By Hook or by Crook: Multifaceted DNA-Binding Properties of MeCP2

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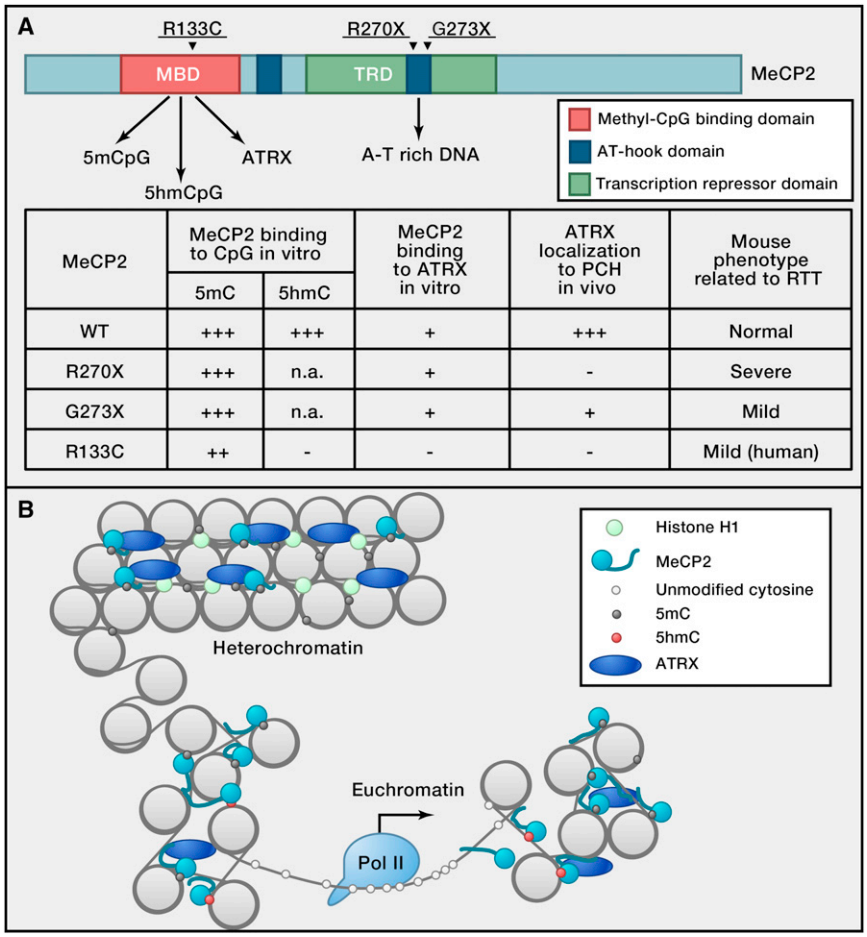
Two new studies reveal novel DNA-binding properties of MeCP2, mutations of which cause Rett syndrome. Baker et al. report critical roles for the AT-hook domain of MeCP2 in chromatin organization and clinical features of Rett syndrome. Mellén et al. find the methyl-CpG-binding domain of MeCP2 interacts with hydroxymethyl-CpG.

Epigenetic mechanisms, including DNA methylation, histone modifications, and chromatin organization, allow eukaryotic cells to define genes and regulate gene expression beyond simple genomic DNA sequence. DNA methylation is critical for development, differentiation, and function of most multicellular organisms. Methyl-CpG-binding protein 2 (MeCP2) is the founding member of the methyl-CpG-binding domain (MBD) protein family that binds to 5-methylcytosine (5mC) (Lewis et al., 1992). MeCP2 is highly abundant in mammalian neuronal nuclei, and overexpression, deletion, or various point mutations of the *MECP2* gene cause Rett syndrome (RTT) (Amir et al., 1999). Understanding MeCP2 function and its underlying mechanism has clinical significance and may reveal fundamental epigenetic regulatory mechanisms. In this and a recent issue of *Cell*, Baker et al. (2013) and Mellén et al. (2012) report mechanistic insights and clinical importance of binding properties of MeCP2 protein toward AT-rich DNA sequences and toward 5-hydroxymethyl-CpG (5hmC) DNA. Both studies suggest a critical involvement of MeCP2 in chromatin structure and shed new light on how MeCP2 dysfunction may contribute to RTT.

