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MeCP2 in Rett syndrome: transcriptional repressor or chromatin architectural protein?

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Rett syndrome is a progressive neurological disorder caused by mutations in the methyl-DNA binding protein MeCP2. The longstanding model depicting MeCP2 as a transcriptional repressor predicts that the Rett syndrome phenotype probably results from misregulation of MeCP2 target genes. Somewhat unexpectedly, the identification of such targets has proven challenging. The recent identification of two MeCP2 targets, *BDNF* and *DLX5,* are suggestive of two very different roles for this protein — one as a classical repressor protein, and the other as a mediator of a complex, specialized chromatin structure.

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Current Opinion in Genetics & Development 2007, **17**:121–125

This review comes from a themed issue on
Chromosomes and expression mechanisms
Edited by Tom Misteli and Abby Demburg

Available online 20th February 2007

0959-437X/$ – see front matter
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DOI 10.1016/j.gde.2007.02.003

Introduction

The recent completion of the human genome sequence has provided a great resource for researchers interested in understanding human disease, because it provides a detailed map of the genes required for proper human development. However, the DNA sequence itself is only part of the equation; each cell type contains the exact same set of directions, but the manner in which they read and follow these directions imparts unique, cellular characteristics. Epigenetic mechanisms, such as DNA methylation, are instrumental in encoding these cell type-specific differences. Although we typically think of DNA sequence mutation as causal in human genetic disease, several diseases that are characterized by defects in the establishment, maintenance or interpretation of epigenetic marks (recently reviewed elsewhere [1]) illustrate the importance of this process.

Rett syndrome, a severe neurodevelopmental disorder affecting mainly females, is one example of a disease resulting from an inability to read and interpret epigenetic information. Rett syndrome results primarily from mutations in *Methyl-CpG-binding protein 2* (*MECP2*) [2], one member of a family of proteins that preferentially bind methylated DNA [3]. In addition to its interaction with methylated DNA, MeCP2 is thought to recruit additional co-repressor complexes such as Sin3a [4,5], Ski [6] and NCoR [7], which in turn remodel chromatin into a repressive state. Although the identification of *MECP2* as the 'disease gene' represented a great advance in Rett syndrome research, this finding by no means provided the key to understanding the pathogenesis of this disorder. Instead, work turned towards identifying the specific genes targeted by the MeCP2 protein. However, microarray analyses comparing gene expression in Rett syndrome patients (or mouse models) to controls failed to identify many genes with robust changes in gene expression [8–12], suggesting that MeCP2 doesn't act as a global regulator of transcription. A set of recent studies has identified specific gene targets that might contribute to the Rett syndrome phenotype through two very different pathways: classical promoter repression (Figure 1a), and an unusual role in establishing silent chromatin domains by promoting chromatin looping (Figure 1b).

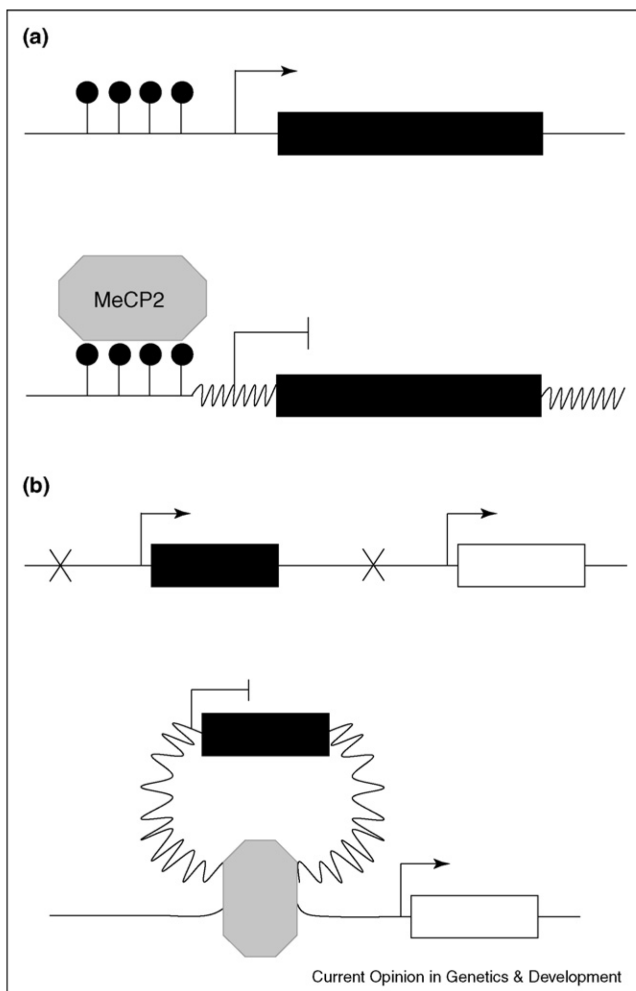
Gene by gene: repression of *BDNF* by MeCP2

