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·Review·

MeCP2: multifaceted roles in gene regulation and neural development

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Methyl-CpG-binding protein 2 (MeCP2) is a classic methylated-DNA-binding protein, dysfunctions of which lead to various neurodevelopmental disorders such as Rett syndrome and autism spectrum disorder. Initially recognized as a transcriptional repressor, MeCP2 has been studied extensively and its functions have been expanded dramatically in the past two decades. Recently, it was found to be involved in gene regulation at the post-transcriptional level. MeCP2 represses nuclear microRNA processing by interacting directly with the Drosha/DGCR8 complex. In addition to its multifaceted functions, MeCP2 is remarkably modulated by post-translational modifications such as phosphorylation, SUMOylation, and acetylation, providing more regulatory dimensions to its functions. The role of MeCP2 in the central nervous system has been studied extensively, from neurons to glia. Future investigations combining molecular, cellular, and physiological methods are necessary for defining the roles of MeCP2 in the brain and developing efficient treatments for MeCP2-related brain disorders.

Keywords: MeCP2; Rett syndrome; central nervous system; gene expression regulation; post-translational modification; post-transcriptional regulation; glia

Introduction

Methyl-CpG-binding protein 2 (MeCP2) was initially identified in 1992 as a classic methyl-CpG-binding protein[1]. Since DNA methylation was considered to be an important epigenetic mechanism for the regulation of gene transcription[2, 3] (Fig. 1A), MeCP2 was then regarded as a transcriptional repressor and the methyl-DNA-binding domain and transcriptional repression domain were revealed by subsequent biochemical studies[4, 5] (Fig. 1B). Furthermore, many proteins such as the Sin3A/HDAC and NCoR/SMRT co-repressor complexes interact with MeCP2[6] (Fig. 1B), confirming its key role in transcription repression.

The role of MeCP2 in the brain was originally discovered in 1999, when Dr. Huda Y. Zoghbi and her team found that MeCP2 mutations are the genetic root of a rare neurodevelopmental disorder named Rett syndrome (RTT, MIM312750)[7]. As *MECP2* is an X-linked gene, it is not surprising that Rett syndrome mainly occurs in females, as loss-of-function mutations of *MECP2* would largely be lethal in males. Classic RTT patients have normal development to 6–18 months of age, and then start to undergo growth arrest and appear to have defects in motor functions such as hand skills. Furthermore, neurological abnormalities including autistic features, seizures, and mental retardation are prevalent in the progression[8]. In accord with the clinical features of RTT patients, expression pattern analysis showed that MeCP2 expression is dominant in brain tissues, emphasizing its crucial role in neural development[9].

The *MECP2* gene is composed of four exons and alternative splicing results in two protein isoforms, MeCP2E1 and MeCP2E2, with different N-termini[10, 11]. *In situ* hybridization in mouse brain showed that *Mecp2e2* expression is restricted to the dorsal thalamus while

