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Review

The Story of Rett Syndrome: From Clinic to Neurobiology

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The postnatal neurodevelopmental disorder Rett syndrome (RTT) is caused by mutations in the gene encoding methyl-CpG binding protein 2 (MeCP2), a transcriptional repressor involved in chromatin remodeling and the modulation of RNA splicing. *MECP2* aberrations result in a constellation of neuropsychiatric abnormalities, whereby both loss of function and gain in *MECP2* dosage lead to similar neurological phenotypes. Recent studies demonstrate disease reversibility in RTT mouse models, suggesting that the neurological defects in *MECP2* disorders are not permanent. To investigate the potential for restoring neuronal function in RTT patients, it is essential to identify MeCP2 targets or modifiers of the phenotype that can be therapeutically modulated. Moreover, deciphering the molecular underpinnings of RTT is likely to contribute to the understanding of the pathogenesis of a broader class of neuropsychiatric disorders.

Introduction

Two severely disabled young girls were relentlessly wringing their hands as they sat in the laps of their respective mothers in the waiting room of a Vienna pediatric clinic. This coincidental occurrence prompted Dr. Andreas Rett to search for more patients with the same unusual behavior. A year later, in 1966, Dr. Rett described similar findings in 22 patients, reporting for the first time a unique clinical entity that now bears his name (Rett, 1966). But it would not be until 17 years later that Rett syndrome (RTT) became recognized in the medical community when Dr. Bengt Hagberg, a Swedish neurologist, and his colleagues reported 35 cases of RTT in the English language (Hagberg et al., 1983), and recognized this clinical entity as a condition "similar to a virtually overlooked syndrome described by Rett in the German literature."

In addition to the worldwide recognition of RTT, the 1980s witnessed major strides in another field, namely DNA methylation. For the first time, a connection was established between DNA methylation and heritable changes in gene expression. Scientists identified the CpG dinucleotide to be the site of almost all DNA methylation in mammalian genomes and began to explore the effects of this modification on gene activity. CpG islands were found to be unmethylated in the native state (with the exception of sequences on the inactive X chromosome), and methylation of CpGs was associated with gene silencing and alterations in chromatin structure. Two mechanisms have been demonstrated for DNA methylation-mediated gene repression. The first suggests that methylation of CpG sites within gene promoters will inhibit sequence-specific binding of transcription factors. In the second, more prevalent mechanism, the repression is mediated by proteins that specifically bind to methylated CpGs (methyl-CpG binding proteins) and alter chromatin structure, rendering it inaccessible to members of the transcription machinery. In 1992, Dr. Adrian Bird and his colleagues identified a novel mammalian protein that binds methylated CpGs, methyl-CpG binding protein 2 (MeCP2) (Lewis et al., 1992). The gene encoding MeCP2 was localized to the X chromosome in the mouse (Quaderi et al., 1994), and the protein repressed transcription in vitro (Nan et al., 1997). Soon afterwards, additional members of the methyl-CpG binding protein family were identified and included methyl-CpG binding domain proteins (MBD) 1–4 (Hendrich and Bird, 1998).

Meanwhile, as the DNA methylation field was deciphering repression mechanisms mediated by methyl-CpG binding proteins, the RTT community was trying to understand the pathophysiology of this puzzling condition. The turning point for RTT research came with the discovery of its genetic basis in 1999. The surprising finding that mutations in the widely expressed *MECP2* are at the root of RTT (Amir et al., 1999) brought together the fields of epigenetics and neurobiology.

Clinical Features of RTT

RTT (MIM 312750) is a postnatal progressive neurodevelopmental disorder that manifests in girls during early childhood; however, not all the symptoms are prominent initially, but rather appear over stages (Figure 1). Patients with RTT appear to develop normally up to 6–18 months

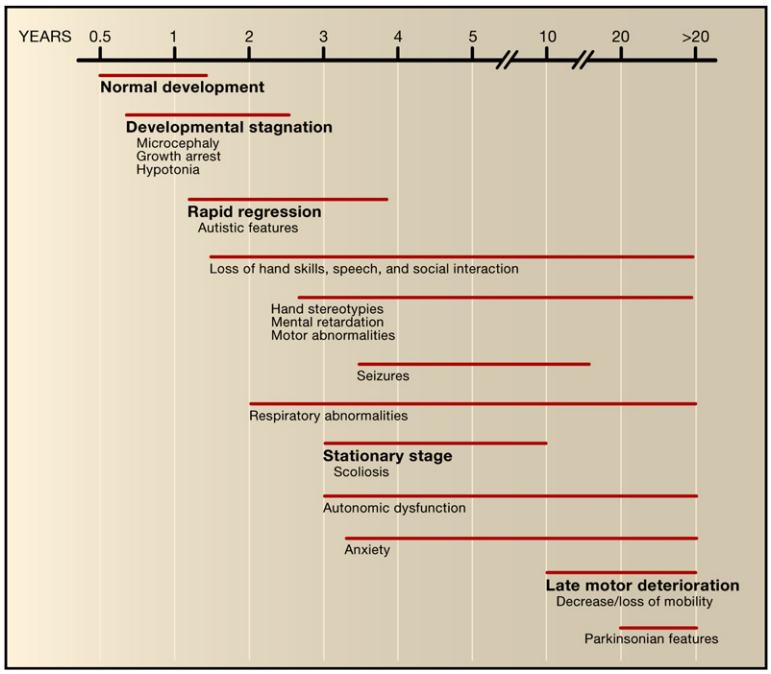

Figure 1. Onset and Progression of RTT Clinical Phenotypes

After a period of normal development, a healthy-looking baby girl falls into developmental stagnation, followed by rapid deterioration, loss of acquired speech, and the replacement of purposeful use of the hands with incessant stereotypies, a characteristic of the syndrome. Patients also develop social behavior abnormalities and are often misdiagnosed as having autism. The condition worsens with loss of motor skills and profound cognitive impairment. In addition, patients suffer from anxiety, seizures, and a host of autonomic abnormalities.

of age. The child with RTT seemingly achieves appropriate milestones, including the ability to walk, and some patients even say a few words. One early indicator of neurological involvement is deceleration of head growth, leading to microcephaly by the second year of life. With the onset of developmental stagnation, the acquired microcephaly is accompanied by general growth retardation, weight loss, and a weak posture brought on by muscle hypotonia. As the syndrome progresses, patients lose purposeful use of their hands and instead develop stereotypic hand wringing or washing movements, and in some cases clapping, flapping, and mouthing of the hands. Social withdrawal and loss of language become apparent in addition to irritability and self-abusive behavior. Other autistic features also manifest, including expressionless face, hypersensitivity to sound, lack of eye-to-eye contact, indifference to the surrounding environment, and unresponsiveness to social cues (Nomura, 2005). The onset of mental deterioration is accompanied by loss of motor coordination and the development of ataxia and gait apraxia. The earliest autonomic perturbation is hyperventilation during wakefulness. Most girls with RTT suffer additional breathing anomalies, including breath-holding, aerophagia, forced expulsion of air and saliva, and apnea. One of the most arduous features of RTT is the occurrence of seizures, which range from easily controlled to intractable epilepsy, with the most common types being partial complex and tonic-clonic seizures (Jian et al., 2006). The seizures tend to decrease in severity after the teenage years and into adulthood, presenting minor problems after the age of forty. Amelioration of the social component of the autistic-like behavior occurs sometime between 5 to 10 years of age. Despite having a normal appetite, patients continue to lose weight and many suffer from osteopenia, scoliosis, and rigidity as they age. Behavioral abnormalities during this postregression phase include teeth grinding, night laughing or crying, screaming fits, low mood, and anxiety episodes elicited by distressful external events (Mount et al., 2001). Patients suffer devastating motor deterioration, generalized rigidity, dystonia, and worsening of scoliosis. Most girls with RTT lose mobility, and are often wheelchair-bound during the teenage years. Additional autonomic abnormalities include hypotrophic, cold blue feet, severe constipation, oropharyngeal dysfunction, and cardiac abnormalities, including tachycardia, prolonged corrected QT intervals, and sinus bradycardia. As patients get older they often develop Parkinsonian features (Hagberg, 2005; Roze et al., 2007). The condition reaches a plateau and some patients survive up to the sixth or seventh decade of life in a severely debilitated physical condition.

