REVIEW PAPER

Rett Syndrome and MeCP2

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Abstract Rett syndrome (RTT) is a severe and progressive neurological disorder, which mainly affects young females. Mutations of the methyl-CpG binding protein 2 (*MECP2*) gene are the most prevalent cause of classical RTT cases. *MECP2* mutations or altered expression are also associated with a spectrum of neurodevelopmental disorders such as autism spectrum disorders with recent links to fetal alcohol spectrum disorders. Collectively, MeCP2 relation to these neurodevelopmental disorders highlights the importance of understanding the molecular mechanisms by which MeCP2 impacts brain development, mental conditions, and compromised brain function. Since *MECP2* mutations were discovered to be the primary cause of RTT, a significant progress has been made in the MeCP2 research, with respect to the expression, function and regulation of MeCP2 in the brain and its contribution in RTT pathogenesis. To date, there have been intensive efforts in designing effective therapeutic strategies for RTT benefiting from mouse models and cells collected from RTT patients. Despite significant progress in MeCP2 research over the last few decades, there is still a knowledge gap between the in vitro and in vivo research findings and translating these findings into effective therapeutic interventions in human RTT patients. In this review, we will provide a synopsis of Rett syndrome as a severe neurological disorder and will discuss the role of MeCP2 in RTT pathophysiology.

Abbreviations

5hmC	5-Hydroxymethylcytosine
5mC	5-Methylcytosine
ALC	Acetyl-L-carnitine
ASD	Autism spectrum disorders
BDNF	Brain-derived neurotrophic factor
CREB	cAMP response element-binding protein
CTD	C-terminal domain
DNMT	DNA methyltransferases
E	Enhancer
EE	Environmental enrichment
ENCODE	Encyclopaedia of DNA elements
FASD	Fetal alcohol spectrum disorders
GA	Glatiramer acetate
HDAC	Histone deacetylases
HMGN1	High mobility group N1 protein
HP1	Heterochromatin binding protein 1
ID	Inter-domain
IGF1	Insulin-like growth factor 1
iPSC	Induced pluripotent stem cells
IRAK1	Interleukin-1 receptor associated kinase gene
lncRNA	Long noncoding RNAs
MBD	Methyl binding domain
MeCP2	Methyl CpG binding protein 2
MeP	*Mecp2* promoter
miRNAs	MicroRNAs
mnt	Months
MRE	miRNA response elements
NG	Nodose cranial sensory ganglial cells

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NSCs	Neural stem cells
NTD	N-terminal domain
PTMs	Posttranslational modifications
RCP	Red opsin gene
RTT	Rett syndrome
S	Silencer
TM	Tamoxifen
TRD	Transcription repression domain
TSA	Trichostatin A
wks	Weeks
yr	Years
ω-3	ω-3 polyunsaturated fatty acids
PUFAs	

Introduction

Rett syndrome (RTT) is a pervasive neurological disorder (Rett 1966; Hagberg et al. 1983), and it is characterized by compromised brain functions, severe mental retardation, language and learning disabilities, repetitive stereotyped hand movements and developmental regression. RTT is predominantly found in young females, with an incidence of 1:10,000–20,000 live births, with rare cases reported in males (Moog et al. 2003). RTT is considered to be a monogenic neurological disorder (Renieri et al. 2003; Amir et al. 1999), because approximately 90 % cases of classical RTT patients harbor loss-of-function mutations of the X-linked methyl-CpG binding protein 2 (*MECP2*) gene (Bienvenu et al. 2000). Rett syndrome is perhaps the best example for contribution of epigenetic mechanisms in disease pathology, mostly due to the involvement of MeCP2, a key epigenetic modulator in the brain. Epigenetic mechanisms include DNA methylation, histone posttranslational modifications (PTMs) and noncoding RNAs, which regulate gene expression without altering the corresponding DNA sequences (Delcuve et al. 2009). Epigenetic mechanisms are involved in controlling embryonic development, stem cell differentiation and have a high impact in human disease (Olynik and Rastegar 2012; Barber and Rastegar 2010). As a well-studied epigenetic factor, MeCP2 controls gene expression and modulates chromatin architecture through binding to methylated DNA (Zachariah and Rastegar 2012).

Considerable progress has been made during the last few decades to understand the role of *MECP2* mutations in RTT pathogenesis, the functional role of MeCP2 PTMs, *MECP2/Mecp2* isoforms, MeCP2 regulation and effective therapeutic strategies for RTT. The focus on this literature review is to discuss the advances in MeCP2 research work with regard to Rett syndrome.

Rett Syndrome

Rett Syndrome in Females and Males

Due to the X-linked nature of *MECP2* gene, RTT is mainly found in females, and it is the leading cause of mental retardation in females (Jedele 2007; Shahbazian and Zoghbi 2002). RTT also affects males in rare cases, and the male RTT patients manifest a range of symptoms including severe encephalopathy, mild mental retardation and dystonia apraxia (Jan et al. 1999).

Causes of Rett Syndrome

The primary cause of Rett syndrome in approximately 90 % of patients is the mutations in the *MECP2* gene (Amir et al. 1999). Studies have shown a genotype–phenotype relationship between RTT phenotypes and *MECP2* mutations (Amir and Zoghbi 2000). For example, truncation mutations within the *MECP2* gene show correlation with more severe RTT phenotypes (Weaving et al. 2003). Other than mutations in the *MECP2* gene, mutations in *CDKL5* and *FOXG1* genes are also associated with approximately 10 % of RTT cases (Evans et al. 2005a; Philippe et al. 2010).

Moreover, since *MECP2* is an X-linked gene, X-chromosome inactivation (XCI) has also been shown to impact the RTT pathology and clinical severity. The XCI causes uneven expression of wild type and mutant *MECP2* alleles within the brain. Even though, the results observed by different groups seem to be complex, the fact that X-chromosome inactivation patterns (either skewed or random, whether the mutant allele is of paternal or maternal origin) are linked to the severity of RTT phenotypes has been established by these groups (Ishii et al. 2001; Gibson et al. 2005; Xinhua et al. 2008). Since the relationship between clinical severity of RTT and XCI is inconclusive, it has been postulated that other factors such as environmental modifiers including epigenetic mechanisms and other MeCP2-related genes might be contributing to RTT clinical severity (Hoffbuhr et al. 2002).

Multiple Stages of Rett Syndrome Progression

The standard criteria of RTT diagnosis describe four major stages of RTT pathogenesis, subsequent to a normal prenatal and perinatal development up to about 5 months of age (Hagberg 2002). *Stage I* of RTT is defined by an early onset of developmental stagnation, which starts approximately from 6 months to 1.5 years of age. During this period, major RTT phenotypes such as microcephaly (reduced brain/head size), reduced growth rate, loss of language and behavioral skills and seizures start to appear

(Chahrour and Zoghbi 2007). *Stage II* is mainly defined by developmental regression with an onset of 1–4 years of age. Affected patients start losing the already acquired skills in communication and behavior and show symptoms of mental retardation (Lee et al. 2013). *Stage III* is usually referred to as pseudo-stationary period, which follows the stage II and approximately spans around 4–7 years. This period is also referred as wake up period because some patients are able to regain certain skills such as communication abilities. However, these patients would still suffer from respiratory problems, disturbed sleeping patterns, scoliosis (abnormal curvature of the spine), anxiety and hand apraxia/dyspraxia. Late motor deterioration is the *Stage IV*, during which patients lose their ability to walk and would become non-ambulatory or completely dependent on a wheelchair for mobility. In more severe cases of RTT, patients may develop Parkinsonian phenotypes at later stages of this period (Roze et al. 2007). The last stage of RTT may last from several years to decades.

Major Phenotypes of Rett Syndrome

As a neurological disorder, the majority of RTT phenotypes are associated with nervous system and brain. A major phenotype observed in brain is the altered size/volume of whole brain, usually reduced brain size, which is also referred to as microcephaly (Reiss et al. 1993; Subramaniam et al. 1997). Studies also show that the volume of specific brain regions, namely cerebellum and cortex is reduced in RTT patients (Reiss et al. 1993; Murakami et al. 1992). Rett syndrome patients generally share many phenotypes with autistic patients, such as impaired social interactions or social withdrawal. For the same reason, several RTT patients are frequently misdiagnosed as autistic patients (Young et al. 2008; Olsson and Rett 1987; Gillberg 1986). Another major feature of this devastating disorder is the repetitive stereotyped hand movements, such as clapping, wringing, squeezing, tapping and loss of purposeful hand movements (Carter et al. 2010). Impaired learning, language and communication skills are also frequently seen in RTT patients. These patients are typically unable to communicate properly and have disabilities in speaking. Some patients also experience sudden loss of speech. However, during stage III, they might regain some of the lost abilities to communicate (Woodyatt and Ozanne 1993).

Apart from major phenotypes associated with nervous system, both human RTT patients and RTT mouse models show phenotypes in other organs. One such phenotype is the breathing abnormalities (Stettner et al. 2008; Katz et al. 2009; Cirignotta et al. 1986). The underlying causes of these breathing abnormalities are linked to MeCP2 dysfunction in specific brain regions such as ventro-lateral

medulla and pons (Ramirez et al. 2013). The reduced life span and higher rates of sudden deaths of RTT patients are associated with cardiac dysrhythmias (Byard 2006). Several impairments in heart function are also reported in RTT cases (Guideri and Acampa 2005; Guideri et al. 2004; Madan et al. 2004) and RTT mouse models (McCauley et al. 2011). The neurological contribution of the cardiac problems is demonstrated by the immature cardiorespiratory neurons (Julu et al. 1997) and impairments in cardiac autonomic nervous system (Acampa and Guideri 2006). Rett syndrome patients also suffer from several bone problems including, osteopenia and difficulties in limb movements, fragile bones, loss of muscle tone (hypotonia) and scoliosis (Budden and Gunness 2003; Heilstedt et al. 2002; Holm and King 1990). Finally, children suffering from Rett syndrome mostly have retarded growth due to the difficulties in feeding and malnutrition (Morton et al. 1997; Motil et al. 2012).

Neurodevelopmental Versus Neurological Disorders

Rett syndrome was primarily discovered as a progressive neurodevelopmental disorder (Rett 1966; Hagberg et al. 1983; Neul and Zoghbi 2004). The high prevalence of RTT in small children and the associated developmental regression support the fact that RTT is a neurodevelopmental disorder. However, recent studies in adult RTT mouse models suggest that it is rather a neurological, not a neurodevelopmental, disorder (McGraw et al. 2011; Guy et al. 2007). This concept was based on the neurological phenotypes caused by the loss of *Mecp2* in adult mice.

MeCP2 Structure, Expression and Functions

MeCP2 Structure

Perhaps MeCP2 is the mostly studied protein of the Methyl-CpG Binding Protein family that binds to methylated DNA through their unique Methyl Binding Domain (MBD) (Hung and Shen 2003; Singh et al. 2008). *MECP2/Mecp2* gene is an X-linked gene that spans $\sim$76 kb in the long arm of the X-chromosome (Xq28). The *MECP2/Mecp2* gene is located in between the Interleukin-1 receptor associated kinase gene (*IRAK1*) and the Red Opsin gene (*RCP*) (Fig. 1a). In both human and mouse, the *MECP2/Mecp2* gene comprises four major exons (exon 1–4) and three introns (intron 1–3) (Fig. 1b-c). MeCP2 protein structure is composed of five major domains, N-terminal Domain (NTD), Methyl Binding Domain, Inter-Domain (ID), Transcription Repression Domain (TRD) and C-terminal Domain (CTD) and is approximately 53 kDa in size (Fig. 1d). Even though the theoretical molecular
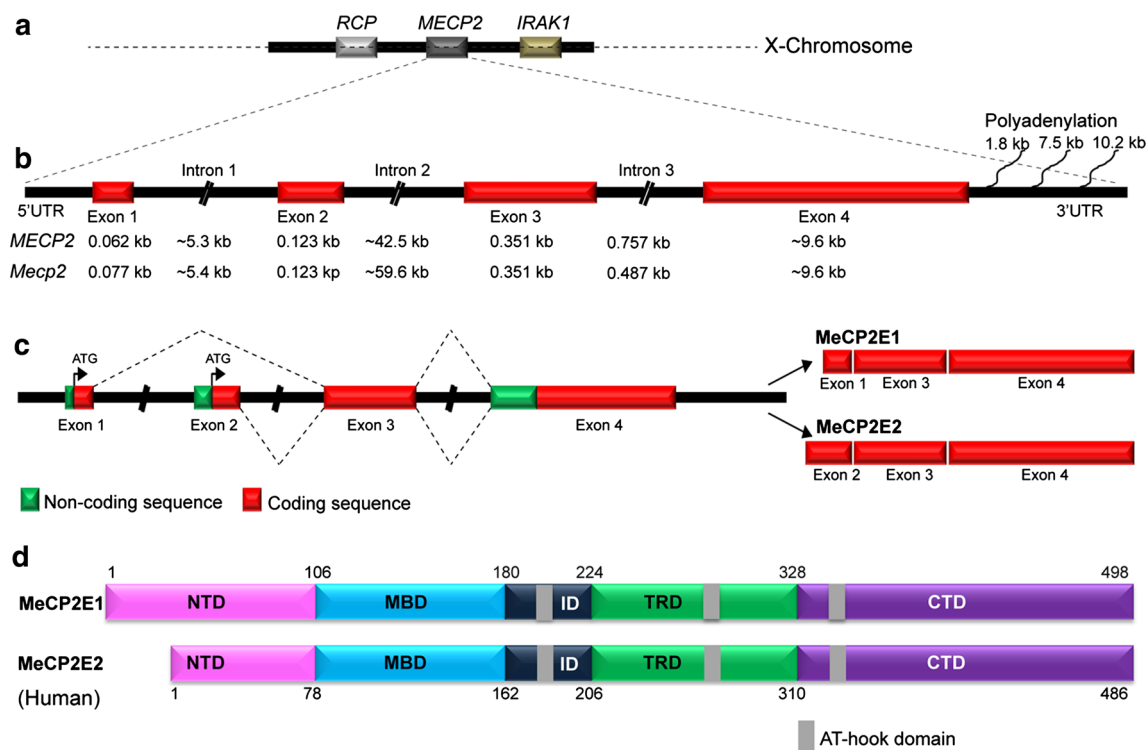
Fig. 1 The structure of the *MECP2/Mecp2* gene and MeCP2 protein. **a** The *MECP2/Mecp2* gene is located in X-chromosome (Xq28), flanked by the *RCP* and *IRAK* genes. **b** The schematic of the *MECP2/Mecp2* gene is shown that is composed of four exons (exon 1–4) and three introns (intron 1–3). The gene has three polyadenylation sites at the 3′UTR. The sizes of each exonic and intronic region in human *MECP2* and mouse *Mecp2* genes are indicated. **c** The generation of two MeCP2 isoforms: MeCP2E1 and MeCP2E2. The translation start site (ATG) for each isoform is indicated by *arrows*. MeCP2E1 isoform is encoded by exons 1, 3 and 4. MeCP2E2 isoform is encoded

