospital, [2]Program in Biological and Biomedical Sciences, Harvard Medical School, Boston, MA 02115, USA. [3]Whitehead Institute for Biomedical Research, [4]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142, USA.

*To whom correspondence should be addressed. E-mail: Michael.Greenberg@tch.harvard.edu

moter, thereby permitting *BDNF* promoter III–dependent transcription.

We used a chromatin immunoprecipitation (ChIP) assay to investigate the association of endogenous MeCP2 with *BDNF* promoters. Chromatin (sonicated to fragments of about 100 to 200 base pairs) was prepared from cultured neurons, incubated with an antibody specific for MeCP2, and immunoprecipitated as described in (*29*). Immunoprecipitated *BDNF* promoter sequences were detected by PCR amplification with primer pairs corresponding to *BDNF* exon I to exon IV as well as *BDNF* promoter III. Among the regions of the *BDNF* gene examined, MeCP2 antibody chromatin immunoprecipitates were found to be most highly enriched with the *BDNF* promoter III sequence immediately 5′ to the transcription initiation site (Figs. 1B and 2A). This enrichment represents a specific association of MeCP2 with the *BDNF* promoter III, because preimmune immunoglobulin G (IgG) failed to immunoprecipitate *BDNF* promoter III sequences (Fig. 2A). In addition, several distinct antibodies against MeCP2 immunoprecipitated *BDNF* promoter III sequences. The specificity of the antibodies against MeCP2 was demonstrated by Western blotting (*29*). The binding of MeCP2 to *BDNF* promoter III appears not to reflect the nonspecific binding of MeCP2 to all DNA sequences, because ChIP analysis failed to detect an association of MeCP2 with the *BDNF* coding sequence (exon V) or with the promoter of the *c-fos* gene. We conclude that, in cultured neurons in the absence of neuronal activity, MeCP2 is specifically bound to *BDNF* promoter III.

Membrane depolarization of cortical neurons by exposure to an increased concentration of extracellular KCl induced release of MeCP2 from *BDNF* promoter III as assessed by ChIP analysis (Fig. 2A). This effect was specific to MeCP2, because the interaction of *BDNF* promoter III with USF1, a transcriptional activator for *BDNF* promoter III (*27*), was not altered by membrane depolarization. The release of MeCP2 was specific to *BDNF* promoter III, because the interaction of MeCP2 with the imprinted gene *H19* (*30*) was not affected by membrane depolarization of cortical neurons (Fig. 2A). We tested whether the release of MeCP2 from *BDNF* promoter III correlated with the ability of extracellular stimuli to induce transcription from this promoter. BDNF stimulation of neurons effectively induces the transcription of IEGs such as *c-fos* but does not induce robust

