Am. J. Hum. Genet. 71:1259–1272, 2002

REVIEW ARTICLE
Rett Syndrome and MeCP2: Linking Epigenetics and Neuronal Function

Mona D. Shahbazian[1] and Huda Y. Zoghbi[1,2,3,4,5]

Departments of [1]Molecular and Human Genetics, [2]Pediatrics, [3]Neurology, and [4]Neuroscience and [5]Howard Hughes Medical Institute, Baylor College of Medicine, Houston

Introduction

Rett syndrome (RTT [MIM #312750]) was discovered when two girls who exhibited the same unusual behaviors happened to be seated next to each other in the waiting room of Andreas Rett, a Viennese pediatrician. It took >30 years after this discovery to determine the genetic basis of RTT, largely because the disease is primarily sporadic in nature and because familial cases are scarce. The discovery that mutations in methyl-CpG–binding protein 2 (*MECP2*) cause RTT and other neurodevelopmental disorders has called attention to the importance of epigenetic modifications in neuronal function.

Features of RTT

Clinical Features

RTT affects ~1/15,000 females worldwide (Hagberg 1985; Kerr and Stephenson 1985; Kozinetz et al. 1993). The classic form of the disease, found almost exclusively in females, follows a distinct developmental course that Andreas Rett described as "both tragic and fascinating" (Rett 1986). Affected girls are born healthy, appear to develop normally until age 6–18 mo, and achieve the expected motor, language, and social milestones (Hagberg et al. 1983). Their neurological development is then arrested and begins regressing in a predictable pattern comprising roughly four stages (Hagberg and Witt-Engerstrom 1986). During Stage I (age 6–18 mo), girls cease to acquire new skills; they display decelerating head growth and autistic features such as emotional withdrawal and diminished eye contact. In Stage II (age 1–4 years), affected children lose learned skills such as speech and purposeful hand use. They develop irregular breathing patterns, truncal and gait ataxia/apraxia, and stereotypical hand wringing; about half the girls also develop seizures. There is some stabilization of the disease during Stage III (age 4–7 years), because loss of skills can continue only to a point. During this stage, girls may learn to communicate preferences using eye pointing. They still have gross cognitive and motor impairments and commonly experience epileptic episodes. Seizures become less frequent during Stage IV (age 5–15 years and older), but motor deterioration continues. Hypoactivity, especially among those who cannot walk, contributes to the frequent development of scoliosis, which can cause the girls to be confined to wheelchairs (Hagberg and Witt-Engerstrom 1986).

Other features are associated with RTT but are not diagnostic. For example, patients with RTT are generally small for their age (Holm 1986). Some have suggested that this growth retardation is due to poor self-feeding abilities and reduced caloric intake (Thommessen et al. 1992), but others have found it to be independent of diet (Rice and Haas 1988). Many patients display an abnormally long QT_c interval (Sekul et al. 1994; Guideri et al. 1999). Although females with RTT often live well into adulthood, the mortality rate is 1.2% per year, with about 1/4 of these deaths described as sudden and unexplained (Kerr et al. 1997). Incidents of sudden death have been postulated to result from breathing dysfunction and cardiac abnormalities (Sekul et al. 1994; Guideri et al. 1999). Finally, patients with RTT often have signs of autonomic dysfunction such as constipation and cold or pale extremities (Hagberg et al. 1983; Naidu et al. 1986).

In addition to classic RTT (summarized in table 1), five distinct categories of atypical cases have been delineated on the basis of clinical criteria (Hagberg and Skjeldal 1994). These variants have some, but not all, diagnostic features of RTT and can be milder or more severe. Milder variants include the *forme fruste* ("worn-down form"), the late regression variant, and the preserved speech variant (Hagberg and Witt-Engerstrom 1986; Zappella 1992; Hagberg and Skjeldal 1994). The more severe forms are the congenital form and the early-seizure-onset variant (Hanefeld 1985; Goutieres and Aicardi 1986; Hagberg and Skjeldal 1994).

Received September 20, 2002; accepted for publication October 1, 2002; electronically published November 19, 2002.

Address for correspondence and reprints: Dr. Huda Y. Zoghbi, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Room T807, MS #225, Houston, TX 77030. E-mail: hzoghbi@bcm.tmc.edu

0002-9297/2002/7106-0003$15.00

Table 1

Phenotypes Associated with *MECP2* Mutations in Human Females

Characteristic	Phenotype for		
	Classic RTT[a]	Mild RTT Variants[b]	Severe RTT Variants[c]
Age at onset	Onset between 6–18 mo	Later onset	Congenital onset
Head/body size	Small head, body	May have small head, body	Small head, body
Seizures	Seizures		Early seizure onset
Speech	Loss of speech	Speech is preserved	
Motor function	Motor deficiencies	Usually ambulatory	Hypotonia, motor deficiencies
Hand use	Stereotypical hand motions	Retain hand use	
Social Interactions	Autistic features		
Intelligence	Mental retardation	Mild or no mental retardation	Mental retardation
Spinal curvature	Scoliosis and/or kyphosis		Scoliosis and/or kyphosis
Respiration	Breathing dysfunction		Breathing dysfunction

[a] Null alleles or severe inactivating mutations, balanced XCI.
[b] Hypomorphic alleles (late truncations) with balanced XCI or null alleles with favorably skewed XCI. (Very late truncations and some missense mutations, such as A140V and Q406X, result in no phenotype in females even when XCI is balanced. These same mutations do, however, produce phenotypes in males.)
[c] Null alleles or severe inactivating mutations, possibly due to unfavorably skewed XCI.

