OXFORD

Briefings in Functional Genomics, 15(6), 2016, 420–431

doi: 10.1093/bfgp/elw023
Advance Access Publication Date: 12 June 2016
Review paper

MECP2, a multi-talented modulator of chromatin architecture

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Abstract

It has been a long trip from 1992, the year of the discovery of MECP2, to the present day. What is surprising is that some of the pivotal roles of MeCP2 were already postulated at that time, such as repression of inappropriate expression from repetitive elements and the regulation of pericentric heterochromatin condensation. However, MeCP2 performs many more functions. MeCP2 is a reader of epigenetic information contained in methylated (and hydroxymethylated) DNA, moving from the 'classical' CpG doublet to the more complex view addressed by the non-CpG methylation, which is a feature of the postnatal brain. MECP2 is a transcriptional repressor, although when it forms complexes with the appropriate molecules, it can become a transcriptional activator. For all of these aspects, Rett syndrome, which is caused by MECP2 mutations, is considered a paradigmatic example of a 'chromatin disorder'. Even if the hunt for *bona-fide* MECP2 target genes is far from concluded today, the role of MeCP2 in the maintenance of chromatin architecture appears to be clearly established. Taking a cue from the non-scientific literature, we can firmly attest that MeCP2 is a player with '*a great future behind it*'*.
**V. Gassmann 'Un grande avvenire dietro le spalle'. TEA Eds*

Key words: MECP2; DNA methylation; pericentric heterochromatin; Rett syndrome; neurological diseases

Introduction

Epigenetic regulation of the genome acts at different levels of complexity and cross-talk, which are responsible for the establishment of cell and tissue identity [1]. Among the major epigenetic modifications, DNA methylation [2] and histone modifications [3] are of particular interest.

Mammalian genomes are globally methylated, except for short hypomethylated regions known as CpG islands [4]. Methylation of cytosine residues (the most characterized DNA methylation site) is catalyzed by the DNA methyltransferase (DNMT) enzymes [5], which can be thought of as the 'writers'. Generally, methylation of CpG sites (mCGs) in the promoters and enhancers is associated with chromatin compaction and

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gene silencing [6, 7], whereas highly expressed genes frequently show hypermethylation in the gene body [8].

DNA methylation is implicated in important biological phenomena, such as genomic imprinting, X-chromosome inactivation in mammals and silencing of transposable elements [9, 10]. In this respect, the novelty of the Bird's hypothesis [11] should still be noted, which links the global repressive effects of DNA methylation, to the suppression of the transcriptional noise that occurs from the spurious expression of retroposons, with the presence of a nuclear membrane that marks the prokaryotes/eukaryotes boundary. The net result is that more genes are tolerated [11, 12].

DNA methylation-mediated-transcriptional repression can occur in two ways: (i) the presence of methyl-cytosine in the binding site of specific transcription factors hampers its interaction with target DNA, and, alternatively, (ii) methylated CpGs act indirectly, by recruiting proteins that can bind methylated DNA [(methyl CpG binding protein 2 (MeCP2) and other methyl binding proteins (MBDs)] through the methyl binding domain (MBD), a protein motif of about 75 amino acids [13, 14]. These proteins induce a repressive state of chromatin by recruiting histone deacetylases (HDACs), methyltransferases and other chromatin-silencing factors, which increase chromatin condensation and prevent transcription [15, 16] (for an updated review on MBDs, see [1]). This evidence suggests that DNA methylation significantly affects the occupancy of a specific genomic region.

Among the protein translators between DNA methylation, histone modifications and chromatin organization, the so-called 'readers' of epigenetic modifications, MeCP2, which was identified by Lewis *et al.* in 1992 [17], is particularly attractive.

MeCP2 mutations are responsible for more than 95% of cases of the Rett syndrome (RTT; OMIM 312750) [18, 19], which is a sporadic and progressive postnatal neurodevelopmental disorder that almost exclusively affects females, with a frequency of 1 in 10 000–15 000, and with complete penetrance [20–23].

Most of the MECP2 mutations are paternally inherited *de novo* mutations that have been found throughout the entire MECP2 gene, as missense, nonsense and frameshift mutations, and C-terminal truncations [24]. The most common MECP2 mutations that cause classical RTT mainly lie within the MBD [25, 26] or in the transcriptional repression domain (TRD, see below) [27]. These are thus likely to alter the ability of MeCP2 to bind methylated DNA or to interfere with the MeCP2 recruitment of its molecular partners.

The phenotypes related to MeCP2 mutations become evident in the central nervous system during postnatal brain development, when MeCP2 expression usually increases [28–30].

Interestingly, the duplication of the MECP2 gene that causes a doubling of the MeCP2 expression is responsible for a neurological disorder [31, 32], which underlines the importance of an appropriate 'dose' of the MeCP2 protein.

MeCP2: the gene and the protein

MeCP2 was the first MBD-containing protein discovered, due to its binding to a single methyl-CpG pair [13, 17]. *In vitro* studies showed that the high affinity binding of MeCP2 to methylated DNA requires four or more A/T sequences adjacent to the methyl-CpGs [33]. MeCP2 also contains: (i) a TRD that mediates its links with the histone modifications and co-repressors [12]; (ii) a nuclear localization signal (NLS) that imports part of the total protein into the nucleus; and (iii) a C-terminal domain that is involved in the interactions with DNA and its protein partners [34]. In addition, three AT-hook-like domains were identified in MeCP2 recently ([Figure 1]). These domains are shared with chromatin-associated proteins of the high-mobility group AT-hook (HMGA) family, and they have roles in DNA binding [36].

The MECP2 gene is located within Xq28 and it is subjected to X inactivation [37, 38]. This gene contains four exons, that code for two protein isoforms that are produced by alternative splicing: MeCP2-e1 (i.e. MECP2B or MeCP2α) and MeCP2-e2 (i.e. MECP2A or MeCP2β). These splicing isoforms differ at their N-terminal regions, although they include both of the two main domains (i.e. MBD and TRD). The most abundant isoform is MeCP2-e1, which includes the exon 1, while the start of translation of the MeCP2-e2 isoform is in the exon 2 [39–41] (Figure 1B). Both of the MeCP2-e1 and MeCP2-e2 isoforms are ubiquitous, although their expression levels are highest in the brain [34].

