MECP2: A Multifunctional Protein Supporting Brain Complexity

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Abstract After more than 20 years from its discovery, MECP2 roles are far from the fully understanding. MeCP2 binds the genome globally, with the need of a single, methylated CG and is enriched in heterochromatic foci. Early hypothesis proposed it as a generalized repressor and modulator of genome architecture that keeps down the transcriptional noise. Its modulation of L1 retrotransposition and the regulation of pericentric heterochromatin condensation might be conceivably associated with this function. Interestingly, MECP2 is mutated in the paradigmatic chromatin disease Rett syndrome, an X linked neurodevelopmental disease affecting females. This highlighted a different function of MECP2, as repressor of downstream genes and the identification of few downstream genes corroborated this hypothesis. Rather recently, however, with the help of high throughput technologies and a number of appropriate mouse models finely dissecting MECP2 functional domains, new and somehow unexpected roles for MECP2 have been highlighted. Expression profiling studies of specific brain areas support a role of MeCP2 not only as a transcriptional silencer but also as activator of gene expression. Beyond its binding to DNA, MeCP2 is also able to influence alternative splicing, promoting inclusion of hypermethylated exons in alternatively spliced transcripts. MeCP2 has been also found to bind non CG methylated residues in brain. Overall, MECP2 appears to be a multifunctional protein, exquisitely adapted to support the functional complexity of the brain.

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1 Mouse Models and Their Contributions to Disentangle MECP2 Functions

MeCP2 (methyl-CG binding protein 2) is an ubiquitous transcription factor encoding two different splicing isoforms, MeCP2A and MeCP2B (Fig. 1a), both containing two main domains, Methyl-Binding Domain (MBD) and Transcriptional Repression Domain (TRD) and predominantly expressed in brain [8]. It is mutated in Rett syndrome (RTT, OMIM 321750), a progressive neurodevelopmental disorder affecting almost exclusively females [36]. The first murine model carrying constitutive ablation of Mecp2 gene showed embryonic lethality [41], but, just after the cloning of RTT causative gene [3] several new mouse models have been generated helping to depict MECP2 biological role. By crossing floxed animals with Cre deleter mice, ubiquitously expressing Cre transgene, mice lacking MeCP2 in all tissues were provided. Floxed mice were crossed also with Nestin-Cre mice, generating a progeny lacking MeCP2 selectively in the brain. Both Mecp2-null constitutive and Nestin-Cre conditional mutants recapitulate symptomatic manifestations of RTT: they are apparently healthy and fertile for the first few weeks of age but develop neurological phenotype at 5–6 weeks and die at 10–12 weeks of age. These findings strongly suggest a primary role of MeCP2 in the brain [13, 18]. Female mice, representing the true RTT model, develop symptoms at 12 weeks of age and survive beyond 12 months. The overall brain structure is conserved in the absence of MeCP2 but brains are smaller than age-matched controls due to reduced neuronal size [13] and neural dendritic arborization [34] as observed in humans. MECP2 absence impairs both excitatory and inhibitory transmission in neurons. Like many other X-linked intellectual disability genes [5], MECP2 impacts also on dendritic spine density and synaptic plasticity.

A helpful model to study the effect of truncating mutation similar to those found in RTT patients is the Mecp2-308/Y mouse, carrying a mutation that introduces a premature stop codon. This model shows progressive RTT-like neurological phenotypes as well, but symptoms onset and age of death are significantly delayed. Moreover, these mice show hyperacethylation of histone H3, suggesting again that MeCP2 dysfunction has an effect on chromatin architecture [38]. Noteworthy, in the last years, murine models have been generated carrying Mecp2 mutations for loss and/or gain of function in specific brain regions or sub-neuronal populations. This detailed analysis allowed to hypothesize that specific phenotypes observed in RTT models may be ascribed to different brain compartments [10].

