INVITED REVIEW

Rett syndrome: disruption of epigenetic control of postnatal neurological functions

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Abstract

Loss-of-function mutations in the X-linked gene Methyl-CpG-binding protein 2 (MECP2) cause a devastating pediatric neurological disorder called Rett syndrome. In males, these mutations typically result in severe neonatal encephalopathy and early lethality. On the other hand, owing to expression of the normal allele in ~50% of cells, females do not suffer encephalopathy but instead develop Rett syndrome. Typically females with Rett syndrome exhibit a delayed onset of neurologic dysfunction that manifests around the child’s first birthday and progresses over the next few years. Features of this disorder include loss of acquired language and motor skills, intellectual impairment and hand stereotypies. The developmental regression observed in patients with Rett syndrome arises from altered neuronal function and is not the result of neurodegeneration. Maintenance of an appropriate level of MeCP2 appears integral to the function of healthy neurons as patients with increased levels of MeCP2, owing to duplication of the Xq28 region encompassing the MECP2 locus, also present with intellectual disability and progressive neurologic symptoms. Despite major efforts over the past two decades to elucidate the molecular functions of MeCP2, the mechanisms underlying the delayed appearance of symptoms remain unclear. In this review, we will highlight recent findings that have expanded our knowledge of MeCP2’s functions, and we will discuss how epigenetic regulation, chromatin organization and circuit dynamics may contribute to the postnatal onset of Rett syndrome.

Introduction

Rett syndrome (RTT; OMIM Entry 312750) is a postnatal neurodevelopmental disorder that results in profound intellectual and motor disability and affects ~1 in 10 000 live female births (1,2). The vast majority of RTT cases result from mutations in Methyl-CpG-binding protein 2 (MECP2), which is located within Xq28 and encodes an epigenetic regulator that is highly expressed in the nervous system (3). Approximately 99% of reported cases of classical RTT are sporadic, and 95% of cases are caused by de novo, loss-of-function mutations in MECP2; however, a handful of cases are familial and display an X-linked dominant inheritance pattern (3,4). Classical RTT is infrequently observed in males because a deleterious mutation in the only copy of MECP2 typically results in severe neonatal encephalopathy and early lethality (5).

RTT is characterized by a period of ostensibly normal development during the first 6–18 months of life; after this time period, patients with RTT undergo a developmental regression marked by loss of acquired language abilities, a slowing of both head and brain growth, impaired motor skills and loss of purposeful hand movements (6). Following this regressive stage, a period of stabilization or even partial recovery of certain skills ensues; after this plateau period, however, comes a later phase of motor deterioration (6). Patients with RTT also exhibit a wide array of phenotypes including abnormal breathing patterns, autistic features, seizures and autonomic nervous system dysfunction (7). The clinical picture produced by a MECP2 mutation can be influenced considerably by favorable X-inactivation patterns as well as the degree of MeCP2 dysfunction conferred by the specific mutation. Hypomorphic alleles typically produce mild or
partial phenotypes in females, and they typically cause psychiatric disorders and intellectual disability without neonatal lethality in males (8–18). Notably, enhanced MeCP2 function caused by duplications of the MECP2 locus also results in neurologic impairment; individuals with MECP2 duplication syndrome present with early-onset infantile hypotonia, delayed cognitive and motor development, severe intellectual disability, epilepsy and progressive spasticity during childhood (19–21). The similarities between these two neurological disorders highlight the importance of having MeCP2 at the appropriate level for proper neuronal function.

