

MSF  
Chromosomal regulation by MeCP2:  
structural and enzymatic considerations

N. J. Bowen<sup>a</sup>, M. B. Palmer<sup>b</sup> and P. A. Wade<sup>b,\*</sup>

<sup>a</sup> Department of Genetics, University of Georgia, Athens, Georgia (USA)

<sup>b</sup> Emory University School of Medicine, Department of Pathology and Laboratory Medicine, Winship Cancer Institute Whitehead Building, Room 142, 615 Michael Street, Atlanta, Georgia (USA), Fax: +1 404 727 8538, e-mail: pwade@emory.edu

**Abstract.** The unique properties of eukaryotic DNA modified via methylation of cytosine residues are believed to result from the action of a conserved family of proteins, the MBD family. The prototype member of this family, MeCP2, was isolated independently in two laboratories. One group isolated MeCP2 as a methylated DNA-binding protein, the second as a sequence-specific DNA-binding protein. Multiple lines of evidence suggest that MeCP2 functions in assembly of specialized chromatin architecture. While initial findings pointed to an

enzymatic mechanism involving histone modification for transcriptional repression mediated by MeCP2, emerging studies clearly provide exceptions to this model. In a recent study, highly compacted, unique chromatin structures were generated by stoichiometric binding of MeCP2 to model chromatin fibers. These findings support the likelihood that MeCP2 can utilize two independent, but not mutually exclusive, mechanisms to repress transcription: enzymatic and structural mechanisms.

**Key words.** MeCP2; histone deacetylase; chromatin; transcriptional repression; DNA methylation.

Introduction

Epigenetic phenomena involve heritable modifications in chromatin. Among such epigenetic signals is methylation of DNA at cytosine residues. In eukaryotes, this modification occurs almost exclusively within the context of CpG dinucleotides. The methylation mark is interpreted as a silencing signal, resulting in reduced expression of associated genes. DNA methylation is correlated with transcriptional silencing in a number of instances, including X chromosome inactivation [1], imprinting [2], proviral silencing [3, 4] and cancer progression [5]. Such repression could either be a direct or indirect effect of the presence of methylation. While methylation may directly interfere with the binding of some transcription factors, indirect repression by proteins that specifically recognize methylated CpGs (mCpGs) is currently believed to be responsible for the majority of methylation-dependent silencing. Supporting this claim is a body of recent work describing a family of proteins that specifically bind

mCpGs. Studies on MeCP2, the founding member of this family, have provided numerous insights into the molecular mechanisms underlying establishment of repressive chromatin architecture at methylated regions of the genome [6, 7].

Historical overview of the discovery of MeCP2

MeCP2, the prototype methyl CpG-binding protein, was independently identified in two experimental contexts. The first was an effort to identify proteins that specifically bind methylated CpGs [8]. The use of southwestern and gel shift assays revealed that MeCP2 is capable of binding oligonucleotide probes containing a single, symmetrically methylated CpG [9]. Furthermore, this binding is independent of sequence surrounding the methylated dinucleotide. These in vitro binding properties are consistent with those exhibited by exogenously expressed MeCP2-bgeo fusion proteins. Indirect immunofluorescence of mouse interphase nuclei showed specific labeling of heterochromatic foci [10], and staining of

\* Corresponding author.

Handwritten notes in Vietnamese at the bottom of the page, including phrases like "Bản thân người ta phải học để được", "Bản thân người ta phải học để được".

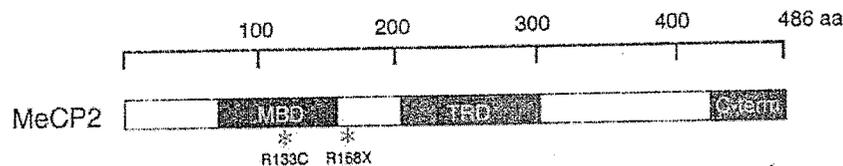


Figure 1. A schematic of the domain structure of human MeCP2 protein. The methyl CpG binding domain (MBD), the transcriptional repression domain (TRD) and the C-terminus are indicated. The mutations used in the study by Georget et al [28] to determine the chromatin-condensing regions of MeCP2 are also shown.

metaphase chromosomes showed the MeCP2 fusion enriched at pericentromeric heterochromatin [11]. This distribution is coincident with repetitive satellite DNA, sequences rich in both mCpGs and repressive chromatin architecture.

