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The Role of MeCP2 in the Brain

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epigenetics, Rett syndrome, methyl-CpG binding domain (MBD), DNA methylation

Abstract

Methyl-CpG binding protein 2 (MeCP2) was first identified in 1992 as a protein that binds specifically to methylated DNA. Mutations in the *MECP2* gene were later found to be the cause of an autism spectrum disorder, Rett syndrome. Despite almost 20 years of research into the molecular mechanisms of MeCP2 function, many questions are yet to be answered conclusively. This review considers several key questions and attempts to evaluate the current state of evidence. For example, is MeCP2 just a methyl-CpG binding protein? Is it a multifunctional protein or primarily a transcriptional repressor? We also consider whether MeCP2, as a chromosome-binding protein, acts at specific sites within the genome or more globally, and in which cell types it is functionally important. Finally, we consider two alternative views of MeCP2 in the brain: as a regulator of brain development or as a factor that helps maintain neuronal/glial function.

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Contents

INTRODUCTION

Methyl-CpG binding protein 2 (MeCP2) is a basic nuclear protein that was identified initially by virtue of its selective binding to DNA sequences methylated at cytosine in the dinucleotide 5′CpG. The protein is present in all vertebrates, including the sea lamprey, a primitive jawless vertebrate (**http://genome.ucsc.edu/cgi-bin/hgGateway?db=petMar1**), but no MeCP2 ortholog has been detected in invertebrate animals or in plants. Among mammals the MeCP2 protein is highly conserved. Sequences from human and mouse, for example, which diverged from a common ancestor ~75 Mya, are 95% identical at the amino acid level. Divergence between mammalian MeCP2 and amphibian or fish MeCP2 is more extensive (33% amino acid identity between human and zebrafish), but conserved domains are apparent. The largest of these is the region implicated in binding to methylated DNA (Nan et al. 1993), which occupies ~100 amino acids near the N terminus. Closely related methyl-CpG binding domains (MBDs) are shared by the MBD protein family, several of whose members also can discriminate methylated from nonmethylated DNA (Hendrich & Tweedie 2003). Another functionally characterized domain is the nuclear localization signal (NLS), which was identified experimentally in rat MeCP2 through deletion analysis and is highly conserved (Nan et al. 1996). A third functionally annotated region corresponds to the transcriptional repression domain (TRD), which was required for repression of a reporter gene construct in transient transfection experiments (Jones et al. 1998; Nan et al. 1997, 1998).

MeCP2 is found in a wide variety of tissues but appears to be most abundant in brain. A quantitative survey normalized against cytoplasmic proteins suggested that lung and spleen are also MeCP2-rich tissues (Shahbazian et al. 2002b). Direct quantification in adult mouse brain has estimated ~16 million molecules of MeCP2 per nucleus in neurons with almost an order of magnitude less in glial cells and 30-fold less in liver cells (Skene et al. 2010). The neuronal MeCP2 level is relatively low at birth but, in the mouse, increases greatly during the first 3 weeks of life before reaching a plateau (Kishi & Macklis 2004, Skene et al. 2010). Because neurogenesis is largely complete before birth, the increase is due to upregulation of MeCP2 expression within a constant number of neurons. These neurons are, however, developmentally active, undergoing synaptogenesis at this time.

The *MECP2* gene is X-linked in mammals and is inactivated on one X-chromosome during dosage compensation in females (Adler et al. 1995). This phenomenon is medically important, as it leads to different outcomes in individuals with *MECP2* mutations depending on gender. As with all sex-linked disorders, *MECP2* mutations affect males much more severely than females owing to hemizygosity. Mutations that severely affect MeCP2 function

usually lead to death of males within 2 years of birth (Schule et al. 2008). These same mutations on one female X chromosome are compatible with life, but mosaic expression of normal and mutated copies of the X-linked *MECP2* gene leads to Rett syndrome (RTT) (Amir et al. 1999). Mosaicism arises owing to random inactivation of one X chromosome; cells silence either the mutated or the normal copy of the gene, which gives rise to a mixed population of functionally MeCP2-positive and MeCP2-negative cells. RTT [Online Mendelian Inheritance in Man (OMIM) #312750] is characterized by apparently normal postnatal development until 6 to 18 months of age. At this stage symptoms appear, including loss of acquired skills such as speech and purposeful hand use, stereotypical movements, acquired microcephaly, seizures, autistic features, and mental retardation (Hagberg et al. 1983). RTT does not normally run in families, as both affected males and females are almost always symptomatic and fail to reproduce. Therefore, the great majority of RTT mutations arise anew in germ cells, usually those of the father (Trappe et al. 2001). In exceptional situations, females are asymptomatic carriers owing to a highly skewed ratio of X-inactivation, whereby the mutant copy of *MECP2* is inactivated in the great majority of cells (Chahrour & Zoghbi 2007, Hoffbuhr et al. 2002). Another rare event is mosaicism in males, which arises owing to acquisition of a new *MECP2* mutation in the early developing embryo, again causing a mixture of MeCP2-positive and -negative cells that results in RTT (Trappe et al. 2001).

MeCP2 is of particular current interest for several reasons. First, RTT is one of a small group of autism spectrum disorders that are overwhelmingly the result of mutations in a single gene (others include fragile X syndrome and tuberous sclerosis). It therefore offers the prospect of a complete account of the disorder from its origin with the molecular lesion to its pathological endpoints. In addition to this biomedical perspective, the role played by MeCP2 is of fundamental interest. The protein appears to link DNA methylation with chromatin structure and therefore mediates epigenetic events that affect genome function. Finally, MeCP2 deficiency affects the brain, which is poorly understood in many respects and is the subject of intense research. Progress is being made on all these fronts (Cobb et al. 2010, Gonzales & LaSalle 2010, Hite et al. 2009, Monteggia & Kavalali 2009), but as things stand, a definitive molecular pathology of RTT remains elusive. This review examines the current state of ongoing efforts, highlighting alternative hypotheses and where possible evaluating them in light of the available evidence.

BINDING TO METHYL-CpG, OR TO CHROMATIN IN GENERAL?

The MBD of MeCP2 shows a strong selectivity for binding symmetrically methylated duplex DNA in vitro (Nan et al. 1993). The X-ray structure of the protein-DNA complex provides a molecular explanation for the preference at atomic resolution (Ho et al. 2008). Specificity for the cytosine methyl group is provided by multiple contacts, several of which are mediated by immobilized water molecules in the major groove of the double helix. A pair of arginine side chains also interacts precisely with G residues within the methyl-CpG dinucleotide, which confirms the specificity of binding to 5-methylcytosine in this DNA sequence context.