Neurophysiology

The electroencephalogram (EEG) is typically normal until 3 years of age (Verma et al. 1986; Glaze et al. 1987). After this period, the background activity remains normal, but repetitive high-amplitude spike-and-wave discharges (focal, multifocal, or generalized) are seen. Later, the background activity becomes abnormally slow (Verma et al. 1986). After 10 years of age, the background activity shows additional slowing, but, consistent with the reduction in seizures during the final stage of the disease, the EEG discharges are reduced in frequency, amplitude, and duration (Niedermeyer et al. 1986; Verma et al. 1986).

Breathing appears to be normal during sleep, but periods of irregular breathing (hyperventilation followed by periods of apnea) often occur during wakefulness, particularly when patients are under emotional or physical stress (Lugaresi et al. 1985; Cirignotta et al. 1986; Southall et al. 1988). Because the respiratory abnormalities in RTT are restricted to the wakeful state, it is thought that the voluntary system for regulating breathing is affected rather than the autonomic system, which controls respiration during non–rapid-eye-movement sleep (Lugaresi et al. 1985).

Neuroimaging

Volumetric magnetic resonance imaging studies show an overall decrease in brain volume, affecting gray matter more than white matter, but reveal no evidence of neurodegeneration (Reiss et al. 1993). The caudate nucleus and frontal cortex show the largest reduction in volume (Reiss et al. 1993; Subramaniam et al. 1997). The volume of white matter does increase with age, however, suggesting that myelinization progresses normally (Reiss et al. 1993).

As measured by single photon emission–computed tomography, global cerebral blood flow (a measure of neuronal metabolism) is 22% lower in patients with RTT (Nielsen et al. 1990). At a regional level, blood flow in the frontal, parietal, and temporal regions is significantly lower than in control individuals, whereas that in the occipital and sensorimotor areas is normal (Nielsen et al. 1990; Lappalainen et al. 1997). Because the blood flow pattern in the brains of patients with RTT resembles the pattern of normal infants before age 6 mo, it has been proposed that blood flow pattern reflects developmental arrest (Nielsen et al. 1990).

Neuropathology

In spite of a deteriorating clinical course and diverse neurological features, the brain pathology in RTT reveals only subtle abnormalities. As one might expect from both imaging studies and the clinical deceleration in head growth, postmortem studies consistently find that brains of patients with classic RTT weigh 14%–34% less than those of control subjects' brains (Jellinger and Seitelberger 1986). To control for the fact that patients with RTT often have a reduced body size, organ weights have been compared with those of normal individuals of the same height to show that there is a selective decrease in the size of the brain (Armstrong et al. 1999).

RTT was first thought to be a neurodegenerative disease because of the developmental regression, but the majority of neuropathological findings argue against this and indicate that it is a disorder of arrested neuronal development (Armstrong 2001). Impaired neuronal development was initially suggested by the observation that the dendrites of pyramidal neurons of the frontal and motor cortex were reduced in length and complexity

(Armstrong 1992; Armstrong et al. 1995). Three-dimensional confocal microscopy studies confirmed these findings and also revealed a reduction in the number of dendritic spines (Belichenko et al. 1994). Other studies showed that neurons of the cerebral cortex, basal ganglia, thalamus, hippocampus, amygdala, and substantia nigra were smaller and more densely packed than in control subjects (Bauman et al. 1995). The observation of reduced pigmentation in the substantia nigra (Jellinger and Seitelberger 1986) also suggests impaired neuronal development, since melanin pigment normally accumulates in these neurons from the 5th postnatal wk of development until age 12–15 years (Fenichel and Bazelon 1968; Spence and Gilles 1971).

A number of molecular changes are consistent with this neuropathology. The dendritic alterations observed in postmortem tissue could be related to reductions in the levels of microtubule-associated protein 2 (an important cytoskeletal component of neuronal dendrites), substance P (a neuropeptide that has been shown to stimulate neurite extension in cultured neuroblastoma cells), and prostaglandin endoperoxide H synthase-2 (the rate-limiting enzyme in prostanoid biosynthesis that localizes to dendritic spines and marks mature neurons) (Whitty et al. 1993; Kaufmann et al. 1995, 1996, 1997; Matsuishi et al. 1997; Deguchi et al. 2000). These pathological observations support the conclusion that, although glial development occurs normally, neuronal development is hindered. Whether the molecular alterations are a cause or a result of the abnormal development has not been determined.