Genetic Basis of RTT

Given that the vast majority of patients with RTT are females, early reports postulated an X-linked dominant mode of inheritance with fateful consequences in hemizygous males. RTT has an incidence of ~1/10,000 female live births. However, since more than 99% of RTT cases are sporadic, it was very hard to map the disease locus by traditional linkage analysis. Using information from rare familial cases, exclusion mapping identified the Xq28 candidate region, and subsequent screening of candidate genes in RTT patients revealed mutations in *MECP2* (Amir et al., 1999). Mutations in *MECP2* are found in more than 95% of classic RTT cases; most arise de novo in the paternal germline and often involve a C to T transition at CpG dinucleotides (Trappe et al., 2001; Wan et al., 1999). The spectrum of mutation types includes

missense, nonsense, and frameshift mutations, with over 300 unique pathogenic nucleotide changes described (Christodoulou et al., 2003), as well as deletions encompassing whole exons (Archer et al., 2006b; Pan et al., 2006; Ravn et al., 2005). Eight missense and nonsense mutations account for ~70% of all mutations, while small C-terminal deletions account for another ~10%, and complex rearrangements constitute ~6%. Several phenotype-genotype correlation studies have been reported and some general conclusions can be made. Mutations affecting the NLS of MeCP2 or early truncating mutations tend to cause more severe phenotypes than missense mutations, whereas C-terminal deletions are associated with milder phenotypes (Smeets et al., 2005). In addition, the R133C mutation causes an overall milder phenotype (Kerr et al., 2006; Leonard et al., 2003; Neul et al., 2007), while the R270X mutation is associated with increased mortality (Bienvenu and Chelly, 2006). Because several mutations introduce premature stop codons throughout the gene and are predicted to result in a null allele, RTT results from loss of MeCP2 function. However, hypomorphic alleles may also occur and result, for instance, in truncated forms of MeCP2 that retain partial function.

Phenotypic Variability in RTT

Atypical forms of RTT that deviate from the classical clinical presentation occur. These variants range from milder forms with a later age of onset to more severe manifestations. Of the milder variants, the "forme fruste" (or "worn-down form") has a later age of onset compared with the classical form, with regression occurring between 1 to 3 years of age; hand use is sometimes preserved with minimal stereotypic movements. The preserved speech variant is another benign form of RTT characterized by the ability of patients to speak a few words, although not necessarily in context. Patients with this variant have a normal head size and are usually overweight and kyphotic (Zappella et al., 2001). The more severe variants include the congenital form that lacks the early period of normal development, and a form of classical RTT with onset of seizures before the age of 6 months. Classical and atypical RTT phenotypes vary in severity and onset between different patients and in the same patient over time.

A major source of the phenotypic variability associated with different *MECP2* mutations in females is the pattern of X chromosome inactivation (XCI). In females, only one of the two X chromosomes is active in each cell and the choice of which X chromosome is active is usually random, such that half of the cells have the maternal X chromosome active and the other half have the paternal X chromosome active. Therefore, a female with a *MECP2* mutation is typically mosaic, whereby half of her cells express the wild-type *MECP2* allele and the other half express the mutant *MECP2* allele. Occasionally, cells expressing the wild-type *MECP2* allele divide faster or survive better than cells expressing the mutant allele, which therefore results in a nonrandom pattern of XCI and amelioration of the RTT neurological phenotypes. Depending on the extent of such favorable skewing, some patients can be mildly affected or are even asymptomatic carriers of *MECP2* mutations. The latter cases are usually identified because of the occurrence of RTT in their offspring. The best examples for illustrating the dramatic effects of XCI patterns in RTT are monozygotic twins who manifest very different phenotypes (Dragich et al., 2000). In addition, skewed XCI patterns occur in brain regions of female mice heterozygous for a mutant *MECP2* allele, where phenotypic severity correlates with the degree of skewing (Young and Zoghbi, 2004). Finally, somatic mosaicism in females with *MECP2* mutations is another rare but important source of phenotypic variability (Bourdon et al., 2001).

MECP2 *Mutations in Males*

Although RTT was initially thought to occur exclusively in females, this dogma was broken with the identification of males presenting with classic RTT (Jan et al., 1999). The discovery of the disease-causing gene led to mutation screening in males with related neurological phenotypes and revealed that *MECP2* mutations in males cause a host of neurological disorders, ranging from mental retardation to severe encephalopathy. *MECP2* mutations that cause classic RTT in females typically lead to neonatal encephalopathy and death in the first year of life in males with a normal karyotype. The same mutations, however, result in an RTT phenotype in males with Klinefelter syndrome (47,XXY) or somatic mosaicism. In addition, some of these mutations have been reported in males with classic RTT and a normal karyotype, suggesting that genetic modifiers suppress the infantile lethality typically seen with loss-of-function *MECP2* mutations (Budden et al., 2005; Dayer et al., 2007; Maiwald et al., 2002; Masuyama et al., 2005). Lastly, some *MECP2* mutations that do not cause RTT in females can cause moderate, nonspecific to profound mental retardation or psychiatric disorders in males. Deficits in language and motor skills, obesity, autistic features, and epilepsy are also common in this category of mutations.

CDKL5 *Mutations Explain a Congenital RTT-like Disorder*

Mutations in the X-linked gene cyclin-dependent kinase-like 5 (*CDKL5*) were identified in patients with a rare congenital disorder with intractable early-onset seizures often accompanied by RTT-like features. Alterations in this gene were originally found to cause early-onset epilepsy and infantile spasms with severe mental retardation (Grosso et al., 2007). Although the majority of patients with *CDKL5* mutations are female, there are rare occurrences in males suffering from severe mental retardation, infantile spasms, or early-onset epilepsy. The protein product of *CDKL5*, previously known as serine/threonine kinase 9 (*STK9*), autophosphorylates itself and phosphorylates MeCP2 in vitro, and this latter activity is eliminated in pathogenic *CDKL5* mutants (Bertani et al., 2006). Disruption of *NTNG1*, which encodes the axon guiding molecule Netrin G1, was described in one female patient with atypical RTT and early-onset seizures (Borg et al., 2005). However, this

Table 1. Phenotypes of MeCP2 Dysfunction

	Sex-Associated Syndromes and Symptoms	
MeCP2 State	Female	Male
Loss of Function	Classic RTT	Infantile encephalopathy
	Atypical RTT	Classic RTT (47,XXY or somatic mosaic)
	Angelman-like syndrome	Mental retardation with motor deficits
	Mental retardation with seizures	Bipolar disease, mental retardation, and tremors
	Mild mental retardation	Juvenile-onset schizophrenia, mental retardation, and tremors
	Learning disability	Mental retardation, psychosis, pyramidal signs, and macroorchidism
	Autism	
	Normal	
Overexpression	Preserved speech variant of RTT	Severe mental retardation and RTT features
		Nonspecific X-linked mental retardation

may prove to be an isolated case because *NTNG1* screening in a cohort of *MECP2* and *CDKL5* mutation-negative patients with RTT failed to identify any pathogenic mutations in this gene (Archer et al., 2006a).

