Synaptic Plasticity and Signaling in Rett Syndrome

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ABSTRACT: **Rett syndrome (RTT) is a disorder that is caused in the majority of cases by mutations in the gene methyl-CpG-binding protein-2 (MeCP2). Children with RTT are generally characterized by normal development up to the first year and a half of age, after which they undergo a rapid regression marked by a deceleration of head growth, the onset of stereotyped hand movements, irregular breathing, and seizures. Animal models of RTT with good construct and face validity are available. Their analysis showed that homeostatic regulation of MeCP2 gene is necessary for normal CNS functioning and that multiple complex pathways involving different neuronal and glial cell types are disrupted in RTT models. However, it is increasingly clear that RTT pathogenetic mechanisms converge at synaptic level impairing synaptic transmission and plasticity. We review novel findings showing how specific synaptic mechanisms and related signaling pathways are affected in RTT models.** © 2013 Wiley Periodicals, Inc. Develop Neurobiol 00: 000–000, 2013

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INTRODUCTION

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that affects primarily girls at a frequency of 1:8000–1:10,000 live female births (Rett, 1966; Hagberg, 1985) and represents the second most common mental retardation in females.

Most (99%) of the RTT cases are sporadic and in up to 97% of classic RTT the disease is associated with mutations in the X-linked methyl-CpG-binding protein 2 (*MECP2*) gene (Amir et al., 1999) suggesting that RTT is caused by mutations in this gene

(Neul et al., 2008). Symptoms can differ substantially among patients, ranging from classic RTT, showing the typical RTT phenotype, to patients with only some RTT features. In the classic form of disease, patients present the first signs of the disease after a period of normal development which may last from 6 to 18 months of age. The typical indicator of neurological involvement is the arrest of head growth, leading to microcephaly by the second year of life. Subsequently, the patients fail to meet psychomotor milestones and eventually regress, losing hand skills and spoken language. As the syndrome progresses, patients develop social behavior abnormalities and are often misdiagnosed as having autism (discussed below). Atypical forms of RTT deviate from the classical clinical presentation. These variants range from milder forms with a later age of onset to more severe manifestations. Of the milder variants, the "forme fruste" (or "worndown form") has a later age of onset compared with the classical form, with regression occurring between 1 and 3 years of age; hand use is sometimes preserved with minimal stereotypic

movements. The preserved speech variant is another benign form of RTT characterized by the ability of patients to speak some words, although not necessarily in context. Patients with this variant have a normal head size and are usually overweight and kyphotic (Zappella et al., 2001). The more severe variants include the congenital form that lacks the early period of normal development, and a form of classical RTT with onset of seizures before the age of 6 months. Classical and atypical RTT phenotypes vary in severity and onset between different patients and in the same patient over time.

Some of these differences of RTT phenotypes are due to the mutation of different genes. Thus classic RTT results from mutations in *MECP2* gene, whereas in the variant forms mutations appear in distinct loci such as in *CDKL5* where patients present early seizures or *FOXG1* which is a congenital variant (Neul et al., 2010). Even within patients carrying mutations of the same gene, symptoms can vary substantially depending on the type of mutation and the pattern of X chromosome inactivation. For instance, the symptom spectrum carried by CDKL5 patients can be so wide that a recent study found that the majority of the patients failed to meet the criteria recently established (Neul et al., 2010) for early-onset variant of RTT (Fehr et al., 2013).

In the DSMIV-TR and ICD10, RTT is classified among the pervasive developmental disorders that include autistic disorder, Asperger's syndrome, pervasive developmental disorder-not otherwise specified, and childhood disintegrative disorder (Levy et al., 2009). Phenotypically, RTT children, similarly to what happens in autism, incur in a phase of regression characterized by symptoms in common between RTT and autism such as screaming fits, avoidance of eye contact, lack of social/emotional reciprocity, and use of nonverbal behaviors to regulate social interaction. Despite this association, clear phenotypic differences exist between autism and RTT, most notably gender, and head growth, and incidence, as well as distinct genetic etiologies. Interestingly, the autistic spectrum disorder (ASD) and RTT patients show some common genetic alterations suggesting that they should share some similarities. Indeed, MeCP2 mutations have been found in 0.8–1.3% of autistic patients (Carney et al., 2003; Coutinho et al., 2007; Campos et al., 2011). Only few of these at later age meet clinical diagnostic criteria for classic or atypical RTT (Lintas et al., 2012). However MeCP2 function could be affected in ASD more widely than expected from incidence of mutations of *MECP2* coding regions. Indeed, MeCP2 expression both at protein and mRNA level was found to be altered in a sub-

stantial percentage of autistic patients (Samaco et al., 2004) suggesting a dysregulation of regulatory mechanisms controlling MeCP2 expression. For instance, an increased frequency of sequence variants within the *MECP2* 3′ untranslated regions has been found in autism (Shibayama et al., 2004) and an autistic patient with mutations in the regulatory region of *MeCP2* has been reported (Nagarajan et al., 2006). A study analyzing MeCP2 expression in the frontal cortex of autistic patients reported a downregulation in 79% of the samples that was correlated with an increase in cytosine methylation on the promoter regions of the *MECP2* gene showing that altered epigenetic mechanisms could mediate MeCP2 dysregulation in autistic patients (Nagarajan et al., 2006, 2008).

The MeCP2 Gene

MeCP2 is a basic nuclear protein able to bind DNA sequences methylated at cytosine 5′CpG. MeCp2 is highly conserved among mammals and includes several domains. The largest of these is the Methyl DNA Binding Domain (MBD) composed of 100 amino acids (Nan et al., 1993). Other domains include a nuclear localization signal (NLS) (Nan et al., 1996) and a transcriptional repression domain (TRB) (Jones et al., 1998; Nan et al., 1998). Almost all mutations in MeCP2 occur *de novo*. The exceptions are familial cases where the mutation has been inherited from a healthy or mildly affected mother; they either have a gonadal mosaicism or favorable XCI.

About 67% of all MECP2 mutations are caused by C>T transitions at 8 CpG dinucleotides (R106, R133, T158, R168, R255, R270, R294, and R306) which are located in the third and fourth exon of the gene. The most common mutation is R168X. Although mutations are dispersed throughout the gene, a clustering of missense mutations occurs in the MBD. A recent analysis revealed the existence of another cluster of missense mutations localized at the C-terminal extremity of the TRD, which includes amino acids 302–306. Strengthening the pathogenetic relevance of these two clusters, neutral mutations are absent from the normal population at these loci. The functional role of the 302–306 cluster has been recently elucidated by showing that it is required for binding of the Ncor/SMRT co-repressor complex and transcriptional repression (Lyst et al., 2013). Nonsense, frameshift, and splicing mutations appear distal to the MBD and result in premature termination of the protein (Vacca et al., 2001; Philippe et al., 2006; Matijevic et al., 2009).

MeCP2 is found in a wide variety of tissues but appears to be most abundant in the brain (Shahbazian

et al., 2002). During embryogenesis, the neuronal MeCP2 level is relatively low but increases progressively during the postnatal period of neuronal maturation (Kishi and Macklis, 2004; Skene et al., 2010). In mouse, MeCP2 is not expressed strongly in the neocortex during embryonic stages, except in Cajal–Retzius cells, one of the earliest-generated populations of neurons in the developing neocortex. Expression of MeCP2 develops progressively from deep cortical layers to superficial layers as development progresses. Thus, there is a clear parallel between neuronal maturation and MeCP2 expression onset (Shahbazian et al., 2002; Kishi and Macklis, 2004). The developmental increase of MeCP2 expression has been confirmed in several brain regions such as the cerebellum and the olfactory epithelium (Cohen et al., 2003; Mullaney et al., 2004) and suggests that MeCP2 has a pivotal role in neuronal maturation throughout the central nervous system (CNS).