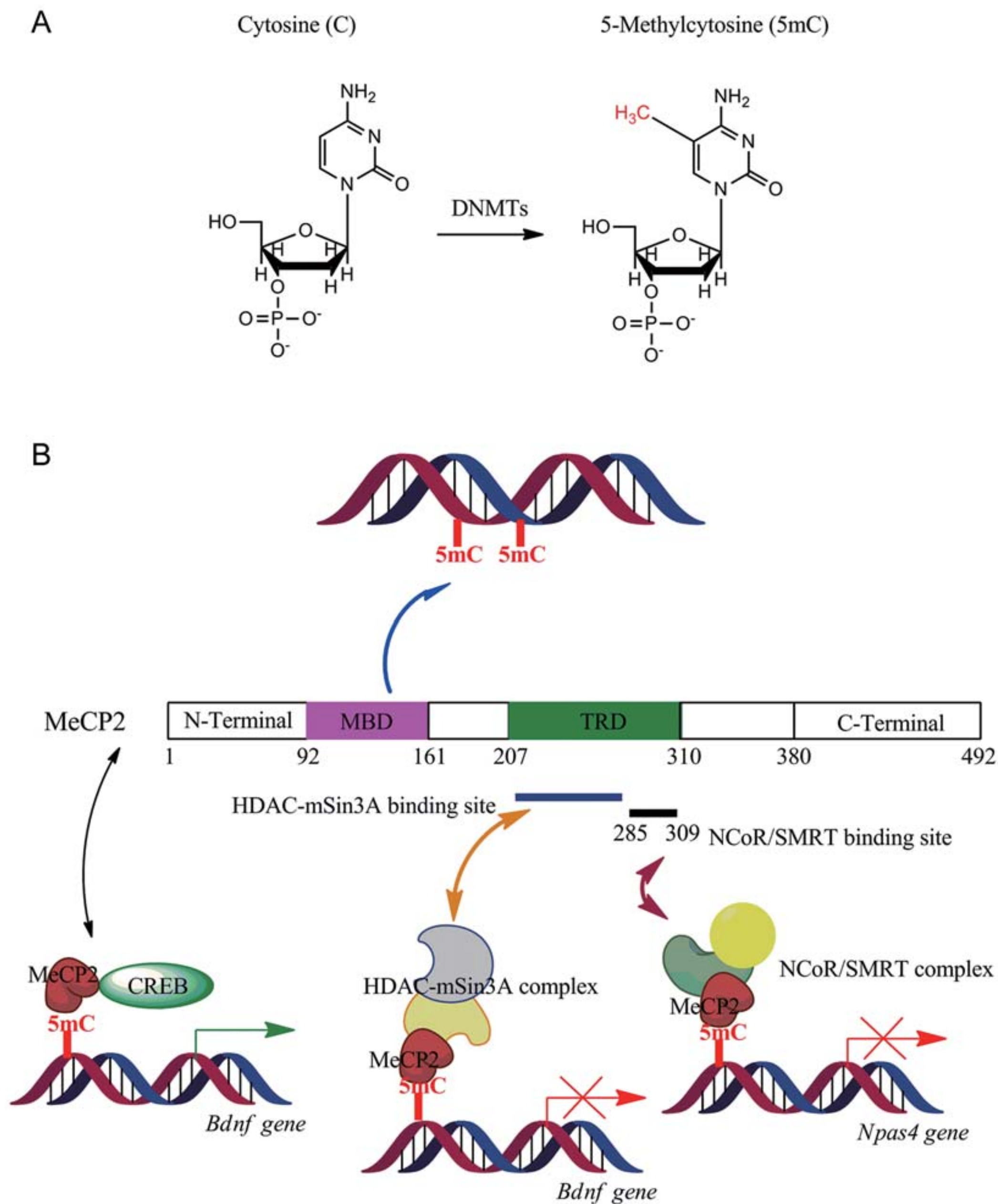

Fig. 1. MeCP2 regulates transcription in a bidirectional manner. A: 5-methylcytosine is the major form of DNA methylation occurring at cytosine. This methylation process is mediated by DNA methyltransferases (DNMTs). B: MeCP2 has two functional domains, a methyl-DNA-binding domain (MBD) and a transcriptional repression domain (TRD). MeCP2 binding to a methylated DNA site is mainly mediated by the MBD. Physical interaction between MeCP2 and co-repressor complexes (HDAC-mSin3A and NCoR-SMRT) is mainly dependent on the TRD. In addition to co-repressor complexes, MeCP2 binding to the transcriptional activator CREB1 has been identified.

Mecp2e1 is the major brain isoform outside the thalamus[12]. Furthermore, expression analysis in human brain tissues revealed that the *MECP2E1* mRNA level is significantly higher than *MECP2E2* in the whole brain or cerebellum[11]. With the development of MeCP2 isoform-specific antibodies, researchers have investigated the expression of both isoforms at the protein level during brain development and in various brain regions, and found

a later onset of MeCP2E2 expression than MeCP2E1[13]. We further showed that the MeCP2E2 expression patterns in different brain regions are quite different while the MeCP2E1 expression pattern is uniform[13]. Although both isoforms have been detected in many cell types including neurons, astrocytes, and oligodendrocytes, MeCP2E1 is significantly higher in neurons than in astrocytes[14]. In addition, MeCP2E1 and MeCP2E2 have quite different functions in neuronal survival[15], embryonic development[16] and responses to drugs[17].