Genetic Aspects of RTT

Mapping of the Mutated Gene

The mode of inheritance of RTT was difficult to determine, because only ~1% of RTT cases are familial and because affected individuals rarely reproduce (Schanen 1999). Nevertheless, the virtual absence of affected males and the presence of families with affected half-sisters suggested an X-linked dominant inheritance pattern with male lethality (Hagberg et al. 1983; Killian 1986). Consistent with an X-linked mutation, analysis of the available families revealed that inheritance through maternal lineages was common (Zoghbi 1988). Furthermore, in families where the mother was an obligate carrier (e.g., families with affected half-sisters or aunt-niece pairs), X-chromosome inactivation (XCI) studies often revealed skewed XCI patterns in the unaffected mother (Zoghbi et al. 1990; Schanen et al. 1997). Some substantiation for the male-lethality aspect of the X-linked hypothesis was provided by males, born into families with RTT, who displayed neonatal encephalopathy with hypotonia, seizures, apnea, and infantile death (Ruch et al. 1989; Schanen et al. 1997, 1998). The identification of males who harbored an extra X-chromosome (47,XXY) and bore features identical to those of RTT was also compatible with X-linked inheritance (Vorsanova et al. 1996).

Conventional genomewide linkage analysis was not feasible in RTT because of the rarity of familial cases and the limited vertical transmission. A focused exclusion-mapping approach on the basis of the hypothesis of an X-linked mutation (Archidiacono et al. 1991; Ellison et al. 1992; Curtis et al. 1993; Schanen et al. 1997; Schanen and Francke 1998; Sirianni et al. 1998) culminated in the exclusion of most of the X-chromosome, leaving only the region distal to Xq27.3. Conventional linkage analysis using the few available families with recurrent RTT revealed linkage to markers in this region with a peak LOD score of 2.9 (Sirianni et al. 1998; Webb et al. 1998; Xiang et al. 1998). A number of genes within Xq27.3-qter were analyzed for mutations in patients with RTT because of their known expression or function in the central nervous system (Wan and Francke 1998; Amir et al. 2000*a*; Xiang et al. 2000), but mutations were finally identified in the methyl-CpG-binding protein 2 gene (*MECP2* [MIM 300005]), a widely expressed transcriptional repressor (Amir et al. 1999). *MECP2* had previously been mapped to human Xq28 and was found to be subject to XCI (Quaderi et al. 1994; Adler et al. 1995; D'Esposito et al. 1996; Vilain et al. 1996).

MECP2 Mutations in Classic RTT

Data from many laboratories have demonstrated that mutations in *MECP2* are the primary cause of RTT, because mutations have been identified in 70%–90% of sporadic cases and ~50% of familial cases (Shahbazian and Zoghbi 2001). Only the coding region has been thoroughly analyzed, however, so mutations in regulatory elements could account for those cases in which no mutation has been identified. Consistent with the prominence of C→T transition mutations at 5′-CG-3′ (CpG) sites in other disease genes, presumably resulting from spontaneous deamination of methylated cytosine residues (Cooper and Youssoufian 1988), almost 70% of the reported mutations result from this transition at only eight different CpG dinucleotides within the *MECP2* gene (Lee et al. 2001).

MECP2 Mutations in RTT Variants

As noted above, there are several variants on the classic RTT phenotype, which itself is complex and variable. One of the first questions to be addressed after the discovery of *MECP2* mutations was whether these RTT variants are truly alternate forms of the same entity. Findings indicate that a majority of females with the preserved speech variant do have mutations in *MECP2*

Table 2

Phenotypic Categories for *MECP2* Mutations in Human Males

Allele Type	Phenotype
Null (early trucating mutations and some missense mutations)	(1) Neonatal encephalopathy, severe hypotonia, seizures, apnea, and infantile death; or (2) RTT-like features with 47,XXY karyotype or somatic mosaicism
Hypomorphic (late truncations and some missense mutations)	(1) Mental retardation, no delayed development of language but macrocephaly, tremors, ataxia, hypoactivity, and seizures; (2) childhood-onset schizophrenia with loss of language; or (3) manic-depressive psychosis, pyramidal signs, and macro-orchidism

(De Bona et al. 2000; Yamashita et al. 2001; Zappella et al. 2001). Mutations have also been identified in a fraction (20%–40%) of patients with other forms of the disease, including the *forme fruste* and congenital variants (Buyse et al. 2000; Cheadle et al. 2000; Huppke et al. 2000; Bourdon et al. 2001; Inui et al. 2001; Nielsen et al. 2001) (see table 1). The lower percentage of identified *MECP2* mutations in patients with atypical RTT could indicate the presence of genocopying mutations or could suggest that RTT variants are more frequently associated with (as-yet-unidentified) mutations in regulatory elements of *MECP2* than the classic form of the disease.

