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Robertson's *Mutator* transposons in *A. thaliana* are regulated by the chromatin-remodeling gene *Decrease in DNA Methylation* (*DDM1*)

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Robertson's *Mutator* transposable elements in maize undergo cycles of activity and then inactivity that correlate with changes in cytosine methylation. *Mutator*-like elements are present in the *Arabidopsis* genome but are heavily methylated and inactive. These elements become demethylated and active in the chromatin-remodeling mutant *ddm1* (*Decrease in DNA Methylation*), which leads to loss of heterochromatic DNA methylation. Thus, DNA transposons in plants appear to be regulated by chromatin remodeling. In inbred *ddm1* strains, transposed elements may account, in part, for mutant phenotypes unlinked to *ddm1*. Gene silencing and paramutation are also regulated by *DDM1*, providing support for the proposition that epigenetic silencing is related to transposon regulation.

[*Key Words*: Transposable element; heterochromatin; epigenetic; DDM1]

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Transposable elements are widespread constituents of all eukaryotic genomes. Discovered first in maize, transposons and retrotransposons occupy 50%–80% of this genome and frequently reach copy numbers of several thousand (SanMiguel et al. 1998; Fedoroff 1999). Most DNA transposons are no longer active and require an autonomous element in *trans* to transpose. In maize, *cis*-acting transposon regulatory mechanisms are thought to include DNA methylation. Transposase promoter sequences from McClintock's *Activator* and *Suppressor-Mutator* transposons, for example, are hypomethylated in the active state, although the rest of the element is methylated constitutively (Banks et al. 1988; Fedoroff 1999). In vitro, transposon DNA binds more efficiently to transposase when hemimethylated than when unmethylated or fully methylated, possibly because this marks recently replicated transposons in vivo (Kunze and Starlinger 1989). For these and other reasons, we have proposed that DNA methylation is a fundamental property of transposons that differentiates them from the remainder of the genome (Martienssen 1998; Rabinowicz et al. 1999).

Robertson's *Mutator* transposons in maize fall into six categories, which share highly similar 200-bp terminal inverted repeats (TIRs; Bennetzen 1996). The autonomous *MuDR* element in maize encodes two genes, *mudrA* and *mudrB*. The *mudrA* gene encodes the MURA transposase, the *mudrB* gene encodes a subsidiary protein (MURB) that is not essential for somatic excision in maize (Lisch et al. 1999; Raizada and Walbot 2000). Two alternatively spliced forms of MURA, 736 and 823 amino acids (aa) in length, are found in maize (Hershberger et al. 1995). The 823 aa MURA protein can effectively bind to a conserved region in the element TIRs (Benito and Walbot 1997) and therefore probably functions as transposase. Nonautonomous elements, further designated as *Mu*-elements, with intact inverted repeats are also mobilized by MURA, which binds to methylated, as well as unmethylated, motifs within the TIRs (Benito and Walbot 1997). Except for sharing similarity between TIRs, *Mu* elements are unrelated to *MuDR* and do not encode functional transposase. Both types of elements, *MuDR* and *Mu*, are heavily methylated in inbred strains of maize. In *Mutator* strains, which show a high degree of transposon activity, the TIRs of *Mu* elements such as *Mu1* and *Mu2*, as well as the TIRs of *MuDR*, are hypomethylated. Because demethylation of TIRs in *MuDR* elements leads to high levels of transposase gene expression, it is thought that they contain the the transposase promoter (Chandler and Walbot 1986; Chomet et al. 1991; Martienssen and Baron 1994; Hershberger et al. 1995; Bennetzen 1996). Autonomous elements can spontaneously lose activity during development, a process accompanied by methylation of TIRs. This results in plants mosaic for cells containing methylated and unmethylated elements (Martienssen et al. 1990; Martienssen and Baron 1994).

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The maize and *Arabidopsis* genomes differ in their organization. Transposable elements in maize (especially retroelements) are the primary constituent of intergenic DNA, outnumbering genes at least four to one (SanMiguel et al. 1998). In contrast, genes outnumber transposons by five to one in *Arabidopsis*, and most transposons are confined to pericentromeric heterochromatin (Lin et al. 1999; Mayer et al. 1999). Recently, an interstitial region of heterochromatin resembling a maize chromomere, or knob, has been completely sequenced (Consortium 2000). The knob was found to be composed of DNA transposons (15%), retrotransposons (35%), and other repeats (21%); the remaining 29% was composed of largely silent genes. Thus, the knob region more closely resembles the maize genome than the remainder of the *Arabidopsis* genome. Transposons and repeats were found to be heavily methylated within the knob as they are in maize.