Human MeCP2 was purified to homogeneity and its structure was characterized in detail by ultracentrifugation, circular dichroism and protease digestion [42]. MeCP2 has a monomeric state under a wide range of conditions, it appears to be intrinsically disordered and it contains at least six distinct domains that are organized in a tertiary structure that is 60% unstructured. Nonetheless, it has also been shown more recently that MeCP2 can form homo-interactions, with itself, both *in vitro* and *in vivo* [43]. The structural autonomy of the different domains that results from the disordered structure of the protein might explain its multi-functionality [42]. Taking these findings together, it is highly likely that the ability of MeCP2 to establish interactions with different molecular partners explains its multi-faceted functions (see below).

MeCP2 protein levels increase during neuronal differentiation in humans, rats and mice [44–46], and this is critical for maturation and maintenance of neurons, rather than for cell fate decision [47].

To study MeCP2 function in detail and to understand how MeCP2 dysfunction impacts on RTT pathogenesis, several mouse models lacking the Mecp2 gene or carrying Mecp2 mutations have been generated. These different models recapitulate the neurological dysfunctions and other aspects typical of RTT to varying extents [48–51]. Interestingly, both the constitutive loss and the conditional ablation of MeCP2 in the whole mouse brain or only in postmitotic neurons can promote many of the RTT phenotypes, and this has emphasized the importance of MeCP2 for the stability of the brain functions, rather than for brain development [49, 50].

MeCP2 as a regulator of the transcription

A large amount of data has been produced to describe the interactions of MeCP2 with transcriptional repressors (see below), which has strongly indicated MeCP2 involvement in gene silencing [52, 53] ([Figure 2A]). However, some evidence has revealed that MeCP2 is not a mere global transcriptional repressor. Indeed, the transcriptional profiles of the Mecp2-null mouse brain have revealed only subtle changes in comparison with age-matched wild-type (WT) mice [54]. Nevertheless, gene expression studies performed in specific brain sub-regions (i.e. hypothalamus and cerebellum) and cell types have revealed changes in the expression levels of thousands of genes, and most of which appeared to be positively regulated by MeCP2 [55–57]. These reports have suggested brain region-specific and cell-type-specific MeCP2-mediated transcriptional regulation, and have revealed an unexpected role of MeCP2 as a gene activator (Figure 2B). These opposite roles might be explained by MeCP2 interactions with co-factors that have alternative roles, such as CREB for transcriptional activation and HDACs/Sin3A (switch-independent 3A) for gene repression [12] (Figure 2A and B). However, a large number of studies have

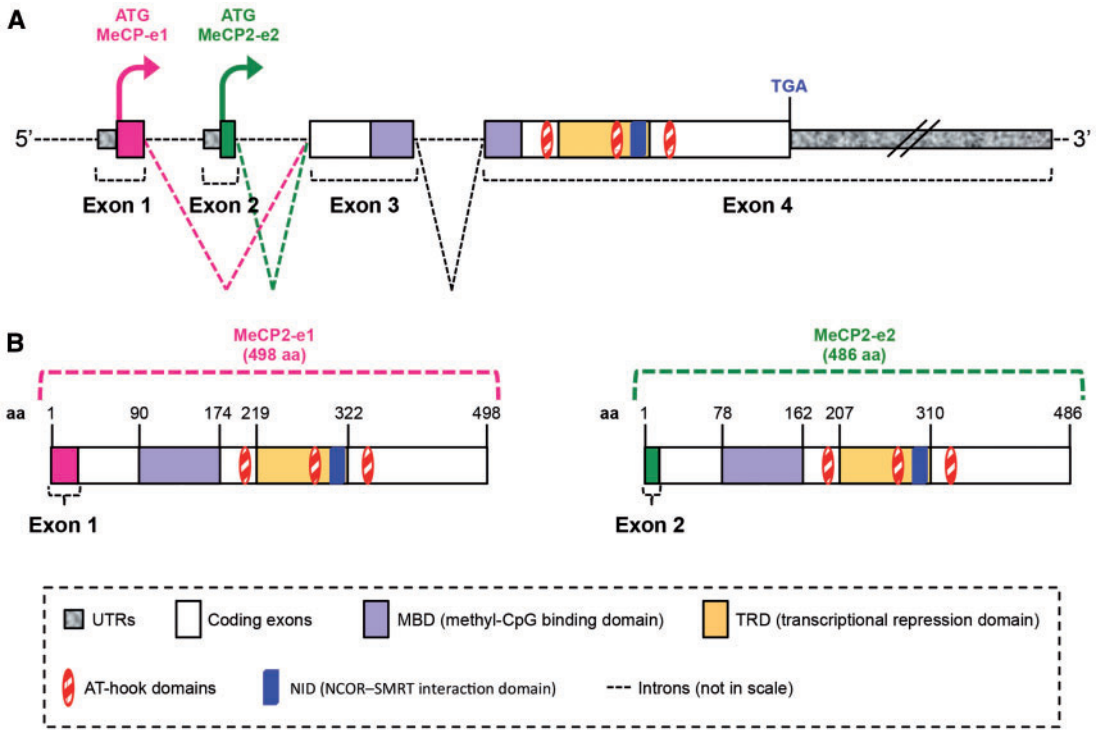

Figure 1. Schematic representation of the human MECP2 gene (**A**) and its main protein variants: MeCP2-e1 (498 amino acids) and MeCP2-e2 (486 amino acids), produced by alternative splicing (**B**). The two variants differ in their N-terminal portion, which include the exon 1 (MeCP2-e1) or the exon 2 (MeCP2-e2). The main domains of MeCP2 are depicted and amino acid position (aa) of MBD [35] and TRD [27] for both of the splicing variants are indicated. (A colour version of this figure is available online at: https://academic.oup.com/bfg)

supported the idea that the primary function of MeCP2 is correlated with transcriptional repression.

