Modeling Rett Syndrome with MeCP2 T158A Knockin Mice

Zhaolan Zhou and Darren Goffin

Introduction

RTT is an autism spectrum disorder (ASD) caused primarily by mutations in the X-linked gene encoding methyl-CpG binding protein 2 (MeCP2) (Amir et al. 1999). Classical RTT patients develop normally for the first 6–18 months of age, after which they enter a period of regression characterized by a partial or complete loss of acquired purposeful hand and spoken language skills and the development of gait abnormalities and hand stereotypies (Neul et al. 2010). Patients then enter a period of recovery or stabilization, frequently followed by the development of seizures, irregular breathing, intellectual disability, autistic behaviors, and autonomic dysfunction (Chahrour and Zoghbi 2007). The temporal dependence of symptom manifestation suggests RTT is a progressive neurodevelopmental disorder.

The clinical manifestations of RTT vary greatly with patients presenting with some but not all the clinical features of RTT. For example, in the preserved speech variant of RTT, patients often show milder motor deficits and intellectual disability, some language abilities, and rarely have seizures or autonomic dysfunction. There are two likely causes for these differences in symptom severity: firstly, different types of mutation within *MECP2* may lead to complete or partial loss of function; and secondly, X-chromosome inactivation (XCI) may result in skewed expression of wild-type or mutant MeCP2. Hundreds of different disease-causing mutations within *MECP2* have been reported in RTT, including missense, nonsense and frameshift mutations (Bienvenu and Chelly 2006), but the correlation between the identity of the *MECP2* mutation and the presence and severity of symptoms is poorly understood.

Z. Zhou (✉)
Department of Genetics, University of Pennsylvania, School of Medicine, Philadelphia, PA, USA
e-mail: zhaolan@mail.med.upenn.edu

D. Goffin
School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
e-mail: dgoffin@mail.med.upenn.edu

V.B. Patel et al. (eds.), *Comprehensive Guide to Autism*,
DOI 10.1007/978-1-4614-4788-7_181,

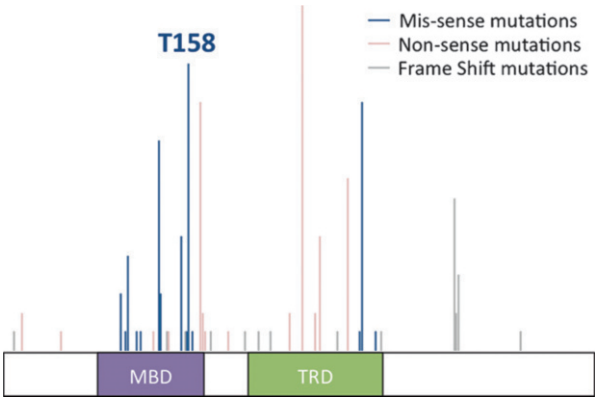

Fig. 1 Schematic of MeCP2 protein showing methyl-CpG binding domain (MBD) and transcriptional repression domain (TRD). The location of missense, nonsense, and frameshift mutations is shown with the height of the bars representing the frequency of the mutation. T158 mutation is the most common mutation observed in RTT (Data obtained from www.mecp2.org.uk)

Since heterozygous RTT female patients are mosaics, skewing of XCI may result in a milder or more severe phenotype, depending on the proportion of cells expressing wild-type MeCP2.

The study of RTT based on genotype or the study of MeCP2 function based on phenotype has been extremely challenging. Therefore, we sought to model RTT with a knockin approach recapitulating RTT-associated mutations in mice. Previous studies of mice lacking *Mecp2* revealed that these mice develop normally in the first 4–6 weeks of life, but later present neurological phenotypes resembling some of those observed in RTT, including motor incoordination, hindlimb clasping, aberrant gait, breathing abnormalities, cognitive deficits, and premature lethality (Chen et al. 2001; Guy et al. 2001). Deletion of MeCP2 from postnatal and adult mice revealed a similar recapitulation of RTT-like phenotypes (McGraw et al. 2011; Cheval et al. 2012). Intriguingly, reintroduction of MeCP2 into behaviorally affected *Mecp2*-null mice is sufficient to ameliorate RTT-like phenotypes (Guy et al. 2007). That RTT-like phenotypes can be ameliorated even in severely phenotypically affected mice suggests that RTT may be a treatable disorder. Thus, an animal model with a faithful RTT-associated mutation would allow us to test the treatment of RTT in a true disease setting.

One of the most common missense mutations associated with RTT occurs at MeCP2 threonine 158 (T158), which is converted to methionine (T158M) or in rare cases alanine (T158A) (Fig. 1). Mutation at T158 is typically associated with severe symptom presentation (Colvin et al. 2004; Schanen et al. 2004; Charman et al. 2005; Fukuda et al. 2005) but has also been reported in mildly affected cases with the preserved speech variant of RTT (Huppke et al. 2000; Vacca et al. 2001a; Kerr et al. 2006). This suggests that the clinical variability associated with these mutations is broad. Both MeCP2 T158M and T158A mutations are believed to impair MeCP2 function through the same mechanism: the loss of a single hydrogen bond from the threonine residue destabilizes MeCP2 structure and reduces the affinity of MeCP2 for methylated DNA (Ho et al. 2008). Indeed, patients carrying MeCP2 T158A or T158M mutation are phenotypically similar in the identity and severity of presented symptoms (Vacca et al. 2001b; Schanen et al. 2004).

The development of this MeCP2 T158A knockin mouse has provided us with the opportunity to assess the causal role for this mutation in the development of RTT, provides mechanistic insight into the biochemical and electrophysiological consequences of this mutation, and provides an invaluable tool for the in vivo testing of preclinical pharmacological agents for the treatment of RTT and/or particular symptoms. The development of future mouse models that recapitulate different mutations observed in RTT will greatly increase our understanding of shared and unique mechanisms in RTT pathogenesis and provide valuable tools for basic and translational research into RTT.

