Rett syndrome: insights into genetic, molecular and circuit mechanisms

Citation: Ip, Jacque P. K. et al. "Rett syndrome: insights into genetic, molecular and circuit mechanisms." Nature Reviews Neuroscience 19, 6 (May 2018): 368–382 © 2018 Macmillan Publishers Ltd., part of Springer Nature.

As Published: http://dx.doi.org/10.1038/s41583-018-0006-3

Publisher: Springer Science and Business Media LLC

Persistent URL: https://hdl.handle.net/1721.1/127274

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

HHS Public Access

Author manuscript

Nat Rev Neurosci. Author manuscript; available in PMC 2019 March 06.

Published in final edited form as:

Nat Rev Neurosci. 2018 June ; 19(6): 368–382. doi:10.1038/s41583-018-0006-3.

Rett syndrome: insights into genetic, molecular and circuit mechanisms

Jacque P. K. Ip[1], **Nikolaos Mellios**[2], and **Mriganka Sur**[1]

[1]Department of Brain and Cognitive Sciences, Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA

[2]Department of Neurosciences, School of Medicine, The University of New Mexico, Albuquerque, NM, USA

Abstract

Rett syndrome (RTT) is a severe neurological disorder caused by mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). Almost two decades of research into RTT have greatly advanced our understanding of the function and regulation of the multifunctional protein MeCP2. Here, we review recent advances in understanding how loss of MeCP2 impacts different stages of brain development, discuss recent findings demonstrating the molecular role of MeCP2 as a transcriptional repressor, assess primary and secondary effects of MeCP2 loss, and examine how loss of MeCP2 can result in an imbalance of neuronal excitation and inhibition at the circuit level along with dysregulation of activity-dependent mechanisms. These factors present challenges to the search for mechanism-based therapeutics for RTT and suggest specific approaches that may be more effective than others.

Introduction

Rett syndrome (RTT; OMIM identifier #312750), first reported by the Austrian physician Andreas Rett, is a devastating neurological disorder that affects brain development and function in females in approximately 1 in 10,000 live births[1,2]. In 90–95% of patients diagnosed with classic RTT, the disease is caused by loss-of-function mutations in the X-linked MECP2 gene, which encodes methyl- CpG binding protein 2 (MeCP2).

MECP2 gene mutations predominantly arise in germ cells from the paternal side[3]. Despite the hundreds of RTT-causing MECP2 mutations that have been identified, eight ‘hotspot’ mutations (encoding the amino acid substitutions R106W, R133C, T158M, R168X, R255X, R270X, R294X and R306C) constitute more than 60% of documented cases[4]. RTT-causing mutations in MECP2 in female patients lead to developmental regression, including a loss of speech and hand skills, after apparently normal development[5]. In females,

Correspondence to: Mriganka Sur.

Author contributions

J.P.K.I., N.M. and M.S. researched data for the article, contributed substantially to the discussion of content, wrote the article and reviewed and edited the manuscript before submission.

Competing interests The authors declare no competing interests.

random X chromosome inactivation results in somatic mosaics with normal and mutant MECP2. MECP2 mutations in males usually lead to severe congenital encephalopathies and death within 2 years[6].

MeCP2 was initially identified as a nuclear protein that binds methylated DNA[7] and was therefore described as a transcriptional repressor. Later, it was described as a multifunctional modulator of gene expression and as a transcriptional regulator with activating or repressing actions depending on the molecular context[8]. Recent evidence points to a role of MeCP2 as a weak repressor but with prominent secondary effects, including post-transcriptional regulation of gene expression through microRNA (miRNA)-mediated mechanisms[9].

A large body of research on RTT produced over two decades has greatly advanced our understanding of the functions and regulation of MeCP2 as well as the mechanisms underlying RTT pathophysiology. Furthermore, recent studies have offered new insights into the roles of MeCP2 at different developmental stages, MeCP2–DNA interactions, the importance of MeCP2 for excitation- inhibition (E/I) balance at the circuit level and the plasticity that underlies RTT. The purposes of this Review are to provide a synthesis of the field, to assess the primary and secondary molecular actions of MeCP2 and to discuss how loss of MeCP2 results in changes in circuit function that are associated with RTT.

Clinical features of RTT

RTT is characterized by apparently normal early development with subsequent regression. However, subtly visible symptoms, such as hypotonia, jerkiness in limb movements and limited social interaction (as reflected by lack of interest in others), are present during early infancy[10]. Stunted head growth between 2 and 4 months of age may be diagnosed as acquired microcephaly and is an early sign of neurologic involvement[10]. The main symptoms often begin at around 12–18 months and include arrested cognitive and motor development and loss of acquired verbal skills. In addition, normal hand function is often replaced with stereotyped repetitive hand movements[10, 11]. This developmental stagnation and regression of acquired skills is characteristic of RTT. Another clinical hallmark is ataxia, which often results in motor disability, rendering affected individuals wheelchair-bound by the teenage years. Autonomic dysfunction and respiratory problems, seizures, anxiety and orthopaedic problems are also common in individuals with RTT[10, 11]. Impaired development of visual processing and reduced visually evoked cortical responses have been reported in some patients[12, 13].

The disease eventually plateaus and stabilizes[10]. Individuals with RTT live into adulthood and often require total care. Individuals with RTT may transiently exhibit autistic-like behaviours, such as social withdrawal and avoidance of eye gaze, during the regression phase, but they often become more interactive and social with age[14]. Notably, RTT is no longer listed as an autism spectrum disorder (ASD) in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V)[15]. However, RTT patients who show autistic-like features may be described as having ASD associated with RTT.

Neural phenotypes of RTT

The gross observation of reduced brain volume in individuals with RTT is associated with abnormally small and densely packed neurons that are reduced in dendritic complexity and spine density[16]. The dendrites of pyramidal neurons in the motor and frontal cortices are considerably shorter than in non-RTT brains[16]. MRI data demonstrate selective reductions in the volume of the parietal and temporal lobes in female RTT patients, with preservation of the occipital cortex[17].

Following the identification of MECP2 as the principal genetic factor underlying RTT[18], enormous effort has been focused on modelling the disease using mouse and other animal models (Box 1) and human induced pluripotent stem cell (iPSC)-derived models (Box 2).

MeCP2 loss affects different cell types in multiple brain regions

MeCP2 is ubiquitously expressed but is enriched in the brain. Brain-specific deletion of Mecp2 (Nestin–Mecp2$^{-/y}$) in mice captures key phenotypes, including reduced movement, abnormal gait, hindlimb clasping, respiratory abnormalities and early lethality, that present in Mecp2-null mice[19, 20]. This finding highlights a crucial role for the specificity of MeCP2 in the brain.

Region-specific knockout in the brain further reveals diverse roles of MeCP2 across various brain regions. For example, deletion of Mecp2 in the brainstem and spinal cord leads to abnormal heart rate and breathing patterns and early lethality[21, 22]; removal from dopaminergic and noradrenergic neurons induces motor incoordination[23]; and removal from serotonergic neurons increases aggression[23]. Evidently, the region-specific loss of Mecp2 induces a subset of RTT phenotypes that correspond to the function of the targeted region. Furthermore, the loss of MeCP2 in all inhibitory neurons (in Viaat–Mecp2$^{-/y}$ mice) strikingly reproduces severe forms of RTT phenotypes, highlighting the important contribution of inhibitory neurons and E/I balance to MeCP2-mediated functions in the brain[24]. Deletion of Mecp2 from astrocytes in mice has relatively minor phenotypic consequences, but restoration of MeCP2 in astrocytes in an otherwise MeCP2-deficient nervous system results in marked amelioration of phenotypes[25]. Transplantation of wild-type microglia to a mouse model of RTT was reported to reverse symptoms[26]; however, multiple attempts to reproduce this finding were not successful[27], pointing to the complexity of non-neuronal, potentially secondary, effects of MeCP2. Microglia have been suggested to contribute to RTT pathogenesis by excessively eliminating presynaptic inputs in fully symptomatic Mecp2-null mice. However, microglial-specific deletion (in Cx3cr1^{CreER}–Mecp2$^{-/y}$ mice) has minimal effects on microglial phagocytosis and synaptic loss, suggesting involvement of other cell types in this effect[28].

Loss of MeCP2 affects multiple developmental stages

Consistent with studies of post-mortem RTT brains, several early studies of mouse models suggested RTT to be a disorder of delayed neuronal maturation and synapse formation. These mouse models revealed reductions in neuronal soma size, dendritic arborization, spine density and postsynaptic density protein 95 (PSD95) immunoreactivity as well as abnormal

spine morphology[29, 30, 31]. Such findings led to the belief that MeCP2 is predominantly involved in the maturation of neuronal function, through processes including synaptogenesis, synaptic plasticity and establishment of functional circuits. In line with the structural defects, alterations in synaptic transmission are observed at the circuit level in Mecp2-mutant mice. Loss of MeCP2 leads to changes in neuronal excitability and deficits in experience-dependent plasticity processes, such as homeostatic synaptic scaling, resulting in altered neuronal connectivity and shifts in E/I balance at the circuit level (see details below). These functional deficits result from a failure of activity-dependent feedback mechanisms that require MeCP2 to establish and maintain functional brain circuits during neuronal maturation and throughout life[32, 33]. However, increasing evidence also suggests that MeCP2 has diverse and distinct roles in all stages of brain development, including the earliest stages of neurogenesis and neuronal migration (Fig. 1).

Initial reports in mouse models reported low MeCP2 expression in neural progenitor cells (NPCs) — with little effect of MeCP2 deficiency incultured neurospheres — and a progressive increase during embryonic and postnatal development[34, 35]. More recent evidence now indicates that loss of MeCP2 affects early developmental stages considerably. Clinical studies report subtle symptoms before 6 months of age[10, 36, 37, 38] and early developmental delays before the full manifestation of RTT[36, 39, 40]. Male babies born with a mutant MECP2 allele, although very rare owing to the predominantly paternal origin of MECP2 mutations, display severe neurological deficits from birth, including disproportionately small frontal and temporal lobes characteristic of a prenatal pathogenesis[6].

Diverse phenotypes of RTT are present during neurogenesis and neuronal migration and during the later stages of synaptic and circuit development and plasticity[41, 42]. Immunohistochemistry studies comparing wild-type with MeCP2-deficient cells suggest that MeCP2 is expressed in the prenatal cortex in mouse[42] and in human iPSC-derived NPCs[43]. The neurogenic function of MeCP2 has been demonstrated in mouse NPC cultures: overexpression of MeCP2 in these cultures inhibited glial differentiation but promoted neuronal differentiation[44]. Conversely, embryonic NPCs extracted at embryonic day 15.5 from Mecp2-null mice exhibit a more proliferative identity and morphological alterations as early as 3 days in vitro[42]. In Xenopus laevis, MeCP2 regulates the transcriptional expression of the neuronal repressor gene XHairy2a (also known as hes4); depletion of MeCP2 protein and expression of a truncated form of MeCP2 (R168X) result in a decrease and an increase in the number of NPCs, respectively[45]. In zebrafish, genetic deletion of mecp2 inhibits neuronal differentiation, whereas Mecp2 overexpression promotes differentiation[46]. Similarly, measurement of the expression of NPC markers and early neuronal markers in the Mecp2-null mouse brain shows an increase in NPC markers and a decrease in neuronal markers, suggesting a possible imbalance in prenatal brain development[42]. Moreover, alterations in human neurogenesis and neuronal differentiation are observed in patient-derived iPSC cultures and cerebral organoids[41]. Even small deflections in the developmental programme may predispose the embryo to complex phenotypes later on. Such reports of an early developmental role for MeCP2 are particularly important when we consider treatment time points for RTT patients. Although postnatal re-expression of MeCP2 at later stages in Mecp2-mutant mice partially rescues several RTT

phenotypes, including lifespan[47, 48], the above findings suggest that earlier treatment would increase its effectiveness.

Earlier and more recent evidence suggests that MeCP2 is required for the maintenance of mature neural networks[49]. Removing MeCP2 during postnatal development or in adulthood leads to rapid progression of RTT-like symptoms, including lethality[50, 51]. Several synaptic proteins, including NMDA receptor (NMDAR) subunits and presynaptic vesicular glutamate transporter 1 (VGLUT1), are reduced upon postnatal removal of MeCP2 (Ref.[50]). Intriguingly, the levels of mRNA encoding these proteins remain unchanged, suggesting that MeCP2 probably regulates the levels of these proteins post-transcriptionally[50]. Further confirming the requirement of MeCP2 in the adult brain, in vivo genome editing by injection of adeno-associated viral vector (AAV)–Streptococcus pyogenes Cas9 and guide RNA targeting Mecp2 in adult mouse primary visual cortex (V1) depleted MeCP2 in 70% of the infected cells and leads to altered visual responses, including reduced orientation selectivity and peak response[52].

Molecular effects

Since the discovery in 1999 that mutations in the MECP2 gene were the underlying cause of RTT[18], tremendous progress has been made in understanding the molecular functions of MeCP2. Interacting with DNA is an essential part of MeCP2 function, as MeCP2 is primarily a chromatin-bound nuclear protein that acts as an epigenetic reader. On the basis of functional studies, MeCP2 has been proposed to regulate gene transcription, chromatin compaction and miRNA processing[53]. This section discusses the molecular effects of MeCP2 and how its loss contributes to neurological deficits in RTT.

Epigenetics of MeCP2–DNA interactions

MeCP2 was originally found to bind specifically to DNA that contains methylated cytosines in CpG dinucleotides (mCG), via its methyl-CpG-binding domain (MBD), thus helping to repress gene expression[7, 54, 55]. However, it was recently reported that MeCP2 also interacts with methylated CA (mCA) sites[56], and another report further specified the binding of MeCP2 to methylated CAC (mCAC) trinucleotides[57]. Intriguingly, mCA is more abundant in postmitotic neurons, accumulates throughout development and seems to promote transcriptional repression[58, 59]. The sites that harbour mCA modifications are frequently found in long genes that have been shown to be preferentially expressed in RTT[59]. mCA sites are concentrated within gene bodies, which further suggests that MeCP2 obstructs transcription within transcribed regions[60]. A recent study suggested that mCA sites are methylated during early postnatal life by DNA (cytosine-5)-methyltransferase 3A (DNMT3A)[61] and that activity-induced sites are less DNMT3A bound and are thus less methylated. This mCA-mediated repressive effect of MeCP2 is believed to mediate cell-type-specific transcriptional fine-tuning[61].

