

Review

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MeCP2 phosphorylation in the brain: from transcription to behavior

Abstract: Methyl-CpG binding protein 2 (MeCP2), a nuclear protein highly expressed in neurons, was identified because of its ability to bind methylated DNA. In association with the transcriptional corepressor proteins Sin3a and histone deacetylases, it represses gene transcription. However, it has since become clear that MeCP2 is a multifunctional protein involved not only in transcriptional silencing but also in transcriptional activation, chromatin remodeling, and RNA splicing. Especially, its involvement in the X-linked neurologic disorder Rett syndrome emphasizes the importance of MeCP2 for normal development and maturation of the central nervous system. A number of animal models with complete or partial lack of MeCP2 functions have been generated to correlate the clinical phenotype of Rett syndrome, and studies involving different mutations of MeCP2 have shown similar effects. Animal model studies have further demonstrated that even the loss of a specific phosphorylation site of MeCP2 (S80, S421, and S424) disturbs normal maturation of the mammalian brain. This review covers recent findings regarding MeCP2 functions and its regulation by posttranslational modification, particularly MeCP2 phosphorylation and its effects on mammalian brain maturation, learning, and plasticity.

Keywords: DNA methylation; intellectual disability; protein phosphorylation; Rett syndrome; X-linked neurologic disorder.

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Introduction

Methyl-CpG binding protein 2 (MeCP2) is a founding member of a family of DNA-binding proteins that selectively bind to methylated cytosine residues. This

transcriptional modulator is ubiquitously expressed throughout the mammalian body with high abundance in the central nervous system (CNS), particularly in postmitotic neurons (Shahbazian et al., 2002). Mutations in the gene encoding MeCP2 have been identified as underlying 95% of classic Rett syndrome (RTT). Approximately 1 out of 10 000 to 15 000 female newborns is clinically diagnosed with RTT, an X-linked neurologic disorder with a wide range of clinical symptoms, initially described by Andreas Rett (Rett, 1966). Classic (or typical) RTT is characterized by apparently typical psychomotor development to 6–18 months of age. The first symptoms appear during a critical developmental window and include loss of spoken language and purposeful hand usage, which is replaced by stereotypic hand movements. Children with RTT show social withdrawal, especially during the regressive stage, and manifest intellectual disability accompanied by loss of motor abilities. The majority of affected people further exhibit growth anomalies, gastrointestinal problems, breathing and cardiac abnormalities, and seizures (revised diagnostic criteria reviewed by Neul et al., 2010; Neul, 2012).

To understand the role of MeCP2 in the pathogenesis of RTT, a number of mouse models carrying different mutations have been generated that display several phenotypes mimicking RTT symptoms (reviewed by Calfa et al., 2011). Like RTT patients, MeCP2 deletion models exhibit reduced brain weight accompanied by decreased volume of distinct brain areas because of shrinkage in neuronal cell volume but without apoptotic loss of cells (Armstrong et al., 1995; Chen et al., 2001; Nguyen et al., 2012). Deletion of MeCP2 solely from neurons produces the same detrimental neurologic phenotype in mice as germline knockout of MeCP2 (Chen et al., 2001). In addition, transgenic mice with loss of MeCP2 in specific neuronal subpopulations develop RTT-like phenotypes covering a couple of signs that are visible after complete MeCP2 knockout (reviewed by Calfa et al., 2011). This outcome stresses the importance of MeCP2 in neurons; however, loss of MeCP2 function in glial cells seems to be involved in RTT pathogenesis as well (Ballas et al., 2009; Maezawa

and Jin, 2010). In this context, reexpression of wild-type MeCP2 specifically in neurons can ameliorate the RTT phenotype (Luikenhuis et al., 2004). Consistently, conditional tamoxifen-induced restoration of MeCP2 preferentially in astrocytes likewise improves general conditions of MeCP2 mutant mice (Lioy et al., 2011). These findings are particularly interesting regarding therapeutic approaches for RTT because they emphasize the reversibility of the pathologic changes occurring in the CNS. A lack of MeCP2 has detrimental effects, but overexpression of wild-type MeCP2 impairs neurodevelopment and affects synaptic plasticity (Collins et al., 2004; Na et al., 2012; Bodda et al., 2013); thus, MeCP2 expression levels must be controlled precisely in the mammalian brain to improve the RTT phenotype. Chao and Zoghbi nicely summarized the impact of MeCP2 dysfunctions in distinct mouse models, clearly demonstrating a correlation between MeCP2 protein levels and phenotype severity (Chao and Zoghbi, 2012).

