Gene silencing

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Reading banned regions of genomes

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The effect of DNA methylation on gene expression has been known for decades. However, the mechanism by which DNA methylation functions to repress transcription has remained a major question in the field. Wang et al. now narrow this gap through their examination of the methylation binding protein MBD2 and expose how DNA methylation is read upstream of transcriptional repression.

Chromatin marks, including DNA methylation, are deposited throughout the genomes of eukaryotic organisms to indicate what regions should be expressed and what regions silenced[1]. Cytosine DNA methylation (particularly of promoter regions) is tightly correlated with the transcriptional silencing of genes, transgenes and transposable elements (TEs) in plants. This is particularly important at TEs, where constant transcriptional repression protects the cell from genome-wide mutagenesis[2]. Methylation itself, however, does not inhibit the transcriptional machinery[3], but is instead a signal that is read and interpreted by the cell to indicate that a region of the genome is 'banned' from transcription. The deposition of DNA methylation by 'writer' proteins and its removal by 'eraser' proteins have been well described[4], but the identity of the 'reader' proteins and the mechanism by which they interpret DNA methylation have remained enigmatic in plants. In this issue of *Nature Plants*, Wang et al.[5], describe the role of methyl-CpG binding domain protein 2 (MBD2) as a key DNA-methylation reader protein.

MBD2 is localized to methylated CpG-context cytosines at heterochromatic TE regions, as well as in the 3′ ends of genes that contain gene-body methylation. Using a zinc-finger-mediated tethering system, Wang et al. found that MBD2 directs transcriptional repression without altering DNA methylation levels. Thus, although MBD2 is localized at regions of the genome where CpG DNA methylation is found, it can neither deposit additional methyl marks nor remove them – which demonstrates that it is a bona fide reader protein.

Wang et al. found that the effect of MBD2 as a reader protein is highly dependent on the genomic context of its binding. When two

