Disease Focus

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Complexities of Rett Syndrome and MeCP2

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Introduction

[1]Rett syndrome (RTT, MIM 312750), originally described by the Austrian pediatrician Andreas Rett, is an X-linked neurodevelopmental disorder that primarily affects girls at a frequency of 1:10,000 live female births (Rett, 1966; Hagberg, 1985; Laurvick et al., 2006; Neul et al., 2010). Greater than 95% of typical RTT is caused by mutations in the gene encoding the transcriptional modulator methyl-CpG-binding protein 2 (*MECP2*) (Amir et al., 1999; Neul et al., 2008). In typical RTT, the child is the product of a normal pregnancy and delivery, and shows apparently normal psychomotor development for the first 6 months of life. Subsequently, these children fail to continue meeting psychomotor milestones and eventually regress, losing hand skills and spoken language. Affected individuals develop repetitive hand stereotypies, autistic features during the regression, and problems with ambulation. After the period of regression, individuals show a post-regression pseudo-stationary phase. Social behavior deficits commonly improve in this stage and in rare cases, some spoken language or hand skills may develop (Zappella et al., 1998; Glaze, 2004). Recent evidence indicates, however, that some autistic behavior may persist (Kaufmann et al., 2011). A variety of additional clinical features also manifest, including anxiety, seizures, growth failure, sleep disturbances, and autonomic dysfunction. Finally, a late motor deterioration stage occurs characterized by scoliosis, worsening dystonia, rigidity, and deterioration in the ability to walk in some people. In many individuals, Parkinsonian-like features such as hypomimia, freezing, and akinesia can develop (Hagberg, 2005; Roze et al., 2007). Longevity studies suggest that despite the overwhelming number of debilitating symptoms, some RTT women may survive until at least the sixth decade of life (Hagberg, 2005; Freilinger et al., 2010; Kirby et al., 2010). Conversely, there clearly is excess mortality in affected individuals, with a yearly death rate of between 1 and 2%, with 25% of all deaths characterized as sudden and unexpected (Kerr et al., 1997).

In addition to those individuals who display the clinically defined classic or typical RTT, there are other atypical or variant RTT individuals who present with some but not all RTT features (Neul et al., 2010). These people commonly have mutations in *MECP2*, such as in the preserved speech variant, but in other variant forms, mutations in distinct genetic loci appear to be causative, such as mutations in *CDKL5* in the early seizure variant or *FOXG1* in the congenital variant (Neul et al., 2010).

Neuropathological studies have not revealed any gross abnormalities in the brains of RTT individuals, although brain weight is reduced and disproportionately smaller in several regions (Reiss et al., 1993; Armstrong, 2005). In addition, the neurons of RTT individuals are smaller and more densely packed, with decreased dendritic complexity (Belichenko et al., 1994; Kaufmann and Moser, 2000; Armstrong, 2005). Importantly, degeneration and atrophy are not observed, establishing the notion that RTT is a postnatal developmental disorder, rather than a neurodegenerative disorder (Jellinger and Seitelberger, 1986; Jellinger et al., 1988; Armstrong, 2005).

Genomic organization and expression pattern of MECP2

The genomic locus of *MECP2* spans ~76 kb, which consists of 4 exons. Alternative 3′-UTR usage leads to a short 1.8 kb and long 10 kb transcript that includes a highly conserved 8.5-kb-long 3′-UTR, with an additional low abundance transcript of ~5–7kb (D'Esposito et al., 1996; Coy et al., 1999; Reichwald et al., 2000). An additional open-reading frame that results in a second *MECP2* 5′-UTR splice variant which encodes the MeCP2e1 iso-

Received Jan. 11, 2011; revised April 1, 2011; accepted April 18, 2011.

This work is supported by the National Institutes of Health (Grant HD062553 to J.L.N.; Grant T32-NS043124 to R.C.S.) and the International Rett Syndrome Foundation. J.L.N. is the Cynthia and Anthony G. Petrello Endowed Scholar of the Jan and Dan Duncan Neurological Research Institute. We are grateful to Christopher McGraw for critical reading of the manuscript.

Sources of additional information include the following: National Institutes of Health–National Institute of Neurological Disorders of the National Institutes of Health (http://www.ninds.nih.gov/disorders/rett/rett.htm), National Institute of Child Health and Human Development (http://www.nichd.nih.gov/health/topics/rett_syndrome.cfm), National Institute of Mental Health (http://www.nimh.nih.gov/health/publications/autism/introduction.shtml), International Rett Syndrome Foundation (http://www.rettsyndrome.org/), and Rett Syndrome Research Trust (http://www.rsrt.org/).