RTT disorder is mainly caused by MeCP2 deficiency, but duplications of *MECP2*-containing loci are also detrimental to neural development and proper brain functions. Patients carrying *MECP2* duplications usually manifest autistic features, mild RTT phenotypes, and mental retardation[18]. All these results underscore the importance of the homeostatic modulation of MeCP2 expression, indicating that the dosage of MeCP2 protein is critical for the development of the central nervous system.

To investigate the underlying pathophysiology of RTT, mouse models in which MeCP2 is either deleted or overexpressed have been established[19-21]. Many clinical features manifested in RTT patients have been reproduced in mouse models and thanks to these models, studies in this field have accelerated and significant valuable insights into the pathogenesis of RTT have been achieved. In addition, RTT has been successfully modeled in cynomolgus monkeys by targeting the *MECP2* gene with TALENs technology[22, 23].

Initially, MeCP2 was thought to be present mainly in excitatory neurons[24]. However, after decades of study, the functions of MeCP2 protein have expanded from transcriptional repression to post-transcriptional regulation, and it is recognized as a key regulator in various cell types including excitatory neurons, inhibitory neurons, and glia. Furthermore, MeCP2 protein undergoes multiple posttranslational modifications such as phosphorylation, SUMOylation and acetylation, which impact its functions. Here, we summarize the multifaceted functions of MeCP2 in the central nervous system, emphasizing its diverse and indispensable roles in brain development and functions. We believe that more functions of MeCP2 remain to be uncovered and these will provide a more comprehensive view of its roles in the brain.

Dual Functions of MeCP2 in the Regulation of Gene Expression

Since methylation of CpG islands is recognized as a hallmark of gene silencing and MeCP2 specifically binds to methylated CpG, much effort has been devoted to identifying gene targets whose expression is repressed by MeCP2. In 2003, two studies showed that MeCP2 binds to the promoter of the *BDNF* (brain-derived neurotrophic factor) gene to repress its expression, while neuronal depolarization decreases the methylation level of *BDNF* regulatory regions and promotes MeCP2 phosphorylation, leading to MeCP2 release from the *BDNF* promoter and the activation of *BDNF* transcription[25, 26]. Recently, it was reported that MeCP2 represses GluR2 expression by binding to the promoter region to modulate synaptic scaling during which neuronal activation increases the expression of MeCP2, leading to further inhibition of GluR2 transcription and a decrease of neuronal excitability[27]. However, MeCP2 is not just a transcriptional repressor, as transcriptional profiling analysis in the hypothalamus and cerebellum of *Mecp2* knockout/transgenic mice revealed a bi-directional change of gene expression[28, 29]. Consistently, protein interaction analysis has shown that MeCP2 interacts not only with co-repressor complexes[6, 30, 31] but also with the transcriptional activator CREB[28] (Fig. 1B). So it was proposed that MeCP2 also serves as a transcriptional activator to stimulate gene expression (Fig. 2). The transcriptional-activation function of MeCP2 has been further confirmed in astrocytes[32]. However, the underlying mechanisms remain to be determined.

More intriguingly, the modulation of specific gene expression by MeCP2 can change dynamically between repression and activation. As noted above, BDNF expression can be repressed by MeCP2 in cultured cortical neurons. However, it has been demonstrated that MeCP2 overexpression promotes BDNF expression in cultured cortical neurons[33]. Furthermore, BDNF expression is upregulated in *mecp2* transgenic mice and downregulated in *mecp2* knockout mice, which suggests that MeCP2 promotes BDNF expression *in vitro* and *in vivo*[28, 33]. Several hypotheses have been proposed to explain the discrepancy between these studies. For example, it has been proposed that different MeCP2 phosphorylation status could recruit

distinct factors to repress or activate BDNF expression[34]. Of course, further experiments are needed to verify these hypotheses.