In vivo, the primary sites of MeCP2 binding in mouse cells are the heterochromatic foci, which are easily visible by 4′,6 diamidino-2-phenylindole (DAPI) staining (Nan et al. 1996). The foci consist of mouse satellite DNA, which composes nearly 10% of the genome and consists of tandem repeats of a 234–base pair element. The major satellite contains approximately 50% of all genomic CpG dinucleotides, and these are heavily methylated (Hörz & Altenburger 1981). MeCP2 is concentrated in the foci, as would be expected of a methyl-CpG binding protein, but becomes dispersed throughout the nucleus when DNA

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methylation is depleted (Nan et al. 1996). Chromatin immunoprecipitation (ChIP) has confirmed this specificity for DNA methylation at a variety of genomic loci. For example, the methylated maternal allele of the imprinted *U2af1-rs1* gene associates with MeCP2, but the unmethylated allele does not (Gregory et al. 2001). Similarly, an integrated viral transgene attracts MeCP2 when methylated, but a nonmethylated version integrated at the same site fails to do so (Lorincz et al. 2001). Recently, genome-wide ChIP sequencing (ChIP-Seq) data from brain have revealed a DNA-binding profile that tracks DNA methylation density throughout the genome (Skene et al. 2010). A limitation of this study is that the depth of sequencing represented only ~0.5-fold coverage of the mouse genome. As a result, it was not possible to examine MeCP2 binding in specific genomic regions at high resolution.

Although the weight of evidence leaves little doubt that, on average, its MBD preferentially targets MeCP2 to methyl-CpGs in genomic DNA, several studies have questioned this generalization. In particular, early evidence suggesting that MeCP2 preferentially interacts with methylated DNA assembled into chromatin in vitro (Nan et al. 1997) is countered by a later study that found that MeCP2 can associate with and compact nucleosomes even when the DNA is in a nonmethylated state (Georgel et al. 2003). This interpretation received support from a genome-wide analysis of MeCP2 binding in a human neuronal cultured cell line; Yasui et al. (2007) found that MeCP2 binds to nonmethylated as well as methylated DNA and, unexpectedly, MeCP2 appeared to track RNA polymerase II loading across genes. These results do not match those of a subsequent ChIP-Seq analysis in mouse brain (Skene et al. 2010), but the antibody and cell types used in the two studies differ. Of potential relevance, the MeCP2 antibody used in the human cell study was reported to discriminate poorly between protein extracts from wild-type and *Mecp2* knockout mice (less than twofold difference in signal), whereas the mouse brain study used a more specific antibody (>15-fold difference in signal). In spite of these reservations, evidence from ChIP of specific genes indicates that MeCP2 can bind where DNA methylation is absent. For example, the *Slc6a2* gene possesses a nonmethylated CpG island promoter, but MeCP2, together with the ATPase-dependent Smarca2 complex, can be immunoprecipitated with these DNA sequences in mouse cortical neuron cultures (Harikrishnan et al. 2010). When neurons are stimulated by KCl, MeCP2 and Smarca2 are lost from the *Slc6a2* promoter, and histone acetylation increases. The imprinted *H19* gene is methylated on the paternal allele, but MeCP2, plus cohesin and ATRX, unexpectedly immunoprecipitates preferentially with the nonmethylated maternal allele (Kernohan et al. 2010). In both of these cases, MeCP2 appears to be associated with other larger proteins, which may predominate over its tendency to bind methylated DNA. Furthermore, MeCP2 contains highly basic regions whose nonspecific affinity for DNA may facilitate these interactions (Adams et al. 2007). In addition to the highly basic N-terminal domain (amino acids 1 to ~80), two consensus A/T-hook motifs, which bind to the minor groove of AT-rich duplex DNA, may contribute to interactions with DNA. To assess these alternative interpretations, there is a pressing need for high-resolution MeCP2 ChIP-Seq studies in a variety of cell types.

TRANSCRIPTIONAL REPRESSOR OR MULTIFUNCTIONAL PROTEIN?

A study of MeCP2 abundance in neurons (Skene et al. 2010) has shed new light on the role it plays in the brain. Quantification in isolated neuronal nuclei unexpectedly revealed that there are almost as many molecules of MeCP2 (1.6×10^7) as there are nucleosomes (3×10^7) and methyl-CpG moieties (5×10^7). Thus, MeCP2 is sufficiently abundant in neurons almost to coat the chromosome. In line with this expectation, ChIP did not show discrete sites of MeCP2 binding but instead a broad distribution that tracks the density of

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5-methylcytosine. The genome-wide distribution of MeCP2 has consequences for global chromatin structure. Enhanced histone acetylation levels in the MeCP2-deficient brain had been observed previously (Shahbazian et al. 2002a), but separation of neuronal and glial nuclei showed that this was entirely owing to a ~2.5-fold increase in neuronal nuclei (Skene et al. 2010). In this study, histone acetylation levels in nuclei from glia were unaffected by the presence or absence of MeCP2, although an earlier report suggested that MeCP2-deficient astrocyte cultures display histone hyperacetylation (Ballas et al. 2009). These findings suggest that highly abundant MeCP2 significantly affects the acetylation level of the entire chromatin complement. This effect may be indirect, but an attractive possibility is that interactions with corepressor molecules are relevant here (see below). Many corepressors mediate silencing, at least in part, through associated histone deacetylases (HDACs). By recruiting such deacetylase complexes throughout the genome, MeCP2 might globally depress histone acetylation. The sensitivity of MeCP2-mediated repression to trichostatin A, an HDAC inhibitor, supports this idea (Nan et al. 1998).

Another global change brought about by MeCP2 absence is a doubling of histone H1 in neuronal nuclei (Skene et al. 2010). Earlier work showed that MeCP2 competes with H1 for binding to chromatin made with methylated DNA in vitro (Nan et al. 1997). Quantitative analysis suggests that competition is not symmetrical, as MeCP2 displaces H1 from nucleosomes efficiently, whereas H1 is relatively inefficient at replacing bound MeCP2 (Ghosh et al. 2010). These findings have been extended to living cells by fluorescence recovery after photobleaching (FRAP), which showed that histone H1 affects the binding kinetics of MeCP2 (Ghosh et al. 2010). Neuronal nuclei have half the amount of histone H1 seen in other cell types (Pearson et al. 1984). Replacement of 50% of neuronal H1 by the uniquely high concentration of MeCP2 in these cells offers a potential explanation for this phenomenon. In the absence of MeCP2, H1 is presumably free to occupy all nucleosomal sites. The functional consequences of the interplay between MeCP2 and H1 are unknown.

