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Dissecting MECP2 Function in the Central Nervous System

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ABSTRACT

Rett syndrome is a neurodevelopmental disorder and an important cause of mental retardation and autistic behavior in girls and in a small group of boys. In 1999, mutation of the methyl-CpG binding protein 2 (MECP2) gene encoding a transcriptional repressor on the X chromosome was found to cause Rett syndrome. Since this discovery, significant research has focused on the elucidation of its specific role in the central nervous system. Recent studies revealed that MECP2 is expressed in more differentiated neurons rather than in less differentiated neuroblasts and that MECP2 is involved in the maturation and maintenance of neurons, including dendritic arborization and axonal projections, rather than in early cell fate decisions in the mammalian brain. In this review, we summarize recent findings regarding regional, temporal, and cell type–specific MECP2 expression in the central nervous system; neurobiologic abnormalities in MECP2–mutant mice; and MECP2 target genes in the central nervous system. (J Child Neurol 2005;20:753–759).
In 1999, Amir and colleagues reported that mutation of the methyl-CpG binding protein 2 (MECP2) gene on the X chromosome is the cause of Rett syndrome; this groundbreaking work provided the foundation for all subsequent analysis of MECP2 function in the central nervous system. MECP2 is a transcriptional repressor that selectively binds methylated CpG dinucleotides in the mammalian genome and mediates transcriptional repression through interaction with histone deacetylase and the corepressor Sin3A (Figure 1). To date, three independent lines of MECP2-deficient mice have been established that display several phenotypic characteristics that mimic the symptoms of patients with Rett syndrome. Although it is evident that phenotypes of MECP2 mutant mice are attributable to a lack of MECP2 in the central nervous system until recently, there was little cellular neurobiologic or neuropathologic understanding of the cellular expression of MECP2 or of the abnormalities in the central nervous system of patients with Rett syndrome or of MECP2-null mice. In this review, we summarize current understanding of MECP2 function on both cellular and molecular levels.

**EXPRESSION PATTERNS OF MECP2**

MECP2 is widely expressed in various human and rodent tissues. MECP2 is relatively highly expressed in brain, lung, and spleen compared with lower-level expression in tissues such as heart and kidney. MECP2 messenger ribonucleic acid (RNA) has three alternatively spliced transcripts (1.9 kb, 7.5 kb, and 10 kb) produced by differential polyadenylation site use in the 3′ untranslated region of eukaryotic mRNA (UTR), suggesting that the MECP2 expression level can be controlled post-transcriptionally. Recent work has highlighted that micro-RNAs have been associated with a post-transcriptional regulation mechanism. Micro-RNAs are endogenous, approximately 22-nucleotide RNAs that are involved in gene regulation by pairing to the messages of protein-coding genes to specify messenger RNA cleavage or repression of productive translation. A computational study predicted two micro-RNAs that potentially regulate the expression of MECP2, although experimental evidence is currently lacking.

In addition, recently, a new form of the protein McP2 (McP2 e1, McP2B, McP2α) that is more prevalent in mouse and human brain than the originally described form (McP2 e2, McP2A, McP2B) was discovered. The transcript of McP2 e1 shares exons 1, 3, and 4 but is missing exon 2. Although exon 1 was thought to be noncoding, exon 1 offers a new open reading frame in the alternatively spliced form consisting of exons 1, 3, and 4. The difference between McP2 e1 and e2 is just 21 amino acids in the N-terminus of human MeCP2 protein (McP2 e1, 408 amino acids; e2, 486 amino acids). The fact that overexpression of McP2 e2 can rescue the phenotype of McP2-null mice suggests that the functions of McP2 e1 and e2 can overlap significantly. However, a deletion mutation in exon 1 is able to cause Rett syndrome without affecting expression of McP2 e2 and the possibility cannot be ruled out that these two isoforms have distinct functions.