Gene Expression Regulated by MeCP2: from mRNAs to Non-coding RNAs

Early studies on the impact of MeCP2 on gene expression mainly focused on the transcriptional level. However, transcripts of protein-coding genes only account for one-fifth of all transcripts in the genome[35]. Indeed, non-coding RNAs including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are abundant in the nervous system and they are critical for neuronal functions and brain development[36, 37]. It is well-known that miRNAs modulate gene expression post-transcriptionally to influence various aspects of neuronal functions from cell fate determination to synaptic plasticity. For example, miR-124a and miR-9 are essential for neural lineage differentiation and are involved in determining neural progenitor differentiation into neurons or glia[38], while miR-134 is located in synapses and is involved in spine development[39]. lncRNAs physically interact with transcriptional factors and chromatin remodelers to modulate gene expression and regulate neuronal functions[40, 41]. For instance, the specific lncRNA RMST interacts with hnRNPA2/B1 and SOX2 to regulate gene transcription and finally influence neuronal differentiation[42]. As the transcriptional process is similar in protein-coding and non-coding genes, and MeCP2 is a transcriptional regulator, it is likely that the transcription of non-coding RNAs is regulated by MeCP2. As expected, deletion of *Mecp2* results in disrupted expression of miRNAs and lncRNAs in the mouse model of RTT[43-45]. These results underscore the essential role of MeCP2 in transcriptional regulation and suggest that MeCP2 might modulate genome transcription globally, independent of RNA type.

Modulation of Gene Expression at the Post-transcriptional Level by MeCP2: miRNA Processing, RNA Splicing, and Protein Synthesis?

The involvement of MeCP2 in regulating miRNA expression was first reported in adult neural stem cells, in which miR-137 was up-regulated after MeCP2 knockdown and proliferation/differentiation balance was impaired[46]. Then, further studies analyzed the expression profiles of miRNAs in the whole brain or cerebellum of *Mecp2*-null mice and found that the levels of many miRNAs were altered[43, 44]. Although studies indicate that the expression of several miRNAs is regulated by MeCP2 at the transcriptional level, recently it has been reported that MeCP2 can also regulate miRNA expression post-transcriptionally[47]. Solexa-based deep sequencing revealed that most mature miRNAs are up-regulated in the hippocampus of MeCP2-knockout mice. Real-time PCR experiments showed that MeCP2 depletion has little effect on primary miRNAs but significantly enhances the expression of precursor and mature miRNAs, indicating a post-transcriptional impact of MeCP2 on miRNA expression. Further analysis revealed that MeCP2 directly interacts with DGCR8, an essential component of the miRNA-processing machinery in the DGCR8/Drosha complex, to inhibit the expression of miRNAs at the post-transcriptional level[47] (Fig. 2).

MeCP2 is also essential for the regulation of RNA splicing. Protein interaction analysis by co-immuno-precipitation revealed that Y box-binding protein 1 (YB-1), a conserved RNA-binding protein involved in the regulation of RNA splicing, interacts directly with MeCP2. Functional studies using splicing mini-gene assays showed that MeCP2 affects the RNA-splicing process and abnormal alternative splicing events have been reported in a mouse model of RTT[48]. Consistently, MeCP2 depletion in non-neuronal cell lines also impairs the process of alternative splicing[49] (Fig. 2). Further studies using mass spectrometry have identified several RNA-binding proteins and splicing factors as MeCP2 partners, providing compelling evidence that MeCP2 is a critical factor in the RNA-splicing process[50].

