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Epigenetic Control of *CACTA* Transposon Mobility in *Arabidopsis thaliana*

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ABSTRACT

Epigenetic mutation, heritable developmental variation not based on a change in nucleotide sequence, is widely reported in plants. However, the developmental and evolutionary significance of such mutations remains enigmatic. On the basis of our studies of the endogenous Arabidopsis transposon *CACTA*, we propose that the inheritance of epigenetic gene silencing over generations can function as a transgenerational genome defense mechanism against deleterious movement of transposons. We previously reported that silent *CACTA1* is mobilized by the DNA hypomethylation mutation *ddm1* (decrease in DNA methylation). In this study, we report that *CACTA* activated by the *ddm1* mutation remains mobile in the presence of the wild-type *DDM1* gene, suggesting that *de novo* silencing is not efficient for the defense of the genome against *CACTA* movement. The defense depends on maintenance of transposon silencing over generations. In addition, we show that the activated *CACTA1* element transposes throughout the genome in *DDM1* plants, as reported previously for *ddm1* backgrounds. Furthermore, the *CACTA1* element integrated into both the *ddm1*-derived and the *DDM1*-derived chromosomal regions in the *DDM1* wild-type plants, demonstrating that this class of transposons does not exhibit targeted integration into heterochromatin, despite its accumulation in the pericentromeric regions in natural populations. The possible contribution of natural selection as a mechanism for the accumulation of transposons and evolution of heterochromatin is discussed.

METHYLATION of cytosine, together with histone modifications, plays a central role in epigenetic gene regulation. Mutations affecting DNA methylation induce transcriptional perturbations and developmental defects in both vertebrates and plants (Li *et al.* 1992, 1995; Finnegan *et al.* 1996; Kakutani *et al.* 1996, 1997; Ronemus *et al.* 1996; Soppe *et al.* 2000; Stancheva and Meehan 2000; Stokes *et al.* 2002; Kankel *et al.* 2003; Kinoshita *et al.* 2004). Interestingly, some of the developmental abnormalities in plants induced by changes in DNA methylation and transcription are inherited over many generations (Jacobsen and Meyerowitz 1997; Soppe *et al.* 2000; Stokes *et al.* 2002). Similar epigenetic variations heritable over generations have also been reported in natural plant populations and in some mouse strains (Cubas *et al.* 1999; Whitelaw and Martin 2001; Rakyan *et al.* 2003). However, the developmental and evolutionary significance of the inheritance of epigenetic information over generations remain unclear.

In addition to the genes silenced at specific developmental stages, many eukaryotes have constitutive heterochromatic chromosomal regions, which are condensed and silent constitutively throughout the life cycle. Notably, the major components of such constitutive heterochromatin are often silent transposons and their derivatives. For example, pericentromeric heterochromatin regions of the flowering plant *Arabidopsis thaliana* contain many copies of a retroelement-related sequence, *Athila*, as well as several other classes of sequences related to retroelements and DNA-type transposons (Pelissier *et al.* 1996; Arabidopsis Genome Initiative 2000). Such transposon-rich regions have been found in the genomes of many plant species, although it is not well understood how the transposon-related sequences accumulate in those regions (Schmidt *et al.* 1995; Pearce *et al.* 1996; Miller *et al.* 1998; Presting *et al.* 1998; Miura *et al.* 2004).

The transposon-related sequences in plants, vertebrates, and some fungi are often marked by the DNA methylation, and it has been proposed that the primary function of DNA methylation may be to protect the genome from the deleterious effects of transposons (Yoder *et al.* 1997; Matzke *et al.* 1999; Selker *et al.* 2003). Consistent with such a "genome defense" hypothesis for the function of DNA methylation, some endogenous Arabidopsis transposons are mobilized by mutations affecting genomic DNA methylation (Miura *et al.* 2001; Singer *et al.* 2001; Kato *et al.* 2003). For example, the endogenous DNA-type transposon *CACTA1* is silent in the wild-type background but transposes in mutants deficient in the *DDM1* (*decrease in DNA methylation*) gene, which encodes a chromatin-remodeling factor (Jeddeloh *et al.* 1999; Brzeski and Jerzmanowski 2003). The

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ddm1 mutation affects DNA methylation, transcription, and transposition of these transposons, although it is unknown whether the *ddm1* mutation affects the specificity of the transposon integration sites (Miura *et al.* 2001, 2004).

In this study, we demonstrated that the *CACTA1* transposon activated by the *ddm1* mutation maintains its mobility in the *DDM1* wild-type background; in this sense, the activated *CACTA1* behaves in a manner similar to epigenetic mutations. In addition, this system allowed us to directly examine the integration site specificity of *CACTA1* in the *DDM1* wild-type background. As the *ddm1*-derived pericentromeric regions still lost the epigenetic marks of heterochromatin, we were able to compare the integration preference for the *ddm1*-derived and wild-type-derived regions. No bias for preferential integration into the heterochromatic regions was detected. On the basis of these results, we discuss the biological meaning of epigenetic inheritance over generations and control of transposons.