We set out to investigate transposon methylation in plants by isolating mutants with decreased DNA methylation (*ddm*) in *Arabidopsis* (Vongs et al. 1993). *DDM1* is required for methylation of tandem repeats at the centromere and at the nucleolar organizer (Vongs et al. 1993), as well as at the heterochromatic knob (Consortium 2000). This gene encodes a SWI2/SNF2 chromatin-remodeling factor (Jeddeloh et al. 1999), and loss-of-function *ddm1* mutations lead to immediate loss of DNA methylation from heterochromatin and gradual loss from euchromatin over successive generations of inbreeding (Kakutani et al. 1999). Here, we examine the impact of *ddm1* on transposon activity.

Mutator*-like elements are widespread constituents of the* Arabidopsis *genome*

We performed text queries and sequence-similarity searches of the *Arabidopsis* genome to catalog all ORFs related to the MURA transposase of *MuDR* from maize. More than 200 individual ORFs were found in 90 Mb of completed sequence. We narrowed our search further to elements structurally related to *MUDR* with long TIRs and harboring intact transposase genes that might still be able to transpose. Therefore, we analyzed the flanking regions of all mined sequences with homology to the *mudrA* gene in order to identify TIR-sequences. Only 22 ORFs encoding a putative MURA transposase were flanked by long TIRs, as is the case with *MuDR* in maize (Table 1A). Unlike maize *MuDR* elements, none of the *Arabidopsis Mutator*-like elements encode a protein resembling MURB.

In a recent study, 108 *Mutator*-like elements were identified in 17.2 Mb of finished *Arabidopsis* sequence (Le et al. 2000). These investigators coined the term MULE (*Mutator*-like element) to describe genes with homology to the *mudrA* transposase gene and related repeats and refer to *Mutator*-like elements with long TIRs as TIR-MULEs (http://soave.bio.mcgill.ca/clonebase/). We have adopted this nomenclature, although only 4 of the 22 TIR-MULEs we identified can be found in their study (T3F12.12 [*AtMu1*] = MULE16, gi2443899; F28J12.70 = MULE3, gi2832639; F1N21.16 = MULE24A; F9D12.2 = MULE24B, gi3319339). We have named individual elements (*AtMu1*, *AtMu2*, etc.) which are capable of transcription or transposition, consistent with the practice in maize, snapdragon, and other plants. Because we were only interested in identifying putatively intact transposons, ~200 MULEs in which no TIRs could be identified were not considered further, and we did not search for *Mu*-like elements with homology only to TIR sequences.

Analysis of TIR and transposase homologies

Cluster analysis of the inverted repeats (Fig. 1A, Table 1B) and of the transposase genes (Fig. 1B) showed that the *Arabidopsis* TIR-MULEs fell into six groups. The individual elements were single-copy, except that two copies of the *AtMu1* element were found on chromosomes 1 (T11I11.3) and 4 (T3F12.12, together designated as subgroup IA), two copies of *AtMu2* were found on chromosome 5 (F14I23, K2K18.2; group II), and element F20D23.2 (group V) has four copies in the Columbia genome (data not shown). Both elements of the *AtMu1* class (T3F12.12, T11I11.3) and both of the *AtMu2* class (F14I23, K2K18.2) share 98% sequence identity on the nucleotide and amino acid level. Bacterial and maize *mudrA* genes share a 25-aa signature sequence [D-x(3)-G-(LIVMF)-x(6)-(STAV)-(LIVMFYW)-(PT)-x-(STAV)-x(2)-(QR)-x-C-x(2)-H] found in a highly conserved 130-aa domain (Eisen et al. 1994). The *AtMu1* (T3F12.12, T11I11.3) and *AtMu2* (K2K18.2, F14I23) elements differ from this signature at a single-residue—E instead of V at position 13 of the motif (Fig. 1B). Because those elements most likely encode functional transposase (see results below), we propose an extended consensus pattern [D-x(3)-G-(LIVMF)-x(6)-(*E*STAV)-(LIVMFYW)-(PT)-x-(STAV)-x(2)-(QR)-x-C-x(2)-H]. The predicted *AtMu1* transposase has 36% similarity and 25% identity to the MURA transposase from maize. The remaining TIR-MULEs might be defective as a result of mutations in the conserved region of MURA or because they encode truncated transposase proteins (Fig. 1B, Table 1A).

The right and left TIR sequences of the TIR-MULEs vary in length (130 bp–356 bp) and show varying degrees of conservation. In pairwise comparisons, all TIRs—with one exception (MJG14.16)—are more closely related to each other than to any other TIR of another element (Fig. 1A). TIRA of the element MJG14.16 is more closely related to the TIRs of F15K19.3 than to its own TIRB (Fig. 1A). TIRs of individual elements share a minimum sequence identity from 67% (T13P21.5) to 97%. (Table 1A). The most conserved TIRs, with 96%–97% sequence identity, are those of the *AtMu1* (T3F12.12, T11I11.3) and *AtMu2* (F14I23, K2K18.2; Fig. 1C). All TIR-MULEs start or end with 1–4 G nucleotides. Figure 1C shows an alignment of the first 100 bp of TIRs of those TIR-MULEs that are transcribed and show the highest sequence similarity between both TIRs.