by exons 2, 3 and 4. *Red* coding sequence. *Green* noncoding sequence. **d** Protein structure of human MeCP2E1 and MeCP2E2. The human MeCP2E1 and MeCP2E2 are 498 and 486 amino acids in size, respectively. In contrast, the mouse MeCP2E1 and MeCP2E2 are 501 and 484 amino acids in size, respectively. *MBD* methyl-CpG-binding domain, *ID* inter-domain, *TRD* transcriptional repression domain, *CTD* C-terminal domain, *NTD* N-terminal domain, *AT-hook* a domain found within the TRD which allows binding to adenine–thymine (AT) rich DNA

weight of MeCP2 protein is 53 kDa, it is usually detected at 75 kDa by Western blot analysis (Zachariah et al. 2012; Olson et al. 2014), probably due to its posttranslational modifications. The known tertiary structure of MeCP2 is composed of 4 % α-helices, 21 % β-sheets and 13 % β-turns. However, the majority (∼60 %) of MeCP2 protein is unstructured. Therefore, MeCP2 is considered to be an intrinsically disordered protein (Adams et al. 2007). Among the aforementioned MeCP2 protein domains, MBD is the only domain that has a definite secondary structure, while the rest of MeCP2 protein domains are highly unstructured (Ghosh et al. 2010b; Hite et al. 2012).

MeCP2 Expression

MeCP2 is widely expressed in many organs, and its highest expression is detected in brain, lung and spleen, compared with which the expression levels are lower in liver, heart, kidney and small intestines (Shahbazian et al. 2002b). However, the brain-specific expression of MeCP2 is

extensively studied, as the majority of RTT phenotypes are neurological. Additionally, abolishing *Mecp2* expression in the embryonic brain results in similar RTT phenotypes caused by *Mecp2* null mutations affecting all murine tissues (Chen et al. 2001; Guy et al. 2001). Nonetheless, non-neuronal RTT symptoms such as scoliosis, breathing/respiratory abnormalities, cardiac problems, difficulty in feeding and limb movements indicate the importance of MeCP2 expression outside the central nervous system (Guideri and Acampa 2005; Ogier and Katz 2008; Nomura and Segawa 1992; Isaacs et al. 2003; Ezeonwuka and Rastegar 2014).

Within the brain, both the distribution and levels of MeCP2 have been shown to be different. For instance, we have recently demonstrated the distribution profile of MeCP2 in the adult murine brain regions, specifically, in the olfactory bulb, cortex, striatum, hippocampus, thalamus, cerebellum and brain stem (Zachariah et al. 2012; Olson et al. 2014). Analysis of whole cell extracts isolated from these brain regions indicated the highest MeCP2

expression in the cortex and cerebellum among the studied brain regions (Zachariah et al. 2012). In contrast, analysis of nuclear extracts from the same brain regions indicated relatively even levels of MeCP2E1 and differential levels of MeCP2E2 (Olson et al. 2014). Implicating the importance of brain region-specific MeCP2 expression in Rett syndrome pathogenesis, others have shown that the expression levels of MeCP2 in different mouse brain regions correlated with impaired behavioral phenotypes in a RTT mouse model (Wither et al. 2013). Several RTT mouse models have been generated by deleting the *Mecp2* expression in specific brain regions and/or specific cell types within the brain regions that show varying degrees of RTT phenotypes. For example, loss of MeCP2 expression in the neurons in basolateral amygdala causes increased anxiety-like behavior and impaired cue-dependent fear learning (Adachi et al. 2009; Wu and Camarena 2009). Several studies have shown abnormal social behaviors, anxiety and also autistic features in RTT mouse models lacking MeCP2 expression in the neurons of forebrain (Gemelli et al. 2006; Chen et al. 2001; Chao et al. 2010). Moreover, MeCP2 deletion from hypothalamic neurons resulted in abnormal physiological stress response, hyper-aggressiveness and obesity (Fyffe et al. 2008).

Within the brain, cellular expression of MeCP2 is predominantly detected in neurons. Other than neurons, MeCP2 expression has also been demonstrated in astrocytes, oligodendrocytes and microglia (Zachariah et al. 2012; Ballas et al. 2009; Rastegar et al. 2009; Derecki et al. 2012; Liyanage et al. 2013; Olson et al. 2014). Our studies also show that MeCP2 is localized to chromocenters in neurons, astrocytes and oligodendrocytes (Zachariah et al. 2012; Rastegar et al. 2009; Liyanage et al. 2013; Olson et al. 2014).

MeCP2 in Neurons

We and others have shown that among the MeCP2-expressing cells, neurons show the highest MeCP2 expression, while lower MeCP2 levels are detected in glial cell types (Zachariah et al. 2012; Ballas et al. 2009). MeCP2 expression in neuronal nuclei is as high as the levels of histone proteins (Skene et al. 2010). For the same reasons, RTT phenotypes are thought to be majorly of neuronal origin (Chen et al. 2001; Guy et al. 2001). Proper MeCP2 expression in neurons is required for neuronal maturation (Kishi and Macklis 2004; Singleton et al. 2011; Thatcher and LaSalle 2006; Shahbazian et al. 2002b) and proper neuronal functions (Nguyen et al. 2012; Shahbazian and Zoghbi 2002). In a previous section (MeCP2 Expression), we described that loss of *Mecp2* in neurons found within specific brain regions of mouse models leads to RTT phenotypes.

MeCP2 in Astrocytes

Even though, astrocytes express lower levels of MeCP2 relative to neurons (Zachariah et al. 2012; Ballas et al. 2009), proper expression of MeCP2 in astrocytes is essential for normal neuronal function. For example, abnormal neuronal morphology in primary neurons can be caused by *Mecp2*-deficiency in the neighboring astrocytes (Ballas et al. 2009; Maezawa et al. 2009). Furthermore, loss of MeCP2 in astrocytes has been shown to cause abnormalities in neurodevelopment and thereby contributing to RTT progression (Maezawa et al. 2009; Okabe et al. 2012; Lioy et al. 2011). A recent study also reported a set of MeCP2 targets in astrocytes which might be important in RTT pathology. The confirmed targets of MeCP2 in astrocytes include *Apoc2*, *Cdon*, *Csrp* and *Nrep*, which the authors suggest to be important for proper astrocytic functions (Yasui et al. 2013).

MeCP2 in Microglia

Microglia is a type of glial cells, which is mainly involved in neuro-inflammatory processes. Similar to other glial cell types, microglia can modulate neuronal morphology through secreting soluble factors. MeCP2-deficient microglia secrete higher levels of glutamate, leading to abnormal neuronal morphology, highlighting the potential role of MeCP2 in microglia that impacts RTT progression (Maezawa and Jin 2010). Further supporting the importance of microglial expression of MeCP2, re-expression of MeCP2 in microglia of *Mecp2*-null mouse model has been shown to restore several RTT phenotypes (Derecki et al. 2012).

MeCP2 in Oligodendrocytes

Mecp2-deficiency in the cortex and hippocampus of a RTT mouse model has been shown to cause reduced CNPase expression, a specific marker for oligodendrocytes (Wu et al. 2012). The role of MeCP2 in regulating myelin-related genes including myelin basic protein, proteolipid protein and myelin-associated glycoprotein has also been shown in a RTT mouse model (Vora et al. 2010). A recent report demonstrated RTT phenotypes such as hindlimb grasping in a mouse model lacking MeCP2 expression in oligodendrocyte lineage cells (Nguyen et al. 2013). Together, these reports suggest that MeCP2 expression is required for proper oligodendrocyte functions and RTT pathology.

MeCP2 Functions

MeCP2 is a multifunctional protein that is involved in transcriptional regulation as well as modulating chromatin

structure. The different domains of MeCP2 have been assigned to facilitate multiple functions through direct DNA binding, interaction with protein partners or by recruiting other factors (Guy et al. 2011). Different MeCP2 functions are described in detail in the following sections.

MeCP2 in Transcriptional Regulation

The role of MeCP2 in transcriptional regulation has been described in several aspects of gene regulation, including transcriptional repression/silencing and activation. However, the precise role of MeCP2 as a transcriptional repressor (Nan et al. 1998) or transcriptional activator (Chahrour et al. 2008) is paradoxical. For example, brain-derived neurotrophic factor (*BDNF*) is one of the most studied MeCP2 target genes. Binding of MeCP2 to the *Bdnf* promoter has been shown to repress its expression, and the removal of MeCP2 from *Bdnf* promoter due to neuronal activity-dependent phosphorylation of MeCP2 caused the de-repression/activation of *Bdnf* expression (Chen et al. 2003; Martinowich et al. 2003; Zhou et al. 2006). In contradiction, the *Mecp2*-deficient mice demonstrated reduced levels of *Bdnf* expression, raising the question whether MeCP2 is a transcriptional activator of *Bdnf* (Abuhatzira et al. 2007).

The transcriptional regulatory role of MeCP2 seems to be dependent on its interacting protein partners. The association of MeCP2 with repressor complexes containing SIN3a and histone deacetylases (HDAC) leads to transcriptional repression. In contrast, MeCP2-mediated transcriptional activation of genes occurs in association with the activator complexes containing cAMP response element-binding protein (CREB) (Chahrour et al. 2008). MeCP2 recruitment to 5hmC-enriched active genomic loci (Mellen et al. 2012) and its association with TET1 protein (Cartron et al. 2013) further support the role of MeCP2 in gene activation. Even though numerous MeCP2 target genes have been reported in multiple cellular systems (Zachariah and Rastegar 2012), the diverse nature of MeCP2 target genes and the opposing effects on the studied genes (activate or repress) pose questions on the exact function of MeCP2 as a transcriptional regulator. Hence, recent studies define MeCP2 as a genome-wide epigenetic modulator rather than a transcriptional regulator (Della Ragione et al. 2012). However, to date, it remains elusive how a single protein can modulate a variety of opposing functions.

MeCP2 in Chromatin Structure

As a major DNA binding protein, MeCP2 is also involved in controlling chromatin structure (Liyanage et al. 2012; Zlatanova 2005; Chadwick and Wade 2007). MeCP2 can modulate chromatin architecture by condensing DNA through regulating the long-range interactions (Horike et al. 2005), formation of higher-order chromatin structures (Georgel et al. 2003; Agarwal et al. 2011), and formation of chromatin loops and DNA bridges (Georgel et al. 2003; Nikitina et al. 2007b; Yasui et al. 2007). The localization of MeCP2 to DAPI-counterstained heterochromatin regions of the nuclei (Zachariah et al. 2012; Craig et al. 2003), which are usually referred to as chromocenters, colocalization of MeCP2 with the heterochromatin-binding protein (HP1) at the chromocenters, and chromocenter-clustering upon MeCP2 overexpression (Brero et al. 2005), further add evidence for the role of MeCP2 as a chromatin architectural protein. Moreover, significant differences have been observed in the sizes and numbers of chromocenters in *Mecp2*-deficient and *Mecp2*-WT neurons, further supporting role of MeCP2 in chromatin organization (Singleton et al. 2011). More importantly, RTT-causing *MECP2* mutations have been shown to disrupt the formation of higher-order chromatin structures (Nikitina et al. 2007a; Agarwal et al. 2011; Kumar et al. 2008).