Aside from classic RTT, some individuals present developmental regression without necessarily manifesting all of the clinical features for a diagnosis of classic RTT. These ‘variant’ or ‘atypical’ forms of RTT are categorized as preserved speech variant, early seizure variant, and congenital variant (Hagberg and Skjeldal, 1994). Almost all patients with the milder preserved speech variant of RTT carry mutations in *MECP2*, but the genetic bases of the early seizure variant are mutations in the gene encoding cyclin-dependent kinase 5 (*CDKL5*), and most cases of the congenital variant are based on mutations in *FOXG1* (reviewed by Neul, 2012). Very few clinical cases involving the early seizure or congenital variants of RTT have been identified with *MECP2* mutations. In this context, it is interesting that *MECP2* mutations causing classic RTT in females usually lead to a more severe clinical course in males, with severe encephalopathy (Zeev et al., 2002). However, some cases involving males carrying *MECP2* mutations have been reported with a clinical phenotype similar to classic RTT in females (Jan et al., 1999; Meins et al., 2005). In contrast to loss-of-function mutations in female RTT patients, *MECP2* gene duplication has been reported to cause a severe syndromic form of intellectual disability in males (Van Esch et al., 2005). Phenotypic features of *MECP2* duplication syndrome include infantile hypotonia, developmental delay, mental retardation, and absent to minimal speech (Ramocki et al., 2010). Furthermore, a recent study has reported that the core behavioral aspects of autism spectrum disorders are related to *MECP2* duplication syndrome (Peters et al., 2013), which is in line with altered social and anxiety-related behaviors in *MECP2* duplication mice (Samaco et al., 2012). The challenge is to determine where the effects of different mutations

converge at the molecular level to induce MeCP2 dysfunction disorders, which will help guide future therapeutic approaches.

MeCP2 as a transcriptional regulator

DNA methylation was thought to be a mechanism for maintaining the repressed state of chromatin and thus permanently silence the expression of certain genes (Bird and Wolffe, 1999). MeCP2 was identified because of its ability to bind DNA-containing methylated CpG dinucleotides (Lewis et al., 1992), mediated by a methyl-CpG binding domain (MBD) (see also Figure 1). The transcriptional repressor domain (TRD) is necessary and sufficient to interact with the corepressor Sin3a, which in turn recruits the histone deacetylases HDAC1 and HDAC2, resulting in compaction of local chromatin structure and subsequent gene repression (Nan et al., 1998). Initially, numerous studies detected only slight changes in the transcriptional profile of murine models or RTT patients (Colantuoni et al., 2001; Tudor et al., 2002). The most prominent finding was the neuronal activity-dependent binding of MeCP2 to rat brain-derived neurotrophic factor (*BDNF*) promoter III, mediating its transcriptional repression (Chen et al., 2003). Together with the defect in neuronal maturation in murine RTT models, these findings led to the hypothesis that MeCP2 represses a few specific genes involved in neuronal maturation. However, this inference was challenged by a study investigating the expression patterns in the hypothalamus of mice with mutations either leading to deletion or overexpression of MeCP2, respectively. Several hundred genes were altered in the hypothalamus of both animal models (Chahrour et al., 2008). Notably, the comparison of loss-of-function and gain-of-function of MeCP2 demonstrated that gene transcription is obviously not only repressed by MeCP2 but also activated and

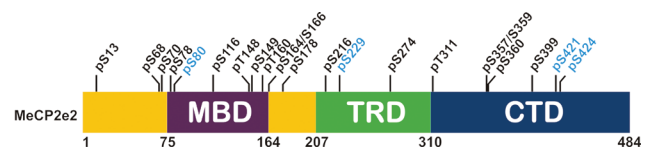


Figure 1 Representation of domains and putative MeCP2 phosphorylation sites of murine MeCP2e2.

CTD, C-terminal domain; MBD, methyl-CpG binding domain; TRD, transcriptional repressor domain. Discussed phosphorylation sites are marked in blue.