Lately, also knock-in (KI) mice carrying disease causing point mutations or MeCP2 derivatives (i.e. protein forms no more phosphorylable in specific amino

acids) have been generated. For example, mice expressing MeCP2 with the common RTT causing mutation R306C or those carrying phosphorylation-defective T308A derivative have been useful to demonstrate the importance of the interaction between MeCP2 and the NCoR complex and how this is regulated by the activity dependent T308 phosphorylation. MeCP2 R306C mutant cannot neither binds the NCoR complex nor be phosphorylated in T308, whereas the MeCP2 T308A phosphorylation-defective derivative constitutively binds the NCoR complex, independently from neuronal activity [15]. It has been noticed that MECP2 R306C KI mice, compatible with a model of MECP2 loss of function, are more dramatically affected than T308A KI mice, compatible with a model of MECP2 gain of function, even if both models exhibit Rett like features [26].

2 MECP2 Deficiency and Changes in Transcriptional Profiling

Despite the hypothesized role of MeCP2 as a transcriptional repressor, transcriptome profiling of total brains from Mecp2-null mice revealed only slight changes in gene expression [11]. On the contrary, dysregulation of thousands of genes came out by profiling specific brain regions relevant to RTT symptoms, such as the hypothalamus and cerebellum of Mecp2-null and -overexpressing animals (Fig. 1b, left panel, i). Interestingly, these studies support a role of MeCP2 not only as a transcriptional silencer but also as an activator of gene expression, through its association with the transcriptional activator CREB1 [6, 12] (Fig. 1b, left panel, ii). The brain-derived neurotrophic factor (Bdnf) gene, encoding a signalling molecule with crucial roles in brain development and neuronal plasticity has been found consistently deregulated in the absence of MeCP2 and is thus considered a bona fide MeCP2 target gene. It has been shown that in resting neurons MeCP2 is bound to methylated promoter of BDNF, while in depolarized neurons, which cause BDNF activation, MeCP2 become phosphorylated in Ser421 and dissociates from BDNF promoter [14, 27]. More recently it has becoming clear that a dual operation model could explain the MeCP2 dependent-BDNF expression control [24].

Not surprisingly, among the MeCP2 targets identified in two different transcriptional profiling studies of Mecp2-null brains there are several non-coding RNAs, including hundreds of miRNAs [19] and long non-coding RNAs (lncRNAs) [35]. Even if a major challenge is to understand the molecular consequences of deregulated lncRNAs, the GABA receptor subunit Rho 2 gene was proposed as an interesting target. Altogether, these findings suggest a global role of MeCP2 in the transcriptional regulation of many classes of genes, underlying the importance of its integrity and the devastating effect of its mutations in RTT patients.

3 MECP2 Global Role in Genome Architecture

A clue to understand the crucial role of MeCP2 for brain functions has been provided by large-scale analysis of MeCP2 distribution. Skene and co-workers [39] reported that in neuronal nuclei, as opposed to other cell-types, MeCP2 is very abundant: its levels approach those of the histone octamer allowing the protein to be genome-wide bound in these cells, tracking methylated-CG moieties (Fig. 1b, right panel, iv). In neurons, MeCP2 may therefore act as a global organizer of chromatin structure, which is also supported by the fact that brains of Mecp2-null mice are characterized by increased histone acetylation and a doubling of histone H1 levels. Moreover, an intriguing finding is that the lack of MeCP2 in neurons from mature brain is responsible for the de-repression of spurious transcription of repetitive elements, such as L1 retrotransposon [32, 39].

The association of MeCP2 with chromatin seems also to be involved in generating higher order chromatin structures. In part, the silencing of an imprinted gene cluster on chromosome 6, including Dlx5 and Dlx6, was proposed to depend on the formation of a MeCP2-dependent chromatin loop enriched in methylated H3 lysine 9 (H3K9), a mark of silent chromatin (Fig. 1b, left panel, iii) [8]. Moreover, MeCP2 accumulates at pericentromeric heterochromatin containing densely methylated major satellite DNA, forming specific chromatin structures called chromocenters [23]. It was revealed a crucial role of MeCP2 and the necessity of the MBD for the condensation of these chromatin structures during myogenic differentiation [9], and later this has been demonstrated also during neural differentiation (Fig. 1b, right panel, v) [7]. Furthermore, mutated MeCP2 forms (carrying different mutations frequently found in RTT) fail to correctly localize in heterochromatin, and many of them are unable to induce a correct chromocenter clustering [2].