Origin of MECP2 mutations

The prediction that most mutations occur de novo (Comings 1986) has been verified with multiple observations that the mutations found in sporadic cases are not present in somatic cells from either parent (Amir et al. 1999; Wan et al. 1999; Bienvenu et al. 2000; Buyse et al. 2000; Erlandson et al. 2001; Monros et al. 2001; Nicolao et al. 2001). Since mutations on the paternal X-chromosome would be inherited by daughters but not sons, some have proposed a high paternal:maternal ratio of de novo mutations to explain the low incidence of affected males (Thomas 1996). This hypothesis is, in fact, correct: haplotype analysis has shown that >95% of *MECP2* mutations originate on the paternal chromosome (Girard et al. 2001; Trappe et al. 2001). A high ratio of male:female germ-cell mutations is not unique to *MECP2*; it has been documented for a number of human disease genes (Crow 2000). It is also known that almost 40% of the mutations responsible for various diseases occur at CpG sites (Cooper and Youssoufian 1988). The higher levels of methylation in male germ cells at early stages of gametogenesis, in addition to the greater number of mitotic divisions in the male germline, could explain sex-specific mutation patterns (Driscoll and Migeon 1990; El-Maarri et al. 1998).

Effect of XCI

Skewed XCI patterns had been postulated to explain the occurrence of unaffected carrier females in families with recurrent RTT (Zoghbi et al. 1990). In support of this idea, skewed XCI (presumably favoring inactivation of the mutant allele) has been observed in females who carry RTT-causing mutations but are asymptomatic or suffer from only mild learning disability (Wan et al. 1999; Amir et al. 2000*b*; Bienvenu et al. 2000; Villard et al. 2000; Hoffbuhr et al. 2001). Although it seems likely that cells expressing mutant *MECP2* would have some growth disadvantage and be selected against, this appears not to be the case: the same mutation is present in the carrier females and their affected daughters. In fact, the majority of affected females have a balanced pattern of XCI, both in peripheral blood and brain tissue (Amir et al. 2000*b*; Shahbazian et al. 2002*b*). Therefore, the skewed XCI pattern in carrier females is probably due to chance.

RTT Mutations in Males

Mutation analysis has also shed light on the question of whether the boys who developed neonatal encephalopathy and died within 1–2 years of birth suffered the consequences of *MECP2* mutations (table 2). Indeed, the same mutations that are present in their sisters with classic RTT proved to be the cause of disease in these boys (Wan et al. 1999; Villard et al. 2000; Hoffbuhr et al. 2001; Geerdink et al. 2002; Zeev et al. 2002). Males bearing a mild *MECP2* mutation that would likely produce a very mild phenotype in girls (i.e., late truncating mutations and some missense mutations) can survive the neonatal period, but they develop severe mental retardation associated with motor abnormalities (table 2). In contrast to these cases, there are several instances of males with RTT-causing *MECP2* mutations that have developed an RTT phenotype. In these cases, some other modifier is present, such as somatic mosaicism for the mutation or a partial or complete Klinefelter (47,XXY) karyotype (Clayton-Smith et al. 2000; Armstrong et al. 2001; Hoffbuhr et al. 2001; Leonard et al. 2001;

Schwartzman et al. 2001; Vorsanova et al. 2001; Topcu et al. 2002). In these situations, the effect of an *MECP2* mutation in a male is mitigated in the same way that it is in a female, that is, by only a fraction of cells expressing the mutant *MECP2* allele.

Genotype-Phenotype Correlation

Several groups have conducted genotype-phenotype correlation studies to determine whether different types of mutations in *MECP2* can account for the variability of clinical features in patients with RTT; these studies have yielded inconsistent results. Two groups found that truncating mutations lead to a more severe overall phenotype than missense mutations (Cheadle et al. 2000; Monros et al. 2001), and one of these studies further showed that late truncations correlate with a less severe outcome than early truncations (Cheadle et al. 2000). In 18 females with the preserved speech variant, all the mutations were either missense or late truncations, further supporting the notion that these types of mutations have milder consequences than the early truncations seen in classic RTT (Zappella et al. 2001). Several other studies, however, have found no significant correlation between the mutation type and the overall severity of clinical features (Amir et al. 2000*b*; Bienvenu et al. 2000; Huppke et al. 2000; Giunti et al. 2001; Yamada et al. 2001; Chae et al. 2002). This discordance likely results from the fact that the pattern of XCI can influence the phenotypic outcome of mutations in females.

In males, the confounding effect of XCI is absent, and the correlation between the type of mutation and the phenotypic outcome is clearer. As mentioned, *MECP2* mutations that cause classic RTT in females lead to neonatal encephalopathy and infantile death in males unless the mutations are mitigated by somatic mosaicism or a Klinefelter phenotype (table 2). In contrast, other mutations in *MECP2*, which are not known to cause RTT in females, have been found in males manifesting various features, some of which are seen in RTT. For example, a Q406X mutation, which eliminates the last 80 amino acids of the protein, was found in two males with delayed development, macrocephaly, seizures, ataxia, and absence of language (Meloni et al. 2000). Females with this same mutation were unaffected, even with balanced XCI patterns (Meloni et al. 2000).