The MECP2 *Duplication Disorder*

Perhaps one of the more intriguing findings regarding RTT and MeCP2 is the fact that loss of function and gain in *MECP2* dosage result in clinically similar neurological disorders. In fact, duplications of Xq28 that span the *MECP2* locus have been reported in males with progressive neurodevelopmental phenotypes. The patients suffer from mental retardation with facial and axial hypotonia, progressive spasticity, seizures, recurrent respiratory infections, and often premature death. In addition, autistic features and RTT phenotypes, including head growth deceleration, motor delay, ataxia, hand stereotypies, teeth grinding, and absence of speech, have been reported in these boys (Friez et al., 2006; Lugtenberg et al., 2006; Meins et al., 2005; Van Esch et al., 2005). A male patient with a triplication of the locus was also described with a worse early-onset neurological phenotype (del Gaudio et al., 2006). Only one duplication case has been identified in females, in a patient with the preserved speech variant of RTT (Ariani et al., 2004). Studies of transgenic mice that express wild-type *MECP2* at twice the normal level reveal that doubling the dosage of this protein leads to a progressive neurological phenotype similar to that observed in human patients (Collins et al., 2004). These observations, together with the finding that *MECP2* is the only common gene shared among all patients with the duplication syndrome, give credence to the notion that *MECP2* is the gene within the ~400 kb duplicated region that is responsible for these phenotypes.

MECP2 *Mutations in Other Neuropsychiatric Disorders*

Disruptions in *MECP2* result in a host of neuropsychiatric disorders, of which classic and variant forms of RTT represent only one chapter (Table 1). On the milder side of the scale are females with mild mental retardation, learning disabilities, and autism spectrum disorders (Carney et al., 2003; Lam et al., 2000). *MECP2* mutations can also cause severe mental retardation with epilepsy and Angelman-like syndrome in females (Milani et al., 2005; Watson et al., 2001). The spectrum of *MECP2* disorders worsens with complex forms of severe mental retardation in males that are associated with epilepsy, ataxia, tremor, hyperactivity, autism, and bipolar disease (Klauck et al., 2002) or juvenile-onset schizophrenia (Cohen et al., 2002). In addition, *MECP2* mutations were identified in males with mental retardation, psychosis, pyramidal signs, Parkinsonian features, and macroorchidism (PPM-X) (Klauck et al., 2002).

The full spectrum of phenotypes in *MECP2* disorders is far from being understood. Mutations that are predicted to result in a null allele suggest that the neurodevelopmental abnormalities are a result of MeCP2 loss of function. However, the *MECP2* duplication disorder demonstrates that an increase in protein levels can be equally detrimental to the nervous system. Studies in loss-of-function and duplication models are beginning to provide insight on how variations in the level of this protein lead to neuronal dysfunction (see below).

MeCP2 Expression and Functions

MECP2 consists of four exons that code for two different isoforms of the protein, due to alternative splicing of exon 2 (Figure 2). The MeCP2 splice variants differ only in their N-termini; the more abundant MeCP2-e1 isoform (encoded by *MECP2α*) contains 24 amino acids encoded by exon 1 and lacks the 9 amino acids encoded by exon 2, whereas the start site for the MeCP2-e2 isoform (encoded by *MECP2β*) is in exon 2 (Dragich et al., 2007; Kriaucionis and Bird, 2004; Mnatzakanian et al., 2004). In addition, *MECP2* has a large, highly conserved 3′-untranslated region that contains multiple polyadenylation sites, which can be alternatively used to generate four different transcripts. Expression studies in mice showed that the longest transcript is the most abundant in brain, with

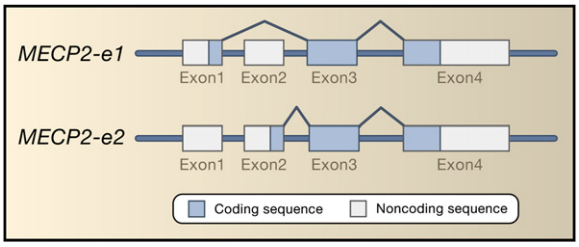

Figure 2. *MECP2* Gene Structure
Alternative splicing of the *MECP2-e1* transcript excludes exon 2, while the *MECP2-e2* isoform is expressed from an ATG in exon 2.

higher expression during embryonic development, followed by postnatal decline, and subsequent increase in expression levels later in adult life (Pelka et al., 2005; Shahbazian et al., 2002b). These different *MECP2* transcripts could be subject to tissue-specific and developmental stage-specific regulation. Although the MeCP2 protein is widely expressed, it is relatively more abundant in the brain, primarily in mature postmigratory neurons (Jung et al., 2003). MeCP2 protein levels are low during embryogenesis and increase progressively during the postnatal period of neuronal maturation (Balmer et al., 2003; Cohen et al., 2003; Kishi and Macklis, 2004; Mullaney et al., 2004; Shahbazian et al., 2002b). The pattern of increasing expression in the cortex follows an inner-to-outer sequence akin to that of cortical development. In the olfactory epithelium, MeCP2 expression coincides with maturation of the olfactory receptor neurons (ORNs) and precedes the onset of synaptogenesis (Cohen et al., 2003). Both MeCP2 isoforms are nuclear and colocalize with methylated heterochromatic foci in mouse cells. A recent report suggests that MeCP2 translocates to the nucleus upon neuronal differentiation (Miyake and Nagai, 2007). Since MeCP2 is expressed in mature neurons and its levels increase during postnatal development, MeCP2 may play a role in modulating the activity or plasticity of mature neurons. Consistent with this, *MECP2* mutations do not seem to affect the proliferation or differentiation of neuronal precursors. Although the mechanisms that regulate the complex *MECP2* expression patterns are unknown, a recent study identified the core promoter and several cis-regulatory elements that drive *MECP2* expression (Liu and Francke, 2006). These regulatory sequences may dictate the spatial and temporal patterns of *MECP2* expression.

MeCP2 is a member of the methyl-CpG binding protein family (Hendrich and Bird, 1998), and is composed of three domains: the MBD, the transcriptional repression domain (TRD), and a C-terminal domain, in addition to two nuclear localization signals (NLS). The MBD specifically binds to methylated CpG dinucleotides, with preference for CpG sequences with adjacent A/T-rich motifs (Klose et al., 2005). MBD also binds to unmethylated four-way DNA junctions with a similar affinity (Galvao and Thomas, 2005), implicating a role for the MeCP2 MBD in higher-order chromatin interactions. The more downstream TRD is involved in transcriptional repression through recruitment of corepressors and chromatin remodeling complexes. The C terminus facilitates MeCP2 binding to naked DNA and to the nucleosomal core, and it also contains evolutionarily conserved poly-proline runs that can bind to group II WW domain splicing factors (Buschdorf and Stratling, 2004). Although the C-terminal region of MeCP2 is not yet well characterized, it is clearly essential for protein function as evidenced by the numerous RTT-causing mutations that involve deletion of this domain, and the fact that a mouse model lacking the MeCP2 C terminus reproduces many RTT phenotypes (Shahbazian et al., 2002a). The function of MeCP2 as a transcriptional repressor was first suggested based on in vitro experiments in which MeCP2 specifically inhibited transcription from methylated promoters (Nan et al., 1997). When MeCP2 binds to methylated CpG dinucleotides of target genes via its MBD, its TRD recruits the corepressor Sin3A and histone deacetylases (HDACs) 1 and 2 (Jones et al., 1998; Nan et al., 1998). The transcriptional repressor activity of MeCP2 involves compaction of chromatin by promoting nucleosome clustering, either through recruitment of HDAC and histone deacetylation (Figure 3A) or through direct interaction between its C-terminal domain and chromatin (Nikitina et al., 2007). In addition, the interaction with Sin3A is not stable and appears to be dependent on MeCP2 being DNA-bound (Klose and Bird, 2004). This suggests that Sin3A is not an exclusive partner of MeCP2 and that other factors may interact with MeCP2 to modulate gene expression or other unknown functions. Additional MeCP2-interacting proteins include the catalytic component of the SWI/SNF chromatin-remodeling complex Brahma (at least in NIH 3T3 cells), the DNA methyltransferase DNMT1, the histone methyltransferase Suv39H1, the transcription factors TFIIB and PU.1, the corepressors c-Ski and N-CoR, LANA, and the SWI2/SNF2 DNA helicase/ATPase responsible for α-thalassemia/mental retardation syndrome X-linked (ATRX) (Harikrishnan et al., 2005; Kaludov and Wolffe, 2000; Kimura and Shiota, 2003; Kokura et al., 2001; Nan et al., 2007). However, the precise functional consequences of these protein-protein interactions remain unknown. To add to the complexity, MeCP2 also interacts with the RNA-binding protein Y box-binding protein 1 (YB1) to regulate splicing of reporter constructs (Figure 3B). The finding of aberrant RNA splicing patterns in a *Mecp2* mutant mouse model of RTT (Young et al., 2005) together with the fact that MeCP2 can form complexes with RNA in vitro independently of its MBD (Jeffery and Nakielny, 2004) suggest that MeCP2 might modulate RNA splicing in vivo. Given that MeCP2 interacts with other proteins, chromatin, DNA, and RNA, it is clearly a multifunctional protein, with roles in chromatin remodeling and RNA splicing.

