Role of CG and Non-CG Methylation in Immobilization of Transposons in Arabidopsis

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Summary

Methylation of cytosine residues in eukaryotic genomes is often associated with repeated sequences including transposons and their derivatives [1, 2]. Methylation has been implicated in control of two potential deleterious effects of these repeats: (1) uncontrolled transcription [2–4], which often disturbs proper expression of nearby host genes [5, 6], and (2) changes in genome structure by transposition and ectopic recombination [2, 7]. Arabidopsis thaliana provides a genetically tractable system to examine these possibilities, since viable mutants in DNA methyltransferases are available. Arabidopsis MET1 (METHYLTRANSFERASE 1, ortholog of mammalian DNA methyltransferase Dnmt1) is necessary for maintaining genomic cytosine methylation at 5′-CG-3′ sites [8, 9]. Arabidopsis additionally methylates non-CG sites using CHROMOMETHYLASE3 (CMT3) [10, 11]. We examined the mobility of endogenous CACTA transposons in met1, cmt3, and cmt3-met1 mutants. High-frequency transposition of CACTA elements was detected in cmt3-met1 double mutants. Single mutants in either met1 or cmt3 were much less effective in mobilization, despite significant induction of CACTA transcript accumulation. These results lead us to conclude that CG and non-CG methylation systems redundantly function for immobilization of transposons. Non-CG methylation in plants may have evolved as an additional epigenetic tag dedicated to transposon control. This view is consistent with the recent finding that CMT3 preferentially methylates transposon-related sequences [12].

Results and Discussion

Changes in transposon activity correlated with cytosine methylation were first described in maize [13–17]. Despite extensive investigation thereafter on both plants and animals (reviewed in [2, 18]), the role of DNA methylation in transposon immobilization remains controversial [2, 19–22].

Arabidopsis thaliana provides an ideal system to examine the role of DNA methylation genetically, since many viable DNA methylation mutants are available. Arabidopsis MET1 and CMT3 DNA methyltransferases are involved in methylation of CG and non-CG sites, respectively [8–11]. A third Arabidopsis gene necessary for DNA methylation is DDM1 (DECREASE IN DNA METHYLATION), which encodes a putative chromatin remodeling factor and which is thought to affect DNA methylation indirectly through changes in chromatin structure [23–26]. The ddm1 mutation affects both CG and non-CG methylation, although some sequences are not affected. A striking feature of ddm1 and met1 mutations is that they induce a variety of developmental abnormalities [9, 27–29]. One of the bases for the developmental abnormalities is transcriptional activation of normally silent genes (such as a homeobox gene FWA and disease-resistance genes) [30, 31]. More extensive genome-wide transcript analysis revealed that the ddm1 mutation induces transcription from many heterochromatic sequences, substantial parts of which are transposon related [32–35]. Similarly, met1 or cmt3 mutations also induce transcription from several sequences including retroelement-related repeats [22, 30, 34, 35]. These results indicate that one role of DNA methylation is to suppress such background transcription [3, 4]. However, the effects of mutations in DNA methyltransferase genes on transposon mobilization (transposition) have remained unexplored.

Through genetic characterization of a ddm1-induced developmental abnormality, we previously identified the mobile endogenous Arabidopsis CACTA family transposons [27, 36]. CACTA and other transposons lose methylation and transpose at high frequency specifically in the ddm1 background [36, 37], consistent with the interpretation that DNA methylation is necessary for transposon immobilization. However, because the primary function of DDM1 is likely to be chromatin remodeling rather than DNA methylation [25, 26], it remains unclear whether ddm1-induced transposition is mediated by loss of DNA methylation or by underlying changes in chromatin structure. Indeed, it has recently been shown that the ddm1 mutation has additional effects to that of DNA methyltransferase mutants; it affects methylation of histone H3 lysine 9 in all loci examined [32, 35] as well as acetylation of histone H4 lysine 16 [38]. These observations suggest that the ddm1 mutation affects histone modifications in pathway(s) independent of DNA methylation [35].

To directly test if loss of DNA methylation is sufficient for mobilization of transposons, we examined mobility of CACTA transposons in mutants of DNA methyltransferase genes. We first tested the effects of a mutation in the major DNA methyltransferase gene MET1. The met1-1 mutation in the Columbia (Col) background in-
Figure 1. Transcription and Transposition of CACTA Elements in met1 and ddm1 Mutants

(A) The CACTA transcript detected by RT-PCR. The MET1 antisense line is in the C24 background, which does not have any CACTA elements (unpublished). Therefore, the MET1 antisense transgenic line was crossed to wild-type Col to examine effects of the transgene on CACTA activity. Total RNA from each genotype was reverse transcribed and amplified as described in Experimental Procedures. PCR reactions from two different dilutions of the cDNA, corresponding to 25 ng and 5 ng of input RNA, are shown for each plant. Length of predicted PCR products: CACTA1, 0.64 kb for cDNA and 0.72 kb for genomic DNA; GapC, 0.54 kb for cDNA and 0.82 kb for genomic DNA.