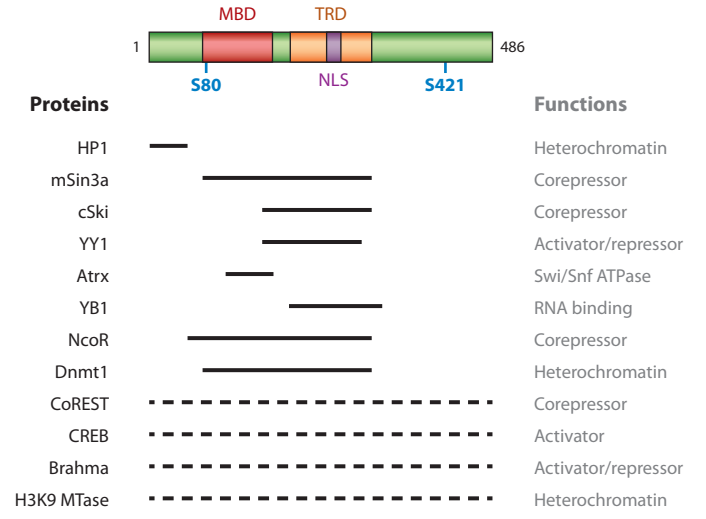

Figure 1

Map of the human methyl-CpG binding protein 2 (MeCP2) showing functionally characterized domains including the methyl-CpG binding domain (MBD), transcriptional repression domain (TRD), and nuclear localization signal (NLS). The diagram is approximately to scale. Below are candidate protein partners of MeCP2 showing the regions (*horizontal lines*) required for the interaction. In no case has the minimal interaction region been defined accurately; these are therefore low-resolution maps. Dashed lines indicate unmapped interactions. The functional significance of each candidate protein is indicated on the right. S80 and S421 indicate sites of serine phosphorylation.

One method to illuminate the function of MeCP2 is to identify the proteins with which it interacts (**Figure 1**). The MeCP2 TRD, which is required for transcriptional repression of transfected reporter genes, binds to several potential MeCP2 partner proteins, including the corepressors mSin3a (Nan et al. 1998) and cSki (Kokura et al. 2001) and the transcription factor YY1 (Forlani et al. 2010). Evidence has been presented that YB1, a protein implicated in transcriptional activation and repression as well as other roles, binds to the TRD via an RNA-dependent interaction (Young et al. 2005). The Swi/Snf family ATPase Atrx binds to MeCP2 within a domain overlapping the MBD, and there is evidence that the A140V mutation, which causes X-linked mental retardation in males, disrupts this interaction (Nan et al. 2007). Localization of Atrx to heterochromatic

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foci in mouse cells is disturbed in the absence of MeCP2. The heterochromatin-binding protein HP1 has also been implicated in binding to the N-terminal domain of MeCP2 (Agarwal et al. 2007). Less well-defined biochemically are reported interactions with the corepressor NcoR, the DNA methyltransferase Dnmt1 (Kokura et al. 2001), and an unidentified H3K9 methyltransferase (Fuks et al. 2002). In addition, binding to the corepressor CoREST (Ballas et al. 2005), the transcriptional activator CREB (Chahrour et al. 2008), and the trithorax-related protein Brahma (Harikrishnan et al. 2005) has been reported but not mapped. Evidence for the last interaction has been questioned (Hu et al. 2006).

As DNA methylation is causally involved in transcriptional repression, methyl-CpG binding proteins are candidates to mediate silencing. Early biochemical evidence supported this notion (Boyes & Bird 1991). In line with this hypothesis, the list of MeCP2 partner proteins includes several corepressors. There is also direct evidence that MeCP2 is able to inhibit transcription both of reporter genes and in natural settings. For example, MeCP2 silenced in vitro transcription of a methylated reporter gene in a HeLa nuclear extract, whereas a nonmethylated reporter remained active. This effect was dependent on an intact MBD (Nan et al. 1997). Using cells from the insect *Drosophila melanogaster*, which contain very little 5-methylcytosine, Kudo (1998) showed that MeCP2 represses transcription in a DNA methylation–dependent manner in vivo. Several additional studies showed that silenced methylated genes are associated with MeCP2, but their reactivation after treatment with the demethylating drug 5-azacytidine coincided with loss of MeCP2 (El-Osta et al. 2002). Recent evidence has strengthened a causal link between MeCP2 binding and transcriptional silencing in vivo. The 5′ untranslated region of L1 retrotransposons binds MeCP2 when methylated, and removal of either MeCP2 or DNA methylation relieves repression (Muotri et al. 2010). Tellingly, MeCP2-deficient mice (see below) exhibit enhanced transposition in the brain, which argues that MeCP2-mediated silencing normally inhibits this process. Repetitive element transcription in general is elevated in the MeCP2-deficient mouse brain, as measured by the abundance of such transcripts in nuclei (Skene et al. 2010). Interestingly, this effect was not detectable in whole-cell RNA, which suggests that these spurious transcripts do not accumulate in the cytoplasm but usually are degraded rapidly.

If the function of MeCP2 is to repress transcription, one might expect its absence to cause overexpression of target genes. As discussed above, elevated transcriptional noise is indeed seen in nuclear RNA from MeCP2-deficient mice. In addition, evidence from several laboratories indicates that histone acetylation is increased in brains from MeCP2-deficient mice (Shahbazian et al. 2002a, Skene et al. 2010). However, analyses of genome-wide mRNA expression, as detailed in the next section, have yielded mixed results. Early data failed to show significant alterations in brain gene expression (Tudor et al. 2002), but later work reported small numbers of misexpressed genes (Jordan et al. 2007, Kriaucionis et al. 2006, Nuber et al. 2005, Urdinguio et al. 2008). More recent expression analysis of mouse hypothalamus and cerebellum detected many increases and decreases in gene expression, most of which were subtle (Ben-Shachar et al. 2009, Chahrour et al. 2008). The majority of these genes was found to be downregulated in the absence of MeCP2 and upregulated when it was overexpressed. The researchers concluded that MeCP2, contrary to expectation, is activating these genes directly or indirectly. In support of this idea, a biochemical interaction between MeCP2 and the transcriptional activator CREB was reported (Chahrour et al. 2008).

Other functional roles have been proposed for MeCP2. One report implied that a long-range interaction between distant regions of the mouse genome was dependent on MeCP2 (Horike et al. 2005). Another showed that MeCP2 interacts with the protein YB1, which has been implicated in regulation of

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alternative splicing. Microarray data supported this hypothesis by showing that alternative splicing is altered in *Mecp2*-null mice (Young et al. 2005). These intriguing proposals have not been pursued in subsequent publications.

A potentially important but poorly understood aspect of MeCP2 is the effect of phosphorylation on its function. Several modified serines and threonines have been identified; of these, two have been investigated further. Serine-421 (see **Figure 1**) is phosphorylated specifically upon neuronal activity (Chen et al. 2003) and has been implicated in dendritic patterning, spine morphogenesis, and the activity-dependent induction of brain-derived neurotrophic factor (*Bdnf*) transcription (Zhou et al. 2006). The interaction between MeCP2 and DNA is altered coincident with S421 phosphorylation, although the mechanisms underlying this effect and its consequences need further investigation. A second site at serine-80, close to the MBD (see **Figure 1**), loses phosphorylation upon neuronal stimulation (Tao et al. 2009). Unlike S421, phosphorylation of this site is not brain specific, but a serine to alanine mutation compromises MeCP2 binding in vivo. Whether this effect is due to the absence of phosphorylation or an effect on protein structure is not known.