Many missense mutations have been identified in males with nonspecific X-linked mental retardation (MRX). In fact, the observed frequency of *MECP2* mutations in a large collection of males with MRX (~2%) is almost as high as the frequency of trinucleotide expansions in the *FMR1* gene in this population (~3%–4%) (Couvert et al. 2001). The A140V mutation has been associated with a number of phenotypes: mental retardation; mental retardation with abnormal gait and speech difficulty; childhood-onset schizophrenia with loss of language; and a syndrome characterized by manic-depressive psychosis, pyramidal signs, and macroorchidism (Orrico et al. 2000; Couvert et al. 2001; Cohen et al. 2002; Klauck et al. 2002). Because males with these mutations survive into adulthood and females with the same mutations are unaffected despite balanced XCI patterns, these types of mutations appear to be less detrimental to MeCP2 function than those causing RTT. Whereas RTT-causing mutations typically lead to either premature truncation or nonconservative amino acid changes, the milder mutations are more often conservative amino acid changes or truncations that preserve a significant portion of the coding region. Given that a single mutation (e.g., A140V) can have disparate effects in different families, other genetic factors probably contribute to this phenotypic variability.

MeCP2 Function

MeCP2 binds preferentially to DNA methylated at CpG sites through an 85–amino acid methyl-CpG–binding domain (MBD) (amino acids 78–162) (Lewis et al. 1992; Nan et al. 1993). Once bound to DNA, MeCP2 is thought to silence transcription of downstream genes by recruiting corepressor complexes through a 104–amino acid transcriptional repression domain (TRD) (amino acids 207–310) (Nan et al. 1997). One complex shown to associate with MeCP2 is the Sin3A corepressor complex (Jones et al. 1998; Nan et al. 1998). This complex contains histone deacetylase (HDAC) 1 and HDAC2 and was originally shown to mediate repression by the DNA-binding heterodimer, Mad-Max (Laherty et al. 1997). MeCP2 also interacts with two other corepressors, the proto-oncoprotein of the Sloan-Kettering virus named after the Sloan-Kettering Institute (c-Ski) and the nuclear receptor corepressor (N-CoR) (Kokura et al. 2001). c-Ski and N-CoR are components of histone deacetylase complexes that can but do not always function together (Heinzel et al. 1997; Nomura et al. 1999). It is interesting that repression by MeCP2 is not completely alleviated by the histone-deacetylase inhibitor, trichostatin A (TSA), suggesting that MeCP2 may also repress transcription in an HDAC-independent manner (Jones et al. 1998; Yu et al. 2000). In support of this idea, the TRD interferes with the assembly of the transcriptional preinitiation complex on naked DNA and interacts directly with transcription factor IIB (TFIIB), a component of the basal transcriptional machinery (Kaludov and Wolffe 2000).

In addition to the MBD and the TRD, there are two other domains of MeCP2 associated with specific functions. First, lying within the TRD is the nuclear localization signal (amino acids 255–271), which is sufficient for transportation of the protein into the nucleus (Nan

et al. 1996). Second, the last 63 amino acids of MeCP2 have been shown to facilitate binding of the protein to both naked and nucleosomal DNA (Chandler et al. 1999). Another study, however, showed that this region decreases binding to methylated DNA but may increase protein stability (Yusufzai and Wolffe 2000).

Effect of Mutations on MeCP2 Function

Missense mutations identified in the *MECP2* gene in patients with RTT cluster in the MBD and TRD, but some are found outside these regions and also in the C-terminus. In vitro studies have demonstrated that many missense mutations within the MBD (R106W, R111G, Y123A, I125A, R133C, F155S, and T158M) significantly reduce the affinity of MeCP2 for methylated DNA (Ballestar et al. 2000; Yusufzai and Wolffe 2000; Free et al. 2001). Consistent with these observations, MeCP2 with the R106W or F155S mutation, when transfected into cells, is impaired in its ability to localize to heterochromatic domains and to repress transcription of a reporter (Kudo et al. 2001). It is interesting that the A140V and E137G mutants (within the MBD), which cause milder phenotypes in humans, localize normally to heterochromatin but have diminished repressive capability (Kudo et al. 2002). This suggests that amino acids in the MBD may have roles outside of binding methyl-CpG sequences or that the heterochromatic localization assay is not sensitive enough to detect the diminished binding affinity. As expected, MeCP2 proteins that retain the MBD but are truncated within the TRD are able to bind methylated DNA but are impaired in their ability to repress transcription (Yusufzai and Wolffe 2000). Less expected is that one missense mutation in the TRD (R306C) does not reduce repressive activity of MeCP2 in a transfection assay, although it may have stronger effects in vivo (Yusufzai and Wolffe 2000). Deletions within the C-terminus of MeCP2, which are common mutations in classic RTT, significantly decrease protein stability (Yusufzai and Wolffe 2000).

