Molecular genetics of Rett syndrome: when DNA methylation goes unrecognized

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Abstract | The discovery that Rett syndrome is caused by mutations that affect the methyl-CpG-binding protein MeCP2 provided a major breakthrough in understanding this severe neurodevelopmental disorder. Animal models and expression studies have contributed to defining the role of MeCP2 in development, highlighting its contribution to postnatal neuronal morphogenesis and function. Furthermore, *in vitro* assays and microrray studies have delineated the potential molecular mechanisms of MeCP2 function, and have indicated a role in the transcriptional silencing of specific target genes. As well as unravelling the mechanisms that underlie Rett syndrome, these studies provide more general insights into how DNA-methylation patterns are recognized and translated into biological outcomes.

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Among the epigenetic mechanisms that are involved in the regulation of mammalian gene expression, DNA methylation is the most widely studied. This modification is catalysed by DNA cytosine-5-methyltransferases (DNMTs) and occurs at the 5′ position of cytosine within CpG dinucleotides, most of which are clustered in the CpG islands that are present at the 5′ ends of about 40% of mammalian genes[1]. These regions are generally unmethylated, but the methylation of specific sites in CpG islands is used as a regulatory mechanism for some genes, where it is usually associated with transcriptional downregulation. DNA methylation is also important for the establishment of epigenetic marks that are involved in the inactivation of the X chromosome and in the allele-specific gene expression that occurs at imprinted genomic regions.

Two mechanisms have been proposed to explain the inhibitory effect of CpG methylation on gene expression. First, it might inhibit the binding of transcription factors to their recognition sites. The second mechanism involves proteins with high affinity for methylated CpGs, such as the methyl-CpG-binding protein MeCP2, the methyl-CpG-binding-domain proteins MBD1, MBD2 and MBD4, and Kaiso. These proteins induce the recruitment of protein complexes that are involved in histone modifications and chromatin remodelling.

The number of diseases that are known to occur when methylation is not properly established, maintained or recognized is increasing. These include cancer, disorders that result from defects in the methylation machinery, imprinting disorders and diseases that are associated with repeat instability[2]. Here, we focus on a fifth group of diseases, Rett syndrome and related neurodevelopmental disorders, which are thought to result from defects in the MeCP2-associated machinery that recognizes and 'reads' methylation marks. The identification of mutations in *MECP2* as being causal in Rett syndrome[3], an X-linked dominant disorder, has led to a rapid increase in understanding the disease. Studies in model organisms have provided insights into how aberrant MeCP2 function leads to specific outcomes, and an understanding of the regulation of gene expression by MeCP2 is beginning to be gained at the molecular level. These studies highlight an emerging theme of interdependence between the pathways that recognize DNA methylation and those that regulate histone modification, chromatin remodelling and splicing, which indicates that these mechanisms interact to have a crucial role in the transcriptional regulation of target genes.

The *MECP2* gene and its products

The protein that is encoded by the X-linked gene *MECP2* (FIG. 1a) belongs to a large family of DNA-binding proteins (including the MBD proteins) that selectively bind 5-methylcytosine residues in symmetrically positioned CpG dinucleotides[1,4]. These dinucleotides occur throughout the genome; however, a recent study has shown that enrichment for A and T bases adjacent to methyl-CpG

dinucleotides is essential for high-affinity binding between MeCP2 and its target sites, indicating a basis for specificity[5]. All proteins of this family have a highly conserved MBD, which in MeCP2 is necessary and sufficient to bind DNA. MeCP2 also contains a central transcriptional repression domain (TRD), which interacts with various co-repressor complexes[6–9], and a nuclear localization signal[10]. The C-terminal domain facilitates the binding of MeCP2 to DNA[11] and contains a WW domain that is predicted to be involved in protein–protein interactions[12].

Two alternatively spliced *MECP2* transcripts have been characterized: *MECP2A* (also known as *MECP2_E2* or *MECP2β*) and the slightly longer *MECP2B* (*MECP2_E1* or *MECP2α*)[13,14], which differ only in their most 5′ regions (FIG. 1b). Although both forms are highly expressed in the brain, they differ in translation efficiency and are expressed at different relative amounts in various tissues, with *MECP2B* being more prevalent in the brain, thymus and lung, and during neuronal differentiation[13]. Additional *MECP2* transcripts with 3′ UTRs of different lengths are also produced by the use of alternative polyadenylation sites. Their expression levels vary between tissues and developmental stages, but the functional significance of this is unknown.

Rett syndrome phenotypes and genotypes

Mutations in *MECP2* underlie most cases of Rett syndrome, which is a progressive neurodevelopmental disorder that occurs almost exclusively in females, with an incidence of between 1/10,000 and 1/15,000 live births and a penetrance of almost 100%. This disease was an enigma for many years; its genetic analysis was hampered by the fact that nearly all cases are sporadic. Furthermore, it was incorrectly believed until recently that Rett syndrome was a dominant X-linked trait that caused prenatal lethality in males.

MECP2 *mutations in females.* Females with Rett syndrome are usually heterozygous for a *de novo* mutation in *MECP2*. After apparently normal development until 6–18 months of age, girls with classical Rett syndrome show regression, with deceleration of head growth, and loss of speech and acquired motor skills. After a period of pseudo-stabilization and then further deterioration, the condition is mainly characterized by severe mental retardation and motor defects. Atypical forms of Rett syndrome have also been described in females, with heterogeneity for age of onset, severity and clinical course.

Over the past few years, more that 2,000 mutations have been reported in females with Rett syndrome[15–18]. There are eight common mutations (FIG. 1a), which arise at CpG hotspots in *MECP2* and result in loss of function due to truncated, unstable or abnormally folded proteins. More recently, large rearrangements that involve *MECP2*, including deletions, were reported in a significant proportion of patients with Rett syndrome[18–23]. Altogether, *MECP2* abnormalities might account for more than 95% of sporadic cases of classical Rett syndrome in females.

