

Original article

MeCP2 expression and function during brain development: implications for Rett syndrome's pathogenesis and clinical evolution[☆]

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Abstract

Most cases of Rett syndrome (RTT) are associated with mutations of the transcriptional regulator *MeCP2*. On the basis of molecular structure, ontogeny, and subcellular and regional distribution, MeCP2 appears to be a link between synaptic activity and neuronal transcription. Integrating data on MeCP2 neurobiology, RTT neurobiology, *MeCP2* mutational patterns in RTT and other disorders, histone profiles of relevance to RTT, and genotype–phenotype correlations in RTT, we update here our synaptic hypothesis of RTT. We postulate that MeCP2 dysfunction leads to abnormal brain development through maladjustment of neuronal gene expression to synaptic and other extra-cellular signals, mainly during the critical period of synaptic maturation. RTT phenotype will develop, only if severe MeCP2 dysfunction is present during early neuronal differentiation. Two models are proposed for explaining general and regional neuronal abnormalities in RTT and the phenotypical outcome of MeCP2 dysfunction, respectively.

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1. Introduction

The identification of mutations in the gene coding for the transcriptional repressor MeCP2 as the genetic basis of Rett syndrome (RTT) [1] represented a major milestone in the characterization of this developmental disorder. A new era of research, which includes studies of MeCP2 and related proteins associated with transcriptional repression [2] and correlations between *MeCP2* mutations and the RTT phenotype [3], complemented and expanded research on the neurobiology and clinical features of RTT [4]. Despite advances in molecular diagnosis, the relationship between *MeCP2* mutations and RTT remains complicated. A small proportion of RTT patients do not have *MeCP2* mutations; conversely, males and females with mutations in this gene do not always present with RTT features [3,5].

Nonetheless, the increasing knowledge on the neurobiology of MeCP2 is key to understanding RTT and other developmental disorders associated with *MeCP2* mutations. In this review, we refine our initial hypotheses about the neurobiology and pathogenesis of RTT by integrating data from different research areas with special emphasis on the most dynamic early stages of the disorder. We will advance the notion that MeCP2 plays a unique role in synaptic plasticity, and that the specific features associated with MeCP2 disruption will determine the development of RTT or other phenotypes.

2. MeCP2 links synaptic activity and transcription: molecular structure and subcellular distribution

Until recently, only one molecular form of the transcriptional regulator MeCP2 was recognized. Two research groups have independently demonstrated that two MeCP2 isoforms of slightly different molecular weight are being expressed in a wide variety of tissues [6,7]. Since most of the published studies to date have employed antibodies targeting regions of the protein common to both isoforms, it is not possible at this point

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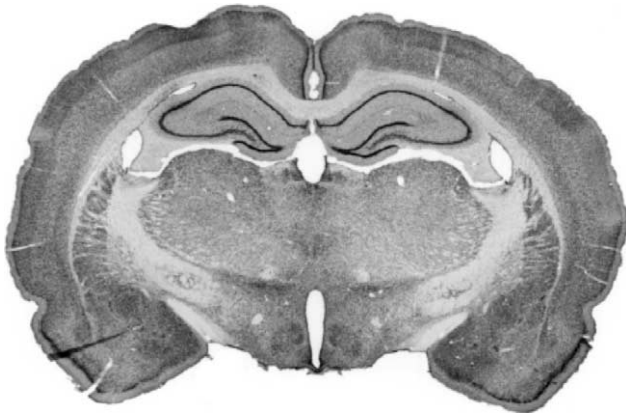


Fig. 1. Pattern of MeCP2 immunostaining in the adult rat brain, characterized by ubiquitous labeling of neuronal nuclei throughout the brain. Low power magnification (from Ref. [10]).

to clearly delineate differential profiles or roles for each MeCP2 isoform. The originally described 486 amino acids MeCP2A (also termed MeCP2 β) and the novel 501 residues MeCP2B (also termed MeCP2 α) [6,7] are generated by alternative splicing of exons 1 and 2. The main difference between MeCP2A and MeCP2B is a polyalanine and polyglycine repeat sequence in the N-terminus of the ‘longer’ MeCP2B. This sequence motif is similar to the one found in the N-terminus of ERK1, a component of the mitogen-activated (MAP) kinase pathway that directly regulates transcription via nuclear translocation [8]. The MAPK signaling cascade is involved in coordinating neuronal responses to extracellular signals, providing the basis for learning paradigms in the limbic system [9]. Whereas the two MeCP2 isoforms are ubiquitous in distribution, the relative abundance of MeCP2 in brain reported by us [10] (Fig. 1) and others [11,12] appears to reflect predominantly MeCP2B immunoreactivity [6,7].

Several features of MeCP2’s pattern of expression suggest that the protein may be involved in linking synaptic activity with transcriptional repression. In addition to MeCP2B’s molecular similarity to the cAMP response element-binding protein (CREB)-regulator ERK1, as other signaling molecules, the two MeCP2 isoforms can be found outside the nucleus [6]. In a previous study, we also demonstrated that MeCP2 immunoreactivity is detected in the post-synaptic

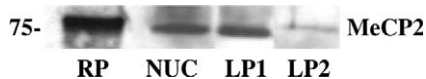


Fig. 2. MeCP2 immunoreactivity in human frontal cortical tissue fractions. MeCP2 immunoreactivity is found in both normalized (in terms of load) nuclear (NUC) and LP1 (pre+post-synaptic) fractions, and minimally in LP2 (pre-synaptic fraction). RP corresponds to human recombinant MeCP2. Molecular weight in kilodaltons is shown on the left (from Ref. [13]).

compartment, along with other transcription factors previously reported in this localization [13] (Fig. 2). It has been well established that CREB and other promoters of transcription are present (as mRNA and/or protein), synthesized, and/or phosphorylated in dendrites [14]. These synaptic transcription factors provide a complementary stream of transcriptional regulation, more directly associated with changes in synaptic activity. It is possible that this parallel synaptic-transcriptional pathway may involve a repressor such as MeCP2. The most direct evidence of MeCP2’s role in linking synaptic activity with transcription was simultaneously reported by Martinowich et al. (2003) [15] and Chen and co-workers (2003) [16]. These investigators demonstrated that neuronal membrane depolarization leads to release of MeCP2 from the brain derived neurotrophic factor (BDNF)’s exon IV promoter (i.e., exon IV in mouse, exon III in rat). Analogous to many signaling proteins, the latter process involves calcium-dependent phosphorylation of MeCP2 [16]. Considering the selective nature of BDNF’s transcriptional control [17], with specific regulatory signals for each promoter region, these data support the notion that MeCP2 has a specific role in regulation of neuronal gene expression.