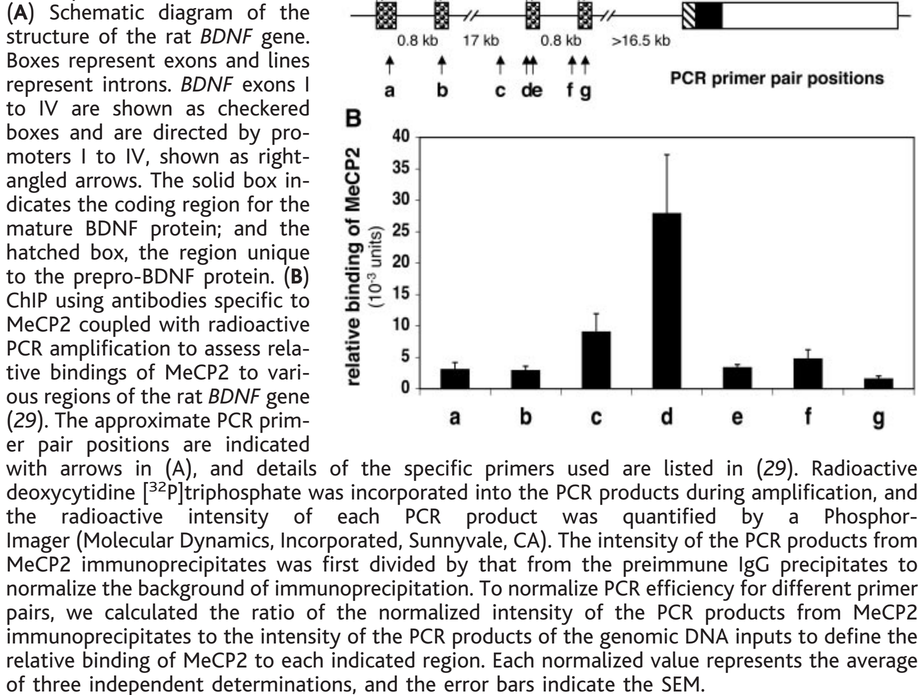

Fig. 1. MeCP2 associates with *BDNF* promoter III in neurons. (**A**) Schematic diagram of the structure of the rat *BDNF* gene. Boxes represent exons and lines represent introns. *BDNF* exons I to IV are shown as checkered boxes and are directed by promoters I to IV, shown as right-angled arrows. The solid box indicates the coding region for the mature BDNF protein; and the hatched box, the region unique to the prepro-BDNF protein. (**B**) ChIP using antibodies specific to MeCP2 coupled with radioactive PCR amplification to assess relative bindings of MeCP2 to various regions of the rat *BDNF* gene (*29*). The approximate PCR primer pair positions are indicated with arrows in (A), and details of the specific primers used are listed in (*29*). Radioactive deoxycytidine [^{32}P]triphosphate was incorporated into the PCR products during amplification, and the radioactive intensity of each PCR product was quantified by a PhosphorImager (Molecular Dynamics, Incorporated, Sunnyvale, CA). The intensity of the PCR products from MeCP2 immunoprecipitates was first divided by that from the preimmune IgG precipitates to normalize the background of immunoprecipitation. To normalize PCR efficiency for different primer pairs, we calculated the ratio of the normalized intensity of the PCR products from MeCP2 immunoprecipitates to the intensity of the PCR products of the genomic DNA inputs to define the relative binding of MeCP2 to each indicated region. Each normalized value represents the average of three independent determinations, and the error bars indicate the SEM.