With respect to the molecular mechanisms of MeCP2 function, many questions are yet to be answered conclusively (Guy et al., 2011). MeCP2 was initially considered a transcriptional repressor whose potency depends on the density and location of methyl-CpGs near a promoter (Nan et al., 1997). MeCP2-mediated gene silencing could occur through chromatin modification mediated by MeCP2 interaction with Sin3A/HDACI or Ski/NcoR/HDACII repression complexes (Jones et al., 1998; Nan et al., 1998; Kokura et al., 2001). These enzymes remodel chromatin, which then becomes inaccessible to the transcriptional machinery (Nan et al., 1998). Further studies showed that MeCP2, in addition to its role as a global repressor, acts as a splicing regulator (Young et al., 2005). The authors identified the RNA-binding protein Y box-binding protein 1 (YB1), a principal component of messenger ribonucleoprotein particles that controls multiple step of mRNA processing, as a MeCP2 binding partner. More specifically, in MeCP2-deficient neurons the splicing is altered, and aberrantly spliced transcripts can arise (Young et al., 2005). Another surprising finding resulted from genome wide analysis of gene expression in MeCP2 mutants showing that MeCP2, contrary to expectation, could activate the expression of many genes directly or indirectly (Chahrour et al., 2008). In support of this idea, a biochemical interaction between MeCP2 and the transcriptional activator CREB was reported (Chahrour et al., 2008). It is clear that further analysis is required to clarify whether MeCP2 is simply a methyl-DNA binding protein involved primarily in transcriptional repression or a multifunctional protein with global or local actions. For instance, recent work demonstrates that MeCP2 binds

broadly throughout the genome, suggesting that MeCP2 functions more as a global regulator of transcription and chromatin remodeling rather than a sequence-specific transcription factor (Skene et al., 2010; Cohen et al., 2011). The current state of our knowledge suggests that MeCP2-related disorders might therefore be the results of misregulation of both transcription and splicing processes. However, MeCP2 deficiency does not appear to cause strong deregulation of gene expression, but instead might have small effects on many genes in a cell context, developmental stage, and stimulus-dependent manner.

Mecp2 and BDNF

Several studies have used candidate gene approaches on samples from both patients and different mouse models (see, Guy et al., 2011; Na et al., 2013 for a discussion of the available MeCP2 mutants) and identified putative MeCP2 targets that might be relevant to the pathogenesis of RTT. One gene that has consistently shown expression changes when MeCP2 is absent is *Bdnf*. Brain derived neurotrophic factor (BDNF) is known to be a key signaling molecule in brain development and plasticity (Greenberg et al., 2009; Cohen-Cory et al., 2010) and alterations of its expression may underlie several psychiatric conditions (Tsankova et al., 2007). Early studies *in vitro* have suggested that MeCP2 represses *Bdnf* gene transcription. Indeed, MeCP2 binds to promoter III of the *Bdnf* gene (Chen et al., 2003; Martinowich et al., 2003) in the absence of neuronal activity and mediates its transcriptional repression. Membrane depolarization in mouse cortical cultures triggers the phosphorylation of MeCP2 at serine 421 through a CaMKII-dependent mechanism, and releases it from promoter to which it is bound allowing transcription (Chen et al., 2003; Zhou et al., 2006). This view, however, has been challenged by subsequent *in vivo* studies. Chang et al. (2006) reported that BDNF protein levels are decreased rather than increased in brains of MeCP2 null mice. Moreover, genome wide analysis in mutants with impaired S421 phosphorylation did not show changes in MeCP2 DNA binding (Cohen et al., 2011). One explanation of this discrepancy could be related to the recently suggested role of MeCP2 as transcriptional activator (Chahrour et al., 2008). However, another possibility is that MeCP2 mutations could influence BDNF levels *in vivo* by reducing the activity state of neuronal networks in the brain. Indeed, BDNF is a highly regulated gene responsive to extracellular stimuli including depolarization and synaptic activation and several studies reported reduced electrophysiological

responses in MeCP2 mutants (see section on synaptic alterations in RTT). The studies that found higher BDNF levels in the absence of MeCP2 were carried out in primary cultures of embryonic or early postnatal neurons, which lack the complex synaptic interactions occurring in brain circuits and could be less sensitive to indirect regulations mediated by changes in network activity.

The reduced BDNF levels in MeCP2 mutants have been repeatedly observed by many labs raising the possibility that BDNF might have therapeutic actions in RTT. This hypothesis was supported also by the presence of phenotypic similarities between MeCP2 and BDNF deficiency. For example, in both models the average brain weight is smaller compared with wild type animals and both display a hind limb clasping phenotype that is thought to mimic stereotypic hand wringing in RTT patients. A direct test of BDNF action in MeCP2 mutants showed that BDNF overexpression improved the locomotor function of MeCP2 mutant mice in the dark cycle running wheel assay and increased firing of layer 5 of cortical neurons (Chang et al., 2006; Greenberg et al., 2009). Furthermore, exogenous BDNF application to brainstem slices was shown to rescue synaptic dysfunction in Mecp2 null mice (Kline et al., 2010). Other data supporting a link between MeCP2 and BDNF include the effect of environmental enrichment (EE) on animal model of RTT (Kondo et al., 2008; Nag et al., 2009; Lonetti et al., 2010). EE is a rearing condition known to enhance synapse formation and plasticity and it is able to increase the levels of BDNF. Lonetti et al. analyzed the effect of early EE from p10 on MeCP2 mutants (Lonetti et al., 2010). They showed that early EE improves motor coordination and motor learning in RTT models. These effects were associated with modifications of excitatory and inhibitory synapses and augmented BDNF levels.

A major obstacle to the use of BDNF for RTT is its inability to cross the blood–brain barrier. Recently, small molecules able to enhance BDNF action and to enter the brain tissue from the periphery have been developed and tested on RTT models. BDNF is a critical regulator of the brainstem circuits involved in respiratory deficits occurring in MeCP2 mutant mice. Treatment of heterozygous MeCP2 female mutants with LM22A-4 for 4 weeks rescued wild-type levels of phosphorylation of the BDNF receptor, trkB, in the medulla and pons and restored wild-type breathing frequency (Schmid et al., 2012). Another study analyzed the effect of Fingolimod, an agonist of sphingosine-1 phosphate receptor 1 (S1P1). This molecule was found to enhance ongoing activity in neurons resulting in enhanced MAPK and CREB phosphorylation and increased BDNF expression. Its systemic administration to MeCP2 homozygous male mutants enhanced activity-dependent production and release of BDNF and promoted morphological and behavioral attenuation of the mutant phenotype (Deogracias et al., 2012). Although Fingolimod might have adverse effects deriving from inhibition of lymphocyte egress from lymph nodes, it is already in clinical use for multiple sclerosis enhancing the translational potential of Fingolimod for RTT. Finally, 7,8-dihydroxyflavone has been suggested to be an agonist of trkB and was shown to exhibit therapeutic efficacy in male homozygous MeCP2 mutants (Johnson et al., 2012). However, whether 7,8-dihydroxyflavone treatment resulted in increased trkB activation in the treated brains was not reported in this study. Taken together, these data indicate that enhancement of BDNF action in the brain could mitigate symptoms of RTT. Pharmacological agents that could be conveniently used in a clinical context are beginning to be available, although the tolerance to the drugs at the doses necessary to ameliorate RTT symptoms is still to be established.