Development of an MeCP2 T158A Knockin Mouse

To investigate the causal role of MeCP2 T158, the most common site for missense mutations observed in RTT, in the etiology of RTT we developed a mouse model with a missense mutation at this site. We generated a mouse model carrying the MeCP2 T158A mutation first instead of the T158M mutation since the bulky side chain of the methionine residue may lead to steric interference. Therefore, we designed an *Mecp2* targeting construct with a floxed neomycin expression cassette in intron III of the *Mecp2* gene (Fig. 2). Site-directed mutagenesis was employed to mutate the ACG sequence encoding threonine at *Mecp2* codon 158 to GCG encoding alanine (T158A). To facilitate screening of properly targeted ES cells, we also incorporated a silent mutation at codon 160 to create a new BstEII restriction enzyme site. Correctly targeted ES cell clones were injected into C57BL/6 blastocysts for chimera production. Chimeras were then bred to *EIIa-Cre* mice for early embryonic deletion of the neomycin cassette leaving a single loxP site within the genomic DNA (Schwenk et al. 1995). Given that our goal was to test a rather subtle mutation on MeCP2 – a single amino acid change of T158A – we also sought to rule out the possibility that manipulation of the *Mecp2* locus could affect MeCP2 expression in unexpected ways. We also engineered knockin mice that contain the silent BstEII restriction enzyme site and loxP insertion but that lacked the T158A mutation, which we refer to here as loxP mice (Fig. 3).

Confirmation of T158A Mutation

Following the development of these mice, we verified the successful mutagenesis of TIS8A at both the RNA and protein level 5. MeCP2 mRNA was extracted from the brain of male wild-type, MeCP2 T158A, and loxP control mice, converted to cDNA and sequenced using primers flanking the T158 sequence. Sequencing verified that both T158A and loxP knockin mice contained the T to C mutation at codon 160 for the generation of the BstEII restriction site, whereas only T158A mice contain the A to G mutation at codon 158 (Goffin et al. 2012). Wild-type MeCP2 mice contained neither of these mutations. To validate that MeCP2 protein contains this T158A mutation, we generated an MeCP2 T158 site-specific antibody. This antibody

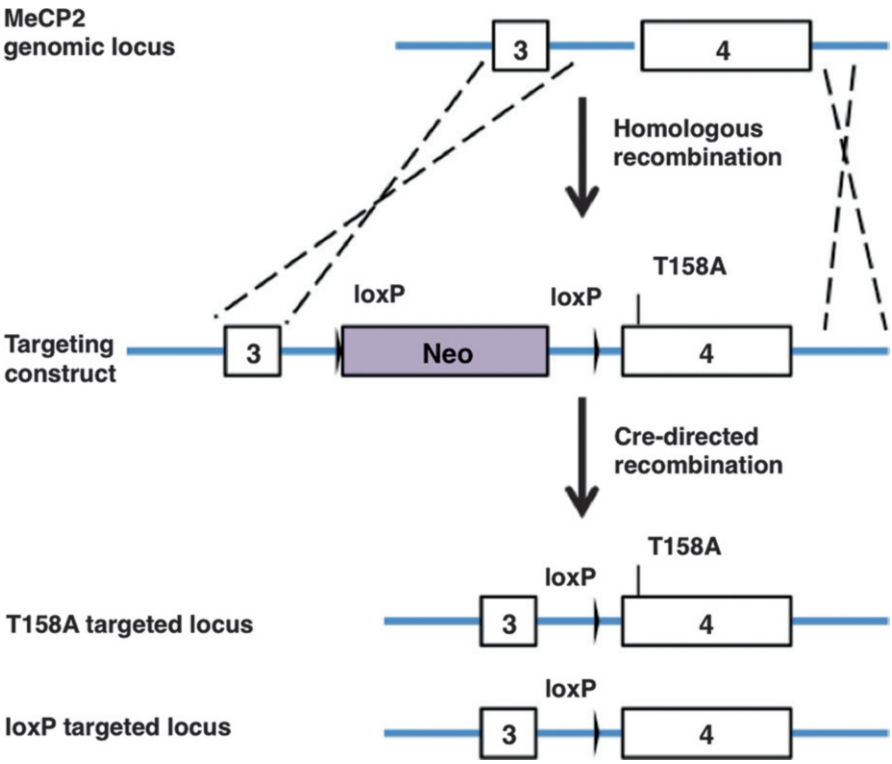

Fig. 2 Targeting strategy to generate MeCP2 T158A and loxP knockin mice. The genomic region surrounding *Mecp2* exons III and IV was targeted for homologous recombination using a construct in which the floxed neomycin positive selection cassette (Neo) was inserted in a non-conserved region of intron III. The location of the mutation in exon IV to create T158A is indicated. Since we were testing for the effects of a single nucleotide mutation, two lines of mice were generated: those with and those without the T158A mutation. Both lines contained floxed loxP sites. Following production of chimera, the Neo cassette was removed using EIIa-cre-mediated excision. The targeted alleles after cre-mediated removal of the Neo cassette are illustrated

recognizes MeCP2 protein from wild-type and loxP control, but not MeCP2 T158A mice. In contrast, an antibody raised against the C-terminus of MeCP2 detects MeCP2 expression in brain lysates from all of these mice (Goffin et al. 2012). These data confirm that we successfully generated knockin mice expressing MeCP2 with a designated T158A missense mutation, and targeting of the *Mecp2* locus did not affect MeCP2 protein expression.

Behavioral Characterization of MeCP2 T158A Mice

RTT is characterized by normal development for the first 6–18 months of age, after which patients enter a period of regression characterized by a partial or complete