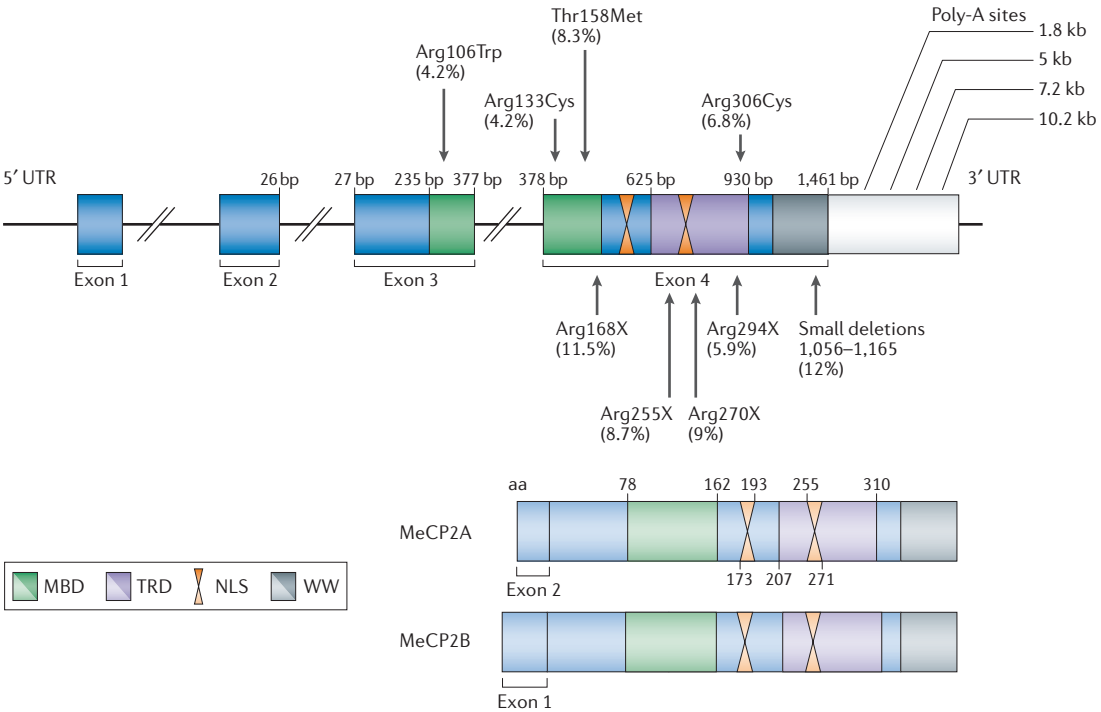

Figure 1 | **The *MECP2* gene and its protein products. a** | *MECP2* (methyl-CpG-binding protein 2) gene structure. The positions and frequencies of the most common mutations that are associated with Rett syndrome in females are indicated. **b** | The two main protein isoforms, MeCP2A (486 amino acids) and MeCP2B (498 amino acids), are produced by alternative splicing of the *MECP2* transcript and differ in their N-terminal regions, which are encoded by exon 2 of the gene in the case of MeCP2A and exon 1 in MeCP2B. MBD, methyl-CpG binding domain; NLS, nuclear localization signal; poly(A), polyadenylation; TRD, transcriptional repression domain; X, stop codon.

Attempts to establish genotype–phenotype correlations in females with Rett syndrome initially gave conflicting results, but patterns have recently begun to emerge. Female patients with mutations in *MECP2* that truncate the protein towards its C-terminal end (late-truncating mutations) have a phenotype that is less severe, and less typical of classical Rett syndrome, than patients who have missense or N-terminal (early-truncating) mutations[24,25]. In addition, the Arg270X mutation (where X is a stop codon), which is predicted to result in a truncated protein, is associated with increased mortality. This is consistent with greater clinical severity in cases with mutations upstream of or within the TRD[26]. However, as missense and late-truncating mutations can lead to either classical or atypical Rett syndrome, it has been suggested that genetic background and/or non-random X-chromosome inactivation in the brain influences the biological consequences of mutations in *MECP2*. Moreover, extreme skewing of X inactivation might account for the existence of rare female carriers with no Rett syndrome symptoms[15–17].

MECP2 *mutations in males.* Mutations in *MECP2* in males were initially thought to be prenatally lethal; however, it has been shown more recently that these mutations actually cause a variable phenotype in male patients[27], who can be divided into three groups (TABLE 1).

Table 1 | **Phenotypic classes of male patients with mutations in *MECP2*, and the corresponding phenotypes in females**

Phenotypic class in males	Amino-acid change	Nucleotide change	Domain affected	Clinical phenotype in females
Severe congenital encephalopathy	Thr158Met	473C>T	MBD	RTT
	Gly163fs	488_489delGG	Interdomain	RTT
	Gly252fs	754_755insC	TRD	RTT
	Gly252fs	755_756insG	TRD	RTT
	Gly269fs	806delG	TRD	RTT
	Pro385fs	1154_1185del32	WW	Not affected (skewed XCI)
Rett-like syndrome*	Arg133Cys	397C>T	MBD	RTT
	Ser134Cys	401C>G	MBD	RTT
	Tyr141X	423C>G	MBD	RTT
	Thr158Met	473C>T	MBD	RTT
	Arg270X	808C>T	TRD	RTT
	Gly273fs	816_822dup7	TRD	Not reported (*de novo*)
	Arg344Trp	1030C>T	WW	Not affected
	Glu455X	1363G>T	WW	Not reported (*de novo*)
Rett-like syndrome‡	Pro56fs	167_168delCC	N terminus	Not reported
	Arg133His	398G>A	MBD	RTT
	Arg270X	808C>T	TRD	RTT
Mild to severe MR§	Glu137Gly	410A>G	MBD	Not affected
	Ala140Val	419C>T	MBD	Not affected (balanced XCI)
	Arg167Trp	499C>T	Interdomain	Not affected
	Ala181Val	542C>T	Interdomain	Not affected
	Pro225Leu¶	674C>T	TRD	Not reported (*de novo*)
	Thr228Ser¶	683C>G	TRD	Not affected
	Lys284Glu¶	850A>G	TRD	Not reported
	Pro387Leu¶	1160C>T	WW	Not reported
	Pro387–Met466del80	1161_1400del240	WW	Not affected (skewed XCI)
	Gln406X	1216C>T	WW	Not affected (balanced XCI)
	Arg453Gln¶	1358G>A	WW	Not reported
	Arg471fs	1411_1412del2	CTS	Not reported (*de novo*)
	Glu472fs	1415_1416delAG	CTS	Not reported (*de novo*)

*Mainly due to X-chromosome aneuploidy. ‡Due to somatic mosaicism of the mutation in *MECP2*. §With or without diverse neurological symptoms. ¶These amino-acid changes were not detected in control individuals, but have not yet been confirmed as disease-causing mutations (see the MeCP2 resource in the online links box). CTS, C-terminal segment; fs, frameshift; MBD, methyl-CpG-binding domain; *MECP2*, methyl-CpG-binding protein 2; MR, mental retardation; RTT, Rett syndrome; TRD, transcriptional-repression domain; WW, WW-domain-binding region; X, stop codon that results from nonsense mutations; XCI, X-chromosome inactivation.

The first group is characterized by severe neonatal encephalopathy that is much more severe than the phenotype in females with classical Rett syndrome. Soon after birth there is severe neurodevelopmental delay, and male patients usually die in early childhood because of a central breathing failure. These patients either carry mutations in *MECP2* that are also found in females with Rett syndrome, or other mutations that have clear deleterious consequences (frameshift mutations or nonsense mutations).

The second group includes patients with symptoms that are highly similar to classical Rett syndrome in females. These cases result either from somatic mosaicism for mutations in MeCP2 such as 241del2, Arg133His and Arg270X[27,28], or occur in cases of Klinefelter syndrome in which mutations in MeCP2 such as Thr158Met, Tyr141X or Arg270X are encoded on one X chromosome[27]. In these patients, only mutations that affect Arg133 (Arg133Cys and Arg133His) — which are considered on the basis of *in vitro* studies to be mild mutations[29] — have been identified in females with Rett syndrome, in whom they result in a mild or atypical form of the disease[30].

The third group of males with mutations in *MECP2* are always mentally retarded, but the phenotype is heterogeneous: even within the same family, the phenotype ranges from mild to severe mental retardation[31–33]. These patients carry mutations that are inherited from their mothers and have never been found in females with Rett syndrome[31]. Apart from the common Ala140Val mutation, most of these mutations encode changes in the WW domain (TABLE 1).

Altogether, these findings indicate that males with mutations in *MECP2* can survive until after birth, but because they only have one copy of the gene they show more severe phenotypes than females with the same genetic defect. The biased distribution of the disease, with an almost exclusive occurrence of Rett syndrome in females, is consistent with the suggestion that most *de novo* mutations in *MECP2* originate from male germline cells[34], a theory that has subsequently been supported by experimental evidence[35,36]. However, although it is known that the mutations arise because of the hypermutability of some *MECP2* CpG sites, it remains unclear why this hypermutability occurs. One theory is that these CpG sites are methylated in the male germ line, and are therefore prone to deamination, which results in a conversion of CpG dinucleotides to TpG and CpA dinucleotides.