Other Genes implicated in RTT Syndrome

Many potential MeCP2 regulated genes have been investigated and we address to recent reviews for a discussion (Guy et al., 2011; Zachariah and Rastegar, 2012). An interesting molecular link between autism and RTT is the interaction between MeCP2 and the activity-dependent immediate early gene early growth response gene-2 (EGR2) (Swanberg et al., 2009). EGR2, the only member of the EGR family restricted to central nervous system neurons, encodes a DNA-binding zinc finger protein important for the cerebral development and synaptic plasticity. Expression of EGR2 and MeCP2 increase during development in mouse and human cortex but is disrupted in autism and RTT. EGR2 expression is decreased in the cortex of individuals with autism or RTT as well as in the cortex of MeCP2 knockout mouse. EGR2 positively regulates MECP2 expression by binding to its promoter, and in turn, MeCP2 binds to an enhancer of EGR2, further upregulating the expression of both genes in a positive feedback loop. Swanberg et al. (2009) proposed that EGR2 and MeCP2 function in a similar feedback loop during postnatal development. Deregulation of this system may play a role in the defects associated with ASDs, with loss or reduction in functional MeCP2 decreasing the response of EGR2, and possibly other immediate early genes, to neural activity and other stimuli that

regulate the maturation of cortical neuronal networks.

Genome wide screening of gene expression has revealed many hundreds of genes with altered expression in overexpressing and knockout MeCP2 mutants (Samaco et al., 2012). A recent study followed this approach, testing whether differential expression of *Oprh* and *Crh* in the amygdala could mediate the effect of MeCP2 mutations (Samaco et al., 2012). Amygdala expression of these genes was found to be downregulated in MeCP2^{Tg1} overexpressing mice. Interestingly, each of these genes was responsible for a subset of the MeCP2 phenotypes. Specifically, *Oprh* dysregulation was involved in the abnormal social behavior displayed by MeCP2 mutants, whereas the *Crh* receptor, *Crhr1*, mediated anxiety-like behavior alterations. This study exemplifies how a complex phenotype can be dissected into single behavioral components, each of which can have a distinct genetic basis and, possibly, a specific treatment.

Synaptic Alterations in RTT

Despite its widespread expression in the body, MeCP2 seems to be more important in the brain that in other tissues. Indeed, the symptoms of MeCP2 mutation in mice and patients are primarily neurologic or psychiatric, and mice that lack MeCP2 only in the brain have the same phenotype as those that are MeCP2-null in all tissues (Chen et al., 2001; Guy et al., 2001). Widespread abnormalities have been observed at the synaptic level in mouse models (see Boggio et al., 2010; Na et al., 2013 for reviews). MeCP2^{Tg1} transgene mutants, that over express MeCP2, show enhanced glutamatergic transmission (Chao et al., 2007). MeCP2 knock-out neurons have lower excitatory postsynaptic currents (EPSCs) both in culture and in mice models (Collins et al., 2004; Chao et al., 2007). Multiple electrophysiological properties of synaptic connections are also abnormal in MeCP2 mutants. Whole-cell patch clamp recordings of layer V pyramidal neurons in the primary somatosensory (S1) cortex revealed a reduction of both spontaneous EPSCs and spontaneous action potential firing in MeCP2-KO male mice (Dani et al., 2005; Chang et al., 2006; Tropea et al., 2009). Intriguingly, in MeCP2-KO animals, excitatory input was reduced whilst the total inhibitory input was enhanced showing that inhibitory synapses might also be affected by MeCP2. Moreover, these data indicate that the lack of MeCP2 induces a shift of the homeostatic balance between excitation and inhibition (E/I) in favor of inhibition (Dani et al., 2005;

Wood et al., 2009). The effects on excitation and inhibition found in specific cell types of the brain might not be the same in all brain circuits. For instance, in contrast to the cortex, a shift in favor of excitation is present in several subcortical nuclei involved in generating the respiratory pattern (Stettner et al., 2007; Medrihan et al., 2008; Taneja et al., 2009; Kline et al., 2010; Kron et al., 2011) and in the hippocampus (Moretti et al., 2006; Zhang et al., 2008; Fischer et al., 2009; Calfa et al., 2011; Nelson et al., 2011). Indeed, recent work analyzing the neuronal activity marker c-fos in the brain of male homozygous MeCP2 mutants revealed a consistent pattern of enhancement and decrease of c-fos positive cells in different nuclei of the brain indicating that some parts of the brain become hyperactive whereas others appear to become hypoactive in the MeCP2 mutant brain (Kron et al., 2012). For example, this study reported forebrain hypoactivity in limbic cortices that project to the ventrolateral periacqueductal gray, and hyperactivity in solitary tract nucleus, a downstream target of the ventrolateral periacqueductal gray, suggesting that the excitatory/inhibitory imbalance across the forebrain–midbrain–hindbrain neuraxis could be a substrate for abnormal behavioral state regulation of respiratory function in RTT.

Several studies have addressed the role in MeCP2 in different neurotransmitter systems by cell-specific genetic interference with MeCP2 using the Cre-LoxP system. It was found that deletion of MeCP2 in gamma aminobutyrric acid (GABA) expressing neurons using the VIAAT promoter to drive the expression of the Cre-recombinase resulted in reduced GABA signaling based on findings of reduced quantal size (Chao et al., 2010) and, more importantly, it reproduced most of the features of RTT (including the stereotyped behavior and premature lethality). When the Dlx5 promoter, that is active only in forebrain GABAergic cells, was used a milder phenotype was observed. A role for MeCP2 in inhibitory cell function was also suggested by the observation that MeCP2 mutants reared in EE do not show the EE induced reduction of inhibitory synaptic density in the cortex typical of wild type mice (Lonetti et al., 2010). By contrast, lack of MeCP2 did not prevent the EE effects on excitatory synaptic contacts. Thus, although the mechanism by which changes in MeCP2 expression result in alterations of synaptic transmission is largely unclear, it seems both glutamatergic and GABAergic synapses can be affected by MeCP2 mutations. It is still to be clarified whether different interneuronal populations are more susceptible to alterations of MeCP2 expression.

A recent paper (Durand et al., 2012) addresses this issue presenting the first *in vivo* report of a progressive shift in cortical E/I balance favoring inhibition. The authors demonstrated that deletion of MeCP2 induces an early upregulation of parvalbumin (PV) positive interneurons and a rapid "hypermaturation" of PV-cell connectivity onto cortical pyramidal neurons. Not all inhibitory circuits were affected by deletion of MeCP2, as this early maturation pattern was not observed in other subsets of GABAergic interneurons. Surprisingly, the authors demonstrated that the upregulation of PV circuits in the visual cortex is particularly evident just after eye opening in anticipation of a dramatic vision loss. Intriguingly, both cortical function and PV hyperconnectivity were strikingly rescued, independently of MeCP2, by early sensory deprivation or genetic deletion of the excitatory N-methyl diaspartate (NMDA) receptor subunit NR2A, suggesting that this early upregulation of PV connectivity could be an initial trigger of visual acuity regression.