MeCP2 in RNA Splicing

As described previously, even though several MeCP2 target genes have been identified, gene expression studies in *Mecp2*-deficient mice did not reveal global changes in gene expression. The broad spectrum of RTT phenotypes could not be explained by the altered expression of few specific genes (Tudor et al. 2002). However, changes in alternative splicing of genes such as *Dlx5*, *Fgf2–5*, *Fut8* and *Nf1* could be observed in a mouse model of RTT (*Mecp2*$^{308/Y}$), implying the potential role of MeCP2 in RNA splicing (Young et al. 2005). Supporting this notion, interactions of MeCP2 with an RNA binding protein YB-1 (Young et al. 2005) and spliceosome-associated protein PRPF3 (Long et al. 2011) have been reported. Moreover, RTT-causing truncating mutations are shown to disrupt the interaction between MeCP2 and spliceosome complex containing PRPF3 (Long et al. 2011). Recently, it has also been shown that the DNA methylation-dependent binding of MeCP2 to exonic sequences modulates alternative splicing (Maunakea et al. 2013). Altered RNA splicing of synaptic genes have been reported in autism as well as Rett syndrome (Smith and Sadee 2011). However, whether MeCP2 plays a role in RNA splicing in autism is currently unknown. MeCP2 is also known to regulate the alternative splicing of NMDA receptor subunit NR1 (Young et al. 2005), splice variants of which are frequently altered in mouse models of fetal alcohol spectrum disorders (FASD) (Brady et al. 2013).

MeCP2 in microRNA Regulation

Apart from the role of MeCP2 in regulating the coding genes, the most recently described function of MeCP2 is the regulation of the expression of microRNAs (miRNAs). MicroRNA-137 (miR-137) is one such miRNA which is involved in regulating neural stem cells (NSCs) proliferation and differentiation. The expression of miR-137 is epigenetically regulated by MeCP2 (activated by *Mecp2*-deficiency) in adult NSCs that undergo neuronal differentiation. The authors also reported the activation of miR-187 and miR-193a and the repression of miR-199a, miR-197, miR-221 and miR-222 in the absence of MeCP2 (Szulwach et al. 2010). In embryonic cortical neurons, binding of MeCP2 to the promoter of miR-184 represses its expression in an activity-dependent manner (Nomura et al. 2008). Interestingly, the role of MeCP2 in regulating miRNA and its contribution to RTT pathology have been demonstrated in mouse models of RTT (which lack MeCP2 expression), by the altered expression of a spectrum of miRNAs [miR-146a, miR-146b, miR-130, miR-122a, miR-342 and miR-409 (downregulated) and miR-29b, miR329, miR-199b, miR-382, miR-296, miR-221 and miR-92 (upregulated)] (Urdinguio et al. 2010). Loss of MeCP2 in the cerebellum of a RTT mouse model also led to altered expression of miRNAs (Wu et al. 2010). The role of MeCP2 in RTT pathology through regulating the expression of miRNAs was further strengthened by the observation of altered transcriptome of long noncoding RNAs (lncRNA) and specific upregulation of AK087060 and AK081227 in the brains of *Mecp2*-null RTT mouse model (Petazzi et al. 2013).

Other Biological Functions of MeCP2

As a multifunctional epigenetic modulator, MeCP2 participates in numerous biological functions both in vitro and in vivo. Within neurons, MeCP2 plays critical roles in neuronal maturation (Kishi and Macklis 2004; Fukuda et al. 2005; Kishi and Macklis 2010), terminal neuronal differentiation (Matarazzo et al. 2004; Tsujimura et al. 2009), modulating the neuronal morphology (Ballas et al. 2009; Belichenko et al. 2009b; Wang et al. 2013a; Rastegar et al. 2009; Belichenko et al. 2009a; Larimore et al. 2009) and synaptic plasticity (Chao et al. 2007; Qiu et al. 2012; Na et al. 2012, 2013; Zhong et al. 2012). Most recent studies demonstrate the role of MeCP2 in regulating protein synthesis, and it is suggested that the reduced protein synthesis in MeCP2-deficient cells is contributing to the RTT phenotypes detected in these cells (Li et al. 2013; Ricciardi et al. 2011). Further confirming the role of MeCP2 in Rett syndrome pathogenesis, the aforesaid functions are impaired in RTT patients (Kim et al. 2011;

Chapleau et al. 2009) and also in RTT mouse models (Asaka et al. 2006; Belichenko et al. 2009).

Mouse Models to Study Rett Syndrome

In order to investigate the molecular mechanisms of RTT pathogenesis and design therapeutic strategies, rodent models, specifically mouse models, are extensively employed. Several different strategies are used to alter/abolish the *Mecp2* expression and/or function in mice that include *Mecp2* knockout, *Mecp2* mutant and *MECP2* knock-in strategies (Calfa et al. 2011; Ricceri et al. 2008, 2013). Most frequently used *Mecp2*-RTT mouse models are summarized in Table 1.

The *Mecp2*-deficient/*Mecp2*-knockout mouse models which are also referred as "null MeCP2" models have been generated through deletion of exon 3 or 4 or 3 and 4 to abolish the expression of whole MeCP2 protein. The examples of such null MeCP2 mouse models include *Mecp2*[tm1.1Bird], *Mecp2*[Jae] and *Mecp2*[neoTam] which demonstrate many RTT phenotypes (Guy et al. 2001; Chen et al. 2001; Pelka et al. 2006). Moreover, a mouse model lacking MeCP2E1 expression recapitulated RTT-like phenotypes (Yasui et al. 2014), in comparison with *Mecp2e2*-knockout model which lacked neurological phenotypes (Itoh et al. 2012). Similarly, the mouse models harboring known RTT-causing *MECP2* mutations have been helpful in determining the role of these mutations in RTT pathology. The examples include, the mutations which result in truncated MeCP2 protein (MeCP2[308] and R168X) (Shahbazian et al. 2002a; Schaevitz et al. 2013; Lawson-Yuen et al. 2007) as well as mutations which cause loss of function or loss of protein/DNA interactions (*MECP2* T308A, *MECP2* R306C and *MECP2* T158A) (Lyst et al. 2013; Goffin et al. 2011; Ebert et al. 2013). The consequences of these mutations, phenotypes and life spans of each mouse model are summarized in Table 1.

Another RTT rodent model was generated with reduced *Mecp2* expression in the rat brain, which showed no significant RTT phenotypes but exhibited transient abnormality in neurobehavior and reduced *Bdnf* expression in brain (Jin et al. 2008). Apart from these rodent models, other animal models are also being used to study the etiology of RTT and MeCP2 functions. A zebrafish model of Rett syndrome has been generated by introducing a C187T transition mutation leading to MeCP2 protein truncation at the position 63 (*Mecp2*Q63*). Unlike most RTT mouse models, this zebrafish RTT model showed milder RTT phenotypes such as motor deficits, however, was viable and fertile (Pietri et al. 2013). Moreover, a *Drosophila* model for RTT was generated using *MECP2* overexpression and known RTT mutations (R106W, R294X, and Δ166), which

Table 1 Mouse models of Rett syndrome

Mouse model	Description (type of mutation)	Altered features of MeCP2 (structure, function, interactions)	Major phenotypes	Life span		References
				M	F	
$Mecp2^{tm1.1Bird}$	Exon 3 and 4 deletion	Expression and function are abolished	Hindlimb grasping, hypoactivity, abnormal gaits and breathing, impaired coordination	7–8 wks	Normal	Guy et al. (2001)
$Mecp2^{Jae}$	Exon 3 deletion	Expression and function are abolished	Reduced growth rate, abnormal anxiety and learning, impaired coordination	10–12 wks	Normal	Chen et al. (2001)
$Mecp2^{tm1Pplt}$ or $Mecp2^{neoTam}$	Exon 3 and 4 deletion	Expression and function are abolished	Premature death, impaired coordination, growth retardation, abnormal anxiety and fear behaviors	5 wks	–	Pelka et al. (2006)
$Mecp2^{tm1Jchr}$	Deletion of MBD	Structural changes (no MBD)	Premature death, hypoactivity, tremors and abnormal stereotypic behaviors	6–8 wks	–	Weaving et al. (2004)
$Mecp2e1^{-/y}$	Exon 1-specific mutation at the translation start codon	Translation of MeCP2E1 is abolished without affecting MeCP2E2	RTT phenotypes such as hindlimb clasping, hypoactivity, anxiety and abnormal social behaviors	7–31 wks	–	Yasui et al. (2014)
$Mecp2^{tm1.1Mitoh}$ or X^{e2-}	Exon 2 deletion	Expression of $Mecp2e2$ is abolished	Embryonic lethality, abnormal placental morphology and apoptosis when allele is inherited maternally. No RTT neurological phenotypes were observed.	normal	Increased embryonic lethality	Itoh et al. (2012)
$Mecp2^{tm1Hzo}$ or MeCP2^{308}	Stop codon in exon 4	Truncated protein and abolished functions	Abnormal behaviors, increased stereotypic behaviors, impaired coordination	10 mnt–1 yr	>1 yr	Shahbazian et al. (2002a)
$Mecp2^{tm1Vnar}$ or $Mecp2^{*a140v}$	Missense mutation A140V	No effects on DNA binding, nuclear localization or transcription repression activity	Females are normal. Infertile males, abnormal cellular morphologies in brain regions. No behavioral phenotypes	>1 yr	>1 yr	Jentarra et al. (2010), Kudo et al. (2002)
$Mecp2^{R168X}$	Nonsense point mutation R168X	Truncated protein	Impaired motor functions, and cognitive functions and reduced anxiety, seizures in some cases, forelimb stereotypes, abnormal breathing and scoliosis	7 mnt–1 yr	>1 yr	Schaevitz et al. (2013), Lawson-Yuen et al. (2007)
Tg(MECP2)1Hzo or MeCP2^{Tg1}	Transgenic	Overexpression of MeCP2 (twofold)	Increased anxiety behaviors and abnormal social behaviors, premature death, seizures, abnormal nervous system phenotypes, increased aggressiveness, abnormal cognitive behaviors	>1 yr or normal	2 wks–1 yr	Collins et al. (2004)
MECP2 T308A	Knock-in mutation	T308A mutations cause loss of interaction with NCoR complex	Neurological RTT phenotypes, seizures, reduced brain weight, abnormal motor functions, hindlimb clasping, reduced activation of activity-dependent gene expression ($Bdnf$)	–	–	Ebert et al. (2013)
MECP2 R306C	Knock-in mutation	Loss of interaction with NCoR/SMRT	Hindlimb clasping, impaired mobility and motor coordination, reduced brain weight and size	<18.5 wks	–	Lyst et al. (2013)

Table 1 continued

Mouse model	Description (type of mutation)	Altered features of MeCP2 (structure, function, interactions)	Major phenotypes	Life span		References
				M	F	
Viaat-*Mecp2*	Cell-type-specific knockout	Abolished expression in GABAergic neurons	Less motility and increased anxiety	∼26 wks	–	Chao et al. (2010)
MECP2 T158A	Knock-in mutation	Loss of DNA binding and protein stability	Lower anxiety, reduced locomotor functions, developmental regression	<16 wks	6 mnt	Goffin et al. (2011)

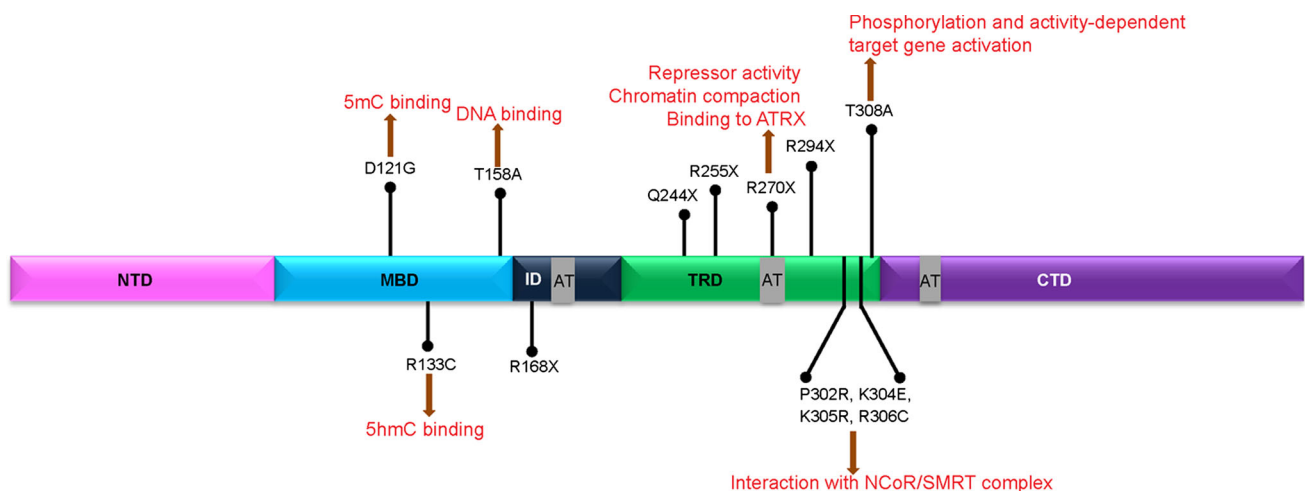*M* male, *F* female, *wks* weeks, *mnt* months, *yr* years

Fig. 2 Distribution of known MeCP2 mutations and the interactions/functions abolished due to mutations. The well-known mutations found in Rett Syndrome patients and the loss of interactions and functions of MeCP2 due these mutations are illustrated. *MBD* methyl-CpG-binding domain, *ID* inter-domain, *TRD* transcriptional repression domain, *CTD* C-terminal domain, *NTD* N-terminal domain, *AT* AT-hook domain, *5mC* 5-methylcytosine, *5hmC* 5-hydroxymethylcytosine. The amino acid numbers are according to the location on MeCP2E2 isoform

resulted in abnormalities in locomotor functions and disrupted eye structure (Cukier et al. 2008).