The proportion of mental retardation in males that is accounted for by mutations in *MECP2* is unclear. In one study, the figure was estimated at 2%[32], but another investigation of 475 males with mentally retardation found a frequency of 0.2–0.4%[37]. More recently, duplications in the Xq28 region that involve *MECP2* have been reported as causes of mental retardation[38,39]. Using X-chromosome-specific array CGH, duplications that affect *MECP2* (as well as another gene, L1 cell-adhesion molecule (*L1CAM*)) were identified in four unrelated male patients with severe mental retardation and spasticity[39]. Interestingly, these duplications seem to be associated with a syndromic, severe form of mental retardation that is distinguishable from that seen in classical Rett syndrome. These findings are consistent with phenotypes of transgenic mice in which *Mecp2* overexpression results in a neurological phenotype that is similar to that caused by *Mecp2* dysfunction[40]. Qualitative and quantitative *MECP2* defects might therefore account for a significant proportion of neurodevelopmental and mental retardation, indicating that *MECP2* analysis should be carried out not only in female patients with Rett syndrome, but also in male patients with severe mental retardation and other neurological symptoms.

Beyond MeCP2: the case of CDKL5. Although mutations in *MECP2* are responsible for almost all known cases of classical Rett syndrome, they have been identified in just 47–50% of atypical cases[23]. Interestingly, genetic defects that affect the X-linked gene cyclin-dependent kinase-like 5 (*CDKL5*) have recently been found to be involved in atypical Rett syndrome; at least 12 point mutations and 2 translocations in this gene have been reported (REFS 41–43 and authors' unpublished observations) (FIG. 2).

As mutations in *MECP2* and *CDKL5* lead to similar phenotypes, their involvement in a common pathway is suspected. Interestingly, recent data have shown that the spatio-temporal expression of CDKL5 during mouse development significantly overlaps with that of MeCP2, and that the two proteins interact *in vitro* and *in vivo*[43]. Moreover, CDKL5 is thought to indirectly mediate MeCP2 phosphorylation[43]. Additional studies are necessary to define this emerging molecular pathway, other components of which could be considered as candidates for targets of mutation in Rett-syndrome-like conditions in which there are no defects in *MECP2* and *CDKL5*.

Developmental roles of MeCP2

The specific neurodevelopmental effects that are seen in Rett syndrome, together with its onset after a period of apparently normal development, indicate that MeCP2 is not required for the early stages of neurogenesis, neuronal migration or neuronal maturation. They also indicate that although DNA-methylation marks are present throughout the genome, and would therefore be predicted to be recognized by MeCP2, the interpretation of this global epigenetic modification is likely to be highly regulated by complementary actions of temporally and spatially regulated proteins to produce specific biological effects. Studies of altered MeCP2 function during mouse development, together with expression studies and functional studies, have provided insights into the developmental roles of this protein.

Insights from mouse models. Several mouse models have advanced our understanding of the function of MeCP2 in development and of the mechanisms that underlie Rett syndrome. Two groups have reported studies in which *Mecp2* (which is also X-linked in the mouse) was deleted[44,45]. Female mice that are heterozygous for *Mecp2* deletion are viable, fertile and seem normal even in early adulthood. However, at about 6 months

Encephalopathy
A degenerative condition of the brain that can be caused by infectious disease, metabolic abnormalities, brain tumours, toxic drug effects or increased intercranial pressure.

Array CGH
A technique that uses comparative genomic hybridization on microarrays to determine copy-number differences between DNA sequences.

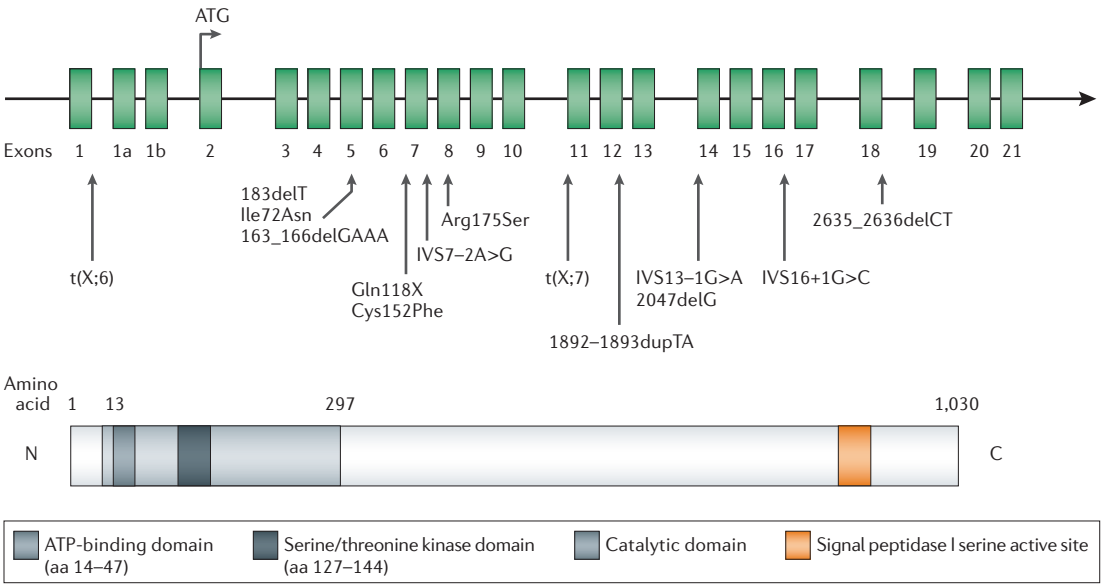

Figure 2 | **Mutations in *CDKL5* that are found in patients with atypical Rett syndrome.** The *CDKL5* (cyclin-dependent kinase-like 5) gene (top) and the protein that it encodes (bottom) are shown. Mutations in this gene that occur in patients with atypical Rett syndrome are indicated. (Gln118X (where X is a stop codon) and 1892_1893dupTA are based on the authors' unpublished data).

of age they begin to show neurological symptoms that are reminiscent of Rett syndrome. So, as in humans, the onset of Rett-syndrome-like symptoms is unlikely to occur during the embryonic and/or fetal stages in mice. Moreover, *Mecp2*-null mice (both male hemizygotes and female homozygotes) are viable and appear phenotypically normal at birth. However, between 3 and 8 weeks of age, these mice develop uncoordinated motor behaviour, reduced activity and breathing irregularities, and usually die by 10 weeks of age.

Interestingly, mice that are null for *Mecp2* only in nestin-positive neural precursor cells show a phenotype that is indistinguishable from that of *Mecp2*-null mice, which demonstrates that Rett syndrome symptoms are caused by MeCP2 deficiency in the CNS. Moreover, in mice in which *Mecp2* was deleted only postnatally and only in cells that expressed calcium/calmodulin-dependent protein kinase II — a kinase that is present only in post-mitotic neurons in the forebrain, hippocampus and brainstem — a postnatal neurological phenotype arises that has some Rett syndrome characteristics[44,46]. These mice lack MeCP2 function in broad forebrain regions, including some regions of the basal ganglia[46]. Their development seems normal until about 3 months of age (later than in the constitutively MeCP2-deficient mice), after which they begin to show symptoms[46]. Moreover, MeCP2 expression in postmitotic neurons under the control of the neuron-specific *tau* promoter rescues Rett syndrome symptoms in mice[47]. Together these data show that postnatal loss of MeCP2 in the forebrain is sufficient to recapitulate the symptoms of Rett syndrome.