MeCP2 mutations result in pathological phenotypes also when induced in neuronal populations producing neuromodulators. MeCP2 was specifically removed from either TH-expressing dopaminergic and noradrenergic neurons or PC12 ETS factor 1 (PET1)-expressing serotonergic neurons using Cre-lox technology to generate conditional knock-out (CKO) animals (Samaco et al., 2009). The lack of MeCP2 in neurons expressing dopamine and noradrenaline, or serotonin caused the appearance of specific phenotypes. TH-CKO animals showed a specific alteration in locomotor activity whereas MeCP2 deletion in serotonergic neurons resulted in aggressive behavior. A caveat in this experiment is that the mouse carrying the floxed allele was hypomorphic for MeCP2 and displayed an attenuated form of a number of symptoms typical of RTT models. The presence of these symptoms could mask deficits caused by cell-specific deletion of MeCP2 resulting in underestimation of the contribution of MeCP2 mutations in neuromodulatory systems to RTT phenotype.

Another way of testing the contribution of a neurotransmitter system to the phenotype of RTT null mice is to reactivate the expression of MeCP2 in a selected population. This approach is based on the seminal observation that reactivation of MeCP2 in all cells even after the onset of symptoms is able to recover behavioral, functional, and morphological phenotypes (Giacometti et al., 2007; Cobb et al., 2010; Robinson et al., 2012). This intriguing observation called into question the classical definition of RTT as a neurodevelopmental disorder, suggesting that

MeCP2 was required, even in the adult, for maintenance of neuronal circuits. In keeping with this hypothesis, further studies showed that loss of MeCP2 in the adult triggers the onset of a RTT-like pathology (McGraw et al., 2011; Cheval et al., 2012). Interestingly, targeted preservation of MeCP2 selectively in catecholaminergic neurons improved the lifespan, ambulatory rate, rearing activity, motor coordination, anxiety, nest-building performances, and cortical epileptiform discharge activity of both male and female Mecp2-deficient mice (Lang et al., in press). This approach has a strong translational value and it is likely that it will be used in the near future by many laboratories to test the ability of different neurotransmitters to promote recovery from RTT symptoms.

Glia and RTT

Although initial studies have strongly supported an exclusively neuronal role for MeCP2 in RTT, it has been shown that other cells, specifically glia, play a major role in the disease. The first demonstration of expression of MeCP2 in astrocytes has been provided by Maezawa et al. (2009). The authors revealed by immunofluorescence assay that MeCP2 protein is expressed not only in neurons but also in astrocytes. In particular, they found that astrocytes carrying MeCP2 deletion are abnormal; they grew slowly, released less interleukin 1 and interleukin 6 in response to an immune challenge, and expressed aberrant levels of BDNF, a target gene of MeCP2. Interestingly, when MeCP2$-$/$+$ astrocytes were co-cultured with wild-type astrocytes, MeCP2 expression in the wild-type astrocytes also decreased in a time-dependent manner. Furthermore, the authors showed that inhibitors of gap junction stop the spread of the deficiency across the astrocytic culture. Overall, this study suggests the intriguing hypothesis that the pathogenesis of RTT syndrome may crucially depend on a progressive decrease of MECP2 expression in astrocytes.

The importance of the expression of MeCP2 in astrocytes was reinforced in another study that demonstrated that MeCP2-null astrocytes had a deleterious impact on both wild-type and MeCP2-null neurons, which develop fewer and shorter dendrites similar to those found in hippocampal pyramidal and granule cells of male MeCP2 null mice (Ballas et al., 2009). Interestingly, conditioned medium from null astrocytes was sufficient to elicit this neuronal phenotype, which points to secreted factors as the causal agent. Consistently, experiments in MeCP2 mutants in which MeCP2 expression was reactivated in

astrocytes significantly improved locomotion and anxiety levels, restored respiratory abnormalities, and prolonged lifespan (Lioy et al., 2011).

Around the same time, researchers demonstrated that microglia cells may also influence the onset and progression of RTT. Microglia play key roles in CNS development, tissue homeostasis, synaptic regulation, and neural plasticity (Ladeby et al., 2005; Hanisch and Kettenmann, 2007).

In RTT syndrome, MeCP2-null microglia was suggested to be toxic to neurons by virtue of upregulated secretion of glutamate. Indeed, neurons cultured in conditioned media from MeCP2-null microglia, which release fivefold higher levels of glutamate than wild-type microglia, developed shorter and thinner dendrites. Inhibition of glutamate synthesis and release from these cells rescued the neuronal defects (Maezawa and Jin, 2010) suggesting that microglia cells have an important role in the pathogenesis or RTT.

To address the role of microglial immunity in RTT, wild type bone marrow of 4-week-old Mecp2-null male was transplanted into irradiation-conditioned MeCP2 null hosts. By 8–10 weeks after bone marrow transplantation, robust engraftment of microglia-like cells from donor mice was evident in brains of MeCP2 null animals. Surprisingly, pathology was halted; mice with an expected lifespan of 8 weeks survived to nearly 1 year (Derecki et al., 2012, 2013). This beneficial effect, however, is abolished if phagocytic activity of microglia is inhibited.

Thus, behavioral deficits in RTT are not restricted to defects in neuronal function but have a contribution from astrocytes and microglia. The importance of these studies goes beyond RTT, indeed they support a pathogenetic mechanism of disease that could be involved also in other neurodevelopmental disease but that remains poorly investigated. Moreover, these data unravel a fundamental role of glia–neuron interaction during synaptic development. Indeed, recent work shows that microglia actively engulf synaptic material and play a major role in synaptic pruning during normal postnatal development in mice (Paolicelli et al., 2011).

Synaptic Plasticity is Affected in RTT

Long-term potentiation (LTP) and long-term depression (LTD) are forms of synaptic plasticity that are believed to underlie long-term memory formation and circuit refinement during development. Impairments in LTP and LTD induction and/or maintenance have been correlated with general learning and memory deficits and point to enduring alterations in synaptic plasticity/connectivity. Electrophysiological studies of mouse models of RTT syndrome (Chen et al., 2001; Guy et al., 2001; Shahbazian et al., 2002; Pelka et al., 2006) showed impairments in long-term synaptic plasticity and excitatory drive. Reduced LTP was observed in hippocampal slices of Mecp2 null mice. Cortical slices from Mecp2$^{308/y}$ mice, a mouse expressing a truncated MeCP2 protein displaying a milder phenotype than the germline knockout (Asaka et al., 2006; Moretti et al., 2006), also showed an LTP impairment suggesting that deficits in synaptic plasticity consistently result from MeCP2 dysfunction. Impairments in synaptic plasticity were also observed in layer II–III synapses of motor and sensory cortex of MeCP2 null mice, indicating that abnormalities of synaptic plasticity extend beyond the hippocampus and involve additional brain regions, including the neocortex (Moretti et al., 2006; Lonetti et al., 2010). MeCP2 overexpression seems to induce an opposite synaptic phenotype: indeed the MeCP2^{Tg1} line expressing twice the normal MeCP2 levels, showed enhanced paired pulse facilitation, an assay of short-term plasticity known to be impaired in MeCP2 loss of function mutants (Asaka et al., 2006; Moretti et al., 2006; Nelson et al., 2011), and increased hippocampal LTP (Collins et al., 2004). Recently, Na et al. characterized a different mouse line with MeCP2 overexpression restricted to neurons (Tau–MeCP2) and replicated the paired pulse facilitation enhancement but found attenuated hippocampal LTP (Na et al., 2012). The different LTP phenotype displayed by Tau–MeCP2 and MeCP2^{Tg1} could be due to factors like neurospecific versus general MeCP2 overexpression, or different MeCP2 overexpression levels (Collins et al., 2004; Na et al., 2013).