Although microarray experiments indicated that MeCP2 did not appear to function as a global repressor, specific gene targets remained elusive until two groups simultaneously identified *Brain-derived neurotrophic factor* (*Bdnf*) as a bona fide MeCP2 target in rodent systems [13,14]. *Bdnf* represented a likely functional candidate for a gene regulated by MeCP2, because it is known to play important roles in normal brain development and in learning and memory, both of which are disrupted in Rett syndrome. Bdnf is encoded by a complex genetic locus containing four well-characterized promoters [15]. One of these promoters, located upstream of exon IV of the mouse gene, is activated upon membrane depolarization in cultured neurons [16–19]. Martinowich *et al.* [14] showed that expression from this promoter was inversely correlated with methylation of several nearby calcium-responsive *cis* elements. This finding led them to question whether methyl-DNA-binding proteins, such as MeCP2, were involved in repressing expression from this promoter. Indeed, chromatin immunoprecipitation (ChIP) experiments performed by both groups demonstrated that MeCP2 (as well as associated co-repressor proteins Histone deacetylase 1 [HDAC1] and mSin3A) specifically associated with the exon IV promoter when cells were not depolarized, but became phosphorylated

[13] and partially dissociated from the promoter after depolarization. As expected, chromatin marks associated with a repressed state correlated with the presence of MeCP2 [13,14,20]. These findings are entirely consistent with MeCP2 acting at Bdnf in much the same manner as well characterized transcriptional repressors that bind specific features in DNA (e.g. DNA sequence elements, or DNA methylation in the case of MeCP2) and recruit additional molecules that establish a repressed state (Figure 1a).

Although these studies conclusively demonstrated that *Bdnf* was a target of MeCP2 regulation, they did not address whether *Bdnf* misregulation in the absence of MeCP2 actually contributed to the neurological phenotype observed in Rett syndrome patients. Two relevant questions remained unanswered: are levels of *Bdnf* transcript impacted by loss of MeCP2 function? And are the phenotypic consequences of loss of Bdnf similar to loss of MeCP2? Recently, Chang *et al.* [21••] addressed the first question by demonstrating that basal levels of Bdnf were reduced in symptomatic *Mecp2*$^{-/-}$ mice relative to wild type — a surprising finding given that MeCP2 acts as a transcriptional repressor. The authors speculated that this apparent discrepancy reflects reduced neuronal activity in *Mecp2*-null mice relative to wild type, resulting in decreased Bdnf expression [22]. These authors also addressed the question of phenotypic similarities between *MeCP2*- and *Bdnf*-null mice. Several phenotypic similarities were observed between *Mecp2*-null mice and those carrying a brain-specific deletion of *Bdnf*, including a reduction in brain weight and hindlimb clasping, both of which are also observed in Rett syndrome patients (hindlimb clasping is likened to the hand wringing behavior characteristic of Rett syndrome). Given that Bdnf levels are only moderately decreased in *Mecp2*-null brains relative to wild type, the authors went on to investigate the phenotypic effects of further modulating *Bdnf* expression in the absence of MeCP2. Did a further reduction in Bdnf exacerbate the Rett syndrome-like phenotype? And could Rett syndrome-like symptoms be alleviated by adding back Bdnf? Through the use of elegant genetic approaches, both of these hypotheses were proven to be correct. The *Bdnf;Mecp2* double knockout exhibited Rett syndrome-like symptoms and lethality even earlier than either of the single mutants alone. Conversely, overexpression of *Bdnf* in *Mecp2*-null mouse brains delayed the onset of Rett syndrome-like symptoms, and enhanced survival rates. These experiments further implicate *Bdnf* misregulation as one factor in the development of Rett syndrome.