The ability of MeCP2 to interact with DNA bearing 5-hydroxymethylcytosine (5hmC) was previously controversial. Several independent studies now show that MeCP2 can bind to hydroxymethylated CA (hmCA) sites, but with a much lower affinity than to

hydroxymethylated CG (hmCG) sites (see Ref.[56] for a more detailed discussion). 5hmC is frequently associated with gene activation (possibly owing to its low binding affinity to MeCP2), whereas the binding of MeCP2 to mCG and mCA is associated with repression[62]; specifically, 5hmC is enriched in genes that are actively expressed in neurons[62]. It is possible that the hmCG modification may result in 'functional demethylation' of mCG sites, as it may promote the detachment of MeCP2 and thus achieve derepression of transcription[63]. One model that synthesizes these recent findings on MeCP2–DNA interaction[56, 60, 61, 63] posits that activity and experience can modulate DNA methylation, through enzymatic actions, for the fine-tuning of MeCP2- regulated gene transcription (Fig. 2).

MeCP2 as a transcriptional regulator

A large body of data on the role of MeCP2 indicates that it is primarily a transcriptional repressor[53], although MeCP2 has also been suggested to act as a transcriptional activator[8].

A role for MeCP2 in transcriptional repression was supported by the negative correlation between mCA levels and transcription of long genes[59]. Moreover, two separate studies emphasized the proportional relationship between DNA methylation levels, MeCP2 binding on the genome and transcriptional repression[57, 60]. MeCP2 is highly abundant (there are an estimated 16 million molecules of MeCP2 per neuronal nucleus, compared with an estimated 32 million nucleosomes per nucleus), suggesting that it binds genome wide[64]. Thus, converging lines of evidence suggest that the repressive effects of MeCP2 are genome wide and positively correlated with the density of methylation of CG and CA sites in gene bodies[65].

Furthermore, microarray profiling and whole-cell RNA sequencing indicate that the repressive effect of MeCP2 is biased towards long genes larger than 100 kb, and expression of mutant MeCP2 leads to upregulation of the expression of long genes[59, 66]. More specifically, highly methylated long genes — and not lowly methylated long genes — are upregulated in MECP2 mutants[59, 60]. A recent study analysing mice carrying specific RTT-associated Mecp2 mutations (namely, those encoding the T158M and R106W substitutions) showed that gene expression changes are largely mutation-specific and cell-type-specific[67]. Nuclear transcriptomes from forebrain neurons of these mutant mice reveal reductions in the nascent transcription of long genes and suggest a functional compensation for the reductions in long gene expression at the whole-cell level via post-transcriptional mechanisms[67]. However, this study did not take into account the methylation status of the long genes affected by MeCP2 loss, which is particularly important because other studies found that only highly methylated long genes are upregulated in Mecp2-knockout mice[59, 60]. Future studies will be required to further delineate the effects of various MECP2 mutations on the transcriptional regulation of long genes in different neuronal cell types and to correlate these effects with the methylation status of long genes.

Considerable progress has recently been made in resolving the molecular mechanisms underlying the repressor role of MeCP2. MeCP2 has been reported to interact with the

histone deacetylase (HDAC)–SIN3A co-repressor complex as well as with the HDAC3-containing nuclear receptor co-repressor (NCoR)-silencing mediator for retinoic acid and thyroid hormone receptor' (SMRT) complex[55, 68]. The recruitment of the NCoR–SMRT complex has been particularly implicated in mediating the repressive effects of MeCP2 (Ref. [68]). The NCoR–SMRT complex contains multiple subunits, including NCoR, SMRT, HDAC3, G protein pathway suppressor 2 (GPS2) and transducin βlike protein 1 (TBL1) or its paralogue TBL1-related protein 1 (TBLR1)[69]. MeCP2 recruits the NCoR–SMRT complex by directly interacting with TBL1 and TBLR1 through its NCoR–SMRT interaction domain (NID) within its transcriptional repression domain (TRD)[69]. In this model, the HDAC3-containing NCoR–SMRT complex is recruited by MeCP2 when MeCP2 is bound to DNA, and modulates chromatin structure by removing acetyl groups from histone lysine residues[55, 68] (Fig. 3). Notably, RTT-associated Mecp2 mutations (such as R306C) that affect the NID of MeCP2 result in severe RTT phenotypes[68], and a recent study demonstrated that the MBD and NID of MeCP2 may be most phenotypically relevant for RTT[70]. Thus, the primary function of MeCP2 is probably to connect methylated DNA with the NCoR- SMRT co-repressor complex. The binding of the NCoR–SMRT complex to MeCP2 is also regulated in an activity-dependent manner (see below) and is required for MeCP2-mediated gene repression[68, 71]. Although the relationship between methylation density and MeCP2 occupancy across the genome is well established, whether the MeCP2–NCoR–SMRT–HDAC3 complex is recruited in a density-dependent way, and is concentrated in the gene bodies of long genes, remains to be determined.

Notably, transcriptome analyses have revealed upregulation and dowru·egulation of different genes in MeCP2-deficient mouse brains(8, 72, 73). As such, MeCP2 has been suggested to also act as a transcriptional activator and is thought to do so via its interaction with cAMP-responsive element-binding protein 1 (CREBl) (8). However, no RTT mutation has yet been reported to interupt MeCP2-CREB1 interaction. One possible explanation for the downregulation of a large numbers of genes in MeCP2-deficient neurons is that the transcriptional machinery does not function optimally, as reflected by the reduction of soma size and total RNA in RTT mouse models and in iPSC-derived neurons from individuals with RTT[57, 74]. This suggestion is consistent with the proposed function of MeCP2 as a multifunctional hub of cellular metabolism[74].

A recent study suggested that MeCP2 might act as a positive regulator for a subset of neuronal genes via its NID[75]. At certain gene promoters, MeCP2 recruits HDAC3 to deacetylate the transcription factor forkhead box protein O3 (FOXO3) to positively regulate transcription (Fig. 3). In support of this mechanism, Hdac3-mutant mice (which show RTT-like phenotypes) and human iPSC-derived NPCs harbouring $MECP2^{R306C}$ show mislocalization of FOXO3 (outside of gene promoters) and downregulation of target genes[75]. Nevertheless, this mechanism is surprising, as methylation density and MeCP2 occupancy are known to be low in transcription start sites, and MeCP2 generally binds within gene bodies[57, 76]. To reconcile these observations, one possibility is that the binding of MeCP2 to the gene body represses transcription, whereas its recruitment of HDAC3 to certain gene promoters positively regulates transcription through FOXO3 deacetylation[75].

An additional model proposes that MeCP2 directly interacts with DNA and thus modifies chromatin architecture globally[64, 77] (Fig. 3). Mecp2-null brains show global alterations in neuronal chromatin architecture, including increases in histone acetylation and elevated levels of linker histone H1 (Ref.[64]). As the NID contains a cluster of basic amino acids, it may act as a DNA-binding domain[78]. In addition, MeCP2 has three AT hooks that could bend DNA and assist chromatin-structure remodelling[77].

Also relevant to a role for MeCP2 in maintaining chromatin structure is the interaction between MeCP2 and the transcription regulating helicase ATRX. MeCP2 and ATRX colocalize on pericentric heterochromatin, and ATRX is mislocalized in MeCP2-deficient neurons[77, 79]. The MeCP2–ATRX interaction may be important for the formation of higher-order chromatin loops[77]. However, more direct evidence is required to demonstrate how MeCP2 may regulate chromatin structure, as well as the role of a loss of MeCP2–ATRX interactions in RTT pathology.

Interplay between MeCP2 and miRNAs

miRNAs are important for many developmental processes, including neurogenesis, brain maturation and synaptic plasticity[80, 81]. miRNAs have been reported to be dysregulated in the (total) brain, hippocampus and cortex of Mecp2-null mice[9, 82, 83, 84, 85]; in mouse MeCP2-deficient neuronal cultures[86, 87, 88, 89]; and in iPSC-derived neuronal cultures and cerebral organoids generated from individuals with RTT[41]. The importance of miRNA- MeCP2 interactions in understanding RTT is being determined by studies of how MeCP2 loss alters miRNA expression[9, 41, 86, 87, 88, 89] and the different MeCP2 domains (discussed above) and RTT-associated MECP2 mutations that could be implicated in miRNA alterations[9, 41].

MeCP2 represses the transcription of primary miRNAs (pri-miRNAs), including miR-137, miR-15a, miR-184 and miR-7b, by binding to mCG sites in the gene promoter regions[86, 87, 88, 89]. MeCP2 deficiency results in increased pri- miRNA transcription, which leads to increased mature miRNA levels. Therefore, RTT-associated mutations in the genetic sequence encoding the MeCP2 MBD and TRD or NID are expected to result in increases in such MeCP2-regulated intergenic miRNAs. Furthermore, MeCP2 can directly inhibit miRNA expression by blocking pri-miRNA processing. MeCP2 may do this by binding to the microprocessor protein DiGeorge syndrome critical region 8 (DGCR8) via the carboxy-terminal domain[9] and thus hindering the interaction of DGCR8 with the other main component of the microprocessor complex, ribonuclease 3 (DROSHA) (Fig. 3). This mode of action has been shown to lead to increased pri-miR-134 processing and mature miR-134 expression in the hippocampus of adult Mecp2-mutant mice[9]. In individuals with RTT who carry MECP2 C- terminal mutations or truncations, the binding between MeCP2 and DGCR8 is expected to be abolished, leaving DROSHA free to bind and activate pri-miR-134 processing (Fig. 3). Intriguingly, MeCP2 loss-mediated increases in miR-137, miR-15a, miR-7b and miR-134 each lead to impaired dendritic maturation[87, 89, 90], a major feature of RTT.

Another set of miRNAs are indirectly regulated by MeCP2. miR-199 is robustly upregulated in iPSC-derived neuronal cultures from individuals with RTT who carry a missense mutation encoding a R106W substitution in the MBD of MeCP2, or a frameshift mutation that results in a truncated transcript (V247X), and in MeCP2-deficient mouse neurons[41]. Bone morphogenic protein 4 (BMP4) activates SMAD signalling, which in turn promotes the processing of pri-miRNAs, and BMP4 is upregulated in certain MeCP2-mutant models[91, 92]. Therefore, the upregulation of mature miR-199 observed in the RTT cultures probably results from elevated BMP4–SMAD signalling (Fig. 3). The upregulation of miR-199 inhibits extracellular-signal-regulated kinase signalling and disrupts early neuronal development and differentiation[41]. Similarly, MeCP2 deficiency results in increased let-7f miRNA expression in the postnatal mouse brain, through reductions in the BDNF-regulated repressor of let-7 miRNA biogenesis, protein lin-28 homologue A (LIN28A)[93, 94]. In turn, this increase in let-7f inhibits the expression of insulin-like growth factor 1 (IGF1) in the postnatal mouse brain[84]. Treatment with the β_2-adrenergic receptor agonist, clenbuterol, restores the affected molecular pathway and ameliorates diverse symptoms of Mecp2$^{-/y}$ mice and Mecp2$^{+/-}$ mice[84]. Taken together, these findings suggest that RTT-relevant mutations[70] could alter diverse miRNA pathways downstream of MeCP2 and result in disturbances in brain development and maturation.

Last, miRNAs may regulate MeCP2 expression. For example, miR-483–5p regulates MeCP2 isoforms through a human-specific target site in the 3′- untranslated region of MECP2 in developing neurons[95]. In addition, miR-200a and miR-302c inhibit MeCP2 expression in human embryonic stem cells[96], and miR-130a regulates neurite and synaptic maturation in the developing cortex through its inhibitory effect on MeCP2 expression[97]. Intriguingly, some miRNAs, such as miR-132 and miR-7b, act in a feedback loop as both targets and regulators of MeCP2 expression to fine-tune MeCP2 levels in the postnatal brain[85, 89, 98].

MeCP2 binds broadly across the genome; therefore, it will remain a challenge to deduce the primary actions of MeCP2 from RNA analyses. Thus, future studies focusing on MeCP2 binding sites (such as methylated DNA) and relating these to miRNA expression will provide crucial information for further analyses of MeCP2 function and about the roles of miRNAs in RTT.

Excitatory and inhibitory circuits

Neurological symptoms in individuals with RTT and in Mecp2-mutant mice result from neural circuit alterations brought on by the loss of MeCP2. In Mecp2- mutant mice, E/I balance in circuits and brain regions is complexly altered; the maintenance of this balance is crucial for nearly every aspect of normal brain function.

Neuronal circuit deficits with MeCP2 loss are region-specific

Somatosensory cortex neurons in brain slices and cultured neurons from Mecp2$^{-/y}$ mice exhibit smaller miniature excitatory postsynaptic current (mEPSCs), with no changes in miniature inhibitory postsynaptic currents (mIPSCs), resulting in an overall reduced excitation[29, 99, 100]. In vivo whole- cell recordings of visually evoked responses in V1

pyramidal neurons reveal a combined reduction of excitatory and inhibitory conductance in Mecp2$^{-/y}$ mice, with a net increase in the E/I ratio[101]. Similarly, there is a shift towards hyperexcitation in the CA1 and CA3 region of the hippocampus in Mecp2$^{-/y}$ mice resulting from reduced mIPSCs[102], and synaptic hyperexcitability is observed in brainstem areas, including the locus coeruleus[103], ventrolateral medulla[104, 105] and nucleus tractus solitarus[106].