MATERIALS AND METHODS

Plant materials and genotyping: The isolation of the *ddm1* mutants in Columbia (Col) background was previously reported by Vongs *et al.* (1993). The wild-type C24 plant was a gift from Jean Finnegan. The *ddm1-1* mutant and the wild-type *DDM1* alleles were distinguished by *Nsi*I digestibility of the PCR product using primers 5′-ATTTGCTGATGACCA GGTCCT-3′ and 5′-CATAAACCAATCTCATGAGGC-3′.

Analysis of transcription, transposition, and methylation of *CACTA* elements: *CACTA1* transcript was detected by RT-PCR, the *CACTA* mobility was examined by Southern analysis, and the cytosine methylation status in the *CACTA1* 5′ region was examined using the bisulfite-mediated sequencing method as described previously (Kato *et al.* 2003).

Characterization of the *CACTA1* integration sites: *Identification of integration site:* Wild-type *DDM1/DDM1* plants were selected from the F_2 families from crosses between *ddm1/ddm1* and *DDM1/DDM1* plants. The genomic locations of the *CACTA1* integration sites were determined by suppression PCR with modification of the conditions described previously (Miura *et al.* 2001). The genomic DNA was digested by *Eco*RV or *Hinc*II before adapter ligation. The *CACTA1* flanking regions were amplified from the ligated product using the primer pairs 5′-GGAT CCTAATACGACTCACTATAGGGC-3′ + 5′-CAGCGACAGATC TTAGCTTTTAGGTTG-3′ and, subsequently, 5′-AATAGGGCT CGAGCGGC-3′ + 5′-GGATTCGACAGATCTAAGGCA-3′. The PCR products obtained were separated by agarose gel and each fragment was directly sequenced with the primer 5′-AGTGTTG GCGCTGAAGTGAAT-3′. The integration sites of transposed *CACTA1* were determined from flanking sequences using BLAST search in The Arabidopsis Information Resource (TAIR, http://www.arabidopsis.org/).

Detection of Col/C24 polymorphism around the integration site: To detect polymorphism between Col and C24, ~2-kb regions around each of the *CACTA1* integration sites were sequenced for these ecotypes. The primers used for amplification and sequencing of these regions are shown in the supplementary data available at http://www.genetics.org/supplemental/.

Determination of the origin of the integration site: The flanking sequences of both the 5′ and the 3′ sides of transposed *CACTA1* were amplified using primers, one from the flanking region and the other from *CACTA1* (shown as solid and open arrows, respectively, in Figure 4A). Finally, the polymorphic sites were sequenced directly to determine whether the integration site was *ddm1* (Col) or wild type (C24) derived. For each of the integration sites, at least two polymorphic sites were examined.

RESULTS

The *ddm1*-derived *CACTA1* remains transcriptionally active in the wild-type background: We first examined whether the *CACTA1* transposon activated by the *ddm1* DNA hypomethylation mutation remains active in the wild-type background. The *ddm1/ddm1* mutants with an active *CACTA1* element were crossed to wild-type *DDM1/DDM1* plants of the C24 strain. The C24 strain does not contain sequence that hybridizes to the *CACTA* probe in Southern blot analysis, which enabled us to follow the *ddm1*-derived *CACTA1* copies after segregation.

The *CACTA1* transcript was detectable in F_1 *DDM1/ddm1* heterozygous plants produced by crosses between wild-type C24 plants and *ddm1* mutants (Figure 1A). The transcript was not detectable in the control F_1 hybrid plants from crossing wild-type C24 to wild-type Col plants. Essentially the same results were obtained from reciprocal crosses (Figure 1A). The transcript was also detectable after intrastrain crosses between Col and *ddm1* (Figure S1 available at http://www.genetics.org/supplemental/). This was not due to heterozygosity of the *DDM1* locus, because the transcript was undetectable in the *DDM1/ddm1* heterozygote in which the *CACTA1* locus had been replaced by repeated backcrossing to Col wild type (Figure 1A). In addition, the transcript was detectable in *DDM1/DDM1* wild-type homozygotes generated by an additional backcross of the F_1 plants to the wild-type plants (Figure 1B). These results indicate that *CACTA1* activated by the *ddm1* mutation remains transcriptionally active even in the wild-type *DDM1* background. The active state was inherited through both male and female meiotic passages.

***ddm1*-derived *CACTA1* remains mobile in the wild-type *DDM1* background:** The *ddm1* mutation induces not only transcription but also transposition of *CACTA1* (Miura *et al.* 2001). We next examined the mobility of the *ddm1*-derived *CACTA1* element in the wild-type *DDM1* background. *DDM1/DDM1* homozygotes were selected from self-pollinated progenies of the F_1 *DDM1/ddm1* (wild-type C24 × *ddm1*). In the Southern hybridization analysis, *DDM1/DDM1* wild-type individuals in the F_2 generation showed several new bands, which were undetectable in their direct parents (F_1 *DDM1/ddm1*, plants A, B, C, and D in Figure 2A). These new bands in the F_2 plants should reflect the transposition events in the F_1 or F_2 generation, suggesting that the *CACTA1* activated by the *ddm1* mutation remained mobile in the presence of the wild-type *DDM1* allele. Furthermore, the *CACTA1* continued to transpose in the next generation in the