Hebbian mechanisms such as LTP and LTD have an important role in shaping synaptic connectivity during development. However, Hebbian plasticity predicts a recursive reinforcement (or depression) of synapses that should destabilize the activity of neural circuits. At the synaptic level such phenomena are countermanded by dedicated pathways dampening Hebbian plasticity and favoring synaptic stability (Turrigiano and Nelson, 2004). This phenomenon is called synaptic scaling, a form of homeostatic synaptic plasticity widely believed to contribute to the maintenance of the proper E/I balance within central neural circuits. Recent work has demonstrated that MeCP2 plays a fundamental role in synaptic homeostasis. Acute and cell-autonomous loss of MeCP2 prevented the usual scaling up of synaptic strengths in response to activity blockade (Blackman et al., 2012), whereas MeCP2 phosphorylation was required for bicuculline-induced synaptic scaling down in hippocampal neurons (Zhong et al., 2012).

Recent work from Qiu et al. has investigated how MeCP2 loss could mediate the synaptic scaling down (Qiu et al., 2012). Increase in neuronal activity upon bicuculline treatment leads to an increased level of MeCP2 expression, which in turn binds to the GluR2 promoter and recruits a repressor complex to inhibit its expression and synaptic availability. Downregulating AMPA receptor GluR2 subunit expression is one direct way to mediate an adaptive response that regulates synaptic strength and prevents recurrent circuit excitation.

Synaptic Role of MeCP2 Phosphorylation

Neuronal activity-induced phosphorylation of MeCP2 could be an important mechanism through which MeCP2 regulates plasticity through activity-dependent gene transcription. Several modified serines and threonines have been identified in MeCP2, two of which have been investigated in some detail (Zhou et al., 2006; Tao et al., 2009; Gonzales et al., 2012). Serine 421 is phosphorylated specifically upon neuronal activity (Chen et al., 2003) and has been implicated in dendritic patterning, spine morphogenesis, and the activity dependent induction of BDNF transcription (Zhou et al., 2006) leading to the hypothesis that S421 phosphorylation could mediate activity-dependent effects of MeCP2 on synaptic development and plasticity. Indeed, recent evidence shows that loss of MeCP2 phosphorylation at S421 affects bicuculline induced homeostatic synaptic scaling down (Zhong et al., 2012). However, the phenotype induced by loss of MeCP2 S421 phosphorylation *in vivo* only partially overlaps with the phenotype of MeCP2 null mice. At the electrophysiological level, layer II–III pyramidal cells of MeCP2 S421 phosphodefective mice displayed enhanced inhibitory currents but did not show alteration of excitatory responses. No abnormalities in social interaction, motor coordination, spatial learning, were observed albeit novel object recognition memory was impaired. On the other hand, selective enhancement of complexity of dendritic tufts of pyramidal cells was observed as opposed to the reduction of dendritic complexity often observed in MeCP2 null mice (see below). Intriguingly, chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) showed that MeCP2 S421 phosphorylation occurs across the genome suggesting that this modification appears not to control the expression of specific genes, but could regulate a genome-wide response of chromatin to neuronal activity. Moreover, MeCP2 DNA binding was not affected by S421 phosphorylation (Cohen et al., 2011). Analysis of a mouse model carrying point mutations in the endogenous MeCP2 gene locus that abolished phosphorylation at both S421 and S424 gave a substantially different outcome showing enhanced hippocampal LTP and hippocampus-dependent learning and memory (Li et al., 2011); a phenotype reminiscent of models of MeCP2 over expression.

Another phosphorylated MeCP2 residue is serine 80, a site close to the MBD that is negatively regulated by neuronal activity. Dephosphorylation of S80 also contributes to the attenuated association of MeCP2 with some of its target chromatin regions (Tao et al., 2009). Very recently, three novel sites of activity-dependent phosphorylation, S86, S274, and T308, have been found using phosphotryptic mapping (Ebert et al., 2013). Phosphorylation of the T308 site is impaired in knockin mice with the missense mutation R306C, a common mutation in RTT patients. The role of defective T308 phosphorylation was confirmed in T308A knockin mice. These mutants showed a decrease in the induction of a subset of activity-regulated genes, and RTT-like behavioral symptoms. Transcriptional alterations present in T308A mice could be due to impaired activity-dependent regulation of MeCP2–NcoR interaction (Ebert et al., 2013). Overall, there is mounting evidence that MeCP2 may play a key role in the activity-dependent regulation of synaptic strength through regulation of MeCP2 activity-dependent phosphorylation. However, a more detailed knowledge of the molecular mechanisms underlying MeCP2 actions is required to understand the role of post-translational modifications of MeCP2 such as phosphorylation and the recently described acetylation (Zocchi and Sassone-Corsi, 2012).

MeCP2 in Synaptic Development

Beginning with classical studies on the neuromuscular junction, experiments performed on a variety of models have shown that formation of neural connections comprises three steps of synapse formation, strengthening and elimination, and activity-dependent plasticity (Kano and Hashimoto, 2009). One fruitful approach to address the role of MeCP2 in these processes has been to monitor the development of synaptic circuits in the visual system. Recent work investigated the maturation of the connection between retinal ganglion cells (RGC) and relay neurons in the dorsal lateral geniculate nucleus (LGN) of the thalamus (Noutel et al., 2011). In this system, the first phase of formation of synaptic contacts lasts until postnatal day (P) 8 when retinal connections are segregated into eye specific zones (Godement et al.,

1984). Throughout the second phase, between P8 and P16, there is a continuous elimination and strengthening of synapses (Chen and Regehr, 2000; Jaubert-Miazza et al., 2005). This process is mainly driven by spontaneous activity and peaks at about eye opening (P12). A third phase of synaptic plasticity occurs after 1 week of visual experience (P20–P34). This developmental phase represents a sensitive period, a time window during which experience is necessary to maintain the refined retinogeniculate circuit and visual deprivation elicits a weakening of RGC inputs (Hooks and Chen, 2006; Hooks and Chen, 2008). In MeCP2 null mice, the initial synapse formation, strengthening, and elimination during the experience-independent phase of development proceeds in a manner similar to wild-type mice. During the third vision-dependent phase however, retinal inputs regresses and experience-dependent plasticity is impaired.

A role of MeCP2 in the final steps of circuit development is also clear by the late deterioration of cortical visual acuity occurring in MeCP2 null mice (Durand et al., 2012). Intriguingly, cortical acuity regression begins after the occurrence of the impairment in retinogeniculate synaptic transmission observed by Noutel et al. (2011), suggesting that alterations in LGN synaptogenesis and visual acuity are not directly related. Durand et al. (2012) also propose that an abnormal early hyperconnectivity of PV positive neurons with their postsynaptic targets in the visual cortex could represent an initial trigger of these late alterations. Whether abnormal development of PV cells is a primary event in the induction of the MeCP2 KO phenotype also in the LGN is still to be ascertained. These studies exemplify the usefulness of adopting the visual system as a model; however other sensory systems are beginning to be investigated. For example, a progressive degradation of auditory evoked potentials was observed in a knock-in mouse model carrying the common T158 residue MeCP2 mutation (Goffin et al., 2011).