The evidence that MeCP2 promotes histone deacetylation, displaces histone H1, and inhibits transcription is derived from several independent laboratories and can be regarded as relatively strong. Key questions remain unanswered, however. What is the role of altered chromatin structure or transcription in the neuron? Is disturbance of these functions at the root of the RTT condition, or are they of secondary importance? We need to learn more about MeCP2 before judging whether its effects occur primarily through transcription, chromatin structure, or other mechanisms as yet uncharacterized.

GENE-SPECIFIC OR GLOBAL CHROMATIN REGULATOR?

Evidence suggests that MeCP2 acts, at least in part, as a transcriptional repressor. Even if it plays a more complex role, the question remains whether its action is important at specific genes or has a more widespread role throughout the genome. The abundance of MeCP2 in the brain and of its binding site, methyl-CpG, makes it unlikely that MeCP2 could act as a classical transcriptional regulator that is targeted to act on a gene by its binding site. However, the requirement for gene-specific binding partners or posttranslational modification of MeCP2 could provide specificity. Target genes would be of great interest, both in determining the molecular causes of RTT and in developing effective treatments.

An alternative view is that MeCP2 binding is important throughout the genome. As it appears to bind wherever DNA methylation occurs (Skene et al. 2010), MeCP2 may be required to reduce aberrant transcriptional events, thus allowing the transcriptional machinery to function efficiently. Aberrant transcription would include apparently random transcription of intergenic regions, genes in cells where expression is not required, and repetitive elements in the genome, which are usually highly methylated and thereby repressed.

The mutation of *MECP2* in RTT and other human disorders has led to intense interest in the expression changes that occur when levels of functional MeCP2 are altered. However, the necessity of using postmortem human brain samples has made it difficult to reach consistent conclusions about gene expression changes in RTT owing to variations in sample quality, brain region, genetic background, and duration of symptoms. These problems have been partially overcome by the development of several mouse models in which the *Mecp2* allele has been modified to prevent production of fully functional MeCP2 protein (**Table 1**). Although mice cannot model all aspects of the human disease, they reproduce many aspects of RTT and are generally accepted as an excellent tool for the study of MeCP2 function. *Mecp2* heterozygous females are the true model for RTT, but most mouse studies have used hemizygous null males, owing to their earlier symptom onset

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Table 1 Mouse models of methyl-CpG binding protein 2 (MeCP2) deficiency and overexpression

Mutation type	Protein produced	Phenotype	References
Targeted exon 3 deletion	mRNA and C-terminal protein fragment can be detected. Lacks MBD, so no longer targeted to methylated DNA.	Hemizygous males have progressive neurological symptoms from 4 weeks, death at 6–12 weeks.	Chen et al. 2001
Targeted exon 3 and 4 deletion	No protein detected; null allele.	As exon 3 deletion.	Guy et al. 2001, Pelka et al. 2006
Targeted nonsense mutation R168X (RTT)	Truncated protein produced; lacks TRD but retains MBD.	As null males.	Lawson-Yuen et al. 2007
Targeted truncation after amino acid 308	Truncated protein produced at approximately wild-type levels. Protein retains MBD and TRD.	Hemizygous males develop progressive neurological symptoms, but 90% survive past 1 year.	Shahbazian et al. 2002a
Targeted missense mutation A140V; non-RTT, X-linked mental retardation in humans	Protein with A140V mutation.	Hemizygous males show no neurological symptoms and normal survival. Increased cell packing density and reduced dendritic arborization in brain.	Jentarra et al. 2010
Targeted floxed *Mecp2* allele	Reduced amount of wild-type protein (approximately 50%).	Mice have late onset of a subset of the null phenotypes but normal life span.	Guy et al. 2001; hypomorphic phenotype described in Kerr et al. 2008, Samaco et al. 2005
Human *MECP2* BAC transgene	Wild-type human MeCP2 protein.	Transgene prevents neurological symptoms on a *Mecp2*-null background. On a wild-type background, overexpression causes neurological symptoms, and 30% of animals die before 1 year.	Collins et al. 2004

Abbreviations: BAC, bacterial artificial chromosome; MBD, methyl-CpG binding domain; RTT, Rett syndrome; TRD, transcriptional repression domain.

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and homogeneous population of MeCP2-negative cells. Despite great advantages over patient samples, the study of MeCP2 in mouse models still provides several challenges. Chief among these is the composition of the brain, as a large proportion of the cells is glia expressing relatively low amounts of MeCP2 (Ballas et al. 2009, Skene et al. 2010). Furthermore, many different types of neurons are interspersed through different brain regions. If MeCP2 has specific targets, but these differ by neuron type, then their identification will prove difficult without further dissection of the tissue.

Several genes are promising candidates for MeCP2 regulation. These include those that have methylation-dependent differential expression such as the imprinted genes (Paoloni-Giacobino & Chaillet 2006). In particular, *UBE3a/Ube3a* has been extensively studied, as it is imprinted in brain (Rougeulle et al. 1998) and there is symptom overlap between RTT and Angelman syndrome, which is caused by UBE3a deficiency (Imessaoudene et al. 2001, Milani et al. 2005). Several conflicting studies have argued for (Makedonski et al. 2005, Samaco et al. 2005) and against (Jordan & Francke 2006, Lawson-Yuen et al. 2007) changes in the expression of *Ube3a* in *Mecp2*-null mice. The *Dlx5/6* gene locus has also proved controversial, both with regard to expression changes in *Mecp2*-null mice and whether this region is actually imprinted

(Horike et al. 2005, Miyano et al. 2008, Schule et al. 2007). Although a role for MeCP2 in regulating certain imprinted genes cannot be ruled out (for example, see Kernohan et al. 2010), MeCP2 does not seem to play a general role in regulating imprinted expression.

One gene, investigated using a candidate approach, that has consistently shown expression changes when MeCP2 is absent is *Bdnf*. BDNF is known to be a key signaling molecule in brain development and plasticity (Cohen-Cory et al. 2010, Greenberg et al. 2009), and its expression is affected in several psychiatric conditions (Tsankova et al. 2007). MeCP2 binds to promoter III of the *Bdnf* gene (Chen et al. 2003, Martinowich et al. 2003), and MeCP2 occupancy is decreased in response to depolarization of primary cultured neurons. Loss of binding, perhaps in response to a site-specific phosphorylation event, may illustrate how global binding of MeCP2 can facilitate gene-specific transcriptional control. Measurement of *Bdnf* transcription rates showed that basal transcription is doubled in the absence of MeCP2, but the level of induced expression is unaffected (Chen et al. 2003). Loss of MeCP2 upon neuronal stimulation therefore makes a relatively minor contribution to *Bdnf* induction. In other systems, MeCP2 has been shown to change its phosphorylation state and/or binding characteristics in response to pain (Geranton et al. 2007, 2008) and early life stress (Murgatroyd et al. 2009). Involvement of MeCP2 in activity-dependent gene regulation in neuronal networks may explain its particular importance in the brain.