The common understanding of MECP2 expression in the central nervous system is that MECP2 is increasingly expressed postnataally, suggesting that MECP2 might have a role in neuronal maturation (Figure 2). Consistent with other studies, experiments from our laboratory revealed that MECP2 is not strongly expressed in the murine neocortex during embryonic stages; expression of MECP2 develops progressively from deep cortical layers to superficial layers as development progresses, suggesting that MECP2 expression increases during postnatal development, following the “inside-out” sequence of cortical laminar development. In addition to our findings in the neocortex, several other groups have reported a similar pattern of increasing MECP2 expression in the other regions of the central nervous system—cerebellum and olfactory epithelium—suggesting that MECP2 is involved in the maturation of neurons throughout the central nervous system. Further, our analysis of cell type–specific expression using cell type– and stage-specific markers revealed that MECP2 is expressed in mature neurons but not in astroglia, oligodendroglia, or immature neuroblasts. In addition, our analysis of potential MECP2 function in early cell fate specification using neural precursors corroborated the results of others that MECP2 is expressed only in the neuronal lineage and that MECP2 expression levels increase as neuronal differentiation progresses.

Usually, MECP2 is exclusively localized to nuclei in neurons, however, one report suggested that MECP2 can be localized in the cytoplasm and in the nucleus. MECP2 staining in the mouse can be categorized into two patterns. The first is “punctate” nuclear staining pattern, and the other is marked by uniform staining of the nucleus. The punctate expression is coincident with the location of heterochromatin that is highly methylated. Uniform chromatin staining is predominant at embryonic and neonatal stages, but the punctate pattern becomes more frequent in the adult brain. This transition from “uniform” nuclear staining with MECP2 to “punctate” nuclear staining of heterochromatin during cortical development is correlated with neuronal maturation. It is thought that localization of MECP2 in the highly methylated heterochromatic region represents MECP2 binding to genomic methylated DNA, supported by the fact that mutation of the methyl-CpG binding domain of MECP2, or reduced DNA methylation, leads to diffuson of MECP2 localization in mouse embryonic stem (ES) cells. However, a recent study demonstrated that MECP2 can also bind to unmethylated nucleosomal arrays and assembles secondary chromatin structures independent of DNA methylation in vitro, suggesting that MECP2 might play a role as a general chromatin condensing factor and a transcriptional repressor to specific...
MECP2 binds to methylated CpGs of the promoter region and recruits histone deacetylase (HDAC) and Sin3A. This complex promotes deacetylation of histone tails, and silences transcription of target genes.

target genes. This binary function of MECP2 might be related to the two different MECP2 localization patterns in mice.

PROPOSED FUNCTIONS OF MECP2 DURING EMBRYONIC STAGES

MECP2 is known to be expressed in the cortex at a low level during embryonic stages or in undifferentiated neural precursors.15,26 Does MECP2 play a role in any aspects of early neurogenesis? Recently, Stancheva and colleagues showed in Xenopus that MECP2 binds to the promoter region of the xHairy2a gene and represses expression of xHairy2a along with the SMART complex.11 The xHairy2a gene, which encodes a neuronal repressor, is a target gene of Notch signaling, which is known to be involved in various cell fate decisions, including neural development.32 Loss of MECP2 in frog embryos by antisense morpholino oligonucleotide injection led to various developmental abnormalities in the head and dorsal axis. Expression of the Hairy2a gene was found to be up-regulated in MECP2-deficient frog embryos. MECP2 binds to methylated CpGs of the promoter region and interacts with the SMART complex via Sin3A, and the MECP2/SMART complex represses the Hairy2a gene. Interestingly, this inhibitory complex leaves the promoter without changing the pattern of methylation, after Notch signaling is on, suggesting that MECP2 association with the promoter of Hairy2a is dynamic and that MECP2 can be displaced from methylated DNA by Notch signaling.

One question that arises from the prior Xenopus work is whether the phenotypes of MECP2-null mice are actually due to abnormal cell fate decisions. If MECP2 mutation leads to abnormal cell fate decisions, the phenotype should be apparent during embryonic stages, similar to mutant mice that lack Notch signaling components.33–36 However, MECP2-null mice begin to display abnormal phenotype at approximately 3 weeks of age, and no phenotype in MECP2-mutant mice has been reported during embryonic stages.11,12 In addition, patients with classic Rett syndrome also develop normally until 6 months of age.