DNA Methylation

Because the function of MeCP2 is strongly tied to DNA methylation, an understanding of the role of methylation is necessary to gain insight into the molecular basis of RTT. Whereas promoters of cellular "housekeeping" genes are relatively rich in CpG yet protected from methylation (Bird et al. 1985), other genes are methylated in unique ways. Tissue-specific genes are often methylated in nonexpressing tissues, X-linked genes are methylated on the inactive X-chromosome, and imprinted genes are methylated according to their parental origin. In addition to cellular genes, satellite DNA, retroviruses, and transposable elements are highly methylated (Yoder et al. 1997). Genomewide methylation is thought to occur early in embryonic development (Lock et al. 1987; Kafri et al. 1992). In the developing germline, methylation patterns are erased, and a second phase of methylation establishes imprinting patterns according to the sex of the embryo (Kafri et al. 1992; Brandeis et al. 1993). DNA methylation is now known to be accomplished by two classes of DNA methyltransferases (DNMTs). DNMT3a and DNMT3b are partially redundant de novo methyltransferases, which establish the methylation pattern (Okano et al. 1998, 1999). DNMT1 is a maintenance methyltransferase, which preserves the methylation pattern through DNA replication by methylating the newly synthesized DNA strand (Bestor and Ingram 1983). Demonstrating the essential role of methylation in gene regulation and development, Dnmt1-deficient mice and mice lacking both Dnmt3a and Dnmt3b die before embryonic day (E) 11.5 (Li et al. 1992; Okano et al. 1999). Because *MECP2* mutations result in a primarily neurological phenotype, it appears that MeCP2 is essential only for the regulation of a subset of methylated genes.

MeCP2 Target Genes

MeCP2 has been shown to bind and/or silence a number of methylated genes, including retroviral, tissue-specific, and imprinted genes. For example, in cell lines infected with the Moloney murine leukemia virus, MeCP2 associates with the provirus in a methylation-dependent manner (Lorincz et al. 2001). LINE-1 but not *Alu* retrotransposons are also repressed by MeCP2 in transfection assays (Yu et al. 2001). Genes methylated in a cell- or tissue-specific manner may also be regulated by MeCP2. For example, the leukosialin gene, which is differentially methylated depending on the tissue, is repressed by MeCP2 in a transfection assay when the promoter is methylated, although whether MeCP2 binds to this promoter in vivo remains to be determined (Kudo 1998). In addition, the multidrug resistance gene promoter in drug-sensitive cells is hypermethylated and bound by MeCP2, whereas in drug-resistant cells, the promoter is hypomethylated, transcriptionally active, and relatively free of MeCP2 (El-Osta and Wolffe 2001; El-Osta et al. 2002). MeCP2 has also been implicated in the regulation of imprinted genes. For example, MeCP2 binds selectively to the paternal allele of the differentially methylated domain of the *H19* gene (Drewell et al. 2002) and to the maternal (methylated) allele of the imprinted *U2af1-rs1* gene (Gregory et al. 2001). It is interesting that several imprinted genes have been implicated in neurological functions, including *Ube3a*, mutated in Angelman syndrome (Kishino et al. 1997; Matsuura et al. 1997), and *Peg1*, an imprinted gene, which, when deleted in mice, causes abnormal maternal behavior and growth retardation (Lefebvre et al. 1998). Whether any

of these genes is abnormally expressed in RTT remains to be determined.

Methyl-CpG–Binding Proteins

Given that methylation is essential for viability in mice, it is not too surprising that a number of proteins other than MeCP2 bind to methylated DNA. A group of proteins, MBD1, MBD2, MBD3, and MBD4, are so named because they contain a methyl-CpG–binding domain similar to that of MeCP2 (Cross et al. 1997; Hendrich and Bird 1998). Another protein, Kaiso, which has homology to the POZ zinc finger family of DNA-binding transcription factors but does not contain the classical MBD motif, has also been shown to specifically bind methylated DNA (Daniel and Reynolds 1999; Prokhortchouk et al. 2001). MBD1, MBD2, and MBD4, like MeCP2, can bind DNA containing only one symmetrically methylated CpG site and can localize to centromeric heterochromatin in transfected cells (Hendrich and Bird 1998; Fujita et al. 1999; Ng et al. 1999). In contrast, Kaiso requires at least two symmetrically methylated CpG sites to bind DNA, and MBD3 lacks the ability to bind methylated DNA in vitro (Hendrich and Bird 1998; Prokhortchouk et al. 2001).

In addition to sharing the ability to bind methylated CpG sites, MBD1, MBD2, and Kaiso are similar to MeCP2 in their ability to repress transcription (Ng et al. 1999; Boeke et al. 2000; Ng et al. 2000; Prokhortchouk et al. 2001). It is interesting to note that Kaiso has no sequence homology to the MBD family members, yet it is able to perform a similar function; thus, the true number of methyl-CpG–binding transcriptional repressors may be much higher than previously suspected. Considering the high level of functional similarity within this group of proteins, it seems likely that there would be at least partial compensation when one member is deleted. The primarily neurological phenotype of *MECP2* mutations, however, leads us to hypothesize that this protein has some function that is specific to neurons and is not compensated for by other members of the MBD family.