No significant similarity to the MURA transposase-binding site in maize predicted by Benito and Walbot

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Table 1A. *Summary of TIR–MULEs in* Arabidopsis.

Group	Accession	TIR–MULE BAC/gene no.	chr.	% identity TIRA & TIRB[a]	Length TIRA/TIRB (bp)	Element position on BAC[b]	Length element (bp)	MURA gene location	Strand	Pre-dicted exons	Pre-dicted protein-length (aa)	Target site duplication	
IA	AC002983.1	T3F12.12 ***AtMu1***	4	97%	294/294	20148-23792	3645	20819-23250	+	3	761	taaaa/ taaat	4/5 bp imperfect
IA	AC012680.3	T11I11.3 ***AtMu1***	1	96%	294/294	11840-15489	3650	12516-14947	+	3	761	tattaaaaa/ tattaaaaa	9 bp perfect
IB	AC007123.1	F21A20_a	5	95%	277/278	42594-46220	3627	45556-43148	-	5	773	ataatataa/ ataatataa	9 bp perfect
IB	AL021710.1	F28J12.70	4	86%	191/161	30842-34611	3769	31243-33492	+	5	633	acaattaatc/ acaattaatt	10 bp imperfect
IB	AC006228.4	F5J5.13	1	85%	289/287	59273-64509	5237	59785-61763	+	3	567	attttaaa/ attttaaa	8 bp perfect
II	AC007399.1	F14I23 ***AtMu2***	5	97%	261/261	49883-53854	3972	52985-49883	-	3	743	tagtatcaac/ tagtatcaac	10 bp perfect
II	AB023031.1	K2K18.2 ***AtMu2***	5	97%	329/330	6738-10748	4011	9880-7481	-	3	743	tagcataatt/ tagcataatt	10 bp perfect
II	AL049876.1	T22B4.180	4	92%	249/244	99243-101373	2131	101266-99528	-	3	461	atcgtcaa/ atcgtcaa	8 bp perfect
III	AC002130.1	F1N21.16	1	93%	275/275	88381-92825	4445	92131-895461	-	3	816	gattctaaa/ gattctaaa	9 bp perfect
III	AF077407.1	F9D12.2	5	88%	285/283	93314-98243	4930	93735-97099	+	5	940	atataaaat/ atataaaat	9 bp perfect
III	AC006438.3	F19G14.19	2	87%	255/257	15946-22558	6613	17506-21460	+	2	545	tttttta/ ttttttg	6/7 bp imperfect
III	AC008016.2	F6D8	1	86%	260/258	45838-50372	4535	46271-48307	+	3	518	ttttt/ ttttt	5 bp perfect
IV	AC006067.3	T13P21.20	2	94%	162/191	16662-18766	2105	17524-18326	+	2	241	107 bp perfect direct repeat	
IV	AB009049.1	MCD7.9	5	77%	185/203	43085-46864	3780	43892-46360	+	5	715	taaaaaata/ taaaaaata	9 bp perfect
IV	AC005398.2	T13P21.5	2	67%	296/301	1682-6206	4525	5767-3406	-	5	430	-	no TSD
V	AC007651.2	F20D23.2[c]	1	76% (96%)	295/266 (131/131)	6902-11712	4811	10148-7273	-	5	622	ataatttaat/ ataatttagt	10 bp imperfect
V	AC006429.3	F15K19.3	2	75%	272/271	9098-13270	4173	12902-11476	-	4	212	ttttcaaaaac/ ttttaaaac	11/9 bp imperfect
V	AB017068.1	MJG14.16	5	75%	155/134	39176-43127	3952	42587-40191	-	5	641	gttttttttc/ gtttttttc	9/10 bp imperfect
VI	AC006528.4	F9B22.8 ***AtMu6***	2	95%	356/356	30888-35590	4703	32219-34323	+	5	597	atattaatat/ atattaatat	10 bp perfect
VI	AC007123.1	F21A20_b	5	90%	294/294	109397-114412	5016	114064-112267	-	3	441	ggatttaaaatag/t gatttaaaatga	13 bp imperfect
VI	AC005693.2	T25N22.6	2	89%	243/243	19540-24231	4692	23513-20653	-	4	558	cattaaaaaaca/ ctttaaaaaaaa	12 bp imperfect
VI	AC007932.2	F11A17.16	1	88%	335/319	58778-61749	2972	68664-66511	-	7	490	tctttaaaaaa/ gctttaaaaaa	11 bp imperfect

[a]The 5′ TIR (TIRA) of each element was compared to the reverse complement of its 3′ TIR (TIRB) over the length (bp) indicated.
[b]Element position refers to 5′ end of TIRA to 3′ end of TIRB.
[c]Percent identity of the first 131 bp between TIRs of this element is 96%.