The view that MeCP2 participates in synaptic maturation throughout postnatal life seems to be consistent with results found in other neural systems. Indeed, monitoring synaptic transmission of cortical and subcortical excitatory projections to the amygdala of MeCP2[308/Y] mutant mice showed that after a period of normal development an exaggerated rate of synaptic elimination and maturation at cortical-LA synapses was observed (Gambino et al., 2010). Interestingly, the excitatory synapses from the thalamus seem to be spared in the case of mutant mice.

Despite the complexity of neural development and the variety of subsystems involved, it seems clear that MeCP2 plays a major role when neural circuit undergoes a rapid maturation in response to environmental stimuli.

MeCP2 Regulates Dendritic Morphology

RTT patients present with a reduced number of axonal and dendritic processes (Armstrong et al., 1998) and a decrease of the levels of microtubule associated protein 2 (MAP2), a crucial protein for dendritic cytoskeleton. In addition, neurons in RTT patients show a decrease of dendritic spine density (Armstrong, 2002; Chapleau et al., 2009). Similar abnormalities in dendritic spines have been seen in human subjects with mental retardation (Purpura, 1974; Takashima et al., 1994) and in animal models of Down syndrome (Kurt et al., 2000; Belichenko et al., 2007; Belichenko et al., 2009b). Abnormal structural properties of dendritic spines might go hand in hand with defects in synaptic contacts. Indeed, dendritic spines are small protrusions from the dendrite that serve as the postsynaptic target for most excitatory synapses in the brain (Bhatt et al., 2009), although recent data suggest that inhibitory synapses showing high levels of plasticity may also be located on dendritic spines (Chen et al., 2012; van Versendaal et al., 2012).

Studies in MeCP2 mutants showed that pyramidal neurons in the cortex of MeCP2 null mice have smaller somas and reduced dendritic arborization (Kishi and Macklis, 2004; Fukuda et al., 2005). MeCP2-deficient neurons also have fewer dendritic spines and reduced arborization and exhibit additional impairments in neuronal maturation in both the hippocampus and in the olfactory system (Zhou et al., 2006; Smrt et al., 2007; Palmer et al., 2008). Alterations in dendritic morphology and decreased cortical thickness have been observed as early as 2 weeks of age with both somatosensory and motor cortices significantly affected by lack of MeCP2 expression. Furthermore, spine density in the apical tuft of dendrites of layer V pyramidal cells was found to be reduced in male MeCP2 null mice at 4 and 6 weeks of age (Landi et al., 2011). Interestingly, reactivation of MeCP2 expression in MeCP2 null mice induces recovery from dendritic structural abnormalities in parallel with behavioral amelioration (Robinson et al., 2012). This strengthens the link between behavioral and morphological alterations in RTT mice. These results also suggest that much of development in these mouse models occurs normally and that those defects that disrupt function are not permanent opening a host of questions into how they may

be therapeutically reversed in animal models and in humans.

In this regard, Chapleau et al. (2012) analyzed the dendritic spines of CA1 pyramidal neurons in the hippocampus of male mutant mice and found that at postnatal-day 7 (P7), well before the onset of RTT-like symptoms, CA1 pyramidal neurons from mutant mice showed lower dendritic spine density than those from wild type littermates. On the other hand, at P15 or even during the symptomatic phase starting at about 5 weeks of age (Chen et al., 2001), dendritic spine density did not differ between mutant and wild type neurons. These results raise caution regarding the use of dendritic spine density in hippocampal neurons as a phenotypic endpoint for the evaluation of therapeutic interventions in symptomatic MeCP2-deficient mice, and suggest that the cellular context could play a crucial role in determining the effect of MeCP2 mutations on neuronal morphology.

To directly assess synaptic alterations in MeCP2 mutants, electron microscopy studies were performed. Cortical neurons from MeCP2 KO mice revealed an increase in premature postsynaptic densities (Fukuda et al., 2005). Postsynaptic density cross-sectional length was also found to be reduced in area CA1 of the hippocampus (Moretti et al., 2006). In superficial cortical layers of 8 weeks old MeCP2 null mice no difference in the density of excitatory or inhibitory synaptic density was observed (Lonetti et al., 2010), however the reduction of dendritic spines present at this age suggest that synapses are mislocalized to dendritic shafts. Overall, these data suggest that functional consequences of the loss of MeCP2 during development include delays in the maturation of neurons and their synaptic connectivity.

Overexpression of MeCP2 can also affect dendritic morphology. Excitatory synapse number was found to be decreased in autaptic hippocampal cultures from MeCP2 null mice and conversely increased in MeCP2 over expressing mice (Chao et al., 2007). However, studies in hippocampal and cortical cultures have shown that overexpression or elimination of MeCP2 decreases dendritic arbor complexity and spine density (Kishi and Macklis, 2004; Zhou et al., 2006; Chapleau et al., 2009). A different mouse model with neuron specific MeCP2 overexpression showed enhanced excitatory currents and LTP and LTD deficits (Na et al., 2012). These observations are highly relevant in that they may help to explain how duplications of the MeCP2 locus and overexpression of MeCP2 can result in RTT-like behaviors in both human and rodents (Archer et al., 2006).

Multiphoton microscopy allows imaging of dendritic spines in the living cortex several hundred micrometers deep from the pial surface (Denk et al., 1990; Theer et al., 2003) over extended periods of time (Holtmaat and Svoboda, 2009). By repeatedly imaging the same spines, this technique allows one to monitor dendritic spine dynamics resolving different cellular processes such as spine formation, motility, stabilization, and retraction. Recently, Landi et al. (2011) employed this technique to image dendritic spines of layer V apical tufts during a 60-min long time window in the somatosensory cortex of MeCP2 male null mice. Neurons in these mice were rendered fluorescent by crossing the null line with a Thy-GFP line (Feng et al., 2000). First, they reassuringly found that the spine density alterations present in fixed tissue were present also in living mice. Second, they found that at 4 weeks of age long spines without heads, called filopodia, that are typically present during the period of intense synaptogenesis were most affected by MeCP2 mutation. Indeed their motility and formation was reduced in MeCP2 mutants suggesting that the reduced spine density present in mutant neurons results from impaired spine formation and maturation rather than enhanced synaptic elimination. Spines with large heads that are generally assumed to be more persistent than thin spines (Holtmaat and Svoboda, 2009) also show dynamic changes of head volume that could involve synaptic cytoskeleton rearrangements that are abnormal in MeCP2 mutants. Forty days postnatally (p40) when the maturation of the connectivity of the somatosensory cortex is complete, the dynamics of dendritic spine formation in MeCP2 null mice becomes similar to that of controls although spine density is still decreased. It is likely that at this age longer times of observation are needed to reveal abnormal spine dynamics in MeCP2 mice.