Modeling RTT and Related Disorders in Mice

To uncover the molecular changes that underlie RTT, three mouse models with different MeCP2 mutations were generated (Table 2). *Mecp2* conditional knockout

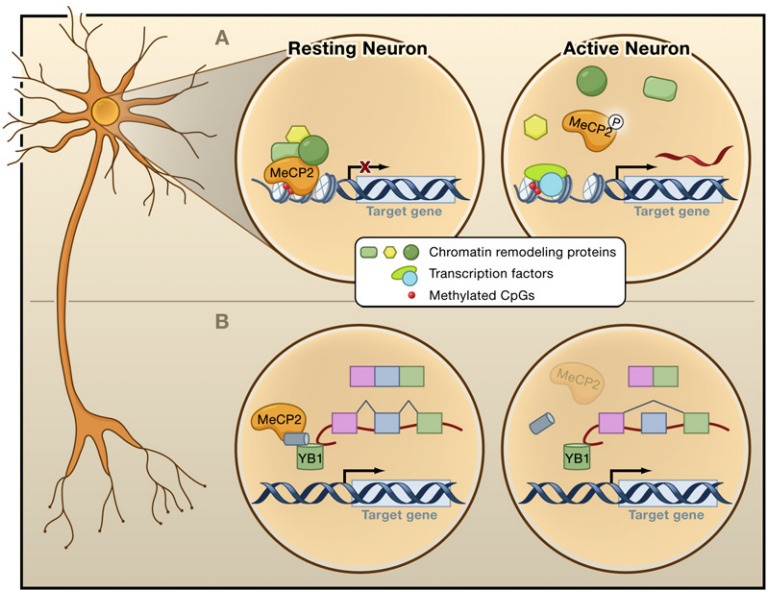

Figure 3. Model for MeCP2 Mechanisms of Action

(A) In resting neurons MeCP2 regulates gene expression by binding to methylated CpG dinucleotides and recruiting the Sin3A-HDAC corepressor complex and chromatin remodeling proteins. This leads to chromatin compaction, making the promoter inaccessible to members of the transcriptional machinery. Neuronal activity induces MeCP2 phosphorylation and leads to its release from the promoter region and dissociation of the corepressor complex. The hyperacetylated chromatin allows access to transcriptional machinery and target gene expression.

(B) MeCP2 interacts with YB1 and regulates alternative splicing of target transcripts. In the absence of MeCP2, these transcripts are aberrantly spliced.

mice, lacking either exon 3 or both exons 3 and 4 (Chen et al., 2001; Guy et al., 2001), undergo a period of normal development, followed by severe progressive neurological dysfunction, leading to death at 8–10 weeks of age. Female *Mecp2*$^{+/-}$ mice have behavioral abnormalities as well, but with a later age of onset. Moreover, an embryonic *Mecp2* deletion only in neurons, using a nestin-Cre transgene, results in a phenotype resembling the ubiquitous knockout, demonstrating that MeCP2 dysfunction in the brain is sufficient to cause the disease (Guy et al., 2001).

Table 2. *Mecp2* Mouse Models

	Mecp2$^{-/Y}$	*Mecp2*$^{308/Y}$	*Mecp2*^{Tg}
MeCP2 aberration	deletion of exon 3; exons 3 and 4; exon 3 and part of exon 4	truncation at amino acid 308	overexpression of *MECP2*
Neurological phenotype	severe	progressive	progressive
LTP	reduced	reduced	enhanced
Hypoactivity	✓	✓	✓
Stereotypies		forepaw rubbing	forepaw clasping
Kyphosis		✓	✓
Spasticity	hindlimb clasping	hindlimb clasping	hindlimb clasping
Tremors	✓	✓	
Seizures	✓	✓	✓
Motor dysfunction	✓	✓	✓
Breathing abnormalities	✓		?
Anxiety	decreased	increased	increased
Learning and memory deficits	✓	✓	
Social behavior abnormalities		✓	
Ataxia	✓	✓	✓
Age of death	8–10 weeks	15 months	7–12 months
References	Chen et al., Guy et al., Pelka et al.	Shahbazian et al.	Collins et al.

The phenotypes described for *Mecp2* null mice and *Mecp2*^{308} mice are based on characterization of male mice. Female mice heterozygous for each of the mutations manifest symptoms at a later age, typically have a milder disease course due to mosaicism, and often have favorable XCI. *Mecp2*^{Tg} mice show phenotypes in both males and females because the extra copy is on an autosome.

Mecp2 null mice display no initial phenotype until 3–6 weeks of age, when male mutant mice develop a stiff, uncoordinated gait, hypoactivity, tremor, hindlimb clasping, and irregular breathing. Symptoms worsen and ultimately lead to weight loss and death by 10 weeks. The respiratory rhythm disturbances observed in the *Mecp2* null mice have been attributed to alterations in bulbar postinspiratory discharges and to malfunction of the Kolliker-Fuse region of the pons that enhances postinspiratory activity and leads to the development of respiratory dysrhythmia and apnea (Stettner et al., 2007). The brains of *Mecp2* null mice are smaller in size and weight than brains of wild-type littermates, but have no detectable structural abnormalities, except for smaller, more densely packed neurons. In addition, the olfactory neurons of *Mecp2* null mice demonstrate abnormalities of axonal targeting in the olfactory bulb, suggesting a function for MeCP2 in terminal neuronal differentiation (Matarazzo et al., 2004). When *Mecp2* is deleted in postmitotic neurons using a calcium-calmodulin-dependent protein kinase II (CaMKII)-Cre transgene, similar but less severe neurological phenotypes are observed with a later age of onset, confirming a critical role for MeCP2 in mature neurons. These mice display gait ataxia, increased anxiety, and impaired social behavior (Chen et al., 2001; Gemelli et al., 2006). In further support of this idea, when *Mecp2* is expressed in postmitotic neurons of *Mecp2* null mice under the control of the endogenous *tau* promoter, the neurological phenotypes of the knockout mice are rescued (Luikenhuis et al., 2004). These studies not only confirmed that neuronal MeCP2 dysfunction is responsible for the neurological phenotype, but also that MeCP2 is not very essential to early brain development. Recently, another *Mecp2* null mouse model was reported in which exon 3 and part of exon 4 were deleted. The mice are hypoactive and display learning deficits and reduced anxiety. The same mutation was also generated on an XO background and resulted in similar phenotypes in $Mecp2^{-/Y}$ and $Mecp2^{-/O}$ mice, indicating that the Y-chromosome has no effect on the phenotypic manifestation in *Mecp2* null mice (Pelka et al., 2006).