Our recent study using neural precursor cultures directly addressed this question.27 To investigate the possibility that subtle, abnormal cell fate decisions during mouse embryonic development lead to the phenotypes observed at the later stages, we examined the proliferation and differentiation ability of neural precursors, which are proliferative and multipotent cells with the capacity to differentiate into neurons and glia, derived from wild-type, MECP2+/−, and MECP2−/− mice. There were no significant differences among the three genotypes in proliferation or differentiation, supporting the in vivo findings that MECP2 mutation does not affect early neural developmental events in mice. Further, two independent studies showed that transgene expression of MECP2 under the control of a neuron-specific promoter rescues the phenotypes, such as postnatal lethality, of MECP2-null mice.21,22,27 Further arguing that loss of MECP2 during embryonic stages does not directly affect the postnatal phenotypes observed in MECP2-null mice.

There are multiple explanations for the difference between mice and Xenopus. One possibility is that there is more redundancy for MECP2 function in mice than in Xenopus. In mice, four additional methyl-CpG binding domain proteins have been identified: MBD1, -2, and -4 and Kaiso.4 Mouse MBD1 is expressed in neural precursors, as well as in mature neurons in the adult hippocampus,37 suggesting that MBD1 might compensate for loss of MECP2 function during embryonic neurogenesis. Another possibility is that target genes of MECP2 might be different between rodents and amphibians. So far, only brain-derived neurotrophic factor (BDNF) and distal-less 5 (DLX5) genes have been identified as MECP2 target genes in the rodent brain,38–40 and these genes are thought to be required for neuronal maturation rather than neural cell fate decisions. Further investigation of MECP2 target genes will be critical to understanding its function in Rett syndrome.

ROLE OF MECP2 IN NEURONAL MATURATION AND MAINTENANCE

After newborn neurons migrate to appropriate positions during embryonic stages, neurons develop axonal projections to targets and extend their dendrites to become fully mature and participate in neuronal circuitry. The fact that MECP2 is expressed in the neuronal lineage, that the expression of MECP2 increases as neuronal maturation progresses (see Figure 2), and that MECP2 mutation does not affect the proliferation and differentiation of neural precursors in mice suggests that MECP2 plays a role in this neuronal maturation process in mammals. However, apart from the reduced brain size of MECP2-null mice, until recently, no specific cellular or structural abnormality had been detected in MECP2-null mice. Two recent studies (one from our laboratory) have now identified subtle but significant cellular phenotypes in MECP2-null mice.27,41

Dendritic arborization is an important maturation step during the formation of neuronal circuitry. Our study found that the neocortex is reduced in thickness in MECP2−/− mice compared with wild-type mice.27 Specifically, layer II/III is most affected. The cell density of the neocortex in MECP2-null mice is significantly higher than that in wild-type mice, suggesting that the reduction in thickness of the neocortex in MECP2−/− mice is due to the reduced size of neurons rather than to loss of neurons. Consistent with this result, Golgi staining, which visualizes the complete structure of individual neurons (Figure 3, A and B), revealed that layer II/III projection neurons in MECP2-null mice have significantly smaller somas than those in wild-type mice (Figure 3E), and Sholl analysis (widely used to assess dendritic complexity and branching) (Figure 3, C and D) revealed that layer II/III pyramidal neurons are significantly less complex than those in wild-type mice (Figure 3F). Consistent with these results, previous findings using human postmortem tissue indicated less complex dendritic arborization and smaller neuronal size in patients with Rett syn-
Our study using MECP2 mutant mice strengthens the evidence that these phenotypes are caused primarily by MECP2 mutation rather than nutritional, respiratory, or other secondary medical issues.