MeCP2 was reported to directly bind the promoters of activated genes. Furthermore, MeCP2 was associated with the transcriptional activator cyclic AMP (cAMP)-responsive element binding protein 1 (CREB1). In this context, MeCP2 binding to promoters of activated genes was not associated with recruitment of the transcriptional corepressor Sin3a, explaining MeCP2-mediated transcriptional activation (Chahrour et al., 2008).

Regarding the large number of genes altered by MeCP2 loss-of-function or gain-of-function, it is interesting that MeCP2 is almost as abundant as the histone octamer in the healthy murine brain (Skene et al., 2010) and binds DNA in a genome-wide manner, tracking the presence of 5-methylcytosine (5mC) residues. MeCP2 is supposed to act as a linker histone promoting the reduction of transcriptional noise (Skene et al., 2010), which supports the notion that MeCP2 plays a global role in neuronal chromatin organization (Figure 2A), in contrast to the assumption of gene-specific repression.

MeCP2 binding to hydroxymethylated cytosines: a new level of complexity

A recent study identified MeCP2 as a major 5-hydroxymethylcytosine (5hmC) binding protein in the mouse brain illustrating a new mechanism for regulating chromatin architecture and gene expression (Mellén et al., 2012). Hydroxymethylation of cytosines is widespread on neuronal euchromatin, and specific enrichment of this epigenetic mark is accompanied by depletion of 5mC within these gene regions. In addition, the relationship between these two cytosine modifications depends on the particular cell type, in agreement with the differential DNA methylation patterns and supporting the maintenance of a cell type-specific expression profile (Jaenisch and Bird, 2003; Mellén et al., 2012). Because levels and distribution of 5hmC are similar in wild-type and MeCP2 deletion animals, MeCP2 is not essential for induction or maintenance of 5hmC but is needed to interpret this cytosine modification. MeCP2 binding to 5hmC alters the chromatin organization, as indicated by an increase in micrococcal nuclease-resistant DNA structures after loss of MeCP2 (Figure 2B). This information supports the idea that MeCP2 binding to 5hmC promotes transcription of actively expressed genes by enhancement of global chromatin accessibility (Figure 2A). Mellén and collaborators studied the effects of RTT-causing mutations of MeCP2 on its ability to bind 5hmC

residues. The RTT-causing substitution Arg133Cys (R133C) exclusively disrupts MeCP2 binding to 5hmC without affecting binding to 5mC (Mellén et al., 2012). This distinction supports the idea of a potential role of the newly identified MeCP2 binding capability in the pathophysiology of RTT. Interestingly, X-ray analysis of the MeCP2 MBD-DNA cocrystal identified R133 as one of three MeCP2-MBD residues (R133, D121, and R111) that directly interact with DNA bases (Ho et al., 2008). Nevertheless, a functional analysis of the MeCP2 R133C mutant failed to detect impairments in heterochromatin accumulation or transcriptional repressor activity, emphasizing the idea that pathogenic effects are linked to MeCP2 binding to 5hmC (Kudo et al., 2001).

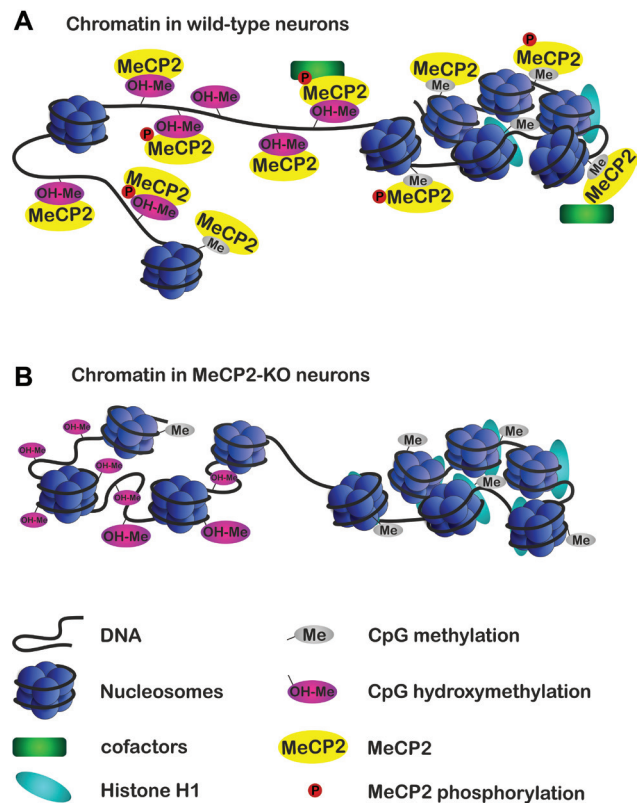


Figure 2 Schematic representation of MeCP2 binding to neuronal chromatin.