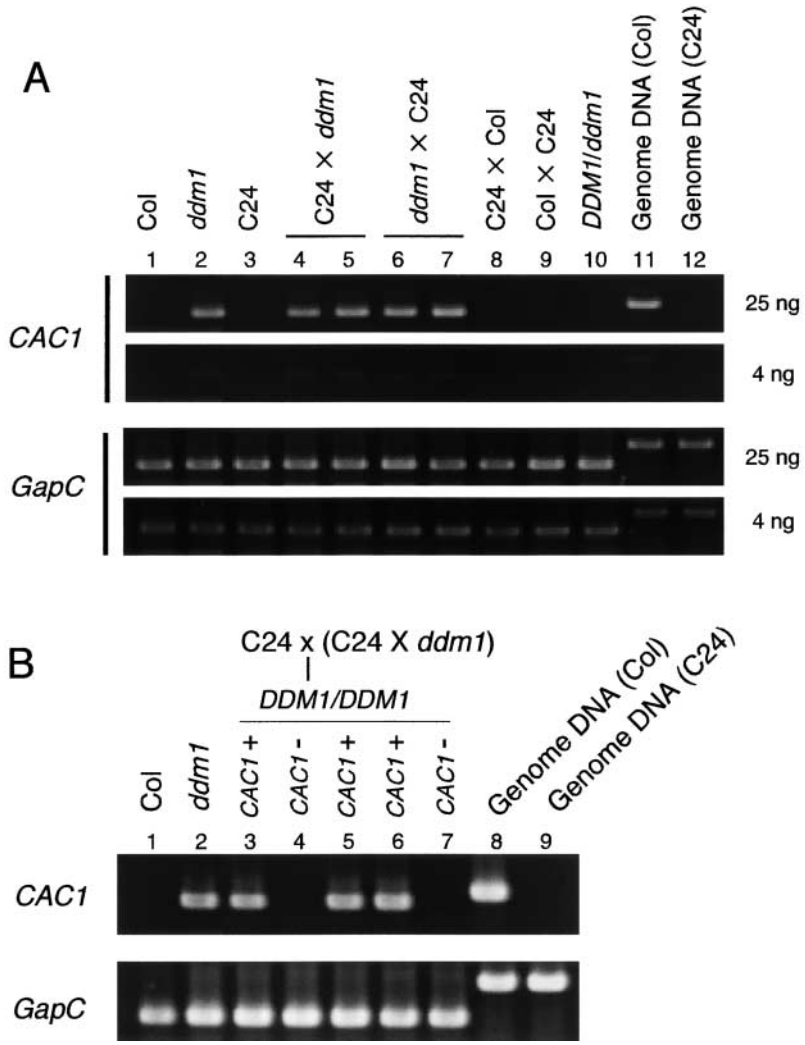
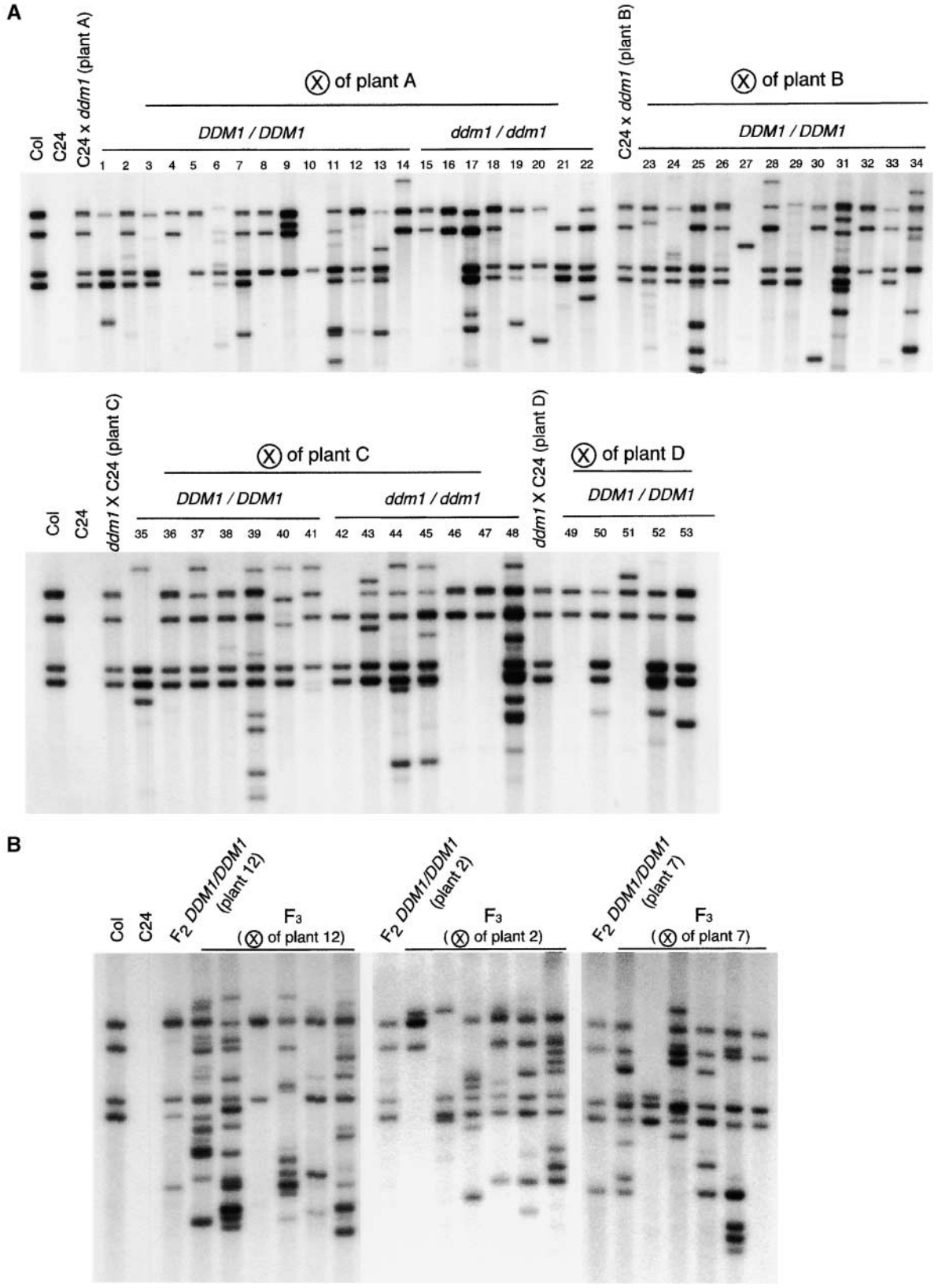