In recent years, genome-wide analyses have promoted the hypothesis of the involvement of MeCP2 in transcriptional regulation of transposons and repetitive elements. Under normal conditions, L1 transposons, major satellite DNA and intracisternal A particles are hypermethylated, and as such, they are MeCP2-bound [30, 62]. In support of this idea, weak up-regulation of the transcripts of these genomic elements (1.6-fold, on average) was observed in neuronal nuclei extracts from MeCP2-null mouse brains, compared with the *WT* mice [30]. In this context, L1 elements are of particular interest, because aberrant L1 retrotransposition can cause insertions and deletions, or new splice-sites formation [63–65], which impacts on correct gene expression and normal cellular functions. Of note, increased genomic content of L1 sequences was reported in MeCP2-null neuroepithelial cells, compared with *WT* cells, although no differences in the L1 content were observed in MeCP2-null fibroblasts. Remarkably, MeCP2-null neural precursor cells (NPCs) that were obtained through differentiation of induced pluripotent stem cells (iPSCs) derived from RTT-fibroblasts showed increased L1 retrotransposition. Accordingly, similar evidence that highlights an MeCP2 function in the repression of L1 retrotransposition [62] was also collected from postmortem RTT brains. Overall, these findings introduce a new function of MeCP2 in the regulation of genomic transcriptional noise and indicate that RTT phenotypes might be ascribed to spurious transcription.

MeCP2 interacts with multiple partners

The interaction of MeCP2 with several partners ([Table 1]) might motivate its multiple roles. MeCP2 is one of the most studied of the epigenetic readers responsible for the link between DNA methylation and histone modifications. Biochemical fractionation allowed the identification, several years ago, of protein complexes that contain both MeCP2 and HDACs. One of the best characterized MeCP2-mediated transcriptional silencing mechanism occurs through TRD recruitment of the HDACs/Sin3A repressor complex and the resulting deacetylation of the closer nucleosomes [53, 71] (Figure 2A). In contrast, other evidence has shown that MeCP2 mediates transcriptional repression also independent of histone deacetylation [73].

In 2003, a link between DNA methylation and histone methylation was described. Indeed, *in vitro* and *in vivo*, MeCP2 associates with an H3-Lys9 histone methyltransferase activity, that promotes the methylation of Lys9 of histone H3 on a DNA-methylated target gene, thus reinforcing the repressive chromatin state [70]. Later, it was shown that the complex that includes MeCP2 and CoREST (co-repressor for element-1-silencing transcription factor) can repress neuronal target genes by recruiting Sin3A and SUV39H1 (suppressor of variegation 3-9 homolog 1), which are responsible for the deacetylation and methylation of histones, respectively [58] (Figure 2C).

MeCP2 is also known to bind the co-repressors SMRT (silencing mediator for retinoid and thyroid receptors) [72], c-Ski (first isolated at Sloan Kettering Institute) and N-CoR (nuclear receptor corepressor 1) [69], which appear to be in complexes that do not include the Sin3 co-repressor [69]. Interestingly, some MeCP2 RTT mutations interfere with these interactions. For instance, the premature truncation of MeCP2 at position 168 (R168X) abolishes the binding between MeCP2 and SMRT in *Xenopus laevis* embryos [72]. Furthermore the missense mutation R306C abolishes the interaction between MeCP2 and the NCoR-SMRT co-repressor complex in *in vitro* assays (Figure 2D),