A second approach led to the isolation of chicken MeCP2 as a sequence-specific DNA-binding protein known as ARBP [12]. This protein was discovered based on a high-affinity interaction ( $K_d \sim 10^{-10}$ ) with matrix attachment regions (MARs), specialized DNA sequences responsible for anchoring chromatin to the nuclear matrix [13]. Although MARs are AT-rich elements, ARBP also binds mouse satellite DNA, an interaction moderately enhanced by CpG methylation [14]. The domains within mammalian MeCP2 and ARBP responsible for interacting with their cognate DNA sequences are found to overlap in a highly conserved region near the N terminus [14]. In MeCP2, this domain was termed the methyl-CpG binding domain (MBD) [15] and provided a starting point toward identifying other MBD-containing proteins.

Searches based on sequence homology led to the definition of the MBD family of proteins [16], whose members possess highly homologous MBDs but are quite divergent outside this domain. An unrelated line of inquiry also led to the identification of MeCP2 as a gene of interest when it was found that a majority of patients with the neurodevelopmental disorder Rett syndrome possess mutations in MeCP2 [17]. These mutations cluster in the MBD, the transcriptional repression domain (discussed below), and the C terminus, and provide valuable tools in the study of MeCP2 function (fig. 1).

The association of MeCP2 with methylated DNA *in vitro* and *in vivo* suggests that MeCP2 may function as an effector of the methylation signal. This hypothesis predicts that artificially tethering MeCP2 to an unmethylated promoter would result in transcriptional repression with mechanistic similarities to that seen at methylated loci. Not only was this found to be the case, but also MeCP2 was demonstrated to be capable of repressing at a considerable distance from the transcription start site [11]. These assays were also used to define the transcription repression domain (TRD, fig. 1) of MeCP2 as a module capable of repression when fused to a heterologous DNA binding domain [11].

### MeCP2 recruits histone-modifying enzymes

The hypothesis that MeCP2 monitors genomic DNA methylation patterns also predicts that MeCP2 is capable of directing the structural alterations of chromatin that accompany methylation. The seminal finding that MeCP2 is physically associated with the transcriptional corepressor Sin3 and with histone deacetylase fulfilled this prediction [18, 19] (fig. 2A). Importantly, MeCP2-dependent repression was shown to be partially sensitive to inhibitors of histone deacetylase (HDAC). Recent evidence suggests that MeCP2 can also recruit histone methyltransferase activity, although the identity of the specific histone methyltransferase involved remains unknown. Whatever enzyme is responsible, its action leads to local H3 lysine 9 methylation, which is itself an epigenetic mark that presumably reinforces the repressive state [20]. These observations provide a rational basis for the widely accepted model that MeCP2 is specifically targeted to mCpGs and establishes repressive chromatin architecture by recruiting histone-modifying enzymes. Recently, this

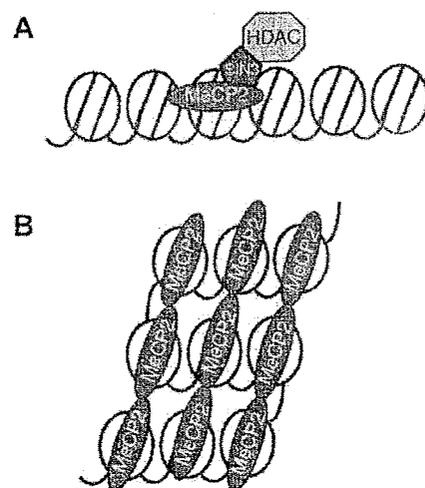


Figure 2. Models of MeCP2-mediated chromatin condensation. (A) The enzymatic model in which MeCP2 recruits histone-modifying enzymes to remodel chromatin. (B) The structural model described in the accompanying text suggests that MeCP2 alone is capable of mediating chromatin condensation by interacting with individual nucleosomes to fold chromatin into novel secondary structures.

model has found substantiation in the case of regulation of a neuron-specific gene, *BDNF*, which may have implications for Rett syndrome pathogenesis [21, 22].