A) Methylation of cytosine residues is highly abundant on heterochromatin, the compact chromatin structure. MeCP2 binding to 5mC induces conformational changes in a nucleosome similar to the histone H1, resulting in transcriptional repression. MeCP2 bound to hydroxymethylated residues in less compact euchromatin is probably associated with active gene transcription. B) Loss of MeCP2 leads to a compensatory increase in H1 expression and binding to heterochromatic structures and compaction of usually more accessible euchromatin. It is not yet clear how MeCP2 binding to methylated or hydroxymethylated cytosine residues is influenced by MeCP2 phosphorylation.

These studies demonstrate the importance of MeCP2 for proper regulation of chromatin organization and precisely controlled gene expression. The detrimental effect of loss-of-function or gain-of-function mutations in MeCP2 emphasizes that especially cells of the CNS rely on appropriate MeCP2 protein levels. Moreover, MeCP2-mediated transcriptional regulation is determined by recruitment of particular protein interaction partners such as Sin3a or CREB1. Apart from that, another important aspect of MeCP2-related transcription, especially in terms of neuronal plasticity and homeostasis, is site-specific phosphorylation following neuronal activity.

Dynamic regulation of MeCP2 by phosphorylation

Neuronal plasticity is essential for learning and memory and forms the basis of processing environmental changes in the brain. The role of transcriptional regulation by MeCP2 in neuronal plasticity is not yet resolved, but neuronal activity directly interferes with MeCP2 at the level of posttranslational modification, particularly protein phosphorylation.

MeCP2 pS80 affect DNA binding, gene expression, and protein interaction partners

In response to neuronal activity MeCP2 becomes transiently phosphorylated at S421. Similarly, neuronal activity has been implicated in dephosphorylation of S80 by a not yet identified phosphatase (nomenclature refers to mouse MeCP2 isoform 2) (Zhou et al., 2006; Tao et al., 2009) (see also Figure 1). Both sites are suggested to be critical for MeCP2 promoter occupancy and are altered by the same stimuli. However, dephosphorylation of MeCP2 pS80 does not necessarily coincide with phosphorylation of S421 or vice versa (Tao et al., 2009). The highly conserved phosphorylation of MeCP2 at S80 is mediated by homeodomain-interacting protein kinase 2 (HIPK2) (Bracaglia et al., 2009) (see also Table 1). The interaction of MeCP2 and HIPK2 has been associated with apoptosis *in vitro* and the combined overexpression of both proteins in wild-type and MeCP2-null mouse embryonic fibroblasts results in apoptotic cell death (Bracaglia et al., 2009). What remains to be uncovered is if HIPK2 and MeCP2

fulfill the same function in the developing or mature neuronal system – an especially interesting question in the context of reexpression of wild-type MeCP2 in the brain of MeCP2 deletion models.

As previously mentioned, neuronal activity regulates phosphorylation of MeCP2 at S80. This dynamic regulation potentially explains why immunohistochemically stained MeCP2 shows only incomplete staining of pS80, suggesting that not every single MeCP2 molecule carries this specific phosphorylation (Gonzales et al., 2012). Interestingly, phosphorylation at S80 seems to enhance the RNA-dependent interaction of MeCP2 and the DNA/RNA-binding protein YB-1 (Young et al., 2005; Gonzales et al., 2012). With this interaction, MeCP2 becomes functionally involved in RNA splicing (Young et al., 2005).

Another study analyzed the effects of the phospho-silent S80A MeCP2 mutant on transcriptional regulation in cortical neurons, in which neuronal activity has been suppressed by tetrodotoxin treatment (TTX) (Tao et al., 2009). Loss of pS80 MeCP2 did not severely affect global transcription in neurons under TTX-mediated resting conditions. However, a corresponding MeCP2 S80A knock-in mouse model displayed decreased locomotor activity (Tao et al., 2009). This phenotype resembles the deficits observed in RTT mouse models, revealing a functional relevance of MeCP2 phosphorylation at S80 in behavioral regulation. It is not yet clear if the mouse phenotype results from decreased DNA binding affinity of S80A MeCP2, leading to transcriptional alterations, or from a disrupted ability to interact with functionally essential protein interaction partners, such as YB-1.