Figure 1



Two functions for MeCP2 in transcriptional repression. **(a)** MeCP2 functions on a gene-by-gene level as a classical repressor recruited by methylated DNA. MeCP2 (grey octagon) binds to methylated CpG islands (filled lollipops) and recruits additional co-repressor complexes (not pictured) to establish a repressive chromatin environment. **(b)** MeCP2 establishes secondary chromatin structures. MeCP2 binding sites are indicated as Xs. MeCP2 binds both of these sites, forming a chromatin loop that, in this example, encompasses one gene. A repressive chromatin environment is established within this loop and does not affect the expression of surrounding genes.

An unexpected role for MeCP2 in genomic imprinting

Several mammalian genes are imprinted and monoallelically expressed in a parent-of-origin-dependent manner. DNA methylation plays an important role in the establishment and maintenance of genomic imprinting. Many imprinted regions harbor differentially methylated regions that differ in their methylation status on each of the parental alleles and are crucial for maintaining expression states [23–27]. Given that MeCP2 is known to bind methylated DNA [28], such genes represent attractive candidates for targets of this protein. Initial analyses did not support this model, because imprinted genes tested did not display expression differences in Rett syndrome cell lines or brain samples relative to controls [29]. Furthermore, detailed analyses of specific imprinted candidate targets, such as *UBE3A*, gave conflicting results; several studies found significant changes

in either overall or allelic expression of *Ube3a* or its associated antisense transcript *Ube3a-AS* in Rett syndrome brains (or those of mouse models) [30•,31•], whereas others did not [32•].

One caveat to using gene expression changes as a marker in identifying imprinted targets is that the predicted 'fold' change in overall expression is relatively low — loss of imprinting would be predicted to result in a twofold increase in expression at most — if the silenced allele was completely reactivated — which might be difficult to reliably detect, especially using microarray technology. Instead, the first evidence of MeCP2 involvement in the regulation of imprinted gene expression came as a result of ChIP-based experiments designed to identify direct genomic targets of MeCP2 in neonatal mouse brain [33••]. This approach identified two MeCP2 binding sites located within a newly identified imprinted gene cluster on mouse chromosome 6 — and within the syntenic region on human chromosome 7q21-22 — containing eight imprinted genes (and several others whose imprinting status is not known) [34]. In keeping with this finding, two genes in this cluster, *Dlx5* (preferentially expressed from the maternal allele) and *Dlx6* (imprinting status not known), showed approximately twofold increases in expression in the brains of *Mecp2*-null mice relative to wild type. In addition, several other genes within the cluster showed significant repression in *Mecp2*-null brains, although the significance of this is not yet clear.

Gene expression changes such as those observed for *Dlx5* might result either from changes in overall expression — *Dlx5* maintains its imprinted status, but transcription from the maternal allele is upregulated in *Mecp2*-null mice or from loss of imprinting (LOI; de-repression of the paternal allele in *Mecp2*-null mice results in equal expression from the two alleles). The authors used expressed polymorphisms to verify that imprinting of *Dlx5* was lost in both *Mecp2*-null mouse brains and Rett syndrome-derived lymphoblastoid cell lines, confirming that MeCP2 binding to these sequences was important for the maintenance of imprinted expression. Rett syndrome patients exhibit a spectrum of mutations, largely concentrated in the methyl-DNA-binding domain (MBD), the transcriptional repressor domain (TRD) and the C-terminal region, as well as a range of phenotypic severity. Thus, some effort was made to correlate *MECP2* genotype with the degree of LOI observed in patient cell lines. However, genotype–phenotype correlations in patients and Rett syndrome-derived cell lines are complicated by the fact that the *MECP2* locus resides on the X chromosome; *MECP2* is subject to X inactivation, so the proportion of cells expressing the mutant copy — and thus the severity of the Rett syndrome phenotype — varies in accordance with the X inactivation pattern in a given individual. Given that only four patient cell lines with four distinct mutations were investigated in this manner, and that only two of these were monoclonal in nature (i.e. all cells expressed the mutant form of *MECP2*), these conclusions are preliminary and would benefit from further study.