It has been reported that protein synthesis is impaired and the translation rate is reduced significantly in *Mecp2* mutant brains[51]. As MeCP2 is located in the nucleus and is not a ribosome-resident protein, the involvement of MeCP2 in the regulation of protein translation may be an indirect effect. Since the AKT/mTOR pathway is critical for the regulation of protein translation and its impairment is implicated in various neurodevelopmental diseases, it is possible that MeCP2 modulates protein translation *via* the AKT/mTOR pathway. Consistent with this hypothesis,

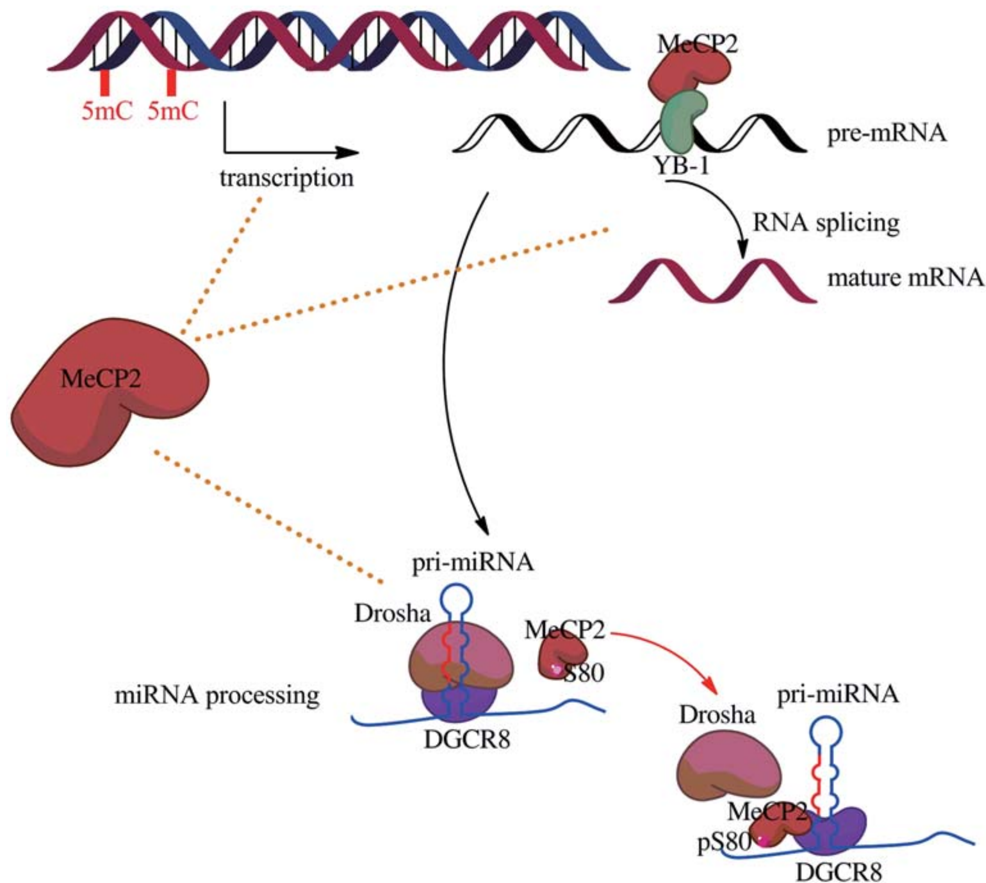

Fig. 2. Multifaceted functions of MeCP2 in the regulation of gene expression. MeCP2 regulates gene transcription bi-directionally. In addition, MeCP2 is involved in the regulation of RNA splicing *via* interaction with YB-1, which is essential for the maturation of pre-mRNA. Furthermore, microRNA (miRNA) processing is also regulated by MeCP2. MeCP2 phosphorylation at the Serine 80 (S80) site interferes with the intra-molecular interaction and promotes its interaction with DGCR8 to inhibit miRNA processing.

phosphorylation of rpS6, an important target of the AKT/mTOR pathway, is reduced in *Mecp2* mutant mice[51].