As mentioned previously, many studies of global gene expression changes have been carried out using *Mecp2*-null mouse models; these have examined both gene transcripts and microRNAs (**Table 2**). In general, these studies have revealed few large expression changes. For most transcriptional regulators, the way to confirm a gene as a target is to look for specific binding using ChIP. However, MeCP2 is likely to be present on any DNA fragment that contains methylated CpG, so finding MeCP2 at a misexpressed gene does not confirm a primary role in its regulation. Expression changes that occur before onset of overt neurological symptoms are most interesting, as these may play a causative role, whereas those observed after symptom onset may be secondary effects owing to structural changes, compensatory mechanisms, or general ill health of the animal. In studies in which progress of symptoms was followed, few gene expression changes occurred before symptom onset (Jordan et al. 2007, Kriaucionis et al. 2006, Nuber et al. 2005). The suggestion that region-specific changes may be diluted away when studying the brain as a whole has prompted studies that look at single brain regions. These have revealed numerous small changes in gene expression in hypothalamus and cerebellum (Ben-Shachar et al. 2009, Chahrour et al. 2008). However, many of these changes were common to the two regions, which argues against MeCP2 regulation of particular subsets of genes in each region. A second study, again looking at cerebellum, found a few hundred expression changes. The changes varied between time points and mouse models and failed to provide a set of genes consistently affected by MeCP2 deficiency (Jordan et al. 2007). To summarize, global analysis has provided few reproducible changes in gene expression. It is not yet clear whether this is because MeCP2 does not act by regulating expression of individual genes or because such changes cannot be detected reliably using current methodology.

Although studies of postmortem brain have been problematic (see above), the isolation of cloned cell lines from patient blood lymphocytes (Delgado et al. 2006) and skin fibroblasts (Nectoux et al. 2010) has allowed comparison between MeCP2-positive and -negative cells from the same patient, thus eliminating the effects of genetic variation and postmortem artifacts. These cell types are not obviously affected in RTT, however, which makes such studies less informative. Recent work in which neuronal cultures have been prepared from patient cells using induced pluripotent stem cell technology may be the precursor to a more promising system to study the role of MeCP2 in material from

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Table 2 Gene and microRNA expression studies using methyl-CpG binding protein 2 (MeCP2)-deficient mice

Comparison	Tissue studied	Method	Main findings	Reference
Male *Mecp2*-null[a] versus wt. Also *Nestin-Cre* and *CamKII-Cre* cKOs	Forebrain, cortex, hippocampus; pre-, early, and late symptom stages	cDNA microarray	No consistent expression changes (>1.5-fold cutoff) across experiments. Optimal predictor gene expression can be used to predict genotype.	Tudor et al. 2002
Male *Mecp2*-null[b] versus wt	Whole brain; pre-, early, and late symptom stages	cDNA microarray	Eleven genes showing expression changes >twofold. Stress-related genes upregulated early.	Nuber et al. 2005
Male *Mecp2*-null[b] versus wt	Whole brain; before and after symptom onset	cDNA differential display	Eleven genes misregulated at late symptomatic stage, none of these altered before symptom onset.	Kriaucionis et al. 2006
Male *Mecp2*-null[b] versus wt	Cortex, midbrain, and cerebellum at 6–10 weeks	cDNA microarray	No significant difference between brain regions and no genes significantly changed across all regions; 53 genes changed >1.5-fold.	Urdinguio et al. 2008
Male *Mecp2*-null[a,b] versus wt	Cerebellum at 2, 4, and 8 weeks	cDNA microarray	Several hundred genes misregulated across all time points and alleles. Few changes common between the two mutant alleles. Clustering analysis showed closer relationship between littermates than mice of same genotype.	Jordan et al. 2007
Male *Mecp2*-null[b] versus wt and *MECP2* BAC transgenic versus wt	Hypothalamus at 6 weeks	cDNA microarray	Approximately 2,500 genes showed small (>1.15-fold) expression changes across both mouse models. Of these, ~85% were decreased in the null and increased in the BAC transgenic.	Chahrour et al. 2008
Male *Mecp2*-null[b] versus wt and *MECP2* BAC transgenic versus wt	Cerebellum at 6 weeks	cDNA microarray	583 genes with expression changes (>1.2-fold) in both mouse models. Of these, ~70% were decreased in the null and increased in the BAC transgenic, and 242 were also altered in hypothalamus (above).	Ben-Shachar et al. 2009
Male *Mecp2*-null[b] versus wt	Whole brain after symptom onset (6–11 weeks)	miRNA microarray	26% of annotated miRNAs showed altered expression (>1.5-fold), 70% down- and 30% upregulated.	Urdinguio et al. 2010
Male *Mecp2*-null[a] versus wt	Cerebellum at 6 and 8 weeks	High-throughput RNA-Seq of small RNAs	Approximately 120 miRNAs upregulated and 60 downregulated at 6 and/or 8 weeks (>1.5-fold). Majority altered at one time point only, and more changed at 6 than at 8 weeks.	Wu et al. 2010

[a] *Mecp2* exon 3 deletion (Chen et al. 2001).
[b] *Mecp2* exon 3 and 4 deletion (Guy et al. 2001).
Abbreviations: BAC, bacterial artificial chromosome; cDNA, complementary DNA; cKO, conditional knockout; miRNA, microRNA; wt, wild type.

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living donors (Hotta et al. 2009, Marchetto et al. 2010). Marchetto et al. uncovered early alterations in RTT neurons differentiated in vitro. These cells were treated with drugs that caused amelioration of synaptic defects.

Although there is scope for further studies using microdissection, culture of homogeneous neuronal populations, or new technologies such as RNA-Seq, current information questions the usefulness of the target gene concept. Recent studies of MeCP2 binding and its apparent substitution for histone H1 in neurons suggest a global role (Skene et al. 2010). Null neurons produce more primary transcripts from transposable elements, although it is not known whether this increase in transcription occurs only from these methylated repeat elements or also from other intergenic regions, which are perhaps made more favorable by the increase in histone acetylation that has been detected in *Mecp2*-deficient neurons. A global distribution and repressive role for MeCP2 need not rule out its involvement in regulation of individual genes. As with *Bdnf*, a background of repression may be present throughout the genome to ensure that transcription occurs only when required. Relief of repression at key regions may occur when MeCP2 is released in response to signaling pathways activated by neuronal activity. The widespread distribution of MeCP2 in neurons and the variability of gene expression changes offer some support for the view that MeCP2 functions globally. It remains to be seen if the binding of MeCP2 in glia, where it has much lower abundance than in neurons, tracks DNA methylation or if a subset of high-affinity sites becomes apparent.

BRAIN REGION–SPECIFIC DEFICITS OR A COMMON CELLULAR DEFECT ACROSS THE WHOLE BRAIN?