More recently, MeCP2 has been proposed to be the major 5-hydroxymethylcytosine (5hmC)-binding protein in brain [31]; the high abundance of 5hmC in neurons and in particular in the gene body of transcribed genes probably ensures a cell specific epigenetic control of MeCP2 on chromatin structure and gene expression (see also Chap. 5). These findings parallel new discoveries suggesting that post-translational modifications of MeCP2 (i.e. phosphorylation) in response to multiple stimuli may provide novel keys to understand how MeCP2 can specifically modulate neuronal chromatin remodelling in response to neuronal activity [15].

4 MECP2 and Regulation of Alternative Splicing

An exciting developing area of research is highlighted by the complex relationships between epigenetics and splicing regulation. 5-hydroxymethylcytosine is highly enriched at the exon-intron junction in the brain, while 5-methyl cytosine (5-mC) is enriched at the exon-intron junction in non-neuronal cells [44]. Interestingly, in non-neuronal context MeCP2 has been found enriched in highly methylated

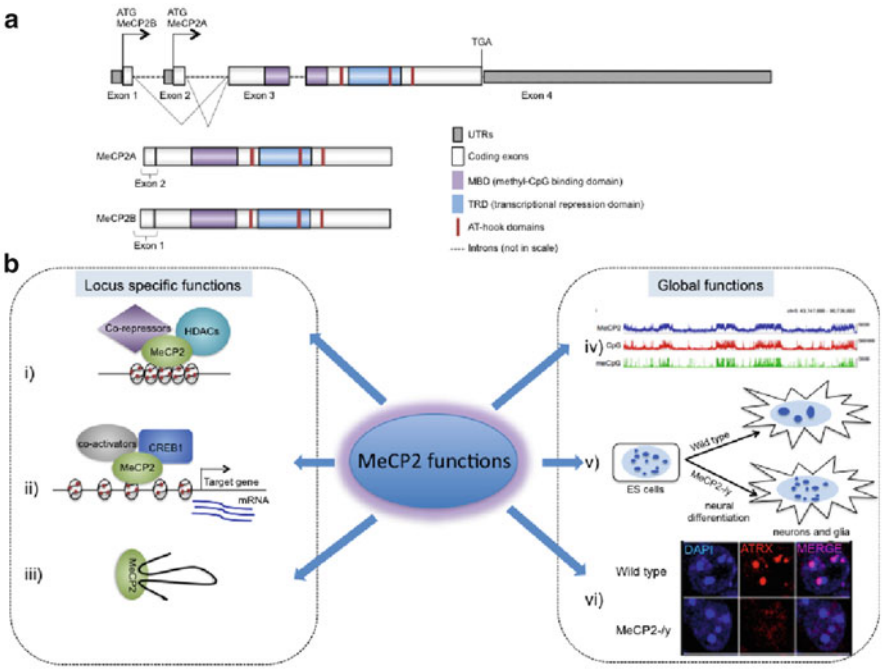

Fig. 1 (**a**) Schematic representation of MECP2 gene structure (*upper panel*). The main protein domains are indicated with *different colors*. Alternative splicing producing the two isoforms, MeCP2A or MeCP2-beta (486 amino acids) and MeCP2B or MeCP2-alpha (498 amino acids), is indicated by *solid lines*. (**b**) Main MeCP2 functions are schematized. The *left panel* reassumes locus-specific roles: MeCP2 is able to repress the transcription of specific target genes by recruiting co-repressors and histone deacetylases (*i*) [14, 22, 33], to activate the transcription of target genes in the hypothalamus by binding co-activators and CREB1 (*ii*) [12] and to silence Dlx5/6 imprinted locus by promoting the formation of a higher order chromatin loop (*iii*) [20]. The *right panel* summarizes the main global roles: MeCP2 is globally distributed in the mouse neurons tracking the methyl-CG density (*iv*) [39], it plays a crucial role for the chromocenter clustering during neural differentiation of mouse ES cells (*v*) [7] and is important for the correct sub-nuclear localization of ATRX protein in the brain (*vi*) [4]