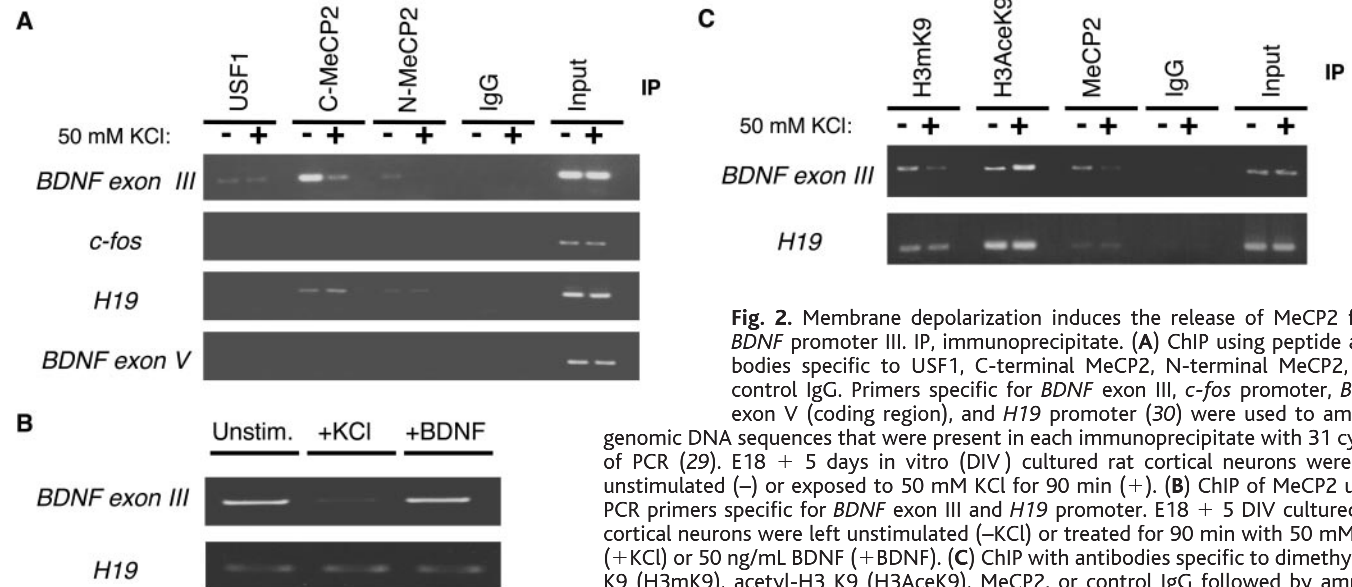

Fig. 2. Membrane depolarization induces the release of MeCP2 from *BDNF* promoter III. IP, immunoprecipitate. (**A**) ChIP using peptide antibodies specific to USF1, C-terminal MeCP2, N-terminal MeCP2, and control IgG. Primers specific for *BDNF* exon III, *c-fos* promoter, *BDNF* exon V (coding region), and *H19* promoter (*30*) were used to amplify genomic DNA sequences that were present in each immunoprecipitate with 31 cycles of PCR (*29*). E18 + 5 days in vitro (DIV) cultured rat cortical neurons were left unstimulated (–) or exposed to 50 mM KCl for 90 min (+). (**B**) ChIP of MeCP2 using PCR primers specific for *BDNF* exon III and *H19* promoter. E18 + 5 DIV cultured rat cortical neurons were left unstimulated (–KCl) or treated for 90 min with 50 mM KCl (+KCl) or 50 ng/mL BDNF (+BDNF). (**C**) ChIP with antibodies specific to dimethyl-H3 K9 (H3mK9), acetyl-H3 K9 (H3AceK9), MeCP2, or control IgG followed by amplification with PCR primers specific for *BDNF* exon III and *H19* promoter. Neurons were either unstimulated (–) or exposed to 50 mM KCl for 90 minutes (+). The specificity of each antibody was assessed by Western blot analysis (*29*).

transcription from *BDNF* promoter III (*31*). Likewise, BDNF stimulation did not lead to release of MeCP2 from *BDNF* promoter III (Fig. 2B).

MeCP2 mediates long-term gene silencing, in part, by recruitment of histone-modifying enzymes such as deacetylases and methyltransferases (*2*, *3*, *32*). We therefore considered the possibility that MeCP2 might regulate promoter III activation by altering chromatin architecture at the *BDNF* gene. ChIP analysis revealed that, before membrane depolarization, *BDNF* promoter III–associated histone H3 (H3) was strongly methylated at Lys^9 (K9) (Fig. 2C), a modification that on the *H19* gene is correlated with gene inactivation (*33*) and facilitated by MeCP2 (*32*). Furthermore, ChIP analysis of *BDNF* promoter III sequences showed reduced methylation of H3 K9 and concurrent increases in acetylation at the same residue within 90 min of membrane depolarization, consistent with the observed release of MeCP2 from the *BDNF* promoter at the same time. The release of MeCP2 from the *BDNF* gene in response to membrane depolarization may allow the assembled transcriptional activator complex to remodel local chromatin structure through the acetylation of specific histone residues, thereby promoting *BDNF* transcription.