MECP2 Mutations and Rett Syndrome

Rett syndrome is caused by de novo mutations in the *MECP2* gene (Christodoulou et al. 2003), which can disrupt molecular functions of MeCP2. Approximately 600 mutations have been detected so far within the *MECP2* gene [(Christodoulou et al. 2003) and (RettBASE: http://mecp2.chw.edu.au)]. These mutations include missense, nonsense and silent mutations, as well as 5′ UTR and 3′UTR variations, intronic variations, insertions (frameshift, in-frame) and deletions (exonic deletions, frameshift, in-frame) (RettBASE: http://mecp2.chw.edu.au). Recent studies have deciphered the impact of *MECP2* mutations in MeCP2 function contributing to RTT pathogenesis.

Figure 2 illustrates distribution of RTT mutation hot spots and the abolished functions/interactions of MeCP2 due to these mutations.

MeCP2 Chromatin Binding

As stated earlier, MeCP2 is a chromatin architectural protein that localizes to the chromocenters, and it is involved in chromocenter-clustering (Liyanage et al. 2012). Supporting the chromatin architectural role of MeCP2, several *MECP2* mutants fail to localize into heterochromatin regions of the nucleus and to cluster heterochromatin (Agarwal et al. 2011). This is not surprising since the majority of *MECP2* mutations are found within the MBD domain, which is required for methylation-dependent chromatin binding (Kudo et al. 2003). Disruption of MBD domain not only results in diffused nuclear staining due to the inability of the MeCP2 protein

localization to heterochromatin, but also causes retention of the protein in the cytoplasm (Stuss et al. 2013). Moreover, T158 amino acid within the MBD domain has been implicated in stabilization of MeCP2 and DNA binding (Ho et al. 2008). A knock-in mouse model harboring T158A mutation demonstrated RTT phenotypes, decreased MeCP2 stability, and reduced DNA binding (Goffin et al. 2011). Recently, it was shown that MeCP2 can bind to both methyl marks: 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) with similar affinities, and it is the major protein that binds to 5hmC in brain (Mellen et al. 2012). Interestingly, *MECP2* missense mutation R133C within the MBD hindered the MeCP2 binding to 5hmC, while D121G mutation inhibited MeCP2 binding to 5mC. The MBD domain recognizes and binds to methylated DNA, whereas the TRD domain mediates binding to AT-rich DNA through an AT-hook domain. AT-hooks are a type of DNA binding motifs, which allow binding to DNA at adenine–thymine (AT)-rich regions through a characteristic RGR domain (Reeves and Nissen 1990). In the MeCP2 protein, the disruption of RGR domain (3 amino acids between 257 and 272) within the AT-hook causes failure of MeCP2 to bind to chromatin. For this reason, the mutations R270X and G273X within the TRD-AT-hook fail to bind to chromatin and was able to disrupt the function of TRD domain and its repressor activity (Baker et al. 2013). MeCP2 recruits ATRX to heterochromatin in association with the Cohesin and CTCF, which are known to regulate certain imprinting genes such as H19 (Kernohan et al. 2010; Nan et al. 2007). The MeCP2 mutation R270X in the AT-hook domain as well as mutations in MBD domain can disrupt localization of ATRX to heterochromatin (Baker et al. 2013; Nan et al. 2007), implicating the potential of this mutation in disrupting chromatin remodeling as well as transcription regulation of MeCP2 target genes. These observations further support the role of MeCP2 mutations in abolishing DNA binding properties of MeCP2, which are required for MeCP2 function as a transcriptional regulator and chromatin architectural protein.

Even though MeCP2 is mostly known as a methyl binding protein, several studies have shown its binding to unmethylated DNA as well (Baubec et al. 2013; Hansen et al. 2010). In the absence of DNA methylation, MeCP2 is localized to genomic regions with open/accessible chromatin structure, independent of MeCP2 protein interacting partners (Baubec et al. 2013). Interestingly, the MBD domain, which is known to mediate the methylated DNA binding with high affinity also has lower affinity to unmethylated DNA (Ghosh et al. 2010a, b). Other than MBD, other domains of MeCP2 including ID, TRD and CTD have been shown to mediate unmethylated DNA

binding (Ghosh et al. 2010b). Moreover, MeCP2 has been shown to cause compaction of chromatin independent of DNA methylation or through binding to unmethylated DNA (Georgel et al. 2003).

MeCP2 Binding to Repressor Complexes

Formation of multi-protein complexes containing repressor proteins such as HDAC, NCoR and SIN3 is required for transcriptional repression of MeCP2 target genes. A recent study demonstrated that neuronal activity-dependent phosphorylation of threonine residue at 308 position (T308) prevents the binding of MeCP2 to NCoR and thus prevents transcriptional repression. Interestingly, R306C, a missense *MECP2* mutation, prevented the phosphorylation at T308, whereas T308A mutation prevented T308 phosphorylation and led to altered expression of MeCP2 target genes such as *Npas4* and *Bdnf* (Ebert et al. 2013). Further confirming the contribution of RTT mutations in disrupting MeCP2 functions and thereby contributing to the RTT pathology, another group discovered a novel cluster of mutations (P302R, K304E, K305R, and R306C) within the TRD domain. These mutations disrupted the interaction of MeCP2 with the repressor complex proteins NCoR and SMRT (Lyst et al. 2013). Together, these studies suggest that the common *MECP2* mutations abolish the bridge between MeCP2, DNA and MeCP2-interacting proteins.

MECP2 Mutations in Non-Rett Syndrome Cases

Although *MECP2* mutations are considered to be the most prevalent cause of Rett syndrome and there is a strong correlation between *MECP2* mutations and RTT phenotypes, several cases of RTT patients are found without *MECP2* mutations (Temudo et al. 2011; Hoffbuhr et al. 2001; Huppke et al. 2005). Similarly, known RTT-causing *MECP2* mutations are found in patients who do not show classical RTT phenotypes (Suter et al. 2013). Other than RTT, *MECP2* mutations have been found in association with neurological/neuropsychiatric disorders such as, schizophrenia (Cohen et al. 2002), FASD (Zoll et al. 2004), PPM-X syndrome (Klauck et al. 2002), autism (Beyer et al. 2002), Prader-Willi syndrome (Tejada et al. 2006) and Angelman syndrome (Watson et al. 2001). These reports emphasize that the diagnosis of RTT as well as other MeCP2-related neurological disorders should not be limited to the detection of *MECP2* mutations, but rather should be carried out in conjunction with clinical features. They also highlight the importance of understanding the function of *MECP2* mutations in RTT as well as other MeCP2-related disorders.

Table 2 MeCP2 phosphorylation	Site of MeCP2 phosphorylation	Affected interactions	Function	RTT mutations	References
	S13	–	Currently unknown	–	Gonzales et al. (2012)
	S78	–	Currently unknown	c.233delC (frameshift)	Dephoure et al. (2008)
	S80	Sin3A, YB-1	Binding to target promoters	–	Tao et al. (2009), Gonzales et al. (2012)
	S86	–	Activity-dependent target gene expression	S86C	Ebert et al. (2013)
	S116	–	Currently unknown	c.345delC (frameshift)	Dephoure et al. (2008)
	S149	–	Currently unknown	–	Gonzales et al. (2012), Dephoure et al. (2008)
	S229	SMC3, Sin3A, HP1	Binding to target promoters	S229L	Gonzales et al. (2012))
	S274	–	Activity-dependent target gene expression	–	Gonzales et al. (2012), Ebert et al. (2013)
	S401	–	Currently unknown	S401N	Gonzales et al. (2012)
	S421	–	Activity-dependent chromatin remodeling, Activity-dependent binding to methylated DNA, Modulating synaptic scaling, Synaptogenesis, Modulate the sensitivity to stressful stimuli	–	Buchthal et al. (2012), Hutchinson et al. (2012), Chen et al. (2003), Zhou et al. (2006), Mao et al. (2011), Li et al. (2011), Cohen et al. (2011)
The amino acid numbers are based on the MeCP2E2 isoform, unless specifically mentioned	S424	–	Modulating synaptic scaling	–	Zhong et al. (2012)
	T308	NCoR	Activity-dependent target gene expression	R306C	Ebert et al. (2013)
RTT-associated *MECP2* mutations were obtained from RettBASE	MECP2E1: S10	–	Currently unknown	c.23_27dupCGCCG c.30delCinsGA	Trinidad et al. (2008)

MeCP2 Posttranslational Modifications

Posttranslational modifications such as phosphorylation, acetylation, methylation and ubiquitination are required for proper function and stability of proteins (Liyanage et al. 2012). Similarly, several PTMs have been detected in MeCP2 protein and shown to be important for its function and stability. These PTMs include phosphorylation, ubiquitination, SUMOylation and acetylation, which are described in detail below.

Phosphorylation

Among the MeCP2 posttranslational modifications, phosphorylation is the mostly studied PTM and has shown implications in neuronal activity-dependent transcriptional regulation and modulating MeCP2 protein–protein interactions. Several serine residues of MeCP2 are phosphorylated, including S13, S80, S149, S229, S274, S401 and S421 (amino acid numbers are based on the MeCP2E2 isoform, unless specifically mentioned). Moreover, phosphorylation at threonine residues is also implicated in MeCP2 activity-dependent functions (Ebert et al. 2013). Examples of such phosphorylation sites, the interactions assigned for some of these phosphorylated serine residues and known RTT mutations corresponding to these sites are summarized in Table 2. A complete list of phosphorylation sites reported so far can be found in the phosphosite Plus/http://www.phosphosite.org. These phosphorylated sites on MeCP2 protein mediate formation of repressor complexes containing Sin3A, HP1, SMC3 and NCoR and allow the subsequent binding to target promoters. Known RTT mutations including S86C, S229L, R306C and S401 N abolish MeCP2 interaction with the repressor proteins. Therefore, it is likely

that disruption of these phosphorylation sites would contribute to RTT disease development.

Acetylation

Acetylation of MeCP2 can modulate its binding to the target gene promoters. The human MeCP2 is acetylated at lysine 461 by p300 (Choudhary et al. 2009). It is also reported that MeCP2E1 isoform is acetylated at lysine 464 in HEK-293 cells and primary mouse cortical neurons by p300 (Zocchi and Sassone-Corsi 2012). The same group showed the interaction of SIRT1 with MeCP2E1 isoform and thereby mediating deacetylation of K464 residue. Interestingly, reduced activity of SIRT1 preventing the deacetylation of MeCP2 induced the binding of MeCP2 to *BDNF* promoter, implying that AcK464 is required for MeCP2 binding to *BDNF* promoter. A recent study discovered two more sites that are acetylated within the TRD (AcK305/307) and CTD (AcK321) domains in human MeCP2E2 in SH-SY5Y cells (Gonzales et al. 2012). Implicating the potential importance of MeCP2 acetylation in RTT pathogenesis, a previously reported RTT mutation (K305E/R) corresponded to AcK305/307 site.

SUMOylation

SUMOylation is a modification which usually regulates nuclear transportation of proteins. However, SUMOylation of MeCP2 protein was reported in both cytoplasm and the nucleus of Neuro2a cells (Miyake and Nagai 2007), questioning whether this modification actually plays a role in nuclear transportation of MeCP2. The authors speculated that the SUMOylation might be involved in determining MeCP2 protein stability and its interaction with other proteins such as HDAC and DNMT. Confirming the role of SUMOylation in MeCP2 function, SUMOylation at the K223 was shown to be required for the recruitment of HDAC1/2 complex and transcriptional repression in mouse primary cortical neurons (Cheng et al. 2013).

Ubiquitination

Protein ubiquitination functions in controlling intracellular signaling events and proteolytic degradation of proteins. So far, approximately 10 ubiquitination sites at the lysine residues of MeCP2 are reported at positions 12, 82, 119, 130, 135, 233, 256, 271, 249, and 321. The functional roles of MeCP2 ubiquitination at different residues are currently unknown, but they might be involved in determining MeCP2 protein stability and degradation. Also, it is also possible that MeCP2 ubiquitination might be contributing to RTT pathogenesis, because known RTT-causing *MECP2* mutations K12N, K82R and K135E correspond to

three of the ubiquitination sites (Gonzales et al. 2012). Moreover, other ubiquitination sites were predicted within two putative PEST domains (enriched for proline, glutamate, serine and threonine). The two PEST domains found in the N- and C-terminal domains of MeCP2 harbored several putative and previously reported phosphorylation, and SUMOylation sites. The authors suggest that the two PEST domains might be important in proteolytic degradation of MeCP2 and thereby modulating the turnover rate of MeCP2 (Thambirajah et al. 2009).