RTT, an autism spectrum disorder (ASD), is a progressive neurodevelopmental disorder characterized by initial normal development, followed by slow brain growth, development of autistic features, stereotypies, and seizures. Zoghbi and colleagues made the groundbreaking discovery that RTT patients have frequent mutations in the X-chromosome-linked *MECP2* gene (Amir et al., 1999). Despite two decades of research, how MeCP2 dysfunction causes RTT remains unclear. MeCP2 was initially cloned by Bird and colleagues as a 5mC-binding protein (Lewis et al., 1992). Their pioneering work defined the MBD and transcription repressor domain (TRD) of MeCP2 (Figure 1A) (Nan et al., 1997), which led to a model that MeCP2 protein binds to 5mC and functions as a transcription repressor (Guy et al., 2011). However, MeCP2 is known to interact with chromatin, and in the brain more than half of MeCP2 molecules reside in the open chromatin, which have lower 5mC levels than heterochromatin. In addition, *MeCP2* knockout mice mostly exhibit decreased expression of many genes, and MeCP2 overexpression mice shows the upregulation of the same set of genes. Thus, MeCP2 function appears to be vastly more complex than had been appreciated (Guy et al., 2011).

Baker et al. (2013) focus on a highly conserved AT-hook domain within the TRD of MeCP2 and present compelling evidence for its role in chromatin organization and disease progression (Figure 1A). They generate male transgenic mice bearing either a R270X or G273X MeCP2 mutation. Strikingly, G273X transgenic mice display significantly later onset and slower disease progression compared to R270X transgenic mice (Figure 1A), resembling features of male RTT patients with G273fs and R270fs mutations, respectively. At the molecular level, however, MeCP2-R270X and MeCP2-G273X both behave similarly to wild-type MeCP2 in many respects, including localization at heterochromatic foci and genome-wide distribution. Both mutations disrupt the TRD and repressor activity. Gene expression profiles are also very similar between the two mutant mice. What then contributes to different phenotypes in these two mutant mice? The answer is that MeCP2-R270X, but not MeCP2-G273X, disrupts a highly conserved AT-hook domain, which binds to AT-rich DNA, regardless of CpG methylation (Figure 1A). MeCP2-R270X mice show significant defects in chromatin compaction and pericentric heterochromatin (PCH) localization of α-thalassemia mental retardation syndrome X-linked (ATRX), a chromatin remodeling protein that interacts with MeCP2 (Figure 1A). The onset of aberrant ATRX localization also coincided with RTT-like phenotypes, further suggesting an important role of ATRX in the function and dysfunction of MeCP2.

In another study, Mellén et al. (2012) discover that the MBD of MeCP2 binds to 5mC and 5hmC in vitro with similar affinity (Figure 1A). 5hmC, a recently discovered oxidation product of 5mC present most abundantly in the brain (Kriaucionis and Heintz, 2009), has been



MeCP2	MeCP2 binding to CpG in vitro		MeCP2 binding to ATRX in vitro	ATRX localization to PCH in vivo	Mouse phenotype related to RTT
	5mC	5hmC			
WT	+++	+++	+	+++	Normal
R270X	+++	n.a.	+	-	Severe
G273X	+++	n.a.	+	+	Mild
R133C	++	-	-	-	Mild (human)

Figure 1. MeCP2 Exhibits Multifaceted DNA-Binding Properties to Regulate Different Chromatin States

(A) A schematic diagram of MeCP2 structure and summary of findings from three different MeCP2 mutations investigated in Baker et al. and Mellén et al. The methyl-CpG-binding domain (MBD) of MeCP2 is involved in the interaction with 5mC, 5hmC and ATRX, whereas the AT-hook domain within the transcription repressor domain (TRD) interacts with AT-rich DNA. MeCP2-R133C loses binding affinity to 5hmC, but retains 5mC-binding affinity. MeCP2-R133C binds very weakly to ATRX, and mutant cells almost completely lose ATRX pericentric heterochromatin (PCH) localization (Nan et al., 2007). Both MeCP2-R270X and MeCP2-G273X retain in vitro ATRX-binding affinity, but ATRX gradually loses its localization to PCH earlier in MeCP2-R270X mutant neurons and later in MeCP2-G273X mutants. The MeCP2 R133C phenotype is from human patients, and the mouse model remains to be examined.

(B) A schematic representation of MeCP2 in heterochromatin (compact chromatin) and euchromatin (open chromatin) in the neuronal nucleus. In neurons, higher amounts of MeCP2 exist in euchromatin than in heterochromatin. Histone H1, which induces highly organized and compact chromatin structure, competes with MeCP2 in binding to linker DNA. In heterochromatin, chromatin is highly organized, DNA is highly methylated, and 5mC-bound MeCP2 mostly interacts with repressive chromatin remodelers and transcription repressors. In euchromatin, chromatin is less compact, DNA is more hydroxymethylated or unmethylated, and 5hmC-bound MeCP2 mostly interacts with transcription activators. ATRX interacts with MeCP2 and loses its localization when MeCP2 bears a mutation disrupting the AT-hook domain in the TRD.