Another RTT mouse model, generated by truncating MeCP2 at amino acid 308, results in a hypomorphic allele that retains the MBD, TRD, and NLS, and only eliminates the C-terminal region of the protein, similar to C-terminal deletions found in RTT patients (Shahbazian et al., 2002a). $Mecp2^{308/Y}$ mice appear normal until 6 weeks of age, when they develop progressive neurological phenotypes, including motor dysfunction, forepaw stereotypies, hypoactivity, tremor, seizures, kyphosis, social behavior abnormalities, decreased diurnal activity, increased anxiety-related behavior, and learning and memory deficits, reminiscent of the clinical picture in human girls with RTT. Female mice heterozygous for the truncation display milder and more variable features. In vivo, the truncated protein maintains normal chromatin localization, but histone H3 is hyperacetylated in the brain, indicating abnormal chromatin architecture (McGill et al., 2006; Moretti et al., 2005; Shahbazian et al., 2002a).

Two-fold overexpression of human *MECP2* under the control of its endogenous promoter in mice ($MECP2^{Tg}$) results in progressive neurological abnormalities with the onset of phenotypes around ten weeks of age. Initially $MECP2^{Tg}$ mice display increased synaptic plasticity, with enhancement in motor and contextual learning abilities. However, at 20 weeks of age, transgenic mice become hypoactive and develop forepaw clasping, aggressiveness, kyphosis, seizures, and motor abnormalities, and die by 1 year of age. In addition, higher levels of MeCP2 expression in other transgenic lines are associated with more severe phenotypes (Collins et al., 2004). Introducing a copy of the *MECP2* transgene into *Mecp2* null mice restores protein levels to normal and rescues the neurological abnormalities of both mouse models, indicating that phenotypes of the transgenic mice are indeed modulated by MeCP2 levels. Overexpression of *MECP2* in adult neurons under control of the *tau* promoter also results in a progressive neurological syndrome. Studies of these mouse models suggest that MeCP2 levels must be tightly regulated even postnatally and that the slightest perturbation results in deleterious neurological consequences (Collins et al., 2004; Luikenhuis et al., 2004). The best illustration of the clinical relevance of such observations is the fact that duplication of the *MECP2* locus in human male patients causes profound mental retardation and progressive neurodevelopmental disorders.

Effects of MeCP2 Dysfunction on the Brain

Neuropathological Changes

Despite the profound neurological phenotype in RTT, the major morphological abnormalities detected in the central nervous system (CNS) are an overall decrease in the size of the brain and of individual neurons. Autopsy studies show a 12%–34% reduction in brain weight and volume in patients with RTT, the effect most pronounced in the prefrontal, posterior frontal, and anterior temporal regions (Armstrong, 2005). The RTT brain shows no obvious degeneration, atrophy, or inflammation, and there are no signs of gliosis or neuronal migration defects (Jellinger et al., 1988; Reiss et al., 1993). These observations indicate that RTT is a disorder of postnatal neurodevelopment rather than a neurodegenerative process. There is a decrease in the size of cortical minicolumns (Casanova et al., 2003), which could correlate with observations of reduced dendritic branching of layers III and V pyramidal neurons in the frontal, temporal and motor regions, and of layers II and IV of the subiculum (Armstrong, 2005). Neurons of hippocampal CA1 and occipital regions are relatively preserved (Armstrong et al., 1995). In addition, dendritic spines of the RTT frontal cortex are sparse and short, with no other apparent abnormalities (Belichenko et al., 1994). Although neuronal size is reduced in the cortex, thalamus, basal ganglia, amygdala, and hippocampus, there is an increase in neuronal cell packing in the hippocampus (Kaufmann and Moser, 2000). Hypopigmentation of the zona compacta of the substantia nigra and reduction in cell number in the nucleus basalis of Meynert

have been reported (Kitt and Wilcox, 1995). Olfactory biopsies from RTT patients reveal that the mature ORNs are dysmorphic, and that there is a marked increase in the ratio of immature to mature ORNs (Ronnett et al., 2003). Delayed neuronal maturation and synaptogenesis were also detected in the *Mecp2*$^{-/Y}$ cerebral cortex (Fukuda et al., 2005). Neuropathological studies in both humans and mice were all performed well after the onset of symptoms. It would be interesting to determine if alterations exist in hippocampal or cortical neurons prior to the onset of symptoms, at least in the existing mouse models. Nonetheless, the results of neuropathological studies suggest a role for MeCP2 in neuronal maturation and maintenance.

Additionally, a variety of neurochemical changes are documented in the cerebrospinal fluid and brain tissue of patients with RTT (Blue et al., 1999; Calamandrei et al., 2001; Guideri et al., 2004; Lipani et al., 2000; Paterson et al., 2005; Riikonen, 2003; Saito et al., 2001; Viola et al., 2007; Wenk, 1997; Zoghbi et al., 1989). However, it should be noted that the majority of these data result from small samples of postmortem tissue and none of these changes are diagnostic or occur in every patient.

Neurophysiological Abnormalities

Neurophysiological studies of patients with RTT suggest that both the CNS and the autonomic nervous system contribute to the pathophysiology of the disease. In RTT, the peripheral auditory and visual systems are normal, as demonstrated from evoked potential studies. Altered somatosensory evoked potentials and abnormal electroencephalogram (EEG) findings of focal, multifocal, and generalized epileptiform discharges, and the occurrence of rhythmic slow theta activity, all suggest altered cortical excitability in the RTT brain. However, the EEG patterns are not diagnostic of RTT, and they vary between patients and at different stages of the disorder (Moser et al., 2007). Electrocardiographic recordings demonstrate long corrected QT intervals and suggest perturbation of the autonomic nervous system (Glaze, 2005). Studies of *Mecp2* mouse models reveal abnormalities in long-term potentiation (LTP) and impaired synaptic plasticity; LTP is reduced in *Mecp2*$^{-/Y}$ cortical slices (Asaka et al., 2006) and in *Mecp2*$^{308/Y}$ cortical and hippocampal slices (Moretti et al., 2006). In contrast, LTP is enhanced with doubling of MeCP2 in *Mecp2*^{Tg1} hippocampal slices (Collins et al., 2004). In addition, loss of MeCP2 in mice results in reduced spontaneous activity in cortical slices due to a decrease in the total excitatory synaptic drive and an increase in the total inhibitory drive (Dani et al., 2005). Cultured hippocampal neurons from *Mecp2* null mice have decreased frequency of spontaneous excitatory synaptic transmission, and an increase in short-term synaptic depression rate (Nelson et al., 2006). Chao et al. (2007) investigated the physiological consequences of either loss or doubling of MeCP2 on an individual neuron using autaptic cultured hippocampal neurons from *Mecp2*$^{-/Y}$ and *MECP2*^{Tg1} mice, and they found an approximately 50% reduction and 100% enhancement of synaptic outputs in glutamatergic *Mecp2*$^{-/Y}$ and *MECP2*^{Tg1} neurons, respectively. They went on to demonstrate that these physiological changes predominantly result from an altered number of glutamatergic synapses rather than dysfunction of the presynapse or postsynapse. Neurons from *Mecp2*$^{-/Y}$ mice have an approximately 40% reduction in VGLUT1-PSD95-positive puncta, whereas those from *MECP2*^{Tg1} mice have a 60% increase in such puncta in comparison with wild-type mice (Chao et al., 2007).

The alterations in glutamatergic synapse number were observed both at the single-neuron level and in vivo during the early or "presymptomatic" disease stage (Chao et al., 2007). This finding is noteworthy in that it suggests that homeostatic compensatory changes must occur in response to altered synapse number. Altogether, these findings indicate that MeCP2 is essential in modulating synaptic function and plasticity, and that MeCP2 function is critical in regulating the number of excitatory synapses during early postnatal development.