MeCP2 Isoforms

As described in a previous section, MeCP2 is a single protein functioning in opposing ways. For instance, MeCP2 functions both as a transcriptional activator and repressor; it binds to methylated and non-methylated DNA; it binds to both 5mC and 5hmC; and either overexpression or its deficiency causes similar neurological problems (Zachariah and Rastegar 2012; Ezeonwuka and Rastegar 2014). One possibility of multifunctional properties of a single protein is to have isoforms with different properties. Alternative splicing of both human and mouse *MECP2/Mecp2* gene generates two isoforms MeCP2E1 (previously described as MeCP2B/α) and MeCP2E2 (previously described as MeCP2A/β) (Fig. 1c–d) (Mnatzakanian et al. 2004; Kriaucionis and Bird 2004). MeCP2E1 isoform uses the translation start site (ATG) in the exon 1 and is comprised of exons 1, 3 and 4. In comparison, MeCP2E2 isoform uses a translation start site in the exon 2 and it is comprised of exons 2, 3 and 4. The two isoforms share the MBD, TRD and CTD domains, while they differ only at their N-termini (Fig. 1c–d).

Differential Distribution of MeCP2 Isoforms

MeCP2E1 isoform is the major isoform found in brain (Kriaucionis and Bird 2004; Zachariah et al. 2012). A previous study has demonstrated distribution of the two isoforms at the transcript levels in three stages of mouse brain development, namely new born (P1), juvenile (P21) and adult (P60) (Dragich et al. 2007). *Mecp2e2* distribution was relatively similar to that of total *Mecp2* at P1, whereas between P21 and P60, *Mecp2e2* localization was restricted to dorsal thalamus and cortical layer V. The difference between the total *Mecp2* detection throughout the adult mouse brain and the restricted detection of *Mecp2e2* in thalamus and cortex layers suggested that *Mecp2e1* might be the predominant *Mecp2* isoform in the adult mouse brain at the transcript levels. A relatively higher expression of *MECP2E2* isoform at the transcript levels is reported in the skeletal muscles, placenta, liver, and prostate gland.

The authors also showed that in the whole human brain, *MECP2E2* transcript levels are approximately 12-fold lower than the levels of *MECP2E*1 transcripts. In the human cerebellum, *MECP2E2* transcript levels are approximately ninefold lower than that of *MECP2E1* (Mnatzakanian et al. 2004).

Until 2012, determination of the distribution and functions of the two MeCP2 isoforms at the protein levels in brain or neural cell types had been hindered by the lack of MeCP2 isoforms-specific antibodies. In 2012, for the first time, we reported the generation of a MeCP2E1-specific antibody and showed that neuronal expression of MeCP2E1 protein is approximately 5 times higher than that of astrocytes. Moreover, distribution of MeCP2E1 isoform in the adult murine brain regions was relatively similar to that of the total MeCP2, which suggested that MeCP2E1 is the major isoform in the brain (Zachariah et al. 2012). Moreover, using a newly generated MeCP2E2-specific antibody, our very recent studies show that similar to total MeCP2 and MeCP2E1, MeCP2E2 protein is localized to the heterochromatin regions of the nucleus (Olson et al. 2014). Within the adult murine brain hippocampus, neurons, astrocytes and oligodendrocytes show the expression of both MeCP2E1 and MeCP2E2 expression within the nucleus. Further analysis of the nuclear expression levels of MeCP2 isoforms in adult brain regions (olfactory bulb, striatum, cortex, hippocampus, thalamus, brain stem and cerebellum), showed relatively uniform expression levels of MeCP2E1 as compared to the differential expression levels of MeCP2E2. During mouse brain development both isoforms showed similar expression profiles, but MeCP2E2 showed a later onset of expression as compared to the MeCP2E1 (Olson et al. 2014).

Presence of Mutations and Relevance to RTT

Approximately 80–85 % of the *MECP2* mutations are localized within the MBD, TRD and CTD domains which are shared by both isoforms. Previously, exon 1 was thought to be a noncoding exon and thus was excluded from the sequencing and mutation analysis studies on Rett syndrome. Since the discovery of MeCP2E1 isoform, scientists have started to re-evaluate the mutation analysis of exon 1. To date, several RTT-causing mutations are reported within exon 1 encoding for MeCP2E1 (Gianakopoulos et al. 2012; Saunders et al. 2009; Chunshu et al. 2006; Quenard et al. 2006; Bartholdi et al. 2006; Saxena et al. 2006; Ravn et al. 2005). Studies conducted in 2005–2006 suggested that mutations in exon 1 are rare in RTT, because they could only detect exon 1 mutations in 0.03–1 % of the tested samples from RTT patients (Amir et al. 2005; Quenard et al. 2006; Evans et al. 2005b). Regardless, another study showed that the RTT patients with mutations in exon 1 had

more severe phenotypes than the patients without exon 1 mutations (Bartholdi et al. 2006). Another study in 2009 showed that mutations found within exon 1 affect the translation of *Mecp2e1*, but not the transcription or translation of *Mecp2e2* (Fichou et al. 2009). These observations also suggest that MeCP2E2 expression alone is unable to compensate for the loss of MeCP2E1 in RTT patients. So far, no mutations are reported in exon 2 encoding for MeCP2E2 isoform. The absence of exon 2 mutations has led to the belief that MeCP2E2 is not important for RTT pathology. Moreover, the *Mecp2e2* isoform was suggested to be dispensable for RTT pathology as loss of *Mecp2e2* in mice did not result in neurological phenotypes that mimic RTT phenotypes (Itoh et al. 2012). However, maternally transferred *Mecp2e2* null allele reduced the embryonic viability implicating that loss of *Mecp2e2* expression and/or function during embryonic development might not lead to a live birth. This might also explain the absence of exon 2-specific mutations in RTT. However, several studies on RTT patients picked up the same 11 bp deletion in the *MECP2* exon 1 (c.47_57del11nt) (Saxena et al. 2006; Ravn et al. 2005; Amir et al. 2005), which was shown to affect the translation of not only *MECP2E1* but also *MECP2E2*, because the deletion disrupts the 5′-UTR of the *MECP2E2* which has been suggested to be essential for its translation (Saxena et al. 2006). A recent study demonstrated that in a girl with typical RTT, a novel mutation activates a splice-donor site upstream of exon 1, leading to premature splicing of *MECP2*. Even though the splice-acceptor site in intron 2-exon 3 was unaffected, the mutation caused a 16 amino acid deletion and a truncated MeCP2E1 protein. This deletion was predicted to cause a shift in the open reading frame for MeCP2E2, which might affect the MeCP2E2 translation efficiency. In the same patient, decreased *MECP2E1* transcript levels and increased *MECP2E2* transcript levels were observed (Sheikh et al. 2013). Therefore, the possibility of the contribution of *MECP2E2* in RTT etiology should not be excluded. Providing more evidence to the altered *MECP2* splice variants in RTT, in a cohort of Israeli patients, mutations in a splice-donor site found within the intron 1 were correlated with significant reduction in both *MECP2E1* and *MECP2E2* in patient's blood samples. Interestingly, not only the splice-donor site-specific mutations, but also different mutations found throughout the *MECP2* gene differentially affected the expression of *MECP2E1* and *MECP2E2*. Moreover, in patients with atypical RTT phenotypes and no *MECP2* mutations, expression of both *MECP2* isoforms was affected (Petel-Galil et al. 2006). The concept on the importance of MeCP2E2 isoform in RTT is strengthened by the ability of *Mecpe2e2* expression alone to rescue RTT phenotypes in mice (Luikenhuis et al. 2004; Jugloff et al. 2008), which also indicates that *Mecp2e2* is required and able to maintain

normal brain functions. However, the efficiency of the rescue of RTT phenotypes differs between the two *Mecp2* isoforms, where *Mecp2e1* is more efficient in rescuing RTT phenotypes than *Mecp2e2* (Kerr et al. 2012). Together, these reports highlight that even though, the exon 1 mutations are found in rare cases of RTT and no mutations are found in exon 2, both *MECP2* isoforms seem to be affected in Rett syndrome patients. Hence, the expression, regulation and the contribution of both *MECP2* isoforms in RTT pathology warrants further investigations.

MeCP2 Isoform-Specific Functions and Interacting Protein Partners

The two MeCP2 isoforms share the majority of the functional domains of MeCP2, and hence, it was assumed that the two isoforms are functionally redundant. However, few recent studies point out that these isoforms might also have non-redundant functions. MeCP2E1 is considered as the major isoform in the brain and is critical for basic brain function. Our previous studies show that MeCP2E1 shows a distribution similar to that of the total MeCP2 in adult murine brain, neurons and astrocytes (Zachariah et al. 2012), and therefore, it is likely that its main functions might be similar to that of total MeCP2. Furthermore, we demonstrated the role of MeCP2E1 isoform in dendrite branching of neurons and the ability of MeCP2E1 in rescuing the aberrant neuronal morphology in RTT neurons (Rastegar et al. 2009). Similarly, the role of MeCP2E2 in early events of neurite formation was demonstrated in PC12 cells (Cusack et al. 2004). As the major protein isoform in brain, MeCP2E1 is suggested to be the relevant isoform for RTT. Even though a direct relationship of MeCP2E2 with RTT has not been shown so far, its function in placental development has been established (Itoh et al. 2012). The authors also showed the misregulation of *Peg-1* in extra-embryonic tissue carrying *Mecpe2e2* null allele, suggesting that *Peg-1* might be a *Mecp2e2*-target gene. Moreover, indicating the proper expression of *Mecp2e2* in neuronal cells, the overexpression of *Mecp2e2,* but not *Mecp2e1* has been shown to cause cerebellar neuronal death (Dastidar et al. 2012). Further supporting the concept of MeCP2 isoform-specific functions, MeCP2E2 has been shown to interact with FOXG1, mutations of which are associated with RTT (Philippe et al. 2010). In comparison, MeCP2E1 isoform interacts with YB-1, Matrin 3 and SFPQ proteins in human neuronal cell-line SH-SY5Y (Yasui et al. 2014). Moreover, a MeCP2E1-specific phosphorylation site (S10) has been reported (Trinidad et al. 2008) and RettBASE mutation database shows that *MECP2* frameshift/deletion mutations (Table 2) can cause alterations in this phosphorylation site (p.S10RfsX45 and p.S10fs). Although a functional role of phosphorylation at the S10 is currently unknown, future studies might lead to discovery of MeCP2E1-specific functions.

Rescue of RTT Phenotypes

Numerous therapeutic strategies are currently under development to treat Rett syndrome. However, alternate therapeutic approaches do not rescue the full spectrum of RTT phenotypes. Independent research groups have shown that introduction of MeCP2 into RTT mouse models can rescue the phenotypes. Interestingly, both MeCP2 isoforms can rescue the RTT phenotypes to different extents, where MeCP2E1 application is more successful as expression of *Mecp2e1* alone can rescue RTT phenotypes (Kerr et al. 2012). However, other studies show that expression of *Mecp2e2* alone can prevent the disease progression in *Mecp2* null mouse models (Luikenhuis et al. 2004; Giacometti et al. 2007; Jugloff et al. 2008). Our previous studies also show that gene therapy delivery of *MECP2E1* to neurons lacking exogenous MeCP2 expression can rescue the aberrant neuronal morphology (Rastegar et al. 2009).

Sensitivity to Environmental Insults/Drugs

MeCP2 is known to modulate sensitivity to different external environmental insults such as exposure to ethanol (Repunte-Canonigo et al. 2013), drugs such as Cocaine (Im et al. 2010) and Methamphetamine (Lewis et al. 2013). On the other hand, MeCP2 expression is also sensitive to these aforementioned stimuli. Implicating the potential role of MeCP2 isoforms in modulating the effects of such environmental insults, a recent study showed differential sensitivity of *Mecp2* isoforms to Bisphenol A, which altered *Mecp2e1* expression at low concentrations (20 μM) and *Mecp2e2* expression at high concentrations (200 μM) (Warita et al. 2013). A recent study from our lab demonstrated that the DNA demethylating agent Decitabine can upregulate *Mecp2e1*/MeCP2E1 expression, but not *Mecp2e2* expression in differentiating neural stem cells (Liyanage et al. 2013), an in vitro model we have established to mimic neural development (Liyanage et al. 2013; Barber et al. 2013; Rastegar et al. 2009). This observation provides insights on inducing a specific *Mecp2*/MeCP2 isoform by treatment with Decitabine (Liyanage et al. 2013). In contrast, withdrawal of Decitabine resulted in downregulation of both *Mecp2* isoforms, where the effect on *Mecp2e1* was greater than that of *Mecp2e2*. Another study showed that the expression of both *Mecp2* isoforms was affected by spinal nerve injury in a study of persistent pain states. The two isoforms responded to the injection of Complete Freund's Adjuvant (CFA) in different ways (Tochiki et al. 2012). Taken together, these studies indicate
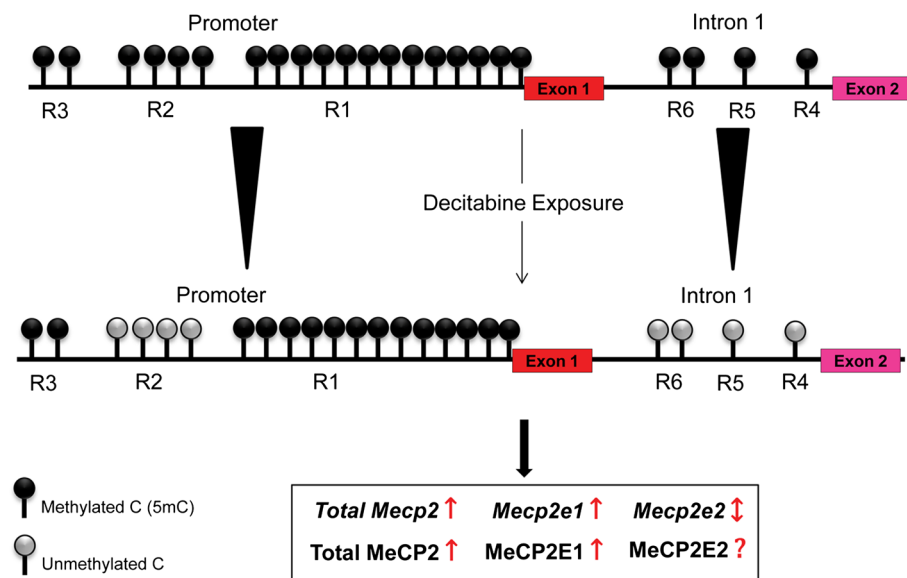
Fig. 3 Summary of DNA methylation-mediated regulation of *Mecp2* isoforms in response to Decitabine exposure during neural stem cell differentiation. DNA methylation at three regulatory elements found within the *Mecp2* promoter (R1-R3) and *Mecp2* intron 1 (R4-R6) was analyzed by bisulfite pyrosequencing, in untreated control and Decitabine-treated neural stem cells. DNA Methylation at specific *Mecp2* regulatory elements (R2 in the *Mecp2* promoter and R4-R6 in the intron 1) were reduced (DNA demethylation), in response to Decitabine exposure. These changes in DNA methylation are correlated with upregulation (↑) of *Mecp2*/MeCP2 (total), *Mecp2e1*/MeCP2E1 and unchanged levels (↕) of *Mecp2e2*. The effects of Decitabine on MeCP2E2 protein levels are unknown (?). The information was extracted from (Liyanage et al. 2013). *R* region, *C* cytosine, *5mC* 5-methylcytosine