Impact of Post-translational Modifications on MeCP2 Functions

Protein functions can be modulated by post-translational modifications such as phosphorylation, acetylation, and SUMOylation. Such modifications alter protein structure and change their physical interactions with other components, leading to changes in protein localization and signal transduction in cells[52]. Various modifications of MeCP2 have been identified (Fig. 3). Repression of BDNF expression by MeCP2 is relieved by membrane depolarization in neurons and such de-repression depends on Ca^{2+}-mediated MeCP2 phosphorylation[25]. And using mass spectrometry, multiple phosphorylation sites in MeCP2 under different conditions have been identified[53]. Of these, Serine-421 (S421) has attracted much attention as its phosphorylation is triggered by physiological stimuli such as neuronal depolarization and behavioral stimuli[54, 55]. The importance of activity-dependent MeCP2 phosphorylation at S421 has been investigated extensively. Indeed, studies in brain slices have shown that S421 phosphorylation is essential for dendritic branching and spine morphogenesis[54]. Furthermore, MeCP2 S421A mutant mice show impaired responses to novelty[56]. Learning and memory tests have further shown that long-term potentiation in the hippocampus and hippocampus-dependent memory are enhanced, while excitatory

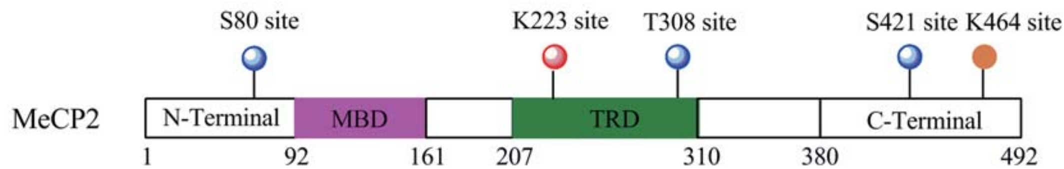

Fig. 3. Post-translational modification (PTM) of MeCP2. Diverse PTMs have been detected in MeCP2 protein. Of these sites, Serine(S) 80, Threonine (T) 308 and S 421 can be phosphorylated (blue spheres); Lysine (K) 223 can be SUMOylated (red sphere); and K 464 can be acetylated (orange sphere).

synaptogenesis is promoted in these mutant mice, implying a critical role of S421 phosphorylation in neuronal and brain circuit development[55]. Exploration of the molecular mechanisms has shown that phosphorylation at the S421 site modulates the binding of MeCP2 to the neuronal genome and in the case of BDNF, S421 phosphorylation blocks MeCP2 binding to the BDNF promoter, leading to the activation of BDNF expression under conditions of neuronal depolarization[25]. Consistently, another study showed that MeCP2 protein with the S421A mutation has a higher binding affinity to multiple target-gene promoters to either enhance or repress the expression of the target gene[55].

The Serine 80 site of the MeCP2 protein can also be phosphorylated. However, MeCP2 S80 is heavily phosphorylated under normal conditions while neuronal activity leads to its dephosphorylation[53]. Functional analysis showed that phosphorylation at S80 is essential for MeCP2 binding to the promoters of specific targets, while dephosphorylation induces the dissociation of MeCP2 from gene promoters. In addition, S80 phosphorylation inhibits the intra-molecular interaction of MeCP2, resulting in enhancement of the physical interaction between MeCP2 and DGCR8, and such interaction interferes with the miRNA processing mediated by the Drosha/DGCR8 complex[47].

Recently, researchers have found that MeCP2 represses gene transcription *via* interactions with NCoR/SMRT co-repressor complexes, and MeCP2 phosphorylation is essential for such interactions[30, 31]. Phosphotryptic mapping has identified a novel site, T308, phosphorylation of which is induced by neuronal activity, and functional analysis has revealed that phosphorylation of T308 interrupts the physical interaction between MeCP2 and the NCoR complex, thus inhibiting the transcriptional suppression effect of MeCP2[30].

Besides phosphorylation, other post-translational modifications such as acetylation and SUMOylation also occur in the MeCP2 protein. And functional analysis has shown that MeCP2 acetylation at K464 promotes its binding to the BDNF promoter and enhances the repressive effect of MeCP2 on BDNF expression. Acetylation and deacetylation of MeCP2 are mediated by p300 and SIRT1, respectively[57]. Furthermore, another modification called SUMOylation, in which MeCP2 is modified by small ubiquitin-like modifiers (SUMOs), is essential for the physical interaction between MeCP2 and the HDAC1/2 complex. SUMOylation at MeCP2 K223 is important for the transcriptional-repression effect of MeCP2 and for synaptic development[58].