(B) Scoring of CACTA transposition. In order to detect independent immobilization events, we examined transposition in self-pollinated progeny from several independently segregating ddm1 or met1 homozygotes. The parental DDM1/ddm1 and MET1/met1 heterozygotes were backcrossed six times to wild-type Col in the heterozygous state. This procedure replaced hypomethylated CACTA elements with normally methylated copies [47]. No transposition was observed in 38 self-pollinated progeny from the (MET1 antisense/H11003 Col) plant, 22 of which had the MET1 antisense transgene as well as a CACTA1 copy (not shown). Details of the Southern analysis are described in Experimental Procedures. The circled \( \times \) indicates self-pollination.

Reduced accumulation of CACTA transcripts (Figure 1A). Similarly, CACTA transcripts were detected in transgenic plants expressing the MET1 gene in antisense orientation (Figure 1A). Despite the transcript accumulation, no transposition was detectable either in the met1 mutants or in MET1 antisense plants (Figure 1B and legend). These results contrast with the high-frequency transposition observed in the ddm1 mutant (Figure 1B) [36].

The ddm1 mutation affects both CG and non-CG methylation, but met1 mutants retain methylation at non-CG sites. We therefore examined the effect of combining mutations in the CG methylase MET1 and the non-CG methylase CMT3 genes. The double heterozygotes (MET1/met1, CMT3/cmt3) were selected from a cross between a cmt3-i11 homozygous mutant in the Wisslewskijä (WS) background and a MET1/met1 heterozygote backcrossed to the Col background, which has potentially active CACTA members [38]. By genotyping self-pollinated progeny from the double heterozygotes, we found several met1/met1-cmt3/cmt3, met1/met1-CMT3/CMT3, MET1/MET1-cmt3/cmt3, and MET1/MET1-CMT3/CMT3 homozygotes. Southern analysis of CACTA elements using the methylation-sensitive restriction enzymes MspI and HpaII revealed that the met1 mutation affected CG methylation, whereas the cmt3 mutation affected non-CG methylation (Figure 2A) as is the case at other loci [10, 11]. The cmt3-met1 double mutation and the ddm1 mutation affected both CG and non-CG methylation (Figure 2A and Figure S1 in Supplementary Material available online).

In each genotype class in the segregating families, we examined CACTA transcript levels by RT-PCR. We detected accumulation of CACTA transcripts in cmt3 as well as met1 mutants (Figure 2B). We next examined transposition of CACTA elements in the segregating F2 generation by Southern analysis using EcoRV, which is insensitive to cytosine methylation. Several faint bands in new positions were detectable in six out of ten cmt3-met1 plants in the F2 families, while no additional bands were detectable in either single mutants or in wild-type in the same segregating families (Figure 2C). The transpositions in the double mutants were confirmed by genomic amplification and determination of the nucleotide
sequence of the region flanking CACTA1 (see Supplementary Material). To estimate the transposition frequency on a larger scale, we examined the self-pollinated progeny from each genotype class. Almost all of the examined double mutant F2 plants showed new bands, most of which were not shared by siblings, reflecting independent transposition events (Figure 3A, Table 1). No transposition was observed in wild-type or met1 single mutants (Figure 3B, Table 1, Supplementary Material), consistent with the results described above. Interestingly, in the cmt3 mutants, bands in new positions were observed in about 10% of the examined F2 plants (Figure 3C, Table 1, Supplementary Material), suggesting that CACTA elements transpose at a low frequency in the cmt3 mutant background. The transpositions in the cmt3 mutants were confirmed by sequencing the region flanking CACTA1 (Supplementary Material). The transposition frequency, however, was much increased in the double mutants (Figure 3A and Table 1). In addition to the increase in the proportion of plants showing transposition, many of the double mutant plants showed multiple new bands reflecting multiple independent transpositions and increase in the transposon copy number (Figure 3A).

The transposition frequency in the double methyltransferase mutant was even higher than that induced by the ddm1 mutation (Table 1), perhaps because the primary function of the DDM1 gene product is chromatin remodeling [25, 26] and the effect of the ddm1 mutation on DNA methylation is secondary and incomplete. Furthermore, the results in the F2 generation (Figure 2C) indicate that the high frequency of transposition in the double mutants was induced somatically, even before passing through the gametophyte stage. Essentially the same results were obtained when we examined double mutants of met1 and another cmt3 mutant allele, cmt3-7, which is in the Landsberg erecta ecotype background; the double mutation specifically induced high-frequency
Figure 3. Transposition in the F3 Generation

For scoring transposition in the F3 generation (Table 1), self-pollinated progeny from plants keeping all the original Col copies (plants 4, 6, 9, 15, 22, 33, and 36 in Figure 2C) were used. For met1-cmt3 double mutants, in addition to plant 4, we used plants 16, 17, and 18. However, we did not use plant 21 because it already showed many transpositions in the F2 generation (Figure 2C).