Although highly speculative and complicated, recent findings might begin to explain some of the molecular mechanisms of this abnormality in MECP2-null mice. Two independent groups identified BDNF as a target gene for MECP2 in the mammalian brain.\textsuperscript{38,40} BDNF is activity dependent, and the protein BDNF plays a variety of roles in neural development, including maintenance of dendritic arborization. The neocortex of forebrain-specific TrkB and BDNF conditional mutant mice have reduced thickness owing to dendritic retraction.\textsuperscript{45,46} Interestingly, BDNF conditional mutant mice\textsuperscript{46} and TrkB\textsuperscript{−/−} neurons\textsuperscript{47} begin to display less complex dendritic arborization at approximately 3 weeks of age, the same age at which MECP2-null mice begin to show a reduced brain size. However, the relationship between BDNF and MECP2 is paradoxical and not so simple because the phenotype of BDNF conditional mutant mice (lacking BDNF) mimics that of MECP2-mutant mice, in which the previous results predict that BDNF might be basally up-regulated. How can we explain this paradox? A number of speculations can be proposed. BDNF has four promoters, and its different transcriptional regulation is not well understood. It is, therefore, possible that MECP2’s transcriptional repression on BDNF might play a critical role in the tight regulation of BDNF. These increased basal levels might disrupt the response to activation.\textsuperscript{38,40} Also, BDNF activity in the developing telencephalon might be such that either low or absent (BDNF conditional mutant mice) or abnormally high levels (owing to a lack of BDNF promoter repression in MECP2 mutant mice) produce aberrant maturation and reduced dendritic arborization. Further experiments to clarify this issue are needed.

Axonal projection is another critical neuronal maturation event that could be potentially affected by MECP2 mutation. Recently, Matarazzo and colleagues offered evidence that MECP2 mutation leads to abnormal axonal projections from the olfactory epithelium to the olfactory bulb.\textsuperscript{51} They found a transient delay in the terminal differentiation of olfactory neurons in MECP2-null mice during the early postnatal stages, although this delay is resolved at later stages. This delay results in abnormal, disrupted axonal targeting to the olfactory bulb, subglomerular disorganization, and smaller glomeruli in MECP2-null mice. Intriguingly, some magnetic resonance imaging studies reported a smaller corpus callosum in patients with Rett syndrome and in some patients with autism, suggesting that MECP2 mutation might affect formation and/or maintenance of these interhemispheric projection neurons.\textsuperscript{46,47} Because the time course of the development of callosal projection neurons, which are responsible for interhemispheric association pathways and are thought to be centrally involved in high-level associative cognitive function, is coincident with the progressive expression of MECP2, it seems plausible that mutation of MECP2 results in some aspects of the cognitive phenotype in Rett syndrome by causing abnormal axonal projections of callosal projection neurons.

Although there is no clear evidence, MECP2 might be involved in other critical events in neuronal maturation. For example, electrophysiologic activity is also crucial for neuronal maturation. Seizures and respiratory problems are often observed both in MECP2 mutant mice and patients with Rett syndrome and are known to be related to mutation in sodium channel genes.\textsuperscript{50,51} Interestingly, the sodium channel type 2 (SCN2A) gene is a target gene for MECP2 in non-neural cells,\textsuperscript{52} and SCN2A-deficient mice die perinatally owing to severe hypoxia, presumably resulting from abnormal respiratory regulation in the brain stem.\textsuperscript{50}

In addition, neurotransmitter release is also an important event in neuronal circuitry. A recent report identified the DLX5 gene as an MECP2 target gene in the central nervous system.\textsuperscript{53} As DLX5 regulates the expression of glutamic acid decarboxylases,\textsuperscript{54} enzymes that synthesize γ-aminobutyric acid (GABA), the dysregulated DLX5 expression might affect GABAergic neuron activity in MECP2-null mice or patients with Rett syndrome.

**MECP2 TARGET GENES**

**IN THE CENTRAL NERVOUS SYSTEM**

Much has been learned about MECP2 biology in the past 5 years since its discovery as the causative gene of Rett syndrome, but much more remains to be elucidated. One of the crucial steps for under-
stands the molecular mechanisms of MECP2 is to identify its cell type–specific target genes, whose abnormal expression directly or indirectly causes the phenotype of MECP2-null mice. It is highly likely that the complex phenotype is caused via subtly abnormal expression of multiple MECP2 target genes. This might prove to be a challenging endeavor. In addition to the classic gene-silencing mechanism by MECP2 through histone deacetylases, a recent study showed a new mechanism for silencing genes by MECP2 in non-neural tissue. In non-neural tissue, a transcriptional repressor Co-REST. The REST–Co-REST complex recruits MECP2 and inactivates target gene transcription via methylation of histone H3 by mammalian histone lysine methyltransferase, SUV39H1. This study directly demonstrated that the role and target genes of MECP2 are tissue- and cell type–specific and reinforces the critical need to find MECP2 target genes in specific affected neuronal populations in the central nervous system to understand the pathogenesis of Rett syndrome.