MeCP2 Expression Pattern

Studies of the expression pattern of *MECP2* indicate that MeCP2 may indeed have a specialized role in neurons. Analysis of the *MECP2* mRNA has been complicated by the presence of three alternatively spliced transcripts (1.9 kb, 7.5 kb, and 10 kb) produced by differential polyadenylation site usage within the 3′-untranslated region (UTR) (Reichwald et al. 2000). These transcripts are found at varying levels in most tissues examined, with no obvious preference for nervous tissue (D'Esposito et al. 1996; Coy et al. 1999; Reichwald et al. 2000). The apparent tissue-specificity of the three *MECP2* transcripts and the presence of distinct 3′-UTRs suggest that the transcripts might have unique roles. To circumvent the difficulty of interpreting the expression pattern from multiple transcripts, however, the distribution of the MeCP2 protein has been analyzed. Unlike the mRNA, the protein is expressed at higher levels in the brain than in many other tissues (LaSalle et al. 2001; Shahbazian et al. 2002*a*). Within the brain, MeCP2 is present at high levels in most neurons but not in glia (Akbarian et al. 2001; LaSalle et al. 2001; Shahbazian et al. 2002*a*).

MeCP2 levels also vary in a neuron-specific manner (LaSalle et al. 2001; Shahbazian et al. 2002*a*). In the developing cerebral cortex of mouse, human, and nonhuman primate embryos, the appearance of MeCP2 correlates with neuronal maturation, with earlier-born neurons expressing MeCP2 before later-born neurons (Akbarian et al. 2001; Shahbazian et al. 2002*a*). These results may partly explain why the brain is most affected in RTT, why the development of white matter progresses normally although neuronal development does not, why certain neuronal populations appear more affected than others, and why the onset of disease is delayed. Understanding the functions of MeCP2 that are specific to mature neurons will be the next challenge in elucidating the pathogenesis of RTT.

Mouse Models of RTT

Tools for studying the in vivo role of MeCP2 have been produced by mutating *Mecp2* in mice (table 3). Male mice with a null mutation in *Mecp2* display stunted body and head growth, hypoactivity, hindlimb-clasping, irregular breathing, and death within 6–10 wk (Chen et al. 2001; Guy et al. 2001) (table 3). Deletion of *Mecp2* in developing neurons results in the same phenotype, demonstrating that the observed deficiencies in the null mice are solely a consequence of neuronal dysfunction and that no overt phenotypes result from MeCP2 dysfunction outside the nervous system, at least within the lifespan of these mice (Guy et al. 2001). These studies also showed that MeCP2 is important not only for developing neurons but also for postmitotic neurons, since deletion of *Mecp2* solely in postmitotic neurons results in a phenotype that is later and milder than that of the null allele (Chen et al. 2001). In *Mecp2*-null mice, concomitant loss of Mbd2 does not worsen the phenotype, suggesting that Mbd2 alone does not compensate for the loss of MeCP2 (Guy et al. 2001). Truncation of the C-terminus of MeCP2 in mice leads to a milder phenotype than the null allele, as revealed by the survival of male mice (Shahbazian et al. 2002*c*). These mice display many features of RTT, including tremors, motor impairments, hypoactivity, anxiety, seizures, kyphosis, and stereotypical forepaw motions (table 3). Elevated levels of acet-

Table 3

***Mecp2*-Deficient Mice**

Characteristic	Male Mice		Female Mice with Hypomorphic Allele[a]
	Null Allele	Hypomorphic Allele	
Age at onset	Appear normal for 5 wk	Appear normal for 6 wk	Appear normal for 6+ wk
Head/body size	Small head, body size		
Involuntary movements	Tremors	Tremors	Tremors
Seizures		Seizures	
Motor function		Motor deficiencies	Motor deficiencies
Motor control	Hindlimb clasping	Stereotypical forepaw motions	Stereotypical forepaw motions
Locomotion	Hypoactivity	Hypoactivity	
Respiration	Irregular breathing		
Spinal curvature		Kyphosis	
Life span	Death at 6–10 wk		
Social interactions		Anxiety and abnormal social interactions	

[a] These female mice have not been thoroughly characterized.

ylated histone H3 in brain tissue from these mice suggest that transcriptional alterations may underlie the phenotype (Shahbazian et al. 2002*c*).

Role of DNA Methylation in Neuronal Function

Because MeCP2 may prove to be important for regulating methylated genes, particularly in neurons, it is worthwhile considering what is known about the role of methylation in neuronal function. It has been questioned for some time why expression levels of the *Dnmt1* maintenance methyltransferase are high in brain, and, more specifically, high in neurons, but are undetectable in glia (Goto et al. 1994). The role of Dnmt1 in neurons has been studied by conditionally deleting the gene in mice. Dnmt1 deficiency in neuronal precursors at E9–E10 allows proper development and survival of neurons through birth but adversely affects neuronal survival after birth (Fan et al. 2001). In contrast, deletion of *Dnmt1* in postmitotic neurons does not affect neuronal survival or methylation of retroviral DNA (Fan et al. 2001). The questions that remain are whether Dnmt1 deficiency in postmitotic neurons leads to changes in the methylation of unique sequences and whether neuronal function is perturbed.