Activity-dependent MeCP2 phosphorylation at S421

In vitro studies have shown that neuronal activity-induced dephosphorylation of pS80 MeCP2 and phosphorylation at S421 are both blocked by treatment with the L-type voltage-sensitive calcium channel (L-VSCCs) antagonist nimodipine (Zhou et al., 2006; Tao et al., 2009). The intracellular calcium increase through L-VSCCs or NMDA receptors evokes phosphorylation of MeCP2 S421 by a calcium/calmodulin-dependent kinase (CamKII/CamKIV)-mediated pathway (Zhou et al., 2006; Tao et al., 2009) (see also Table 1).

Previous studies have implied a dynamic control of MeCP2 binding to target promoters by the CNS-specific phosphorylation of S421. This inference predominantly originates from the observation that phosphorylation

Table 1 Summary of functionally relevant MeCP2 serine residues modified by phosphorylation and corresponding animal models.

p-site	Kinase	Changes in phosphorylation status	Protein interaction partner	Chromatin association	Transcriptional regulation	Mouse model phenotype	References
S80	HIPK2	Dephosphorylation upon neuronal activity	YB-1	Flag MeCP2 ^{S80A} decreased promoter binding; <i>Pomc</i> , <i>Gtl2</i> , <i>Rab3d</i> , <i>Vamp3</i> , and <i>Igsf4b</i>	Flag MeCP2 ^{S80A} in TTX-treated cortical neurons: 56 genes ↓ and 149 genes ↑	MeCP2 ^{S80A} -KI: hypoactive	Young et al., 2005; Zhou et al., 2006; Braccaglia et al., 2009; Tao et al., 2009; Huttlin et al., 2010; Gonzales et al., 2012
S229	N.A.	N.A.	SMC3, HP-1	N.A.	N.A.	–	Zhou et al., 2006; Agarwal et al., 2007; Gonzales et al., 2012
S421	CamKII	Phosphorylation upon neuronal activity; behavioral training, cocaine, amphetamine, citalopram, imipramine	N.A.	MeCP2 ^{S421A} -KI: no changes in binding profile detected by ChIP sequencing	MeCP2 ^{S421A} -KI: no differences in number, extent, or time-course of induction of activity-dependent mRNAs	MeCP2 ^{S421A} -KI: increased cortical dendritic complexity, enhanced cortical inhibition, deficit in processing novelty, no antidepressant-induced behavioral adaptation	Zhou et al., 2006; Tao et al., 2009; Huttlin et al., 2010; Cohen et al., 2011; Hutchinson et al., 2011, 2012
S421/S424	N.A.	Phosphorylation upon neuronal activity	N.A.	Flag MeCP2 ^{S421A/S424A} -KI: increased promoter binding; <i>Bdnf</i> , <i>Bmp4</i> , <i>Mef2c</i> , and <i>Grim1</i>	Flag MeCP2 ^{S421A/S424A} -KI: <i>Bdnf</i> ↑, <i>Bmp4</i> ↑ and <i>Grim1</i> ↓	MeCP2 ^{S421A/S424A} -KI: hyperactive, enhanced hippocampal-dependent learning, LTP, and excitatory synaptogenesis	Tao et al., 2009; Huttlin et al., 2010; Li et al., 2011

KI, knock-in; N.A., not analyzed. For details, see text.

precedes the release of MeCP2 from the promoter of *BDNF* resulting in derepression of *BDNF* transcription (Chen et al., 2003; Zhou et al., 2006). Recently, Cohen and collaborators reported that neuronal activation evokes phosphorylation of MeCP2 bound across the genome not only at specific genomic sites, as shown by phosphosite-specific chromatin immunoprecipitation (ChIP) analysis (Cohen et al., 2011).