(1997) could be identified in any of the TIR-MULE sequences. According to the cluster analysis of multiple-sequence alignments, the TIR-MULEs can be classified in two main lineages: one class comprising groups I–II, the other class containing groups III–VI (Fig. 1A). Elements belonging to neighboring groups share ~50% sequence identity (Table 1B). Assuming that highly conserved pairs of TIRs are an essential requirement for transposase binding and, hence, transposition, elements with a low level of sequence identity in their TIR sequences might no longer be transactivated and therefore are probably nonfunctional. For that reason and the observed divergence between TIR-groups, the tree very likely does not reflect an evolution of different functional groups of elements but rather different stages of degeneration. We, therefore, cannot derive a meaningful consensus sequence from multiple-sequence alignments of all TIRs in order to determine conserved MURA-binding sites. Experimental analysis of TIR sequences of elements still capable of transposition, however, will undoubtedly reveal motifs important for MURA binding. Nearly all of the TIR-MULEs (20 of 22) were inserted between short direct repeats, which varied between 8 bp and 13 bp (Table 1A). Five of the TIR-MULEs were flanked by perfect 9-bp repeats (Table 1A), similar to the 9-bp target-site duplications (TSD) found in maize. One

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element (T13P21.20) was flanked by identical 107 bp repeats on either side. Imperfect TSDs may have resulted from local rearrangement following transposition (Taylor and Walbot 1985; Das and Martienssen 1995) or random mutation after the transposition event.

The locations of each of the TIR-MULEs are shown in Figure 2. Six TIR-MULEs are distributed along chromosome I. On chromosomes 2, 4, and 5, 13 out of 16 TIR-MULEs were found within 2 Mb of the centromeric repeats. On chromosome 4, one copy of *AtMu1* was found where pericentromeric heterochromatin has been cytologically defined (Fransz et al. 2000). This bias toward heterochromatin was even more pronounced among non-TIR elements (Consortium 2000).

MULEs are regulated by DDM1

For further analysis, at least one representative element from each group was selected that had either long or well conserved TIRs (85%–97% sequence identity over at least 130 bp) or encoded potentially full-length transposase (743–773 aa). TIR-MULE F15K19.3, however, has only 75% sequence identity between TIRs and encodes very likely a truncated protein of 212 aa. In total, we tested 12 elements: two from group IA (T3F12.12, T11I11.3), two from group IB (F21A20_a, F5J5.13), three from group II (F14I23, K2K18.2, T22B4.180), one from group III (F9D12.2), one from group IV (T13P21.20), two from group V (F20D23.2, F15k19.3), and one from group VI (F9B22.8; Tables 1A, 2). DNA gel blots indicated that all elements were partially methylated at *Hpa*II and *Eco*RII restriction sites in the Columbia and Landsberg ecotypes (Fig. 3; data not shown). Overexposure of these blots indicated that *AtMu1* was less methylated in Landsberg *erecta* (Fig. 3A). In both Columbia and Landsberg background, all 12 elements were hypomethylated in *ddm1* mutants (Fig. 3B; data not shown). Hypomethylation was apparent in pooled F3 seedlings from self-pollinated homozygous mutants in the F_2 generation and did not change further in subsequent generations. Thus, TIR-MULEs are a primary target of *DDM1*.

Transcription of TIR-MULE transposase genes was examined by RT–PCR. In wild-type Columbia plants, no transcripts could be detected for any of the elements. In *ddm1* mutant plants, both copies of *AtMu1* (Fig. 4A), both copies of the group II element *AtMu2*, and one other