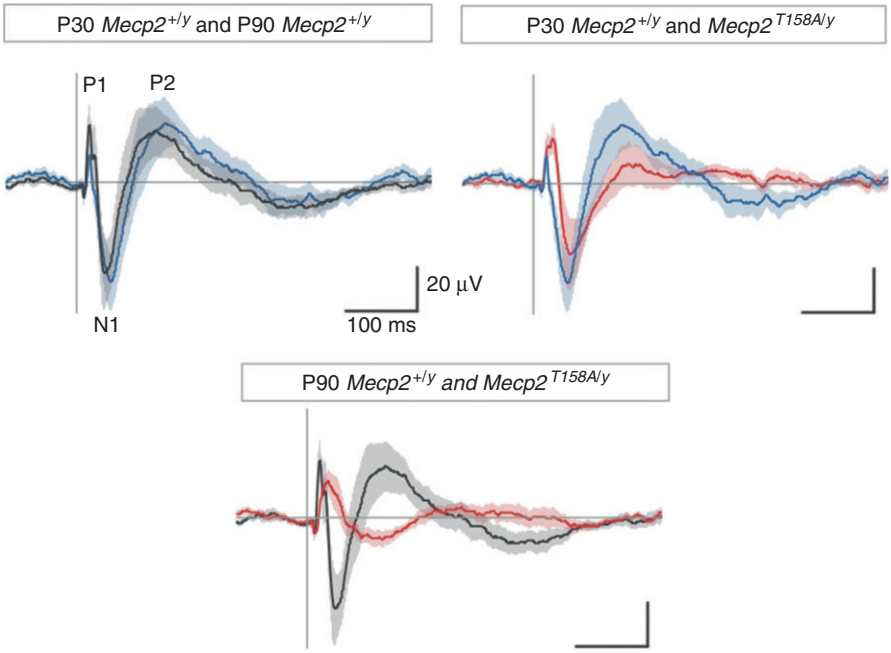

Fig. 3 Event-related potentials (ERPs) following 85-dB auditory stimulation in wild-type and MeCP2 T158A mice at postnatal days 30 (P30) and P90. ERPs are not substantially different between wild-type and MeCP2 T158A mice at P30 but are significantly altered at P90 in MeCP2 T158A mice. MeCP2 T158A mice do not exhibit development changes in ERPs calculated using time-amplitude analysis (Adapted from Goffin et al. 2012)

loss of acquired purposeful hand and spoken language skills and the development of gait abnormalities and hand stereotypies (Neul et al. 2010). Similarly, *Mecp2*-null mice acquire RTT-like phenotypes at 4–6 weeks of age following normal development, with phenotypes including hypoactivity, abnormal gait, decreased motor coordination, cognitive deficits, and breathing abnormalities, leading to death around 12 weeks of age (Chen et al. 2001; Guy et al. 2001). Heterozygous female mice also develop RTT-like phenotypes at around 6 months of age, considerably later than in male mice. The symptoms are less severe in female mice with no changes in their survival.

Phenotypic Scoring of RTT-Like Phenotypes

Perhaps the most important diagnostic criterion for RTT is normal development followed by regression. Therefore, we suggest that this is also an important proxy for assessing RTT-like phenotypes in mouse models of RTT. To understand the phenotypic expression and course of RTT-like phenotypes in MeCP2 T158A mice,

a scoring system that is amenable to regular testing was used (Guy et al. 2007). Mice were scored on a weekly basis beginning postnatal week 1 and assessed for the following phenotypes:

1. Locomotor ability
2. Gait
3. Hind limb clasping
4. Tremor
5. Breathing
6. Overall condition

Mice are scored on a scale of 0–2 for the presence and severity of each of these phenotypes and the aggregate score calculated. We demonstrated that male MeCP2 T158A mice develop normally for the first 4–5 weeks, after which RTT-like phenotypes increase in number and severity. Male MeCP2 T158A mice die on average around 16 weeks of age (Goffin et al. 2012). Heterozygous females containing the MeCP2 T158A mutation show normal development until about 17 weeks of age when RTT-like symptoms begin to manifest with increasing severity. No alterations in longevity were observed in female heterozygous TIS8A mice, similar to that observed in female heterozygous MeCP2 knockout mice.

Decreased Brain Weight and Soma Size

A common symptom observed in RTT is a deceleration of head growth leading to microencephalopathy within the first 2 years of life (Chahrour and Zoghbi 2007). Although this symptom is no longer required for the diagnosis of RTT, its appearance often directs physicians towards the disorder. Male *Mecp2*-null mice consistently show decreased brain weight and size together with decreased soma size (Chen et al. 2001; Guy et al. 2001). Male MeCP2 T158A mice also show decreased brain size and weight at both pre- and post-symptomatic stages (Goffin et al. 2012).

Behavioral Assessment

The development of new mouse models that recapitulate mutations observed in RTT has tremendous utility in understanding both the endogenous function of MeCP2 and the pathogenic mechanisms that lead to RTT-like symptoms. To properly understand the similarities and differences between new mouse models and previously established models, it is important that side-by-side behavioral comparisons are performed on age-matched and genetic background-matched animals.

Using age-matched male MeCP2 T158A and *Mecp2*-null mice on a congenic (>95 %) C57BL/6 background, behavioral experiments were performed to assess motor activity and motor skills, anxiety, and learning and memory capabilities. Behavioral experiments used to test these phenotypes are as follows:

1. Open field
2. Elevated zero maze and elevated plus maze
3. Accelerating rotarod
4. Context- and cue-dependent fear conditioning

These experiments revealed that MeCP2 T158A mutation leads to a less severe phenotype in age-matched mice compared to *Mecp2*-null mice but gets progressively worse with increasing age. Locomotor and rotarod tests demonstrated that both MeCP2 T158A and *Mecp2*-null mice show clear deficits in motor activity and motor skills compared to their wild-type littermates, but *Mecp2*-null mice were more severely affected than MeCP2 T158A mice. A fear conditioning paradigm used to assess learning and memory revealed that MeCP2 T158A mice show a profound reduction in both context- and cue-dependent learning and memory. A direct comparison with *Mecp2*-null mice was not possible due to severe basal hypoactivity in these mice.

Sensory Information Processing Deficits in MeCP2 T158A Mice

To understand the neuronal basis of the behavioral abnormalities in RTT, we turned our attention to assessing sensory information processing. The onset of RTT symptoms occurs during the establishment and refinement of neural networks in early postnatal life and has been suggested to occur due to alterations in the excitatory/inhibitory (E/I) balance in the brain. Neurons receive a balance of excitatory and inhibitory membrane currents during ongoing and stimulated activity. The balance of equal amounts of incoming depolarizing and hyperpolarizing currents is essential for maintaining stability in cortical networks and facilitates rapid responses to small changes in synaptic input following sensory stimulation thus allowing for efficient sensory information processing. Disturbances in these neuronal networks and their ability to process sensory stimuli have been implicated in a number of neurological disorders including schizophrenia, autism, and RTT (Bader et al. 1989; Stauder et al. 2006; Uhlhaas et al. 2009; Gandal et al. 2010). It was not known however until recently whether similar disturbances were also observed upon MeCP2 dysfunction in mice.