In the case of phosphorylation-mediated dissociation of MeCP2, the binding profile of MeCP2 to the genome of depolarized neurons would have to be different from that in the resting state. The authors detected no such difference, leading to the conclusion that phosphorylation of MeCP2 at S421 is not enough to induce MeCP2 dissociation from DNA. Moreover, activity-dependent gene expression such as *BDNF* was not affected in a knock-in mouse in which S421 of MeCP2 was mutated to alanine, demonstrating that MeCP2 dissociation is not essential for transcriptional activation. The authors infer that the phosphorylation might contribute to structural changes in chromatin architecture, thus supporting activity-dependent gene expression (Cohen et al., 2011).

Is MeCP2 phosphorylation involved in learning and memory?

Phosphorylation of MeCP2 S421 is essential for normal development of neuronal circuits because loss of activity-dependent phosphorylation of MeCP2 in mice increases the dendritic complexity of cortical neurons and alters the excitation-inhibition balance towards inhibition, which is similar to MeCP2 knockout mice (Dani et al., 2005; Cohen et al., 2011). These changes are accompanied by impaired behavioral responses to novel versus familiar mice or objects (Table 1). Because MeCP2^{S421A} mice show appropriate interest in novel mice, the authors suggested that MeCP2^{S421A} mutation alters processing of novel experience rather than causing a deficit in social recognition. Loss of MeCP2 phosphorylation at S421 did not affect anxiety-related behavior and spatial learning and memory (Cohen et al., 2011).

In this context, another study recently reported a robust increase in MeCP2 S421 phosphorylation in the hippocampus, caused by hippocampus-dependent behavioral training (Li et al., 2011). Mass spectrometric examination of seizure-induced phosphorylation of MeCP2 resulted in the two adjacent sites S421 and S424 becoming newly phosphorylated. A distinct knock-in mouse model was generated, carrying serine-to-alanine

substitutions at both sites (Tao et al., 2009). In contrast to MeCP2^{S80A} knock-in animals, MeCP2^{S421A/S424A} mice exhibit increased locomotor activity but resemble wild-type littermates in anxiety-related behavior or motor coordination (Tao et al., 2009; Li et al., 2011). The behavior of MeCP2^{S421A/S424A} mice was compared with wild-type animals in context-dependent fear conditioning and in spatial learning abilities using Morris water maze test. Surprisingly, in both tests, elimination of neuronal activity-induced phosphorylation resulted in enhanced learning ability. This behavioral phenotype correlates with significantly strengthened NMDA receptor-dependent Schaffer collateral-CA1 long-term potentiation (LTP) and NMDA receptor-independent mossy fiber-CA3 LTP induction in hippocampal slices of MeCP2^{S421A/S424A} mice, accompanied by an increased number of excitatory synapses in cultured hippocampal and cortical neurons. On the molecular level, serine substitutions in MeCP2 to a phospho-silent S421A/S424A mutant increased the promoter occupancy of MeCP2 for distinct genes (Table 1). Notably, the increased promoter occupancy resulted not only in decreased glutamate receptor 1 (*Grm1*) and myocyte enhancer factor 2c (*Mef2c*) transcript levels but also increased *Bdnf* and bone morphogenetic protein 4 (*Bmp4*). This effect is in accordance with the above-mentioned study by Cohen et al. (2011) showing that gene expression does not necessarily require MeCP2 dissociation. Interestingly, Li and coauthors note that the behavioral phenotype and alterations in gene expression resemble those of mice overexpressing MeCP2 (MeCP2^{Tg}) (Collins et al., 2004; Chahrour et al., 2008; Li et al., 2011). MeCP2 overexpression, however, enhances hippocampal LTP only transiently before animals develop pathological symptoms such as seizures and hypoactivity (Collins et al., 2004). It remains unclear if MeCP2^{S421A/S424A} mice develop a similar phenotype with increasing age.

Although behavioral training increases the level of pS421 MeCP2 in wild-type mice, as described in the aforementioned study, the single phospho-silent MeCP2^{S421A} mice exhibited only a behavioral deficit in processing novelty but were otherwise indistinguishable from wild-type animals in spatial learning and memory. As already mentioned, elimination of phosphorylation at S421 and S424 actually results in enhanced hippocampus-dependent learning in MeCP2^{S421A/S424A} mice. Altogether, these data show that further studies are needed to elucidate the relationship between learning and memory and phosphorylation of MeCP2 S421. These findings pose questions regarding the implications of MeCP2 phosphorylation for cellular adaptations.