MeCP2 Target Genes and Relevance to RTT Pathogenesis

Since RTT results from dysfunction of a putative transcriptional repressor, it is logical to pursue the transcriptional targets of MeCP2 to gain insight into disease pathogenesis. Transcriptional profiling studies using brain tissue from *Mecp2* null mice did not reveal major gene expression changes, suggesting that MeCP2 may not be a global transcriptional repressor as previously thought, and that loss of MeCP2 must lead to subtle gene expression changes (Tudor et al., 2002). Alternatively, if MeCP2 regulates the expression of a select number of genes in different subsets of neurons, such transcriptional changes would be missed in samples of brain tissue containing a host of vastly different neuronal types. Several studies have used candidate gene approaches or samples from both human and mouse tissues and identified putative MeCP2 targets that might be relevant to the pathogenesis of RTT. Some of these targets have been confirmed while others yielded different results in different laboratories. A list of the targets that have been studied more extensively is shown in Table 3. In this section we will limit the discussion to a handful of targets that might provide insight about the function of MeCP2, RTT pathogenesis, or both.

MeCP2 Regulates Gene Expression in an Activity-Dependent Manner

Brain-derived neurotrophic factor (BDNF) was identified as a target of MeCP2 through a candidate gene approach (Chen et al., 2003; Martinowich et al., 2003). Rat *Bdnf* is under the control of four promoters, one of which, promoter III, is activated by calcium influx through L-type voltage-sensitive calcium channels (Tao et al., 1998). Chen et al. (2003) found that in the absence of neuronal activity in wild-type cultured neurons, MeCP2 is bound to the rat *Bdnf* promoter III and mediates its transcriptional repression. Membrane depolarization triggers the phosphorylation of MeCP2 at serine 421 (S421) through a CaMKII-dependent mechanism, and releases it from the promoter,

Table 3. MeCP2 Target Genes

Gene	Function	References
Bdnf	neuronal development and survival	Chen et al., Martinowich et al.
xHairy2a	neuronal repressor	Stancheva et al.
DLX5/ Dlx5	neuronal transcription factor	Horike et al.
Sgk1	hormone signaling	Nuber et al.
Fkbp5	hormone signaling	Nuber et al.
Uqcrc1	mitochondrial respiratory chain	Kriaucionis et al.
ID1-3/ Id1-3	neuronal transcription factors	Peddada et al.
FXYD1/ Fxyd1	ion channel regulator	Deng et al.
IGFBP3/ Igfbp3	hormone signaling	Itoh et al.
Crh	neuropeptide	McGill et al.
UBE3A	ubiquitin ligase	Samaco et al.
GABRB3	GABA-A receptor	Samaco et al.

For some of these targets (*DLX5* and *UBE3A*), the data were not reproduced in independent studies (Hogart et al., 2007; Jordan and Francke, 2006; Makedonski et al., 2005; Samaco et al., 2005; Schule et al., 2007).

thus allowing transcription (Chen et al., 2003; Zhou et al., 2006) (Figure 3A). In neurons expressing an S421A mutant form of MeCP2, the neuronal activity-dependent transcription of the mouse *Bdnf* promoter IV (which corresponds to rat promoter III) was impaired, suggesting that the unphosphorylatable form of MeCP2 cannot be released from the *Bdnf* promoter in response to membrane depolarization (Zhou et al., 2006). Another study found that the depolarization-induced increase in BDNF expression in mouse cortical cultures correlates with a decrease in methylation of CpGs in the *Bdnf* promoter IV region. Moreover, the increase in *Bdnf* transcription involved dissociation of MeCP2 and its associated corepressor complex from *Bdnf* promoter IV (Martinowich et al., 2003). So while these cortical culture studies provide evidence that MeCP2 binds the *Bdnf* promoter and regulates transcription of *Bdnf* in an activity-dependent manner, in vivo studies provide intriguing findings that question the role of MeCP2 as a repressor of *Bdnf* expression. Chang and colleagues reported that BDNF protein levels are decreased rather than increased in brains of symptomatic *Mecp2*$^{-/Y}$ mice. Moreover, conditional deletion of *Bdnf* in postmitotic neurons of mice mimicked some of the phenotypes observed in *Mecp2* null mice, including hindlimb clasping, reduced brain weight, and reduced olfactory and hippocampal neuronal sizes. Furthermore, forebrain-specific deletion of *Bdnf* in *Mecp2*$^{-/Y}$ mice resulted in earlier onset of locomotor dysfunction and reduced lifespan, while forebrain-specific overexpression of *Bdnf* in *Mecp2*$^{-/Y}$ mice improved locomotor function and extended their lifespan (Chang et al., 2006). These results demonstrate in vivo interaction between *Mecp2* and *Bdnf*, and a correlation between altered BDNF levels and neurologic impairment in *Mecp2* null mice. One possible explanation for the discrepancy in observed BDNF levels between the in vivo and cell culture data is that neuronal activity was experimentally controlled in isolated cortical cultures (Chen et al., 2003), whereas in brains the neurons are inherently at different activity states (Chang et al., 2006). Since *Bdnf* is known to be upregulated in response to neuronal activity, the reduced cortical activity in *Mecp2* null mice is expected to negatively affect *Bdnf* expression, hence masking the expected upregulation that would normally result from loss of repression in resting cortical neurons that lack MeCP2 (Chang et al., 2006). A recent study found that although *Mecp2*$^{-/Y}$ nodose ganglia and cortical neurons express less *Bdnf* mRNA and contain lower levels of the protein compared with wild-type neurons, they secrete a greater percentage of their total BDNF content. This enhanced secretion is due to an increase in BDNF stores available for release. Catecholamine release from *Mecp2*$^{-/Y}$ adrenal chromaffin cells is similarly affected, indicating general neurosecretory alterations in *Mecp2* null mice (Wang et al., 2006). The fact that *Bdnf* null mice display a subset of phenotypes observed in *Mecp2* null mice raises the possibility that the two proteins have certain overlapping effects. It is possible that MeCP2 dysfunction reduces overall neuronal activity and indirectly results in decreased BDNF. Alternatively, the alleviation of some of the *Mecp2*$^{-/Y}$ phenotypes upon BDNF overexpression may be a consequence of the general benefits of this neurotrophic factor. More studies are needed to determine the effects of MeCP2 on *Bdnf* expression in vivo and the role of BDNF in the initial stages of RTT pathogenesis. Irrespective of the effects of MeCP2 on *Bdnf* expression, there is compelling evidence that the phosphorylation of MeCP2 at S421 occurs in response to activity and that this phosphorylation leads to displacement of MeCP2 from the *Bdnf* promoter (Chen et al., 2003; Zhou et al., 2006). The finding that MeCP2 is phosphorylated at S421 in the suprachiasmatic nucleus in response to light exposure in mice after they are placed in 48 hr of darkness is an elegant demonstration of a role for this protein in activity-dependent modulation of gene expression.

MeCP2 as a Modulator of Gene Expression

The role of MeCP2 in regulating the expression of some genes that are involved in the stress response is consistent with its critical role in the context of altered neuronal activity. Stress is clearly an altered physiologic state that calls for dynamic changes in the expression of a variety of genes that control the activity of the hypothalamic-pituitary-adrenal axis, and the effector genes that are needed to modulate the activity of steroids and their receptors. Gene expression studies using RNA from *Mecp2* null mouse brains reveal upregulation of the glucocorticoid-regulated genes, serum glucocorticoid-inducible kinase 1 (*Sgk1*) and FK506-binding protein 5 (*Fkbp5*). The reported expression changes were observed in

presymptomatic, early symptomatic, and late symptomatic *Mecp2* null mice. It is interesting that although these genes are induced in response to stress, the basal plasma glucocorticoid levels in these animals were not elevated, suggesting that the induced differences are not a result of high hormone levels. In addition, ChIP analysis showed that MeCP2 binds to the *Fkbp5* and *Sgk1* genes in wild-type brain. These findings raised the possibility that at least some RTT symptoms arise from the disruption of MeCP2 regulation of stress-responsive genes (Nuber et al., 2005).