A flurry of experiments employing genetic and pharmacological approaches indicates that actin rearrangements drive morphological plasticity of dendritic spines (Matus, 2000). Thus, it could be possible that alterations of pathways regulating actin dynamics could underlie dendritic spine abnormalities engendered by MeCP2 loss or gain of function. Moreover, the correlation between spine head volume, postsynaptic density size, and synaptic strength that is normally observed (Holtmaat and Svoboda, 2009) suggests that morphological alterations could result in functional impairments. In agreement with this notion, it was recently found that the bacterial toxin CNF-1 that activates the RhoGTPase pathway, a key regulator of actin dynamics, can ameliorate the phenotype of fully symptomatic MeCP2[308] mice (De Filippis et al., 2012). Other studies show that the spine motility deficits present in MeCP2 mutants
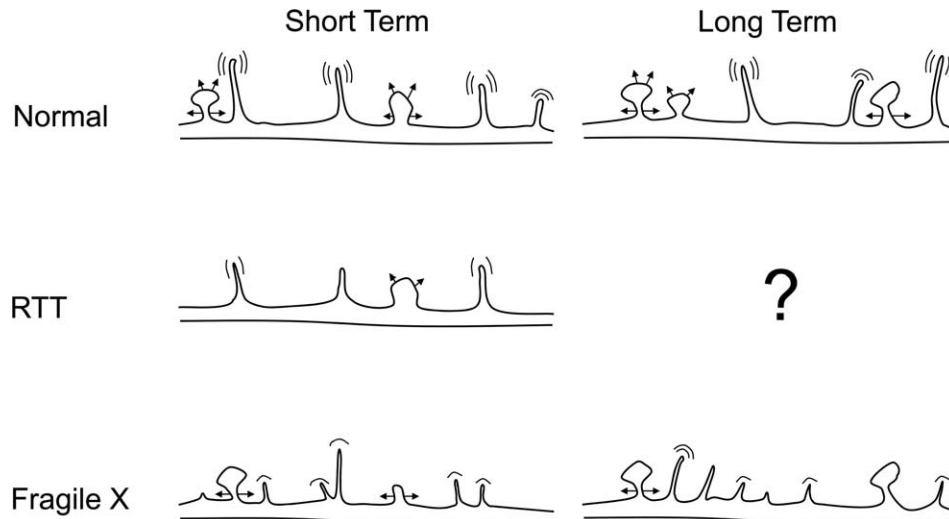
Figure 1 In wild type mice, short-term dynamics are mostly represented by filopodia motility and changes in the volume of spine heads (arrows). In the long-term, new spines are formed and pre-existing spines undergo elimination or stabilization. In mice models of RTT, short-term dynamics is strongly affected. Filopodia production is much reduced and motility is impaired. Long-term dynamics have not been studied. In contrast to RTT, Fragile X models show a deficit in spine maturation resulting in an exaggerated turnover causing the appearance of higher than normal numbers of immature spines at any age.

could be rescued by an injection of insulin-like growth factor-1 (IGF-1) (Landi et al., 2011) which was previously shown to be necessary for spine maintenance in wild type mice. In agreement with this, Tropea et al. (2009) showed that using a fragment of IGF-1 in systemic treatment of MeCP2 mutant mice could ameliorate their phenotype.

Substantial alterations in either spine morphology or density are present in a number of psychiatric and neurological diseases such as addiction, anxiety, and depression that are often linked to environmental factors including malnutrition, abnormal hormone levels, and chronic drug abuse (Benitez-Bribiesca et al., 1999; Robinson et al., 2001; Shors et al., 2001; Chen et al., 2008). These alterations may result from specific impairments of the distinct mechanisms underlying dendritic spine dynamics. In fragile X syndrome (FXS), for example, which is the most frequent form of inheritable mental retardation (Marin-Padilla, 1976; Takashima et al., 1981, 1994; Ferrer and Gullotta, 1990) and that often includes autistic features, spines are found in much higher density and display a more immature, long, and thin form. Chronic imaging of FXS mice showed that a higher degree of spine turnover in mutants (Cruz-Martin et al., 2010) was responsible for the presence of more immature-appearing spines at any point in time. Interestingly, once spines were fully mature, their long-term stability was not affected by the mutation. Furthermore,

formation of new spines and the elimination of existing ones were less sensitive to modulation by sensory experience (Pan et al., 2010). These studies exemplify how *in vivo* imaging analysis can probe into the cellular mechanisms underlying the synaptic defects in RTT and in other disorders (Fig. 1).

AKT/Mammalian Target of Rapamycin (mTOR): A Pathway Implicated in Rett Syndrome

The more characteristic feature of RTT neurons is a reduced complexity of dendritic arbors and reduced cell soma size (Kishi and Macklis, 2004; Belichenko et al., 2009). Alteration of cell and nuclear size has been taken as a cellular phenotype that could be used to monitor the effects of RTT mutations and treatments in neurons obtained from embryonic stem cells or induced pluripotent stem cells (iPSC) derived from RTT patients (Marchetto et al., 2010; Kim et al., 2011; Yazdani et al., 2012). Therefore, dysfunctions in signaling pathways regulating these aspects of neuronal maturation are good candidate as possible molecular substrates of RTT. Following this reasoning, the Akt/mTOR pathway has been recently investigated in MeCP2 mutants (Ricciardi et al., 2011). The authors showed reduced levels of phosphorylation of Akt, mTOR, p70S6K, and rpS6, but not of
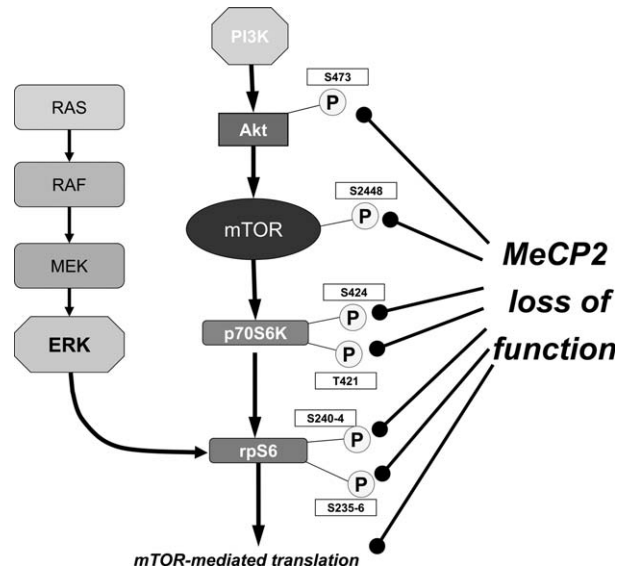
Figure 2 MeCP2 mutant mice show hypophosphorylation of Akt, mTOR, p70S6K, and S6, but not ERK1/2. These defects are associated with lower rates of translation. Ball-headed lines indicate inhibition.

ERK1/2, in symptomatic male MeCP2 null mice. These alterations were associated with a severe deficit in protein synthesis (Fig. 2). Importantly, decreased rpS6 phosphorylation and protein synthesis were present before symptom onset suggesting that they could be involved in the early stages of the disease.

Further analyses indicated that reduced rpS6 phosphorylation was present also in heterozygous MeCP2 mutant females. Since MeCP2 gene is located on X chromosome, heterozygous MeCP2 mutant females are a mosaic of MeCP2 positive and negative cells. The authors exploited this feature to ask whether rpS6 phosphorylation was affected by MeCP2 mutation cell-autonomously. Double staining for MeCP2 and phosphorylated rpS6 revealed that rpS6 phosphorylation was impaired both in cells carrying MeCP2 deletion and in neighboring MeCP2 positive cells, suggesting that rpS6 phosphorylation is affected by MeCP2 mutation in a non cell-autonomous manner. This might be caused by the reduced activation present in MeCP2 mutant networks and/or the aberrant secretion from mutant neurons or glial cells of soluble factor(s) with neurotoxic effects. Indeed, MeCP2 mutant astrocytes as well as microglia were found to induce multiple defects in WT neurons including the dendritic patterning, synapse formation, and microtubule stability (Ballas et al., 2009; Maezawa and Jin, 2010).