Time-Amplitude Analysis

Sensory information processing can be measured in vivo through EEG recordings during the performance of a cognitive, sensory, or motor task. The manifestation of these brain activities is recorded as a series of amplitude deflections in the EEG as a function of time and is referred to as an event-related potential (ERP). Compared to background activity, ERPs are small and can be difficult to resolve. To circumvent this, single ERPs are averaged across trials and the mean ERP is calculated. Any change in the EEG unrelated to the event is lost through averaging leaving only

those changes temporally related to the event. Following averaging, three polarity peaks emerge defined by their polarity and latency: an initial positive peak (P1), followed by a negative peak (N1), and a subsequent second positive peak (P2) (Fig. 3). This method of analysis of ERPs is referred to as time-amplitude analysis and is the traditional method for analyzing changes in ERP. The amplitude and latency of the polarity peaks are believed to reflect the strength and timing of the cognitive processes related to the event. Notably, RTT patients, as well as patients with schizophrenia and autism, are reported to show alterations in both the amplitudes and latencies of ERP (Bader et al. 1989; Stauder et al. 2006; Uhlhaas et al. 2009; Gandal et al. 2010).

To assess sensory processing in MeCP2 T158A mice, auditory-evoked ERP measurements were performed in response to the presentation of a series of white-noise auditory events. Auditory-evoked ERP assessments were performed since they can be performed on freely mobile mice and are not confounded by the motor or attentional deficits associated with MeCP2 dysfunction. They are therefore readily translated for study in RTT patients. ERP measurements were performed on male MeCP2 T158A mice at two developmental time points: postnatal day 30 (P30) when RTT-like phenotypes were absent and P90 when RTT-like phenotypes were overt. ERP measurements revealed significant alterations in the ERPs of MeCP2 T158A mice at P90 but not P30, suggesting a disruption in sensory information processing at P90 (Fig. 3). Analysis of ERP responses in symptomatic *Mecp2*-null mice revealed similar disturbances suggesting a common deficit in neuronal network function.

Time-Frequency Analysis

A limitation of the time-amplitude analysis of ERPs is that considerable information is lost through averaging. Only those oscillatory events that are temporally locked to the sound presentation and occur following all or most of the events are preserved after averaging. Any signals that show variability in time or appearance, but are nevertheless induced by sound presentation, will be lost using this method. To circumvent this, it is possible to separate the EEG signal into the time and frequency domains allowing for the analysis of changes in the power and phase following presentation of sensory information (Fig. 4). The protocol for this is briefly outlined below:

1. Create a set of real-valued bandpass filtered signals. The raw EEG signal is separated into bands with center frequencies ranging from 2 Hz to 140 Hz, in 1 Hz steps with 2 Hz bandwidths. All filtering is done with a two-way least-squares FIR filter to ensure that phase information is not altered.
2. Each bandpass filtered signal is normalized by subtracting the temporal mean and dividing by the temporal standard deviation. This normalization procedure is performed to allow comparison between different frequency bands.
3. The Hilbert transform is applied to each normalized signal to create a set of normalized complex-valued analytical signals.

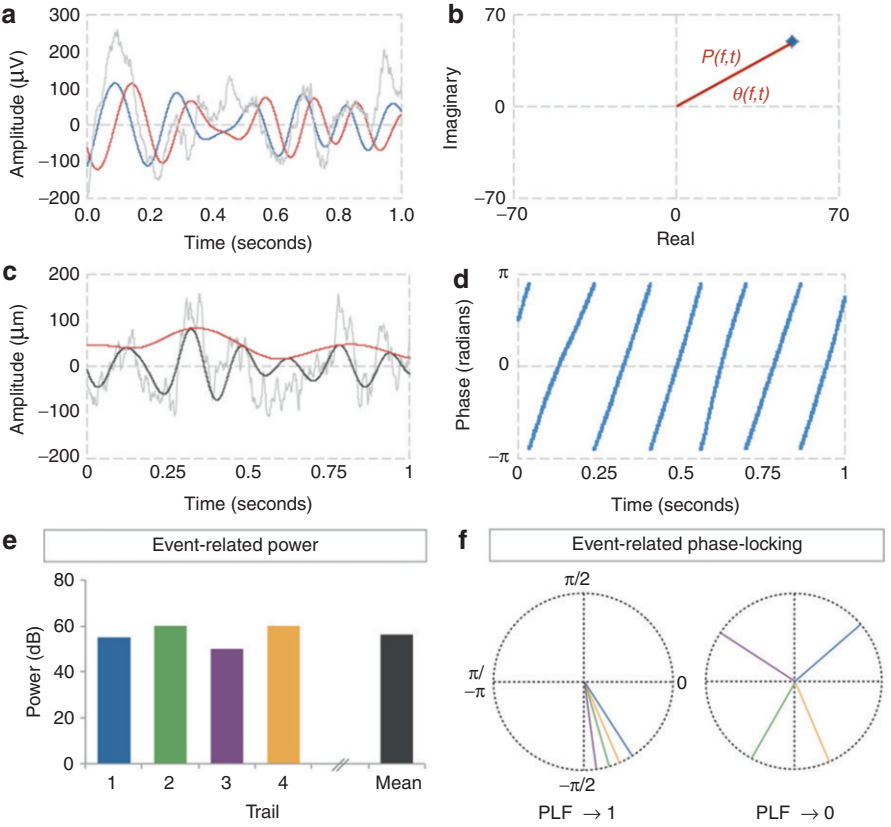

Fig. 4 Time-frequency analysis. (**a**) One second of the raw EEG trace (*gray*) is filtered in desired range (2–4 Hz in this example; blue). Hilbert transform of the raw EEG signal creates an imaginary transform of the signal (*red*). (**b**) The real (raw EEG signal) and imaginary components at each time point and each frequency are plotted on creating a vector that is used to calculate the magnitude of the signal (length of the vector squared) and the phase of the signal (angle of vector). (**c**) The filtered EEG signal (*black*) and the magnitude of the filtered EEG signal (*red*) are shown for each time point across this 1 s of EEG trace (*gray*). (**d**) The phase of the filtered EEG signal is shown in blue showing phase changes from $-\pi$ to π across this 1-s time period. (**e**) To calculate event-related power, the magnitude values calculated as described are squared, and the mean value across trials is taken. This is performed for all time points and at all frequencies. (**f**) To calculate the event-related phase-locking factor (PLF), the phase values are calculated for each trial as above and the circular variance is taken. The PLF is then calculated as 1 – circular variance. Shown are two possible results showing an example where phase values are similar between trials (*left*) which would create a PLF that tends to 1 and an example where phase values across trials are dissimilar (*right*) which would create a PLF that tends to 0

4. The absolute value of this complex analytical signal is taken to produce the set of normalized analytic amplitude time series. This is then squared element wise to produce a set of normalized instantaneous power time series.
5. The phase at each time point is extracted to create the analytic phase time series with values from $-\pi$ to π.