Following a phenotype-driven approach, McGill et al. identified a MeCP2 target gene that is potentially relevant to a specific RTT feature (McGill et al., 2006). The RTT mouse model, $Mecp2^{308/Y}$, carrying a truncated *Mecp2* allele, displays increased anxiety-like behavior (McGill et al., 2006; Shahbazian et al., 2002a). This is reminiscent of clinical reports of frequent high-anxiety episodes in RTT patients (Mount et al., 2001). To determine if stress underlies the anxiety in $Mecp2^{308/Y}$ mice, McGill and colleagues evaluated the physiological stress response in these animals and found that $Mecp2^{308/Y}$ mice have an enhanced physiologic response to stress manifested as increased corticosterone levels. This finding led to the evaluation of *Crh* as a target gene given that it encodes the neuropeptide corticotropin-releasing hormone (CRH), which modulates behavioral and physiologic responses to stress (Bale and Vale, 2004; Bruhn et al., 1984). RNA in situ hybridization and quantitative real-time RT-PCR on the paraventricular nucleus of the hypothalamus, the central amygdala, and the bed nucleus of the stria terminalis, three brain regions critical to the stress response, revealed increased *Crh* levels in $Mecp2^{308/Y}$ mice. ChIP analysis revealed that MeCP2 binds the *Crh* promoter, which is enriched for methylated CpGs. The enhanced *Crh* expression detected in $Mecp2^{308/Y}$ mice is due to a functional defect in the truncated $MeCP2^{308}$ protein itself, since there were no differences in *Crh* promoter methylation patterns between the wild-type and mutant mice (McGill et al., 2006). This study suggests that overexpression of a specific MeCP2 target might explain a specific RTT phenotype. *Crh* is an attractive candidate in the investigation of therapeutic intervention strategies in RTT given that Crh receptor 1 (Crhr1) antagonists have been shown to reduce anxiety (Bale and Vale, 2004). It is noteworthy that the increase in *Crh* RNA levels results from upregulation of expression specifically where it is normally expressed, rather than from ectopic expression in neurons where it is normally repressed. These data argue that MeCP2 is not a silencer that turns off its targets where they are not normally expressed, but rather functions as a modulator of gene expression depending on the physiologic state of the organism. Metaphorically speaking, MeCP2 may be best thought of as the dimmer that regulates the amount of light rather than the switch that turns the lamp on and off. The facts that MeCP2 undergoes phosphorylation upon light exposure (a new physiologic state) and that it regulates RNA levels of a neuropeptide whose levels fluctuate according to the level of stress are consistent with this model.

Rescuing RTT in Mouse Models

The absence of neuronal degeneration in MeCP2 disorders begs the question of whether restoring *MECP2* expression would restore normal neuronal function and reverse the resulting disease phenotypes. Recent work by Guy et al. (2007) and Giacometti et al. (2007) provide evidence supporting the feasibility of disease reversibility in mouse models of RTT. Guy et al. created a mouse in which endogenous *Mecp2* is silenced by insertion of a *Lox-Stop* cassette and can be conditionally activated through Cre-mediated deletion of the cassette. The $Mecp2^{lox\text{-}Stop}$ allele behaved as a null mutation, and its activation was controlled by a tamoxifen-inducible (TM-inducible) Cre transgene. Acute TM injections caused sudden activation of *Mecp2* and led to either rapid death or complete phenotypic rescue of the null mice. The associated toxicity resembled that caused by MeCP2 overexpression and was not due to the TM injections. A more gradual approach for induction using smaller and repeated doses of TM eliminated the toxicity and reversed the late-onset neurological phenotype of adult $Mecp2^{lox\text{-}Stop/+}$*; cre* heterozygotes. The results indicate that MeCP2-deficient neurons are not permanently damaged, since *Mecp2* activation leads to robust abrogation of advanced neurological defects in both young and adult animals. The authors propose a model in which neuronal MeCP2 target sites are defined by DNA methylation patterns that are preserved in its absence and that guide newly synthesized MeCP2 to its correct chromosomal positions. MeCP2 then resumes its role as interpreter of the DNA methylation signal required for normal neuronal function (Guy et al., 2007). Although the results do not provide immediate therapeutic strategies for RTT, they do establish that consequences of MeCP2 loss of function are reversible, and suggest that the neurological defects in RTT, and other *MECP2* disorders, are not impervious to therapeutic possibilities.

In an independent study, Giacometti et al. (2007) demonstrated partial disease rescue by postnatal reactivation of MeCP2 in mutant animals. The investigators targeted a transgene carrying the mouse *Mecp2-e2* cDNA downstream of a LoxP-Stop-LoxP (LSL) cassette to the *Col1a1* locus, which provides a strong ubiquitous promoter. The LSL-*Mecp2* transgene was then placed on a *Mecp2* null background to determine if it could rescue upon excision of the Stop signal. Four different Cre transgenes, as follows, were tested to activate *Mecp2*: Nestin-Cre in neuronal and glial precursors; *Tau*-Cre in postmitotic neurons during embryogenesis; CaMKII-Cre 93 (C93) in the forebrain, hippocampus, midbrain, and brainstem at ~postnatal day (P) 0–P15; or CaMKII-Cre 159 (C159) in the forebrain at ~P15–P30. Although activation of the LSL-*Mecp2* transgene prolonged the lifespan and delayed motor deterioration of $Mecp2^{-/Y}$ mice, the extent of rescue directly correlated with the time, level, and site of

Cre expression. The most efficient symptomatic rescue was obtained in lines that provided early and wide Cre expression in most neurons (Nestin-Cre and *Tau*-Cre), extending the lifespan of mutant mice to 8 months. Postnatal MeCP2 activation in C93 and C159 lines extended the life span by 4 weeks, with the earlier C93 expression giving a more efficient rescue than the later C159 expression (Giacometti et al., 2007). Inappropriate *Mecp2* expression levels resulting from use of nonendogenous promoters could account for the partial disease rescue obtained in this study, since this was not seen by Guy et al. when *Mecp2* was activated under the control of its endogenous promoter. In fact, MeCP2 levels were much lower than the expected wild-type levels when induced by the C159 line, and neither line expressed the protein in the cerebellum. The findings from the C93 and C159 lines argue that it is critical that MeCP2 levels are restored to the expected wild-type levels and done so in all neurons, or at least in neurons outside of CaMKII domains, to achieve rescue. This conclusion is bolstered by another study in which induction of MeCP2 expression using a CaMKII promoter failed to rescue the lethality in MeCP2 null mice (Alvarez-Saavedra et al., 2007). Altogether, these data underscore the importance of proper expression of MeCP2, probably throughout the CNS or at least beyond the CaMKII expression domain, to achieve rescue. What is exciting, however, is that once proper expression of MeCP2 is achieved, symptoms can be reversed even in adult animals, implying that neurons might not be permanently damaged.

New Questions and Challenges

The past 8 years have witnessed many exciting discoveries that are relevant to MeCP2 function and RTT, yet many of these discoveries have inspired new questions and unveiled unexpected challenges. In the following section we will pose some of the questions that are worthy of consideration and potential new areas of investigation.