A mouse model has been developed that expresses a truncated form of MeCP2 with a stop codon at the position that corresponds to amino acid 308, which is predicted to have effects that are similar to the Arg294X mutation that is frequently observed in classical human Rett syndrome[48]. Males that were hemizygous for this mutation seemed phenotypically normal for about 6 months, but then developed a progressive neurological disease that included many features of Rett syndrome. Heterozygous females had impaired motor features at 35–39 weeks of age, and showed phenotypic variability, similar to the situation in human cases. In this model, X-chromosome inactivation was found to be unbalanced, favouring the expression of the wild-type allele in most mutant females. This indicates that the activation status of the two X chromosomes in females influences the phenotypic outcome of MeCP2 defects in the mouse; this probably also occurs in humans[49]. As well as motor and behavioural abnormalities, these animal models (both MeCP2-null and MeCP2[308]) were shown to have learning and memory defects, and some impairment of synaptic plasticity[50,51].

A transgenic mouse model has recently been developed that expresses human MeCP2 at about twice the level at which the wild-type mouse protein is expressed[40]. Intriguingly, early in life, these mice show increased learning ability and increased synaptic plasticity. They seem otherwise normal until 10–12 weeks of age, but then show neurological symptoms, such as seizures, forepaw clasping, hypoactivity and spasticity. Between 30 weeks and 1 year of age, seizures become more frequent and mice develop kyphosis, become hypoactive and ataxic, and many die prematurely. Overexpression of MeCP2 in neurons is therefore also detrimental, indicating that levels of MeCP2 in the CNS are tightly regulated and crucial for neuronal function. Therefore, any therapeutic strategy that is aimed at increasing the levels of MeCP2 must be considered cautiously.

Nestin
A class VI intermediate-filament protein that is expressed during early embryonic development in mammals but is absent from nearly all mature CNS cells.

Basal ganglia
Clusters of neurons that are located deep in the brain that relay messages between the most anterior part of the cortex that is involved in problem solving and complex thought, and the lower motor and sensory areas; includes the striatum.

Kyphosis
Abnormal curvature of the thoracic spine.

Astrocyte
A star-shaped neuroglial cell that surrounds and supports neurons in the CNS.

Oligodendrocyte
A type of non-neuronal cell that lacks axons and dendrites and insulates axons so that they can send electric impulses. These are also myelin-producing cells.

Expression studies of MeCP2. Several studies have analysed the spatio-temporal distribution of MeCP2 in the brains of mice[52], macaque monkeys[53] and humans[52,54]. All these data indicate that MeCP2 expression is low or absent in immature neurons, astrocytes and oligodendrocytes, then steadily increases during neuronal maturation to reach a high level that is maintained throughout life. Other studies have revealed the presence of at least two types of MeCP2-expressing cell in normal human brains: the high ($MeCP2^{hi}$) and low ($MeCP2^{lo}$) subtypes. The $MeCP2^{hi}$ population expresses more of the MeCP2B isoform than the $MeCP2^{lo}$ cells in both children and adults[55]. Interestingly, $MeCP2^{hi}$ cells acquire this expression pattern mainly during postnatal stages of development, indicating that a high level of MeCP2 might be required for postnatal cellular processes, which is consistent with the late onset of Rett syndrome[55,56].

Expression studies also indicate that MeCP2 is involved in the differentiation of neuronal cells, including dendritic arborization and the development and maintenance of dendritic spines, rather than in cell-fate decisions[57–59]. Consistent with this, a recent study[60] showed that downregulation of *MECP2β* expression using antisense morpholinos markedly inhibits the formation of neuritic processes in PC12 cells. In addition, studies of human postmortem tissue have shown less complex dendritic arborizations, smaller neurons and a reduction of dendritic spines of cortical neurons in patients with Rett syndrome[57,58], which could be due to defects in either the maturation of neurons or the maintenance of their differentiated state. The most dramatic decreases in the complexity of dendritic arborization were in regions such as the cerebral motor and prefrontal cortex, consistent with Rett syndrome symptoms[57,58].

Consistent with these findings, lower levels of several dendrite-specific and synaptic proteins have been found in brain tissue from patients with Rett syndrome[61–63]. The most significant reductions were observed for the microtubule-associated proteins MAP2 and MAP5, which are markers of early dendritic differentiation and branching. A potential role of reduced levels of these proteins in the pathogenesis of Rett syndrome is supported by the identification of a patient with Rett-syndrome-like symptoms who carried a chromosome-2 deletion that encompassed *MAP2* (REF. 64). These presynaptic and postsynaptic markers are not known as direct targets of MeCP2, and their reduced level is probably secondary to downstream effects of MeCP2 deficiency, such as defects in neuronal differentiation and maturation.

The evidence that is outlined above indicates a mechanism that could explain the late onset of Rett syndrome symptoms. It is likely that MeCP2 dysfunction leads to the impaired maturation of most neurons. However, late-developing neural cells for which maturation continues during postnatal stages (for example, in the frontal cortex) would suffer from cumulative effects, which could be both cell-autonomous and non-autonomous. The latter defects could result from a lack of early afferent neuronal connections and branching, which normally induce additional synaptic changes, including synapse formation and stabilization.