(A) Transposition in self-pollinated progeny from met1/met1-cmt3/cmt3 plants (plants 4, 18, 16, and 17 of Figure 2C).
(B) Self-pollinated progeny from a met1/met1-CMT3/CMT3 plant (progeny from plant 22 in Figure 2C).
(C) Self-pollinated progeny from a MET1/MET1-cmt3/cmt3 plant (progeny from plant 9 in Figure 2C). Arrowheads indicate unique bands.

mobilization of CACTA elements in the segregating F2 family (not shown).

Interestingly, although the transposition frequency in single methyltransferase mutants (met1 or cmt3) was much less than that induced in the double mutant, we could detect significant accumulation of CACTA transcript in both met1 and cmt3 single mutants (Figures 1A and 2B). Although our semiquantitative estimation of the transcript level suggests that the double mutant accumulated more transcript than the single mutants, elevation of the transposition frequency in the double mutants is much greater than the increase in transcript levels. One possible explanation for this apparent discrepancy is that accumulation of the transcript over a threshold level might induce the high frequency of transposition observed in the double mutant. Alterna-

Table 1. Transposition of CACTA Elements in ddm1, met1, cmt3, and met1-cmt3 Double Mutants

<table>
<thead>
<tr>
<th>Family Number* and Parental Genotype</th>
<th>F2 Genotype</th>
<th>CACTA1 Transcript</th>
<th>F2: Self-Pollinated Progeny from F2</th>
<th>Number Examinedb</th>
<th>Number of Transparents^</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, DDM1/ddm1</td>
<td>ddm1/ddm1</td>
<td>+</td>
<td>29</td>
<td>17 (59%)</td>
<td></td>
</tr>
<tr>
<td>2, MET1/met1</td>
<td>met1/met1</td>
<td>+</td>
<td>42</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3+4, MET1/met1-CMT3/cmt3</td>
<td>cmt3-met1</td>
<td>+</td>
<td>39</td>
<td>38 (97%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMT3-met1</td>
<td>+</td>
<td>45</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cmt3-MET1</td>
<td>+</td>
<td>42</td>
<td>4 (10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CMT3-MET1</td>
<td>−</td>
<td>43</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*All results of Southern analysis in F2 plants in families 3 and 4 are shown in the Figure S2 of the Supplementary Materials available online.

bTotal number of plants examined.

^Number of plants with new bands, which reflect transpositions.
tively, DNA methylation might also affect transposition efficiency through steps other than transcript accumulation. For example, accessibility of transposase to transposon ends might be affected by DNA methylation [39]. In addition, if the host recombination/repair machinery is involved in transposon excision and integration, this machinery might also be affected by DNA methylation [7].

Our results indicate that loss of DNA methylation is sufficient for transposon mobilization. However, changes in chromatin structure may function as downstream factors. It was recently shown that met1 or cmt3 mutations caused reduced histone H3 lysine 9 methylation on some retrotransposon sequences, although not on untranscribed heterochromatic sequences [35]. In addition, cytogenetic studies indicate that the met1 mutation also affects heterochromatin formation in pericentromeric sequences, where the CACTA1 sequence is located [36]. A combination of these effects on chromatin might also affect transposon mobility. Irrespective of mechanisms downstream of DNA methylation to suppress transposons, the high-frequency transposition in the double methyltransferase mutant strongly suggests that CG and non-CG methylation redundantly contribute as epigenetic tags for immobilization of transposons.

Arabidopsis and mouse mutants with reduced CG methylation display several types of developmental defects, which are associated with changes in host gene transcription [9, 27–30, 40–42]. In contrast, Arabidopsis cmt3 mutations do not induce any morphological phenotype, despite the global loss of non-CG methylation [10, 11]. Interestingly, CMT3 preferentially methylates transposon-related sequences [12], suggesting that CMT3-mediated non-CG methylation might have evolved specifically to reinforce transposon control. Although CHROMOMETHYLASE genes have been found only in the plant kingdom, non-CG methylation is connected specifically to reinforce transposon control. Although DNA methylation might also contribute to more universal epigenetic processes such as RNA interference and histone methylation [43, 44], which might also contribute to transposon control [45].

Experimental Procedures

Plant Materials and Genotyping

The isolation of the ddm1-1, cmt3-i11, and cmt3-7 mutants was previously reported by Vong et al. [23], Bartee et al. [11], and Lindroth et al. [10], respectively. The cmt3/cmt mutant (cmt3/1) is in the WS background. It is a presumed null mutation that causes missplicing of elements in maize. In Epigenetic Mechanisms of Gene Regulation, A. Int, R. Martienssen, and V. Russo, eds. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 575–592. Supported by Grant-in-Aid for Creative Scientific Research 14GS0321.

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References


Note Added in Proof

The cmt1-11 single mutation mobilized CAC elements only when the transposons were introduced from MET1/met1 heterozygotes, not when introduced from wild-type Col plants (Figure S3 in the Supplementary Material), further supporting our proposal that MET1 and CMT3 function redundantly in the immobilization of the CAC elements.