To test the idea that MeCP2 functions as an activity-regulated repressor of *BDNF* promoter III–dependent transcription, we compared the amount of *BDNF* exon III–containing mRNA transcripts in cultured neurons from wild-type or MeCP2 knockout mice (*29*). Before membrane depolarization, *BDNF* exon III–containing mRNA levels were significantly higher in the MeCP2-deficient cultures than in the cultures isolated from wild-type mice ($n = 6$ pairs of animals, $P < 0.05$) (Fig. 3). By contrast, under conditions of membrane depolarization that release MeCP2 from *BDNF* promoter III and effectively activate *BDNF* transcription, the amounts of *BDNF* exon III–containing transcripts were similar in neurons prepared from wild-type and MeCP2-deficient mice. This increase in *BDNF* transcripts in MeCP2-deficient neurons before membrane depolarization appears not to reflect a general increase in gene expression under these conditions, because the basal level of expression of the activity-regulated gene *c-fos* was similar when MeCP2-deficient and wild-type neurons were compared. We conclude from these experiments that one function of MeCP2 is to suppress *BDNF* promoter III–dependent transcription in neurons in the absence of neuronal activity.

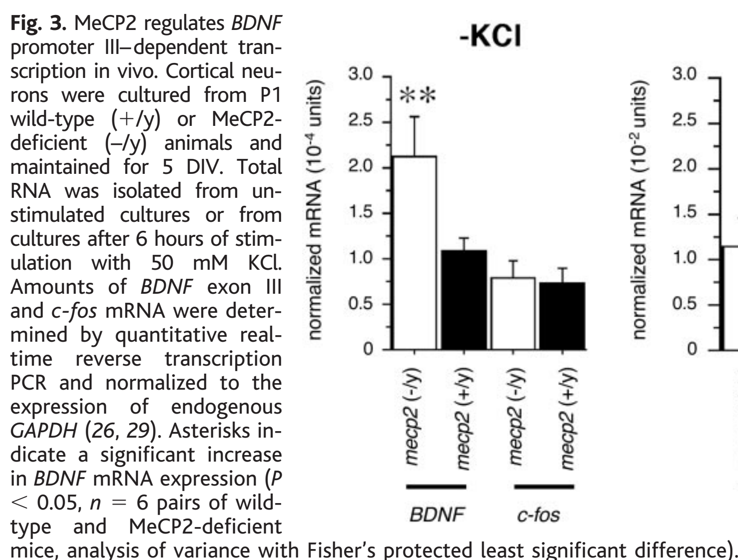

Fig. 3. MeCP2 regulates *BDNF* promoter III–dependent transcription in vivo. Cortical neurons were cultured from P1 wild-type (+/y) or MeCP2-deficient (–/y) animals and maintained for 5 DIV. Total RNA was isolated from unstimulated cultures or from cultures after 6 hours of stimulation with 50 mM KCl. Amounts of *BDNF* exon III and *c-fos* mRNA were determined by quantitative real-time reverse transcription PCR and normalized to the expression of endogenous *GAPDH* (*26*, *29*). Asterisks indicate a significant increase in *BDNF* mRNA expression ($P < 0.05$, $n = 6$ pairs of wild-type and MeCP2-deficient mice, analysis of variance with Fisher's protected least significant difference).