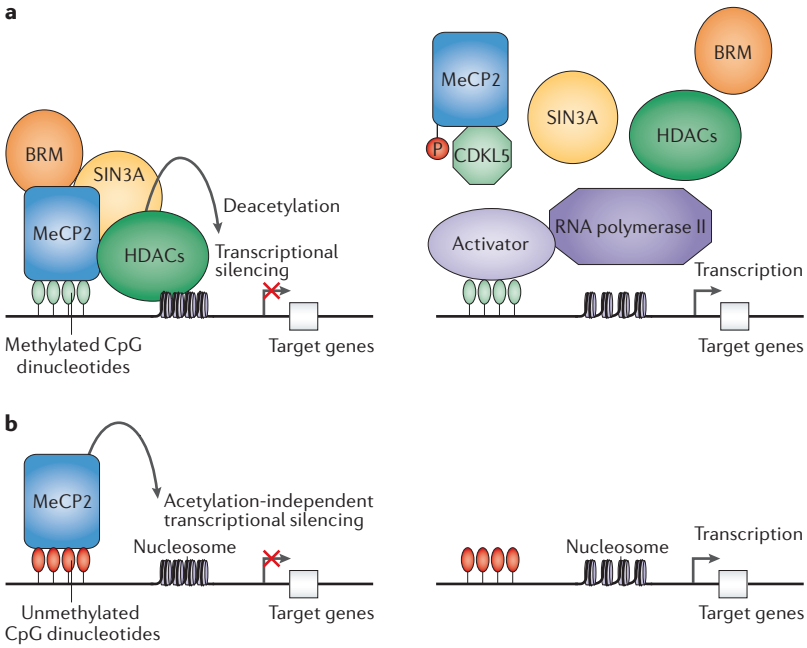

Figure 3 | **MeCP2 regulation of chromatin remodelling and transcription.** **a** | Transcription is suppressed in promoter regions containing methylated CpGs that are bound by MeCP2 (methyl-CpG-binding protein 2), for example, in the promoter of the gene that encodes BDNF (brain-derived neurotrophic factor)[77,78], as shown in the left panel. MeCP2 binds methylated DNA and recruits chromatin-remodelling complexes that contain SIN3A (a transcriptional co-repressor), BRM (a SWI/SNF-related chromatin-remodelling protein) and histone deacetylases (HDACs). This leads to chromatin condensation owing to histone deacetylation, which results in a limited accessibility of the transcriptional machinery to promoter regions. When MeCP2 is not bound to methylated DNA (right panel), the complex that usually contains MeCP2, BRM, SIN3A and HDACs is not recruited. This lack of MeCP2 binding to DNA could be due to the activity of CDKL5 (cyclin-dependent kinase-like 5), which is thought to bind and contribute to the phosphorylation of MeCP2, resulting in the inability of MeCP2 to bind its methylated binding site (as shown). Alternatively, a similar effect could result from a missense mutation in the methyl-CpG-binding domain or loss of expression of MeCP2, for example, because of an early-truncating mutation (these scenarios are not shown here). In each of these cases, histones remain acetylated and the DNA at the promoter remains in an open conformation, allowing transcription factors to bind DNA and initiate transcription. **b** | MeCP2 is also a potent chromatin-condensing protein and can repress gene expression independently of DNA methylation, at least *in vitro*[71] (left panel). At promoters where this DNA-methylation-independent function of MeCP2 is involved in regulating expression, a deficiency or absence of MeCP2 leads to a disorganization of chromatin structure (indicated here by increased spacing between nucleosomes), making transcription more likely to occur (right panel).

MeCP2 as a transcriptional regulator

The results that are described above provide strong evidence that MeCP2 regulates genes that are involved in neuronal maturation. Considerable effort has been put into understanding how MeCP2 regulates genes and into identifying its potential targets.

MeCP2 was initially shown to repress transcription through the interaction of the TRD with a co-repressor, the SIN3A complex, which contains histone deacetylase 1 (HDAC1) and HDAC2 (FIG. 3a). These enzymes remodel chromatin, which becomes inaccessible to the transcriptional machinery[7,65]. MeCP2 also interacts with other co-repressors, such as the SKI viral proto-oncoprotein and the nuclear receptor

Dendritic arborization
The process that leads to the production of the numerous dendrites that characterize many neurons, and the branches that project from these dendrites.

Dendritic spine
Mushroom-shaped structures on neuronal dendrites that receive synaptic input and are the sites of postsynaptic densities. Changes in spine shape are thought to be important for modulating synaptic strength.

Morpholinos
Oligonucleotides that are efficient gene-silencing reagents, and that can either block translation initiation in the cytosol or modify pre-mRNA splicing in the nucleus (by targeting splice junctions).

PC12 cells
A clonal cell line that responds reversibly to nerve growth factor.

co-repressor, NCOR[9], both of which are components of HDAC complexes.

However, the link between histone deacetylation and MeCP2-mediated transcriptional repression is not straightforward. Increased acetylation of histone H4 (at lysine 16) is seen in lymphoblasts that carry the common Rett syndrome Arg168X nonsense mutation[66], whereas a recent study of lymphocytes from patients with Rett syndrome showed an unexpected decrease in histone H3 acetylation, mainly at lysine 14 (REF. 67). Moreover, mice with an *MECP2* nonsense mutation showed increased acetylation of histone H3 in the brain, but no changes in acetylation of histone H4 (REFS 48,52). In addition, in both mammalian and *Xenopus* systems, HDAC inhibitors do not completely restore levels of transcription of a reporter that is repressed by the MeCP2 TRD[8]. Although further investigations are required to assess the effects of MeCP2 deficits on histone acetylation and gene expression, these studies indicate either temporal- and tissue-specific regulation by MeCP2, and/or that MeCP2-mediated transcriptional repression involves local recruitment of specific combinations of proteins that include HDACs.

Methylation is another key post-translational modification of histones, and represents an important epigenetic mechanism for the organization of chromatin structure and the regulation of gene expression[68]. In particular, methylation at lysine 9 of histone H3 (H3K9) is associated with gene silencing. Recently, it has been shown that MeCP2 is involved in histone methylation *in vitro* and *in vivo*. At the *H19* gene, MeCP2-associated methylation is specific for H3K9, and is probably mediated by a complex that has not yet been identified[69,70].

Another link between MeCP2 and histone methylation is the function of MeCP2 in the repressor-element-1-silencing transcription factor (REST)–Rest-corepressor-1 (RCOR1) repressor complex. This complex, which binds to repressor elements of target-gene promoters (such as that of the sodium channel type II (SCN2A) gene), recruits MeCP2 and inactivates transcription through H3K9 methylation, which is carried out by the histone lysine methyltransferase suppressor of variegation 3-9 homologue 1 (SUV39H1)[69,70]. However, the exact function of MeCP2 in this complex is unknown. Altogether, these studies indicate that MeCP2 contributes to the establishment of various epigenetic marks, and highlight the potential involvement of these MeCP2-related epigenetic modifications in transcriptional repression.

More recently, MeCP2 has also been shown to mediate the assembly of novel chromatin secondary structures independently of its binding to methylated DNA[71], which provides another possible explanation for the finding that MeCP2-mediated transcriptional repression is only partially relieved by HDAC inhibitors[7,8] (FIG. 3b). Analysis of the effect of mutations in *MECP2* on chromatin condensation localized the responsible domain to the TRD and/or C-terminal regions, but the mechanism has yet to be elucidated.

MeCP2: a specific or global regulator?

Does MeCP2 interpret DNA-methylation marks to function as a global gene regulator, or does it regulate specific target genes with neuronal functions? Furthermore, are the clinical symptoms of Rett syndrome caused by global or specific transcriptional deregulation? With the availability of various mouse models of the loss or overexpression of MeCP2, several studies have examined the genes that are abnormally expressed in neurons from patients with Rett syndrome or mice that are deficient in MeCP2 (TABLE 2).

Global studies of MeCP2-regulated changes in gene expression. Six studies have investigated global gene-expression changes in brain, neuronal and non-neuronal tissue from mice that lack MeCP2 and/or from patients with Rett syndrome. However, as only small and unreproducible differences in gene expression were observed, most of these studies failed to pinpoint convincing target genes that are involved in Rett syndrome pathogenesis[72–74]. Here, we summarize the most significant results from these

Table 2 | **MeCP2 target genes**

Gene	Species	Unigene cluster	Function	Tissue in which gene is expressed	Change in expression level caused by MeCP2	References
BDNF	Mouse	Mm.1442	Survival, neuronal plasticity	Cultured neurons	Upregulated ~2-fold	77,78
hairy2	*Xenopus*	Xl.25977	Neuronal differentiation	Whole embryo	Downregulated ~2-fold	93
Fkbp5	Mouse	Mm.276405	Hormonal signalling	Brain (74 days of age)	Upregulated 2.26-fold	76
IGF2	Human	Hs.147470	Cell proliferation	Lymphoblastoid cells	Upregulated 2.21-fold	74
DLX5	Human	Hs.99348	Transcription factor	Lymphoblastoid cells	Upregulated ~2-fold	88
Dlx5	Mouse	Mm.4873	Transcription factor	Brain	Upregulated ~2-fold	88
Dlx6	Mouse	Mm.5152	Transcription factor	Brain	Upregulated ~2-fold	5
Ube3a	Mouse	Mm.9002	Proteolysis	Brain	Downregulated ~2-fold	87
UBE3A	Human	Hs.22543	Proteolysis	Brain (2–20 years of age)	Downregulated ~2-fold	87
Sgk1	Mouse	Mm.28405	Cellular stress response	Brain (74 days of age)	Upregulated 3.44-fold	76
MPP1	Human	Hs.305360	Signal transduction	Lymphoblastoid cells	Upregulated 3.32-fold	74

BDNF, brain-derived neurotrophic factor; DLX, distal-less homeobox; Fkbp5, FK506-binding protein 5; IGF2, insulin-like growth factor 2; MeCP2, methyl-CpG-binding protein 2; *MPP1*, palmitoylated membrane protein 1; *Sgk1*, serum/glucocorticoid kinase 1; Ube3a, ubiquitin protein ligase E3A.

studies that contribute to understanding the function of MeCP2 and the pathophysiology of Rett syndrome.