Histologically, the brains of RTT patients have smaller, more closely packed neurons with decreased dendritic complexity; despite these changes in connectivity and cell morphology, there is no evidence of neurodegeneration or gross malformations (22,23). Efforts to unravel the biological functions of MeCP2 have benefited from the generation of numerous mouse models with disease-causing Mecp2 mutations that reproduce many aspects of RTT, including altered dendritic branching and decreased brain weight (24–28). MeCP2 is expressed in the majority of cell types within the body, is highly expressed within the central nervous system, and its expression increases during neuronal maturation (29–32). The extensive expression of MeCP2 throughout the brain helps explain the breadth of symptoms seen in RTT. Similar to findings in human patients, mouse models also exhibit a broad spectrum of symptom severity and phenotypes. Female mice heterozygous for a Mecp2-null allele show a delayed onset of behavioral deficits around 4 months of age or older (24,33,34). Male mice with a Mecp2-null allele are more severely affected than heterozygous females, with overt symptoms observable at 3–4 weeks of age and death occurring at ∼8–10 weeks of age (24,33). Consistent with patient data, male mice with hypomorphic alleles have milder phenotypes and variable increases in longevity depending on the specific mutation (25,35–37).

One of the most intriguing and, as of yet, incompletely understood features of RTT is the period of seemingly normal early development prior to the onset of progressive neurologic dysfunction. This belated appearance of symptoms has been recapitulated in mouse models and begs the following question: how does altered MeCP2 function lead to delayed disease manifestation? In this review, we will discuss the pathophysiological mechanisms that might account for this postnatal onset of neurological dysfunction by examining recent advances in our understanding of MeCP2 function at the molecular, nuclear and network levels. First, we will consider the epigenetic mechanisms that might contribute to the timing of symptom appearance by discussing the dynamic interaction of MeCP2 with alternatively methylated forms of DNA. Then, we will move to the nuclear level to look at the chromatin-organizing abilities of MeCP2 and the progressive chromatin architectural changes observed in mouse models. Finally, we will discuss the ramifications of the molecular and nuclear changes on neuronal network function.

**Impaired epigenetic transcriptional regulation**

Originally characterized as having a high affinity for methylated cytosines that are followed by a guanine nucleotide (mCG) (38), recently MeCP2 has also been found to bind two alternatively methylated forms of DNA: methylated cytosine followed by a nucleotide other than guanine (mCH, where H = A, C or T) and hydroxymethylcytosine (hmC) (39,40). Both mCH and hmC are enriched in human and mouse brain samples, and, in contrast to most methylated CpG dinucleotides, these marks accumulate postnatally during neuronal maturation (40–42). Coinciding with these changes in the DNA methylation landscape of neuronal chromatin is a surge in MeCP2 expression, which also correlates with the maturation of neurons (29). In general, mCH appears to be a repressive mark that acts to inhibit gene expression (40,42), whereas the presence of hmC within gene bodies has been associated with active gene expression in neurons (39,42). However, it is important to note that the impact of DNA methylation on transcription is context dependent, as methylation has been shown to either inhibit or promote gene expression depending on the location of the mark (43). Given the timing of the concurrent increase in both MeCP2 and its methylated DNA substrate, as well as the role of these marks in transcriptional regulation, it is possible that binding of MeCP2 at mCH and hmC regulates gene expression in mature neurons (Fig 1). Consistent with this idea is the finding that overt symptoms of RTT typically appear around the end of the first year of life. This timing coincides with increased synaptogenesis, metabolic activity and maturation of the neuronal circuitry (44,45), as well as correlates with the period during which mCH accumulates most rapidly in the frontal cortex in humans (42). Notably, many of the genes whose transcriptional profiles are altered in mouse models of RTT are enriched for mCH (46,47), and mice that overexpress MeCP2 show enhanced MeCP2 binding at mCH in the genes that are misregulated (47). Impaired binding of MeCP2 to hmC has also been implicated as a contributor to the symptoms of RTT. One of the most common missense mutations, Arg133Cys (11), has been shown to specifically affect MeCP2 binding to hmC in vitro (39). However, adult knockout of MeCP2 has demonstrated that the timing of symptom onset does not track precisely with the loss of MeCP2; rather, the change in the behavioral phenotype of the mice emerges a few weeks after the change in MeCP2 protein levels (48,49). If MeCP2 binding at these marks directly mediates the behavioral impairment observed in the adult knockout, we would anticipate that symptoms appear as MeCP2 protein levels declined. Given the delay between changes in MeCP2 levels and behavioral findings, it is likely that binding to mCH and hmC is only one component of the process that results in the delayed onset of RTT symptoms, and that loss of MeCP2 may initiate a series of events that eventually lead to disease presentation.