that the two MeCP2 isoforms respond to drugs at different doses, and thus, future drug therapies will have to be designed with caution not to exert detrimental effects on the two isoforms.

Regulation of *Mecp2*/*MECP2* Isoforms

The studies on the regulation of *Mecp2*/*MECP2* were limited to *Mecp2*/*MECP2* in total (without differentiating between the two isoforms). Recently, we reported for the first time the potential role of DNA methylation at the regulatory elements within the *Mecp2* promoter and intron 1 in regulating *Mecp2* isoforms during in vitro neural differentiation (Liyanage et al. 2013). In response to Decitabine-mediated DNA demethylation at the *Mecp2* promoter and intron 1 elements, *Mecp2e1*/MeCP2E1, but not *Mecp2e2* expression was elevated significantly (Fig. 3). The nature of the correlation (positive or negative) between *Mecp2* isoform-specific expression and DNA methylation at regulatory elements was dependent on specific stages of NSC differentiation, suggesting that DNA methylation might have differential impact on the *Mecp2* isoform-specific expression depending on the type of cells and stages during neural development (Liyanage et al. 2013). Furthermore, the differential expression of transcript *Mecp2* isoforms in the adult mouse brain regions (olfactory bulb, striatum, cortex, hippocampus, thalamus, brain stem and cerebellum) was associated with significantly different changes in DNA methylation patterns at previously reported the regulatory elements found within the *Mecp2* promoter and intron 1 (Olson et al. 2014). Therefore, our studies show the potential impact of DNA methylation at *Mecp2* regulatory elements in isoform-specific *Mecp2* expression in both in vitro and in vivo.

Regulatory Mechanisms of *MECP2* in Brain

Previous research studies on MeCP2 have shown that both deficiency (Nagarajan et al. 2006; Squillaro et al. 2012; Smrt et al. 2007) and overexpression (Bodda et al. 2013; Na et al. 2012; Collins et al. 2004) of MeCP2 lead to severe neurological complications. However, expression and function of MeCP2 in neurodevelopmental disorders are the focus of most MeCP2-related research work. Even though therapeutic strategies to restore the expression of MeCP2 are advancing, any therapeutic application should be administered with caution. In order to maintain proper expression levels of MeCP2, a thorough knowledge on *MECP2*/*Mecp2*/MeCP2 regulation is required. However, studying *MECP2*/*Mecp2*/MeCP2 regulatory mechanisms is perhaps the least studied topic by the MeCP2-RTT research community, yet is an essential aspect to be understood. The *MECP2*/*Mecp2* gene is regulated at
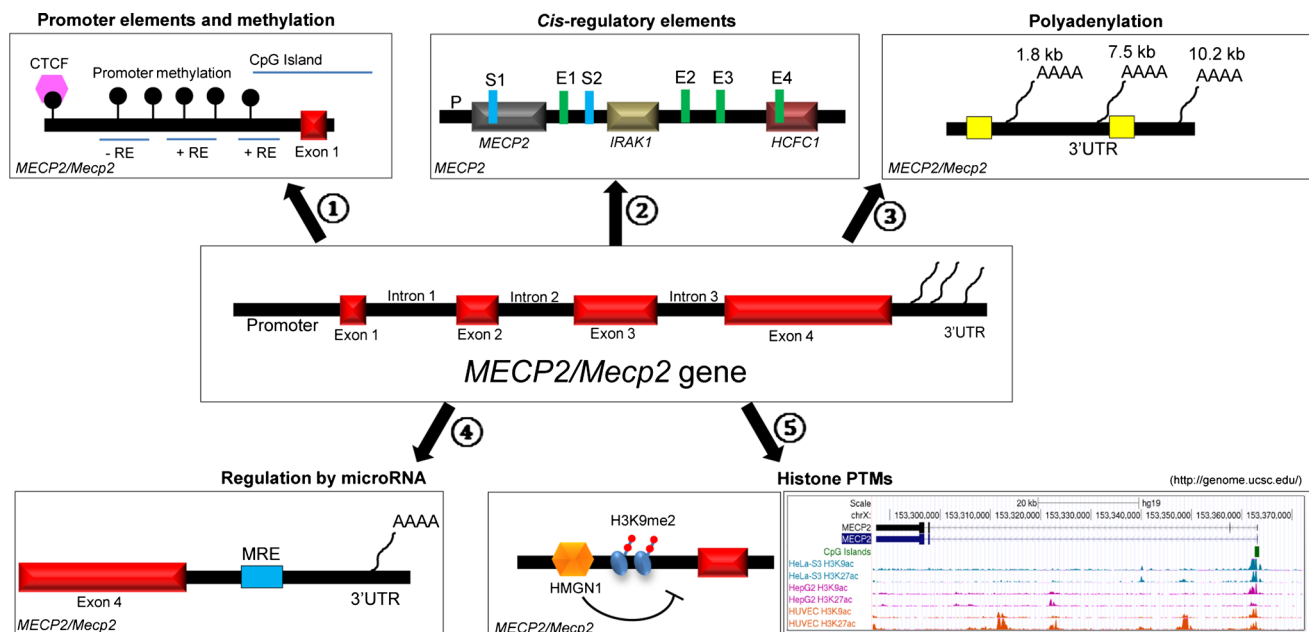
Fig. 4 Known regulatory mechanisms of *MECP2/Mecp2* gene. The *MECP2/Mecp2* gene is regulated transcriptionally and posttranscriptionally by multiple mechanisms. *(1)* The *MECP2* promoter contains positive and negative regulatory elements (+RE and -RE) which are known to regulate *MECP2* expression. Both *MECP2/Mecp2* genes harbor CpG islands and several CpG dinucleotides regulated by DNA methylation. *(2)* Cis-regulatory elements are found in intragenic regions of *MECP2* gene as well as intragenic regions. There are two silencer elements (S1–S2) and four enhancer elements (E1–E4). *P* promoter. *(3)* *MECP2/Mecp2* 3′UTR contains polyadenylation sites and *Cis*-acting elements (*yellow boxes*) which harbor binding sites for trans-acting factors involved in polyadenylation. There transcript

variants are generated by polyadenylation (1.8, 7.5 and 10.2 kb). *(4)* Binding of miRNAs to miRNA response elements (MRE) at the 3′UTR involves in posttranscriptional regulation of *MECP2/Mecp2*. *(5)* Changes in histone modifications in response to binding of regulatory proteins regulate *Mecp2/MECP2* expression. For example, binging of HMGN1 induces modifications in histones and thereby changes the chromatin structure. There is a differential enrichment of histone posttranslational modifications (PTMs) across the *MECP2/Mecp2* gene. As an example, the distribution of active histone marks H3K9Ac and H3K27Ac across the *MECP2* gene in three cell types (HeLa, HepG2 and HUVEC) is shown (data extracted from http://genome.ucsc.edu/; February 2009 assembly)

transcript level as well as posttranscriptionally by different mechanisms (Fig. 4).

Promoter Elements

The human *MECP2* core promoter lies between −179 and −309 (relative to transcription start site) and harbors several transcription factor binding sites such as SP1 and STAT (Liu and Francke 2006). The authors also reported the presence of two negative regulatory elements [(−309 to −370) and (−553 to −681)] and one positive regulatory element (−847 to −1,071) in the promoter region upstream of the *MECP2* exon 1 (−1,071 to +9), activity of which are cell-type specific (Liu and Francke 2006). Later on, another study on the encyclopedia of DNA elements (ENCODE) [http://genome.ucsc.edu/] for *MECP2/Mecp2* gene showed that the same promoter region and upstream of that region harbors binding sites for transcription factors such as SP1, SP3, TAP1, RNA Pol II, C/EBP, E2F1 and CTCF, binding of which were confirmed by chromatin immunoprecipitation experiments in different cell lines (Singh et al. 2008). Adachi et al., in 2005 reported that only a segment of

Mecp2 promoter (−677/−56) is essential to drive the *Mecp2* expression in neuronal cell lines and cortical neurons (Adachi et al. 2005). However, the same region does not drive the expression of *Mecp2* in non-neuronal cells such as astrocytes. The extracts obtained from mouse brain cortex, striatum, cerebellum and brain stem showed binding of transcription factors such as SP1 within this promoter region. The *Mecp2* promoter is a TATA-less promoter, and it does not have any canonical TATA-box and thus resembles the nature of a house keeping gene (Adachi et al. 2005). Reporting the first preclinical *MECP2* isoform-specific gene therapy vectors, we previously showed that this internal mouse *Mecp2* promoter (MeP) is able to drive the *MECP2* expression only in neurons and glia, but not in neural stem cells (Rastegar et al. 2009).

Cis-Regulatory Elements

Human *MECP2* gene is regulated at transcript level by six *cis*-regulatory elements: four enhancers and two silencers (Liu and Francke 2006). The two silencers are found within intron 1 (Silencer 1: S1) and *MECP2* 3′UTR (S2). The S2

harbors binding sites for regulatory proteins such as REST, Brn2 and BCL6. The S1 silencer element within intron 1 was proposed to be important for tissue-specific *MECP2* expression or its alternative splicing. However, binding proteins for S1 were not identified in this study. Providing evidence for the potential role of this silencer element in alternative splicing, we recently showed that the expression of *Mecp2* isoforms during NSC differentiation is dynamically correlated with DNA methylation at this silencer element. Furthermore, DNA demethylation of this silencer element was associated with upregulation of *Mecp2e1*, but not *Mecp2e2* in differentiating NSCs (Liyanage et al. 2013). Moreover, our recently published studies show preferential correlation of *Mecp2* isoforms with the DNA methylation at the regulatory elements found within this silencer element. For example, *Mecp2e2* expression negatively correlates with the DNA methylation within this silencer in striatum, brain stem and hippocampus, in contrast to the positive correlation between *Mecp2e1* and DNA methylation at the silencer element only in the olfactory bulb (Olson et al. 2014).

The four enhancer elements (E1–E4) are located within the *MECP2* 3′UTR (E1), downstream intergenic region between *IRAK1* and *CXorf12* genes (E2 and E3) and within *HCFC1* intron 1 (E4). These enhancers harbor binding sites for Brn3-4, STAT, SP1, OLF1 and MYT1 (Liu and Francke 2006). The presence of *cis*-regulatory G-quadruplexes at the *MECP2* 5′UTR was described by a recent bioinformatics-based study. These conserved G-quadruplexes have been proposed to be important in regulating many features of *MECP2* including translation, polyadenylation and modulating *cis/trans* interactions (Bagga and D'Antonio 2013).

Polyadenylation

There are several 3′polyadenylation sites in the *MECP2/Mecp2* gene, which generates transcripts with different lengths (1.9, 7.5 and 10 kb) and regulate its expression in different tissues (Coy et al. 1999; Reichwald et al. 2000). The distribution of these transcript variants is tissue-specific. For instance, the short product ($\sim$8.5 kb) is the most abundant transcript, which is found in tissues such as lymphoid system and muscles, while the longer product ($\sim$10 kb) is predominantly found in the brain (Coy et al. 1999; Pelka et al. 2005). Studies in control and autistic patients suggest that there are numerous variations in 3′UTR between them, and these transcripts have different translational efficiencies. Some of these transcript variants are more unstable and undergo transcriptional degradation (Coutinho et al. 2007). Newnham et al., reported the presence of *cis*-acting elements which can regulate the polyadenylation of human *MECP2* gene and mutations of which

can reduce the polyadenylation efficiency. The putative *Cis*-acting regulatory elements include, CstF binding site, G-rich element and upstream sequence elements. Moreover, binding of the *trans*-acting factor hnRNP F to these *cis*-regulatory elements (G-rich element) can further regulate the polyadenylation (Newnham et al. 2010).