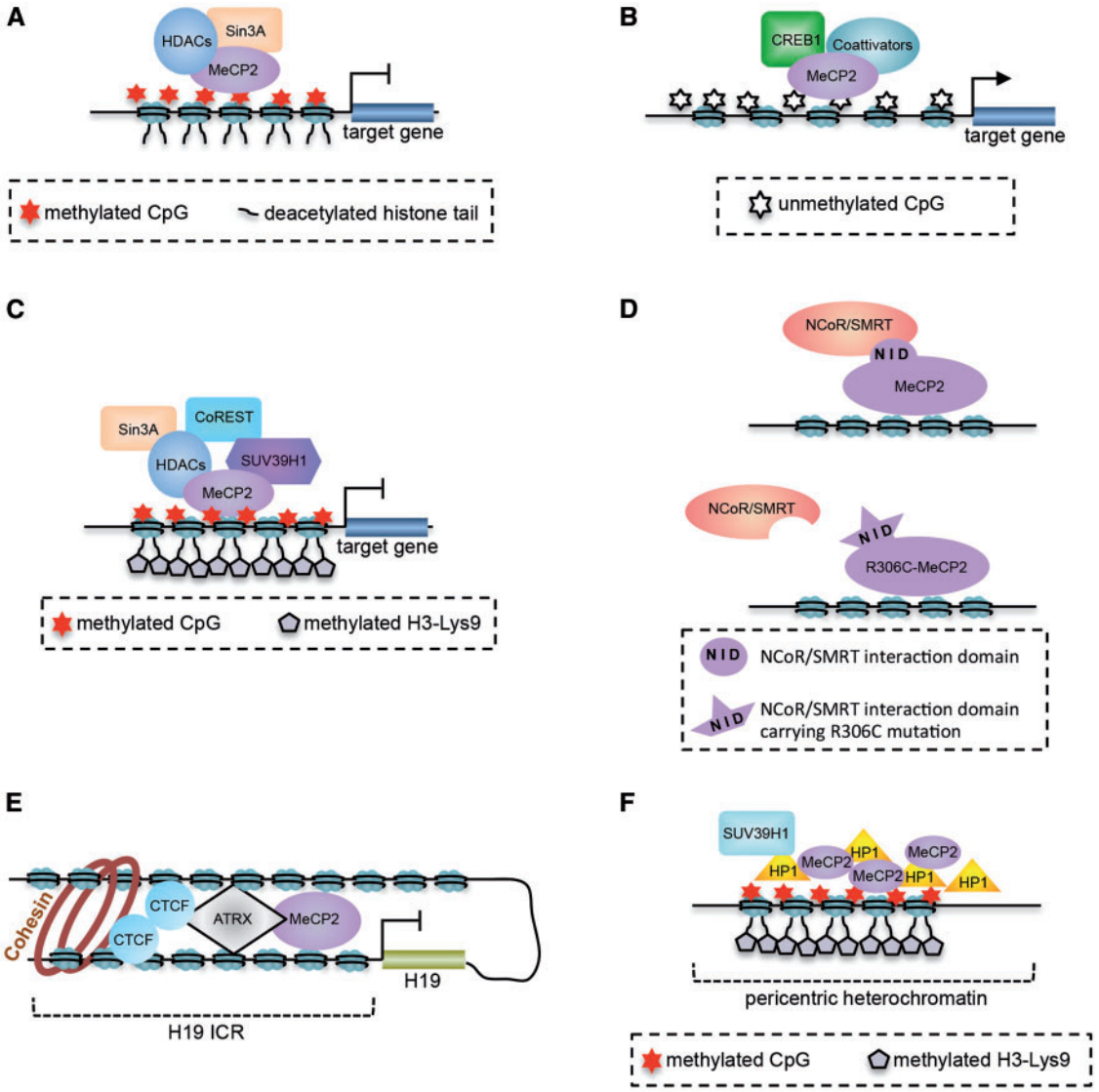

Figure 2. MeCP2 forms complexes with several molecular partners. (**A**) MeCP2 mediates transcriptional silencing through binding with methylated CpGs and the recruitment of a HDAC-containing complex [53, 54]. (**B**) MeCP2 activates transcription of target genes in specific brain regions and cell types [55–57]. (**C**) MeCP2 and CoREST repress neuronal genes by recruiting a HDAC-containing complex and SUV39H1, to promote deacetylation and methylation of histones, respectively [58]. (**D**) MeCP2 recruits the NCoR-SMRT co-repressor complex to chromatin by NID domain (upper panel). The RTT mutation R306C, which lies within the NID, abolishes this interaction (lower panel) [59]. (**E**) MeCP2, ATRX and cohesin bind the maternal allele of H19 ICR, and promote CTCF binding and formation of higher-order chromatin loops. These events induce allelic-repression of the imprinted locus [60]. (**F**) MeCP2 facilitates binding of HP1 on pericentric heterochromatin during myogenic differentiation, and promotes the recruitment of SUV39H1 methyltransferase [61]. (A colour version of this figure is available online at: https://academic.oup.com/bfg)

which suggests that MeCP2 mediates the interplay between DNA and co-repressors; thus, these authors defined a new MeCP2 domain, the NCoR/SMRT interaction domain (NID), which is crucial for this binding (Figure 1). The loss of these interactions that can be caused by RTT mutations (e.g. R306C) might contribute to some RTT phenotypes [59] (Figure 2D).

Another protein that interacts with MeCP2 is the chromatin remodeling protein α-thalassemia/mental retardation syndrome X linked (ATRX) [66]. ATRX is a SWI/SNF (SWItch/Sucrose Non-Fermentable) family ATPase that has key roles in brain development and is mutated in the ATR-X syndrome, a severe intellectual disability disorder [74, 75].

MeCP2 and ATRX co-localize on pericentric heterochromatin (PCH), and this binding is crucial for correct ATRX accumulation on PCH in the central nervous system. Indeed, correct ATRX accumulation is impaired in Mecp2-null neurons and in transgenic mice carrying the A140V missense MeCP2 mutation [66, 67] and increased in MeCP2-overexpressing models [36]. Moreover, Baker *et al.* [36] highlighted the involvement of the AT-hook 2 domain of MeCP2 in this MeCP2-mediated ATRX PCH localization. A complex that includes MeCP2, cohesin and ATRX is involved in the silencing of a sub-set of imprinted genes in the mouse brain [67], which acts on a higher-order chromatin structure. Later, the same team identified the repression mechanism that is mediated by the MeCP2/ATRX complex, in the neonatal brain [60]. In particular, Kernohan *et al.* [60] showed that MeCP2 is required for the recruitment of ATRX at the H19 maternal imprinting control region (ICR). Accordingly, in MeCP2-null forebrains, they observed an increased nucleosome occupancy at a sub-set of ICRs, which included H19 ICR,

Table 1. MeCP2 interaction with molecular partners

MeCP2 interacting partner	Function	Reference
ATRX	Correct localization of ATRX at PCH	[35, 66, 67]
	Transcriptional repression of a sub-set of imprinted genes	[67, 60]
Brahma	Transcriptional repression	[68]
Cohesin	Transcriptional repression of a sub-set of imprinted genes	[67, 60]
CoREST	Transcriptional repression of neuronal genes	[58]
CREB	Transcriptional activation	[56]
c-Ski	Transcriptional repression	[69]
H3-Lys9 histone methyltransferase	Transcriptional repression	[70]
HDACs	Transcriptional repression	[53, 71]
HP1s	Transcriptional repression	[61]
MBD2	Heterochromatin organization	[43]
NCoR	Transcriptional repression	[69]
Sin3A	Transcriptional repression	[53, 71]
SMRT	Transcriptional repression	[72]
SUV39H1	Transcriptional repression	[58]

that causes an altered CCCTC-binding factor (CTCF) binding, as already observed in ATRX-null samples [67]. Moreover, in ATRX-null and MeCP2-null brains, there is a strong decrease in long-range chromatin interactions between H19 ICR and other imprinted regions, compared with *WT* brains. Kernohan *et al.* [60] thus proposed a model in which MeCP2 and ATRX control nucleosome positioning at certain CTCF binding sites, which promotes the formation of local chromatin loops and the consequent repression of imprinted genes in the neonatal brain (Figure 2E).

In 2005, a MeCP2-mediated silencing mechanism of methylated genes was identified, through the recruitment of Brahma (Brm), a catalytic component of the SWI/SNF-related chromatin-remodeling complex. The mechanism was studied in mouse fibroblasts and human T-lymphoblastic leukemia cells, and it requires DNA methylation at specific promoters and involves nucleosome remodeling [68]. These findings underline an interesting connection between an epigenetic reader and an ATP-dependent chromatin remodeler. However, the physical interaction between MeCP2 and Brm and their sub-nuclear co-localization were not confirmed by another team [76]; thus, the formation and the function of a complex including Brm and MeCP2 is still questioned.