included alternatively spliced exons [28]. Also histone modifications can influence alternative splicing: it was proposed that loss of HDAC1 activity increased histone H4 acetylation surrounding alternative exons [44]. In Rett patients, expression of MECP2 mutated alleles is specifically associated with an increased mono-acetylation level of H4 [42]. In turn, this phenomenon may result in over-expression of MeCP2 target genes providing functional implications in RTT pathogenesis.

Interestingly, tri-methylation of H3 lysine 9 (H3K9-me3) is a functional histone mark to recruit the heterochromatin protein HP1 and foster the inclusion of alternative exons [44]. Remarkably, MeCP2 physically interacts with HP1 proteins [1] and H3K9-me3 is mainly enriched at pericentric heterochromatin, an already known landscape for MeCP2 binding.

Recent findings highlighted that chromatin remodeling is mediated also by lncRNAs [37]. LncRNAs are involved, indeed, in the recruitment of epigenetic factors to specific genomic loci [43]. In brain tissues MeCP2 binds a number of lncRNAs as RNCR3 and MALAT1, this latter interacts with splicing factors too [29]. These data provide evidence that MeCP2 could be a bridge between epigenetic modification and alternative splicing regulation, taking also into account that MeCP2 binds several spliceosome components [29].

5 MECP2 Functions and the Brain DNA Methylation Landscape

Emergence of new experimental approaches analyzing the genome-wide single base resolution profiling of DNA methylation and hydroxymethylation [25] has made feasible to reconsider reading-mechanisms of DNA methylation signature. Not surprisingly, spotlight has been focusing on MECP2 to better define its role both in physiological and pathological conditions, such as Rett syndrome.

Lister and co-workers report an extensive DNA methylation re-assessment during postnatal mouse development. New roles are emerging for non CG methylation, such as CH methylation (mCH, in which H = A, C or T) and hydroxymethylation. In the latter case, genes losing CG methylation thus acquiring hmC signature, become active. Conversely, CH-methylation in neurons is depleted in expressed genes, representing an additional marker of gene repression.

In human and mouse CNS neurons mCH level significantly rises during brain postnatal development, reaching levels as abundant as methylated CGs [21, 25]. DNA hydroxymethylation is also enriched in neurons, 10 times more than in embryonic stem cells, with a postnatal increase. 5hmC profiling revealed its enrichment in gene bodies of expressed genes concomitantly to depletion around transcriptional start sites. If 5hmC represents a stable epigenetic mark or an intermediate molecule, tagging active sites of DNA demethylation, remains to be clarified [21, 25].

Quantitative modulation of CG and non-CG methylation in brain mirrors those of specific epigenetic factors, primarily methyl binding proteins. For instance, MeCP2 level increases synchronously with mCH and 5hmC rate [21, 39]. Additionally, MeCP2 is capable to bind mCH and repress transcription [17], in contrast to earlier experiments reporting a strong preference for mCG [30]. This discrepancy has been recently clarified by testing the binding capacity of MeCP2 towards all known forms of methylated DNA. Gabel and colleagues demonstrated that MeCP2 binds efficiently mCG and poorly 5hmCG in contrast to what reported previously [31]; actually, it shows higher affinity binding to mCA and hmCA [16]. Furthermore, MeCP2 binding to mCA is biased towards long genes expressed in brain; these genes become up-regulated upon MECP2 mutations, possibly causing neurological symptoms of Rett syndrome [16]. Similarly, neuronal overexpression of long genes has been already noticed in a MeCP2-loss of function mouse model [40]. Thus, transcriptional up-regulation of long genes is becoming a specific feature of Rett brain, over other neurological pathologies [16].

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