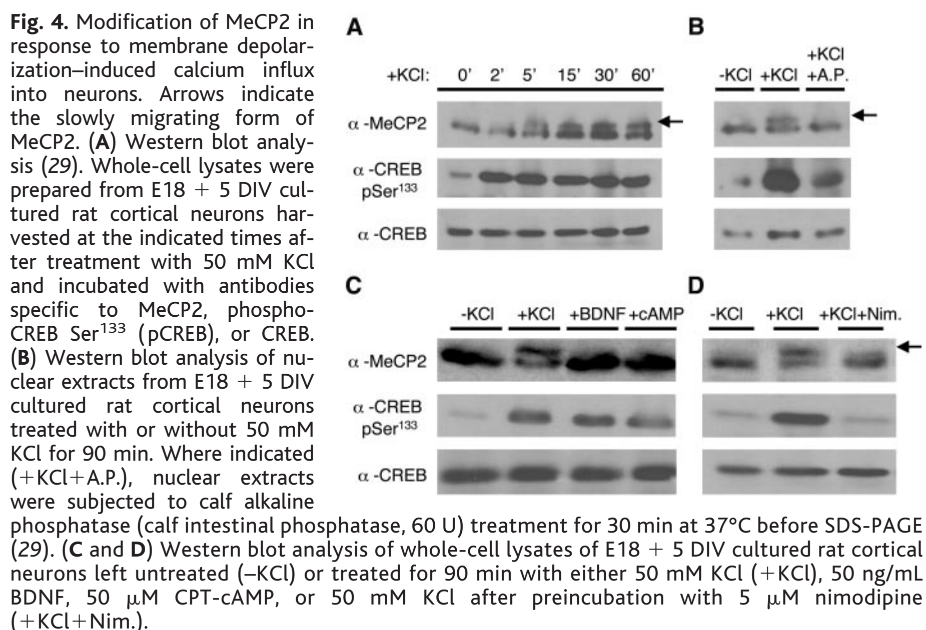

Fig. 4. Modification of MeCP2 in response to membrane depolarization–induced calcium influx into neurons. Arrows indicate the slowly migrating form of MeCP2. (**A**) Western blot analysis (*29*). Whole-cell lysates were prepared from E18 + 5 DIV cultured rat cortical neurons harvested at the indicated times after treatment with 50 mM KCl and incubated with antibodies specific to MeCP2, phospho-CREB Ser^{133} (pCREB), or CREB. (**B**) Western blot analysis of nuclear extracts from E18 + 5 DIV cultured rat cortical neurons treated with or without 50 mM KCl for 90 min. Where indicated (+KCl+A.P.), nuclear extracts were subjected to calf alkaline phosphatase (calf intestinal phosphatase, 60 U) treatment for 30 min at 37°C before SDS-PAGE (*29*). (**C** and **D**) Western blot analysis of whole-cell lysates of E18 + 5 DIV cultured rat cortical neurons left untreated (–KCl) or treated for 90 min with either 50 mM KCl (+KCl), 50 ng/mL BDNF, 50 μM CPT-cAMP, or 50 mM KCl after preincubation with 5 μM nimodipine (+KCl+Nim.).

We investigated the mechanism by which membrane depolarization–induced calcium influx into neurons triggers the release of MeCP2 from *BDNF* promoter III. We considered the possibility that membrane depolarization might trigger the post-translational modification of MeCP2 so that MeCP2 no longer interacts with *BDNF* promoter III. Western blotting of neuronal lysates with antibodies to MeCP2 (*29*) revealed that within minutes of membrane depolarization a previously unknown form of MeCP2 was present that migrated more slowly during SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 4A). The more slowly migrating form of MeCP2 appeared to be a result of phosphorylation, because alkaline phosphatase treatment of nuclear extracts from membrane-depolarized neurons caused the disappearance of this MeCP2 species (Fig. 4B) (*29*). The phosphorylation of MeCP2 occurs more slowly than does phosphorylation of CREB at Ser^{133}. CREB Ser^{133} phosphorylation is detected as early as 1 min after membrane depolarization (*34*, *35*) and is critical for the induction of *BDNF* promoter III–dependent transcription (*21*, *28*). In contrast, MeCP2 phosphorylation was not maximal until 15 to 30 min after membrane depolarization. Given that the initiation of transcription from *BDNF* promoter III also lags membrane depolarization by at least 30 min (*21*), the similarly delayed kinetics of the MeCP2 modification suggest that MeCP2 phosphorylation may be required to relieve repression of *BDNF* promoter III, allowing for transcriptional activation.