The only MECP2 target genes that have been identified in the central nervous system and well characterized are BDNF and DLX5 in mammals and Hairy2α in Xenopus. The identification of MECP2 target genes offered new insights for the field. For example, two independent groups identified MECP2 as a negative regulator of the BDNF gene. MECP2 binds selectively to the BDNF promoter III in rat neurons (promoter IV in mouse), which is activated in response to neuronal depolarization and represses BDNF expression. Although it was thought that gene silencing by MECP2 is semipermanent, these studies revealed that MECP2 binding to the promoter is reversible, and electrophysiologic stimuli trigger release of MECP2 from the promoter. This suggests that one MECP2 function is tight regulation of genes necessary for neuronal maturation, in addition to silencing of genes that are unnecessary for neuronal maturation. Although the mechanism of release of MECP2 is not well characterized, calcium-dependent phosphorylation of MECP2 and a decrease in CpG methylation within the promoter region are potential mechanisms.

In addition, the recent identification of the DLX5 gene as a MECP2 target gene also raises interesting issues. The DLX5 gene, which encodes a distal-less–related DNA binding homeobox protein, is an imprinted gene that is preferentially transcribed from the maternal allele in mice (DLX5 is expressed specifically from the maternal allele in humans). MECP2 deficiency leads to loss of relaxed imprinting of DLX5, and DLX5 expression increases approximately twofold. Surprisingly, the CpG island of DLX5 is unmethylated, and MECP2 preferentially binds to a genome region 10 kb away from the DLX5 locus. MECP2 regulates DLX5 by forming a silent chromatin-associated 11 kb chromatin loop at the DLX5 locus, suggesting that MECP2 can regulate gene expression not only by MECP2 binding to the promoter region of target genes but also by formation of a silent chromatin loop.

**CONCLUSION**

The identification of MECP2 mutation as the cause of Rett syndrome by Zoghbi and colleagues in 1999 enabled a new era of cellular and molecular analysis and understanding of the pathophysiology of Rett syndrome. Between 1966, when Andreas...
Rett described the syndrome that bears his name, and 1999, when the MECP2 mutation was discovered as its cause, Rett syndrome was defined only by its clinical features. With the MECP2 link, we have entered an exciting stage of dissecting the pathophysiology of Rett syndrome at a fundamental neurobiologic level. We now know that MECP2 appears to function predominantly in the maturation, dendritic elaboration, and axonal outgrowth of populations of central nervous system neurons. One important goal will be to identify the specific target genes of MECP2 in individual affected neuronal populations. A number of laboratories are already making progress in this direction. Deeper understanding of the specific normal roles of MECP2 and deleterious effects of MECP2 mutation during central nervous system maturation will provide insight into the pathophysiology of Rett syndrome and possible therapeutic strategies.

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References


**Original Article**

**Rett Syndrome and Neuronal Development**

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**ABSTRACT**

The clinical signs of Rett syndrome, as well as neuropathology and brain imaging, suggest that the disorder disrupts neuronal circuits. Studies using receptor autoradiography demonstrate abnormalities in the density of excitatory glutamate and inhibitory γ-aminobutyric acid (GABA) synaptic receptors in postmortem brain from young female subjects with Rett syndrome. MeCP2, the protein that is abnormal in most female individuals with Rett syndrome, is expressed predominantly in neurons and appears during development at the time of synapse formation. Studies of nasal epithelium from patients with Rett syndrome show that the maturation of olfactory receptor neurons is impeded prior to the time of synapse formation. Recent reports indicate that MeCP2 controls the expression of brain-derived neurotrophic factor and the DNA-binding homeobox protein Dlx5. Brain-derived neurotrophic factor enhances glutamate neurotransmission at excitatory synapses, whereas Dlx5 is expressed in most GABAergic neurons and stimulates the synthesis of GABA. Taken together, this information supports the hypothesis that Rett syndrome is a genetic disorder of synapse development, especially synapses that use glutamate and GABA as neurotransmitters. (*J Child Neurol* 2005;20:759–763).