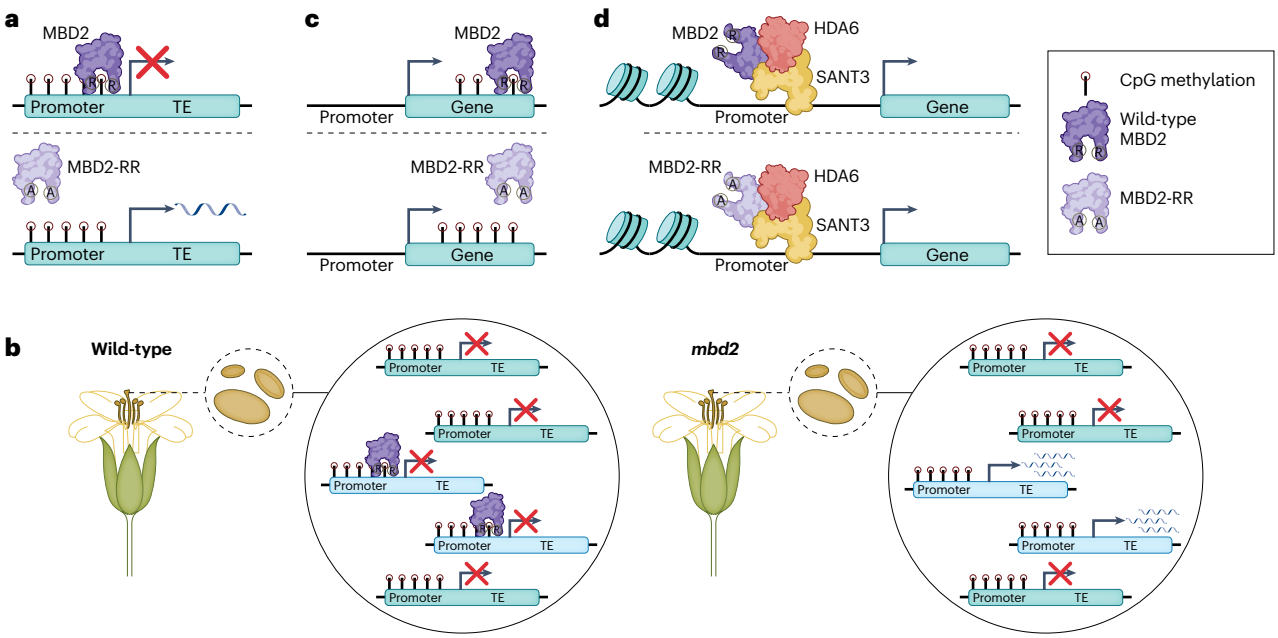

Fig. 1 | Context-dependent roles of the MBD2 protein. a, At CpG-methylated TEs, MBD2 binds to methylated DNA using conserved arginine residues (R) in the MBD2 methyl-binding domain. When these arginines are mutated in the MBD2-RR mutant (bottom), the DNA-binding function of MBD2 is lost and transcriptional activation occurs without changes in DNA methylation at some select TEs. **b**, Loss of MBD2 (in *mbd2* mutants) results in the activation of a small subset of primarily LTR–Gypsy family TEs from the pollen vegetative nucleus, where TEs are already partially reactivated. **c**, MBD2 also binds CpG-methylated gene bodies. At these genic regions, the loss of MBD2 binding in the MBD2-RR mutant does not affect gene expression (thus the function of MBD2 at these loci is unknown). **d**, MBD2, along with HDA6, localizes to non-methylated genes in a manner that is dependent on the protein SANT3 and independent of its methylated DNA-binding ability. The function of this methylation-independent localization is not understood.

conserved arginine residues were mutated in the methyl-binding domain, MBD2 could no longer bind DNA methylation at TE promoters (Fig. 1a), and a small subset of TEs were transcriptionally reactivated (Fig. 1b). These TEs are primarily long-terminal-repeat (LTR)–Gypsy elements located deep in heterochromatin (the so-called banned region of the genome), which indicates that MBD2 actively represses transcription when binding to select TEs (Fig. 1b). By contrast, the function of MBD2 binding in methylated bodies of genes is less understood. Wang et al. demonstrated that MBD2 binds to gene-body methylation and that, similar to the TEs, this binding is dependent on the conserved arginine residues (Fig. 1c), but loss of MBD2 binding had no observable effect on expression of those genes.

MBD2 is a member of a family of DNA-methylation reader proteins, two of which (MBD5 and MBD6) were previously known to repress the expression of some TEs in pollen[6,7]. Using single-cell RNA sequencing, Wang et al. revealed that – similar to its family members – MBD2 functions in the repression of TE expression in pollen, specifically in the somatic pollen vegetative nucleus (Fig. 1b). This cell type is particularly sensitive to the loss of silencing machinery owing to a lack of dimethylation on leucine 9 of histone H3 (H3K9me2), resulting in a decompacted state of the heterochromatin[8]. As several members of the MBD family of reader proteins function in pollen, Wang et al. next examined whether there are redundancies between MBD2 and the other MBD proteins. They found that there was some degree of redundancy between the three proteins with regard to which TEs were reactivated in pollen from *mbd2*, *mbd5 mbd6* double-mutant and *mbd2 mbd5 mbd6* (*mbd256*) triple-mutant plants. Specifically, a larger number of TEs were reactivated in *mbd256* compared with *mbd2* and *mbd5 mbd6* plants. Therefore, MBD2, MBD5 and MBD6 are all active DNA-methylation reader proteins and can regulate different subsets of TEs when the ban on TE expression is already partially lifted in pollen.

In addition to the now-understood role of MBD2 in reading DNA methylation, MBD2 is also found at some non-methylated regions and may have a more general role in gene regulation that is independent of its DNA-methylation binding ability (Fig. 1d). When the conserved arginines that are responsible for binding DNA methylation were mutated, Wang et al. found that the localization and function of MBD2 at these non-methylated genes are independent of its ability to bind methylated DNA (Fig. 1d). Although MBD2 is found at non-methylated regions, its function there is not understood. MBD2 may have some effect on histone acetylation levels at these non-methylated regions, as MBD2 interacts with the histone deacetylase protein HDA6 at these same regions[9,10] (Fig. 1d). Another MBD protein (MBD3) is a known member of the nucleosome remodelling and deacetylase (NuRD) complex[11], and therefore MBD2 may have a role in chromatin organization at these genes. Future studies will need to overcome potential functional redundancies with other MBD proteins and identify changes in gene regulation to determine the role of MBD2 at non-methylated genes.

In summary, Wang et al. provide key insights into the function of MBD2 as a DNA-methylation reader protein that begin to answer the long-standing question of how DNA methylation leads to transcriptional repression. This work has opened up many interesting avenues for future research; for example, exploring what other protein or proteins bind MBD2 and dictate transcriptional repression at TEs, but not the 3′ regions of methylated genes. This may work through a series of protein interactions that result in the eventual modification of histone marks that are known to regulate transcription in promoters but not gene bodies[12]. An additional question is how MBD2 is directed to and functions only on a subset of the CpG-methylated banned regions of the genome. These insights into MBD2 will result in the further identification of DNA-methylation reader proteins and the eventual understanding of their affinity for specific sites. This work represents an important step towards understanding the mechanism that plant cells use to construe DNA methylation into transcriptional repression.

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Competing interests

The authors declare no competing interests.