As a direct demonstration of the region-specific changes in activity in Mecp2$^{-/y}$ mice, expression of the immediate early gene Fos is decreased in the forebrain and increased in the hindbrain[106]. Gene expression analyses of different neuronal cell types in Mecp2-knockout mouse brains showed variation in different sets of genes in these different cell populations[66]. Clinically, selective volume reductions in the dorsal parietal and anterior frontal lobe regions in individuals with RTT also suggest that some brain regions are more affected than others[17].

Overall, these studies highlight an intriguing region specificity of disruption to neural circuits that may explain the clinical features of RTT. For example, reduced excitation and connectivity in forebrain motor areas probably contributes to motor dysfunction in RTT, whereas hyperexcitation in the brainstem region probably leads to abnormalities in breathing patterns[21]. Similarly, hyperexcitability in the hippocampus probably contributes to the limbic seizures observed in Mecp2-mutant mice and people with RTT[107].

Although the mechanisms underlying this region specificity remain unclear, two non-mutually exclusive possibilities can be extrapolated. First, MeCP2 may differentially affect different brain regions. Second, although MeCP2 may function similarly in different regions, some neurons or circuits may be more vulnerable to MeCP2 dysfunction than others, owing to their connectivity (see below).

MeCP2 loss affects excitatory and inhibitory neurons

Although the molecular effects of MeCP2 seem to be primarily mediated by its MBD and NID (see previous section), the functional effects of MeCP2 are region-specific and cell-type-specific (for example, selective for exhibitory or inhibitory neuron subtypes). The functional effects of MeCP2 loss depend on the disruption of E/I imbalance within neuronal circuits[101], and neuronal circuits seem to be particularly sensitive to MeCP2 loss from inhibitory neurons[24]. We therefore suggest that the molecular effects of MeCP2 are not equal across functional cell classes and functional brain regions. Deletion of MeCP2 from functional cell types can provide insights into the primacy of MeCP2 function in specific cell types. One study that used cell-type-specific biotin tagging of MeCP2 in wild-type mice, Mecp2$^{T158M/y}$ mice and Mecp2$^{R106W/y}$ mice to profile nuclear transcriptomes revealed distinct sets of differentially regulated genes in excitatory and inhibitory neurons in the Mecp2 mutants[67]. This finding is consistent with the observation that the landscape of DNA methylation varies between excitatory and inhibitory neurons[61, 108]. If the methylation landscape varies across cell types, MeCP2 may exert different molecular actions in different cell types, leading to cell-specific transcriptional alterations with MeCP2 mutations.

Several symptoms of whole-brain Mecp2 deletion, including ataxia, repetitive behaviours, deficits in motor coordination and obesity, are strikingly recapitulated in mice with Mecp2 deletion in inhibitory neurons (Viaat-Mecp2$^{-/y}$ mice). In addition, the reductions in GABA synthesis and mIPSCs in Viaat- Mecp2$^{-/y}$ mice[24] are also observed in whole-brain Mecp2 deletion[101, 109]. Mice lacking MeCP2 in forebrain excitatory neurons exhibit milder behavioural phenotypes, including tremor and anxiety-like behaviours, which are less evident in mice lacking MeCP2 in inhibitory neurons[110, 111]. Furthermore, re- expression of MeCP2 in inhibitory neurons, but not in excitatory neurons, rescues motor coordination deficits in Mecp2-knockout mice[110, 112]. These studies indicate that primary deficits in excitatory signalling or inhibitory signalling caused by cell-type-specific MeCP2 loss lead to distinct neurological phenotypes.

Inhibitory neurons and RTT

GABAergic inhibitory neurons account for up to 20% of the total neurons in the brain[113]. In particular, subtypes of inhibitory neurons are known to have very different developmental programmes, circuit influences and effects on activity- dependent plasticity[113, 114]. Among the interneuron populations are three major subclasses that directly influence pyramidal neurons: soma-targeting parvalbumin (PV)-expressing neurons, dendrite-targeting somatostatin (SOM)- expressing neurons and vasoactive intestinal polypeptide (VIP)-expressing neurons[113, 115, 116]. SOM^{+} neurons integrate large numbers of inputs[117] and seem to be crucial for feedback or top-down modulation[118]. VIP^{+} neurons are a subset of the serotonin receptor (5-HT$_{3A}$)-expressing subclass of inhibitory neurons and receive top-down inputs; they disinhibit pyramidal neurons by inhibiting other inhibitory neurons[113, 119]. PV^{+} neurons form intricate nests of synaptic contacts on the soma of target pyramidal neurons, providing fast control over their excitability[115]. PV^{+} neurons, which mainly subserve feedforward inhibition and gain control[120, 121], have been suggested to be the major drivers of E/I balance in cortical circuits[122].

Intriguingly, Mecp2 deletion from different GABAergic neuronal subtypes generates differential and non-overlapping symptoms and phenotypes; mice lacking MeCP2 in PV^{+} neurons show sensory, motor and memory deficits, whereas mice lacking MeCP2 in SOM^{+} neurons exhibit seizures and stereotypies[123]. These different effects of Mecp2 deletion in inhibitory neuron subtypes possibly reflect the different developmental and integrative circuit properties of these subtypes.

Anatomically, Mecp2$^{-/y}$ mice, particularly juveniles, exhibit more PV^{+} puncta and increased PV^{+} neuron connections in the visual cortex compared with wild- type mice[109, 124]. In addition, Mecp2$^{-/y}$ mice show alterations in NMDAR subunit expression, with a greater NR2A:NR2B ratio in PV^{+} neurons, suggesting abnormally accelerated NMDAR maturation[125]. Reducing NR2A expression in Mecp2$^{-/y}$ mice (achieved by crossing heterozygous Mecp2$^{+/-}$ mice with NR2A- deficient males) reduces the PV^{+}-cell hyperconnectivity and prevents the regression of visual acuity[109, 125]. However, reductions in inhibition — including reduced PV^{+} neuron responses — have consistently been reported in V1 of adult Mecp2$^{-/y}$ mice, Viaat-Mecp2$^{-/y}$ mice (which lack MeCP2 from all GABAergic cells) and PV^{+} cell-specific Mecp$^{2-/y}$ mice[24, 101]. In addition, brain-wide

Mecp$2^{-/y}$ mice and mice lacking Mecp2 from PV^+ cells display impaired processing of complex visual stimuli by neurons in the visual cortex, consistent with severely reduced inhibition in vivo[101, 126].

One proposed explanation to reconcile reports of hyperconnectivity of PV^+ neurons with reduced functional inhibition of pyramidal neurons in Mecp2- mutant mice is that the effectiveness of GABA as an inhibitory neurotransmitter may be altered in RTT mouse models. Indeed, recent studies (see below) demonstrate that GABA is less hyperpolarizing in Mecp2-mutant mice[101] and in iPSC-derived neurons generated from RTT patients[127]. Thus, PV^+ neuron hyperconnectivity may represent a homeostatic, compensatory response to the reduction in inhibition in cortical circuits (Fig. 4). Neurodevelopmental disorders are marked by germline or early genetic mutations and a cascade of changes that are superimposed on developmental programmes of growth and plasticity; altogether, these factors lead to altered neuronal wiring and function. Thus, direct and indirect effects are deeply intertwined in creating functional as well as dysfunctional neural circuits[33].

KCC2:NKCC1 imbalance in RTT

The neuronal cation–chloride co-transporters KCC2 (a K^+/Cl^- exporter) and NKCC1 (a $Na^+/K^+/Cl^-$ importer) set the transmembrane chloride gradient and determine the maturation of GABAergic inhibition[128, 129]. During typical brain development, GABAergic currents shift their polarity from depolarization to hyperpolarization as the KCC2:NKCC1 ratio increases. GABA typically hyperpolarizes (inhibits) mature neurons, as they have very low intracellular chloride levels, whereas GABA has less inhibitory, or even depolarizing (excitatory), effects on immature neurons, which show high intracellular chloride concentrations[130]. Increased intracellular chloride concentrations and KCC2- NKCC1 imbalance have been reported in animal models of fragile X syndrome and in other neurological and neuropsychiatric disorders[131, 132].

Recent evidence suggests that such alterations are also present in RTT. Cerebrospinal fluid samples from individuals with RTT have revealed that levels of KCC2 relative to NKCC1 are reduced[133]. The expression of KCC2 in iPSC- derived neuronal cultures generated from individuals with RTT, and in V1 of Mecp$2^{-/y}$ mice, is also reduced and this reduction causes the reversal potential of GABA to be less negative than in controls[101, 127]. Overexpression of KCC2 in iPSC-derived neurons generated from individuals with RTT rescues this functional deficit[127]. IGF1 treatment, which partially rescues behavioural and synaptic deficits in mutant mouse models of RTT[29, 134], also increases the KCC2:NKCC1 ratio by promoting KCC2 expression and restoring the inhibitory action of GABA[135]. Thus, the circuit effects of MeCP2 loss crucially include reduced inhibition owing to reduced KCC2 expression and a reduced KCC2:NKCC1 ratio (Fig. 4).

Activity-dependent plasticity

Neuronal circuits are subject to experience-driven[136, 137] or learning- driven[138, 139, 140] changes that cause neurons within a microcircuit or an ensemble to modify their functional connectivity and responses[141]. The development of excitatory[142] and

inhibitory[143] microcircuits and the maintenance of E/I balance[122] require activity-dependent mechanisms, including Hebbian and homeostatic plasticity. Whereas the term 'Hebbian plasticity' describes associative and input-specific potentiation or depression of the strength of a synapse, homeostatic plasticity describes a negative feedback- driven mechanism that maintains appropriate levels of overall activity and synaptic strength in neurons and circuits[144]. Together, these mechanisms enable neuronal activity to induce pleotropic effects on gene expression to regulate synaptic molecule levels and function, and allow neuronal circuits to respond dynamically to experience.

Impaired homeostatic machinery resulting in altered experience-dependent refinement of circuits may be a core functional mechanism underlying autism and RTT[33, 145, 146]. Recent evidence indicates that early-life experience or changes in activity, such as that induced by dark rearing, alter methylation, which influences MeCP2 binding and hence gene expression[61]. The reverse is also true: Mecp2 mutations result in changes in experience-dependent plasticity, including (but not limited to) homeostatic plasticity[147, 148, 149]. For example, retinogeniculate synapses in Mecp2-null mice show a lack of experience-dependent refinement, despite an apparently normal initial development of synaptic connectivity[150]. Mecp2-mutant mice also show substantial impairments in experience-dependent plasticity in the visual cortex[29, 134]. Closure of one eye during a critical period of development can cause ocular dominance plasticity, which leads to decreased and increased visual cortex responses to the deprived and non-deprived eye, respectively[151]. In Mecp2- mutant mice, the critical period during which ocular dominance plasticity can be induced is prolonged and extends into adulthood, mainly because adult mice still show a persistent homeostatic upscaling of cortical responses to stimulation of the non-deprived eye[134]. Similarly, PV^+ cell-specific $Mecp2^{-/y}$ mice exhibit deprivation-induced plasticity in adulthood, accompanied by a reduction of inhibition[101]. This persistent experience-dependent plasticity is corrected by treatment with IGF1, which has crucial roles in the maturation of PV^+ circuits as well as GABAergic inhibition[29, 101]. A critical level of inhibition is also required to initiate plasticity. PV^+-cell-specific $Mecp2^{-/y}$ mice fail to show ocular dominance plasticity during the critical period, consistent with a reduction of inhibition, and restoring inhibition through intracortical infusion of the GABA agonist diazepam restores plasticity[126].

The activity-induced phosphorylation of MeCP2 at the NID is important for activity-dependent plasticity. Neuronal activity induces phosphorylation of MeCP2 at several sites, including T308 (Ref.[71]). Phosphorylation at T308 blocks the interaction of MeCP2 with the NCoR–SMRT complex, thus suppressing the ability of MeCP2 to repress transcription[71]. A human RTT- associated missense mutation in MECP2, leading to a R306C substitution, abolishes phosphorylation of MeCP2 at T308 and the interaction of MeCP2 with the NCoR–SMRT complex[68, 71]. Importantly, both $Mecp2^{R306C}$ and $Mecp2^{T308A}$ knock-in mice show RTT-like behavioural phenotypes, although $Mecp2^{R306C}$ knock-in mice display a more severe phenotype[68, 71]. A subset of activity-regulated genes that influence inhibitory neurons, including Npas4 and Bdnf, are downregulated in $Mecp2^{T308A}$ mice[71], possibly providing a molecular mechanism for the alterations in inhibition in these animals. Thus, dysregulation of activity-dependent signalling that is

normally mediated by the NID of MeCP2 likely contributes crucially to RTT pathophysiology.

Conclusions and perspectives

MeCP2 has spatiotemporally diverse effects depending on brain regions, phases of development and cell types. MeCP2 regulates multiple stages of brain development, including prenatal neurogenesis, postnatal development of synaptic connections and function, and experience-dependent refinement of neural circuits. Like many other brain disorders, RTT is associated with dysfunction reflective of functional failure at several levels, including the levels of gene regulation, molecular expression, synaptic function and neuronal circuitry, and at various developmental stages.

Accumulating evidence indicates that the primary role of MeCP2 is transcriptional repression, with the MBD and NID of MeCP2 connecting methylated DNA with the NCoR–SMRT co-repressor complex. However, it remains unclear how certain missense mutations, such as that encoding the T158M substitution in the MBD, confer more severe clinical symptoms than do mutations affecting the NID (such as that encoding the R306C substitution)[152]. Examining transcriptomes associated with different RTT mutations could provide critical insights into the differences in clinical severity.

At the molecular level, one major question is how MeCP2 loss produces the subtle yet widespread effects on neuron-enriched long genes, key activity- dependent signalling pathways and a large array of miRNAs. All of these effects may reflect the ability of MeCP2 to interact widely across the genome. To better understand the functions of MeCP2, an important next step will be to evaluate, in detail, the molecular events that occur following MeCP2 binding to the gene bodies across the genome. Another feature of RTT to be resolved is a reduction in total RNA[57, 74], which is potentially due to suboptimal functioning of transcriptional machinery and which may explain the large number of downregulated genes in RTT.