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DOI:10.1523/JNEUROSCI.0169-11.2011

 0270-6474/11/317951-09$15.00/0

form has also been reported (Mnatzakanian et al., 2004).

MeCP2, is a member of the methyl-CpG-binding domain (MBD) family of proteins due to the presence of an MBD (Quaderi et al., 1994; D'Esposito et al., 1996). MBD proteins function to bind symmetrically methylated CpG sites (Lewis et al., 1992; Meehan et al., 1992; Nan et al., 1993). MeCP2 also contains a nuclear localization signal (NLS), and a transcriptional repression domain (TRD) that is important in generating a physical association with the transcriptional corepressor Sin3a, which recruits the histone deacetylases HDAC1 and HDAC2. These histone deacetylases remove acetyl groups from histones, resulting in a compact chromatin structure that represses local gene expression (Nan et al., 1997, 1998; Jones et al., 1998) (Fig. 1*A*). Finally, MeCP2 contains a C-terminal domain for which the molecular function is not entirely defined, but which appears critical due to the large number of disease-causing human mutations that disrupt this region of the protein (Neul et al., 2008). The various protein domains and transcript variants of MeCP2 have been well documented in previous reviews (Singh et al., 2008; Hite et al., 2009).

MeCP2 protein expression is found throughout the body, with abundant expression in the CNS. The onset of MeCP2 expression occurs in a defined pattern during perinatal development, first becoming apparent in the most ontogenetically ancient parts of the brain, such as the brainstem and thalamic regions, and then expressed in progressively more rostral structures during development (LaSalle et al., 2001; Shahbazian et al., 2002b; Braunschweig et al., 2004). No independent function has yet been ascribed to the MeCP2e1 isoform, although it appears to be more highly expressed in human brain and in cultured neurons from mouse compared with other human and mouse tissues, and in the adult mouse brain, it appears to be more highly expressed in the hypothalamus compared with the *Mecp2e2* isoform (Mnatzakanian et al., 2004; Dragich et al., 2007).