Another intriguing observation, suggesting that MeCP2 may have a complex regulation by synaptic activity, concerns the predominantly extra-nuclear detection of a novel MeCP2 immunoreactivity. The approximately 100 kd MeCP2-like band (vs. \sim 75 kd of the ‘typical MeCP2A/B’) is found in peripheral lymphoid cells and brain, including in synaptic fractions [18] (Fig. 3). Whether the 100 kd MeCP2 is a modified MeCP2 isoform or a related molecule is yet unknown; nonetheless, its presence in association with intermediate molecular weight (i.e., 75–100 kd) MeCP2 immunoreactive bands in lymphoblasts suggests that MeCP2 may exist in multiple molecular forms linked to cell signaling.

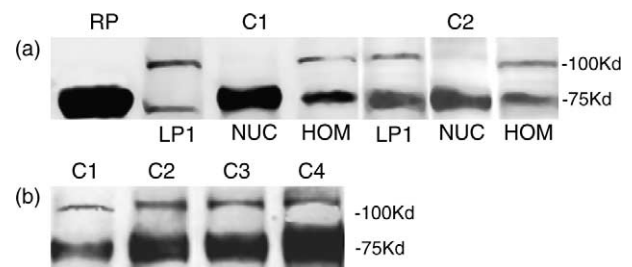


Fig. 3. Higher molecular weight MeCP2-like immunoreactivity. (a) Immunoblotting assays of whole homogenate (HOM), nuclear (NUC), and pre+post-synaptic (LP1) cortical fractions from normal subjects (C1, C2) demonstrate, as expected, the presence of a strong 75 kd immunoreactive band in all three HOM, NUC, LP1. However, in HOM and LP1 there is also a less abundant \sim 100 kd band. (b) Similarly, in lymphocyte whole cell lysates there is in addition to the 75 kd MeCP2 a less abundant \sim 100 kd band. RP corresponds to human recombinant MeCP2 (from Ref. [18]).

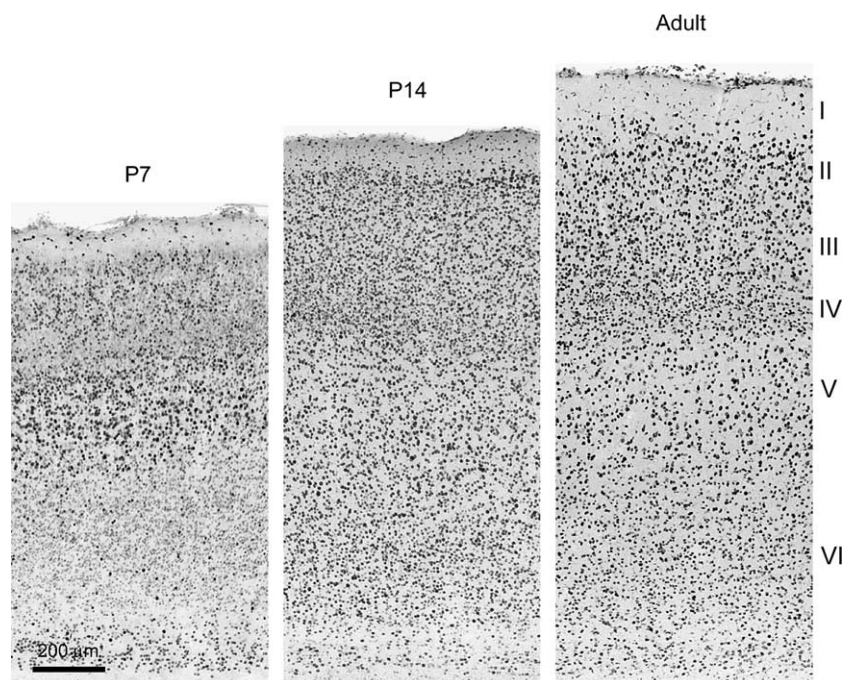


Fig. 4. Developmental changes in MeCP2 expression in the rat neocortex. The intensity of MeCP2 immunocytochemical staining increases staining in layers II/III and VI between postnatal day (P)7 and P14. At the latter stage, the pattern is similar to the adult brain (right panel). (Adapted from Ref. [24]).

3. Spatial and temporal profiles of MeCP2 expression: further evidence for a role in synaptic regulation of transcription

In normal adult rodents, monkeys, and humans, MeCP2 is widely distributed in neuronal populations throughout the CNS with virtually no *in vivo* expression in glial cells [10–12,19] (Fig. 1). This generalized pattern of MeCP2 immunostaining superficially resembles that of the neuron-exclusive transcription factor NeuN, which labels fully differentiated neurons [20]; however, differences in the levels of MeCP2 immunoreactivity between strata or cellular groups within a region and between neurons of the same cell population are analogous to those of activity-regulated transcription factors (e.g., *zif268*) [21]. Based on intensity of immunoreactivity, fluorescent cytometry has revealed two types of MeCP2-expressing cells with unique temporal and spatial profiles. ‘Low’ type cells (MeCP2^{lo}) are the most abundant in brain and other tissues, particularly in humans, while the ‘high’ subtype (MeCP2^{hi}) is characteristic of layer IV of the neocortex, cerebellar molecular layer, and hippocampal pyramidal layer [22]. We also observed that neurons with perikaryal MeCP2 immunoreactivity tended to have high intensity of nuclear staining [13], suggesting that they are MeCP2^{hi} cells.