**Altered chromatin architecture**

At the nuclear level, a contributing factor to the delayed appearance of RTT symptoms may be the time it takes for the chromatin organization to change upon the loss or restoration of MeCP2. Specifically, it is possible that alterations in chromatin architecture could affect gene transcription, leading to neuronal dysfunction and symptom onset. The interaction of MeCP2 with chromatin has been found to extend beyond its ability to bind methylated DNA using its methyl-CpG-binding domain (MBD). The protein sequence of MeCP2 contains three basic clusters that bear homology to the high-mobility group AT hook family of chromatin-associated proteins (35). Using these basic residues, known as AT hooks because of their high affinity for AT-rich DNA sequences, MeCP2 is able to compact chromatin and influence the higher-order structure of the DNA (35,36,50). In doing so, MeCP2 appears to be able to impact the ability of other nuclear proteins to interact with the chromatin, such as the chromatin remodeling protein alpha thalassemia/mental retardation syndrome X-linked (ATRX), which has been shown to lose its normal localization at heterochromatic foci in MeCP2-null mice (35,51). MeCP2-null mice, as well as early truncating mutations (Arg270X and Gly273X) that disrupt one or more of these AT hooks, but leave the putative ATRX interaction domain of MeCP2 intact (51),
also show a gradual loss of ATRX from the heterochromatin, indicating that the mislocalization of ATRX is not due to a loss of direct interaction with MeCP2 (35). Both truncated mutants display altered chromatin states, and the loss of ATRX from heterochromatin is cell autonomous and correlates with disease onset and progression (35). Taken together, these discoveries suggest that the gradual onset of chromatin disorganization, as visualized by delayed ATRX mislocalization, may be one of the contributing factors to the delayed onset of RTT symptoms as well as disease progression (Fig. 2). Consistent with the idea that the basic clusters are important for MeCP2 function, five of the six most common MeCP2 mutations cataloged in the IRSA MECP2 Variation Database (Arg168X, Arg255X, Arg270X, Arg294X and Arg306Cys) (52) either lack a portion of these basic residues or show impaired function of the residues. However, it is important to note that the chromatin-organizing capabilities of these basic clusters are likely dependent on MeCP2 first binding to methylated DNA via its MBD, as mutations that specifically disrupt MBD function cause loss of MeCP2 from the chromatin (36,53,54). Consequently, any mutation that disrupts binding to methylated DNA (including mCH or hmC) is also predicted to impair MeCP2 binding via its basic residues.

**Neuronal circuit dysfunction**

The third aspect in the discussion of the timing of RTT onset pertains to the impact of MeCP2 dysfunction at the level of the
neuronal circuit. The progressive molecular and nuclear changes that accrue in MeCP2-deficient neurons can reasonably be expected to impair neuronal function (Fig. 3 middle panel) and ultimately culminate in altered neuronal network dynamics (Fig. 3 bottom panel). As a result, the time required for a neuronal circuit to manifest dysfunction can also contribute to the delayed onset of RTT symptoms. Altered connectivity has been suggested by the diminished dendritic arborization of cortical neurons (23) and by the altered neurotransmitter profiles observed in patients, such as decreased aminergic metabolites (55) and reduced tyrosine hydroxylase staining in the substantia nigra and hypothalamus (56). Electroencephalogram (EEG) recordings from patients have demonstrated changes in cortical circuit activity, including cortical hyperexcitability that is consistent with a decrease in inhibitory synaptic control (7,57). Interestingly, EEG abnormalities are typically not observed during the pre-regression (presymptomatic) stage of RTT; rather, the development of aberrant cortical activity patterns appears to coincide with the regressive stage of RTT (7). This finding supports the theory that atypical network properties may directly lead to the appearance of symptoms. Furthermore, this finding also indicates that circuit dysfunction develops over time. Consistent with this idea is the discovery that female mice heterozygous for a Mecp2-null allele have deficits in hippocampal long-term potentiation, which do not arise until the mice are symptomatic (58).