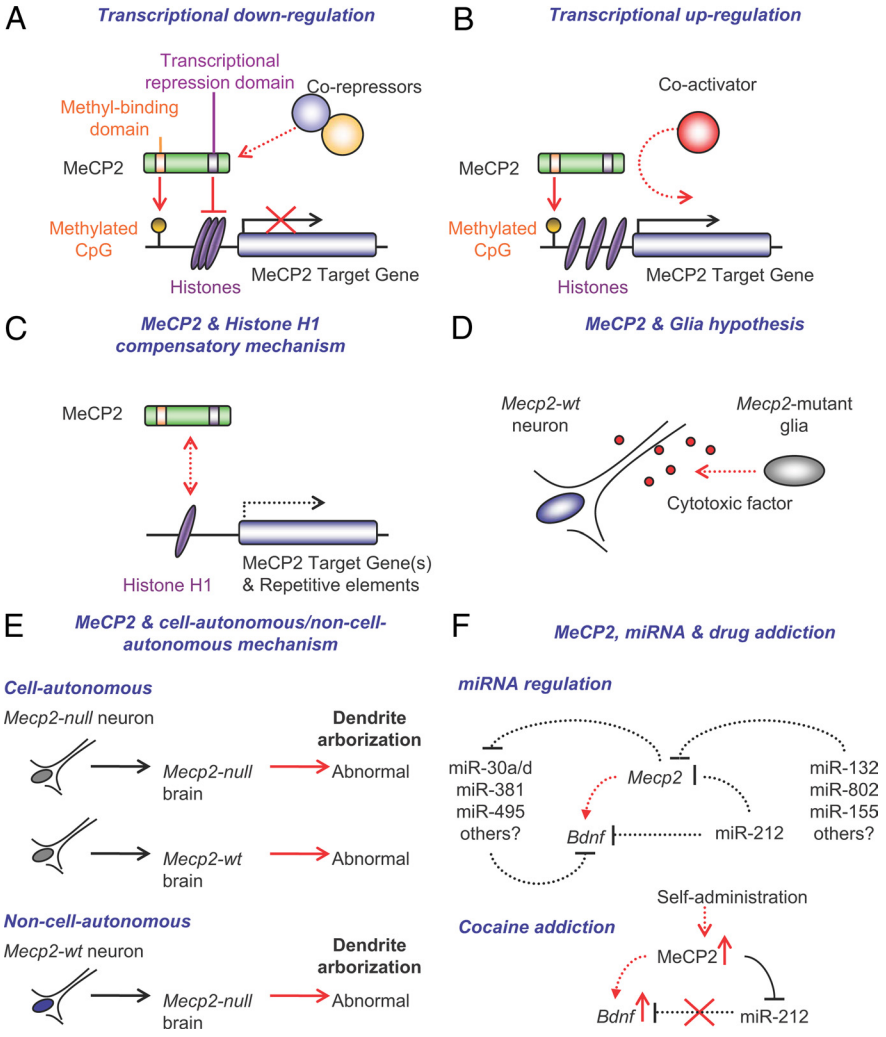

Figure 1. Vignettes of MeCP2 function. ***A***, MeCP2 as a transcriptional repressor. MeCP2 binds to methylated CpG upstream of the transcriptional start site of a MeCP2 target gene. This recruits repressive cofactors to presumably cause local chromatin compaction and transcriptional downregulation. ***B***, MeCP2 as a transcriptional activator. In this case, MeCP2 recruits a transcriptional coactivator to cause the transcriptional upregulation of a target gene. ***C***, MeCP2 and histone H1. The regulation of repetitive elements and perhaps MeCP2 target gene expression may by coordinated by a dynamic interplay between MeCP2 and histone H1. ***D***, MeCP2 and glia. A mutant MeCP2-expressing glial cell (*Mecp2*-mutant) may secrete a cytotoxic factor. This may be deleterious to the normal function(s) of wild-type MeCP2-expressing neurons (*Mecp2*-wt). ***E***, MeCP2, and cell- and non-cell-autonomous mechanisms. Shown is one experimental example of how both cell- and non-cell-autonomous mechanisms are important in the manifestation of RTT phenotypes. The transplant experiments are described in the text. ***F***, MeCP2, miRNAs, and drug addiction. miRNAs have an emerging role in the regulation of both *Mecp2* and *Bdnf*, a MeCP2 target gene. Arrows and blocks indicate activation and repression, respectively. Recent evidence also demonstrates that MeCP2 and miR-212 play important roles in cocaine addiction.

Model systems of MeCP2 function

Mouse models

A number of transgenic mouse models that alter MeCP2 expression have been generated to study the *in vivo* function of MeCP2 (Tate et al., 1996; Chen et al., 2001; Guy et al., 2001; Shahbazian et al., 2002a; Collins et al., 2004; Lawson-Yuen et al., 2007; Tao et al., 2009; Jentarra et al., 2010; Brendel et al., 2011). The neurological phenotypes associated with some of these mouse models have been reviewed previously (Ricceri et al., 2008). The three most widely used animal models of RTT are the *Mecp2*$^{tm1.1Bird}$, *Mecp2*$^{tm1.1Jae}$, and *Mecp2*^{tm1Hzo} mouse models. The *Mecp2*$^{tm1.1Bird}$ and *Mecp2*$^{tm1.1Jae}$ mouse models have been used to study the consequences related to the complete absence of MeCP2 function, although the *Mecp2*$^{tm1.1Jae}$ allele still produces a truncated and modified protein product (Chen et al., 2001; Guy et al., 2001). The *Mecp2*^{tm1Hzo} mouse model was generated to study the effects of partial MeCP2 loss-of-function and harbors a truncating nonsense mutation at amino acid position 308, which spares the MBD, TRD, and NLS (Shahbazian et al., 2002a).

It is noteworthy that a majority of work in the field has focused on the loss of MeCP2 in male mice, which develop a condition similar to that observed in boys with *MECP2* mutations (Schüle et al., 2008). The primary reason for this is because some evidence suggests that non-random X chromosome inactivation (XCI) may lead to phenotypic variability in female animals (Young and Zoghbi, 2004). In light of the fact that typical RTT

is a female disorder, our understanding of the disease would benefit from further efforts to elucidate the complete spectrum of behavioral abnormalities in *Mecp2*-deficient female mice.

Cellular models

A significant challenge in the development of pharmacotherapy for neurodevelopmental disorders such as RTT is the lack of a viable cell-based system to use in high-throughput screening (Jain and Heutink, 2010). Knocking down MeCP2 either in slice culture (Larimore et al., 2009) or in primary neuronal culture (Chapleau et al., 2009) caused changes in spine density and dendritic arborization; however, these primary cultures are not suitable for high-throughput screening. Recently, induced pluripotent stem cells (iPSCs) derived from human RTT patients have been generated and differentiated into neurons (Marchetto et al., 2010). These RTT iPSC-derived neurons have decreased glutamatergic synapses, decreased spine density, and decreased soma size. Treating these cultured neurons with insulin-like growth factor 1 (IGF1) (see below for more details on rationale) improved some of these features. Furthermore, Marchetto et al. (2010) showed that gentamycin can improve some of these structural features in one of the RTT iPSC-derived neuron cultures that possesses a nonsense mutation (Q244X). Aminoglycosides (AGs) such as gentamycin are capable of suppressing such nonsense mutations, allowing the read-through of premature stop codons and the subsequent production of full-length protein. This work demonstrated the proof-of-concept that such RTT iPSC-derived neurons may be useful to test drugs; however, it needs to be determined whether this system can be scaled to the level required for true high-throughput screening methods.