Epigenetic mechanisms such as promoter DNA methylation, activity of miRNAs and histone PTMs are also involved in regulating *Mecp2/MECP2* expression.

Promoter DNA Methylation

As stated earlier, *MECP2/Mecp2* gene undergoes changes according to X-chromosome inactivation (D'Esposito et al. 1996). Analysis of *MECP2* promoter regions spanning between -531 and -243 bp showed increased overall promoter methylation (2–2.5 %) in the cerebral cortex of male autistic patients, relative to control brains. This significantly increased promoter methylation negatively correlated with reduced *MECP2* expression, implying the potential involvement of promoter methylation in *MECP2* downregulation. Within the 15 CpG sites analyzed in this study, two CpG sites (-496 and -445) showed significantly higher methylation over other CpG sites. Moreover, the same promoter region shows binding sites for RNA polymerase II, as well as transcription factors SP1 and SP3 (Nagarajan et al. 2006). The same research group later demonstrated that X-chromosome inactivation by DNA methylation influences *MECP2* expression in males through modulating DNA methylation over a boundary between *MECP2* promoter and heterochromatin regions (-700 to -509 bp). The binding of CTCF to this methylation boundary (-595 to -389) prevents *MECP2* silencing in human neuronal cells (Nagarajan et al. 2008). A comparison of *MECP2* promoter methylation in RTT monozygotic twins had been conducted before, which indicated no differences in the *MECP2* promoter methylation between the twins (Miyake et al. 2013). However, to conclude that promoter methylation does not play a role in RTT, a direct comparison of RTT patients to age- and gender- matched healthy individuals is necessary.

The potential role of *Mecp2* promoter methylation in regulating *Mecp2* gene transcription was demonstrated by the association of increased promoter methylation at individual CpG sites (2–5 %) over a 164 bp region (1 to -164 bp) with reduced *Mecp2* expression in different samples collected from pups, which underwent stress due to maternal separation. These samples were sperms and brain cortex in three generations (F1 to F3). DNA methylation changes at the studied promoter region were observed in these generations in association with reduced *Mecp2* expression, indicating that the epigenetic memory

of early stress is transmitted through generations and results in altered expression of *Mecp2* (Franklin et al. 2010).

Further strengthening the role of DNA methylation in regulating *Mecp2* expression, we recently showed a dynamic relationship between *Mecp2* expression and DNA methylation at three regulatory elements found within the *Mecp2* promoter, and three regulatory elements found within the *Mecp2* intron 1 in in vitro differentiating neural stem cells (Liyanage et al. 2013). The reported intron 1 regions overlap with a previously described silencer element (Liu and Francke 2006). Treatment with DNA demethylating agent Decitabine upregulated *Mecp2* expression in an isoform-specific manner, in association with demethylation of specific elements within the promoter and intron 1 (Fig. 3). In contrast, Decitabine withdrawal caused downregulation of both *Mecp2* isoforms without significant changes in promoter and intron 1 DNA methylation. We observed a negative correlation between *Mecp2* isoform-specific expression and *Mecp2* promoter methylation. The correlation between the expression of *Mecp2* isoforms and intron 1 DNA methylation was dynamic (negative or positive correlation) depending on the stage of NSC differentiation, and our results suggested that DNA methylation may play dynamic roles at different stages of neural development (Liyanage et al. 2013). Similar positive/negative correlations between *Mecp2* isoform-specific expression and DNA methylation at the *Mecp2* regulatory elements were also observed in vivo in the adult mouse brain regions, confirming our finding in in vitro studies (Olson et al. 2014). Taken together, these results suggest the involvement of DNA methylation at these studied *Mecp2* regulatory elements in controlling *Mecp2* expression in a brain region-specific manner.

In addition to these reports, treatment with DNA demethylating agents has been shown to downregulate *Mecp2* expression both in vivo and in vitro. Delivery of intrathecal 5-azacytidine to rats following chronic constriction injury in the spinal cord resulted in downregulation of *Mecp2*/MeCP2 in association with reduced global DNA methylation within the spinal cord cells (Wang et al. 2011). Treatment of lens epithelial myofibroblasts with Zebularine, another DNA demethylating agent also resulted in reduced levels of MeCP2 (Zhou et al. 2011). However, it is unknown whether these Zebularine- or 5-azacytidine-mediated changes in global DNA methylation led to any changes of gene-specific DNA methylation at the *Mecp2* promoter causing the reduced levels of MeCP2.

Regulation by MicroRNAs

Activity of several miRNAs has been shown to influence the expression of MeCP2 in different systems. The 3′UTRs of *MECP2*/*Mecp2* gene have been predicted to provide different effects on the translation efficiency (Reichwald et al. 2000). The long 3′UTR part of the transcripts harbors binding sites for miRNAs (miRNA response elements/ MRE), which can regulate expression of *MECP2*/*Mecp2* expression posttranscriptionally (Klein et al. 2007).

The MREs found within the long *MECP2* 3′UTR in fetal human brains provide binding sites for miR-483-5p and suppress the expression of human MeCP2 during human brain development as well as in cells obtained from Beckwith–Wiedemann syndrome patients (Han et al. 2013). In schizophrenic brain neocortex, miR130b has been shown to target 3′UTR of *MECP2* (Burmistrova et al. 2007). Both miR-155 and miR-802 can downregulate MeCP2 expression in Downs Syndrome brains and thereby cause altered expression of MeCP2 target genes *Creb1* and *Mef2c* (Kuhn et al. 2010). Under ischemic conditions, MeCP2 protein, but not transcript expression, is regulated by miR-132, in the mouse cortex (Lusardi et al. 2010). The same miRNA (miR-132) is involved in homeostatic feedback loop regulation of MeCP2 in rat neurons through binding to the *Mecp2* 3′ UTR miRNA recognition element (Klein et al. 2007). Suggesting the potential involvement of miRNAs in regulating MeCP2 expression in fetal alcohol syndrome, MeCP2 was detected as a common target for miR-152, miR199a-3p and miR-685 in cortical neurons exposed to ethanol (Guo et al. 2012). Even though irrelevant for MeCP2 expression in brain and neurodevelopmental disorders, miR-212, miR-224, miR-452, miR-181c and miR-340 have been shown to impact MeCP2 expression in gastric cancer conditions (Wada et al. 2010; Hashimoto et al. 2013). Furthermore, MeCP2 is a direct target of miR-30a-3p in human umbilical vein endothelial cells, which can significantly reduce MeCP2 protein levels (Volkmann et al. 2013).

Histone Posttranslational Modifications

The binding of high mobility group N1 protein (HMGN1) to *MECP2*/*Mecp2* promoter in human and mouse brain extracts acts as a negative regulator for *MECP2*/*Mecp2*, downregulating its expression upon HMGN1 binding. The binding of HMGN1 induces chromatin structural changes at the binding sites within the promoter. HMGN1 binding reduced the enrichment of active histone mark H3K9Ac, while increasing the enrichment of H3K9me2, an inactive histone mark (Abuhatzira et al. 2011). Chromatin immunoprecipitation results shown in ENCODE demonstrate unique distribution of histone PTMs throughout the *MECP2*/*Mecp2* gene. For example, active histone marks H3K4me2 and H3K4me3 are clustered around the transcription start sites of *MECP2*/*Mecp2* [(Singh et al. 2008) and (http://genome.ucsc.edu/)]. Further showing the role of

histone acetylation in regulating *MECP2* expression in glioma cells, inhibition of HDAC activity by valproic acid induced *MECP2* transcript expression in a time-dependent manner (Kim et al. 2008). However, during synapse maturation in hippocampal neurons, treatment with Trichostatin A (TSA) resulted in downregulation of *Mecp2* transcript and MeCP2 protein levels (Akhtar et al. 2009). These reports also suggest that *MECP2/Mecp2* regulatory mechanisms might be cell-type specific and thus should be taken into consideration when regulatory mechanisms are used in therapeutic interventions.

Even though majority of these regulatory mechanisms are not directly linked to RTT physiology, it is important to investigate whether any of these mechanisms contribute in reduced expression and/or function of *MECP2* in RTT patients. This is significantly important especially in the absence of known RTT-causing *MECP2* mutations.

Advances in Therapeutic Approaches for Rett Syndrome

Currently, Rett syndrome has no cure. However, giving more hope for the patients suffering from RTT, intensive efforts from multiple research groups have proven that certain RTT phenotypes can be rescued in mouse models of RTT (Ricceri et al. 2013), and thus, there might be possible effective treatments for RTT. The proposed therapeutic strategies for RTT include restoring the expression and function of MeCP2 and/or targeting downstream targets of MeCP2. Moreover, for translating the preclinical mice model research into human, there are multiple clinical trials taking place in human RTT patients. Examples of some of the therapeutic strategies occupied so far in RTT mouse models as well as in human RTT patients are described in Table 3.

MeCP2 and Other Human Diseases

Autism Spectrum Disorders

Autism spectrum disorders (ASD) are a spectrum of pervasive neurodevelopmental disorders, which are characterized by impaired social interactions and repetitive stereotyped behaviors (Rapin and Tuchman 2008). Altered expression of *MECP2* is found in patients with autism (Nagarajan et al. 2006; Samaco et al. 2004). Reduced *MECP2* expression in autistic patients has been associated with increased *MECP2* promoter DNA methylation (Nagarajan et al. 2006, 2008). Moreover, *MECP2* mutations within the coding regions (Carney et al. 2003; Beyer et al. 2002; Lam et al. 2000; Loat et al. 2008) and sequence

variants within the *MECP2* 3′UTR (Shibayama et al. 2004; Coutinho et al. 2007) are also found in autistic patients, indicating the potential involvement of these sequence variations in altered expression of *MECP2* in autism. Not only reduced *MECP2* expression, but also overexpression of *MECP2* is found in ASD patients (Kuwano et al. 2011). Recent studies also demonstrate that the patients with *MECP2* duplication (*MECP2* duplication syndrome) share similar features to ASD (Peters et al. 2013; Xu et al. 2012b).

Fetal Alcohol Spectrum Disorders

Prenatal exposure to ethanol leads to a spectrum of neurodevelopmental disorders referred to as FASD. Demonstrating a potential link between MeCP2, RTT and FASD, a mutation (R270X) within the TRD-AT-hook domain was found in a FASD patient with combined phenotypes of both RTT and FASD (Zoll et al. 2004). With the accumulating evidence on the role of epigenetic mechanisms in FASD pathogenesis, several recent studies have investigated the expression patterns of MeCP2 in rodent FASD models. These studies show the aberrant expression of MeCP2 in rodent brains exposed to ethanol, where the effect of ethanol on MeCP2 expression seems to be dependent on multiple factors such as amount of ethanol, time of exposure as well as duration of ethanol exposure (Chen et al. 2013; Kim et al. 2013; Romano-Lopez et al. 2012; Repunte-Canonigo et al. 2013; Tunc-Ozcan et al. 2013). Moreover, the role of MeCP2 in sensitivity to alcohol and addiction to alcohol have also been demonstrated using a RTT mouse model [MeCP2$^{308/Y}$] (Repunte-Canonigo et al. 2013). These reports implicate the potential role of MeCP2 in other neurodevelopmental disorders and hence highlight the critical role of MeCP2 in nervous system function.