In RTT and its mouse models, all tissues have the same primary defect: They lack MeCP2 expression in approximately 50% of cells (100% in hemizygous null males). This mutation seems to be more important in the brain than in other tissues, as mice that lack MeCP2 only in brain have the same phenotype as those that are *Mecp2*-null in all tissues (Chen et al. 2001, Guy et al. 2001). Another question to be addressed is whether all cell types in the brain and nervous system (both neurons and glial cells) are equally affected by MeCP2 deficiency or whether certain cell types or brain regions are more sensitive. RTT patients and MeCP2-deficient mice show a range of overlapping symptoms that are not linked functionally to a few specific regions of the brain. However, because the brain functions via large networks of neurons and glia, a defect in one cell type is likely to result in deficiencies and compensatory changes in other cells that may not be affected directly by the mutation itself. This problem of cause and effect makes it difficult to untangle which cells are primarily affected by lack of MeCP2.

Anatomically, MeCP2 deficiency causes reduced brain size. This may be due to size decreases in major brain regions such as the frontal and temporal lobes, caudate nucleus, thalamus, midbrain, and cerebellum, all of which have been documented in RTT patients (Armstrong 2001, Reiss et al. 1993, Saywell et al. 2006, Subramaniam et al. 1997). At the cellular level, the neuronal soma is smaller in the absence of MeCP2, and cells are more densely packed (Armstrong 2005, Chen et al. 2001, Kaufmann & Moser 2000, Taneja et al. 2009). More specific abnormalities have been observed at the synapses: Postmortem brain samples from RTT patients or MeCP2-deficient mice present postsynaptic morphological defects such as reduced dendritic branching, reduced dendritic spine density, and defects in spine morphology (Armstrong 2001; Armstrong et al. 1995; Belichenko et al. 1994, 2009; Chapleau et al. 2009; Fukuda et al. 2005; Kishi & Macklis 2004; Schule et al. 2008; Smrt et al. 2007). Presynaptically, lack of MeCP2 is associated with an abnormal number of axons (Belichenko et al. 2009) and a defect in axonal targeting (Belichenko et al. 2009, Matarazzo et al. 2004, Palmer et al. 2008). These results imply a decrease in the number of synapses in RTT brains, which has been confirmed for glutamatergic

synapses in primary neuronal cultures (Chao et al. 2007). Overall, the structural defects described at the synapse would suggest that loss of MeCP2 triggers alterations in the functioning of the synapses and, consequently, of the neuronal networks. However, morphological analyses have focused on neurons and specific cerebral regions, namely the hippocampus and various cortical regions. Therefore, even though it is tempting to assume that the morphological defects described are the same throughout the entire brain, all major brain regions have not been studied systematically.

Analysis of neurotransmission associated with loss of MeCP2 provides further evidence for synapse dysfunction. Postmortem analysis in RTT brains showed altered levels of neurotransmitters such as glutamate and biogenic amines as well as changes in the abundance of some neurotransmitter receptors. Several studies (Armstrong 2001, 2005) contradict these results, however. These discrepancies may be due to technical issues associated with human postmortem brain analysis (see above). In mice, reduced levels of serotonin (5-hydroxytryptamine), adrenaline, and dopamine have been found in the *Mecp2*-null brain (Ide et al. 2005, Isoda et al. 2010, Samaco et al. 2009, Santos et al. 2010). These are associated with regional defects in the expression of key rate-limiting enzymes tyrosine hydroxylase and tryptophan hydroxylase 2 in the brainstem, the substantia nigra, and the raphe nuclei (Samaco et al. 2009, Taneja et al. 2009, Viemari et al. 2005). Analysis of spontaneous miniature excitatory and inhibitory postsynaptic currents indicated a shift in the excitatory/inhibitory (E/I) balance, with increased excitatory and decreased inhibitory neurotransmission in the hippocampus and cortex (Chao et al. 2007, Dani et al. 2005, Nelson et al. 2006, Wood & Shepherd 2010, Zhang et al. 2008). This is supported by data showing pre- and postsynaptic defects of GABA (gamma-aminobutyric acid)-ergic, and therefore inhibitory, neurotransmission in the brainstem (Medrihan et al. 2008). Consistent with the idea of disturbance of the E/I balance, long-term potentiation (LTP) is also altered in the hippocampus of symptomatic MeCP2-deficient mice (Asaka et al. 2006, Guy et al. 2007, Weng et al. 2011). These data, added to the morphological studies, imply that loss of MeCP2 causes malfunction of numerous synapses throughout the brain, which creates less efficient neuronal networks and gives rise to RTT-like phenotypes.

Several mouse models have been used to examine the function of MeCP2 in specific cell types and regions. Cre-LoxP technology has been invaluable in creating mouse *Mecp2* alleles in which the gene can be turned from on to off or vice versa using *Cre* transgenics (**Table 3**). Conditional knockout experiments have combined a floxed *Mecp2* allele (Chen et al. 2001, Guy et al. 2001) with a *Cre* transgene to remove MeCP2 from specific cells, on a background of wild-type cells. *Nestin-Cre* acts in all neurons and glia, and this conditional knockout broadly reproduced the phenotype of the null mouse (Chen et al. 2001, Guy et al. 2001), although breathing defects were significantly less severe (Bissonnette & Knopp 2006). Note that the early lethality of the null mouse prevents the detection of any later symptoms that could result from lack of MeCP2 in tissues other than brain. However, these studies have shown that the cause of the progressive neurological symptoms and death seen in the males originates in the brain.

The severity of the null phenotype may also mask more subtle neurological defects arising from MeCP2 deficiency in particular cell types in the brain. Region-specific conditional knockout studies have revealed subsets of the null phenotype, and also some novel phenotypes not seen in the nulls, that broadly relate to the cells from which MeCP2 was deleted (see **Table 3**). For example, knockout in forebrain excitatory neurons (*CamKII-Cre*) did not affect life span but caused impaired motor coordination, increased anxiety, and impaired social behavior (Chen et al. 2001, Gemelli et al. 2006). In contrast, deletion in regions of the hypothalamus, using *Sim1-Cre*, affected stress response and appetite as well as increasing stranger aggression (Fyffe et al. 2008).