The molecular mechanisms of MeCP2 loss manifest as dysfunction in brain circuits. The wiring and function of brain circuits depend on proper functioning of inhibitory and excitatory neurons and maintenance of E/I balance, all of which require MeCP2. A major functional contributor to RTT phenotypes is alteration of inhibition, owing to altered gene expression in GABAergic neurons and a reduction in inhibitory drive onto pyramidal cells. Importantly, the NID mediates activity-dependent functions of MeCP2 and hence probably influences activity- dependent maturation of inhibitory neurons and the function of GABA receptors.

A key outstanding question is how a loss of the molecular functions of MeCP2 leads to the functional circuit deficits in RTT phenotypes. Are there cell-type- specific effects of the loss of the normal molecular mechanisms of MeCP2? In particular, it will be important to establish the effects of mutations affecting the MBD or NID on PV^+ neurons and to investigate how these domains shape the properties of PV^+ neurons and hence functional circuits. Experimentally, combining single-cell RNA sequencing and physiology offers an

opportunity to dissect such alterations, thus bridging molecular dysregulation and circuit dysfunction following MeCP2 loss.

The development of treatment strategies (Box 3) must take into account the functional properties of MeCP2 discussed in this Review and consider the therapeutic target, brain region, cell type and time of application. More directly targeted therapeutics that are administered as early as possible are likely to be the most effective. Gene therapy is a potentially powerful approach (Box 3), although notably, the protein level of MeCP2 must be tightly titrated, as overdosage of MeCP2, similar to MECP2 duplication, would lead to severe neurological consequences[153, 154, 155]. A fuller understanding of RTT based on our increasing knowledge of the neurobiology and function of MeCP2, together with rapidly advancing genome-editing tools, could lead to an effective treatment for RTT.

Acknowledgements

The authors thank H. Tsang for thoughtful discussions and critical reading of the manuscript. The authors apologize to colleagues whose research was not cited owing to the broad scope and space limitations of this Review. The authors' research is supported by a Human Frontier Science Program Long-Term Fellowship (J.P.K.I.), and by US National Institutes of Health grants MH085802 and EY007023, and the Simons Foundation Autism Research Initiative through the Simons Center for the Social Brain (M.S.).

Glossary

Classic RTT
The most common form of RTT. Several atypical forms of RTT have been described. The classic and atypical forms may differ by their symptoms or by the specific gene mutation

Random X chromosome inactivation
Random inactivation of one X chromosome in early embryonic cells in females.

Somatic mosaics
The presence of two populations of cells with different genotypes (mutation-positive and mutation-negative) in one individual.

Hypotonia
A state of low muscle tone (the amount of tension or resistance to stretch in a muscle), often resulting in muscle weakness.

Guide RNA
A short synthetic RNA composed of a 20-nucleotide sequence, homologous to the gene of interest, and a Cas9 nuclease-recruiting sequence for CRISPR–Cas9 gene editing.

Epigenetic reader
A protein that reads specific epigenetic marks on DNA.

Long genes
Genes with length greater than 100kb.

Gene bodies
Regions of DNA from the transcription start site to the end of the transcript.

Transcription start sites
The location where transcription starts at the 5′ end of a gene sequence. Promoters are located near the transcription start sites of genes.

AT hooks
A DNA-binding motif that binds specifically to AT-rich DNA. Chromatin loops When stretches of genomic sequence in the same chromosome are physically closer together than to the intervening sequences. These are involved in regulating chromatin organization and gene expression.

Primary miRNAs
(pri-miRNAs). Large primary precursor, hairpin-structured, microRNA transcripts that are subsequently cleaved to generate intermediate precursor microRNAs and mature microRNAs.

Stereotypies
Diagnostic repetitive behaviours. Patients with RTT develop repetitive hand movements, a diagnostic criterion for RTT. Mouse models of RTT also develop repetitive behaviour.

Fragile X syndrome
A genetic disorder, caused by mutations in the FMR1 gene, that includes developmental problems such as learning disabilities and cognitive impairment.

Reversal potential
The membrane potential at which a given ion has no net current flow through the cell membrane.

Retinogeniculate synapses
Synaptic connections projecting directly from retinal ganglion cells in the retina to the neurons in the lateral geniculate nucleus.

References

1. Chahrour M & Zoghbi HY The story of Rett syndrome: from clinic to neurobiology. Neuron 56, 422–437 (2007). [PubMed: 17988628]
2. Laurvick CL et al. Rett syndrome in Australia: a review of the epidemiology. J. Pediatr 148, 347–352 (2006). [PubMed: 16615965]
3. Trappe R et al. MECP2 mutations in sporadic cases of Rett syndrome are almost exclusively of paternal origin. Am. J. Hum. Genet 68, 1093–1101 (2001). [PubMed: 11309679]
4. Neul JL et al. Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. Neurology 70, 1313–1321 (2008). [PubMed: 18337588]
5. Neul JL et al. Rett syndrome: revised diagnostic criteria and nomenclature. Ann. Neurol 68, 944–950 (2010). [PubMed: 21154482]
6. Schüle B, Armstrong DD, Vogel H, Oviedo A & Francke U Severe congenital encephalopathy caused by MECP2 null mutations in males: central hypoxia and reduced neuronal dendritic structure. Clin. Genet 74, 116–126 (2008). [PubMed: 18477000]
7. Lewis JD et al. Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. Cell 69, 905–914 (1992). [PubMed: 1606614]
8. Chahrour M et al. MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 320, 1224–1229 (2008). [PubMed: 18511691]

9. Cheng TL et al. MeCP2 suppresses nuclear microRNA processing and dendritic growth by regulating the DGCR8/Drosha complex. Dev. Cell 28, 547–560 (2014). [PubMed: 24636259]
10. Dolce A et al. Rett syndrome and epilepsy: an update for child neurologists. Pediatr. Neurol 48, 337–345 (2013). [PubMed: 23583050]
11. Leonard H, Cobb S & Downs J Clinical and biological progress over 50 years in Rett syndrome. Nat. Rev. Neurol 13, 37–51 (2016). [PubMed: 27934853]
12. von Tetzchner S et al. Vision, cognition and developmental characteristics of girls and women with Rett syndrome. Dev. Med. Child Neurol 38, 212–225 (1996). [PubMed: 8631518]
13. LeBlanc JJ et al. Visual evoked potentials detect cortical processing deficits in Rett syndrome. Ann. Neurol 78, 775–786 (2015). [PubMed: 26332183]
14. Neul JL The relationship of Rett syndrome and MECP2 disorders to autism. Dialogues Clin. Neurosci 14, 253–262 (2012). [PubMed: 23226951]
15. American Psychiatric Association (eds). Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (American Psychiatric Publishing, 2013).
16. Armstrong D, Dunn JK, Antalffy B & Trivedi R Selective dendritic alterations in the cortex of Rett syndrome. J. Neuropathol. Exp. Neurol 54, 195–201 (1995). [PubMed: 7876888]
17. Carter JC et al. Selective cerebral volume reduction in Rett Syndrome: a multiple-approach MR imaging study. AJNR Am. J. Neuroradiol 29, 436–441 (2008). [PubMed: 18065507]
18. Amir RE et al. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat. Genet 23, 185–188 (1999). [PubMed: 10508514]
19. 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 27, 327–331 (2001). [PubMed: 11242118]
20. Guy J, Hendrich B, Holmes M, Martin JE & Bird A A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat. Genet 27, 322–326 (2001). [PubMed: 11242117]
21. Ward CS et al. MeCP2 is critical within HoxB1-derived tissues of mice for normal lifespan. J. Neurosci 31, 10359–10370 (2011). [PubMed: 21753013]
22. Huang T-W et al. Progressive changes in a distributed neural circuit underlie breathing abnormalities in mice lacking MeCP2. J. Neurosci 36, 5572–5586 (2016). [PubMed: 27194336]
23. Samaco RC et al. Loss of MeCP2 in aminergic neurons causes cell- autonomous defects in neurotransmitter synthesis and specific behavioral abnormalities. Proc. Natl Acad. Sci. USA 106, 21966–21971 (2009). [PubMed: 20007372]
24. Chao H-T et al. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature 468, 263–269 (2010). [PubMed: 21068835]
25. Lioy DT et al. A role for glia in the progression of Rett's syndrome. Nature 475, 497–500 (2011). [PubMed: 21716289]
26. Derecki NC et al. Wild-type microglia arrest pathology in a mouse model of Rett syndrome. Nature 484, 105–109 (2012). [PubMed: 22425995]
27. Wang J et al. Wild-type microglia do not reverse pathology in mouse models of Rett syndrome. Nature 521, E1–E4 (2015). [PubMed: 25993969]
28. Schafer DP et al. Microglia contribute to circuit defects in Mecp2 null mice independent of microglia-specific loss of Mecp2 expression. eLife 5, e15224 (2016). [PubMed: 27458802]
29. Tropea D et al. Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice. Proc. Natl Acad. Sci. USA 106, 2029–2034 (2009). [PubMed: 19208815]
30. Fukuda T, Itoh M, Ichikawa T, Washiyama K & Goto Y Delayed maturation of neuronal architecture and synaptogenesis in cerebral cortex of Mecp2-deficient mice. J. Neuropathol. Exp. Neurol 64, 537–544 (2005). [PubMed: 15977646]
31. Smrt RD et al. Mecp2 deficiency leads to delayed maturation and altered gene expression in hippocampal neurons. Neurobiol. Dis 27, 77–89 (2007). [PubMed: 17532643]
32. Cohen S & Greenberg ME Communication between the synapse and the nucleus in neuronal development, plasticity, and disease. Annu. Rev. Cell Dev. Biol 24, 183–209 (2008). [PubMed: 18616423]

33. Nelson SB & Valakh V Excitatory/inhibitory balance and circuit homeostasis in autism spectrum disorders. Neuron 87, 684–698 (2015). [PubMed: 26291155]
34. 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 11, 115–124 (2002). [PubMed: 11809720]
35. Kishi N & Macklis JD MECP2 is progressively expressed in post- migratory neurons and is involved in neuronal maturation rather than cell fate decisions. Mol. Cell Neurosci 27, 306–321 (2004). [PubMed: 15519245]
36. Fehr S et al. Altered attainment of developmental milestones influences the age of diagnosis of Rett syndrome. J. Child Neurol 26, 980–987 (2011). [PubMed: 21543746]
37. Einspieler C, Kerr AM & Prechtl HFR Is the early development of girls with Rett disorder really normal? Pediatr. Res 57, 696–700 (2005). [PubMed: 15718369]
38. Einspieler C, Kerr AM & Prechtl HFR Abnormal general movements in girls with Rett disorder: the first four months of life. Brain Dev. 27, S8–S13 (2005). [PubMed: 16182501]
39. Marschik PB et al. Changing the perspective on early development of Rett syndrome. Res. Dev. Disabil 34, 1236–1239 (2013). [PubMed: 23400005]
40. Neul JL et al. Developmental delay in Rett syndrome: data from the natural history study. J. Neurodev. Disord 6, 20 (2014). [PubMed: 25071871]
41. Mellios N et al. MeCP2-regulated miRNAs control early human neurogenesis through differential effects on ERK and AKT signaling. Mol. Psychiatry 23, 1051–1065 (2018). [PubMed: 28439102]
42. Bedogni F et al. Defects during Mecp2 null embryonic cortex development precede the onset of overt neurological symptoms. Cereb. Cortex 26, 2517–2529 (2016). [PubMed: 25979088]
43. Kim KY, Hysolli E & Park IH Neuronal maturation defect in induced pluripotent stem cells from patients with Rett syndrome. Proc. Natl Acad. Sci. USA 108, 14169–14174 (2011). [PubMed: 21807996]
44. Tsujimura K, Abematsu M, Kohyama J, Namihira M & Nakashima K Neuronal differentiation of neural precursor cells is promoted by the methyl-CpG-binding protein MeCP2. Exp. Neurol 219, 104–111 (2009). [PubMed: 19427855]
45. Stancheva I et al. A mutant form of MeCP2 protein associated with human Rett syndrome cannot be displaced from methylated DNA by notch in Xenopus embryos. Mol. Cell 12, 425–435 (2003). [PubMed: 14536082]
46. Gao H et al. Mecp2 regulates neural cell differentiation by suppressing the Id1 to Her2 axis in zebrafish. J. Cell Sci 128, 2340–2350 (2015). [PubMed: 25948585]
47. Guy J, Gan J, Selfridge J, Cobb S & Bird A Reversal of neurological defects in a mouse model of Rett syndrome. Science 315, 1143–1147 (2007). [PubMed: 17289941]
48. Giacometti E, Luikenhuis S, Beard C & Jaenisch R Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. Proc. Natl Acad. Sci. USA 104, 1931–1936 (2007). [PubMed: 17267601]
49. McGraw CM, Samaco RC & Zoghbi HY Adult neural function requires MeCP2. Science 333, 186 (2011). [PubMed: 21636743]
50. Nguyen MVC et al. MeCP2 is critical for maintaining mature neuronal networks and global brain anatomy during late stages of postnatal brain development and in the mature adult brain. J. Neurosci 32, 10021–10034 (2012). [PubMed: 22815516]
51. Du F et al. Acute and crucial requirement for MeCP2 function upon transition from early to late adult stages of brain maturation. Hum. Mol. Genet 25, 1690–1702 (2016). [PubMed: 26908602]
52. Swiech L et al. In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. Nat. Biotechnol 33, 102–106 (2014). [PubMed: 25326897]
53. Lyst MJ & Bird A Rett syndrome: a complex disorder with simple roots. Nat. Rev. Genet 16, 261–275 (2015). [PubMed: 25732612]
54. Meehan R, Lewis JD & Bird AP Characterization of MECP2, a vertebrate DNA binding protein with affinity for methylated DNA. Nucleic Acids Res. 20, 5085–5092 (1992). [PubMed: 1408825]
55. Nan X et al. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393, 386–389 (1998). [PubMed: 9620804]