Current perspectives on MeCP2 function

MeCP2 as a transcriptional repressor. . . or activator(?)

The prevailing view of MeCP2 function at the transcriptional level is largely based on a model of methylation-dependent binding and subsequent induction of transcriptional repression (Fig. 1A). In some cases, MeCP2 has been shown to promote transcriptional repression of genes through its recruitment to protein complexes consisting of both the RE-1 silencing transcription factor (REST) and its corepressor, CoREST (Lunyak et al., 2002; Ballas et al., 2005). This is particularly critical for the regulation of the gene expression in non-neuronal cells, and in the cellular transition from a non-neuronal to neuronal state (Lunyak et al., 2002; Ballas et al., 2005). Another important aspect of MeCP2-related transcriptional repression in neurons involves the phosphorylation of specific serine resides of MeCP2. In particular, the highly conserved Ser 80 residue, which can be phosphorylated by the homeodomain-interacting protein kinase 2 (Bracaglia et al., 2009), and the Ser 421 residue, which is phosphorylated via a CaMKII/CaMKIV-dependent mechanism (Zhou et al., 2006), are critical for MeCP2 promoter occupancy of specific target genes and have direct relevance to RTT phenotypes, such as locomotor function (Chen et al., 2003; Zhou et al., 2006; Tao et al., 2009).

In regard to MeCP2 target genes, the initial attempts to identify such targets were heavily influenced by the model that MeCP2 acts solely as a transcriptional repressor, such that only genes whose expression increased in response to the loss of MeCP2 function were characterized. The identification of target genes was an important step toward understanding how mutations in a transcription factor could lead to such devastating and broad clinical features. A number of candidate gene studies were performed based on this rationale; these findings have been reviewed by Chahrour and Zoghbi (2007), and more recently by LaSalle and Yasui (2009).

In an attempt to identify putative target genes in a high-throughput, genome-wide manner, transcriptional profiling studies were performed. These early gene expression studies using either human postmortem tissue or whole brain tissue from *Mecp2*-deficient animals, however, identified only a few, modest gene expression alterations (Colantuoni et al., 2001; Traynor et al., 2002; Tudor et al., 2002; Nuber et al., 2005). Although these data failed to support a role for MeCP2 in genome-wide transcriptional repression, Jordan et al. (2007) reasoned that the sensitivity of detecting gene expression changes may be brain region specific, and may arise in an anatomical region pertinent to the motor abnormalities present in RTT. Transcriptional profiling of RNA obtained from the cerebellum of both *Mecp2*$^{tm1.1Jae/y}$ and *Mecp2*$^{tm1.1Bird/y}$ animals identified several hundred upregulated and downregulated gene expression changes. This was the first *in vivo* report to show a large number of transcriptional changes in the absence of MeCP2. It is noteworthy, however, that analyzing specific brain regions did not always result in uncovering hundreds of gene expression changes; transcriptional profiling of the dentate gyrus granule cell layer of *Mecp2*$^{tm1.1Jae/y}$ resulted in only 13 altered genes (Smrt et al., 2007).

Aside from the fact that the loss of MeCP2 did not cause genome-wide dysregulation of gene expression, one highly significant, yet underappreciated finding from the transcriptional profiling studies was the consistent observation of downregulated genes. Collectively, these studies argued against a single role for MeCP2 in transcriptional regulation, as suggested by Peddada et al. (2006). Given the findings of downregulated gene expression in the absence of a proposed transcriptional repressor, it was conceivable that MeCP2 may also play either a limited or direct role in transcriptional activation (Fig. 1B). In fact, early evidence suggesting this possibility was demonstrated *in vitro* using a reporter construct in which the TRD of MeCP2 could enhance transcriptional activity (Yu et al., 2001). Furthermore, the expression of a reporter construct was positively affected in the presence of both methylation and full-length MeCP2 (Yu et al., 2001). The recent evidence providing solid support for the role of MeCP2 in transcriptional activation was demonstrated by two studies. In the first study, a meta-analysis of transcriptional changes, methylation alterations and MeCP2 promoter occupancy showed that a majority of activated genes were associated with an enrichment of MeCP2 promoter occupancy in SH-SY5Y cells (Yasui et al., 2007). A more disease-related observation was reported in the second study, which used transcriptional profiling of the hypothalamus, a brain region proposed to be responsible for autonomic phenotypes in the mouse models of RTT (Chahrour et al., 2008). The expectation from using both loss- and gain-of-function MeCP2 mouse models (*Mecp2*$^{tm1.1Bird/y}$ and *MECP2*-TG, respectively) was that primary MeCP2 targets would display gene expression patterns that were directionally opposite in the two mouse models. In this context, the effect of MeCP2 on the transcriptional status of a gene could then be genetically defined; genes that were downregulated in the presence of excess MeCP2 and upregulated in the absence of MeCP2 were defined as repressed target genes, whereas genes that were upregulated in the presence of excess MeCP2 and downregulated in the absence of MeCP2 were defined as activated target genes.