#### Additional MeCP2 activities

While considerable data exist to support the concept that MeCP2 alters local chromatin structure by recruiting histone-modifying enzymes, there is also evidence to support additional, alternative mechanisms of transcriptional repression by MeCP2 that are independent of DNA methylation and histone modifications. For example, MeCP2 clearly has the capacity to bind unmethylated DNA in a high-affinity, sequence-specific fashion [12, 13] as well as in a non-specific, low-affinity manner [23]. Correspondingly, MeCP2 can repress transcription *in vitro* from both methylated and unmethylated naked DNA templates [11, 23, 24]. In addition, while the TRD of MeCP2 is thought to be responsible for recruiting histone-modifying activity, the MBD of MeCP2 alone is capable of repressing transcription *in vivo* in a heterologous system that is devoid of DNA methylation [25]. Moreover, the carboxyl-terminal 191 amino acids (including the TRD) of MeCP2, when artificially tethered by fusion to the Gal4 DNA binding domain, are able to repress the SV40 enhancer/promoter *in vivo* even in the presence of trichostatin-A (TSA) [26]. This observation supports a histone deacetylase-independent mechanism of repression. Finally, MeCP2 can associate with mononucleosomal DNA, and this association is dependent on both the MBD and a 63-amino acid carboxyl-terminal region of the protein [27]. These examples suggest that in addition to the MBD, other regions of MeCP2 are capable of interacting with DNA. They also suggest that recruitment of HDACs by the TRD of MeCP2 is not the only mechanism by which MeCP2 is able to repress transcription.

#### MeCP2 as a structural component of chromatin

A recent report [28] provides an alternative physical mechanism for HDAC-independent chromatin condensation by MeCP2. In this study, MeCP2 alone was capable of mediating chromatin compaction regardless of the methylation state of DNA. When prescribed amounts of highly purified, recombinant human MeCP2 were combined in solution with compositionally defined sea urchin 12-mer nucleosomal arrays [29], the assembly of novel secondary chromatin structures was observed. Analysis of data acquired using solution-based biophysical assays (electrophoretic mobility shifts and analytical ultracentrifugation) and electron microscopy (EM) revealed an unexpected and novel nucleoprotein complex. Increasing

the molar ratio of MeCP2 from 0.25 to 2.0 MeCP2/nucleosome resulted in a progressive reduction in mobility of the nucleoprotein complex in gel shift assays. At molar ratios near 1.0 MeCP2/nucleosome, a predominant species appeared as a discrete, slowly migrating band.

When the corresponding species were visualized using shadowed EM, a full range of nucleosomal array structures was observed beginning with the linear 'beads on a string' conformation in the absence of MeCP2. At 0.5 MeCP2/nucleosome, local areas of compaction were seen in the nucleosomal arrays. Remarkably, at 1.0 MeCP2/nucleosome an extensively condensed, novel 'ellipsoid' structure was seen, corresponding to the predominant electrophoretic band mentioned above. Additionally, interaction between these compacted structures can be seen as oligomers of the ellipsoidal species. Ultracentrifugation revealed that the ellipsoidal structure corresponded to a near homogeneous 60 S species. When maximally folded in high salt, the 12-mer nucleosomal arrays are known to sediment at 55 S [30]. Thus, MeCP2 compacted the nucleosomal arrays into similarly condensed structures. Unlike all other chromatin-condensing proteins, this compaction is achieved under low ionic conditions, permitting detailed biophysical analysis of the resulting structures. The striking stoichiometry of 1.0 MeCP2/nucleosome further suggested a specific interaction between each MeCP2 molecule and an individual nucleosome.

Nuclease digestion was used to further probe the nature of the molecular interactions that contribute to the stability of the 60 S complexes. Digestion of EcoRI sites conveniently located within the linker regions of the nucleosomal arrays indicated that the linker DNA between individual nucleosomes was not protected by the presence of MeCP2. However, electrophoresis of the digested chromatin under native conditions revealed that the structure of the 60 S species remained essentially intact. These observations indicate that interactions between molecules of MeCP2 and the nucleosomes are able to stabilize the 60 S particles and that these interactions do not involve the linker DNA.

Finally, in order to map the chromatin-condensing regions of MeCP2, similar experiments were conducted with known Rett syndrome-associated MeCP2 mutants. A truncation mutation that contains the N-terminus and MBD, but lacks the TRD and far C-terminus was unable to form the stable 60 S particles. This indicated that regions other than the MBD are necessary to assemble the secondary chromatin structures. In support of this hypothesis, a second mutation that substitutes a cysteine for a key arginine in the MBD, but leaves the rest of the protein intact, does not interfere with the ability of MeCP2 to form the 60 S particles. This further advances the idea that compaction of the arrays is not dependent on the ability to productively interact with methylated DNA.