6. Four-second epochs centered for each event with time t = 0 representing the presentation of the 250 sound stimuli are extracted for power and phase measurements.
7. The power measurements are averaged across all epochs to produce the event-related power as a function of time and frequency.
8. The circular variance of the phase information is also determined across trials. Phase-locking factor (PLF) is then determined as 1 – circular variance.

Developmental Increase in Event-Related Power Is Absent in MeCP2 T158A Mice

Performing time-frequency analysis on ERP recordings described above revealed novel insights into the development of ERP responses in wild-type and MeCP2 T158A mice. Comparing event-related neuronal responses in wild-type mice at P30 and P90 revealed a developmental increase in event-related power and phase locking of neuronal oscillations (Fig. 5). This likely reflects the maturation and refinement of the underlying neuronal circuitry allowing for greater contrast for discrimination of sensory inputs. In contrast, MeCP2 T158A mice do not show this developmental increase and instead show similar event-related power and phase-locking responses at both P30 and P90. This suggests that MeCP2 dysfunction leads to a disruption in the refinement and functioning of neuronal networks, leading to a reduction in sensory information processing. Furthermore, since these experiments are easily translatable into humans, they may provide a novel biomarker to RTT and potentially other ASDs and monitoring the efficacy of therapeutic treatments.

Biochemical Consequences of MeCP2 T158A Mutation

The development of RTT-like phenotypes and neuronal network dysfunctions confirms the causal role of MeCP2 T158A in RTT symptom manifestation. Next, we examined the molecular mechanisms underlying the partial loss of function of MeCP2 by T158A mutation.

MeCP2 is a highly abundant nuclear protein that preferentially binds to 5'-methyl-cytosine residues in CpG dinucleotides through a conserved methyl-CpG binding domain (MBD) (Lewis et al. 1992; Nan et al. 1997). The interaction between the transcriptional repressor domain (TRD) within MeCP2 and a repressor complex that includes histone deacetylases (HDACs), Sin3a, cSki, and N-CoR, is believed to repress gene transcription (Jones et al. 1998; Nan et al. 1998; Kokura et al. 2001; Lunyak et al. 2002) and dampen transcriptional noise (Skene et al. 2010). MeCP2 has also been suggested to act as a transcriptional activator (Chahrour et al. 2008) and regulator of mRNA spicing (Young et al. 2005) and miRNA production (Szulwach et al. 2010; Wu et al. 2010).

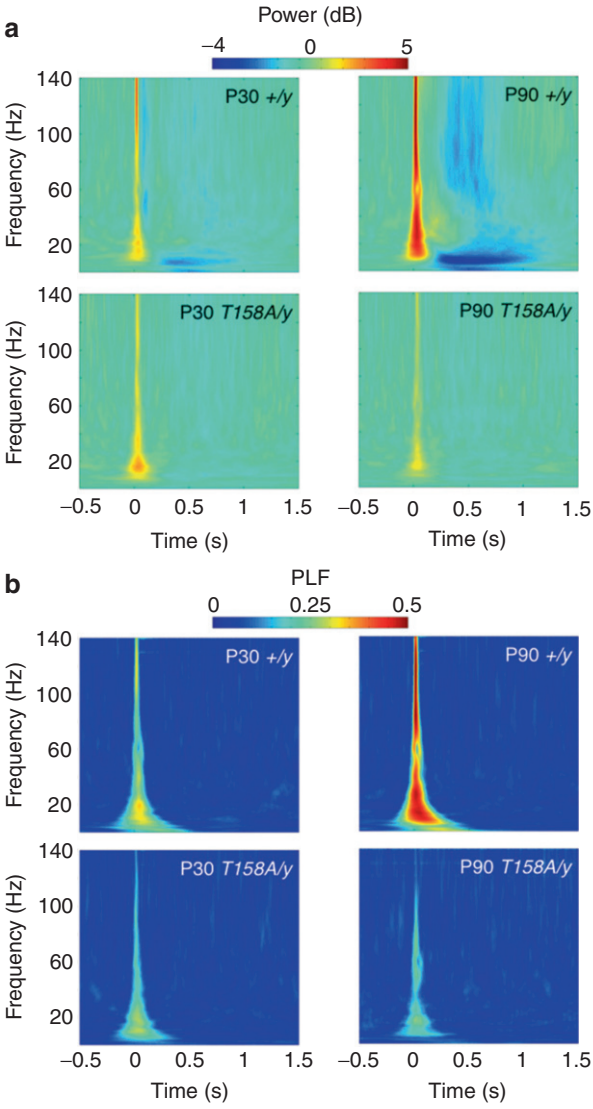

Fig. 5 (**a**) Time-frequency plots showing changes in event-related power in response to 85-dB auditory stimulation in wild-type (+/*y*; n = 8) and MeCP2 T158A (*T158A*/*y*; n = 7) mice at postnatal days 30 (P30) and P90. Color represents mean power with warmer colors corresponding to an increased power and cooler colors representing decreased power compared to pre-stimulus baseline. Wild-type mice show clear changes in event-related power from P30 to P90, whereas MeCP2 T158A mice show no such change. (**b**) Time-frequency plots showing changes in event-related phase locking showing development increase in wild-type mice but not MeCP2 T158A mice (Adapted from Goffin et al. 2012)

In vitro binding assays and transfection experiments in heterologous cells demonstrated that MeCP2 T158A or T158M mutation decreases the affinity of MeCP2 to methylated DNA but to a lower extent than other point mutations (Ballestar et al. 2000; Ghosh et al. 2008; Ho et al. 2008). However, patients with either T158A or T158M mutation show typical RTT symptoms suggesting additional roles for T158 in MeCP2 function (Bienvenu and Chelly 2006). MeCP2 T158A knockin mice provided the first opportunity to determine whether T158A mutation disrupts MeCP2 binding to methylated DNA in vivo and identify any other biochemical consequences of this mutation.