FIGURE 1.—(A) Transcription of *ddm1*-derived *CACTA1* after crosses to wild-type plants. Semiquantitative RT-PCR reactions corresponding to 25 ng and 4 ng of input total RNA are shown for each plant. C24 and Col represent wild-type (*DDM1/DDM1*) plants in those ecotypes. The *DDM1/ddm1* heterozygote in lane 10 was derived from a *ddm1/ddm1* Columbia plant, which was backcrossed six times to the wild-type Columbia plant in the heterozygous state (KAKUTANI *et al.* 1999). (B) Transcription of *ddm1*-derived *CACTA1* after two successive backcrosses to C24 wild-type plants. Because C24 does not have *CACTA1*, plants with and without *CACTA1* segregate after the second backcross (lanes 3, 5, and 6, and lanes 4 and 7, respectively). The plants in lanes 3 and 4 and lanes 5–7 are siblings. The constitutively expressed *GapC* gene was used as a control. Length of the predicted PCR product: *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.

DDM1/DDM1 background; we examined self-pollinated progeny from some of the F_2 *DDM1/DDM1* wild-type homozygotes and found additional bands, which were undetectable in the previous generation (F_2 wild-type *DDM1/DDM1* parents; Figure 2B). Mobilization of *CACTA1* was not due to the interstrain crosses between Col and C24, because transposition of *CACTA1* was not detected in the F_2 wild-type hybrid from crosses between two wild-type strains (Figure S2 at http://www.genetics.org/supplemental/). In addition, we were able to detect transposition in *DDM1/DDM1* plants segregating from intrastrain crosses between *ddm1* and wild-type Col plants (Figure S3 at http://www.genetics.org/supplemental/). These observations indicate that the *CACTA1* element activated by the *ddm1* mutation remained mobile for at least two generations in the presence of the wild-type *DDM1* gene.

Hypomethylation in *CACTA1* promoter region is inherited in the presence of the wild-type *DDM1* allele: We next examined whether the *CACTA1* hypomethylated by *ddm1* mutation remained hypomethylated in the wild-type background. We examined DNA methylation in ~300 bp of the 5′ terminal region, which includes the entire upstream region from the transcriptional starting site (K. WATANABE and T. KAKUTANI, unpublished results). In wild-type Col, this region was heavily methylated, especially at the CpG sites. In contrast, DNA methylation was almost completely lost in this region in the *ddm1* mutant (Figure 3; Figure S4 at http://www.genetics.org/supplemental/; KATO *et al.* 2003). The F_1 *DDM1/ddm1* plant (wild-type C24 × *ddm1*) has one *CACTA1* copy derived from the *ddm1* mutant parent. The *CACTA1* copy in this F_1 plant remained hypomethylated for both CpG and non-CG sites. In the F_1 hybrid from a control interstrain cross (wild-type C24 × wild-type Col), the *CACTA1* copy was normally methylated (Figure 3; Figure S4 at http://www.genetics.org/supplemental/. Essentially the same methylation patterns were obtained from reciprocal crosses (data not shown). These observations suggest that the *ddm1*-induced hypomethylation in this region was meiotically and mitotically transmitted even in the presence of the wild-type *DDM1* copy. This observation is consistent with the inheritance of transposon mobility.