MeCP2 phosphorylation in drug addiction

Recent work indicates a role for MeCP2 in drug addiction. Drugs of abuse affect neuroplasticity, leading to long-lasting adaptations in the brain reward circuits at the cellular and synaptic levels mediated by altered gene expression (reviewed by McClung and Nestler, 2008). Initially, chronically administered cocaine and extended, unlimited, self-administration of cocaine were reported to increase MeCP2 expression in a number of brain regions, whereas lentiviral-induced knockdown of striatal MeCP2 restricted cocaine intake, suggesting MeCP2 involvement in uncontrolled drug use (Cassel et al., 2006; Im et al., 2010). Cocaine-induced expression of MeCP2 may influence cellular adaptations to repeated drug exposure by different mechanisms. Repeated cocaine treatment, on the one hand, changes the promoter methylation of *CDKL5* and reduces expression in a MeCP2-mediated way, potentially resulting in plastic changes of nucleus accumbens neurons (Carouge et al., 2010). On the other hand, another study presented a model in which cocaine results in transcriptional repression of the microRNAs miR212 and miR132 (Im et al., 2010). The resulting reduction in miRNA-dependent repression of *BDNF* may promote cocaine self-administration by enhanced reward stimuli (Im et al., 2010).

Interestingly, a single acute cocaine injection influences MeCP2 at the level of phosphorylation, eliciting a transient increase in pS421 MeCP2 in the striatum, particularly the caudate putamen and nucleus accumbens (Mao et al., 2011). The same is true for acute injection of amphetamine, resulting in MeCP2 S421 phosphorylation in the nucleus accumbens (Deng et al., 2010). A detailed analysis revealed that MeCP2 is phosphorylated exclusively in the small population of fast-spiking GABAergic interneurons (expressing glutamic acid decarboxylase-67 and parvalbumin). Because psychostimulants such as cocaine and amphetamine act on the monoamine neurotransmitter system, the differential roles of dopamine (DA), serotonin (5-HT), and norepinephrine (NE) have been investigated. Although enhanced NE signaling failed to induce pS421 MeCP2, individual activation of DA or 5-HT sufficiently evoked MeCP2 phosphorylation (Deng et al., 2010; Hutchinson et al., 2011). It turned out that DA and 5-HT independently induce pS421 MeCP2 in selective brain regions and a combination of agents targeting specific classes of DA and 5-HT receptors could alone reproduce the pS421 MeCP2 pattern of amphetamine. The authors suggested that the combinatorial signaling through DA

and 5-HT receptors is integrated at the level of intracellular cAMP, because intrastriatal infusion of forskolin was sufficient to induce pS421 MeCP2 (Hutchinson et al., 2011).

MeCP2 pS421 in mood disorders

Serotonergic neurotransmission plays a central role in behavioral control and emotion. RTT patients experience alterations in aggressive and anxiety-related behavior and emotional conditions (Sansom et al., 1993; Fyffe et al., 2008) accompanied by changes in the activity of aminergic neurons and reduced levels of 5-HT (Samaco et al., 2009). Studying the aminergic system in MeCP2 knock-out animals demonstrated that loss of MeCP2 directly decreases levels of 5-HT and DA (Ide et al., 2005; Samaco et al., 2009). Interestingly, Hutchinson et al. (2012) analyzed the effect of the tricyclic antidepressant imipramine on MeCP2 in wild-type and MeCP2^{S421A} mice. Imipramine induces an increase in 5-HT neurotransmission, and acute injection results in a temporarily increased level of MeCP2 phosphorylation. Like amphetamine treatment, imipramine selectively affects MeCP2 in GABAergic interneurons of the nucleus accumbens. Chronic treatment of wild-type animals results in altered pS421 MeCP2 in the lateral habenula. Furthermore, data from this study indicate that MeCP2 phosphorylation is essential for antidepressant-induced behavioral adaptation, because MeCP2^{S421A} knock-in mice failed to respond to chronic antidepressant treatment (Hutchinson et al., 2012). Because MeCP2^{S421A} animals lack the specific phosphorylation throughout the whole brain, it is not entirely clear if the missing response to chronic imipramine treatment results solely from loss of pS421 MeCP2 in fast-spiking GABAergic interneurons of the nucleus accumbens.