The identification of impaired signaling pathways in RTT mice provides crucial information to drive the choice of therapeutic strategies. Moreover, the impaired mTOR pathway in MeCP2 mutants gives mechanistic support to the beneficial effects of activation of BDNF and IGF-1 signaling, that includes the mTOR pathway, in MeCP2 mutants. Indeed, it was recently shown that Akt is hypophosphorylated in the pons and medulla of MeCP2 mutants and that the positive effect of the trkB agonist LM22A-4 is associated with restoring normal levels of phosphorylated Akt (Schmid et al., 2012).

CDKL5 and FOXG1 in Atypical RTT

Little is known about the mechanisms involving *CDLK5* and *FOXG1* in atypical forms of RTT (Ariani et al., 2008; Guerrini and Parrini, 2012). CDKL5 is a ubiquitous protein but is expressed at high levels in the brain (cerebral cortex, hippocampus, cerebellum, striatum, and brainstem), thymus, and testes (Lin et al., 2005). In the developing mouse brain, CDKL5 is expressed in the early postnatal stages and in the adult brain is present in mature neurons. This expression profile suggests that CDKL5 protein is important for neuronal maturation (Rusconi et al., 2008). Moreover, it has been found that CDKL5 is able to act on complexes called nuclear speckles and affects their disassembly and redistribution (Ricciardi et al., 2009). It seems that this kinase works in a molecular pathway common to that of MeCP2: indeed CDKL5 and MeCP2 interact both *in vivo* and *in vitro*, but
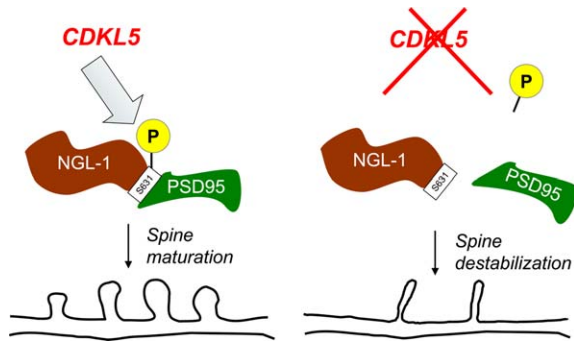
Figure 3 CDKL5 is necessary for correct genesis and maintenance of dendritic spines and for synapse formation by regulating NGL-1–PSD95 interaction. CDKL5-dependent phosphorylation on Ser 631 controls the association of NGL-1 with the postsynaptic molecule PSD95 and this ensures correct synaptic activity. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

there are some controversial results regarding the functional role of this interaction. *In vivo* phosphorylation of MeCP2 has been reported (Mari et al., 2005), but the result has not been confirmed (Lin et al., 2005). Interestingly, recent data show that CDKL5 mutations could be linked to synaptic alterations. By studying CDKL5 in the mouse and in a human cellular model (iPSC-derived neurons) derived from patients carrying CDKL5 mutations, it was shown that CDKL5 is located at excitatory synapses and contributes to correct dendritic spine structure and synapse activity (Ricciardi et al., 2012). The authors found that CDKL5 phosphorylated NGL-1, a protein important for synaptic homeostasis (Hering and Sheng, 2001; Kim et al., 2006; Han and Kim, 2008; Woo et al., 2009). In turn, NGL-1 phosphorylation promotes binding to PSD95, a key protein for the organization of the postsynaptic density (Fig. 3). These data indicate that CDKL5 is very important in spine development and synapse morphogenesis and confirm that synaptic alterations are major substrates of RTT. Studies in mouse models are required to advance our knowledge about CDKL5 role in pathogenesis. This need is beginning to be fulfilled. Indeed, a knockout mouse model of CDKL5-related disorders was generated (Wang et al., 2012) and showed autistic-like deficits in social interaction, as well as impairments in motor control and fear memory but, surprisingly, it did not show an epileptic phenotype.

FOXG1 is a gene implicated in the congenital variant of RTT (Ariani et al., 2008) syndrome. Patients with *FOXG1* mutation meet criteria for atypical RTT (Florian et al., 2012). The *FoxG1* gene encodes a winged-helix transcriptional repressor important for early development of the ventral telencephalon dorso-ventral patterning by integrating several signaling centers (Hanashima et al., 2004; Danesin et al., 2009). Additionally, FOXG1 controls production of specific neuronal subtypes and regulates the balance between neural progenitor cell proliferation and differentiation in the telencephalon (Shen et al., 2006; Hanashima et al., 2007; Miyoshi and Fishell, 2012). FOXG1 is expressed from the earliest stages of telencephalic development through adulthood. Interestingly, using a CKO mouse of FOXG1, Tian et al. were able to demonstrate that FOXG1 is involved in the development of the postnatal dentate gyrus affecting different processes of adult neurogenesis from proliferation to differentiation and neuronal survival (Tian et al., 2012). Moreover, in differentiated post mitotic neurons, FOXG1 promotes cell survival through the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and has a protective effect against the neurotoxic effect of MeCP2 (Dastidar et al., 2011, 2012). Interestingly, Dastidar et al. studied the roles of two previously identified MeCP2 splice variants, MeCP2-e1 and MeCP2-e2, in cultured mouse cerebellar neurons (CCNs). The authors demonstrated that MeCP2-e1 and MeCP2-e2 exhibit different expression profiles and play divergent functional roles in CCNs. When cells were subjected to apoptosis-inducing conditions, mRNA expression of the e2 isoform was upregulated and expression of the e1 isoform was downregulated. Overexpression of MeCP2-e2 promoted apoptosis in healthy cells, whereas MeCP2-e2 knockdown rendered cells resistant to the apoptosis-inducing treatment. In contrast, neither overexpression nor knockdown of MeCP2-e1 affected cell viability. This study further showed that FoxG1 preferentially interacts with MeCP2-e2 and that this interaction restricts the neurotoxic effects of MeCP2-e2. The authors concluded that MeCP2-e2 promotes neuronal cell death when unchecked by FoxG1. This article provides the first evidence of a functional difference between MeCP2 isoforms, and underscores a possible link between MeCP2 and FoxG1.

Final Remarks

The understanding of the cellular basis underlying RTT has greatly improved in the last years and several papers have achieved the previously unthinkable goal of reverting or at least attenuating RTT symptoms. On the other hand, it is clear that many questions are still unanswered. For instance the molecular function of MeCP2 is still far from clear, and how the

multifaceted ensemble of cellular deficits determines RTT symptoms needs clarification. Moreover, we are only at the beginning of clarifying CDKL5 and FOXG1 actions in the disease. However, the National Institute of Neurological Disorders and Stroke (NINDS), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), the International Rett Syndrome Foundation (IRSF), and the Rett Syndrome Research Trust (RSRT) recently organized a workshop to set the standards for preclinical research on RTT sending a clear signal that the time for testing experimental therapies has come (Katz et al., 2012).

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