Further consistent with this possibility, we found that treatment of neurons with agents such as cAMP and BDNF that fail to induce *BDNF* promoter III–dependent transcription (*31*) also failed to induce MeCP2 phosphorylation, even though these agents led to phosphorylation of CREB at Ser^{133}

(Fig. 4C). By contrast, membrane depolarization induces both MeCP2 phosphorylation (Fig. 4A) and *BDNF* promoter III–dependent transcription (Fig. 3). Membrane depolarization of cultured neurons induces *BDNF* promoter III–dependent transcription by a process that requires influx of calcium through L-VSCCs (*21*). Likewise, we found that calcium influx through L-VSCCs contributed to the induction of MeCP2 phosphorylation. Incubation of cells with the L-VSCC antagonist nimodipine blocked the ability of membrane depolarization to induce the appearance of the slowly migrating species of MeCP2 (Fig. 4D). Taken together, these experiments demonstrate that the calcium-dependent modification of MeCP2 correlates with *BDNF* promoter III activation, raising the possibility that the phosphorylation of MeCP2 is required for release of MeCP2 and activation of *BDNF* promoter III.

Because MeCP2 phosphorylation appears to correlate with its release from *BDNF* promoter III, we sought to test directly whether membrane depolarization-induced phosphorylation of MeCP2 affects the binding of MeCP2 to *BDNF* promoter III. Toward this end, we first identified CpG sequences within *BDNF* promoter III that are methylated and therefore capable of binding MeCP2. We examined the methylation status of the *BDNF* promoter III by sodium bisulfite genomic-modification sequencing (*29*). This procedure results in the conversion of nonmethylated cytosines to thymidines but does not convert methylated cytosines, so that upon sequencing of the DNA it is possible to distinguish methylated from nonmethylated CpGs. Genomic DNA was extracted from cortical neurons before or 180 min after membrane depolarization to induce the release of MeCP2. After exposure to proteinase K to digest DNA-bound proteins, the DNA was treated with sodium bisulfite to convert nonmethylated cytosines to thymidines. *BDNF* promoter III DNA was then amplified by the polymerase chain reaction (PCR), and the PCR products were cloned and sequenced. This analysis revealed the presence of a methylation site termed the –148 bp CpG site and located 148 base pairs (bp) 5′ to the site of initiation for *BDNF* promoter III–dependent transcription (Fig. 5A). By sequencing individual clones obtained from the bisulfite mutagenesis procedure, we found that 83% of a total of 53 clones sequenced from four independent experiments were methylated at the –148 bp CpG in unstimulated neuronal cultures (Fig. 5B). Sixty-three percent of a total of 19 clones sequenced from three independent experiments were methylated at the –148 bp CpG site 3 hours after membrane depolarization. These data suggest that the frequency of methylation at this site decreases somewhat upon membrane depolarization; however, further analysis will be required to determine whether this reduction contributes to the activity-dependent release of MeCP2 from *BDNF* promoter III. In addition to the –148 bp CpG, several other CpG sites (–111 bp, –109 bp, –24 bp, +19 bp, and +35 bp) within *BDNF* promoter III were found to be methylated, although at lower frequencies (less than 20% in untreated neurons) [Supporting Online Material (SOM) Text]. The functional significance of methylation at these sites to *BDNF* promoter III–mediated transcription remains to be determined.

We found, with the use of an electrophoretic mobility shift assay (EMSA) (*29*), that MeCP2 specifically bound to a double-stranded methylated oligonucleotide corresponding to the –148 bp CpG and surrounding sequences (Fig. 5C). This binding was dependent on the methyl-CpG site, because MeCP2 binding was effectively competed away by the addition of an excess of unlabeled double-stranded oligonucleotides that include sequences corresponding to the –148-bp methylated CpG site but not by the addition of unmethylated –148 bp CpG sequences or unmethylated sequences surrounding and including the +19 bp CpG. The ability of MeCP2 to bind to methylated CpG sequences within *BDNF* promoter III was affected somewhat by the DNA sequence surrounding the methylated CpG, because the addition of an excess of unlabeled methylated +19 bp CpG sequence or an unlabeled synthetic methylated CpG se-