In 2007, further important cross-talk between DNA methylation and histone modifications was characterized, which was based on the interaction between MeCP2 and HP1s (Heterochromatin protein 1) [61]. Three isoforms of HP1 have been characterized to date (i.e. α, β, γ). These bind trimethylated H3-Lys9, which is a repressive histone modification that is enriched on PCH [77, 78], and they recruit the histone methyltransferase Suv39H1 [79]. Analysis of the sub-nuclear localization of HP1s during myogenic differentiation showed no changes in overall protein levels, although increased accumulation on PCH was observed (especially for HP1γ), which correlated with the presence of MeCP2. Interestingly, the chromo shadow domain (CSD) of HP1s interacts *in vivo* with the N-terminal portion of MeCP2 (i.e. from amino acids 1-55), and this interaction appears to promote association of HP1s with PCH. These findings reinforce the idea of cooperation between HP1s and MeCP2, to form repressive compartments that are involved in gene silencing, and to reduce transcriptional noise [61] (Figure 2F). Physical interactions between MeCP2 and MBD2, another member of the MBDs family, have also been demonstrated both *in vitro* and *in vivo*, which have promoted the hypothesis of cooperation between these two epigenetic readers in heterochromatin organization (see above) [43, 80].

Although many MeCP2 interactors with different molecular roles have been reported, the exact composition of the different molecular complexes in which MeCP2 participates is still incomplete to date.

Genomic distribution of MeCP2

MeCP2 is predominantly expressed in the brain, and especially in neurons [47], where it reaches its maximal expression during the postnatal period [28]. MeCP2 was initially described as a protein that binds methylated and unmethylated DNA with a repressive function, and with a preference for the methylated CpG contexts [43, 81, 82]. Surprisingly, recent genome-wide analyses have questioned these results. Chromatin immunoprecipitation (ChIP)-on-chip assays in the human SH-SY5Y neuronal cell line have revealed that 60% of the MeCP2 binding sites lie within intergenic regions, and many of these are located more than 10 kb from the nearest gene. Interestingly, two-thirds of the genes strongly bound by MeCP2 are actively transcribed, and only the 2.2% of highly methylated genes show MeCP2 binding. This finding is in contrast to the hypothesis of MeCP2-mediated and methylation-dependent transcriptional silencing [83]. Some years later, the first ChIP-sequencing analysis that was carried out in mouse neurons showed that MeCP2 was widely distributed in the neuronal genome, and most importantly, that its binding tracks methyl-CpG distribution, which is consistent with global MeCP2-mediated silencing [30].

DNA methylation is extensively re-modulated during postnatal development [84]. Interestingly, non-CpG methylation such as CH methylation (mCH; with H = A, T, C) and DNA hydroxymethylation of CGs (hmC) appear to have important roles here. DNA hydroxymethylation is believed to be involved, as an intermediate, in active DNA demethylation [85]. HmC levels increase during postnatal development, and genes that lose CG methylation and acquire an hmC signature become transcriptionally active. Conversely, mCH, which is the primary form of DNA methylation in adult neurons, is depleted in neuronally expressed genes, which suggests a role in gene silencing [84].

HmC profiling has revealed enrichment of this signature in the gene body of expressed genes, and its depletion around

transcriptional start sites. Whether methylation of hydroxylcytosines is a stable epigenetic marker that tags active sites of DNA demethylation or whether it simply represents an intermediate molecule is a question that needs to be clarified [84, 85].

The re-modulation of DNA methylation in the brain is correlated with that of specific epigenetic factors, and primarily with methyl binding proteins. Of note, the increase in MeCP2 levels follows the rate of mCHs and hmCs [85]. In contrast to earlier reports that indicated a strong preference of MeCP2 for mCG [81], recent data have shown that MeCP2 can bind mCH and repress transcription in neurons *in vivo* [86]. One year later, Gabel *et al.* demonstrated that MeCP2 efficiently binds mCGs and poorly binds hmCGs, in contrast to what had been reported previously [87]. Moreover, MeCP2 binds mCAs (methylated CA dinucleotides), hmCAs and mCGs with a similar high affinity [88]. The same team showed that MeCP2 preferentially binds and represses long genes in the brain [88], as previously observed [57]. In addition, they demonstrated that this transcriptional repression is mediated by the binding of mCA, which is enriched in the neurons, within the gene body of the repressed genes [88]. The up-regulation of these genes when Mecp2 was mutated appears to contribute to the neurological symptoms of Rett syndrome. Thus, transcriptional up-regulation of long genes is a specific feature of the RTT brain, over other neurological pathologies [88].

MeCP2 and chromatin organization

In recent years, it has become evident that MeCP2 participates in higher-order chromatin structure, through promotion of both transcriptional repression and activation [89].

MeCP2 and chromatin fibers

In 2003, it was shown that MeCP2 can assemble secondary chromatin structures independent of DNA methylation ([Figure 3A]). Of note, assays performed with RTT-related MeCP2 mutant proteins highlighted that the region of MeCP2 implicated in this function is located outside the MBD. Indeed, the MeCP2-R168X mutant protein (which contains the MBD) cannot compact 60S ellipsoidal particles and assemble oligomeric supra-structures (Figure 3A), whereas the MeCP2-R133C protein (which carries a missense mutation in the MBD) shows only a weak impact on this ability. These findings have promoted the hypothesis that MeCP2-mediated silencing can be ascribed to its role in chromatin organization [90]. Interestingly, it was shown 10 years later that an MeCP2-mutant protein with a truncation in the AT-hook 2 domain failed to induce high-order chromatin structures starting from nucleosomal arrays, which underlines a role of this portion of the protein in this phenomenon [36] (Figure 3A).

A study carried out by Nikitina *et al.* [82] showed that indeed human MeCP2-e2 induces compaction of nucleosomal arrays, represented by tandem repeats of DNA that were 208-bp-long and that included 12 CpG units assembled with histones. This was independent of the methylation status of the substrate, which confirmed previously reported data [90]. The model of this interaction indicates that each MeCP2 molecule simultaneously binds to two different nucleosomes to form a 'sandwich' [82] (Figure 3B). Experiments performed with MeCP2 proteins with severe RTT mutations showed reduced induction of compaction, as compared with the *WT* protein. Furthermore, assays performed with the MeCP2-R294X mutants highlighted that the C-terminal portion of MeCP2 (from amino acids 295 to 486) is essential for its interactions with chromatin, and suggested that impaired chromatin binding might account for the clinical symptoms observed in patients with RTT who carry the R294X mutation [82]. The same team identified nucleosomal linker DNA as a key binding partner of MeCP2, and revealed different alterations in the MeCP2-chromatin interactions according to different RTT mutations [91].