56. Kinde B, Gabel HW, Gilbert CS, Griffith EC & Greenberg ME Reading the unique DNA methylation landscape of the brain: non-CpG methylation, hydroxymethylation, and MeCP2. Proc. Natl Acad. Sci. USA 112, 6800–6806 (2015). [PubMed: 25739960]
57. Lagger S et al. MeCP2 recognizes cytosine methylated tri-nucleotide and di-nucleotide sequences to tune transcription in the mammalian brain. PLoS Genet. 13, e1006793 (2017). [PubMed: 28498846]
58. Guo JU et al. Distribution, recognition and regulation of non-CpG methylation in the adult mammalian brain. Nat. Neurosci 17, 215–222 (2013). [PubMed: 24362762]
59. Gabel HW et al. Disruption of DNA-methylation-dependent long gene repression in Rett syndrome. Nature 522, 89–93 (2015). [PubMed: 25762136]
60. Kinde B, Wu DY, Greenberg ME & Gabel HW DNA methylation in the gene body influences MeCP2-mediated gene repression. Proc. Natl Acad. Sci. USA 113, 15114–15119 (2016). [PubMed: 27965390]
61. Stroud H et al. Early-life gene expression in neurons modulates lasting epigenetic states. Cell 171, 1151–1164.e16 (2017). [PubMed: 29056337]
62. Mellén M, Ayata P, Dewell S, Kriaucionis S & Heintz N MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system. Cell 151, 1417–1430 (2012). [PubMed: 23260135]
63. Mellén M, Ayata P & Heintz N 5-Hydroxymethylcytosine accumulation in postmitotic neurons results in functional demethylation of expressed genes. Proc. Natl Acad. Sci. USA 114, E7812–E7821 (2017). [PubMed: 28847947]
64. Skene PJ et al. Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. Mol. Cell 37, 457–468 (2010). [PubMed: 20188665]
65. Shah RR & Bird AP MeCP2 mutations: progress towards understanding and treating Rett syndrome. Genome Med. 9, 17 (2017). [PubMed: 28212680]
66. Sugino K et al. Cell-type-specific repression by methyl-CpG-binding protein 2 is biased toward long genes. J. Neurosci 34, 12877–12883 (2014). [PubMed: 25232122]
67. Johnson BS et al. Biotin tagging of MeCP2 in mice reveals contextual insights into the Rett syndrome transcriptome. Nat. Med 23, 1203–1214 (2017). [PubMed: 28920956]
68. Lyst MJ et al. Rett syndrome mutations abolish the interaction of MeCP2 with the NCoR/SMRT co-repressor. Nat. Neurosci 16, 898–902 (2013). [PubMed: 23770565]
69. Kruusvee V et al. Structure of the MeCP2-TBLR1 complex reveals a molecular basis for Rett syndrome and related disorders. Proc. Natl Acad. Sci. USA 114, E3243–E3250 (2017). [PubMed: 28348241]
70. Tillotson R et al. Radically truncated MeCP2 rescues Rett syndrome- like neurological defects. Nature 550, 398–401 (2017). [PubMed: 29019980]
71. Ebert DH et al. Activity-dependent phosphorylation of MECP2 T308 regulates interaction with NCoR. Nature 499, 341–345 (2013). [PubMed: 23770587]
72. Ben-Shachar S, Chahrour M, Thaller C, Shaw CA & Zoghbi HY Mouse models of MeCP2 disorders share gene expression changes in the cerebellum and hypothalamus. Hum. Mol. Genet 18, 2431–2442 (2009). [PubMed: 19369296]
73. Tudor M, Akbarian S, Chen RZ & Jaenisch R Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. Proc. Natl Acad. Sci. USA 99, 15536–15541 (2002). [PubMed: 12432090]
74. Li Y et al. Global transcriptional and translational repression in human-embryonic-stem-cell-derived Rett syndrome neurons. Cell Stem Cell 13, 446–458 (2013). [PubMed: 24094325]
75. Nott A et al. Histone deacetylase 3 associates with MeCP2 to regulate FOXO and social behavior. Nat. Neurosci 19, 1497–1505 (2016). [PubMed: 27428650]
76. Ciernia AV & LaSalle J The landscape of DNA methylation amid a perfect storm of autism aetiologies. Nat. Rev. Neurosci 17, 411–423 (2016). [PubMed: 27150399]
77. Baker SA et al. An AT-hook domain in MeCP2 determines the clinical course of Rett syndrome and related disorders. Cell 152, 984–996 (2013). [PubMed: 23452848]

78. Heckman LD, Chahrour MH & Zoghbi HY Rett-causing mutations reveal two domains critical for MeCP2 function and for toxicity in MECP2 duplication syndrome mice. eLife 3, e02676 (2014).
79. Nan X et al. Interaction between chromatin proteins MECP2 and ATRX is disrupted by mutations that cause inherited mental retardation. Proc. Natl Acad. Sci. USA 104, 2709–2714 (2007). [PubMed: 17296936]
80. Krol J, Loedige I & Filipowicz W The widespread regulation of microRNA biogenesis, function and decay. Nat. Rev. Genet 11, 597–610 (2010). [PubMed: 20661255]
81. Mellios N & Sur M The emerging role of microRNAs in schizophrenia and autism spectrum disorders. Front. Psychiatry 3, 39 (2012). [PubMed: 22539927]
82. Wu H et al. 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 107, 18161–18166 (2010). [PubMed: 20921386]
83. Urdinguio RG et al. Disrupted microRNA expression caused by Mecp2 loss in a mouse model of Rett syndrome. Epigenetics 5, 656–663 (2010). [PubMed: 20716963]
84. Mellios N et al. β2-Adrenergic receptor agonist ameliorates phenotypes and corrects microRNA-mediated IGF1 deficits in a mouse model of Rett syndrome. Proc. Natl Acad. Sci. USA 111, 9947–9952 (2014). [PubMed: 24958851]
85. Klein ME et al. Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat. Neurosci 10, 1513–1514 (2007). [PubMed: 17994015]
86. Nomura T et al. MeCP2-dependent repression of an imprinted miR-184 released by depolarization. Hum. Mol. Genet 17, 1192–1199 (2008). [PubMed: 18203756]
87. Gao Y et al. Inhibition of miR-15a promotes BDNF expression and rescues dendritic maturation deficits in MeCP2-deficient neurons. Stem Cells 33, 1618–1629 (2015). [PubMed: 25639236]
88. Szulwach KE et al. Cross talk between microRNA and epigenetic regulation in adult neurogenesis. J. Cell Biol 189, 127–141 (2010). [PubMed: 20368621]
89. Chen Y, Shin BC, Thamotharan S & Devaskar SU Differential methylation of the micro-RNA 7b gene targets postnatal maturation of murine neuronal Mecp2 gene expression. Dev. Neurobiol 74, 407–425 (2014). [PubMed: 24039126]
90. Smrt RD et al. MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. Stem Cells 28, 1060–1070 (2010). [PubMed: 20506192]
91. Davis BN, Hilyard AC, Lagna G & Hata A SMAD proteins control DROSHA-mediated microRNA maturation. Nature 454, 56–61 (2008). [PubMed: 18548003]
92. Li H, Zhong X, Chau KF, Williams EC & Chang Q Loss of activity-induced phosphorylation of MeCP2 enhances synaptogenesis, LTP and spatial memory. Nat. Neurosci 14, 1001–1008 (2011). [PubMed: 21765426]
93. Huang Y-WA, Ruiz CR, Eyler ECH, Lin K & Meffert MK Dual regulation of miRNA biogenesis generates target specificity in neurotrophin-induced protein synthesis. Cell 148, 933–946 (2012). [PubMed: 22385959]
94. Piskounova E et al. Lin28A and Lin28B inhibit let-7 microRNA biogenesis by distinct mechanisms. Cell 147, 1066–1079 (2011). [PubMed: 22118463]
95. Han K et al. Human-specific regulation of MeCP2 levels in fetal brains by microRNA miR-483–5p. Genes Dev. 27, 485–490 (2013). [PubMed: 23431031]
96. Rodrigues DC et al. MECP2 is post-transcriptionally regulated during human neurodevelopment by combinatorial action of RNA-binding proteins and miRNAs. Cell Rep. 17, 720–734 (2016). [PubMed: 27732849]
97. Zhang Y et al. MiR-130a regulates neurite outgrowth and dendritic spine density by targeting MeCP2. Protein Cell 7, 489–500 (2016). [PubMed: 27245166]
98. Im HI, Hollander JA, Bali P & Kenny PJ MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. Nat. Neurosci 13, 1120–1127 (2010). [PubMed: 20711185]
99. Dani VS et al. Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. Proc. Natl Acad. Sci. USA 102, 12560–12565 (2005). [PubMed: 16116096]

100. Nelson ED, Kavalali ET & Monteggia LM MeCP2-dependent transcriptional repression regulates excitatory neurotransmission. Curr. Biol16, 710–716 (2006). [PubMed: 16581518]
101. Banerjee A et al. Jointly reduced inhibition and excitation underlies circuit-wide changes in cortical processing in Rett syndrome. Proc. Natl Acad. Sci. USA 113, E7287–E7296 (2016). [PubMed: 27803317]
102. Calfa G, Li W, Rutherford JM & Pozzo-Miller L Excitation/inhibition imbalance and impaired synaptic inhibition in hippocampal area CA3 of Mecp2 knockout mice. Hippocampus 25, 159–168 (2015). [PubMed: 25209930]
103. Taneja P et al. Pathophysiology of locus ceruleus neurons in a mouse model of Rett syndrome. J. Neurosci 29, 12187–12195 (2009). [PubMed: 19793977]
104. Abdala APL, Dutschmann M, Bissonnette JM & Paton JFR Correction of respiratory disorders in a mouse model of Rett syndrome. Proc. Natl Acad. Sci. USA 107, 18208–18213 (2010). [PubMed: 20921395]
105. Medrihan L et al. Early defects of GABAergic synapses in the brain stem of a MeCP2 mouse model of Rett syndrome. J. Neurophysiol 99, 112–121 (2008). [PubMed: 18032561]
106. Kron M et al. Brain activity mapping in Mecp2 mutant mice reveals functional deficits in forebrain circuits, including key nodes in the default mode network, that are reversed with ketamine treatment. J. Neurosci 32, 13860–13872 (2012). [PubMed: 23035095]
107. Calfa G et al. Network hyperexcitability in hippocampal slices from Mecp2 mutant mice revealed by voltage-sensitive dye imaging. J. Neurophysiol 105, 1768–1784 (2011). [PubMed: 21307327]
108. Mo A et al. Epigenomic signatures of neuronal diversity in the mammalian brain. Neuron 86, 1369–1384 (2015). [PubMed: 26087164]
109. Durand S et al. NMDA receptor regulation prevents regression of visual cortical function in the absence of Mecp2. Neuron 76, 1078–1090 (2012). [PubMed: 23259945]
110. Meng X et al. Manipulations of MeCP2 in glutamatergic neurons highlight their contributions to Rett and other neurological disorders. eLife 5, (2016).
111. Gemelli T et al. Postnatal loss of methyl-CpG binding protein 2 in the forebrain is sufficient to mediate behavioral aspects of Rett syndrome in mice. Biol. Psychiatry 59, 468–476 (2006). [PubMed: 16199017]
112. Ure K et al. Restoration of Mecp2 expression in GABAergic neurons is sufficient to rescue multiple disease features in a mouse model of Rett syndrome. eLife 5, e14198 (2016). [PubMed: 27328321]
113. Rudy B, Fishell G, Lee SH & Hjerling-Leffler J Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev. Neurobiol 71, 45–61 (2011). [PubMed: 21154909]
114. Kepecs A & Fishell G Interneuron cell types are fit to function. Nature 505, 318–326 (2014). [PubMed: 24429630]
115. Fishell G & Rudy B Mechanisms of inhibition within the telencephalon: ‘where the wild things are’. Annu. Rev. Neurosci 34, 535–567 (2011). [PubMed: 21469958]
116. Pfeffer CK, Xue M, He M, Huang ZJ & Scanziani M Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. Nat. Neurosci 16, 1068–1076 (2013). [PubMed: 23817549]
117. Adesnik H, Bruns W, Taniguchi H, Huang ZJ & Scanziani M A neural circuit for spatial summation in visual cortex. Nature 490, 226–231 (2012). [PubMed: 23060193]
118. Gentet LJ et al. Unique functional properties of somatostatin-expressing GABAergic neurons in mouse barrel cortex. Nat. Neurosci 15, 607–612 (2012). [PubMed: 22366760]
119. Lee S, Kruglikov I, Huang ZJ, Fishell G & Rudy B A disinhibitory circuit mediates motor integration in the somatosensory cortex. Nat. Neurosci 16, 1662–1670 (2013). [PubMed: 24097044]
120. Olsen SR, Bortone DS, Adesnik H & Scanziani M Gain control by layer six in cortical circuits of vision. Nature 483, 47–52 (2012). [PubMed: 22367547]
121. Wilson NR, Runyan CA, Wang FL & Sur M Division and subtraction by distinct cortical inhibitory networks in vivo. Nat. Biotechnol488, 343–348 (2012).