Network hyperexcitability has also been implicated in the generation of specific symptoms, including seizures (59,60) and breathing abnormalities (61,62). Furthermore, pharmacologic

![Figure 3](https://academic.oup.com/hmg/article-abstract/24/R1/R10/670857)

**Figure 3.** Model of the cellular and circuit changes that occur in Rett syndrome. Schematic diagram showing a normal circuit (top panel) and two time points in the disease course: (1) (middle panel) cell-autonomous changes arise only in mutant neurons and impair their functioning. These changes include altered neurotransmitter content, decreased dendritic complexity, impaired epigenetic regulation and changes in chromatin architecture; (2) (bottom panel) over time, both the wild-type and mutant neurons are affected, causing overt circuit dysfunction and manifestation of symptoms.
interventions that directly decrease the synaptic excitability of respiratory cells within the nucleus tractus solitarius (nTS) of the brainstem have demonstrated improvement in the breathing parameters of MeCP2-null mice (63,64). Changes in neuronal firing and circuit dynamics have been demonstrated in both RTT patients and mouse models, and this altered connectivity appears to produce at least a portion of the symptoms associated with RTT. Moreover, perturbations in neuronal networks may also explain the variability in disease progression observed with different MeCP2 alleles. Specifically, individuals with hypomorphic alleles, such as the Ala140Val mutation, have a less severe phenotype and typically develop symptoms more slowly (8,17,65). Because MeCP2 retains some activity with hypomorphic mutations, we would expect that network dysfunction would occur more gradually, which is consistent with the clinical picture of individuals with these types of mutations.

Mouse models of RTT recapitulate other synaptic morphological and physiological features of this disorder, including increased cell packing density, decreased brain weight and reduced dendritic branching (25,30,66,67). EEG abnormalities and altered synaptic plasticity have also been described in numerous mouse models (27,28,58,59,68–72). Furthermore, many of the electrophysiological findings in RTT mouse models reveal an imbalance between excitation and inhibition in neuronal circuits. Selective loss of MeCP2 expression in either GABAergic or glutamatergic cortical neurons can alter circuit function and result in the appearance of specific RTT-associated phenotypes (59,69,73–75). Along these same lines, the characteristic progression of neurologic symptoms seen in RTT patients (76) hints at the possibility that certain neuronal subtypes and networks are more vulnerable to the loss of MeCP2. As a result, symptoms derived from the dysfunction of these more susceptible neuronal circuits would be predicted to manifest earlier in the course of disease.

Future Perspectives

The delayed presentation of symptoms is a striking feature of Rett syndrome, and recent research has provided insight into the potential mechanisms leading to postponed disease onset. At the molecular and nuclear levels, MeCP2 binding to alternatively methylated DNA and chromatin remodeling have been implicated as contributing factors in the timing of symptom presentation. However, it remains unclear whether these processes are impaired in all cases of RTT or only in patients with specific MECP2 mutations. To better understand these functions of MeCP2, it will be important to establish whether the specific loss or gain of MeCP2 binding at mCH/hmC can reproduce or rescue all symptoms of RTT, respectively. In addition, a thorough evaluation of the three-dimensional structure of the chromatin in mouse models bearing different MeCP2 mutations would help illuminate the role of disordered chromatin in RTT. Finally, the symptoms of RTT can also be viewed in light of the underlying circuit dysfunction that produces them. A critical next step for understanding these neuronal network changes will be elucidation of the precise series of molecular and nuclear events that culminate in aberrant circuit dynamics.

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