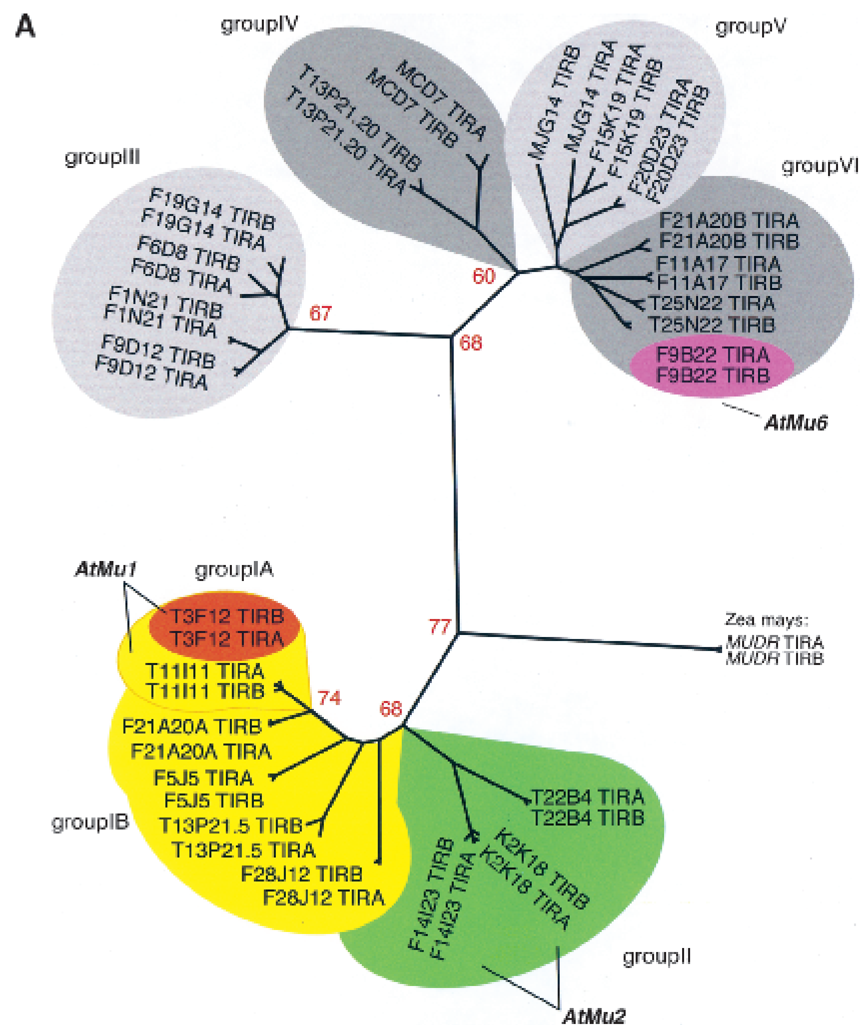

Figure 1. Analysis of *Mutator*-like elements with long terminal inverted repeats (TIR-MULEs) in *Arabidopsis*. (*A*) Unrooted distance tree of the 22 TIR-MULEs identified in this study (Table 1A,B) based on terminal inverted repeat (TIR) sequences. *Mutator*-like elements with TIRs (TIR-MULEs) have been grouped by similiarty according to the cluster analysis. Elements that transpose in *ddm1* strains are highlighted in red (*AtMu1*) and purple (*AtMu6*). Bootstrap values are indicated at nodes. Subgroups IA and IB (yellow) and group II (green) contain elements which are transcribed in *ddm1* mutants (see Table 2). (*B*) Alignment of a 97-aa conserved region of the *Arabidopsis* MURA-like transposases with the maize MURA protein. Identical amino acids are boxed in dark grey, similar in light grey. Identical amino acids from the PROSITE signature pattern are shown below the corresponding sequence. (*C*) Alignment of TIR sequences of transcribed elements. Identical nucleotides are boxed in grey (TIRA, 5′ TIR; TIRB, 3′TIR).