MeCP2 expression parallels the process of postmigrational neuronal differentiation, particularly during cortical histogenesis [11,12,19,23,24]. We [24] and others [12] have reported that MeCP2 is initially expressed in the cortical preplate-derived populations, the Cajal-Retzius and

subplate neurons. Then MeCP2 expression extends radially from deep to superficial cortical layers (Fig. 4), in a pattern that follows histogenetic gradients [25]. Monoaminergic and other brainstem nuclei are also among the earliest MeCP2-expressing neuronal populations [12]. While MeCP2 immunoreactivity appears relatively late in the thalamus and basal ganglia, brainstem, cerebellar, and cortical MeCP2 expression increases markedly and steadily from early fetal stages to late childhood [12,24]. The aforementioned MeCP2 developmental profile differs from that of other transcription factors, such as NeuN, which follow maturational gradients throughout the CNS [20]. Furthermore, levels of MeCP2 immunoreactivity do not parallel degree of cytodifferentiation as in the case of NeuN. For instance, most mature Purkinje cells are MeCP2^{lo} whereas the majority of cerebellar molecular layer interneurons are MeCP2^{hi} [12,22]. Balmer and co-workers (2003) [26] have reported that both the proportion of MeCP2 positive neurons and the percentage of MeCP2^{hi} neurons increase markedly during human postnatal life. These changes in MeCP2 levels seem to be highly regulated since the same authors have shown that the utilization of the longer brain-enriched 10 kb *MeCP2* transcript, which contains 3′ UTR sequences in exon 4 [27], decreases over time in correspondence with the increase in MeCP2 expression [26]. MeCP2 developmental pattern is also different from that of typical synaptic activity-dependent transcriptional regulators, such as the immediate early genes *zif268* and *c-fos*, which show dramatic increases in their levels at the time of synaptic maturation with subsequent decline in levels during adulthood [21].

In terms of percentage of MeCP2-expressing neurons, there is a continuous postnatal increase that plateaus in adulthood [12,24].

The studies reviewed in the preceding paragraphs suggest that, although linked to neuronal maturation, levels of MeCP2 are not exclusively a reflection of the degree of cellular differentiation. Evidence from the mouse olfactory system [28] and our data on cerebellar ontogeny [24] indicate that the onset of MeCP2 expression is not dependent on the establishment of synapses, but that maintenance of mature levels of MeCP2 requires synaptic activity. Only mature olfactory receptor neurons express MeCP2; ablation of these cells leads to decrease in MeCP2 immunoreactivity that returns to pre-lesioning levels after newly generated neurons differentiate [28]. In contrast, removal of the target of these olfactory neurons leads to cell death, neurogenesis, and maturation without complete recovery of MeCP2 levels [28]. Some of this reduction in MeCP2 immunostaining is due to olfactory neuron death due to absence of synaptic target. We have observed that, in the mouse cerebellar granule cell layer, MeCP2 immunoreactivity begins to be detected towards the end of granule cell migration and increases markedly with granule cell synaptogenesis to a peak in adulthood [24] (Fig. 5). In this case, MeCP2 expression was minimal despite granule cell differentiation and only reached significant levels after mossy fiber-granule cell synapse formation was under way [24]. Additional evidence suggesting MeCP2 involvement in synaptic plasticity was provided by Francis and co-workers (2002) [29], who showed that perforant pathway (hippocampal) kindling leads to a delayed increase in MeCP2 in the dentate gyrus. This response was distinctive to MeCP2, when compared with other methyl-binding proteins; however, it resembled the late induction of MBD1

(i.e., the methyl-binding protein with closer molecular homology to MeCP2; [30]) after amygdala kindling [29].

The relative importance of MeCP2 for human brain development is underscored by the fact that patients with marked deficit in MeCP2 (e.g., males with RTT) die in utero or present with severe neonatal encephalopathy [3]. Mild to moderate reductions in MeCP2 function, as those present in most cases of RTT patients and in some patients with Angelman syndrome, are viable but lead to severe neurologic involvement with a frequent period of regression [3,31]. These observations, and the data reviewed in the preceding paragraphs, suggest that a minimum level of MeCP2 expression is needed for the survival of many neuronal populations shortly after they complete basic cytodifferentiation. However, it is unclear at this point what is the necessary level of MeCP2 function required for long-term adequate neural activity. Two types of *MeCP2* null mice are viable; these animals show neurologic impairment at 5–6 weeks and premature death at approximately 12 weeks [32,33]. Prenatal, neuroblast postmitotic deletion of the gene leads to a similar outcome [32,33], 2001), which is milder if *MeCP2* is deleted postnatally [32]. These findings support the postmitotic/postmigrational role of MeCP2 in neurons; however, the relatively less severe mouse phenotype raises the question of whether MeCP2 is essential for neuronal maturation or survival. LaSalle (2004) [34] postulated that mice are able to handle MeCP2 deficiency better than humans because of their less complex neurologic function and behavior. We agree with this explanation that, in our opinion, supports the concept that MeCP2's role is greater in more complex neural systems. As opposed to simpler subcortical neural networks, the highly elaborated cortical and limbic neurons and circuits seem to have a critical requirement of MeCP2 for the appropriate completion of the process of cytodifferentiation. This dependency on MeCP2 is even higher for the predominantly postnatal phenomenon of synaptic plasticity, which involves the precise integration of multiple afferents and other extra-cellular signals. Table 1 summarizes the evidence for MeCP2's role in synaptic plasticity.