122. Xue M, Atallah BV & Scanziani M Equalizing excitation-inhibition ratios across visual cortical neurons. Nature 511, 596–600 (2014). [PubMed: 25043046]
123. Ito-Ishida A, Ure K, Chen H, Swann JW & Zoghbi HY Loss of MeCP2 in parvalbumin-and somatostatin-expressing neurons in mice leads to distinct Rett syndrome-like phenotypes. Neuron 88, 651–658 (2015). [PubMed: 26590342]
124. Krishnan K et al. MeCP2 regulates the timing of critical period plasticity that shapes functional connectivity in primary visual cortex. Proc. Natl Acad. Sci. USA 112, E4782–E4791 (2015). [PubMed: 26261347]
125. Mierau SB, Patrizi A, Hensch TK & Fagiolini M Cell-specific regulation of N-methyl-d-aspartate receptor maturation by Mecp2 in cortical circuits. Biol. Psychiatry 79, 746–754 (2016). [PubMed: 26185009]
126. He LJ et al. Conditional deletion of Mecp2 in parvalbumin-expressing GABAergic cells results in the absence of critical period plasticity. Nat. Commun 5, 5036 (2014). [PubMed: 25297674]
127. Tang X et al. KCC2 rescues functional deficits in human neurons derived from patients with Rett syndrome. Proc. Natl Acad. Sci. USA 113, 751–756 (2016). [PubMed: 26733678]
128. Blaesse P, Airaksinen MS, Rivera C & Kaila K Cation–chloride cotransporters and neuronal function. Neuron 61, 820–838 (2009). [PubMed: 19323993]
129. Kaila K, Price TJ, Payne JA, Puskarjov M & Voipio J Cation- chloride cotransporters in neuronal development, plasticity and disease. Nat. Rev. Neurosci 15, 637–654 (2014). [PubMed: 25234263]
130. Ben-Ari Y NKCC1 chloride importer antagonists attenuate many neurological and psychiatric disorders. Trends Neurosci. 40, 536–554 (2017). [PubMed: 28818303]
131. He QE, Nomura T, Xu J & Contractor A The developmental switch in GABA polarity is delayed in fragile X mice. Eur. J. Neurosci 34,446–450 (2014).
132. Ben-Ari Y et al. Refuting the challenges of the developmental shift of polarity of GABA actions: GABA more exciting than ever! Front. Cell. Neurosci 6, 35 (2012). [PubMed: 22973192]
133. Duarte ST et al. Abnormal expression of cerebrospinal fluid cation chloride cotransporters in patients with Rett syndrome. PLOS ONE 8, e68851 (2013). [PubMed: 23894354]
134. Castro J et al. Functional recovery with recombinant human IGF1 treatment in a mouse model of Rett Syndrome. Proc. Natl Acad. Sci. USA 111,9941–9946 (2014). [PubMed: 24958891]
135. Kelsch W et al. Insulin-like growth factor 1 and a cytosolic tyrosine kinase activate chloride outward transport during maturation of hippocampal neurons. Eur. J. Neurosci 21, 8339–8347 (2001).
136. Ko H et al. The emergence of functional microcircuits in visual cortex. Nature 496, 96–100 (2013). [PubMed: 23552948]
137. Espinosa JS & Stryker MP Development and plasticity of the primary visual cortex. Neuron 75, 230–249 (2012). [PubMed: 22841309]
138. Chen SX, Kim AN, Peters AJ & Komiyama T Subtype-specific plasticity of inhibitory circuits in motor cortex during motor learning. Nat. Neurosci 18, 1109–1115 (2015). [PubMed: 26098758]
139. Cichon J & Gan W-B Branch-specific dendritic Ca2+ spikes cause persistent synaptic plasticity. Nature 520, 180–185 (2015). [PubMed: 25822789]
140. Hayashi-Takagi A et al. Labelling and optical erasure of synaptic memory traces in the motor cortex. Nature 525, 333–338 (2015). [PubMed: 26352471]
141. Turrigiano GG & Nelson SB Homeostatic plasticity in the developing nervous system. Nat. Rev. Neurosci 5, 97–107 (2004). [PubMed: 14735113]
142. Sur M, Nagakura I, Chen N & Sugihara H Mechanisms of plasticity in the developing and adult visual cortex. Prog. Brain Res 207, 243–254 (2013). [PubMed: 24309257]
143. Hong EJ, McCord AE & Greenberg MEA Biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. Neuron 60, 610–624 (2008). [PubMed: 19038219]
144. Yee AX, Hsu Y-T & Chen L A metaplasticity view of the interaction between homeostatic and Hebbian plasticity. Philos. Trans. R. Soc. Lond. B Biol. Sci 372, 20160155 (2017). [PubMed: 28093549]

145. Mullins C, Fishell G & Tsien RW Unifying views of autism spectrum disorders: a consideration of autoregulatory feedback loops. Neuron89, 1131–1156 (2016). [PubMed: 26985722]
146. Ebert DH & Greenberg ME Activity-dependent neuronal signalling and autism spectrum disorder. Nature 493, 327–337 (2013). [PubMed: 23325215]
147. Blackman MP, Djukic B, Nelson SB & Turrigiano GG A critical and cell-autonomous role for MeCP2 in synaptic scaling up. J. Neurosci 32, 13529–13536 (2012). [PubMed: 23015442]
148. Qiu Z et al. The Rett syndrome protein MeCP2 regulates synaptic scaling. J. Neurosci 32, 989–994 (2012). [PubMed: 22262897]
149. Zhong X, Li H & Chang Q MeCP2 phosphorylation is required for modulating synaptic scaling through mGluR5. J. Neurosci 32, 12841–12847 (2012). [PubMed: 22973007]
150. Noutel J, Hong YK, Leu B, Kang E & Chen C Experience- dependent retinogeniculate synapse remodeling is abnormal in MeCP2- deficient mice. Neuron 70, 35–42 (2011). [PubMed: 21482354]
151. Tropea D, Van Wart A & Sur M Molecular mechanisms of experience-dependent plasticity in visual cortex. Philos. Trans. R. Soc. Lond. B Biol. Sci 364, 341–355 (2009). [PubMed: 18977729]
152. Brown K et al. The molecular basis of variable phenotypic severity among common missense mutations causing Rett syndrome. Hum. Mol. Genet 25, 558–570 (2016). [PubMed: 26647311]
153. Samaco RC et al. Crh and Oprm1 mediate anxiety-related behavior and social approach in a mouse model of MECP2 duplication syndrome. Nat. Genet 44, 206–211 (2012). [PubMed: 22231481]
154. Sztainberg Y et al. Reversal of phenotypes in MECP2 duplication mice using genetic rescue or antisense oligonucleotides. Nature 528, 123 (2015). [PubMed: 26605526]
155. Van Esch H et al. Duplication of the MECP2 region is a frequent cause of severe mental retardation and progressive neurological symptoms in males. Am. J. Hum. Genet 77, 442–453 (2005). [PubMed: 16080119]
156. Samaco RC et al. Female Mecp2+/− mice display robust behavioral deficits on two different genetic backgrounds providing a framework for pre- clinical studies. Hum. Mol. Genet 22, 96–109 (2013). [PubMed: 23026749]
157. Goffin D et al. Rett syndrome mutation MeCP2 T158A disrupts DNA binding, protein stability and ERP responses. Nat. Neurosci 15, 274–283 (2011). [PubMed: 22119903]
158. Lamonica JM et al. Elevating expression of MeCP2 T158M rescues DNA binding and Rett syndrome–like phenotypes. J. Clin. Invest 127, 1889–1904 (2017). [PubMed: 28394263]
159. Veeraragavan S et al. Loss of MeCP2 in the rat models regression, impaired sociability and transcriptional deficits of Rett syndrome. Hum. Mol. Genet 25, 3284–3302 (2016). [PubMed: 27365498]
160. Chen Y et al. Modeling Rett syndrome using TALEN-edited MECP2 mutant cynomolgus monkeys. Cell 169, 945–955.e10 (2017). [PubMed: 28525759]
161. Khwaja OS et al. Safety, pharmacokinetics, and preliminary assessment of efficacy of mecasermin (recombinant human IGF-1) for the treatment of Rett syndrome. Proc. Natl Acad. Sci. USA 111, 4596–4601 (2014). [PubMed: 24623853]
162. Li W & Pozzo-Miller L BDNF deregulation in Rett syndrome. Neuropharmacology 76, 737–746 (2014). [PubMed: 23597512]
163. Katz DM et al. Rett syndrome: crossing the threshold to clinical translation. Trends Neurosci. 39, 100–113 (2016). [PubMed: 26830113]
164. Chang Q, Khare G, Dani V, Nelson S & Jaenisch R The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. Neuron 49, 341–348 (2006). [PubMed: 16446138]
165. US National Library of Medicine. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT02061137 (2014).
166. US National Library of Medicine. ClinicalTrials.gov https://clinicaltrials.gov/ct2/show/NCT02153723 (2014).

167. El-Husseini AED, Schnell E, Chetkovich DM, Nicoll RA & Bredt DS PSD-95 involvement in maturation of excitatory synapses. Acta Crystallogr. B 290, 1364–1368 (2000).
168. Sheng M & Kim MJ Postsynaptic signaling and plasticity mechanisms. Acta Crystallogr. B 298, 776–780 (2002).
169. Yoshii A & Constantine-Paton M BDNF induces transport of PSD-95 to dendrites through PI3K-AKT signaling after NMDA receptor activation. Nat. Neurosci 10, 702–711 (2007). [PubMed: 17515902]
170. Zheng WH & Quirion R Comparative signaling pathways of insulin- like growth factor-1 and brain-derived neurotrophic factor in hippocampal neurons and the role of the PI3 kinase pathway in cell survival. J. Neurochem 89, 844–852 (2004). [PubMed: 15140184]
171. Chao H-T, Zoghbi HY & Rosenmund C MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. Neuron 56, 58–65 (2007). [PubMed: 17920015]
172. Farra N et al. Rett syndrome induced pluripotent stem cell-derived neurons reveal novel neurophysiological alterations. Mol. Psychiatry 17, 1261–1271 (2012). [PubMed: 22230884]
173. Marchetto MCN et al. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell 143, 527–539 (2010). [PubMed: 21074045]
174. Williams EC et al. Mutant astrocytes differentiated from Rett syndrome patients-specific iPSCs have adverse effects on wild-type neurons. Hum. Mol. Genet 23, 2968–2980 (2014). [PubMed: 24419315]
175. Yazdani M et al. Disease modeling using embryonic stem cells: MeCP2 regulates nuclear size and RNA synthesis in neurons. Stem Cells 30, 2128–2139 (2012). [PubMed: 22865604]
176. Lancaster MA et al. Cerebral organoids model human brain development and microcephaly. Nature 501, 373–379 (2012).
177. Quadrato G et al. Cell diversity and network dynamics in photosensitive human brain organoids. Nature 545, 48–53 (2017). [PubMed: 28445462]
178. Di Lullo E & Kriegstein AR The use of brain organoids to investigate neural development and disease. Nat. Rev. Neurosci 18, 573–584 (2017). [PubMed: 28878372]
179. Sahin M & Sur M Genes, circuits, and precision therapies for autism and related neurodevelopmental disorders. Science 350, aab3897 (2015). [PubMed: 26472761]
180. Luikenhuis S, Giacometti E, Beard CF & Jaenisch R Expression of MeCP2 in postmitotic neurons rescues Rett syndrome in mice. Proc. Natl Acad. Sci. USA 101, 6033–6038 (2004). [PubMed: 15069197]
181. Hao S et al. Forniceal deep brain stimulation rescues hippocampal memory in Rett syndrome mice. Nature 526, 430–434 (2015). [PubMed: 26469053]
182. Lu H et al. Loss and gain of MeCP2 cause similar hippocampal circuit dysfunction that is rescued by deep brain stimulation in a Rett syndrome mouse model. Neuron 91, 739–747 (2016). [PubMed: 27499081]
183. Chiken S & Nambu A Mechanism of deep brain stimulation: inhibition, excitation, or disruption? Neuroscientist 22, 313–322 (2016). [PubMed: 25888630]
184. Garg SK et al. Systemic delivery of MeCP2 rescues behavioral and cellular deficits in female mouse models of Rett syndrome. J. Neurosci 33, 13612–13620 (2013). [PubMed: 23966684]
185. Brendel C et al. Readthrough of nonsense mutations in Rett syndrome: evaluation of novel aminoglycosides and generation of a new mouse model. J. Mol. Med 89, 389–398 (2011). [PubMed: 21120444]
186. Vecsler M et al. Ex vivo treatment with a novel synthetic aminoglycoside NB54 in primary fibroblasts from Rett syndrome patients suppresses MECP2 nonsense mutations. PLoS ONE 6, e20733 (2011). [PubMed: 21695138]
187. Sripathy S et al. Screen for reactivation of MeCP2 on the inactive X chromosome identifies the BMP/TGF-β superfamily as a regulator of XIST expression. Proc. Natl Acad. Sci. USA 114, 1619–1624 (2017). [PubMed: 28143937]
188. Carrette LLG et al. A mixed modality approach towards Xi reactivation for Rett syndrome and other X-linked disorders. Proc. Natl Acad. Sci. USA 115, E668–E675 (2018). [PubMed: 29282321]

189. Sinnamon JR et al. Site-directed RNA repair of endogenous Mecp2 RNA in neurons. Proc. Natl Acad. Sci. USA 114, 201715320 (2017).

Box 1 Translational insights from mice

Research into Rett syndrome (RTT) received a major impetus through the generation and wide availability of methyl-CpG-binding protein 2 (Mecp2)-mutant mice[19, 20]. Mecp2-null ($Mecp2^{-/y}$) male mice are used more often than female heterozygous ($Mecp2^{+/-}$) mice, as the former display early-onset, severe symptoms similar to those in patients with RTT; namely, breathing irregularities and motor abnormalities, along with early lethality[19, 20]. Female $Mecp2^{+/-}$ mice, which provide a more accurate genetic representation of the disorder, should be used as well as $Mecp2^{-/y}$ mice for preclinical studies for potential therapeutics[112], even though their neurological symptoms develop later and they show higher phenotypic variability (owing to X chromosome inactivation) [156].