Using a proteomics approach to investigate temporal and regional expression patterns, one recent study detected 13 proteins of various functions (such as chromatin structure, energy metabolism, cell signalling and neuroprotection) as being differentially expressed in the olfactory systems of wild-type and MeCP2-deficient mice[75]. For a few genes, such as the one that encodes the calcium-binding protein calretinin, differences were seen at the protein level but not at the RNA level, which indicates a deregulation of post-translational modifications that remains difficult to reconcile with the known functions of MeCP2.

In another study[76], gene expression was compared in *Mecp2*-null and wild-type mice, both at early postnatal stages — before the onset of the neurological manifestation — and at a later stage in mice that had developed symptoms. This study identified 11 genes that are misexpressed in *Mecp2*-null mice, 5 of which are regulated by glucocorticoid hormones that are secreted in response to stress. The most significant changes were observed for FKBP5 (FK506-binding protein 5) and SGK1 (serum/glucocorticoid kinase 1), which showed between two- and threefold elevation of expression in *Mecp2*-null brains before the onset of symptoms[76]. SGK1 has a role in activating specific potassium, sodium and chloride channels, and could therefore be involved in the regulation of several processes, including cell survival and neuronal excitability, whereas FKBP5 might modulate steroid-receptor function through its association with the molecular chaperone HSP90 (heat-shock protein 90). Misregulation of these genes seems to be an early event that occurs during important stages of postnatal brain development, but a contribution to the Rett syndrome phenotype remains to be demonstrated.

BDNF as an MeCP2 target. As well as these large-scale studies, candidate-gene approaches to identifying MeCP2 target genes have also been carried out and have identified the gene that encodes brain-derived neurotrophic factor (BDNF) as a genuine MeCP2 target (TABLE 2). Two groups[77,78] showed that MeCP2 is bound to methylated CpG sites near the promoter III region of BDNF in resting neurons. However, when the neurons were exposed to potassium chloride — which causes membrane depolarization, calcium influx and BDNF activation — MeCP2 dissociated from the *BDNF* promoter. This displacement has been suggested to result from reduced CpG methylation in the relevant region of the activated promoter[77]. An alternative mechanism has been suggested in a study that showed a time-dependent increase in MeCP2 phosphorylation when neurons were stimulated with potassium chloride, which might influence the affinity of MeCP2 for the methylated promoter site[78]. As CDKL5 interacts with MeCP2 both *in vivo* and *in vitro* and is thought to indirectly induce its phosphorylation, this protein might link neuronal depolarization to MeCP2 displacement.

SIN3A, an MeCP2 co-repressor, is also displaced from the repressor complex after potassium chloride treatment[77] and loss of MeCP2 is accompanied by changes in histone modification, which results in a transcriptionally permissive chromatin state. Subsequently, one study showed that *BDNF* transcription in resting cells is twofold higher in neurons of MeCP2-deficient mice than in those from wild-type animals. However, this difference was not seen in depolarized cells, indicating that MeCP2 represses basal levels of *BDNF* expression. It is therefore unsurprising that microarrays failed to detect significant differences in *BDNF* expression, as the *in vivo* situation corresponds to a mixture of both active and inactive neuronal cells. Further evidence for MeCP2 function as a regulator of *BDNF* expression was provided by postmortem studies of brains from patients with Rett syndrome. BDNF levels were found to be higher in the 'Rett-syndrome-affected' prefrontal cortex and lower in the 'Rett-syndrome-spared' occipital cortex, particularly after childhood[79].

Although the specific functions of BDNF have not been completely elucidated, it is clear that, as well as its neurotrophic effects, it has an important function in regulating synaptic plasticity in various brain areas, including the hippocampus[80,81]. A deficit in MeCP2 function and in its downstream effects on BDNF expression, especially in postnatal stages, might therefore account for the neurobiological and cellular defects that are observed in Rett syndrome. In support of this, transgenic mice that overexpress BDNF develop symptoms that are similar to those in MeCP2-deficient mice[82,83]. In addition, mice in which BDNF deletion is triggered early in development and specifically in the forebrain show less complex dendritic arborization at 3 weeks of age, the same age at which *Mecp2*-null mice begin to manifest neurological symptoms and cessation of brain development[84,85]. Therefore, it appears that deregulation of BDNF expression (either through repression or overexpression) results in abnormal neuronal morphogenesis and maturation, as well as disrupted synaptogenesis and neural circuitry.

Most recently, studies in mice have provided the first *in vivo* evidence for a functional interaction between BDNF and MeCP2 (REF. 86). The results of this study were in some ways surprising: decreased levels of BDNF protein in the brains of MeCP2-deficient mice were observed, in contrast to the previous finding by the same group that the *Bdnf* gene is a target of repression by MeCP2 (REF. 78). To explain these conflicting results, the authors emphasized the differences between the experimental designs of the *in vitro* and *in vivo* studies, which examined BDNF mRNA and protein levels, respectively; they argue that these differences prevent a direct comparison of the results. Because BDNF expression depends on neuronal activity, they favour the hypothesis that MeCP2 deficiency reduces neuronal activity, thereby indirectly causing decreased BDNF protein levels. However, examination of MeCP2-deficient mice showed that forebrain-specific knockout of *Bdnf* in these animals exacerbates the Rett-syndrome-like phenotype, whereas forebrain-specific overexpression of BDNF rescued a subset of Rett-syndrome-like phenotypes. The overall data strongly indicate that there is a functional interaction between MeCP2 and BDNF and that brain BDNF levels can modulate Rett syndrome disease progression.

MeCP2 regulation of imprinted genes. An involvement of MeCP2 in the regulation of imprinted genes was first indicated by studies that showed subtly altered expression of *UBE3A* (ubiquitin protein ligase E3A), *GABRB3* (γ-aminobutyric-acid receptor β3) and *Dlx5* (distal-less homeobox 5) — all of which are imprinted — in patients with Rett syndrome and in MeCP2-deficient mice[87–89]. Subsequent studies have provided insights into the mechanisms that are involved.