MeCP2 T158A Mutation Reduces MeCP2 Binding to Methylated DNA

To investigate whether MeCP2 T158A mutation disrupts MeCP2 binding to methylated DNA in vivo, chromatin immunoprecipitation (ChIP) assays that assess the binding of proteins to DNA were employed. MeCP2 T158A mutation led to decreased binding at the *Bdnf* locus with binding tracking methyl-CpG sites (Skene et al. 2010; Goffin et al. 2012). In males, there is a single copy of the *Xist* promoter, which is highly methylated and bound by MeCP2 (Skene et al. 2010). The imprinted *Snrpn* locus has an unmethylated paternal allele and a methylated maternal allele (Sutcliffe et al. 1994) with MeCP2 binding predominantly to methylated maternal allele (Skene et al. 2010). MeCP2 T158A was shown to destabilize the binding of MeCP2 to all of these loci with binding decreased to approximately 75 % of that observed using wild-type MeCP2 (Goffin et al. 2012). Thus, MeCP2 T158A mutation was observed to decrease MeCP2 binding to methylated DNA in vivo.

Visualizing MeCP2 Binding to Methylated DNA Using Immunohistochemistry

In mouse cell nuclei, heterochromatin-dense foci representing regions of high DNA methylation and low transcription levels are clearly visualized as strong punctate staining. MeCP2 immunoreactivity is readily observed in association with these heterochromatic foci, resulting in a characteristic punctate pattern of nuclear staining that is dependent upon the presence of both the MBD and methylated DNA (Nan et al. 1996). This method can be used to visualize whether MeCP2 mutation interferes with MeCP2 binding to methylated DNA in vivo. MeCP2 T158A was shown to disrupt the association of MeCP2 with heterochromatin-dense foci, highlighting the requirement for this residue in binding methylated DNA (Fig. 6).

MeCP2 T158A Mutation Decreases MeCP2 Protein Stability

MeCP2 protein levels in vivo are very low at birth and increase steadily during the first 3–4 weeks of age, reaching their maximal levels around 5–10 weeks of age (Shahbazian et al. 2002; Skene et al. 2010). The onset of overt RTT-like phenotypes at 5–10 weeks of age in *Mecp2*-null mice suggests a strong correlation between MeCP2 protein levels and MeCP2 protein function. Indeed, increased or decreased protein levels lead to the manifestation of neurological symptoms, highlighting the importance of maintaining proper MeCP2 protein levels (Chen et al. 2001; Guy et al. 2001; Collins et al. 2004). Assessment of MeCP2 protein levels in MeCP2 T158A mice revealed a hitherto unknown function of T158: the stabilization of the MeCP2 protein levels. Western blot analysis of brain protein extracts revealed a 40–50 % reduction in MeCP2 protein levels compared to wild-type littermates. These alterations were not attributable to transcriptional changes since *Mecp2* T158A mRNA levels remained

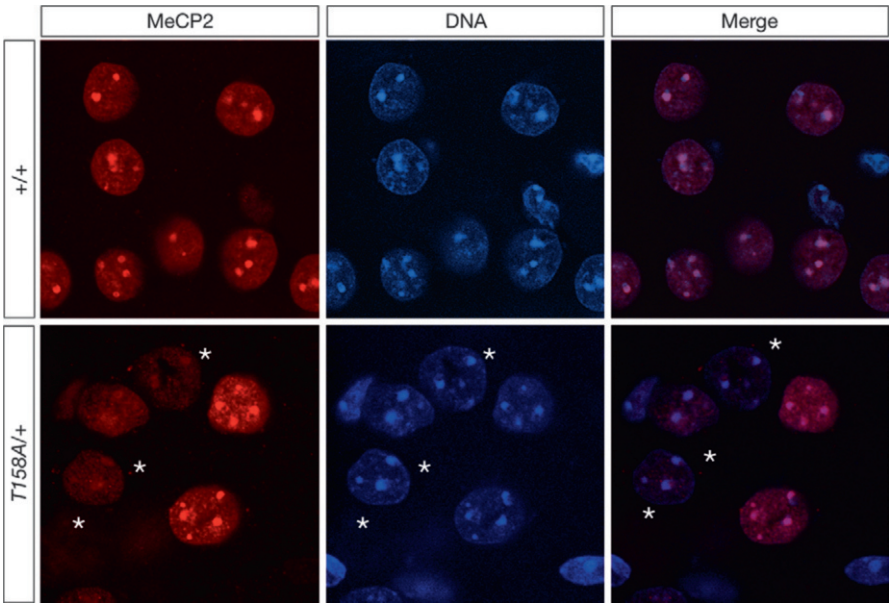

Fig. 6 MeCP2 immunoreactivity in nuclei obtained from female wild-type (+/+) or female heterozygous MeCP2 T158A (*T158A*/+) mice at P90. MeCP2 shows clear colocalization with DNA with particular enrichment at heterochromatin-dense foci. In contrast, MeCP2 T158A colocalization with DNA is absent in 50 % of cells (marked with *asterisks*) (Adapted from Goffin et al. 2012)

equivalent to wild type. Furthermore, fibroblasts obtained from a RTT patient carrying the MeCP2 T158M mutation revealed a similar reduction in MeCP2 protein levels. Embryonic neuronal cultures from MeCP2 T158A mice and their wild-type littermates revealed these reduced MeCP2 protein levels were due to decreased protein stability since blocking new protein synthesis with cyclohexamide led to a downregulation of MeCP2 protein in cultures derived from MeCP2 T158A mice but not wild-type littermates. The observation that wild-type MeCP2 protein levels were not reduced is not surprising since the half-life of MeCP2 in vivo is thought to be around 2 weeks (Cheval et al. 2012). Thus, MeCP2 T158A mutation leads to a destabilization of MeCP2 protein stability and a downregulation of protein levels. We concluded that MeCP2 T158A mutation disrupts neural circuit function, leading to RTT-like phenotypes through reduced methyl-DNA binding and decreased MeCP2 protein stability.