Integration specificity of the *CACTA* transposon in the wild-type *DDM1/DDM1* background: DNA methylation is necessary for immobilization of *CACTA* and other transposons, consistent with the transcriptional activation of a variety of transposons and transposon-related sequences in DNA methylation mutants (Hirochika *et al.* 2000; Steimer *et al.* 2000; Lindroth *et al.* 2001; Miura *et al.* 2001; Singer *et al.* 2001; Johnson *et al.*



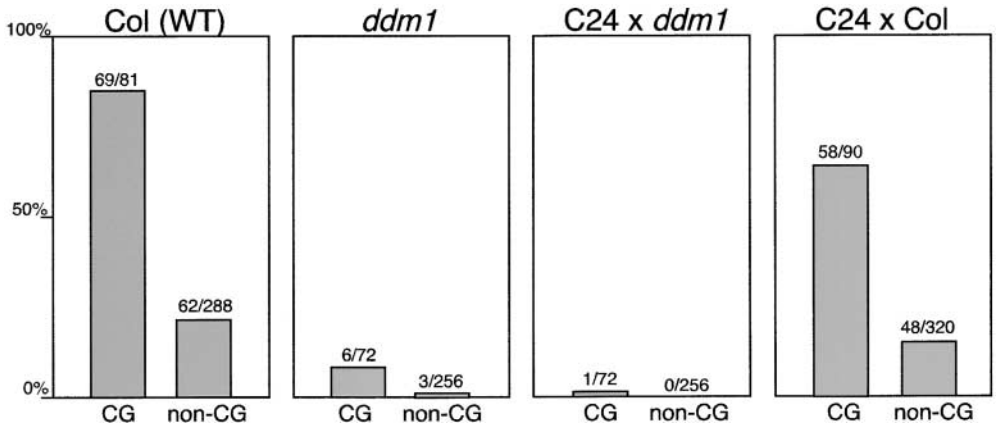


FIGURE 3.—DNA methylation status of *ddm1*-derived *CACTA1* in the presence of a wild-type *DDM1* allele. The DNA methylation pattern of the bottom strand of the 5′ terminal 310-bp sequence of *CACTA1* was determined by the bisulfite-mediated sequencing method. A total of 8–10 independent clones were examined for each plant. Distribution of the methylation in each clone is shown in Figure S4 at http://www.genetics.org/supplemental/.

2002; KATO *et al.* 2003; LIPPMAN *et al.* 2003). On the other hand, it is not known whether DNA methylation and the associated heterochromatin formation in the transposon integration site affect transposition efficiency. In other words, it is not known whether any of the Arabidopsis transposons preferentially integrate into heterochromatic regions.

We have previously shown that all five loci for *CACTA* transposons in the Col genome and 11 polymorphic integration sites of the related sequences in other ecotypes are localized near the centromeric heterochromatin (MIURA *et al.* 2004). In contrast, they transpose throughout the genome in the *ddm1* mutant background. The difference in integration sites between natural populations and *ddm1* could be due to loss of pericentromeric heterochromatin mark(s) in the *ddm1* mutant background (MIURA *et al.* 2001, 2004). However, it is not clear whether the *ddm1* mutation affects integration site specificity of the transposon.

We examined the integration sites of transposed *CACTA1* in F_2 *DDM1/DDM1* wild-type plants derived from the crosses between *ddm1* mutant and wild-type C24 plants. Self-pollinated progeny of F_1 without strong additional bands (plants A and B in Figure 2A) was used to avoid detecting transposition in the F_0 (*ddm1*) generation. The detected transposition should occur in the F_1 (*DDM1/ddm1*) or F_2 (*DDM1/DDM1*) generation. We confirmed each of the insertions by PCR using primers from flanking sequences and transposon sequences. The *CACTA1* integration sites in the F_2 *DDM1/DDM1* background distributed to chromosomal arms as well as to pericentromeric regions (Figure 4B). This is in contrast to the pericentromeric distribution of *CACTA*-like sequences fixed in natural populations (MIURA *et al.* 2004). In addition, although most of the *CACTA*-related sequences distribute in transposon-rich regions in natural populations, the integration sites for transposition induced in the laboratory did not show such a bias. Even in the *DDM1* wild-type background, they were distributed in both transposon-rich and gene-rich regions (Table 1).

The pericentromeric heterochromatic regions of Arabidopsis are detectable by DAPI staining as condensed structures and show a high degree of methylation in cytosine and lysine 9 (K9) of histone H3, which depend on the *DDM1* gene; the *ddm1* mutation abolishes DNA and H3K9 methylation and reduces the DAPI-stained chromocenter size (SOPPE *et al.* 2002). Interestingly, all these effects of the *ddm1* mutation on the pericentromeric regions are heritable; in the F_1 hybrid between *ddm1* and wild type, half of the chromocenters show decondensation and reduced methylation of DNA and H3K9, while the other half are indistinguishable from the normal wild-type chromocenter (SOPPE *et al.* 2002). This observation suggests that the *ddm1*-induced loss of pericentromeric heterochromatin, associated with loss