One interesting facet of these experiments is that the ribosomal DNA (rDNA) used was not methylated. Therefore, it is not surprising that a mutation in a residue involved in recognizing methylated DNA would have no effect on the chromatin-condensing properties. In separate control experiments, MeCP2 was shown to bring copies of the non-nucleosomal-containing naked rDNA into close proximity where they appear to be 'cross-linked' into complex structures. Clearly, MeCP2 is capable of interacting with unmethylated DNA under these conditions. These observations suggest that MeCP2 mediated compaction of methylated chromatin could spread to adjacent unmethylated regions of the genome, with the methylated regions serving as nucleation points for extensive MeCP2-mediated chromatin compaction. This study illustrating the ability of MeCP2 to compact chromatin, independent of histone modifications, posits a plausible structural rationale for the HDAC- and DNA methylation-independent transcriptional repression ability of MeCP2 discussed earlier. The picture that emerges is one where a single molecule of MeCP2 is capable of interacting with a single nucleosome and initiating further interaction with neighboring nucleosomes. The resulting condensed chromatin structure is maintained even in the presence of linker DNA digestion. Whether this is mediated by MeCP2-nucleosome or MeCP2-MeCP2 interactions is not known.

#### Models of MeCP2 and chromatin

Taking into consideration all of the data discussed so far, one can envision at least two distinct mechanisms by which MeCP2 compacts chromatin. The first is the widely accepted model that MeCP2 is specifically targeted to mCpGs and establishes a repressive chromatin architecture by recruiting histone-modifying enzymes (enzymatic). The second model suggests that MeCP2 alone possesses intrinsic chromatin-compacting abilities (structural). These models might predict that in tissues where MeCP2 is limiting, the enzymatic model of transcriptional repression would be more likely to function at select methylated genomic loci (fig. 2A). However, in tissues such as the brain, where MeCP2 is known to be abundant, the structural method of chromatin compaction may be more likely. In this scenario, the local concentration of MeCP2 would be greater and might favor MeCP2-MeCP2 or MeCP2-nucleosome interactions that lead to altered secondary chromatin structure not seen in other tissues to an equivalent extent (fig. 2B). It is important at this point to indicate that the two models (enzymatic vs structural) of MeCP2-mediated transcriptional repression are by no means mutually exclusive. One can envision a scenario whereby MeCP2 is targeted to mCpGs and establishes repressive chromatin architecture through local

recruitment of histone-modifying enzymes (enzymatic). The modified histones could nucleate the recruitment of additional MeCP2 molecules, further altering the local secondary chromatin structure (structural). In this fashion, the recruitment of histone modifying enzymes by MeCP2 would work in concert with the establishment of compact chromatin structures by the incorporation of MeCP2 itself.

#### Future challenges

The current data suggest multiple roles for MeCP2 in the organization of chromatin. The ability to recognize methylated DNA gives MeCP2 and other MBD-containing proteins a unique role in our current understanding of the biology of DNA methylation in vertebrates. The recently described ability to compact nucleosomal arrays into novel secondary structures supports an additional role for MeCP2 in the biology of chromatin. Future studies will need to address the precise nature of the interaction between MeCP2 and nucleosomal arrays. Perhaps additional mutagenesis will reveal key residues required for MeCP2-MeCP2 or MeCP2-nucleosome interactions. Whether or not other MBD-containing proteins behave similarly in the presence of nucleosomal arrays is also an interesting question. MBD1 has been shown to be localized predominantly to the pericentromeric regions of chromosome 1 and to telomeric regions of several chromosomes in HeLa cells [31]. The dense accumulation of MBD1 in these highly structured regions of human chromosomes may also indicate an ability of MBD1 to create local regions of specialized chromatin. As we learn more about the genomic targets of MeCP2, it will also be important to substantiate whether MeCP2 is able to create specialized secondary chromatin structures *in vivo* that resemble those observed *in vitro*.

*Acknowledgements.* Work in our laboratory is supported by grants from the NIH (DK065961 to P.A.W.; 5T32GM008367-14 to M.B.P.) and the Georgia Cancer Coalition Distinguished Cancer Clinician and Scientist Program (to N.J.B.).

- 1 Avner P. and Heard E. (2001) X-chromosome inactivation: counting, choice and initiation. *Nat. Rev. Genet.* 2: 59-67
- 2 Ferguson-Smith A. C. and Surani M. A. (2001) Imprinting and the epigenetic asymmetry between parental genomes. *Science* 293: 1086-1089
- 3 Jahner D., Stuhlmann H., Stewart C. L., Hatberis K., Lohler J., Simon I. et al. (1982) De novo methylation and expression of retroviral genomes during mouse embryogenesis. *Nature* 298: 623-628
- 4 Walsh C. P., Chaillet J. R. and Bestor T. H. (1998) Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. *Nat. Genet.* 20: 116-117
- 5 Feinberg A. P. and Vogelstein B. (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* 301: 89-92