Concluding Remarks

In recent years, the development of mouse models leading to the constitutive or conditional deletion of MeCP2 has provided valuable insight in the etiology

of RTT. However, these mice do not recapitulate mutations observed in RTT and do not allow us to fully appreciate how specific MeCP2 mutations lead to the pathogenesis of RTT. There is considerable need to develop mouse models of RTT that faithfully recapitulate disease-associated mutations. The development of MeCP2 T158A mice elucidated the causal role for this mutation in the etiology of RTT. Analysis of these mice demonstrated that T158 mutation leads to a decrease in MeCP2 protein stability and a reduction in MeCP2 binding to methylated DNA. Since MeCP2 function is not completely lost in these mice, only severely compromised, this has provided new evidence showing that the level of MeCP2 correlates with disease severity. The development of these mice and future mouse lines will provide an invaluable tool for further research into RTT as well as providing an animal model system for the preclinical evaluation of pharmacological therapeutics.

Key Terms

Chromatin Immunoprecipitation. Biochemical technique to assess the endogenous interaction between a protein and DNA (or in some cases RNA) in vivo.

DNA Methylation. A DNA modification consisting of the addition of a methyl group (CH_3) to cytosine residues located adjacent to guanine residues (in a so-called CpG orientation). It is generally thought to repress gene transcription.

Electroencephalography (EEG). A technique for recording electrical signals arising from the scalp. The coordinated activity of thousands or millions of neurons is recorded as changes in voltage.

Event-Related Potential (ERP). The averaged EEG response that is time locked to sensory stimuli, which is used as a measure of how well the brain responds to these external events.

Heterozygous. Under normal circumstances, an individual has two copies of every gene, one from the father and one from the mother. If the sequence of these genes or of a particular genetic locus is different, the organism is said to be heterozygous for that gene or locus.

Hemizygous. Since males contain only one X-chromosome, under normal circumstances they have only one copy of each gene, or genomic locus, located on the X-chromosome; the organism is therefore hemizygous for that gene or locus.

Microencephalopathy. Decreased size or growth of head.

Missense Mutations. A mutation in the nucleotide sequence of a protein-coding gene that changes a codon to one encoding a different amino acid.

Rett Syndrome (*RTT*). An autism spectrum disorder affecting 1 in 10,000 girls caused by mutations within MeCP2.

X-Chromosome Inactivation (*XCI*). Female mammals have two X-chromosomes, while males have only one (and a Y-chromosome). In females, one X-chromosome is transcriptionally silenced (inactivated). The identity of the particular X-chromosome that is inactivated is random in mice and humans. On average, 50 % of cells in an individual will have a silenced paternal

X-chromosome, and the other 50 % of cells will have a silenced maternal X-chromosome. However, the percentage of cells in an individual with a particular X-chromosome silenced can be skewed in either direction.

Key Facts

Key facts about methyl-CpG binding protein 2 (MeCP2):

- MeCP2 was first cloned in 1992 as a methyl-CpG binding protein.
- MeCP2 is an extremely abundant nuclear protein.
- Mutations in MeCP2 were identified as the major cause of Rett syndrome in 1999.
- Doubling the levels of MeCP2 leads to severe neurological symptoms in patients.
- Loss of MeCP2 from mice leads to phenotypes that closely resemble those observed in Rett syndrome.
- The deletion of MeCP2 from postnatal or adult mice leads to phenotypes resembling those observed if MeCP2 is constitutively absent.
- The reintroduction of MeCP2 into mice can rescue the Rett syndrome-like phenotypes in mice that are otherwise lacking MeCP2.
- Deletion of MeCP2 leads to small changes in gene transcription, but many of those genes have been associated with autism or other neurological dysfunctions.

Summary Points

- This chapter focuses on mutation at MeCP2 T158 – the most common site for missense mutations in Rett syndrome.
- We generated a knockin mouse model that recapitulates the RTT-associated MeCP2 T158A mutation.
- MeCP2 T158A knockin mice recapitulate RTT-like symptoms observed in *Mecp2*-null mice.
- Wild-type mice show a developmental increase in event-related power and phase locking of auditory events. This is absent in MeCP2 T158A mice and *Mecp2*-null mice.
- MeCP2 T158A mutation leads to reduced MeCP2 binding to methylated DNA and decreased MeCP2 protein stability.

References

Amir RE, van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet. 1999;23:185–8.