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Table 3 Region- and cell-type-specific methyl-CpG binding protein 2 (*Mecp2*) knockouts and rescues

Name/type	Cells targeted	Phenotype	References
Nestin-cKO	Neurons and glia (from embryonic stages)	As *Mecp2*-null except breathing phenotype	Bisonnette & Knopp 2006, Chen et al. 2001, Guy et al. 2001
CamKII-cKO	Forebrain neurons (postnatally)	Normal life span Impaired motor coordination, increased anxiety, abnormal social behavior	Chen et al. 2001, Gemelli et al. 2006
Sim1-cKO	Neurons in regions of the hypothalamus; lateral olfactory tract (amygdala)	Normal life span Abnormal stress response, hyperphagia, stranger aggression	Fyffe et al. 2008
TH-cKO	Dopaminergic and noradrenergic neurons	Normal life span and mild phenotype (hypoactivity) Reduced DA and NA concentration via reduced expression of TH	Samaco et al. 2009
Pet1-cKO	Serotonergic neurons	Normal life span Increased aggression, hyperactivity Reduced 5-HT concentration	Samaco et al. 2009
Viaat-cKO	GABAergic neurons	Symptom onset at 5 weeks Reduced life span (~50% survival at 26 weeks) Similar phenotypes to null plus overgrooming/self-mutilation phenotype	Chao et al. 2010
Dlx5/6-cKO	Forebrain GABAergic neurons	Normal life span Impaired motor coordination	Chao et al. 2010
Tau-MECP2-rescue	Neurons	*MECP2* knock-in rescues *Mecp2*-null phenotype Neurological phenotype owing to overexpression on wt background	Luikenhuis et al. 2004
Human *MECP2* BAC	All MeCP2-expressing cells	Rescues *Mecp2*-null phenotype Neurological phenotype owing to overexpression on *Mecp2*-wt background	Collins et al. 2004
Removal of floxed Stop cassette from *Mecp2* locus; reactivation of endogenous gene	All MeCP2-expressing cells	Reactivation at 3 weeks prevents symptom onset Reactivation after symptom onset reverses some or all phenotypes	Guy et al. 2007
Activation of floxed Stop-*Mecp2* transgene with various Cre transgenes	*Nestin-Cre*: all neurons and glia *Tau-Cre*: postmitotic neurons *CamKII-Cre*: postnatal forebrain neurons	Life span extended (*Nestin-* and *Tau-Cre* >8 month survival; *CamKII-Cre* lines only 4 weeks longer survival) All rescue lines had improved motor activity at 5 weeks but not at 15 weeks	Giacometti et al. 2007
Activation of Tet-inducible *MECP2* transgene with *Eno2*- or *CamKII*-Tet-VP16 transgenes	*Eno2*: neurons and neuroendocrine cells in striatum and cerebellum *CamKII*: postnatal forebrain neurons	No effect on life span Marginal increase in motor activity for *CamKII* rescue	Alvarez-Saavedra et al. 2007
$Mecp2^{+/-}$ and *CamKII-Mecp2* transgene	MeCP2-positive and -negative forebrain neurons (postnatally)	Combination of transgene with heterozygous *Mecp2*-null allele alleviates symptoms	Jugloff et al. 2008

Abbreviations: 5-HT, 5-hydroxytryptamine; BAC, bacterial artificial chromosome; cKO, conditional knockout; DA, dopamine; NA, noradrenaline; TH, tyrosine hydroxylase.

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Mice that lack MeCP2 in specific neuronal subtypes that reflect neurotransmission systems also have been analyzed. Interestingly, the lack of MeCP2 in neurons expressing dopamine, adrenaline, or serotonin caused the appearance of only a small number of mild phenotypes (Samaco et al. 2009), whereas MeCP2 deficiency in GABAergic neurons (*Viaat-Cre*) recapitulated most of the features displayed by *Mecp2*-null mice, including altered synaptic activity and plasticity (Chao et al. 2010). A possible interpretation is that altered GABAergic neurotransmission is a direct consequence of MeCP2 deficiency that causes a shift in the E/I balance and thereby Rett-like symptoms. Other defects such as altered levels of other neurotransmitters and morphological defects at the synapses would be indirect phenotypes caused by the loss of MeCP2 in GABAergic neurons. Although GABA-positive neurons make up less than 10% of the total, GABA is the major inhibitory neurotransmitter in the brain (Watanabe et al. 2002); therefore, a defect in all inhibitory neurons is likely to affect most neuronal networks, thereby mimicking the situation in the *Mecp2*-null mouse, where all neurons could be defective. In fact, a defect in only inhibitory neurons, rather than both inhibitory and excitatory neurons, may have a more extreme effect on E/I balance and brain function, thus giving rise to the additional phenotypes, such as overgrooming and self-mutilation, that are seen in the *Viaat-Cre* knockout. A specific role for MeCP2 in GABAergic neurons could be confirmed by using the *Viaat-Cre* transgene to reactivate *Mecp2* expression (see below). A resulting robust rescue of most phenotypes would strongly support a specific role for GABAergic neurotransmission in the pathology of MeCP2 deficiency.

Whereas all conditional knockout experiments have been performed in a similar way, studies in which MeCP2 expression was restored have used several different methodologies with varying success and sometimes contradictory results (see **Table 3**). Rescue experiments using either a human *MECP2* genomic bacterial artificial chromosome transgene (Collins et al. 2004) or a *MECP2* complementary DNA (cDNA) knocked into the *Tau* locus (Luikenhuis et al. 2004) were able to rescue the null phenotype. When present on a wild-type background, these transgenes caused neurological symptoms, showing that MeCP2 overexpression is detrimental. Similarly, an increased level of MeCP2, caused by duplication of the gene, is known to cause disease in humans (Ramocki et al. 2010). The *Tau* rescue also suggests that MeCP2 is required only in neurons (but see below).

A floxed Stop allele of *Mecp2* allowed restoration of MeCP2 to wild-type levels, using its own promoter, after Cre excision of the Stop cassette. Restoration of expression in approximately 80% of brain cells at 3 weeks prevented symptom onset, whereas reactivation after symptom onset resulted in a reversal of some or all symptoms as well as a normal life span (Guy et al. 2007). In contrast to reactivation of *Mecp2* from its endogenous locus, studies using inducible transgenes to express MeCP2 after Cre-mediated excision of a Stop cassette were less successful, even when using widely expressed *Nestin-Cre* and *Tau-Cre* transgenes (Giacometti et al. 2007). This indicates that the exact level of MeCP2 protein in each cell type may be crucial. The combination of inducible *Mecp2* transgenes with forebrain-restricted *Cre* transgenes resulted in little or no improvement in symptoms or longevity (Alvarez-Saavedra et al. 2007).

Studies involving the manipulation of MeCP2 levels in mice have not yet demonstrated that any one cell type or region is supremely important in the pathology of MeCP2 deficiency. On the contrary, these studies suggest that expressing MeCP2 at the correct level, in as many cells as possible, is the key to correct brain function. Defects in neuronal morphology and neurotransmission also do not appear to be region specific, but further regional analysis is needed to confirm this. GABAergic neurotransmission seems to be involved in RTT, but no data as yet confirm

whether this disorder is caused specifically by a dysfunction of this subtype of neurons only or by a global dysfunction of most neurons. In either case, efficiency of neuronal networks in the entire brain would be diminished.

IS METHYL-CpG BINDING PROTEIN 2 IMPORTANT IN NEURONS, GLIA, OR BOTH?

Until recently, RTT was regarded as solely neuronal in its pathology. This neuron-centric view arose primarily as a result of two pieces of evidence: Expression of MeCP2 in postmitotic neurons was sufficient to rescue RTT in mice (Luikenhuis et al. 2004), and MeCP2 was not detected in glial cells (Akbarian et al. 2001, Coy et al. 1999, Kishi & Macklis 2004, Shahbazian et al. 2002b). Recent publications have indicated that glial cells may in fact play a role in the pathology of RTT.