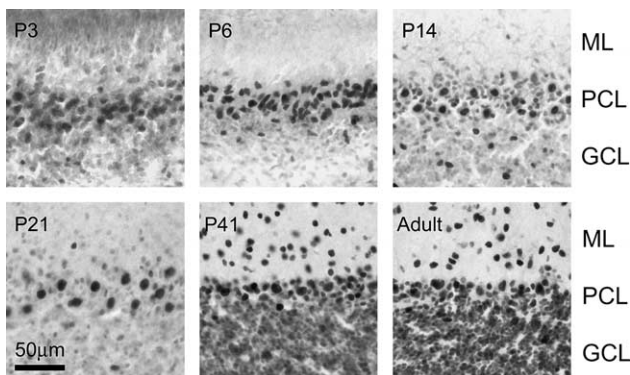


Fig. 5. Ontogeny of MeCP2 staining in the rat cerebellum. MeCP2 expression patterns change dramatically with age; early postnatal ages exhibit significant staining only in Purkinje cells and Golgi cells within the granule cell layer (GCL). Granule cells exhibited minimal MeCP2 staining up through the first three postnatal weeks, but were darkly stained by postnatal day (P) 41. Abbreviations: ML, molecular layer; PCL, Purkinje cell layer. (From Ref. [24]).

Table 1
Evidence supporting MeCP2's role in synaptic plasticity

Feature	Reference(s)
ERK1-like N-terminal motif (MeCP2B)	6,7
Extra-nuclear localization (MeCP2A, MeCP2B)	6
Post-synaptic localization	13
Membrane depolarization-dependent BDNF silencing	15,16
100 kd MeCP2-like band in synaptic fractions	18
Neuronal group-specific pattern of expression	12,22,24
High-expressing neuronal subtype (η postnatally)	22,26
Levels regulated by deafferentation (olfactory system)	28
Induction by hippocampal kindling	29

4. MeCP2 dysfunction during brain development: global and selective effects on neuronal differentiation determine the RTT phenotype

The previous sections have analyzed findings supporting the notion that MeCP2 plays a unique role in neuronal maturation and developmental synaptic plasticity. Here, in order to delineate a model of RTT pathogenesis, we integrate the abovementioned information with data on the neurologic phenotype of RTT and the pattern of expression of MeCP2 in this disorder. We will use the neuroanatomical features of RTT as the prototype phenotype, because they have been extensively delineated and provide clues about the fundamental abnormalities in the disorder. In correspondence with MeCP2 expression in differentiating neuroblasts, RTT is characterized by a reduction in neuronal size that involves both the soma and the dendritic tree [35, 36]. Based on neuroimaging and postmortem anatomical surveys, these neuroanatomical features are distributed throughout the brain leading to a marked reduction in gray matter and, to a lesser extent, white matter volumes [35,36]. Despite this diffuse pattern, some regions are relatively either more affected or spared [35]. In the cerebral cortex, motor and prefrontal regions exhibit the most dramatic decreases in dendritic trees. In contrast, superior temporal and occipital cortices display milder changes in neuropil [35]. Studies using different molecular approaches reveal that, as expected in a disorder of neuronal differentiation, a number of major synaptic-related genes/proteins are detected at lower levels in RTT brains [37,38]. These pre- and post-synaptic markers are not necessarily direct targets of MeCP2, but genes that are affected by MeCP2 deficiency and its downstream events. Probably, the most illustrative example is that of the major dendritic constituent microtubule-associated protein 2 (MAP-2). In RTT neocortex, there is reduction in *MAP-2* transcript [37] and a selective decrease in MAP-2 immunoreactivity [38]. MAP-2 is not only a distinctive marker of mature neurons, but also a protein that is dynamically regulated by a variety of neurotransmitter and synaptic signals during development and adult life [38]. Several of the neurotransmitter systems modulating MAP-2 expression are known to be disrupted in RTT; of special interest are the glutamate receptors. We found that in the frontal cortex *N*-methyl-D-aspartate (NMDA) receptors were elevated in density in younger RTT patients (<8 years), while the opposite was true for older RTT subjects [39]. These findings suggest a persistence of an immature pattern of NMDA receptors, in agreement with the overall phenotype of RTT and the changes in MAP-2 and other synaptic proteins. They also indicate that MeCP2 deficit in subcortical pathways (e.g., monoaminergic brainstem nuclei) [12], which regulate early cortical development, may result in cortical neurotransmitter imbalance and compensatory responses that further deteriorate neural function. Consequently, lower MeCP2 function in RTT would lead to impaired maturation of most

Table 2
Changes in gene expression (mRNAs) in postmortem RTT cerebral cortex

Increased >1.5×control	Decreased >1.5×control
NMDA Receptor NR1 subunit	MAP-2
Metabotropic mGluR1 receptor	Synapsin II
Glial EAAT1 Transporter	Synaptogyrin 3
Type 3 GABA transporter	Synaptotagmins 1, 5
	Syntaxin 1A
	Annexin VI

Adapted from Refs. [10,37].

neurons; however, late developing neural pathways (e.g., frontal cortex) would suffer the superimposed effect of disrupted afferent connections secondary to early MeCP2 deficit. This mechanism may also account for the greater vulnerability of certain brain regions and the life-long disturbance in neural function in RTT. Table 2 lists genes coding for synaptic proteins that are abnormally expressed in RTT.