Mouse models for common missense mutations (including those encoding T158A, T158M and R306C substitutions) recapitulate RTT-like phenotypes[68, 157, 158]. T158A and T158M affect the methyl-CpG-binding domain (MBD), reduce MeCP2 protein stability and cause reductions in the amplitude of event response potentials in the auditory cortex at the circuit level[157, 158]. R306C is a mutation that affects the nuclear receptor co-repressor (NCoR)–silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) interaction domain (NID) and abolishes the interaction of MeCP2 with the NCoR–SMRT complex[68, 69]. As common missense mutations are highly relevant to RTT pathology, these models are valuable tools for preclinical studies.

One limitation of transgenic mouse models is that the onset of neurological symptoms occurs much later in development than in humans. Moreover, RTT- related phenotypes may be less severe in rodents. Other factors, including genetic background and rearing conditions, should be considered when analysing expressed phenotypes. Furthermore, RTT models in other species, including rats and non-human primates, have been developed. These recapitulate key features of RTT and offer unique behavioural features, including regression of a learned psychomotor skill in rats[159] and unique eye-tracking measures in primates[160]. Such models could enable cross-species validation in developing preclinical outcome measures in the future[159].

RTT animal models have helped to establish biomarkers to track disease progression and amelioration. Several behavioural assessments have been established to track the onset and severity of RTT in mouse models[156]. Unbiased, quantitative biomarkers of brain function and disease progression are needed. A recent study used frontal alpha-band electroencephalogram asymmetry, an index of anxiety and depression, in humans to evaluate the effects of insulin-like growth factor 1 (IGF1) treatment[161]. Other measurable phenotypes have been suggested: $Mecp2^{-/y}$ mice exhibit regression of visual acuity in adulthood (as opposed to progressive increase of acuity during normal development)[109], and $Mecp2^{+/-}$ mice and individuals with RTT exhibit decreases in the amplitude of cortical visually evoked potentials (VEPs)[13]. Therefore, a standardized VEP analysis might be useful in assessing preclinical and clinical therapeutic outcomes. The ideal biomarker may combine behavioural and electrophysiological assessments.

Mouse models have provided the basis for preclinical studies and therapeutic strategies for RTT (Box 3). For example, several signalling pathways, including the brain-derived neurotrophic factor (BDNF)[162] and IGF1 (Ref.[29]) pathways, are altered in RTT mouse models and are currently being targeted for potential therapeutics[163]. BDNF has important roles in synaptic maturation and plasticity. BDNF levels are reduced in Mecp2-mutant mice[164], and candidate agents that increase BDNF levels (namely, glatiramer acetate and fingolimod) are in clinical trials[165, 166] in RTT[163]. IGF1 regulates the phosphoinositide 3-kinase (PI3K)–AKT–postsynaptic density protein 95 (PSD95) pathway, a major pathway of excitatory synaptic maturation that is deficient in RTT model mice[29, 84] and in individuals with RTT[161]. PSD95 is a highly abundant synaptic protein crucial for synaptic maturation and plasticity[167, 168] and is upregulated by BDNF[169] and IGF1 (Refs[29, 170]). PSD95 is deficient in Mecp2-null mice[171] and is restored following systemic treatment with IGF1[29, 134]). Indeed, IGF1 treatment has shown efficacy in a phase I/IIa trial[161]. NNZ-2566, a synthetic analogue of IGF1, has been claimed to improve breathing and motor behaviour in patients with RTT; NNZ-2566 will soon advance to phase III trials.

Box 2 Modelling RTT in human cells

Intact human brains cannot be subjected to irreversible genetic manipulations or high-throughput drug screening, and human brain samples can be obtained only post-mortem and represent only the end point of the disorder. Hence, induced pluripotent stem cell (iPSC)-derived cell culture models of human brain development have been important for elucidating specific cellular aspects of disease pathology. Genome editing using transcription activator-like effector nuclease (TALEN) and CRISPR technologies, together with human neuronal cultures derived from people with Rett syndrome (RTT), has revealed structural, molecular and physiological RTT-related phenotypes during early developmental stages[41, 43, 74, 172, 173, 174, 175].

Isogenic controls are crucial for iPSC analyses. In RTT, neurons expressing only the non-mutated methyl-CpG-binding protein 2 (MECP2) allele can be generated from patients' cells using random X chromosome inactivation, such that controls share the same genetic background as the patients[41, 43]. Similar to RTT mouse models and post-mortem human patient samples, RTT iPSC-derived neurons demonstrate reductions in soma size, spine density, neuronal firing and activity- dependent signalling[173]. In addition, these cells show various signs of impaired neuronal maturation, including reduced expression of the neuronal marker TUJ1 (neuron-specific class III β-tubulin) and excitatory and inhibitory neuronal markers, aberrant electrophysiological properties and deficits in activity-dependent transcription[43, 74, 172, 175]. Moreover, RTT iPSC-derived cell cultures show impaired neuronal differentiation and migration[41], and differentiated astrocytes derived from RTT patients have adverse effects on the morphology and function of wild-type neurons[174].

Cerebral organoids are stem-cell-derived in vitro 3D structures, with a certain self-organizing capacity, that mimic, at least in part, in vivo brains. Although cerebral organoids currently can be used only to model early development of the human brain, several key features of in vivo brain organogenesis are recapitulated by in vitro organoids, which thus provide robust models for studying neurological disorders[176]. RTT patient-derived cerebral organoids have recently been generated and exhibit abnormalities in neuronal proliferation and differentiation as well as reduced maturity — phenotypes also observed in human MeCP2-deficient monolayer cultures[41]. However, to avoid the variability driven by random X chromosome inactivation, cerebral organoids are generated primarily from cells that express only the mutant copy of MECP2; thus, the effect more closely mimics human male mutations, which are more severe. Therefore, it will be of great interest to generate cerebral organoids that more closely mimic female RTT brains, with heterozygous expression and random X inactivation.

One intriguing difference between mouse models and human RTT is their disease severity. The effects of MeCP2 deficiency are milder in rodents than in humans: $Mecp2^{-/y}$ mice survive for several weeks postnatally, and $Mecp2^{+/-}$ mice show late symptom onset. This difference puts human iPSC-derived models in a unique position for studying RTT pathophysiology. With rapid advances in organoid- culturing strategies, cerebral organoids can develop over an extended period (more than 9 months), establishing late-developmental and even mature features, including dendritic spine

formation and active neuronal networks[177]. If or when cell-type-specific differentiation becomes a routine feature of these models, they and human-derived 2D cultures will be very useful for studying the interaction between excitatory and inhibitory neurons in the context of RTT[178]. In the meantime, cerebral organoids are suitable models to investigate early-developmental events, including neurogenesis and migration. As a future direction for precision medicine, drugs could potentially be tested on neuronal cultures derived from an individual with RTT before deciding whether they can be administered to the patient[179].

Box 3 Therapeutic strategies for RTT

There currently exist two major objectives in the development of therapeutic strategies: therapeutic targets downstream of methyl-CpG-binding protein 2 (MeCP2) and gene therapy. Postnatal restoration of MeCP2 in Mecp2-mutant mice rescues several Rett syndrome (RTT) phenotypes, emphasizing the reversibility of this devastating disorder[47, 48, 180]. Several treatment strategies that are based on targeting signalling downstream of MeCP2, such as increasing brain-derived neurotrophic factor (BDNF) or insulin-like growth factor 1 (IGF1), have improved certain RTT features in preclinical tests and are in clinical trials[161, 163] (Box 1). Given the diverse functional targets of MeCP2, it is likely that no single signalling pathway can explain disruptions resulting from MeCP2 dysfunction or loss. Rather, the development of novel treatment strategies must take into account not only the validity and specificity of the therapeutic target (that is, the mechanism of drug action and its relevance to MeCP2 mechanisms) but also the diversity of the effects of MeCP2 dysfunction or loss on different brain regions and cell types and during different developmental phases as well as on the time course of disease progression.

Furthermore, therapeutic effectiveness is influenced by various factors, including the stratification and validity of patient populations and their match to preclinical drug validation models, drug dosage, trial onset and duration, effectiveness of biomarkers and choice of end points. Considering the temporal and regional features of RTT discussed in the main text, and the issues mentioned above, the most effective therapeutics will probably target mechanisms as directly, and as early, as possible. Current therapeutic strategies for RTT, including those in clinical trials, have been reviewed recently[163].

As inhibitory dysfunction is a major feature of RTT, its modulation is a feasible approach to a functional treatment for RTT. As excitation is also reduced in forebrain areas in Mecp2-mutant mice, enhancing excitation should also be considered for treatment strategies. Strikingly, deep brain stimulation (DBS) targeting the hippocampus rescues memory deficits in Mecp2-mutant mice[181,182], presumably by modulating the excitation and inhibition of local circuits[183]. This finding opens a potential new avenue for RTT therapy. Mechanism-based approaches, such as IGF1 treatment (Box 1), restore inhibition and excitation in Mecp2-mutant mice[101]. Ketamine, an NMDA receptor antagonist, improves specific functions, including forebrain activity (as indicated by FOS expression) and prepulse inhibition of the acoustic startle in $Mecp2^{-/y}$ and $Mecp2^{+/-}$ mice, respectively[106]. MeCP2-deficient mice and neurons derived from individuals with RTT show a lower K^+/Cl^- exporter 2 (KCC2):$Na^+/K^+/Cl^-$ symporter (NKCC1) ratio, leading to abnormal GABA- induced changes in membrane response and thus reduced inhibition. The US Food and Drug Administration (FDA)-approved NKCC1 antagonist bumetanide could thus be a potential candidate[101, 130], although the development of molecules that enhance KCC2 function would be an important advance.

Owing to the complexity of MeCP2 dysfunction in RTT, gene therapy is possibly the most direct approach for treating the disorder. In principle, gene therapy would need to be attempted as early as possible, but the level of MeCP2 would have to be accurately titrated, as overdosage of MeCP2 or duplication of MECP2 would lead to severe

neurological consequences[153, 154, 155]. Current evidence supports four potential gene therapy strategies[11, 163]. First, delivery of Mecp2 gene using an adeno-associated virus serotype 9 (AAV9) vector through intravenous injection partially rescues RTT-like features in Mecp2- mutant mice[184]. Second, molecules such as aminoglycoside antibiotics (for example, gentamyicin) can enable ribosomes to read across MeCP2-encoding RNA despite nonsense mutations[185, 186] and could therefore potentially be used to target nonsense mutations that generate truncated MeCP2. Third, small molecules can activate normal MECP2 alleles on the inactive X chromosome (Xi)[187]. A recent study used a mixed-modality approach, combining brain-specific deletion of X-inactivation master regulator Xist and a small-molecule inhibitor of DNA methylation, to selectively reactivate Xi in mice in vivo[188]. Fourth, gene editing using CRISPR–Cas9 techniques may be used to correct point mutations. As a complementary proof of principle, the CRISPR–Cas9 technique has been shown to be able to remove 70% of MeCP2 protein in the mouse hippocampus[52]. Site-directed RNA editing mediated by double-stranded RNA-specific editase 1 (ADAR2; also known as ADARB1) can correct disease-causing mutations affecting the mouse MeCP2 DNA-binding domain in vitro, repairing 72% of Mecp2 mRNA[189]. Although many technical barriers remain, the potential for gene therapy for RTT warrants further exploration.

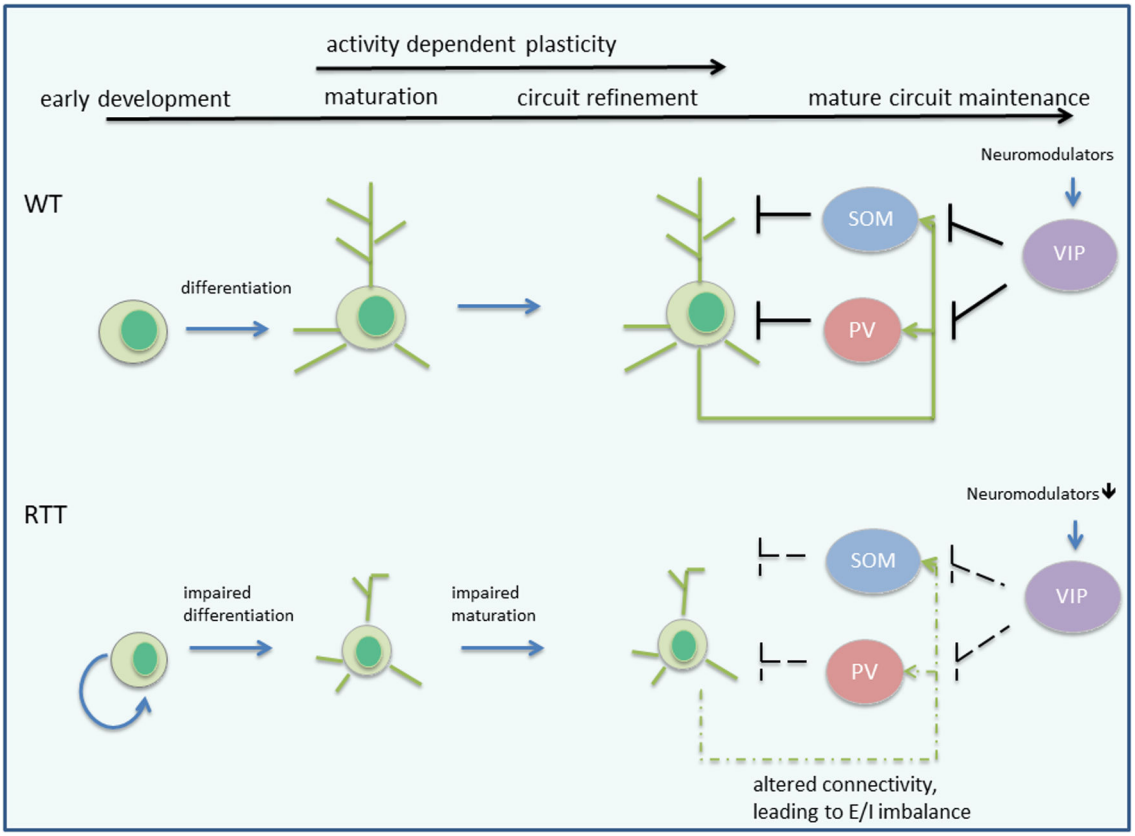

Fig. 1. Effects of MeCP2 loss at different stages of brain development.