Surprisingly, an overwhelming number of genes were found to be altered using this genetic approach, with ~400 genes identified as repressed targets and ~2000 genes identified as activated targets. Some of the target genes that were identified in this study, such as *somatostatin*, *κ-opioid receptor*, and *guanidinoacetate methyltransferase* are involved in processes important for brain function (Reisine and Bell, 1995; Chahrour et al., 2008; Le Merrer et al., 2009; Béard and Braissant, 2010). These target genes are highly interesting, given that their encoded proteins function in pathways that may be targeted by pharmacological means to improve RTT phenotypes.

The identification of both upregulated and downregulated gene expression changes in the hypothalamus has led to an emerging paradigm shift in terms of MeCP2 function. Elucidating the transcriptional function of MeCP2 is further complicated by the fact that recent evidence suggests that MeCP2 may in fact act as a linker histone in neurons, as its levels are as abundant as histone proteins (Skene et al., 2010). An important observation from this study is that neurons only have half the amount of histone H1 compared with non-neurons; in the absence of MeCP2, histone H1 increases in neurons to the level of non-neuronal cells. This is accompanied by a global increase in acetylation state, and the derepression of repetitive elements. These findings indicate a complex interplay between gene expression regulation in neurons that may involve compensatory mechanisms between MeCP2 and histone H1 (Fig. 1C) Although these findings may, to some degree, suggest that the modest gene expression alterations reported by Chahrour et al. (2008) are simply due to the loss of a global transcriptional dampener, they do not explain how genes downregulated in the loss-of-function mouse model are in fact upregulated in the gain-of-function mouse model. More work is needed to determine the relationship between gene expression changes and MeCP2's role as a histone-like protein, if any, and the biological significance of these two major findings in light of disease phenotypes.

MeCP2 in neurons and glia

Mouse models confirmed that the crucial tissue in the pathogenesis of RTT is the CNS because the specific deletion of *Mecp2* from brain using a *Nestin*-Cre resulted in animals that were indistinguishable from the global deletion of *Mecp2* (Guy et al., 2001). Additional experiments removing MeCP2 from specific neuronal populations reproduced several biochemical and phenotypic abnormalities observed in *Mecp2*-null animals (Gemelli et al., 2006; Fyffe et al., 2008; Samaco et al., 2009; Chao et al., 2010). In contrast, restoring MeCP2 function solely within postmitotic neurons rescued the physical appearance, body weight abnormalities, and hypoactivity of animals lacking MeCP2 (Luikenhuis et al., 2004). Together, these experiments demonstrated that MeCP2 function is critical within neurons.

Despite the evidence that MeCP2 plays an important role in neurons, recent work has raised questions about the primary cell types affected in brain that lead to the manifestation of RTT phenotypes. Studies show that MeCP2 may be important within the other cell types within the CNS, specifically in astrocytes and microglia (Ballas et al., 2009; Maezawa et al., 2009; Maezawa and Jin, 2010), despite the low abundance of MeCP2 in these cell types (Skene et al., 2010). In an *in vitro* coculture system, astrocytes or microglia lacking MeCP2 and grown in culture produce a toxic factor that inhibits dendritic arborization from neurons (Fig. 1D). Glutamate is the proposed toxic factor that appears to be overproduced by *in vitro* microglia cell cultures; the molecular details of the proposed toxic factor produced by astrocytes is currently unknown. Additionally, there is *in vitro* evidence that the loss of MeCP2 within astrocytes leads to a gap junction-dependent failure of affected astrocytes to properly support dendritic development. Although these *in vitro* studies present compelling and novel ideas for additional non-cell-autonomous roles of MeCP2 function in the development of pathogenesis, further *in vivo* studies need to be performed to validate the significance of such findings.