Greater involvement of both early (e.g., motor) and late (prefrontal) developing cortices could not be explained only on the basis of maturational gradients of MeCP2 deficit. Regional differences in synaptic and other extracellular influences are the most likely mechanism underlying these differences. In support of this is the fact that concentrations of BDNF, the best-characterized direct target of MeCP2 [15,16], are variable across cortical regions. BDNF levels are higher in the ‘RTT-affected’ prefrontal cortex and lower in the ‘RTT-spared’ occipital cortex, particularly after childhood [40]. BDNF’s effects on the maturation of certain cortical layers [41] may also explain the selectivity of changes in RTT (e.g., prefrontal layer III>layer V) [35]. An example of the interplay between neurotransmitter imbalance and BDNF dysfunction in RTT is the frequent presence of respiratory irregularities, specifically hyperventilation and breath holding, during early stage III (2–4 years) of the disorder [4]. Regulation of breathing in the form of long-term facilitation of ventilation, in particular in response to hypoxia, depends on the precise integration of serotonergic, glutamatergic, and BDNF signals at the medullary respiratory network-phrenic nerve synapse [42]. Considering the increased medullary serotonin receptor binding reported in postmortem RTT samples [35], we propose that elevated serotonergic and NMDA receptor [39] activities, both due to early MeCP2 deficit, in conjunction with postnatal BDNF dysregulation would constitute the basis for the distinctive breathing abnormalities in RTT. This type of interaction between neurotransmitter and growth factor abnormalities, some direct and other indirect consequences of MeCP2 dysfunction, could be considered a prototype for the pathogenesis of selective phenotypical features of RTT.

Fig. 6 depicts a model of RTT neuronal pathogenesis, using as example the pyramidal neurons of the prefrontal cortex. We propose that MeCP2 deficit will affect dendritic

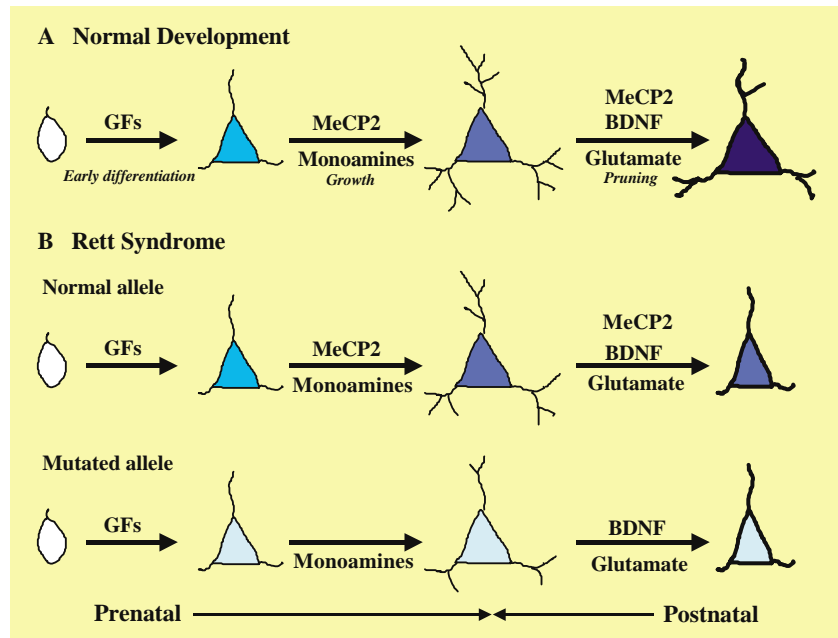


Fig. 6. Model of neuronal pathology in Rett syndrome based on dendritic development in the prefrontal cortex. During normal development, onset of MeCP2 expression coincides with early neuronal differentiation. Levels of MeCP2 function, depicted as intensity of blue label, increase steadily after afferents (e.g., monoamines) begin to influence cortical neuronal differentiation. Direct targets of MeCP2, such as BDNF, in conjunction with other synaptic signals have a particularly strong effect on the process of dendritic pruning. Marked reduction in MeCP2 function and deficient afferent input, in neurons carrying a *MeCP2* mutated allele, impairs appropriate dendritic expansion. The abnormality extends and worsens during dendritic pruning because of the abnormally high levels of MeCP2 targets (i.e., BDNF) and additional neurotransmitter disturbances (\uparrow glutamate receptor activity). The ultimate neuronal phenotype is characterized by a smaller cell with markedly decreased MeCP2 expression and dendritic arborizations. Rett neurons carrying the normal allele are also affected. Because of decreased local (neighboring neurons with mutated allele) and distant (monoaminergic) synaptic signals, and secondary abnormalities such as increases in BDNF and glutamatergic activity, these neurons are unable to reach normal soma and dendritic size and remain as low-expressing (*MeCP2*^{lo}) cells. Abbreviation: GFs, growth factors. (For interpretation of the reference to color in this legend, the reader is referred to the web version of this article.)

growth and pruning, but not the initial steps of dendritic formation, in neurons carrying the mutated *MeCP2* allele. MeCP2 deficit will prevent normal dendritic expansion, while excess of BDNF and neurotransmitter abnormalities (i.e., elevated NMDA receptor activity) will lead to increased pruning and further reduction of dendritic trees. Similar mechanisms would prevent neuronal soma growth. The molecular basis of this phenomenon would be a combination of MeCP2-directly regulated genes and synaptic activity-dependent proteins that are affected by the changes triggered by MeCP2 dysfunction. Neurons with the normal *MeCP2* gene would also show disturbed development; in this case, the process of dendritic growth is affected by changes in cortical afferents (e.g., monoamines). In these genetically normal cells, dendritic pruning would be relatively more involved than dendritic growth because of the influence of neurons with abnormal alleles through factors such as elevated BDNF levels and NMDA activity. Moreover, the levels of MeCP2 expressed by neurons with normal alleles would also be reduced due to their dependence on local and distant synaptic influences.