Cancer

MeCP2 is mainly discussed in association with neurological disorders such as RTT and autism. Interestingly, recent reports also show the epigenetic role of MeCP2 and other MBD proteins in different cancers (Parry and Clarke 2011). This is not surprising, because many cancer-related genes are silenced by epigenetic mechanisms, specifically promoter hypermethylation (Fukushige and Horii 2013). The relation between MeCP2 and breast cancer is well established, where MeCP2 is involved in epigenetic regulation of breast cancer-related genes (Mirza et al. 2013; Ray et al. 2013; Sapkota et al. 2012; Muller et al. 2003). Moreover, the expression and potential importance of MeCP2 in prostate cancer were shown previously (Shu et al. 2011; Yaqinuddin et al. 2008; Bernard et al. 2006). MeCP2

Table 3 Therapeutic strategies for Rett syndrome

Therapeutic target	Type of therapy Drug/genetic	Experimental model Mouse/human/cell lines	Description of treatment	Time of treatment Neonatal/adult/age of patients/pre-symptomatic	Success/improvement of phenotypes	References
MeCP2	Gene therapy and/ or introduction of *Mecp2* gene by genetic engineering	*Mecp2*$^{lox-stop/y}$	Activation of *Mecp2* transgene, which is expressed as Cre-ER fusion product and retains in cytoplasm. Injection with estrogen analog Tamoxifen (TM) translocate the protein to nucleus	5 TM injections per day for 3–4 weeks	Re-expression of MeCP2 protein in brain. The RTT neurological phenotypes were rescued. However, some mice died due to *Mecp2* overexpression toxicity	Guy et al. (2007)
				One TM injection per week for 5 weeks	Reduced toxicity. Several RTT phenotypes including hindlimb grasping, breathing irregularities were rescued.	
		Mecp2 knock-in mice (*Tau-Mecp2* ki/+) and *Mecp2*$^{1lox/+}$ (null mice) (Chen et al. 2001)	The *Mecp2* knock-in mice was bred with null mice to activate the *Mecp2* gene in null mice	N/A	Many RTT phenotypes were rescued including general health, fertility, life span, reduced weight, smaller head and reduced neuronal size	Luikenhuis et al. (2004)
		Mecp2 transgenic mice overexpressing HA-tagged *Mecp2* in forebrain neurons	Targeted delivery of *Mecp2* into forebrain neurons to induce *Mecp2* overexpression phenotypes and cross the transgenic mice with *Mecp2* to rescue phenotypes	N/A	RTT phenotypes of female *Mecp2* mice were rescued. Examples: Improved mobility and locomotion	Jugloff et al. (2008)
		Mecp2-deficient mouse model (Guy et al. 2001)				
		Mecp2 null mice (Luikenhuis et al. 2004)	Delivery of *Mecp2e2* transgene to ES cells to generate transgenic mice with specific reactivation of *Mecp2e2* in brain	*Mecp2e2* was activated at postnatal 1–2 weeks (early) or 2–4 weeks (late)	Early activation of *Mecp2* improved life span, brain growth more efficiently than late activation	Giacometti et al. (2007)

Table 3 continued

Therapeutic target	Type of therapy Drug/genetic	Experimental model Mouse/human/cell lines	Description of treatment	Time of treatment Neonatal/adult/age of patients/pre-symptomatic	Success/improvement of phenotypes	References
MeCP2	Gene therapy and/or introduction of Mecp2 gene by genetic engineering	Neural stem cells (NSCs) isolated from 1 year old Mecp2^tm1.1Bird+/− female mice	Delivery of MECP2 isoforms-specific retroviral vectors (preclinical gene therapy vectors)	Differentiation of transduced NSCs for 3–6 weeks	Successful rescued expression of MeCP2 in differentiated adult NSCs from Mecp2^tm1.1Bird+/− mice. Rescue of aberrant dendrite branching seen in Mecp2^tm1.1Bird+/− NSCs by MeCP2E1.	Rastegar et al. (2009)
		Mecp2^stop (Guy et al. 2007) and Mecp2^Bird.knockout (Guy et al. 2001) (female RTT mice model)	Delivery of scAAV9 virus with MECP2 cDNA under the control of scAAV9/MeCP2 promoter.	Vascular delivery: Viral solution was injected intravenously. Cranial delivery: Viral solution was injected to striatum, thalamus and deep cerebellar nuclei	Rescues RTT phenotypes in both male and female Mecp2^stop mice and rescues neuronal soma size. Induces neuronal MeCP2 expression and milder improvements in behavior	Garg et al. (2013)
	Compounds to read through nonsense mutations	HeLa cells transfected with R168X, R255X, R270X, and R294X MECP2 constructs	Treatment of cells with aminoglycosides to suppress nonsense mutations: gentamicin and geniticine (G418)	Cells were treated for 24 h	Successful read through of MECP2 mutation with different efficiencies	Brendel et al. (2009)
		Mouse model carrying R168X mutation (Mecp2^R168X) and HeLa cells transfected with mutant MECP2	Administration of aminoglycosides to suppress nonsense mutations: gentamicin NB30, NB54, and NB84	Cells were treated daily for 4 days	Successful read through of R168X mutation in both fibroblasts cells obtained from the mouse model and HeLa cells	Brendel et al. (2011)
		HEK293 cells transfected with MECP2 constructs with R168X, R255X, and R294X	Administration of aminoglycosides to suppress nonsense mutations: Geniticine, Amikacin, Paromomycin	Cells were treated daily for 2 days Cells were treated for 4 days and 12 days.	Successful read through of MECP2 mutation with different efficiencies and in a dose-dependent manner	Popescu et al. (2010)
		RTT patient-derived fibroblast cell-line harboring R294X, R270X and R168X mutations	Administration of aminoglycosides to suppress nonsense mutations: NB54 and Gentamicin	Cells were treated for 5 days	Successful read through of MECP2 mutation with different efficiencies and in a dose-dependent manner. Lead to increased expression of BDNF	Vecsler et al. (2011)
		RTT iPS cells with Q244X differentiated to neurons	Administration of aminoglycosides to suppress nonsense mutations: Gentamicin	Cells were treated for 1 week	Successful expression of MeCP2 protein and increased number of synapses	Marchetto et al. (2010)
	Activate silenced MECP2	RTT patient-derived fibroblast cell line, GM(11272)	Used epigenetic drugs [inhibitors of DNA methyl transferases (DNMT): Decitabine, 5-Azacytidine and inhibitors of histone deacetylases (HDAC): Trichostatin A, SAHA and Valproic acid]	Cells were treated for 72 h.	The pilot study was unable to activate MECP2 expression	Yu et al. (2011)

Table 3 continued

Therapeutic target	Type of therapy Drug/genetic	Experimental model Mouse/human/cell lines	Description of treatment	Time of treatment Neonatal/adult/age of patients/pre-symptomatic	Success/improvement of phenotypes	References
Downstream MeCP2 targets	BDNF as a growth factor	Mecp2^tm1-1Jae mice (Chen et al. 2001)	Exogenous delivery of BDNF	Bath application of BDNF for 15 min	Rescues synaptic dysfunctions found in Mecp2 null mice	Kline et al. (2010)
		Brain cortex and Hypothalamus from B6.129S-Mecp2^tm1Hzo/J mice	Administration of Glatiramer acetate (GA) to induce BDNF	GA was injected daily to 16 weeks old mice for 14 days	Significantly induced BDNF expression in cortex	Ben-Zeev et al. (2011)
		Cultured E18 hippocampal neurons with knocked down Mecp2.	Overexpression of Bdnf	N/A	Bdnf overexpression rescued abnormal neuronal morphology in Mecp2-knockdown neurons. (Bdnf increased dendritic and axonal length)	Larimore et al. (2009)
		Neurons were transfected with Mecp2e2 harboring 106 W and T158 M missense mutations.			Partial rescue of neurite length in neurons bearing RTT mutations	
		Nodose cranial sensory ganglial cells (NG) from Mecp2^tm1-1Jae mice (Chen et al. 2001)	Ampakine treatment to induce BDNF	Treatments started at P25 with saline treatments for 3 days, followed by Ampakine injections till P35	Ampakine significantly induced BDNF expression in NG cells and rescued respiratory dysfunctions observed in Mecp2-deficient mice	Ogier et al. (2007)
		Mecp2-null mice	Conditional overexpression of Bdnf in brain	N/A	BDNF overexpression caused delayed onset of RTT phenotypes, with increased lifespan, better locomotor activities and increased brain weight	Chang et al. (2006)
		Mecp2^Bird.knockout (Guy et al. 2001)	Treatment with fingolimod (sphingosine-1 phosphate receptor agonist)	I.p. injection of fingolimod to mutant Mecp2 mice every 4 days for 4 weeks	Increased BNDF levels in brain regions, increased brain weight, improved locomotor functions, and increased life span	Deogracias et al. (2012)
	Insulin-like Growth Factor 1 (IGF1)	Mecp2-null mice	Administration of IGF-1	IGF-1 was administered daily via i.p. injections, starting from at P15–18 and continued for 2 weeks	Rescue of RTT phenotypes such as increased extends the life span, improved breathing and heart rate, locomotor function and synaptic maturation	Tropea et al. (2009)

Table 3 continued

Therapeutic target	Type of therapy Drug/genetic	Experimental model Mouse/human/cell lines	Description of treatment	Time of treatment Neonatal/adult/age of patients/pre-symptomatic	Success/improvement of phenotypes	References
Downstream MeCP2 targets	Insulin-like growth factor 1	Human RTT girls with confirmed *MECP2* mutations (4–11 years old)	Treatment with IGF1	IGF1 was administered subcutaneously twice/day for 6 months	Improved growth rate, breathing, communication skills and bone density in some patients. No improvement in heart rate. No side effects reported	Pini et al. (2012)
		Human RTT-iPSC-derived neurons	Treatment with IGF1	IGF1 was added to neuronal cultures for 7 days	Rescue neuronal phenotypes such as increased number of glutamatergic synapses	Marchetto et al. (2010)
	Catecholamine signaling	$Mecp2^{Bird.knockout}$ (Guy et al. 2001)	Administration of Desipramine	Desipramine treatments started at P40 and the drug was administered through i.v. daily for 3–14 days	Improved life span and breathing patterns	Roux et al. (2007)
				Desipramine treatments Started at P30 through drinking water (oral treatment)	Improved life span and breathing patterns, but did not rescue the microcephaly and altered locomotor functions	Zanella et al. (2008)
Dietary interventions	ω-3 polyunsaturated fatty acids (ω-3 PUFAs)	Stage I RTT patients (early stage)	Administration of PUFA in the form of fish oil	Twice a day for 6 months	Improved communication skills and reduced breathing abnormalities	De Felice et al. (2012)
	Dietary administration of Acetyl-L-carnitine (ALC)	$Mecp2^{llox}$ mice (Chen et al. 2001)	Administration of ALC	Subcutaneous injection of ALC from P1 to P47	Increased levels of carnitines, improved general health, metabolic functions, motor and cognitive functions and rescued abnormal dendritic morphology	Schaevitz et al. (2012)
	Folinic acid supplementation	RTT girls at stages III-IV	Treatment with Folinic acid	Folinic acid for 1 year followed by placebo for 1 year	Reduced seizures and epileptic activities	Hagebeuk et al. (2011)
	Dietary supplementation of choline	$Mecp2^{llox}$ mice (Chen et al. 2001)	Supplementation with choline	Choline was administered through drinking water from P1 to P22.	Choline did not rescue the aberrant brain size. But, it increased BDNF and NGF expression in a brain region-specific manner and thereby improved motor functions.	Nag et al. (2008)
	Dietary treatment of Curcumin	$Mecp2^{tm1-1Jae}$ mice (Chen et al. 2001)	Treatment with Curcumin through food sources	6 weeks old male and 6 months aged female were given Curcumin for 21 days	Rescued vascular/endothelial system alterations	Panighini et al. (2013)
Targeting bones	Transplantation of wild-type bone marrow	$Mecp2^{tm1.1Jae}$ and $Mecp2^{tm2Bird}$	Transplantation of wild-type bone marrow	Injection of wild-type syngeneic bone marrow from intravenously at P28	Increased life spans and weight of brain, improved breathing and locomotor functions. But the abnormal neuronal soma size was not rescued.	Derecki et al. (2012)

Table 3 continued

Therapeutic target	Type of therapy Drug/genetic	Description of treatment	Experimental model Mouse/human/cell lines	Time of treatment Neonatal/adult/age of patients/pre-symptomatic	Success/improvement of phenotypes	References
Environmental enrichment	Enhance environmental quality/ Environmental enrichment (EE)	Compared with the standard housing, the tested mice were provided with large, spacious cages with running wheels and nesting materials	$Mecp2^{tm1Tam}$ mice (Pelka et al. 2006)	EE started at 4 weeks of age till the end of the study	Improved locomotor functions observed in females but not in males. Increased BDNF expression in different brain regions	Kondo et al. (2008)
			$Mecp2^{tm1-1Jae}$ mice (Chen et al. 2001)	EE started at P10.	Improved motor functions, learning and synaptic density and long-term potentiation. Reduced anxiety and memory problems	Lonetti et al. (2010)
			$Mecp2^{Bird.knockout}$ (Guy et al. 2001)	EE started at 3 weeks of age and continued for 14 days	Improved locomotor activities and behaviors, but no improvement in life span or body weight. Downregulation of synaptic plasticity-related genes	Kerr et al. (2010)

regulates cell proliferation, cell growth and apoptosis in prostate cancer cells (Bernard et al. 2006; Yaqinuddin et al. 2008). Apart from these reports, MeCP2 has also been linked to cervical cancer (Wang et al. 2013) and ductal carcinomas (Xu et al. 2012a). Altered MeCP2 expression in gastric cancer has been shown to be mediated by the action of miR-212 (Wada et al. 2010). Also, showing a link between brain tumors and Rett syndrome, one study reported the presence of brain-stem tumor in a small girl with RTT phenotypes (Vanhala et al. 1998).

Unanswered Questions and Closing Remarks

Despite the tremendous progress that has been made with regard to understanding the molecular mechanisms by which *MECP2* mutations and MeCP2 expression and/or functional abnormalities cause RTT and to design potential therapeutic strategies, there are numerous unanswered questions yet to be disclosed. To design an effective therapeutic strategy for Rett syndrome, comprehensive understanding of the mechanisms by which *Mecp2*/MeCP2 expression (total and isoform-specific) is regulated in brain, and in specific cell types of the brain is critical. Moreover, it is still unclear how a single protein can perform opposing functions such as transcriptional activation and repression and how a single protein can bind to un-methylated and methylated DNA (5mC and 5hmC). Further, it is also important to investigate whether this multifunctional property can be explained by the nature of the two MeCP2 isoforms, the MeCP2 interacting protein partners or conformational changes of the protein. Most of the proposed therapeutic approaches are designed to be used in developing animal models. It is important to know whether any of these therapies can be studied in adult RTT patients. The next challenge would be to translate the knowledge obtained from animal models to human. Even though the mouse and human *Mecp2*/MECP2 are highly conserved, differences between mouse and human brain structure, immune system and response to environmental factors would challenge the knowledge translation.

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