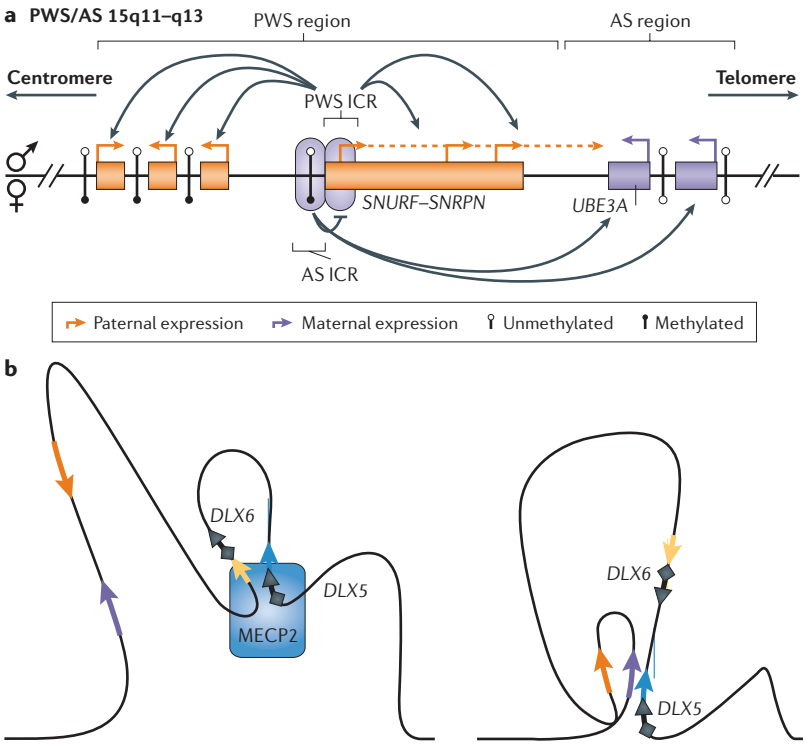

Figure 4 | **Regulation of imprinted regions by MeCP2. a** | The imprinted gene *UBE3A* (ubiquitin protein ligase E3A) lies within the Prader-Willi syndrome (PWS)–Angelman syndrome (AS) locus. The methylation status of the paternal and maternal alleles are indicated above and below the chromosome, respectively. The imprinting control region (ICR) for the PWS–AS locus consists of two parts, with the more centromeric component functioning as the AS ICR. The two ICRs direct the allele-specific expression of imprinted genes within these regions (indicated by arrows; not all genes in the region are shown). The *SNRPN* (small nuclear ribonucleoprotein polypeptide N)–*SNURF* (SNRPN upstream reading frame) gene produces a long and complex transcript that leads to the expression of not only SNURF–SNRPN, but also several small nucleolar RNAs (snoRNAs). This transcript is also thought to inhibit the expression of *UBE3A* from the paternal allele through an antisense mechanism. *UBE3A* expression has been shown to be regulated by MeCP2 (methyl-CpG-binding protein 2), as *UBE3A* mRNA levels are reduced in MeCP2-deficient cells. This downregulation of expression correlates with a biallelic production of *UBE3A* antisense RNA and changes in chromatin structure, with increased acetylation and methylation of H3K4 (histone 3 lysine 4), and reduced methylation of H2K9 at the PWS–AS imprinting centre[87]. This indicates that MeCP2 might mediate the interpretation of imprinted DNA-methylation marks in this region, in combination with other chromatin-binding proteins. **b** | MeCP2 can also regulate gene expression and maternal imprinting through formation of a silent chromatin loop. The yellow and blue arrows indicate MeCP2-interacting sequences that have been identified by chromatin immunoprecipitation. When MeCP2 is present (left panel), it interacts with sequences that are near the imprinted *DLX5* (distal-less homebox 5) and *DLX6* genes and define the boundaries of an 11-kb chromatin loop, the formation of which is induced by MeCP2 binding[88]. This leads to an integration of *DLX5* and *DLX6* into a loop of silent, methylated chromatin, and represses their expression. In neurons that are deficient for MeCP2 (right panel), the chromatin in this region is structured into a distinct conformation that corresponds to active chromatin loops, which are delimited by sequences (indicated by purple and orange arrows) that interact with chromatin factors. Therefore, in MeCP2-deficient neurons, the expression of *DLX5* and *DLX6* is no longer repressed, which results in the overexpression of these genes. Part **b** is modified with permission from *Nature Genetics* REF. 88 © (2005) Macmillan Publishers Ltd.

UBE3A is located in an imprinted region, defects in which have a causal role in Prader-Willi syndrome (PWS) and Angelman syndrome (AS). The imprinted expression of genes in this region involves a complex regulation that requires *cis*-acting elements called imprinting control regions (ICRs), which coordinate the expression of several neighbouring genes within a domain (FIG. 4a). The ICRs direct expression of genes within this region from the allele that is derived from one parent only, based on allele-specific epigenetic modifications of chromatin. These modifications are DNA-methylation marks at differentially methylated regions (DMRs), histone acetylation and histone methylation, and are thought to result in allele-specific accessibility to the transcriptional machinery and to DNA-binding proteins that might function as enhancers or insulators (reviewed in REFS 2,90).

UBE3A encodes the ubiquitin ligase E3A and is maternally expressed and imprinted only in the brain, and mutations in the maternal copy account for about 10% of cases of AS. Regulation of *UBE3A* expression involves both a region that is located upstream of the nearby small nuclear ribonucleoprotein polypeptide N (*SNRPN*)–SNRPN upstream reading frame (*SNURF*) locus, known as the PWS–AS imprinting centre, and the recently identified small nucleolar RNAs that overlap with *UBE3A* and are transcribed in the opposite direction. These *UBE3A* antisense transcripts have been suggested to inhibit *UBE3A* transcription from the paternal allele[2,90].

UBE3A mRNA and protein levels are slightly reduced in human and mouse MeCP2-deficient brains (TABLE 2). This correlates with a biallelic production of *UBE3A* antisense RNA and changes in chromatin structure, with increased acetylation and methylation of H3K4 and reduced methylation of H3K9 at the PWS–AS imprinting centre[87]. This indicates that MeCP2, the expression of which is also tightly regulated, might mediate the interpretation of imprinted DNA-methylation marks, in combination with other chromatin-binding proteins. This might produce temporally and spatially defined gene-expression patterns, such as that of *UBE3A*. Moreover, as maternal mutations in *UBE3A* (or repression of the maternal allele) give rise to AS, it is tempting to speculate that deregulation of UBE3A expression that results from MeCP2 loss of function might contribute to the clinical manifestations of Rett syndrome, such as mental retardation, seizures, muscular hypotonia and acquired microcephaly, that are common to both conditions[87].

In a separate study[88], chromatin immunoprecipitation has been used to identify MeCP2-binding sites in mouse brain, revealing several sequences that are located within an imprinted gene cluster on chromosome 6, which includes *Dlx5* and *Dlx6*. Expression of these genes is roughly two times higher in the brains of *Mecp2*-null mice than in those of wild-type mice. Interestingly, the *Dlx5*-imprinting pattern is disrupted in the brains of *Mecp2*-null mice. MeCP2 was shown to mediate the formation of an 11-kb chromatin loop at the *Dlx5–Dlx6*

Prader-Willi syndrome
A genetic disorder that is caused by loss of paternally expressed genes that are located in the 4-Mb imprinted region of 15q11–q13. Features of the disorder include excessive eating (hyperphagia), obesity, short stature, mental retardation or learning disabilities, and behavioural problems.

Angelman syndrome
A genetic disorder that is caused by several genetic mechanisms that inactivate or disrupt the maternally derived *UBE3A*. Symptoms include hyperactivity, ataxia, problems with speech and language, and an unusually happy demeanour.

Ubiquitin ligase
A protein that is involved in polyubiquitylation that covalently attaches ubiquitin to a lysine residue on a target protein. Polyubiquitylation marks proteins for degradation by the proteasome.

γ-Aminobutyric acid
A major inhibitory neurotransmitter in the mammalian CNS that participates in the regulation of neuronal excitability through interaction with specific receptors.

locus that is enriched in methylated H3K9, which is usually present in silent chromatin. This loop was absent in the brains of *Mecp2*-null mice, where *Dlx5* and *Dlx6* form distinct loops that are associated with active chromatin (FIG. 4b). This highlights a previously unrecognized mechanism of gene regulation by MeCP2, which is thought to be mediated by its involvement in conformational changes in chromatin structure. The relevance of these findings to Rett syndrome was supported when it was shown that *DLX5*, which is maternally expressed, shows a loss of imprinting in lymphoblastoid cells from individuals with Rett syndrome[88]. As DLX5 regulates the production of enzymes that synthesize γ-aminobutyric acid (GABA), loss of imprinting of *DLX5* might alter GABA-dependent neuron activity and contribute to the clinical manifestations of Rett syndrome.