Relevance of posttranslational modifications other than pS80, pS421, or pS424

As noted, Cohen and colleagues postulated that single phosphorylation of MeCP2 is not sufficient to release MeCP2 from the DNA (Cohen et al., 2011). Hence, the roles that other phosphorylation sites of MeCP2 play in transcriptional regulation remain to be determined (Table 2). For instance, phosphorylation of MeCP2 at S229 mediates the interaction of MeCP2 and heterochromatin protein-1 (HP-1) and SMC3, respectively, and loss of pS229 MeCP2

Table 2 Summary of MeCP2 phosphorylation sites identified by mass spectrometric analysis.

p-site	Origin	References
S13	Human Flag-MeCP2	Gonzales et al., 2012
S68	Human	Shiromizu et al., 2013
S70	Mouse	Huttlin et al., 2010
S78	Mouse	Huttlin et al., 2010
	Mouse renal mpkCCD	Rinschen et al., 2010
	Mouse brain	Tweedie-Cullen et al., 2009
	TSC-KO mouse embryonic fibroblast	Yu et al., 2011
S116	HeLa (mitotic phosphorylation)	Dephoure et al., 2008
T148	Mouse brain	Tao et al., 2009
S149	Human Flag-MeCP2	Gonzales et al., 2012
	Mouse	Tao et al., 2009
T160	Mouse brain	Tweedie-Cullen et al., 2009
S164	Mouse brain	Tao et al., 2009; Tweedie-Cullen et al., 2009
S166	Mouse	Huttlin et al., 2010
S178	Human	Shiromizu et al., 2013
S216	Human	Shiromizu et al., 2013
T228	Human embryonic stem cell	Rigbolt et al., 2011
S274	Human Flag-MeCP2	Gonzales et al., 2012
	Mouse brain	Tweedie-Cullen et al., 2009
T311	Mouse	Huttlin et al., 2010
	Human	Shiromizu et al., 2013
S357	Human skin fibroblast cell	Yang et al., 2006
S359	Human skin fibroblast cell	Yang et al., 2006
S360	Human	Shiromizu et al., 2013
	Human skin fibroblast cell	Yang et al., 2006
S399	Human Flag-MeCP2	Gonzales et al., 2012
	Mouse/rat brain	Tao et al., 2009

alters transcriptional properties of MeCP2 in SH-SY5Y cells (Gonzales et al., 2012). The regulation of this specific site of phosphorylation and all remaining target sites of MeCP2 is not yet clear. In addition to phosphorylation, a number of distinct posttranslational modifications have been identified for MeCP2, including acetylation and ubiquitination (Gonzales et al., 2012). Recently, MeCP2 acetylation at K464 (MeCP2e1 isoform) has been demonstrated to occur in cultured cortical neurons (Zocchi and Sassone-Corsi, 2012). This modification is removed by the nicotinamide-adenine dinucleotide (NAD⁺)-dependent histone deacetylase SIRT1. Inhibition of SIRT1 increases MeCP2 acetylation *in vitro*, and ChIP experiments performed on SIRT1 deletion animals showed increased MeCP2 binding to the *BDNF* exon 4 promoter, suggesting that K464 acetylation of MeCP2 affects the association of MeCP2 with the DNA (Zocchi and Sassone-Corsi, 2012). It remains to be investigated how phosphorylation, acetylation, and ubiquitination further influence MeCP2 functions regarding DNA-binding and protein interaction and thus transcriptional regulation.

Conclusion

Although the detailed work on MeCP2 and RTT-causing mutations has provided insights into the pathogenesis of RTT, at the same time, the findings raise new questions regarding the basic functions and the pathologic effects of up-regulation or down-regulation of MeCP2. MeCP2 phosphorylation at S80 and S421 were assumed to alter chromatin association, but recent studies have reported conflicting results. MeCP2 binds in a genome-wide manner, suggesting that it is involved in the global regulation of gene activation and repression. The newly identified binding of MeCP2 to hydroxymethylated DNA might play a major role in MeCP2-mediated transcriptional activation, presumably influencing global chromatin architecture rather than regulating specified genes selectively. Nevertheless, phosphorylation of MeCP2 is essential for proper protein function, and how distinct patterns of phosphorylation alter MeCP2 binding to 5mC and 5hmC residues remains to be investigated.

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