FIGURE 2.—Transposition of *ddm1*-derived *CACTA1* in a wild-type *DDM1* background. Genomic DNAs were digested with *Eco*RV, which is insensitive to cytosine methylation, and examined by Southern analysis with a probe corresponding to the 5′ end of *CACTA1* (probe B of MIURA *et al.* 2001). C24 and Col represent wild-type (*DDM1/DDM1*) plants of those ecotypes. The C24 strain does not contain sequence that hybridizes to this probe. (A) The *DDM1/ddm1* F_1 plants (plants A–D) were derived from crosses between *ddm1/ddm1* and wild-type (C24) *DDM1/DDM1*. From self-pollinated progeny of each of the F_1 plants, *DDM1/DDM1* and *ddm1/ddm1* segregants were selected and their band patterns were compared to those of their direct parents. A circled X represents self-pollination. We initially examined five F_1 plants, but one of them was not used for the F_2 analysis, because it showed a strong new band, possibly reflecting transposition in the F_0 *ddm1/ddm1* generation. Cleavage by another enzyme, *Hin*dIII, suggests that plant C has a weak additional band possibly reflecting somatic transposition (not shown). (B) The wild-type *DDM1/DDM1* plants in the F_3 generation were derived by self-pollination of *DDM1/DDM1* wild-type F_2 plants, which correspond to plants 12, 2, and 7.