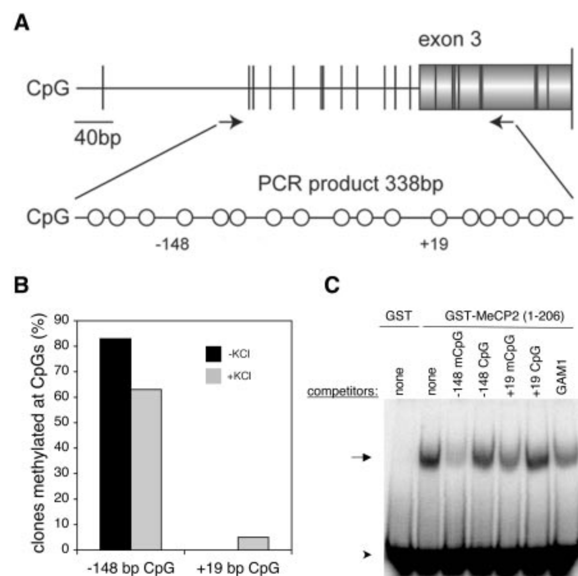

Fig. 5. Identification of a methyl-CpG site in *BDNF* exon III that is capable of binding MeCP2. (**A**) Locations of CpG sites in *BDNF* promoter III region relative to the transcription initiation site. *BDNF* exon III is shown as a shaded box. The vertical lines and open circles indicate the location of CpG sites. Arrows represent PCR primer set I as described in SOM. (**B**) Mapping of the methylation status of the proximal region of *BDNF* promoter III by bisulfite sequencing of genomic DNA. E18 + 5 DIV rat cortical neurons were cultured in 10% calf serum and treated with or without 50 mM KCl for 3 hours (*29*). For the –KCl culture, a total number of 53 individual clones from four independent cultures were sequenced, and 83% of them were methylated at the –148 bp CpG and none at the +19 bp CpG. For the +KCl culture, a total number of 19 individual clones from three independent cultures were sequenced, and 63% of them were methylated at –148 bp CpG and one at the +19 bp CpG. (**C**) EMSA and competition assays using a radiolabeled methylated *BDNF* exon III double-stranded oligonucleotide (–148 bp mCpG) and recombinant MeCP2 (*29*). –148 bp mCpG, –148 bp CpG, +19 bp CpG, and +19 bp mCpG indicate the addition of an excess of unmethylated or methylated *BDNF* exon III DNA sequences. GAM1 indicates the addition of an excess of a synthetic methylated CpG DNA sequence (*36*) used for competition. The arrow indicates the complex formed by MeCP2 and the radiolabeled –148 bp mCpG probe. The arrowhead indicates the free radiolabeled –148 bp mCpG probe.

Fig. 6. Decreased binding of the slowly migrating form of MeCP2 to the –148 bp mCpG site. Nuclear protein extracts from E18 + 5 DIV cultured rat cortical neurons that were treated with or without 50 mM KCl for 90 min, after SDS-PAGE separation and transfer to nitrocellulose membranes, were incubated with ^{32}P-labeled methylated *BDNF* exon III DNA (–148 bp mCpG in Fig. 5C) and visualized on a PhosphorImager after overnight exposure to a phosphor-imaging screen. The same nitrocellulose membrane blot was probed with an antibody specific to MeCP2 and visualized by enhanced chemiluminescence (*29*).

quence, GAM1 (*36*), did not compete away MeCP2 binding to the –148 bp CpG oligonucleotides as effectively as an excess of unlabeled double-stranded –148 bp mCpG sequences. The findings that the –148 bp CpG is methylated in neurons before membrane depolarization and that MeCP2 shows somewhat enhanced binding to the –148 bp CpG are consistent with the ChIP analysis showing that MeCP2 binds within this region in vivo (Fig. 1B) and raise the possibility that the –148 bp mCpG mediates the interaction of MeCP2 with the *BDNF* gene. However, given the complexity of the methylation pattern of *BDNF* promoter III revealed by bisulfite mutagenesis, it is possible that MeCP2 also binds to other methylated CpGs within *BDNF* promoter III.