What Is the Full Spectrum of MECP2 *Disorders?*

The findings that *MECP2* mutations cause RTT as well as a spectrum of neuropsychiatric phenotypes and that either loss or doubling of MeCP2 causes such phenotypes raise the possibility that regulatory mutations or posttranscriptional alterations that slightly alter MeCP2 levels might cause more restricted phenotypes such as autism, seizures, Parkinsonian features, or cognitive impairment (Coutinho et al., 2007). Given the importance of MeCP2 for postnatal neuronal function, it is conceivable that hypomorphic mutations might compromise neuronal plasticity such that overt phenotypes will become apparent only upon aging. It will take coordinated and large-scale efforts to establish if variations in *MECP2* contribute to late-onset neurological disorders involving cognition and motor control.

What Are the Functions of MeCP2 and How Are They Affected by the Various Mutations?

Based on various in vivo studies, a few conclusions can be safely made about the function of MeCP2. It binds DNA at specific sites and this binding is affected by its phosphorylation state at S421, which in turn depends on neuronal activity. The finding that the expression changes in some of its targets are small argues that the protein modulates expression in response to activity and that such modulation is critical for normal neuronal function. Such modulatory effects would be easily missed when profiling RNA from heterogeneous neuronal populations. This is particularly true for targets that are expressed in specific neurons.

The finding that either loss or doubling of MeCP2 results in progressive postnatal neurological disorders that have overlapping features is quite intriguing. The recent discovery that such loss and doubling result in decreases and increases (respectively) in excitatory synapse numbers argues that MeCP2 plays an important role in determining excitatory synapse number in early development. Whether this is achieved by modulating expression or splicing of factors critical for synapse formation (or both) remains to be determined. The finding, however, that loss and doubling of MeCP2 have opposing effects on synapses argues that certain expression or splicing changes might go in opposite directions in *Mecp2* null versus duplication mice. Thus, these two models might provide an excellent resource to uncover some of the primary targets of MeCP2, which in turn would be an excellent framework to evaluate the functional consequences of some of the other RTT-causing *MECP2* mutations. There are several alleles that alter one amino acid outside of the MBD or cause late truncations, raising the possibility that some of these might lead to a gain-of-function or hypermorphic effect as observed for the duplication.

What Is the Anatomical Origin of Various RTT Phenotypes and What Are the Associated Molecular Changes?

Given the diversity of phenotypes associated with MeCP2 dysfunction, a key question is whether individual phenotypes result from dysfunction of the protein in specific neurons, whether the phenotypes result from dysfunction of the entire neuronal network (the more compromised the network, the more phenotypes become apparent), or a combination of both (Figure 4). The identification of isolated partial phenotypes that are not always the same among patients with favorable XCI supports a model whereby dysfunction in specific neurons could explain one or more clinical features. The starting point of the disease mechanism could be cell-autonomous changes occurring during postnatal development that subsequently cause specific symptoms. Such select neuronal changes together with altered experiences might eventually lead to malfunction of an entire network, which is then manifested as additional neurobehavioral abnormalities. The molecular corollary of such a model is that the neuropsychiatric features of RTT and related disorders result from misregulation of some genes expressed in a specific set of neurons. Studies of large numbers of patients with essentially any one of the RTT phenotypes might identify females with partial symptoms and favorable XCI. Eventually, the contributions of specific neurons versus the

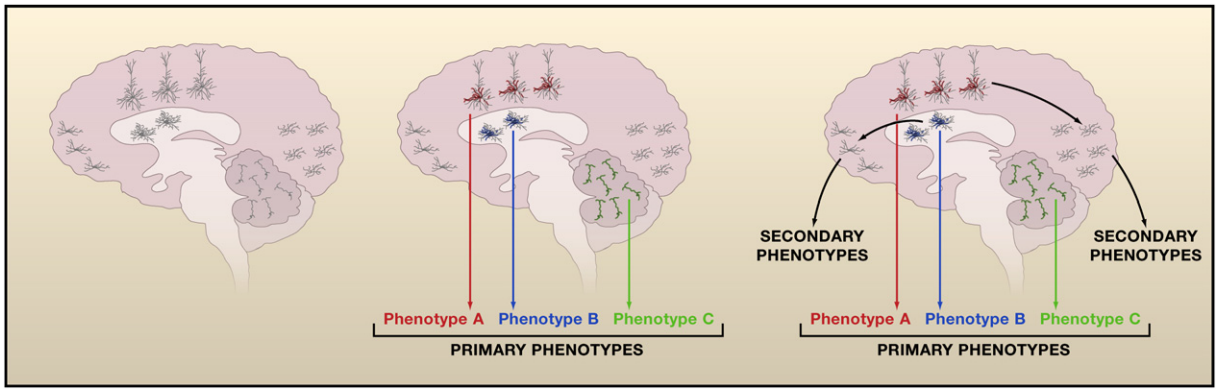

Figure 4. RTT: A Disorder of Cell-Autonomous Abnormalities versus Complex Network Defects
In this model, MeCP2 dysfunction leads to misregulation of target genes expressed in specific neuronal populations. These neuron-specific perturbations result in individual phenotypes that constitute some of the key features of RTT. Eventually, cell-specific changes will impact the function of other neurons, leading to network abnormalities and possibly secondary neurobehavioral phenotypes.

networks of neurons to the RTT phenotype could be addressed in the mouse using neuron-specific deletions of *Mecp2*. The finding that anatomical changes, such as the decrease in glutamatergic synapses, are only detected in the presymptomatic stage (Chao et al., 2007) argues that compensatory changes might mask the primary consequences of MeCP2 dysfunction. Therefore, to get at some of the primary pathogenic events, one needs to study presymptomatic as well as early symptomatic animals.

The Challenge of Developing Therapies for *MECP2* Disorders

The recent studies demonstrating that neurological deficits resulting from loss of MeCP2 can be reversed upon restoration of gene function are quite exciting. The next phase of research needs to assess how complete the recovery is. Clearly, lethality, level of activity, and hippocampal plasticity are rescued, but are the animals free of any other RTT symptoms such as social behavior deficits, anxiety, and cognitive impairments? Since postnatal rescue results in viability, it will be important to evaluate if even the subtler phenotypes of RTT and *MECP2* disorders are rescued when protein function is restored postnatally. This is particularly important given emerging data about early neonatal experiences and their long-term effects on behavior in adults (Plotsky et al., 2005; Sullivan et al., 2006). The genetic rescue data are promising because they show that neurons that have suffered the consequences of loss of MeCP2 function are poised to regain functionality once MeCP2 is provided gradually and in the correct spatial distribution. This provides hope for restoring neuronal function in patients with RTT. However, the strategy in humans will require providing the critical factors that function downstream of MeCP2 because of the challenges in delivering the correct MeCP2 dosage *only* to neurons that lack it, given that the slightest perturbation in MeCP2 level is deleterious. Thus, therapeutic strategies necessitate the identification of the molecular mechanisms underlying individual RTT phenotypes and picking out the candidates that can be therapeutically targeted. Although the data are minimal, it is quite likely that there will be many genes whose expression is sufficiently altered to cause neuronal dysfunction, probably due to loss of normal homeostatic responses. While it is conceivable that some of these molecules could be investigated for potential therapeutics, it might prove challenging to restore levels of tens of targets.

An alternative approach will be to identify proteins or pathways that suppress MeCP2 dysfunction phenotypes, which might prove easier to target therapeutically. The fact that there are human patients with milder phenotypes in spite of severe mutations (Dayer et al., 2007) argues that some variant in another protein or proteins might subdue the disease. Identifying such modifiers using various mouse models might prove very helpful. Lastly, given that loss and gain of MeCP2 have opposing effects on neurons, it will be important to establish whether certain mutant *MECP2* alleles are functionally null or hyperactive when contemplating therapeutic trials.

In closing, the RTT story started in the clinic, but today has inspired many exciting basic science studies in neurobiology and epigenetics. It is anticipated that the next chapter in this story will involve translating some of the discoveries back to the clinic to benefit patients with RTT and patients with related neurological disorders.

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