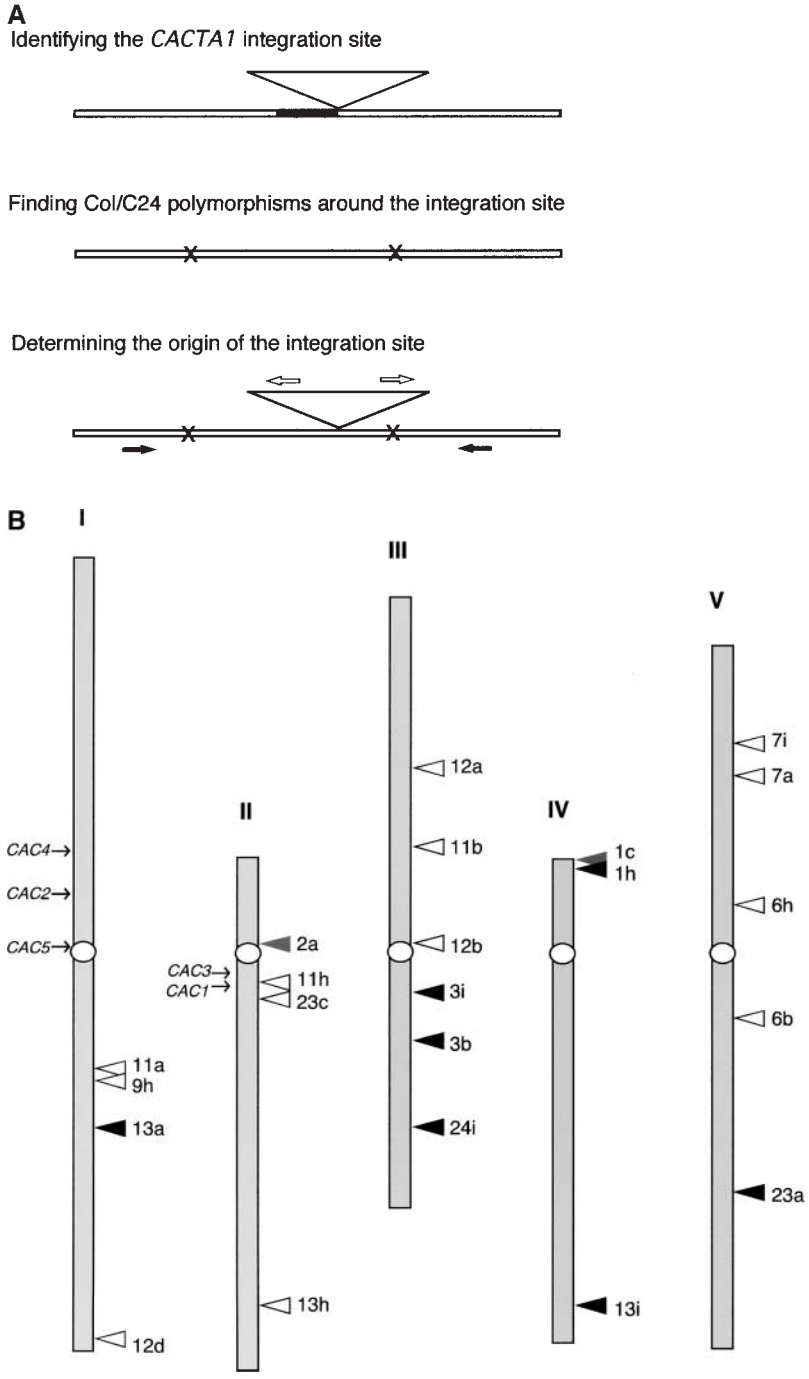


FIGURE 4.—Distribution of integration sites of the *CACTA1* element in a wild-type background. (A) Procedures to characterize integration sites of *CACTA1*. (Top) Regions flanking the 5′ side of *CACTA1* were sequenced by suppression PCR. Identified flanking regions are shown as solid. (Middle) The regions spanning 2 kb (1 kb upstream and 1 kb downstream) surrounding the integration sites were amplified from the Col and C24 genomes and sequenced. Polymorphism in the nucleotide sequence between Col and C24 is indicated by an "x." (Bottom) Sequences flanking the 5′ side and 3′ side of transposed *CACTA1* were amplified with primers near the integration site (solid arrow) and from the transposon (open arrows). The parental origin of the integration site was identified using Col/C24 polymorphism (indicated by an "x"). (B) Integration sites of transposed *CACTA1* were examined in 11 wild-type *DDM1* F_2 plants derived from C24 WT × *ddm1* (plants 1, 2, 3, 6, 7, 9, 11, 12, 13, 23, and 24 in Figure 2A). The integration sites of *CACTA1* are shown by arrowheads. Open and solid arrowheads represent the integration into the Col (*ddm1*-derived) and C24 (wild-type-derived) genomes, respectively (shaded arrowheads: unclassified integration sites). For each of the integration sites, the number corresponds to the plant number in Figure 2A. The position of each integration site was estimated using theTAIR MapViewer (http://arabidopsis.org/servlets/mapper).

of DNA and H3K9 methylations, is heritable as an epigenetic imprint in the presence of wild-type *DDM1* copy (SOPPE *et al.* 2002). This offers a useful system to examine whether *CACTA1* has integration preference between the heterochromatic and euchromatic regions.

We examined whether *CACTA1* integrates preferentially into the wild-type-derived pericentromeric region. The wild-type-derived and the *ddm1*-derived chromosomal regions were distinguished using nucleotide sequence polymorphism between the two parental ecotypes, Col and C24 (detailed procedures are shown in Figure 4A and MATERIALS AND METHODS). Instead of using molecular markers already available, we surveyed Col/C24 polymorphisms by sequencing 2-kb regions around each integration site to minimize possible ambiguity due to meiotic recombination with linked markers. Within the 22 *CACTA1* integration sites, we could identify the Col/C24 polymorphisms in 20 loci. We examined the origin of alleles for each of these sites. A total of 13 insertions were on *ddm1*-derived chromosomes, while 7 were on wild-type-derived chromosomes (Figure 4B; Table 1). For each of the integration sites, identity was confirmed

TABLE 1

Distribution of the integration sites of *CACTA1* fixed in natural populations or induced in the laboratory in *ddm1* or *DDM1*

	Gene-rich regions[a]	Transposon-rich regions[b]	Total
Natural populations[c]	1	10	11
Laboratory			
ddm1[d]	11	6	17
DDM1[e]	9	13	22
To *ddm1*-derived regions	5	8	13
To *DDM1*-derived regions	3	4	7
Unclassified	1	1	2

By chi-square test, the difference between natural populations and *ddm1* is significant ($P < 0.004$). Although the difference between natural populations and *DDM1* is not significant ($P = 0.06$), the difference is significant ($P = 0.007$) if 100-kb regions with only one sequence annotated to be transposon related are classified as gene rich rather than transposon rich.

[a] Insertion into regions without sequences annotated to be transposon, transposase, or reverse transcriptase related within 50-kb + 50-kb window.

[b] Insertion into regions other than those mentioned in footnote *a*.

[c] Miura *et al.* (2004).

[d] Miura *et al.* (2001).

[e] This work.

by examining more than two polymorphic sites. These results are not biased by segregation, because the *ddm1*- and wild-type-derived centromeric regions segregated randomly in the F_2 generation (Table S1 at http://www.genetics.org/supplemental/). Taken together, these results demonstrate that *CACTA1* integration is not biased toward wild-type-derived heterochromatic regions.

DISCUSSION

In the wild-type Col ecotype, the *CACTA1* is localized within a pericentromeric region and remain silent. However, it is mobilized by mutations abolishing genomic DNA methylation (Miura *et al.* 2001; Kato *et al.* 2003). We showed in this study that the *CACTA1* transposon mobilized by the DNA hypomethylation mutation *ddm1* remained mobile in *DDM1* wild-type backgrounds. The results are striking considering that *CACTA1* is completely silent in original wild-type Col plants. We have never found movement of this transposon in >100 wild-type Col plants examined (A. Miura, M. Kato and T. Kakutani, unpublished results). In addition, six Col accessions, Col-0, -1, -2, -3, -4, and -5, all showed the same band pattern of *CACTA* (Miura *et al.* 2004). *CACTA* did not transpose in the F_2 generation from a cross between Col and C24 (Figure S2 at http://www.genetics.org/supplemental/). In recombinant inbred lines between Col and Landsberg *erecta* (Lister and Dean 1993), only bands corresponding to the parental ecotypes segregated, suggesting that *CACTA* did not transpose during the initial interstrain cross nor the subsequent repeated self-pollinations (A. Miura and T. Kakutani, unpublished results).