The complex interaction of MeCP2 with both DNA and nucleosomes was analyzed in detail later using electron microscopy imaging. MeCP2 binds DNA in a cooperative fashion, and its binding affinity strongly correlates with mCG density and the proximity to A/T repeats. As for MeCP2-DNA binding, the methylation of CpGs also increases the MeCP2-binding affinity for nucleosomes. Furthermore, MeCP2 induces compaction of tetra-nucleosomes, to form an architectural motif that is similar to that formed by histone linker H1. Interestingly, fluorescence anisotropy assays (*in vitro*) and fluorescence recovery after photobleaching (FRAP)-based assays (*in vivo*) have shown that MeCP2 and H1 compete for nucleosome binding, with much stronger displacement of H1 by MeCP2 than the opposite; this suggests that H1 and MeCP2 share common nucleosome-binding sites [93].

In the same year, Skene *et al.* [30] studied the abundance of MeCP2 in purified neuronal nuclei from mature brains. Here, they demonstrated that the amount of MeCP2 is similar to the number of histone octamers, thus promoting the hypothesis of almost complete saturation of the genome [30]. Interestingly, in neurons, histone H1 represents half of the total amount of H1 contained in other cell types [94, 95], while in MeCP2-null neurons, this quantity is doubled. These quantitative analyses suggest that neuronal MeCP2 can compensate for reduced amounts of H1 [30] and they support data reported by Ghosh *et al.* [93].

MeCP2 and chromatin loops

A new MeCP2-mediated silencing mechanism that is based on the induction of higher-order chromatin structure was identified for the transcriptional regulation of the imprinted Dlx5-Dlx6 (distal-less homeo box 5/6) locus in mouse brain [96]. Dlx5 and Dlx6 were up-regulated in MeCP2-null brains due to the postulated loss of imprinting. Human cells from patients with RTT indeed showed biallelic expression of the *DLX5* gene, although inter-individual variability has also been documented. In Mecp2-null mouse brains, DNA binding of HDAC1 in the Dlx5-Dlx6 locus is almost completely lost and acetylation of H3-Lys9/Lys14 at the same site is increased, while dimethylation of H3-Lys9 is strongly reduced. MeCP2 induces formation of higher-order for the 11-kb silent chromatin loop at the Dlx5-Dlx6 locus, which is absent in the MeCP2-null mouse brain [96]. Nonetheless, 2 years later, this silencing mechanism was questioned by a study that showed the non-imprinted nature of the DLX5-DLX6 human locus and the high variability of their expression in mouse [97].

MeCP2 and pericentric heterochromatin organization

PCH is constitutive heterochromatin that is located in proximity to the centromeres, and in mouse PCH is composed of arrays of AT-rich major satellite repeats [98]. PCH is important for several biological functions, such as the genome integrity, repression of transposable elements and correct chromosomal segregation [99–101]. PCH is involved in silencing of euchromatic genes following their repositioning adjacent to PCH [102, 103]. This phenomenon can operate in both *cis* and in *trans* [104]. PCH is