postulated to function as an intermediate of active DNA demethylation and as an independent epigenetic mark (Guo et al., 2011b). Mellén et al. start with quantitative, genome-wide analyses of 5hmC, 5mC and gene expression in vivo. Using an elegant approach with genetically modified animals, they were able to perform RNA-seq and MeDIP analyses from distinct neural cell types, thus eliminating complications from cell heterogeneity. They not only confirm early reports of neuron-specific anticorrelation between gene body CpG methylation and gene expression (Guo et al., 2011a), but also find a strikingly clear positive correlation between gene expression and 5hmC/5mC ratio. Probably the most exciting result is direct mass spectrometric detection of MeCP2 in both 5mC- and 5hmC-bound nuclear protein fractions. Using electrophoretic mobility shift and surface plasmon resonance assays, they confirm similar in vitro binding affinity of the MBD of MeCP2 toward 5mC and 5hmC. These results raise an important question: how does each of these MeCP2 populations, either 5mC- or 5hmC-bound, contribute to the development of RTT? Interestingly, the MeCP2-R133C mutation, which causes a milder form of RTT in patients, specifically ablates 5hmC binding but leaves 5mC binding relatively intact (Figure 1A). Finally, a chromatin accessibility assay shows that 5hmC is enriched in highly accessible chromatin, which is attenuated without MeCP2.

Both studies identify novel DNA-binding features of MeCP2 protein and together suggest a new model for the multifaceted role of MeCP2 in neurons depending on its genomic locations (Figure 1B). In somatic cells, MeCP2 mostly resides in heterochromatin with histone H1, which forms regular and compact chromatin structure arrays. In neurons, where the level of MeCP2 is an order of magnitude higher, MeCP2 largely substitutes for histone H1 and is distributed throughout the genome. MeCP2 can form complexes with either transcription activating or suppressing factors (Guy et al., 2011). 5hmC-bound MeCP2 in euchromatin may be coupled with transcriptional activators to turn on gene expression, whereas MeCP2 in heterochromatin is likely to bind to transcriptional repressors to shut down gene expression.

Several interesting questions arise. First, both studies support the model that MeCP2 regulates chromatin organization, yet the exact features of chromatin structures that are altered by MeCP2 dysfunction are not clear. Genome-wide chromatin analyses, such as Hi-C or ChIA-PET, combined with different MeCP2 mutant animal models, may provide a more complete picture. Second, what is the mechanism underlying disrupted MeCP2-ATRX interaction in the RTT

mouse model, despite normal in vitro binding of mutant MeCP2 to ATRX? Third, what is the structural basis for MeCP2 binding to 5hmC and 5mC? It is intriguing that other MBD family members showed no or much weaker binding to 5hmC, although R133 is highly conserved among the MBD family. The in vitro binding properties of MeCP2 to 5hmC also beg further confirmation of interaction in vivo. Fourth, do posttranslational modifications of MeCP2, which are known to affect MeCP2 function (Guy et al., 2011), regulate binding of MeCP2 to 5hmC and/or AT-rich DNA? Both studies, while investigating neurons only in the basal state, raise the possibility of dynamic interactions between MeCP2 and different binding partners to regulate chromatin structure, which can be corroborated with dynamic changes of 5mC and 5hmC in neurons in response to neuronal activity (Guo et al., 2011a, and 2011b). Rapidly accumulating evidence supports the contribution of diverse chromatin remodeling factors to ASD. Baker et al. and Mellén et al. highlight the importance of incorporating complex and dynamic chromatin structures into our understanding of RTT and other ASDs. By identifying molecular events triggered by MeCP2 dysfunction, we will be able not only to identify therapeutic targets for RTT and ASD patients, but also to elucidate fundamental epigenetic regulatory mechanisms in the brain.

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Evolution of Cell Division: From Shear Mechanics to Complex Molecular Machineries

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Cell division depends on sophisticated molecular machinery. However, wall-less forms of bacteria use a much simpler mechanism that mimics spontaneous division of synthetic lipid vesicles. Mercier et al. (2013) show that this "mechanical" division can be activated by increased lipid synthesis. Conceivably, the first cells divided via this route.

Cell division, even in the relatively simple bacterial and archaeal cells, is mediated by highly complex, elaborate molecular machinery. However, the cell-wall-deficient L forms to which many bacteria convert when cell wall biogenesis is inhibited, in particular by cell-wall-targeting antibiotics, bypass these mechanisms and instead divide via a much simpler mechanism that involves shape perturbations, including blebbing, tubulation, and vesiculation (Errington, 2013). In this issue of *Cell*, Mercier et al. (2013) show that the switch to this "biophysical" mode of division can be triggered by an increased lipid synthesis that results in an increased cell surface to volume ratio. The first cells, up to the stage of the last universal cellular