In sum, phosphorylation, acetylation, and SUMOylation are just three types of post-translational modification and other kinds of modification exist. Exploring their relationships with MeCP2 is important and will deepen the understanding of MeCP2 functions in neuronal development. Furthermore, exploring and evaluating the role of aberrant MeCP2 modifications in RTT pathogenesis will pave the way for the development of treatment for RTT.

MeCP2 in Neurons: Importance for Excitatory and Inhibitory Neurons and Glia

Initially, studies on the effect of MeCP2 on neuronal functions mainly focused on excitatory neurons[24]. Electrophysiological studies showed that long-term potentiation recorded in cortical/hippocampal slices is reduced in *Mecp2*-null mice[59] and enhanced in *Mecp2* transgenic mice[21]. The magnitude of synaptic output in MeCP2-null and transgenic neurons showed that the amplitude/frequency of excitatory postsynaptic currents is inhibited/enhanced under MeCP2 knockout/overexpression conditions[24]. Consistent with these results, further

immunohistochemical analysis revealed that glutamatergic synapse formation is indeed regulated by MeCP2.

In addition to glutamatergic neurons, MeCP2 is also critical for dopaminergic and serotoninergic neurons, as MeCP2 deletion in either type leads to motor impairment and increased aggression, respectively[60]. Furthermore, though researchers initially thought that MeCP2 was only expressed in excitatory neurons, subsequent studies revealed that mice with MeCP2 depletion in GABAergic neurons manifest many of the phenotypes of RTT[61]. All these results confirm that MeCP2 is critical for normal functions of various neuronal types rather than being limited to excitatory neurons.

Neurons are not the only cell type in the central nervous system. In fact, the major type in brain is glia: astrocytes, oligodendrocytes, and microglia. MeCP2 expression was initially thought to be limited to neurons. However, subsequent studies showed that MeCP2 is expressed in both neurons and glia. Importantly, deletion of MeCP2 in astrocytes impairs neural development and functions in a non-cell-autonomous manner[62, 63]. And in support of these observations, MeCP2 re-expression solely in astrocytes in MeCP2-null mice alleviates many of the abnormal features of RTT mouse models, implying the involvement of astrocytes in the pathogenesis of RTT. Thus, targeting astrocytes is promising for the treatment of RTT patients. In addition to astrocytes, dysfunctions of two other glial types, oligodendrocytes and microglia, also contribute to RTT neuropathology[64]. Surprisingly, transplantation of wild-type bone marrow, the source of microglia, markedly arrests the progression of RTT symptoms and further investigations have shown that impaired phagocytic activity in microglia is critical for the development and progression of RTT symptoms[65]. This study also supports the idea that bone marrow transplantation is feasible for the treatment of RTT patients.

Concluding Remarks

Taken together, the findings show that MeCP2 is widely expressed in the central nervous system and not limited to specific cell types, indicating that the pathogenesis of RTT is not just a disruption of neuronal functions but is rather caused by multiple impairments of the central nervous system. The mechanisms behind RTT neuropathology are complicated and what we know is still limited. In addition, the nervous system is composed of brain and spinal cord and the brain is composed of different regions. The functions of MeCP2 in these different structures remain to be elucidated. As the expression of MeCP2 can also be assessed in tissues other than the nervous system, investigating their functions in these tissues will provide more valuable insights into the pathology of RTT.

Initially recognized as a transcriptional repressor, the functions of MeCP2 have been extensively expanded. Multi-layer control of gene expression by MeCP2 is now widely accepted. We believe that a comprehensive understanding of MeCP2 would not only benefit RTT patients but accelerate studies of neural development and other neurodevelopmental disorders, paving the way for the development of effective treatments for patients with neurodevelopmental disorders.

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