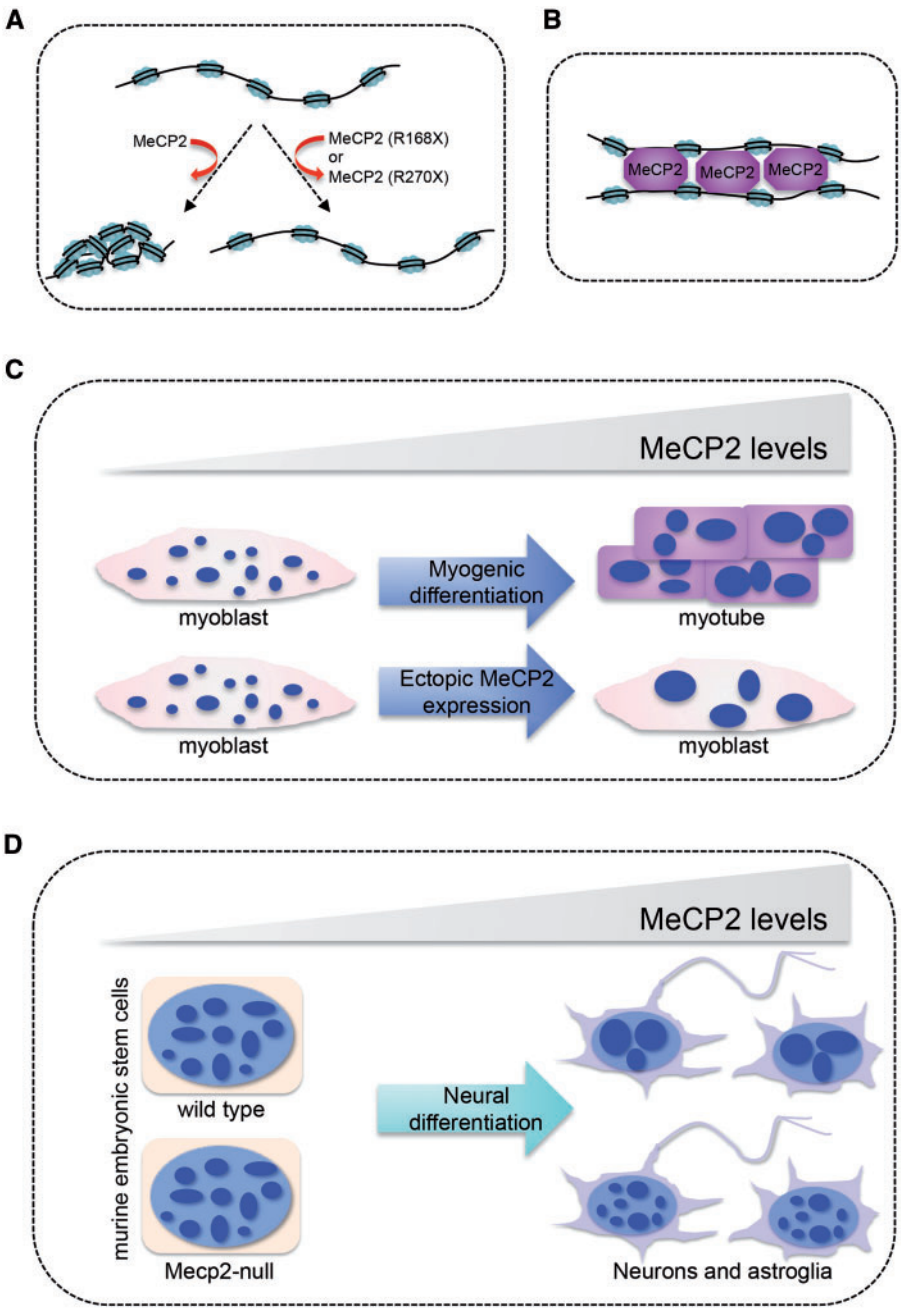

Figure 3. MeCP2 has crucial functions in chromatin architecture. (**A**) MeCP2 promotes compaction of nucleosomal arrays to form 60S ellipsoidal particles (left) [90]. The R168X and R270X MeCP2 mutations that are found in patients with RTT impair formation of the higher-order chromatin structure (right) [36, 90]. (**B**) A single MeCP2 molecule brings together two DNA molecules, represented by nucleosomal arrays, to form a DNA-MeCP2-DNA sandwich [82]. The linker DNA appears to be the key-binding site for MeCP2 [91]. (**C**) MeCP2 promotes condensation of pericentric heterochromatin (blue spots) during myogenic differentiation or under ectopic MeCP2 over-expression in myoblast cells [80]. (**D**) MeCP2-mediated chromocenter clustering during neural differentiation. Upon differentiation, WT mouse embryonic stem cells (mESCs) show strong pericentric heterochromatin condensation. On the contrary, this phenomenon is significantly affected during the differentiation of Mecp2-null mESCs [92]. (A colour version of this figure is available online at: https://academic.oup.com/bfg)

characterized by specific epigenetic markers, which include DNA methylation, histone variants, hypoacetylation and methylation of H3-Lys9, which is critical for recruitment of the HP1 proteins. In interphase nuclei, PCH of different chromosomes tends to aggregate to form specific structures known as chromocenters. These structures might generate silent sub-nuclear compartments where silencing factors are concentrated, to thus maintain the heterochromatic status. The chromocenter organization is established early during the development, and it is extremely dynamic and can differ in different cell types and at different developmental stages [105, 106].

For many years, it has been known that MeCP2 accumulates at chromocenters in mouse cells [17, 27]. However, recent data have shown that only ~30% of MeCP2 accumulates at chromocenters, and highlight additional MeCP2 clusters with lower intensities in euchromatin and nucleoplasm [107]. The binding of MeCP2 on PCH depends on DNA methylation and requires the MBD [108].

In 2005, the involvement of MeCP2 in PCH organization was established [80] (Figure 3C). Increased MeCP2 expression and methylation of CpGs was shown on PCH during myogenic differentiation, concomitant with a reduction in the number of chromocenters and an increase in their size, due to their clustering (Figure 3C, upper panel). Experiments of MeCP2-YFP overexpression in myoblasts revealed a negative correlation between MeCP2-YFP amount and chromocenter number, suggesting that increasing MeCP2 levels during differentiation may give rise to the chromocenter clustering (Figure 3C, lower panel). This phenomenon requires the MBD of MeCP2 [80]. These data agree with *in vitro* data on the compaction of chromatin fibers [82, 90, 91].

Afterwards, an analysis of the number of nucleoli and chromocenters in developing MeCP2-null neurons revealed delayed nuclear maturation. Indeed, samples from MeCP2-deficient mice showed higher numbers of nucleoli that were smaller in comparison with the *WT* nuclei, which supports a role for MeCP2 in the re-organization of nucleoli in neurons. Moreover, the chromocenter size did not change in depolarized MeCP2-null neurons, while it increased following depolarization of *WT* neurons. This phenomenon underlines the importance of MeCP2 in activity-dependent chromatin re-organization during postnatal neuronal maturation, which possibly acts through induction of chromocenter clustering [109]. Subsequently, the role of MeCP2 in PCH architecture was analyzed during neural differentiation, taking advantage of a new MeCP2-null murine embryonic stem cell (mESC) line [92]. This cellular model differentiates toward various neuronal sub-types and astrocytes, without significant differences compared with *WT* cells, consistent with the concept that MeCP2 is not required for neural differentiation. Increased MeCP2 levels have been observed in *WT* cells during neural differentiation, concomitant with remarkable chromocenter clustering, whereas this PCH compaction was strongly impaired in MeCP2-null cells (Figure 3D) [92]. These findings testify that PCH re-organization is MeCP2-dependent and that this is shared among a variety of differentiation pathways.

The ability of MeCP2 to bind to and re-organize PCH is affected by missense RTT mutations. Agarwal *et al.* [110] observed that 20 out of 21 MeCP2 mutant proteins retain their binding to chromocenters, even though this occurs with different strengths. Moreover, two out of three of these mutant forms showed impaired induction of chromocenter clustering [110]. Interestingly, forcing the impaired binding of some RTT MeCP2 mutant proteins (i.e. R133L, R111G) to PCH is sufficient to rescue the chromocenter clustering, which suggests that these RTT mutations only affect PCH binding, and not the aggregation of heterochromatin. The same assay performed with other RTT MeCP2 mutant proteins (i.e. P101H, P152R), where the clustering was altered but not the binding, did not provide rescue of the phenotype, which indicated that these mutated residues are directly implicated in the PCH condensation. On the other hand, forcing the F155S MeCP2 mutant protein (which shows defects in both binding and clustering) to bind PCH did not rescue chromocenter aggregation, which indicates that this residue is pivotal for both of these functions [111].