One possible explanation for the mobility of the *ddm1*-derived *CACTA1* could be that the *CACTA1* transposed into a euchromatic arm region in the *ddm1* mutant background, and it therefore remained mobile. However, even *CACTA1* in the original pericentromeric region remained mobile in the *DDM1* wild-type background; *DDM1/DDM1* plants without any additional band to the original Col pattern also generated progeny with new bands (plant 2 and its progeny in Figure 2B). These results suggest that the *ddm1* mutation induces change in a heritable epigenetic mark(s), which is critical for transposon mobility.

The inheritance of epigenetic change of transcription over generations has been reported in several plant genes (Jacobsen and Meyerowitz 1997; Soppe *et al.* 2000; Cubas *et al.* 2001; Stokes *et al.* 2002). Two of them, *FWA* and *BAL*, are due to ectopic transcription, which can be induced by the *ddm1* or *met1* mutation (Soppe *et al.* 2000; Stokes *et al.* 2002; Kankel *et al.* 2003). *CACTA1* behaved in a similar manner. It has recently been shown that other transposons (or transposon-like sequences) are transcribed in the *ddm1* mutant and continue to be transcribed after a cross to a wild-type plant (Lippman *et al.* 2003).

The transposition of *CACTA1* in the *DDM1* wild-type background allowed us to directly examine its integration specificity in the wild-type background. We have previously shown that all *CACTA* transposons in the Col ecotype and most of the related sequences in other ecotypes tend to be localized in pericentromeric heterochromatin (Miura *et al.* 2004). This genomic distribution could be due to selective integration near the centromere. In the present study, we showed that *CACTA1* did not transpose preferentially into pericentromeric regions even in the *DDM1* background. In addition, *CACTA1* did not show preferential integration into wild-type-derived chromosomal regions compared to *ddm1*-derived regions (Figure 4B). These observations suggest that *CACTA1* does not preferentially integrate into heterochromatic regions.

The reason that *CACTA* elements tend to be localized near the centromere rather than near the chromosomal-arm regions in natural populations remains unknown. One possible mechanism to account for the accumulation of *CACTA1* in pericentromeric regions is natural selection; even though *CACTA1* integrates into chromosome arm regions, the resultant chromosome may be eliminated from the natural population by purifying selection. It is possible that *CACTA* insertions into gene-rich regions sometimes reduce host fitness by direct gene disruption. In fact, Tc transposon insertions

fixed in the *Caenorhabditis elegans* genome show a bias against insertion into coding regions, while the high-frequency insertions induced in the laboratory in the *mut-7* mutant background show a more random distribution (Rizzon *et al.* 2003). This observation suggests natural selection against gene disruption. In addition to the direct gene disruption by insertion, transposon insertion sometimes disturbs the proper function of nearby host genes by affecting their transcription (Fedoroff 1996; Martienssen 1996). These mechanisms may also contribute to differentiation of gene-rich and transposon-rich regions in the plant genome. An alternative explanation is that the transposition is random, but the low frequency of transposon excision in the heterochromatic region results in net accumulation of "cut-and-paste" type transposons. Meiotic recombination rates tend to be low in the heterochromatin region, which would interfere with recombination-based mechanisms to remove transposons. However, recent analysis revealed that transposon abundance does not generally correlate with the low meiotic recombination rate in the Arabidopsis genome (Wright *et al.* 2003).

Another important question is whether integration of other classes of transposons is controlled in a similar manner. Although accumulation near the centromere is conserved among many classes of transposons, the underlying mechanisms might differ. With regard to transcriptional activation, different transposons respond differently to mutations affecting DNA and histone modifications (Johnson *et al.* 2002; Lippman *et al.* 2003). Despite the extensive investigation of transcription of many endogenous Arabidopsis transposon families, epigenetic control of integration specificity has been examined in only two of them: *CACTA* (this work and Miura *et al.* 2001) and AtMu (Singer *et al.* 2001). AtMu integrates throughout the genome, as is the case in *CACTA*, at least in the *ddm1* mutant background (Singer *et al.* 2001).

Inheritance of differential epigenetic states over generations is an enigmatic phenomenon found in plants. Similar inheritance of epigenetic variations has also been reported for some alleles of mammalian genes (Whitelaw and Martin 2001; Rakyan *et al.* 2003). Interestingly, the affected mammalian alleles have transposon insertions compared to the wild-type allele. We propose that inheritance of epigenetic silencing is used, at least for some sequences, for transgenerational genome defense against deleterious genome rearrangement induced by transposon movement. It has long been known that maize transposons sometimes switch between active and inactive states (McClintock 1958). Such active or inactive states are also often inherited over multiple generations. Correlation between reversible transposon activity and DNA methylation has been found on a variety of transposons (Chandler and Walbot 1986; Schwartz and Dennis 1986; Banks *et al.* 1988; Martienssen *et al.* 1990). If one of the major functions of DNA methylation and the epigenetic modification system is a defense against the deleterious effects of transposons and other invasive elements (Yoder *et al.* 1997; Matzke *et al.* 1999), the heritable property would be advantageous, because the silencing is maintained at every stage of development, including early development before *de novo* silencing is established.

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