Changes in gene expression mediated by DNA methylation are thought to be important for neuronal maturation. Studies of a model system of neuronal differentiation—PC12 pheochromocytoma cells induced to differentiate with nerve growth factor (NGF)—have shown that gene methylation is required for neurite outgrowth (Persengiev and Kilpatrick 1996). Consistent with this finding, expression levels of several genes are reduced during differentiation of PC12 cells but only in the presence of DNA methyltransferase activity (Persengiev and Kilpatrick 1997). Substantiating the link between neuronal differentiation and chromatin modifications is the observation that the histone deacetylase inhibitor, TSA, also inhibits NGF-induced neurite outgrowth of PC12 cells (Futamura et al. 1995). In vivo evidence supports the role of epigenetic modifications in neuronal development, as several neuronal gene promoters have been found to undergo developmental changes in methylation. For example, the methylation pattern of the neuronal-specific gene *Stac* changes during mouse development (Suzuki et al. 1996). In addition, *GFAP* becomes methylated in neurons but not glia of the rat brain (Barresi et al. 1999). Because both methylation and histone deacetylation appear to be involved in neurite extension of PC12 cells and because dendrites appear to be underdeveloped in RTT brains, it is conceivable that MeCP2 plays a role in mediating the chromatin modifications necessary for dendritic outgrowth.

In addition to changes in neuronal chromatin structure that may occur during development, it is worth considering whether there are changes in chromatin structure in fully differentiated neurons. The fact that *Dnmt3a*$^{-/-}$ mice are normal at birth but show impaired growth and die at 4 wk of age (Okano et al. 1999) suggests that de novo methylation by this enzyme is either important during the embryonic stage for postnatal survival or that it is important after birth. Because *Dnmt3a* is expressed only in a few adult tissues, one of which is the brain (Okano et al. 1998), it is possible that de novo methylation is important in the brain after birth. There is already some evidence to suggest this, since DNA methylation levels increase in neurons after cerebral ischemia induced by temporary blockage of the cerebral artery (Endres et al. 2000). Thus, in postmitotic neurons, neuronal methylation patterns (and, presumably, gene expression) are regulated in response to at least one environmental factor. This leads to the question of whether methylation plays a role in neuronal responses to other types of stress or experiences.

The fact that both *Mbd2* and *Mecp2* mutant mice show primarily or solely neuronal phenotypes that manifest after birth may suggest that these MBD proteins

are specialized to mediate methylation events that occur in mature neurons. *Mecp2* mutant mice display abnormal responses to a novel environment; they are hypoactive and more anxious than wild-type mice, further indicating that methylation might be regulated in response to the environment (Shahbazian et al. 2002*c*). It is noteworthy that a light pulse applied to mice induces phosphorylation of serine 10 on histone H3, specifically in neurons of the hypothalamic suprachiasmatic nucleus that function as a circadian clock (Crosio et al. 2000). Serine 10 phosphorylation of histone H3 is known to enhance acetylation of the nearby lysine 14 (Cheung et al. 2000). These results suggest that environmental signals can, indeed, dynamically affect chromatin architecture in neurons.

To understand the molecular basis of RTT and the role of epigenetics in neuronal function, it is essential to identify genes that are targets of MeCP2 repression and determine whether their expression is altered in patients or mouse models. Gene-expression analysis of postmortem RTT brain tissue has revealed reduced expression of a number of neuronal-specific genes and upregulation of some glial-specific genes, although these changes likely reflect secondary changes that occur long after the initial insult (Colantuoni et al. 2001). Now that mouse models of RTT are available, it will be easier to dissect the primary changes in gene expression and neuronal function that underlie the RTT phenotype.

Concluding Remarks

It is becoming evident that MeCP2, a protein originally thought of as a global transcriptional repressor, is actually specialized for a function in neurons of the central nervous system. It is remarkable that mouse models have reproduced virtually every aspect of RTT, including the highly specialized hand-wringing behaviors, suggesting that the pathways leading from dysfunctional MeCP2 to each of these features are conserved between humans and mice. Gene expression analyses in these mouse models may help elucidate the neuronal-specific functions of MeCP2 that are misregulated in RTT. To understand the basis for the selective function of MeCP2, it may be worthwhile to search for additional interacting factors. Given that, in humans, the phenotypic outcome of *MECP2* truncation mutations depends on the position of the truncation, different regions of MeCP2 may interact with particular proteins or complexes. Uncovering the molecular alterations that lead to RTT will not only provide insight into the disease pathogenesis but may also shed light on the normal role of epigenetic modifications in the function of developing and mature neurons.

Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for RTT [MIM 312750] and MeCP2 [MIM 300005])

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