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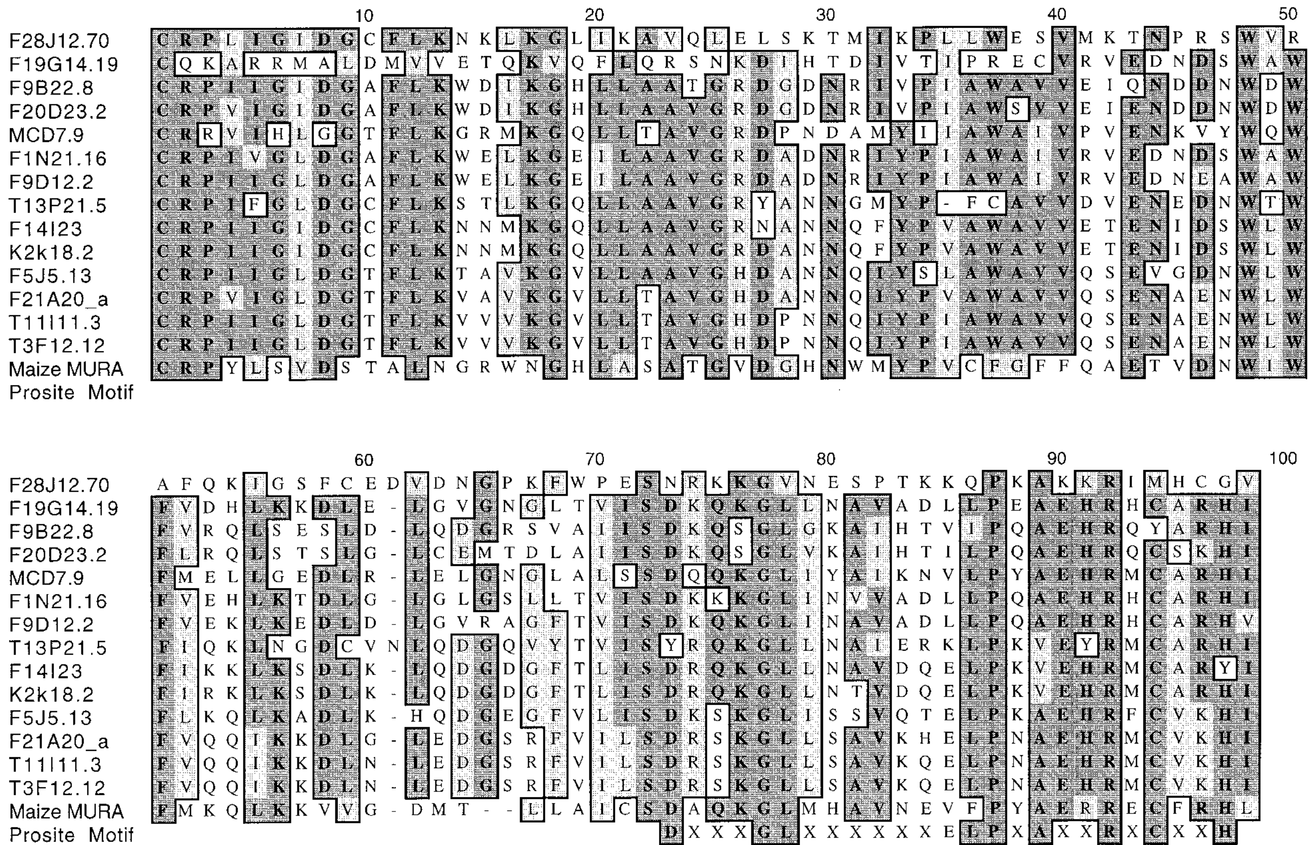
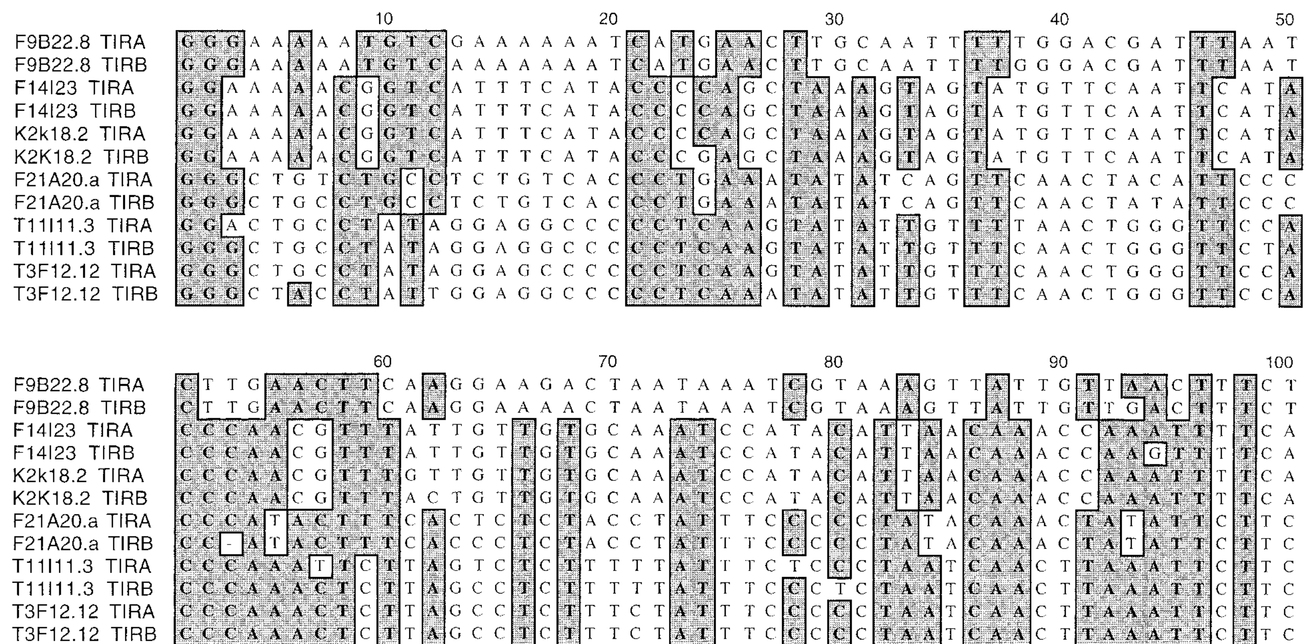
(*Figure 1 continued*)





group IB element (F21A20_a) were transcribed (Fig. 4D; Table 2; data not shown). Bona fide transcription was confirmed by sequencing the RT–PCR products from *AtMu1* and showing accurate splicing (see Materials and