Many new reports have highlighted the role of posttranslational modifications (PMTs) in MeCP2 functions [112–115]. Of particular interest, in the mouse brain, MeCP2 is subject to PARP-1-mediated poly(ADP-ribosyl)ation. In PARP-$1^{-/-}$ mouse fibroblasts, that ectopically express MeCP2, and in myoblasts, that express a MeCP2 form that lacks the poly(ADP-ribosyl)ated regions, there is an increased chromocenter clustering, which suggests that this PMT negatively modulates MeCP2-induced PCH re-organization. Interestingly, poly(ADP-ribosyl)ation of MeCP2 reduces its chromatin binding affinity. These data suggest that MeCP2-poly(ADP-ribosyl)ation has a modulatory effect on MeCP2-mediated chromocenter clustering and they highlight the complex interplay between the MeCP2 domains [112].

The set-up of new quantitative high-resolution imaging technologies has allowed the comparisons of cell-type-specific differences in chromatin architecture in the brain from *WT* and Mecp2-null female mice [107]. This mouse model is a mosaic for loss of MeCP2 due to dosage compensation, and it represents an ideal tool to analyze the chromatin architecture in normal (i.e. expressing MeCP2) and MeCP2-null neurons in the same animal. Using array tomography (AT) imaging, an increase in heterochromatin compaction and a redistribution of trimethylated H4-Lys20 into PCH was observed in MeCP2-null hippocampal neurons, but not in other neuronal types. These findings highlighted the importance of a cell-type-specific analysis [107], as already observed for MeCP2-mediated transcriptional regulation [55, 56].

Although the mechanisms that underlie the binding of MeCP2 to PCH and the following higher-order chromatin re-organization are still not clear, recent data have illustrated the importance of MBD in these phenomena. The truncated MeCP2 protein coded for by the $Mecp2^{tm1.1}$Jae [49] recombinant gene and lacking most of the MBD shows a diffused distribution in brain nuclei, in contrast with the accumulation of *WT* MeCP2 on PCH, which suggests a function of this domain in the binding with the chromocenters. These data suggest that the RTT-like phenotype observed in the $Mecp2^{tm1.1}$Jae mouse model might arise from impaired chromatin binding, defective nuclear transport and accelerated protein turnover [116]. On the other hand, MBD is not the only domain that is required for chromatin binding. Indeed, another study showed the importance of the AT-hook 2 domain, which is located in the TRD, for the binding of MeCP2 to major satellite DNA [36].

A recent study analyzed the correlation between gene expression and the proximity to chromocenters or to the nuclear periphery, to understand the position effects of heterochromatin in gene silencing [117]. Transcriptional profiling analyses combined with three-dimensional DNA fluorescent *in situ* hybridization and three-dimensional distance measurements were performed using two cellular models of chromatin re-organization: myoblasts differentiating into myotubes, and myoblasts ectopically expressing MeCP2. In both of these cellular systems, the chromocenter clustering was associated with increased MeCP2 expression [61, 80]. They found a weak, mostly not statistically significant, positive correlation between gene expression changes and the proximity to chromocenters, and they concluded that even if gene activity was not related to proximity to PCH in their model systems, the observed general tendency might have some biological meaning [117].

Conclusions

MeCP2 is a multi-talented protein. Its roles range from transcriptional regulation of specific target genes, with both positive and negative effects, to the silencing of spurious transcription and organization of higher-order chromatin structure. Its peculiar multi-functionality can be ascribed to its intrinsic disordered structure, which confers functional autonomy to its different domains.

It has now been ascertained that MeCP2 represses or activates gene expression depending on the cellular context. This can be justified by diverse MeCP2 interactions with both activators and inhibitors of transcription. In addition, a new function in the silencing of transposable and repeated elements has recovered the old hypothesis that assigned to DNA methylation a crucial role in repression of transcriptional noise [11] and introduced the hypothesis of the involvement of spurious transcription in the pathogenesis of RTT.

The complex and tissue-specific roles of MeCP2 can be explained also according to its affinity for different DNA-methylated forms (e.g. mCGs, hmCGs, mCA, etc.) that are differentially enriched in tissues.

Importantly, to date we can affirm that one of the main functions of MeCP2 is related to chromatin architecture. Evidence of the wide distribution of MeCP2 in the genome primarily supports this idea. In addition, several reports have demonstrated that this epigenetic reader can induce the compaction of different forms of chromatin *in vitro*. Most importantly, direct involvement of MeCP2 in the higher-order chromatin organization was observed in different ways, especially during differentiation.

In the light of these data, we can hypothesize that, together with its molecular partners, MeCP2 contributes to the establishment and the maintenance of silent compartments on PCHs where genes to be silenced are brought. This hypothesis is partially encouraged by the findings of Jost *et al.* [117], who reported weak correlations between the position of transcriptionally silent genes and PCH domains, although this did not reach statistical significance. However, to date, the mechanism by which MeCP2 operates this biological phenomenon and the MeCP2 molecular partners involved are still obscure.

In the future, it will be interesting to investigate the role of MeCP2 in regulation of chromatin architecture particularly in human samples, to unravel the pathogenic mechanisms of RTT. Findings here might provide the opportunity to correctly manipulate MeCP2 functions with therapeutic aims.

Acknowledgements

The authors gratefully acknowledge Mr. Christopher Berrie for accurate English profreading. GP is the recipient of a Neuromed Fellowship.

Funding

This work was supported by Associazione Italiana Rett, project title: “Studio di due approcci farmacologici per ripristinare i difetti molecolari, morfofunzionali e neurocomportamentali in modelli di laboratorio preclinici sperimentali della sindrome di Rett”, Epigenomics Flagship Project EPIGEN MIUR-CNR, project title: “Chromatin diseases e disordini dell’imprinting: modelli per lo studio dell’interazione dinamica dei complessi multi-proteici che controllano l’epigenoma” and Progetto MIUR PON03_PE00060_7, project title: “Sviluppo preclinico di nuove terapie e di strategie innovative per la produzione di molecole ad azione farmacologica’.

Key Points

- MECP2 is mutated in Rett syndrome, an autism spectrum disorder (ASD) and in other neurological diseases.
- MECP2 can act as a transcriptional repressor or activator, depending on its associated partners.
- MECP2 regulates genome stability by repressing inappropriate transcription from repetitive elements.
- MECP2 binds to pericentric heterochromatin and regulates its condensation, shaping genome architecture.

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