Bader GG, Witt-Engerström I, Hagberg B. Neurophysiological findings in the Rett syndrome, II: visual and auditory brainstem, middle and late evoked responses. Brain Dev. 1989;11:110–4.
Ballestar E, Yusufzai TM, Wolffe AP. Effects of Rett syndrome mutations of the methyl-CpG binding domain of the transcriptional repressor MeCP2 on selectivity for association with methylated DNA. Biochemistry. 2000;39:7100–6.
Bienvenu T, Chelly J. Molecular genetics of Rett syndrome: when DNA methylation goes unrecognized. Nat Rev Genet. 2006;7:415–26.
Chahrour M, Zoghbi HY. The story of Rett syndrome: from clinic to neurobiology. Neuron. 2007;56:422–37.
Chahrour M, Jung SY, Shaw C, Zhou X, Wong STC, Qin J, Zoghbi HY. MeCP2, a key contributor to neurological disease, activates and represses transcription. Science. 2008;320:1224–9.
Charman T, et al. Dimensional phenotypic analysis and functional categorisation of mutations reveal novel genotype-phenotype associations in Rett syndrome. Eur J Hum Genet. 2005;13:1121–30.
Chen RZ, Akbarian S, Tudor M, Jaenisch R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. Nat Genet. 2001;27:327–31.
Cheval H, Guy J, Merusi C, De Sousa D, Selfridge J, Bird A. Postnatal inactivation reveals enhanced requirement for MeCP2 at distinct age windows. Hum Mol Genet. 2012;21:3806–14.
Collins AL, Levenson JM, Vilaythong AP, Richman R, Armstrong DL, Noebels JL, David Sweatt J, Zoghbi HY. Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. Hum Mol Genet. 2004;13:2679–89.
Colvin L, Leonard H, de Klerk N, Davis M, Weaving L, Williamson S, Christodoulou J. Refining the phenotype of common mutations in Rett syndrome. J Med Genet. 2004;41:25–30.
Fukuda T, Yamashita Y, Nagamitsu S, Miyamoto K, Jin J-J, Ohmori I, Ohtsuka Y, Kuwajima K, Endo S, Iwai T, Yamagata H, Tabara Y, Miki T, Matsuishi T, Kondo I. Methyl-CpG binding protein 2 gene (MECP2) variations in Japanese patients with Rett syndrome: pathological mutations and polymorphisms. Brain Dev. 2005;27:211–7.
Gandal MJ, Edgar JC, Ehrlichman RS, Mehta M, Roberts TPL, Siegel SJ. Validating γ oscillations and delayed auditory responses as translational biomarkers of autism. Biol Psychiatry. 2010;68:1100–6.
Ghosh RP, Horowitz-Scherer RA, Nikitina T, Gierasch LM, Woodcock CL. Rett syndrome-causing mutations in human MeCP2 result in diverse structural changes that impact folding and DNA interactions. J Biol Chem. 2008;283:20523–34.
Goffin D, Allen M, Zhang L, Amorim M, Wang I-TJ, Reyes A-RS, Mercado-Berton A, Ong C, Cohen S, Hu L, Blendy JA, Carlson GC, Siegel SJ, Greenberg ME, Zhou Z. Rett syndrome mutation MeCP2 T158A disrupts DNA binding, protein stability and ERP responses. Nature Neurosci. 2012;15:274–83.
Guy J, Hendrich B, Holmes M, Martin JE, Bird A. A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat Genet. 2001;27:322–6.
Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. Science. 2007;315:1143–7.
Ho KL, McNae IW, Schmiedeberg L, Klose RJ, Bird AP, Walkinshaw MD. MeCP2 binding to DNA depends upon hydration at methyl-CpG. Mol Cell. 2008;29:525–31.
Huppke P, Laccone F, Krämer N, Engel W, Hanefeld F. Rett syndrome: analysis of MECP2 and clinical characterization of 31 patients. Hum Mol Genet. 2000;9:1369–75.
Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet. 1998;19:187–91.
Kerr AM, Archer HL, Evans JC, Prescott RJ, Gibbon F. People with MECP2 mutation-positive Rett disorder who converse. J Intellect Disabil Res. 2006;50:386–94.
Kokura K, Kaul SC, Wadhwa R, Nomura T, Khan MM, Shinagawa T, Yasukawa T, Colmenares C, Ishii S. The Ski protein family is required for MeCP2-mediated transcriptional repression. J Biol Chem. 2001;276:34115–21.

Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, Bird A. Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. Cell. 1992;69:905–14.

Lunyak VV, Burgess R, Prefontaine GG, Nelson C, Sze S-H, Chenoweth J, Schwartz P, Pevzner PA, Glass C, Mandel G, Rosenfeld MG. Corepressor-dependent silencing of chromosomal regions encoding neuronal genes. Science. 2002;298:1747–52.

McGraw CM, Samaco RC, Zoghbi HY. Adult neural function requires MeCP2. Science. 2011;333:186.

Nan X, Tate P, Li E, Bird A. DNA methylation specifies chromosomal localization of MeCP2. Mol Cell Biol. 1996;16:414–21.

Nan X, Campoy FJ, Bird A. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell. 1997;88:471–81.

Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature. 1998;393:386–9.

Neul JL, Kaufmann WE, Glaze DG, Christodoulou J, Clarke AJ, Bahi-Buisson N, Leonard H, Bailey MES, Schanen NC, Zappella M, Renieri A, Huppke P, Percy AK, RettSearch Consortium. Rett syndrome: revised diagnostic criteria and nomenclature. Ann Neurol. 2010;68:944–50.

Schanen C, Houwink EJF, Dorrani N, Lane J, Everett R, Feng A, Cantor RM, Percy A. Phenotypic manifestations of MECP2 mutations in classical and atypical Rett syndrome. Am J Med Genet A. 2004;126A:129–40.

Schwenk F, Baron U, Rajewsky K. A cre-transgenic mouse strain for the ubiquitous deletion of loxP-flanked gene segments including deletion in germ cells. Nucleic Acids Res. 1995;23:5080–1.

Shahbazian MD, Antalffy B, Armstrong DL, Zoghbi HY. Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. Hum Mol Genet. 2002;11:115–24.

Skene PJ, Illingworth RS, Webb S, Kerr ARW, James KD, Turner DJ, Andrews R, Bird AP. Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. Mol Cell. 2010;37:457–68.

Stauder JEA, Smeets EEJ, van Mil SGM, Curfs LGM. The development of visual- and auditory processing in Rett syndrome: an ERP study. Brain Dev. 2006;28:487–94.

Sutcliffe JS, Nakao M, Christian S, Orstavik KH, Tommerup N, Ledbetter DH, Beaudet AL. Deletions of a differentially methylated CpG island at the SNRPN gene define a putative imprinting control region. Nat Genet. 1994;8:52–8.

Szulwach KE, Li X, Smrt RD, Li Y, Luo Y, Lin L, Santistevan NJ, Li W, Zhao X, Jin P. Cross talk between microRNA and epigenetic regulation in adult neurogenesis. J Cell Biol. 2010;189:127–41.

Uhlhaas PJ, Pipa G, Lima B, Melloni L, Neuenschwander S, Nikolić D, Singer W. Neural synchrony in cortical networks: history, concept and current status. Front Integr Neurosci. 2009;3:17.

Vacca M, et al. Mutation analysis of the MECP2 gene in British and Italian Rett syndrome females. J Mol Med. 2001a;78:648–55.

Vacca M, et al. MECP2 gene mutation analysis in the British and Italian Rett Syndrome patients: hot spot map of the most recurrent mutations and bioinformatic analysis of a new MECP2 conserved region. Brain Dev. 2001b;23 Suppl 1:S246–50.

Wu H, Tao J, Chen PJ, Shahab A, Ge W, Hart RP, Ruan X, Ruan Y, Sun YE. Genome-wide analysis reveals methyl-CpG-binding protein 2-dependent regulation of microRNAs in a mouse model of Rett syndrome. Proc Natl Acad Sci USA. 2010;107:18161–6.

Young JI, Hong EP, Castle JC, Crespo-Barreto J, Bowman AB, Rose MF, Kang D, Richman R, Johnson JM, Berget S, Zoghbi HY. Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. Proc Natl Acad Sci USA. 2005;102:17551–8.