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Table 1B. *Comparison of TIRs between related TIR–MULE groups*

% identity of TIRs (100 bp)

TIR-MULE group	group IA (ATMU1)	T3F12.12	mean
group IB	F21A20_a	70%	
	F5J5.13	58%	
	T13P21.5	55%	
	F28J12.70	47%	**58%**
	group IB	**F28J12.70**	
group II	F14I23/K2K18.2	53%	
	T22B4.180	43%	**48%**
	group II (ATMU2)	**K2K18.2/F14I23**	
group III	F19G14.19	53%	
	F6D8	50%	
	F1N21.16	49%	
	F9D12.2	45%	**50%**
	group III	**F9D12.2**	
group IV	T13P21.20	50%	
	MCD7.9	55%	**53%**
	group IV	**T13P21.20**	
group V	F20D23.2	46%	
	MJG14.16	46%	
	F15K19.3	48%	**47%**
	group V	**MJG14.16**	
group VI	F21A20_b	52%	
	T25N22.6	53%	
	F11A17.16	54%	
	F9B22.8	50%	**52%**

TIR sequences of one representative element of each group (typed in bold in the second vertical column) were compared to TIR sequences of all of the elements of the next closest related group (designated in the first column) according to the evolutionary tree. The percentage of identity is given. Mean is the average sequence identity between groups of elements.

Methods). Interestingly, although only one *AtMu1* element (T3F12.12) was present in Landsberg *erecta* (Fig. 4B), it was transcribed in *DDM1*+ plants (Fig. 4D). The same was true of *AtMu2*, which is located near the centromere of chromosome 5 (Table 2). Thus, Landsberg *erecta* and Columbia differ in the regulation of TIR-MULE transcripts.

None of the other transposase genes tested by PCR was transcribed by this assay. Expressed sequence tags (AC701558554 and H7A1T7) were found corresponding to a group-VI element (F9B22.8, *AtMu6*) located near the centromere of chromosome 2, which was not tested by RT–PCR (Table 2). In pairwise comparisons, all the transcribed elements share at least 95% similarity between both TIRs (Table 1A). Because multiple elements are transcribed, it is not possible to assign any one element as the source of functional transposase in *ddm1* mutants.

DNA gel blots revealed that *AtMu1* was stable in >80 individual wild-type plants from Columbia (Table 2). Thirteen percent of individual *ddm1* plants had novel *AtMu1* bands suggestive of transpositions (Fig. 4B,C). These bands were not found in parental Columbia DNA (Fig. 4B,C). In Landsberg, rare transpositions (1%) were detected in wild-type plants (consistent with transcription in this ecotype) but were much more frequent (16%) in *ddm1* mutants (Table 2). These frequencies of transposition are comparable to rates found for individual *Mu*-elements in minimal Robertson's *Mutator*-lines in maize, which are equivalent to those described here (Lisch et al. 1995). In more active maize lines with multiple elements, transposition frequencies are 2–5 times higher, and *Mu* elements are also found as extrachromosomal circles (Sundaresan and Freeling 1987). We tested for such circles in *Arabidopsis* by DNA gel blot analysis of undigested *Arabidopsis* DNA but failed to detect them (data not shown). One possibility is that circular forms are transposition intermediates that require the MURB that is only found in maize. Alternatively, low copy number may prohibit detection.

In plants that did have transposed elements, no evidence of germinal excision was observed, either by Southern blotting or PCR among 25 progeny carrying the transposed element (data not shown). This suggests that germinal insertions occur without germinal excision, just as they do in maize. Further experiments using selectable assays for germinal excision are needed to make this conclusion more robust. The *AtMu6* element F9B22.8 was transcribed but transposed only rarely in *ddm1* mutants (Table 2). The transposase gene in this case is lacking the first 190 amino acids and may be nonfunctional, suggesting that this element is activated in *trans*.

Flanking sequences were amplified from 10 *AtMu1* transpositions by adapter-ligation PCR (AIMS) and sequenced to determine their location in the genome. Insertions were verified by DNA gel blot analysis of progeny plants using the flanking sequence (data not shown) and *AtMu1* as probes (Fig. 4). Amplification with element-specific primers and primers from either side of the *AtMu1* insertion site, followed by sequencing of the PCR products, confirmed the location of insertions. In all cases examined, it was the *AtMu1* element on chromosome 4 (T3F12.12) that had transposed. For eight of these sequences, the insertion site could be mapped to the nucleotide, and they all generated 9-bp insertion-site duplications (Table 3). One transposition integrated between T3H13.5 (*HY4*) and T3H13.6, only 176 kbp distal from the *AtMu1* copy on chromosome 4. The other transpositions were unlinked to the parental *AtMu1* element. Consistent with these results, *Mutator* elements in maize do not preferentially transpose to linked sites (Lisch et al. 1995). In this small sample, there were no biases against integration within repeats, promoters, or retrotransposons. None of the transpositions disrupted predicted coding regions, but integration of 6 out of 10 *AtMu1* elements within 200 bp–1500 bp upstream of predicted start codons indicated possible promoter disruption (Table 3).