Despite the fact that MeCP2 is believed to function through an interaction with methylated DNA, this study did not identify any sequences in the region of identified MeCP2 binding sites that were differentially methylated on the two parental alleles. This surprising finding led to an alternative model for MeCP2 function in regulating genomic imprinting in this region — could MeCP2 be involved in establishment of specialized complex chromatin structures? Looping of chromatin into sub-domains is emerging as an important mechanism in regulating gene expression, possibly by influencing promoter–enhancer interactions or by acting as an insulator between different chromatin states. *In vitro* biochemical studies and electron microscopy have suggested such a role for MeCP2 — the addition of MeCP2 to unmethylated reconstituted nucleosomal arrays resulted in significant chromatin compaction and apparent nucleosome–nucleosome interactions [35]. These data suggest that MeCP2 has an incredible capacity to organize chromatin, independent of its ability to bind methylated DNA, a role which it might be playing in the *Dlx5* region. The authors tested this hypothesis using a modified version of the 3C (chromosome conformation capture) assay combined with ChIP [33••]. This approach demonstrated several MeCP2-associated interactions between sequences within the *Dlx5* region that resulted in the formation of chromatin loops. Furthermore, one loop was detected only in wild type brains, not in *Mecp2* null mouse brains, demonstrating that formation of this structure was dependent upon the presence of MeCP2. Further analysis showed that this loop was marked by histone H3 dimethylated at lysine 9, a histone modification characteristic of silent chromatin. Thus, MeCP2 establishes a chromatin loop structure that is associated specifically with inactive chromatin, which is required to maintain imprinted expression of *Dlx5*.

Looking to the future

The identification of the gene responsible for a given disease is always met with great fanfare and high hopes for possible therapies, although the path from gene to treatment is rarely straightforward. In the case of Rett syndrome, things turned out to be even more complex — the genes misregulated by MeCP2 disruption were responsible for the phenotypic abnormalities observed rather than the gene itself, and thus represented more appropriate therapeutic targets. Unfortunately, identifying genes affected by *MECP2* mutation has proven challenging. This line of research continues, with several other targets being recently identified [36•,37•]. The majority of successes have occurred as the result of direct analyses of MeCP2 binding (such as ChIP) rather than by searching for targets on the basis of altered gene expression. The

availability of genome-wide arrays for ChIP hybridization (ChIP-on-chip) might result in the identification of further targets.

The biological example of Bdnf strongly suggests that MeCP2 binds with high selectivity to methylated DNA and recruits accessory molecules that effect transcriptional repression. This model (Figure 1a) depicts action by MeCP2 as being analogous to a multitude of transcriptional repressors that interact in a specific manner with DNA and influence transcriptional outcome in a conventional manner.

Dlx5 presents an entirely different biological context for gene regulation by MeCP2 — it acts as a chromosomal architectural element. This model (Figure 1b) is consistent with *in vitro* biochemical data wherein MeCP2 association with nucleosomal arrays can induce formation of unique structures. This property is strongly influenced by concentration [35], suggesting that the architectural properties of MeCP2 at Dlx5 reflect thermodynamic properties of the interaction of the protein with chromatin. In this respect, MeCP2 action could be considered similar in mechanism to the Polycomb group proteins of the PRC1 complex, which also creates specialized, repressive chromatin architecture at genetic targets [38].

How can a single protein participate in such mechanistically disparate regulatory mechanisms? The situation is reminiscent of the classic parable describing blind men encountering an elephant. Each man, touching a different part of the elephant, provides a unique description of a different animal. Is it possible that the biological descriptions of Bdnf and Dlx5 regulation by MeCP2 are analogous? If so, this suggests that MeCP2 is actually quite adaptable in its potential responses to gene regulatory and chromatin architectural issues. While an elephant inherently knows what its trunk can do that its tail cannot, it is unclear how a chromatin-associated protein such as MeCP2 adopts the appropriate characteristics for a given biological context. How does the protein distinguish between situations dictating specific interaction with methylated DNA versus nucleation of a specialized chromatin structure? Are there definable differences between the MeCP2 molecules localized at Bdnf versus Dlx5? What regions of the protein impart crucial properties used in different biological contexts? Does MeCP2 interaction with other nuclear factors influence its mode of interaction with the chromatin fiber? Are these properties biologically regulated and how do these events influence disease states like Rett syndrome? The answers to these and other outstanding questions will provide us with a better picture of a very interesting elephant, MeCP2.

Acknowledgements

We gratefully acknowledge the members of the Wade laboratory for helpful discussion in the preparation of this work. We apologize to our colleagues whose work could not be cited here, owing to space considerations. This work was supported by the Intramural Research Program of the National Institute of Environmental Health Sciences, National Institutes of Health.

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