Cell- and non-cell-autonomous effects of MeCP2 deficiency

The experiments demonstrating the influence of MeCP2 within non-neuronal cells in the CNS also raise the issue of whether MeCP2 dysfunction leads to strictly cell-autonomous problems or whether there are significant non-cell-autonomous effects in the absence of MeCP2. Solid evidence supports the cell-autonomous function of MeCP2 in the genesis of the molecular, neurochemical, and whole-organism phenotypes (Samaco et al., 2009; Chao et al., 2010). Cellular level abnormalities observed in the global absence of MeCP2 function are also reproduced in a cell-autonomous fashion. For example, in a neuronal transplant study of *Mecp2*-null neurons into the brains of either wild-type or *Mecp2*-null animals, *Mecp2*-null neurons developed dendritic arborization defects regardless of the recipient cortical environment (Kishi and Macklis, 2010) (Fig. 1E). Another example showing the cell-autonomous effects of MeCP2 deficiency was observed in the smaller and hyperexcitable *Mecp2*-null neurons in the locus ceruleus of $Mecp2^{+/-}$ females; these defects were comparable to those observed in *Mecp2*-null neurons from male animals completely lacking MeCP2 (Taneja et al., 2009).

In contrast, there is growing evidence that loss of MeCP2 function also confers a non-cell-autonomous effect. In the same transplant study, dendritic arborization of wild-type neurons transplanted into the brains of *Mecp2*-null animals is abnormal compared with wild-type neurons transplanted into brains of wild-type animals (Kishi and Macklis, 2010). This non-cell-autonomous role for MeCP2 function can also be observed in female animals that are heterozygous for the *Mecp2* mutation, and in brains from RTT individuals. Female brains are mosaic for MeCP2 expression due to non-random XCI, leading to expression of either the normal or mutant copy of MeCP2 (wild-type vs mutant MeCP2-expressing cells). In the brains of *Mecp2* female animals, wild-type MeCP2-expressing neurons have decreased dendritic arborization (Belichenko et al., 2009). In addition, there is evidence in mouse and human that the levels of MeCP2 protein is reduced in wild-type MeCP2-expressing neurons (Braunschweig et al., 2004). Furthermore, although there is evidence for a cell-autonomous role for MeCP2 in the regulation of tyrosine hydroxylase (TH) within the locus ceruleus of $Mecp2^{+/-}$ animals, wild-type MeCP2-expressing neurons have a decreased level of TH (Taneja et al., 2009).

Because the primary role of MeCP2 appears to reside in the nucleus, likely as some kind of transcriptional regulator, it would be expected that there should be a strong cell-autonomous role for this protein. Additional studies, however, are needed to clarify the non-autonomous effects of the loss of MeCP2. It is conceivable that the loss of neurotrophic support from other neurons is responsible for these defects. Decreased levels in the gene encoding brain-derived neurotrophic factor (BDNF) has been reported in *Mecp2*-deficient animals (Chang et al., 2006; Chahrour et al., 2008), which may play a critical role in dendritic arborization, circuit formation and reinforcement, and

function of connected neurons. Another possible source of non-cell-autonomous effects may be due to the loss of MeCP2 in non-neuronal lineages within the CNS (Ballas et al., 2009; Maezawa et al., 2009; Maezawa and Jin, 2010).

MeCP2 in development and CNS patterning

One role of MeCP2 function that has not been extensively explored is the possible role in development and patterning of the nervous system. The reason this has not been explored in depth is that the brain anatomy is grossly normal, albeit reduced in size or smaller, in affected individuals and in the mouse models. However, the fact that obvious developmental abnormalities or degenerative changes have not been reported may be misleading. One aspect of RTT that may be the most challenging problem to address is the phenomenon of developmental regression, which is a key feature of the disorder (Neul et al., 2010). It is conceivable that there is a specific requirement for MeCP2 between 6 months and 1 year of age; in the case of RTT, the brain's demand for MeCP2 during this critical period is unmet due to the absence or lack of functional MeCP2. As a result, the outward clinical manifestation is the loss of acquired skills and the diagnosis of regression. Alternatively, perhaps regression manifests due to the accumulation of MeCP2-related insults during early life, i.e., the adverse molecular and cellular consequences that occur in the absence or lack of functional MeCP2 from conception. The fact that regression occurs in other neurological disorders such as autism (one-fourth to one-third of cases) (Parr et al., 2011), and childhood disintegrative disorder (Homan et al., 2011), may also suggest that such a phenomenon is not specific to MeCP2 dysfunction, but rather, the loss of normal brain activity during this phase of development. Further studies using *Mecp2* mouse models may be useful in addressing such possibilities.