Although long regarded as the "glue" of the brain, over the past 20 years an increasing body of evidence has highlighted the role of glial cells in dendritic pruning, tripartite synapse formation, synaptic plasticity, and neurotransmitter release and uptake (Barker & Ullian 2010, Wenker 2010). Karadottir et al. (2008) even suggested that glia are able to generate action potentials. The hypothesis that the neurological phenotypes in mice are solely neuronal was tested by targeting an MeCP2 cDNA to the neuron-specific *Tau* locus (Luikenhuis et al. 2004). The resultant gene knock-in gave rise to levels of MeCP2 expression that were approximately threefold higher than those of endogenous MeCP2 in the brain. Expression of Tau-MeCP2 and appropriate localization were demonstrated in neurons throughout the brain. Overexpression of MeCP2 in animals homozygous for the knock-in transgene was deleterious, as the mice developed a complex neurological phenotype. Appropriate levels of MeCP2 expression, however, appeared to comprehensively rescue the *Mecp2*-null phenotype. Rescued animals survived beyond 6 months of age and did not show any signs of the hypoactivity or tremors seen in null littermates. Expression of Tau-MeCP2 in glia was not investigated and therefore cannot be discounted. Given that reports have detailed Tau expression in some classes of glial cells (Klein et al. 2002), full characterization of the Tau-MeCP2 expression pattern in these mice is important to verify the conclusion that neuronal deficiency of MeCP2 is the exclusive cause of RTT. Behavioral analyses extending beyond 1 year may also be required to establish complete phenotypic rescue.

Ballas et al. (2009) reported MeCP2 expression in glial cells using a novel antibody to facilitate the detection of low levels of MeCP2 in astrocytes. In vitro coculture studies demonstrated that *Mecp2*-null astrocytes had a deleterious effect on both wild-type and *Mecp2*-null neurons, namely fewer and shorter dendrites. Interestingly, conditioned medium from null astrocytes was sufficient to elicit the neuronal phenotype, which points to a secreted factor as the causal agent. This non-cell-autonomous toxic effect could be compatible with RTT pathogenesis; the secreted factor or its depletion could damage both the null and normal neurons in the RTT brain and thus manifest the symptoms. The nature of the secreted neurotoxic factor has not been determined, and in two follow-up papers, Jin and coworkers (Maezawa & Jin 2010, Maezawa et al. 2009) were unable to reproduce the effects seen using conditioned medium from *Mecp2*-null astrocytes. These studies did, however, confirm that MeCP2 is expressed in astrocytes, at 25% of the level seen in neurons, and that coculture of neurons with defective astrocytes had detrimental effects on the neurons. They also reported that the level of MeCP2 in heterozygous long-term astrocyte cultures was only 20% of that seen in wild-type astrocytes, rather than the anticipated 50% level.

Whereas conditioned medium from a *Mecp2*-null mixed glial population (containing both astrocytes and microglia) was able to affect neurons adversely in coculture, conditioned media from a pure astrocyte culture had no

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such effects (Maezawa & Jin 2010). Neurons cultured in conditioned media from *Mecp2*-null microglia, which release fivefold higher levels of glutamate than wild-type microglia, developed shorter and thinner dendrites. Inhibition of glutamate synthesis and release from these cells rescued the neuronal defects. The reason for discrepancies between these studies is uncertain, but both implicate glial cells in the pathogenesis of MeCP2 deficiency.

IS METHYL-CpG BINDING PROTEIN 2 REQUIRED FOR DEVELOPMENT OR MAINTENANCE OF THE BRAIN?

A key question regarding a potential cure for RTT is whether restoring MeCP2 to the brain is sufficient for cells to function normally or whether development in the absence of MeCP2 causes irreversible abnormalities. In the latter case, restoration of MeCP2 may have no effect or may cause further malfunction in a brain that has partially compensated for its absence. MeCP2 seems to be dispensable for early growth and differentiation. In its absence both humans and mice develop apparently normally until after birth, and brain structures appear grossly normal (Chen et al. 2001, Guy et al. 2001, Schule et al. 2008). In vitro, *Mecp2*-null neuronal precursors are no different from wild-type cells in their ability to differentiate into various neuronal and glial cell lineages (Kishi & Macklis 2004). In addition, the accumulation of high levels of MeCP2 only in postmitotic neurons suggests a function in synaptogenesis or maintenance of neuronal function rather than in the initial growth of the brain. The onset of RTT, at 6 to 18 months, coincides with a period of widespread synaptogenesis in the human brain (Huttenlocher & Dabholkar 1997), which is compatible with the view that RTT could be caused by failure to form synapses appropriately. Evidence supporting a role for MeCP2 in synapse formation includes altered glutamatergic synapse numbers in vitro and (transiently) in vivo, abnormal axonal targeting by olfactory neurons, and changes to neuronal morphology in some brain regions (see above). These findings suggest that long-term changes occur in neuronal networks in the MeCP2-deficient brain.

Although the timing of symptom onset in RTT implies a role for MeCP2 in brain development, at least from synaptogenesis onward, mouse models of *Mecp2* deficiency suggest otherwise. In particular, *Mecp2*-heterozygous mice develop normally until adulthood and are able to breed and rear litters, but then develop symptoms as mature adults (Guy et al. 2001, Jugloff et al. 2008). LTP appears normal before symptoms appear at 4 to 12 months but becomes defective thereafter (Guy et al. 2007). The onset of symptoms outside the early developmental period argues for a failure to maintain neuronal function rather than a defect in the developmental setup of neuronal networks in the brain. When comparing mice and humans, the presymptomatic period for female heterozygotes appears to be similar in real time but not developmental time. One possible explanation for these findings is that *Mecp2*-deficient neurons (or neuronal networks) are unable to maintain a fully functional state over time. Once the proportion of poorly functioning neurons reaches a threshold level, symptoms begin to appear and, in the case of heterozygotes, plateau once all MeCP2-negative neurons are affected. The reason for this functional decline is not yet clear and requires further investigation of MeCP2 function at the molecular level, as discussed in previous sections.

The possibility that the effects of MeCP2 deficiency may manifest after development of the brain is complete raises the prospect that restoring MeCP2 protein to these cells could improve their function, even after an absence of months or years of life. Experiments described earlier in this review, using mice that are able to inducibly reactivate *Mecp2* expression, have shown that it is indeed possible to ameliorate the symptoms of deficiency in this way. In addition to reduction of neurological symptoms, these animals show improvements in measures of brain function such as LTP (Guy

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et al. 2007) as well as recovery of neuroanatomical measurements, such as cortical thickness, soma size, dendritic complexity, and dendritic spine density, to wild-type values (S. Cobb, personal communication). This suggests that the adult mouse brain may have sufficient plasticity for it to return to a wild-type state once MeCP2 has been restored.

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