Methyl-CpG-binding protein 2 (MeCP2) regulates multiple stages of brain development and function. Loss of MeCP2 results in impaired neurogenesis and differentiation in early development, abnormal neuronal maturation, reduced circuit connectivity and excitation–inhibition (E/I) imbalance — all of which potentially contribute to Rett syndrome (RTT) pathophysiology. Neural progenitor cells (left) differentiate into mature neurons through the development of dendrites and synapses and through experience-dependent and activity-dependent refinement. The proper development of microcircuits comprising pyramidal neurons and inhibitory neurons, including cells expressing somatostatin (SOM), parvalbumin (PV) or vasoactive intestinal peptide (VIP), is required for proper E/I balance. In RTT, the differentiation of neural progenitors is impaired at an early stage of development. At postnatal stages, RTT neurons show smaller soma sizes and underdeveloped dendritic arbors. Altered connectivity and reduced excitation and inhibition lead to E/I imbalance in RTT and RTT models; dashed lines represent altered connection strengths. WT, wild type.

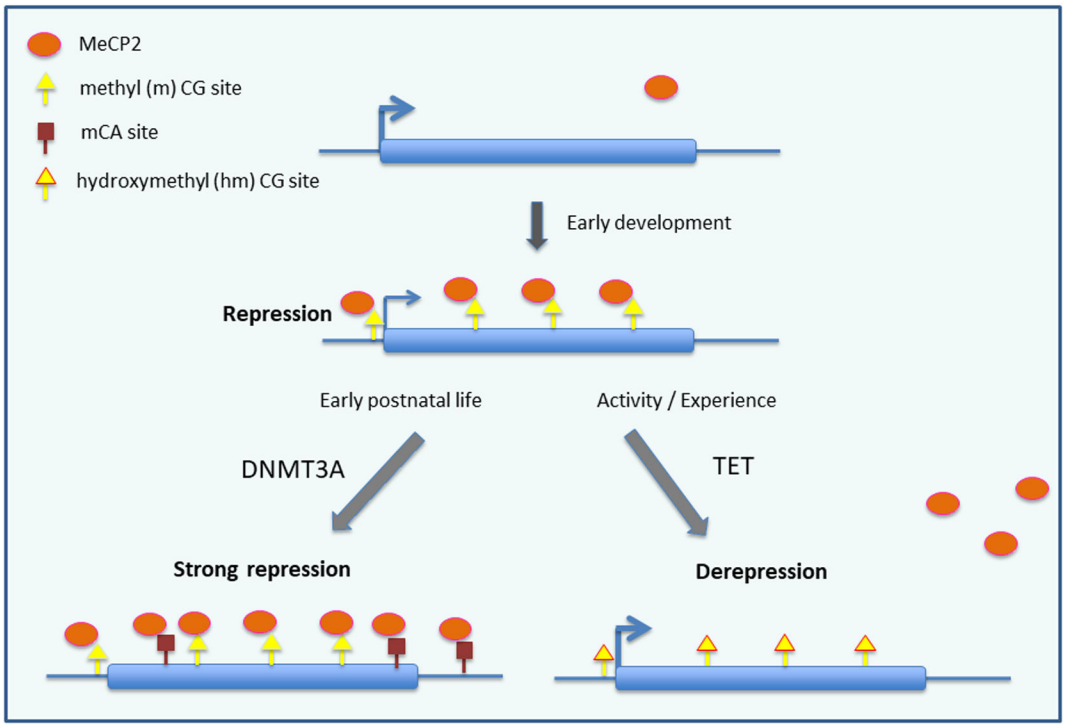

Fig. 2. A model of MeCP2–DNA interaction

During early development, methyl-CpG-binding protein 2 (MeCP2) binds to genomic methylated CG (mCG) dinucleotide sequences to repress gene expression. During early postnatal life, DNA (cytosine-5)-methyltransferase 3A (DNMT3A) binds across transcribed regions of genes that show low expression and dictates DNA methylation at CA sequences (producing methylated CA (mCA) modifications)[61]. Once methylated, mCA is bound by MeCP2, leading to strong repression of gene transcription[61]. Passive ‘functional demethylation’ can occur through oxidation of mCG to hydroxymethylated CG (hmCG) by the ten-eleven translocation (TET) enzyme. As MeCP2 has a low affinity for hmCG, this modification results in the detachment of MeCP2, leading to derepression of transcription[63].

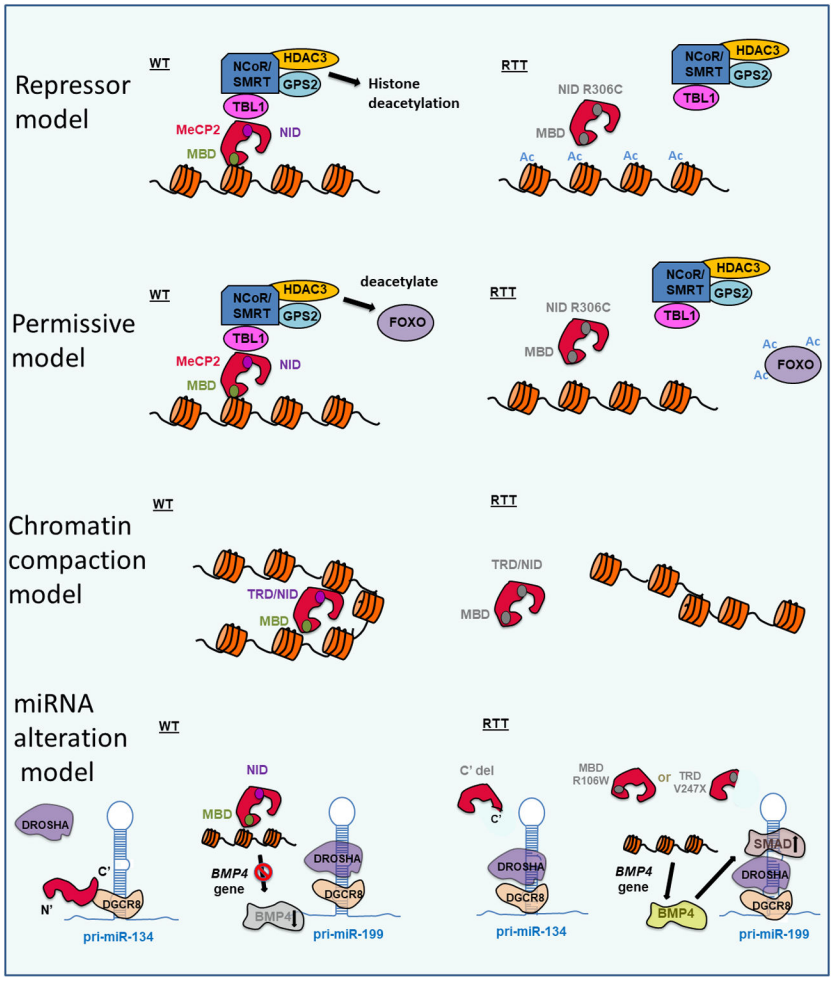

Fig. 3. Proposed molecular effects of MeCP2 and their alterations in RTT.

Methyl-CpG-binding protein 2 (MeCP2) has been proposed to recruit the nuclear receptor co-repressor (NCoR)–silencing mediator of retinoic acid (SMRT) complex to methylated DNA to repress transcription[68] or permit transcription[75] through the action of histone deacetylase 3 (HDAC3) depending on different genomic contexts. MeCP2 interacts directly with transducin β-like protein 1 (TBL1) in the NCoR–SMRT complex via its NCoR–SMRT interaction domain (NID)[69]. This function is abolished in NID variants of MeCP2, such as R306C MeCP2. MeCP2 has also been suggested to compact chromatin structure through a basic amino acid cluster in its NID as well as via three AT hooks (not shown)[77]. The carboxyl terminus of MeCP2 binds to the microprocessor complex protein DiGeorge syndrome critical region 8 (DGCR8) and inhibits ribonuclease 3 (DROSHA) from binding; thus, MeCP2 may inhibit the processing of pri-miR-134 (Ref.[9]). In Rett syndrome (RTT) patients with MECP2 mutations that change or truncate the C terminus (for example, the MeCP2 380 truncation), it is expected that MeCP2 binding to DGCR8 is abolished and DROSHA is free to bind and activate pri-miR-134 processing. Other methyl-CpG binding domain (MBD)-affecting or truncation- inducing mutations in MECP2 also lead to upregulations of pri-miR-199 indirectly, via aberrant bone morphogenetic protein 4 (BMP4) and SMAD signalling[41]. Ac, acetyl group; C´, carboxyl terminus; FOXO3, forkhead box

protein O3; GPS2, G protein pathway suppressor 2; miRNA, microRNA; TRD, transcriptional repression domain, WT, wild type. Figure adapted from Ref.[53], Macmillan Publishers Limited.

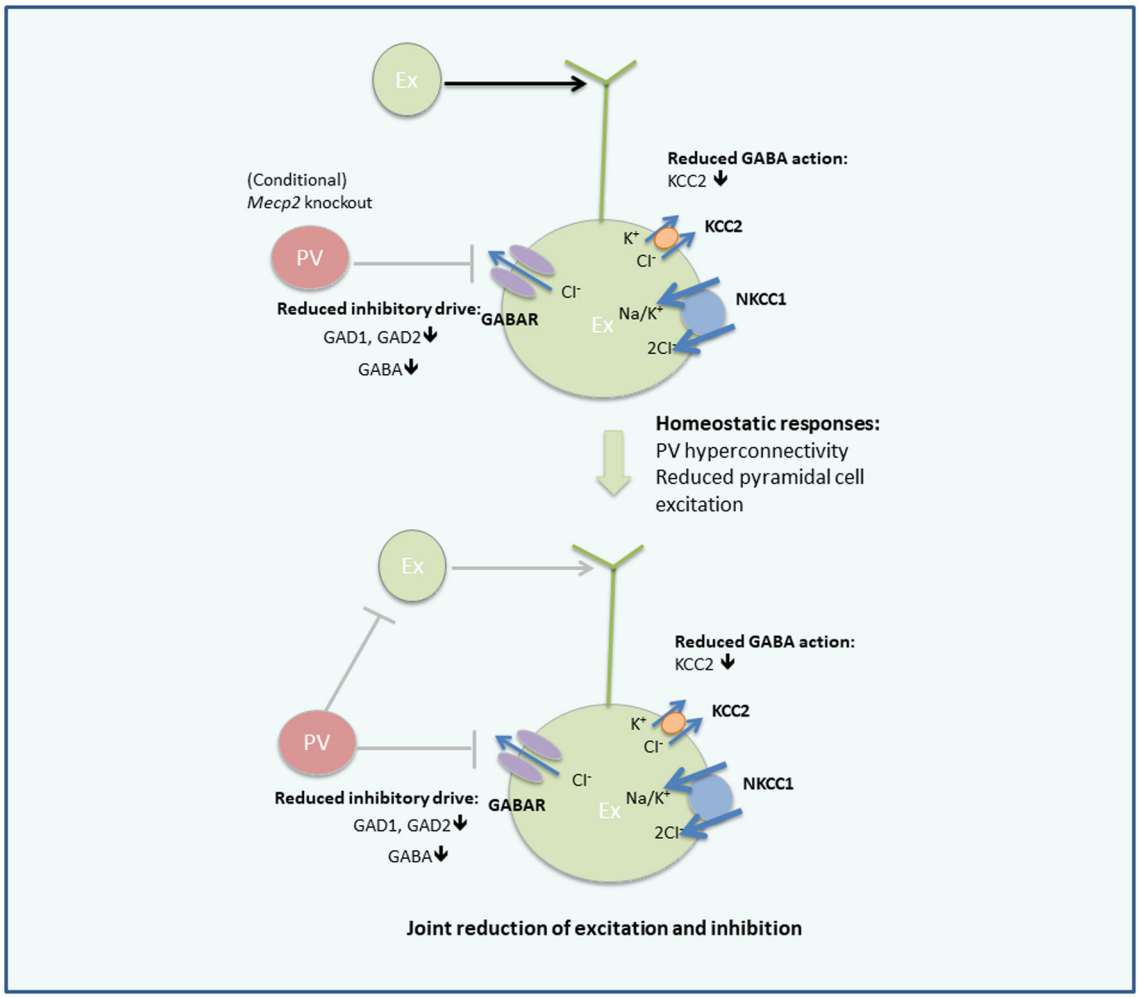

Fig. 4. Effects of deficiency in inhibitory neurons and MeCP2 excitation–inhibition balance

Deletion of methyl-CpG-binding protein 2 (MeCP2) in all forebrain neurons or only in inhibitory neurons (such as parvalbumin (PV)-positive neurons) reduces GABA synthesis, glutamate decarboxylase 1 (GAD1) and GAD2 levels and miniature inhibitory postsynaptic currents, and thus reduces inhibition onto excitatory pyramidal neurons (green cells). The effectiveness of GABA as an inhibitory neurotransmitter is also altered in MeCP2-deficient neurons. Reduced levels of the K^+/Cl^- exporter KCC2 relative to the $Na^+/K^+/Cl^-$ symporter NKCC1 have been found in cells with mutations in the gene encoding MeCP2, resulting in neurons with higher levels of intracellular chloride, and hence a reduction in the reversal potential for GABA and reduced inhibitory drive to pyramidal neurons. We suggest that homeostatic compensation for the reduction in inhibition leads to hyperconnectivity of PV^+ neurons and reduced excitatory drive onto pyramidal neurons, resulting in a joint reduction of excitation and inhibition in cortical circuits. Dashed arrows depict reduced function and solid arrows depict normal function. Ex, excitatory; GABAR, GABA receptor.