In terms of patterning, a recent detailed analysis within the olfactory system has found that axonal outgrowths are at least transiently abnormal in the absence of MeCP2 function (Degano et al., 2009). Additionally, some axonal pathfinding molecules such as semaphorins are misexpressed in these brains (Chahrour et al., 2008; Degano et al., 2009). Ongoing detailed characterization of specific developmental abnormalities in clearly defined experimental models will be needed to determine the role of MeCP2 in these kinds of developmental decisions.

Mecp2 in addiction

It is now understood that MeCP2 dysfunction is critical in a number of neuropsychiatric disorders (Chahrour et al., 2008). More recent evidence suggests that MeCP2 may also play a role in drug addiction. For example, in a rat model of drug addiction, self-administration of cocaine led to an increase in striatal MeCP2 expression and a concurrent decrease in a microRNA (miRNA). miRNAs are small ~22 nucleotide non-coding regulatory RNAs that bind to recognition sequences located in the 3′-UTR of their cognate protein-coding mRNA target genes to cause mRNA degradation or translational repression (Krol et al., 2010). By decreasing miR-212 levels, BDNF expression was derepressed, presumably enhancing cocaine self-administration in this model system (Im et al., 2010). In addition to miR-212, several other MeCP2-regulated miRNA that can also repress BDNF expression, such as mir-30a/d, mir-381, and mir-495, were recently identified (Mellios et al., 2008; Wu et al., 2010), indicating the possibility of additional miRNA that may play a role in MeCP2-related phenotypes such as addiction (Fig. 1F). Further support for a role for MeCP2 in drug addiction was shown in a complementary study using *Mecp2*^{Hzo}-mutant animals (Deng et al., 2010). *Mecp2*^{Hzo} mice chronically exposed to amphetamine displayed altered behavioral reward responses. Additionally, wild-type mice chronically exposed to amphetamine show increased phosphorylation of MeCP2 at serine 421 in GABAergic inhibitory neurons of the nucleus accumbens. These findings suggest that increasing phospho-MeCP2 levels in this specific neuronal population could lead to an imbalance in synaptic plasticity driving the stimulant-induced behavioral abnormalities. More work is needed to further delineate the role of MeCP2 in drug addiction, and perhaps in other neuropsychiatric conditions that involve obsessive-compulsive behavior.

Therapeutic approaches for RTT

The ultimate goal of RTT research is to sufficiently understand the disease to design methods of treatment. To date, only three controlled clinical trials in RTT individuals have been reported, and all studies showed some, but no dramatic, effects. These trials involved the administration of the opioid antagonist naltrexone (Percy et al., 1994), the amino acid L-carnitine, important in fatty acid metabolism (Ellaway et al., 1999), and folate-betaine, which increases the available pool of methyl-donors (Glaze et al., 2009). Here, we discuss some areas of research in the field that could potentially lead to the development of promising beneficial therapies.

Work in MeCP2 mouse models has been instrumental not only in understanding the function of MeCP2, but in suggesting possible therapeutic options by identifying neurochemical and molecular changes that occur in the absence of MeCP2 function. For example, mice lacking MeCP2 have decreased expression of BDNF, and genetically increasing BDNF levels improves survival in these animals (Chang et al., 2006), which has led to the exploration of treating animals with BDNF. To pharmacologically increase BDNF, *Mecp2*-null animals treated with the ampakine drug CX546, which increases BDNF levels in the nodose cranial sensory ganglia, caused an improvement in respiratory frequency and minute volume (Ogier et al., 2007). Also with the notion of increasing BDNF levels, administration of a tripeptide form of IGF1, which can regulate BDNF levels, caused global improvements in the overall condition and longevity of *Mecp2*^{Jae} mice (Tropea et al., 2009). Because serotonin levels are decreased in people with RTT and animal models (Samaco et al., 2009), the role of serotonin receptor 1a agonist 8-OH-DPAT combined with a GABA reuptake inhibitor, NO-711, was tested. This combination improved the breathing abnormalities in *Mecp2*-deficient animals (Abdala et al., 2010). Last, chronic L-dopa administration, with the goal to correct the dopamine and norepinephrine deficits (Samaco et al., 2009), improved the motor deficits in *Mecp2*-null mice (Panayotis et al., 2011).