We next asked whether membrane depolarization–induced phosphorylation of MeCP2 affected the ability of MeCP2 to interact with *BDNF* promoter III sequences that surround and include the –148 bp CpG. For this purpose, we prepared nuclear extracts from cortical neuron cultures before and after membrane depolarization (*29*). The nuclear extracts were resolved by SDS-PAGE, transferred and renatured on a nitrocellulose membrane, and then probed with a radiolabeled oligonucleotide corresponding to the –148 bp mCpG (*27*) or the +19 bp mCpG (*31*). The methylated sequences bound strongly to a single protein that comigrated by SDS-PAGE with the rapidly migrating form of MeCP2 that is present in unstimulated neurons (Fig. 6). By contrast, the slowly migrating phosphorylated form of MeCP2 showed little binding to the methylated –148 bp CpG or the +19 bp CpG. In addition, alkaline phosphatase treatment of neuronal nuclear extracts converted MeCP2 to a form that showed enhanced binding to the methylated CpG sequences (*31*). These findings suggest that the membrane depolarization–induced phosphorylation of MeCP2 decreases the affinity of MeCP2 for mCpGs within *BDNF* promoter III, which appears to facilitate activation of *BDNF* transcription.

Taken together, our results demonstrate that MeCP2, in addition to its long-term gene-silencing role, also functions in the dynamic neuronal activity–regulated expression of *BDNF*. Our results suggest that neuronal activity regulates MeCP2 target genes in a promoter-specific manner, because depolarization induces the release of MeCP2 from *BDNF* promoter III yet has no apparent effect on the association of MeCP2 with the promoter of the imprinted gene *H19*. This distinction may be attributable to the different transcriptional regulatory complexes that associate with these two genes. In the absence of neuronal activity, transcriptional activators such as USFs are already associated with *BDNF* promoter III, and this association is not affected by calcium influx induced by neuronal activity (Fig. 2A). The binding of MeCP2 adjacent to transcriptional activators such as USFs in the absence of neuronal activity may act to recruit histone-modifying enzymes, which temporarily silence the promoter. Calcium influx–induced release of MeCP2 from *BDNF* promoter III may then lead to relief of repression of the promoter. However, this relief is not sufficient to fully activate promoter III–dependent transcription, because in MeCP2-deficient neurons we detected only a twofold increase in *BDNF* promoter III–dependent transcription whereas membrane depolarization leads to at least a 100-fold increase in *BDNF* promoter III–driven transcripts (Fig. 3). These data indicate that, in addition to the release of MeCP2 from *BDNF* promoter III, other activity-dependent events, such as the phosphorylation of promoter III–bound CREB and possibly USFs and CaRF, are required for full induction of *BDNF* promoter III.

It will be important to determine whether MeCP2 plays a more general role in repressing activity-dependent gene expression. It is interesting that induced expression of the activity-regulated gene *c-fos* was independent of MeCP2. One major distinction between the *BDNF* and *c-fos* genes is the kinetics of transcriptional activation. Activation of the *c-fos* promoter by neuronal activity occurs within 5 min of stimulation (*37*, *38*), whereas the activation of *BDNF* promoter III requires at least 30 min (*21*). Membrane depolarization–induced modification of MeCP2 does not peak until 30 min after stimulation (Fig. 4A), raising the possibility that MeCP2 may preferentially regulate the expression of activity-dependent genes that have slower activation kinetics.

Because activity-dependent transcription plays important roles in synapse development and plasticity, our findings suggest that MeCP2 may itself regulate aspects of synaptic development and maturation. This might explain the observation that, although MeCP2 is expressed in many different cell types, mutations of MeCP2 in RTT specifically affect neurons at a time when synaptic development and maturation are taking place. It is possible that deregulation of activity-dependent gene transcription that occurs when MeCP2 is mutated could affect synaptic development and contribute to the pathology of RTT.

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Materials and Methods
SOM Text
Figs. S1 to S4
References and Notes

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