In addition to the data reviewed above, the proposed model is supported by a variety of observations. A recent

communication by Pescucci and co-workers (2003) reported a patient with RTT-like features and a chromosome 2 deletion encompassing the *MAP-2* gene [43], emphasizing the role of this activity-dependent gene in the pathogenesis of RTT. The limited available data on mouse models and cell lines from RTT subjects indicate that genes involved in dendritic and axonal formation are preferentially affected in MeCP2 deficiency and RTT. For instance, in mice with postmitotic deletion of *MeCP2*, the most differential transcript was Rho GDI γ [44]. As a GDP-dissociation inhibitor (GDI), this down-regulated gene is involved in neurite/synapse formation and rearrangement through the modulation of Rho GTPases [45]. Interestingly, two non-syndromic X-linked mental retardation disorders have been associated with the mutation of its homologue Rho GDI α [45]. Similar to MAP-2, Rho GDI expression/function is regulated by extra-cellular signals such as neurotrophins and glutamate receptors. Surveys of RTT peripheral cell lines have reported the upregulation of neuronal signaling and dendritic genes, such as the high molecular weight neurofilamentin protein and 5-lipoxygenase [46]. The implications of our model of RTT pathogenesis for the clinical evolution of the disorder are presented in the following section.

5. *MeCP2* mutations, MeCP2 dysfunction, and the RTT phenotype: severe maladjustment to synaptic signals

The different types of *MeCP2* mutations that characterize RTT should ultimately lead to a reduction in MeCP2 function, via truncated and/or unstable proteins (i.e., nonsense mutations, frameshifts) or through proteins of abnormal configuration (i.e., missense mutations, frameshifts) [2]. To date, our knowledge on the functional consequences of *MeCP2* mutations is quite limited. Although the primary role of MeCP2 appears to be to promote histone deacetylation and, perhaps also, methylation of histone H3 [2], systematic evaluations of *MeCP2* mutations have focused on assessments of chromatin targeting and transcriptional repression of reporter genes [47,48]. These studies have focused exclusively on missense mutations, using in vitro assays of transfected cell lines and not of cell samples from RTT patients [47,48]. The evaluations have confirmed nonetheless that, in the absence of skewed X chromosome inactivation (not introduced into the experimental model), certain missense mutations in the DNA methyl-binding domain lead to a greater functional deficit than others (e.g., T158M > R133C) [48] in correspondence with genotype–phenotype correlations [49–51]. Despite these general associations between specific mutations and MeCP2 dysfunction [48], the contribution of specific mutations and gene dosage to MeCP2 deficit and phenotypical outcome is still unknown. For instance, certain in vitro ‘mild’ mutations [48] are seen in patients with severe classic RTT [5,50]. On the other hand, the relatively in vitro ‘mild’ R133C mutation is also clinically less severe with later onset, better preservation of gait and speech, and less prominent hand stereotypies [49]. Although evaluations of X inactivation patterns in peripheral lymphoid cells and brain indicate the process is balanced in most RTT patients [52], studies of males indicate that gene dosage is important. Boys with common *MeCP2* mutations are characterized by severe encephalopathy, whereas males with the same type of mutations but mosaicism (somatic or 47,XXY karyotype) show a RTT phenotype [5]. On the other hand, X inactivation does not appear to influence the severity of

the R133C mutation [49] (probably, the only comprehensively studied *MeCP2* mutation). Non-RTT phenotypes are also associated to *MeCP2* mutations; a subgroup of males with *MeCP2* mutations presents with non-syndromic mental retardation [5] and some patients with Angelman syndrome and no 15q11-13 abnormalities have mild *MeCP2* mutations (e.g., C-terminus frameshifts) [31]. Nevertheless, the *MeCP2* mutations found in these phenotypically unusual subjects tend to be rare or never described in RTT.

The data discussed above suggest that environmental factors, or other yet unknown genetic variables, might be responsible for the final outcome of *MeCP2* mutations. For these reasons, direct assessments of MeCP2 dysfunction in affected RTT individuals are needed. Initial studies of histone profiles in RTT patients focused on understanding the effect of specific mutations independent of X inactivation; hence, they employed cloned lymphoid cells. Data from lymphoblasts carrying a common nonsense mutation (i.e., R168X) showed an increase in acetylated histone H4 (AcH4) [53]. In contrast, cloned T lymphocytes did not display any change in the acetylation status of core histones H3 and H4 [54]. We recently performed a similar study on RTT lymphocytes directly isolated from peripheral blood. In this polyclonal cell population, we found a reduction in the levels of AcH3 and minimal changes in AcH4 or in methylated residues of histone H3 [55] (Table 3). The magnitude of reduction in AcH3 levels, but not MeCP2 levels or mutational parameters, also correlated with severity of head growth deceleration [55]. Although the direction of the changes we detected in acetylated histones is opposite to the expected one, our study suggests a critical role for histone H3 in MeCP2 deficiency. This is in agreement with a previous report on MeCP2-mediated proviral transcriptional silencing, showing changes in AcH3 but not in AcH4 [56]. The characterization of a mouse model of *MeCP2* nonsense mutation also demonstrated an elevation in brain AcH3 levels but no changes in AcH4 [57]. While the significance of global changes in histone acetylation/methylation is not fully understood, there is increasing evidence that global profiles of histone modifications may be good indicators of chromatin organization

Table 3
Levels of acetylated histones H3 and H4 in RTT lymphocytes

	AcH3/NonAcH3 ^a (Mean ± SE ^b)	<i>P</i>	AcH4/NonAcH4 ^a (Mean ± SE ^b)	<i>P</i>
Control	3.69 ± 2.14	0.03^c	0.96 ± 0.33	0.21
RTTALL ^d	0.49 ± 0.14		3.01 ± 1.06	
RTTPos ^e	0.52 ± 0.21	0.81	3.35 ± 1.46	0.72
RTTNeg ^f	0.44 ± 0.11		2.39 ± 1.55	

Note: There were no significant differences between the two RTT subgroups for any acetylated histone. Adapted from Ref. [55].

^a Acetylated histones were measured as ratios of acetylated (AcH) to non-acetylated (Non-AcH) histones.

^b SE: Standard Error.

^c Significant difference.

^d All RTT subjects, regardless of mutation status: RTTALL.

^e Mutation positive RTT patients: RTTPos.