An alternative hypothesis — MeCP2-regulated splicing. Another mechanism by which alterations in MeCP2 function might contribute to the specific defects that are seen in Rett syndrome is through disruption of alternative splicing in the brain[91]. Y-box-binding protein 1 (YB1; also known as p50 and EF1A), which has many cellular functions that are mediated through its involvement in the regulation of DNA- and RNA-dependent events, was identified as an MeCP2 binding partner (FIG. 5). The functional significance of this interaction was investigated by determining whether the MeCP2–YB1 complex affects mRNA processing and splice-site selection. In a cell-based assay, MeCP2 modified the splicing pattern of a reporter gene in a sequence-dependent manner. The *in vivo* role of the MeCP2–YB1 complex was then investigated by examining its function in the regulation of alternative splicing of candidate genes, including the NMDA receptor subunit 1 (NR1). The expression of NR1 is regulated by an alternative splicing event that is dependent on neuronal activity and generates two functional variants[92], and a comparison of wild-type and *Mecp2*-null mice showed significant differences in the relative amounts of the two variants[91].

In the same study, the expression of alternative transcripts in the cerebral cortex of wild-type and *Mecp2*$^{308/y}$ mice was determined by microarray analysis, which showed differences in the splicing patterns of several genes. Interestingly, one of these was *Dlx5*, which, as described above, is also a direct target of MeCP2 transcriptional silencing. MeCP2-related neurodevelopmental disorders might therefore be the result of misregulation of both transcription and splicing, although genes that are affected by alternative-splicing alterations that contribute to the Rett syndrome phenotype in humans have yet to be identified. It is tempting to speculate more generally that the various functions of MeCP2 in splicing and transcriptional regulation are coordinated, so that when a promoter becomes reactivated by the dissociation of the MeCP2-related repressor complex from its target, splicing of the nascent transcript is modulated by MeCP2. This remains to be demonstrated; however, if this were the case, an efficient coordinated contribution of MeCP2 to transcriptional and splicing regulation might be expected to depend on the molecular context, specific target genes and/or the spatio-temporal pattern of expression of interacting proteins.

How many MeCP2 targets are there? So far, attempts to identify MeCP2 target genes have had limited success and have led to the conclusion that MeCP2 is not a global repressor of transcription. However, one potential limitation of the approaches that have been taken is the heterogeneity of brain tissue and the possibility that MeCP2 regulates different sets of genes in different cell types. A limited number of MeCP2 targets have been identified, including *hairy2*, *BDNF*, *Dlx5* and several glucocorticoid-regulated genes[76,86,88,93] (TABLE 2). However, because the functional relevance of these targets to Rett syndrome pathogenesis has not been established, an important question is whether misregulation of these genes alone contributes to Rett syndrome. It is conceivable that the altered expression of a few key genes is responsible for manifestations of Rett syndrome, and for each of the targets that have been identified a case can be made that misregulation would have neurological consequences that could give rise to specific symptoms. However, an alternative hypothesis is that Rett syndrome symptoms are due to a combination of many milder defects in gene expression and/or splicing regulation, only a few of which are currently known. As discussed above, this issue has now been partially addressed by the first *in vivo* evidence for a functional interaction between BDNF and MeCP2, and the finding that brain BDNF levels can modulate Rett syndrome progression, which seems to tip the balance of evidence away from MeCP2 being a global regulator.

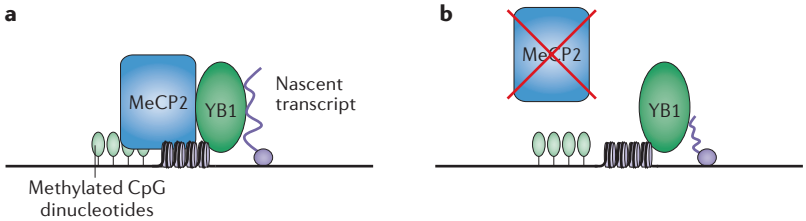

Figure 5 | **Regulation of alternative splicing by MeCP2. a** | MeCP2 (methyl-CpG-binding protein 2) interacts with YB1 (Y-box protein 1), a principal component of messenger ribonucleoprotein particles that controls multiple steps of mRNA processing, including the selection of alternative splice sites [91]. **b** | In neurons that are deficient for MeCP2, the splicing of nascent transcripts is altered and aberrantly spliced transcripts can be produced.

Outlook

Although much has been learnt about the biology of Rett syndrome since the discovery of mutations in *MECP2* as the main underlying genetic defect, there are several areas in which extensive investigation is needed in the future. First, identification of MeCP2 targets remains an important issue that should be addressed by improving the design of future differential-expression experiments, which should, for example, take into account the possibility that MeCP2 is regulated differently in resting and active neurons. Second, the precise role of MeCP2 in neuronal nuclei remains unclear, although there is evidence for a functional link between MeCP2 and the expression of BDNF, and a role in postnatal neuronal morphogenesis,

maturation and synaptic activity has been suggested. Further investigations are required to define the cellular pathways that lead to BDNF regulation and the subsequent neuronal effects, and to address the apparent discrepancy in mouse models between the increased level of *BDNF* mRNA in resting neuronal cells and the decreased level of BDNF protein in MeCP2-deficient mouse brains. In addition, experiments that dissect cell-autonomous and non-autonomous BDNF-mediated consequences of MeCP2 deficits, and the functional interaction between MeCP2 and BDNF, should advance our understanding of Rett syndrome pathogenesis.

Much is also still to be learned about the functions of MeCP2 at the molecular level. As many studies support the coupling of transcription and pre-mRNA processing[94], it would be relevant to assess whether MeCP2 functions in the regulation of these processes in a coordinated manner or through independent molecular mechanisms. Target genes such as *BDNF*, *DLX5* and *NR1* that are known to be regulated by MeCP2 and are thought to contribute to Rett syndrome pathogenesis could be used as models to address this issue. Finally, it is hoped that investigations that are based on both animal models of Rett syndrome and cells from patients will improve our understanding of the consequences of MeCP2 dysfunction and provide insights for the development of therapeutic approaches.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BDNF | calretinin | *CDKL5* | *Dlx5* | *Dlx6* | FKBP5 | *GABRB3* | *H19* | H3 | H4 | *hairy2* | HDAC1 | HDAC2 | HSP90 | Kaiso | *L1CAM* | MAP2 | MAP5 | MBD1 | MBD2 | MBD4 | MeCP2 | NCOR | NR1 | RCOR1 | REST | SCN2A | SGK1 | SIN3A | SKI | *SNRPN* | *SNURF* | SUV39H1 | *UBE3A* | YB1
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
AS | PWS | Rett syndrome

FURTHER INFORMATION
French Association for Rett Syndrome: http://www.afsr.net
International Rett Syndrome Association: http://www.rettsyndrome.org
InterRett - IRSA Rett Phenotype Database: http://www.ichr.uwa.edu.au/rett/irsa
MeCP2 resource: http://www.mecp2.org.uk
Rett Syndrome Association UK: http://www.rettsyndrome.org.uk
SyReNe Rett Syndrome Network: http://afsr.in2p3.fr/RETT
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