Another possible approach in treating the symptoms of RTT includes strategies to increase the levels of MeCP2. Postnatal reactivation of MeCP2 restored the general health condition, LTP defects, and viability in mice, raising the possibility that gene therapy approaches to increase MeCP2 levels in RTT individuals may be beneficial (Guy et al., 2007). However, the observations that overexpression as well as the partial loss of function of MeCP2 in cells can be detrimental has raised concerns about the viability of gene therapy in RTT, given that affected females have a proportion of cells that still express a normal version and amount of MeCP2 due to XCI (Collins et al., 2004; Samaco et al., 2008).

Attempts to control the increase in expression of MeCP2 in a mosaic population of wild-type and mutant MeCP2-expressing cells could be challenging. Therefore, an indirect approach to increase MeCP2 expression may be necessary, perhaps through decreasing the levels of miRNA that target MeCP2. Thus far, several miRNAs have been demonstrated to target MeCP2, including miR-132, miR-212, mir-802, and miR-155 (Klein et al., 2007; Im et al., 2010; Kuhn et al., 2010; Wada et al., 2010). The long 3′-UTR of MeCP2 harbors >50 putative miRNA binding sites (Hon and Zhang, 2007). By reducing the expression of such miRNAs in wild-type MeCP2-expressing neurons of *Mecp2*$^{+/-}$ animals, one could test the hypothesis that increasing the levels of MeCP2 protein may partially compensate for the loss of MeCP2 function in mutant MeCP2-expressing neurons. The non-cell-autonomous increase in MeCP2 expression may not, however, be beneficial in a mosaic population of cells. Furthermore, given that neuronal phenotypes are sensitive to MeCP2 levels, such an approach must be well controlled to normalize MeCP2 expression.

Recent studies have also shown that a number of miRNAs are dysregulated in *Mecp2*-null animals (Urdinguio et al., 2010; Wu et al., 2010). Although the biological significance of these changes may also reflect the transcriptional noise that occurs in the absence of MeCP2 as a transcriptional dampener, it is possible that a few miRNAs may cause a cascade of transcriptional changes relevant to disease pathogenesis. One could envision that designing therapies that target the downstream targets of affected miRNAs in these studies could possibly improve RTT phenotypes.

Another method to increase the expression of MeCP2 relies on the previously described "read-through" compounds that suppress nonsense mutations and allow the production of full-length protein. Because a significant fraction of RTT disease-causing mutations in *MECP2* are nonsense mutations, this strategy could prove valuable. AGs such as gentamycin are capable of suppressing nonsense mutations. Recent work has demonstrated that AGs can suppress such nonsense mutations in *MECP2* both in cell culture and in fibroblasts derived from a mouse expressing an allele of *Mecp2* containing a nonsense mutation (Brendel et al., 2011). Among the unfortunate side-effects of AGs, however, are nephrotoxicity and ototoxicity (Houghton et al., 2010). Further work will be needed to determine whether these or other novel compounds with reduced toxicity but conserved read-through properties can work sufficiently well *in vivo* to rescue the phenotypic abnormalities of this mouse model.

As detailed *in vivo* work on the role of MeCP2 progresses, one of the striking observations is the variation in the cellular phenotypes observed, depending on the specific cell studied as well as the overall cellular milieu. For example, although somatosensory cortical neurons show decreased firing rates and decreased miniature EPSCs (mEPSCs), neurons within the brainstem, such as the locus ceruleus, and neurons in the nucleus of the solitary tract show increased firing rates and increased mEPSCs (Dani et al., 2005; Kline et al., 2010). These differential findings raise a note of caution both for the overgeneralization of specific cellular phenotypes observed in one neuronal population and, importantly, for the consideration of possible therapeutic strategies. For example, one might postulate that because there appears to be a decrease in the excitatory/inhibitory balance within the sensory cortex, therapies should be targeted toward decreasing inhibitory signaling or increasing excitatory signaling. However, while such a strategy might prove beneficial for cortical functioning, it might be detrimental for critical brainstem functions such as breathing.

Future perspectives

Dramatic progress has occurred in the decade since the discovery of RTT-causing gene, providing insight into the pathogenesis of the disease as well as animal and cellular models that are useful in testing possible therapeutic options. However, a number of important basic as well as clinically relevant questions remain. For example, what is the cause of one of the most distinctive features of RTT, the regression? Furthermore, do the animal models have any regression, or do they simply display fixed phenotypic abnormalities? On a cellular level, what is the nature of the non-cell-autonomous effects observed, and how does MeCP2 function in non-neuronal cells within the CNS to modulate disease? Finally, what is the exact nature of the molecular function(s) of MeCP2? Although these broad questions will take intensive investigations to fully understand the complexities of RTT, the progress made thus far offers hope that many of these questions will be tractable and that the understanding that arises from this knowledge will help guide future treatment opportunities.

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