^f Mutation negative RTT subjects: RTTNeg.

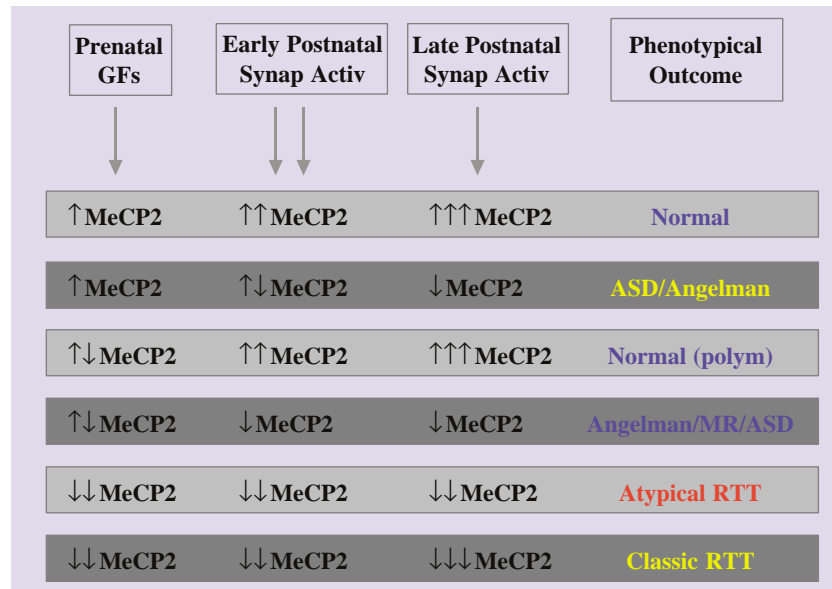


Fig. 7. Model of phenotypical outcomes secondary to MeCP2 deficiency. During normal development, onset of MeCP2 expression (\uparrow) coincides with early neuronal differentiation directed by specific signals (i.e., growth factors [GFs]). Levels of MeCP2 expression/function increase ($\uparrow\uparrow$), for most cortical and limbic regions, in early postnatal life and are strongly modulated by synaptic activity during the critical period of synaptic maturation. In the same regions, MeCP2 levels continue to increase ($\uparrow\uparrow\uparrow$) into adulthood. If developmental synaptic activity or other factors (e.g., 15q11-13 abnormality) regulating MeCP2 expression in early postnatal life are disturbed, levels of MeCP2 could decrease and a phenotype of Angelman syndrome or autism spectrum disorder (ASD) may develop. If *MeCP2* polymorphisms or mild prenatal MeCP2 deficits ($\uparrow\downarrow$) occur, depending on the genetic compensatory capacity of the subject or X inactivation skewing, no phenotype (polymorphism [polym]) or a non-RTT disorder with mild MeCP2 deficiency (\downarrow) may arise. This situation will explain the majority of non-RTT phenotypes associated with *MeCP2* mutations, including Angelman syndrome, ASD, and non-syndromic mental retardation (MR). If MeCP2 dysfunction takes place early and is severe ($\downarrow\downarrow$), as in most patients with pathogenic *MeCP2* mutations, development of subcortical pathways will be affected. These secondary/compensatory neurotransmitter changes, in combination with insufficient MeCP2-dependent response to synaptic signals during the critical postnatal period, will perpetuate MeCP2 deficiency. If 'facilitating' factors (e.g., genetic polymorphisms, unfavorable X inactivation skewing) are also present, a more severe classic RTT phenotype with severe MeCP2 deficit ($\downarrow\downarrow\downarrow$) will emerge. Otherwise, MeCP2 function will remain at a moderately low level ($\downarrow\downarrow$) and an atypical/variant RTT phenotype will develop. Note that this model does not distinguish between RTT patients with or without *MeCP2* mutations, since the postulates are based on MeCP2 function that could be impaired by other genes functionally associated with *MeCP2*. Abbreviations: GFs, growth factors; Synap Activ, synaptic activity. The intensity of the gray shading symbolizes the presence (darker) or absence (lighter) of negative or 'facilitating' factors that lead to a more severe phenotype.

and overall transcriptional activity [34]. For instance, intense synaptic stimulation via electroconvulsive seizures leads to changes in ACh4 that parallel levels of mRNA for several major neuronal genes (e.g., CREB, BDNF) [58]. In contrast, changes in the levels of ACh3, particularly of histone H3 acetylated at lysine 14, were associated with the transcription of selected genes by activation/silencing of specific promoters [58] as reported for MeCP2 regulation of BDNF [15,16]. Interestingly, our findings in RTT lymphocytes indicated that changes in ACh3 were driven by lysine 14 [55]. In line with MeCP2's potential role as integrator of extra-cellular signals, levels of ACh3 (lysine 14) more than other histone modifications seem to be a direct reflection of signal transduction activity, in particular of the MAP kinase pathway [59].

MeCP2 dysfunction could arise from mutations in non-coding sequences of the *MeCP2* gene, an area that is not systematically surveyed in mutational analyses. Several lines of evidence also indicate that abnormal MeCP2 levels may play a role in the pathogenesis of non-RTT phenotypes. A number of studies have failed to demonstrate RTT-like mutations in the coding region of *MeCP2* in large cohorts of

patients with idiopathic autism [60–62]. However, two recent reports have raised the possibility that reduced levels of structurally normal MeCP2 could be present in some patients with autism. Shibayama and collaborators (2004) [63] described two autistic subjects with 3'-UTR *MeCP2* variants, while Samaco and co-workers (2004) found mainly reduced MeCP2 levels in postmortem brain samples from 6 patients with autism spectrum disorders (2 had increased MeCP2) and no *MeCP2* mutations [64]. Decreased brain MeCP2 immunoreactivity was also detected in one subject with Angelman syndrome and two patients with the diagnosis of Prader-Willi syndrome [64]. Given the clinical similarities between RTT and both Angelman syndrome and idiopathic autism, associations between *MeCP2* mutations and specific disorders should also consider the dynamics of the phenotype. The cases of Angelman syndrome with *MeCP2* mutations reported by Watson et al. (2001) were notable for their developmental regression [31], which is not typical of this disorder. Zappella and co-workers (2003) studied 19 girls with the diagnosis of autism; only the two who also fulfilled criteria for the atypical RTT form termed preserved speech variant

(PSV) had pathogenic *MeCP2* mutations [65]. These patients with PSV had a different course from their autistic counterparts; they developed the first three stages of RTT, but their abilities increased over the years and were no longer autistic by early adolescence [65].

The data reviewed in this and precedent sections allow us to propose a second model, illustrated in Fig. 7, regarding the role of MeCP2 and other factors in determining the RTT phenotype. We postulate that a basic requirement for developing RTT, either the classic condition or one of its variants, is a marked deficit in MeCP2 expression/function (most likely, but not necessarily due to *MeCP2* mutation) at the earliest stages of neuronal maturation. If this deficit occurs in the context of a ‘facilitating’ genetic background and/or unfavorably skewed X inactivation, the individual will develop a more severe phenotype such as classic RTT; otherwise, the patient will be less affected and diagnosed as atypical RTT. It is important to emphasize that this hypothesis assumes that early postnatal (prenatal for cortical regions that develop earlier) synaptic activity is the most critical influence on mature levels of MeCP2 expression and function. A failure to respond to these developmental signals that modulate synaptic maturation, particularly in the presence of negative modulating factors (e.g., polymorphism of a neurotransmitter transporter, skewed X inactivation), will lead to further reduction in MeCP2 as delineated in Fig. 6. Subjects with mild or borderline prenatal MeCP2 function will present with non-syndromic mental retardation, idiopathic autism, or Angelman syndrome only if other genetic or environmental variables are unfavorable; if not, they will be able to compensate and the mutation will probably be characterized as a polymorphism (e.g., A140V in males) [5]. Finally, in the absence of *MeCP2* mutations, MeCP2 deficit can occur if early postnatal synaptic stimulation fails to increase MeCP2 levels due to a primary disturbance in connectivity or in factors that regulate MeCP2 transcription (i.e., largely unknown [66]). In this situation, mild MeCP2 dysfunction will be a feature (perhaps pathogenic) of a wide variety of developmental disorders such as idiopathic autism or typical Angelman syndrome (i.e., 15q11-13 abnormality). We emphasize that the defining process underlying a MeCP2-related phenotype is an abnormal ‘MeCP2-developmental synaptic activity interaction’. For this situation to evolve into an RTT-like disorder, MeCP2 disruption during early neuronal differentiation must also be present (probably because it produces a severe imbalance in neurotransmitters). Supporting this hypothesis is the fact that only patients with Angelman syndrome or autism-RTT preserved speech variant overlap *and MeCP2* mutations display an RTT-like regressive period [31,65]. These and other distinctive features of RTT could be explained by the unique developmental dynamics of neocortical and limbic regions that are highly dependent on MeCP2. Disturbances in areas such as the prefrontal cortex, which are

characterized by early postnatal dendritic growth and minimal pruning [25,67] and higher BDNF levels [40], could account for the normal head circumference at birth and rapid growth deceleration during the first year [4]. Regions that follow the ontogenetic pattern of the motor cortex, with more protracted dendritic development and substantial pruning after the second year [25,68], may be the basis for functional regression. The fact that mice over-expressing BDNF, a presumably up-regulated growth factor in RTT [16], show dendritic instability and spine retraction [41] lends further support to this dendritic-synaptic theory.

6. Concluding remarks

Recent developments in MeCP2 and RTT neurobiology provide the basis for a re-evaluation of mechanisms underlying RTT pathogenesis and clinical evolution. We have previously proposed that RTT is a disorder of synaptic formation, resulting from the combination of specific gene abnormalities and secondary compensatory effects in the CNS. Here, we update this hypothesis by proposing that MeCP2 dysfunction leads to abnormal brain development through maladjustment of neuronal gene expression to synaptic and other extra-cellular signals, mainly during the critical period of synaptic maturation. Whether the resulting phenotype is RTT or not, or whether it is a more severe or a milder form of RTT, depends on a number of factors that include the prenatal non-synaptic driven period of MeCP2 expression and the individual’s genetic background. In this regard, RTT and related phenotypes belong to the growing category of developmental disorders of synaptic development. As in Fragile X syndrome and other X-linked mental retardation syndromes [69], there is a direct or indirect disruption of proteins involved in synaptic plasticity. Since these molecular complexes participate in the two main forms of plasticity, the one linked to synaptic maturation and the one responsible for learning and complex behavior in the mature brain, their disruption represents a ‘double jeopardy’ phenomenon. Initial abnormal synaptic maturation leads to abnormal synaptic structure that, in turn, further intensifies the maturational disruption. The combination of anomalously configured synaptic circuits and continuous molecular deficiency ultimately determines the severity of the neurobehavioral phenotype beyond late childhood. ‘Double jeopardy’ represents also the opportunity of ameliorating the effects of the molecular defect in adulthood, since even a defective synaptic structure would benefit of ‘corrected’ synaptic molecular complexes. Although these general principles appear to operate in several disorders, the challenge is to identify the features unique to each condition. For instance, in RTT the neurotransmitter systems abnormalities secondary to early MeCP2 dysfunction seems to play an important role in

‘setting the stage’ for a highly disrupted process of synaptic maturation. Significant variability in regional MeCP2 expression and increasing levels of this protein into adulthood are other examples of features to be considered, if neurobiologically-based interventions for RTT are to be developed. Unrelenting integration of data from areas as diverse as molecular genetics and synaptic physiology will continue to provide the framework for understanding this complicated disorder.

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