Role of transposons in epigenetic regulation and genome organization

DDM1 is required for silencing of methylated and re-

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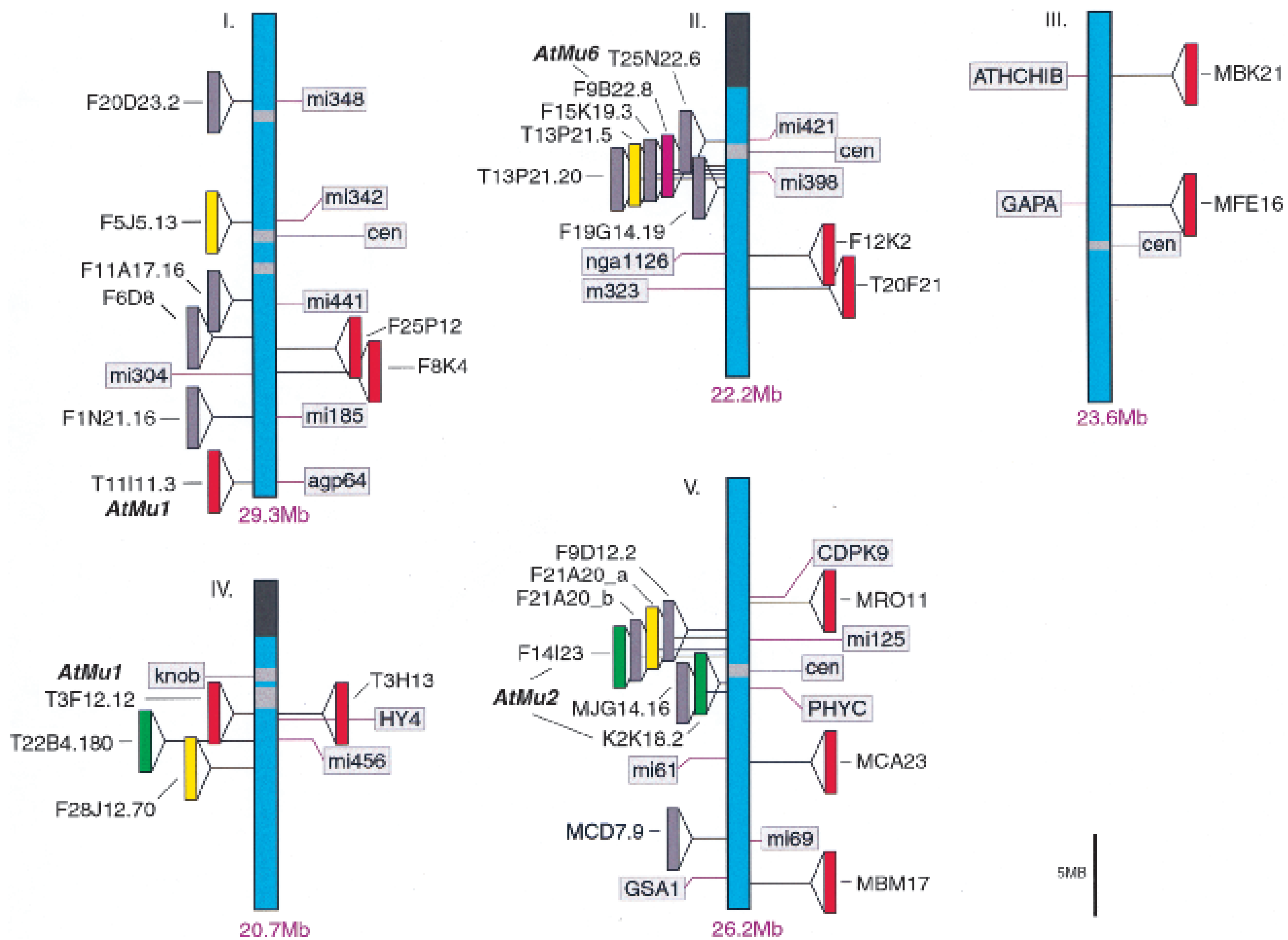

Figure 2. Genomic locations of TIR-MULE transposons. Potentially intact elements with TIRs in the Columbia ecotype are shown to the left of each chromosome. Transposed *AtMu1* elements in *ddm1* strains are shown on the right (Table 3). Genetic markers and map positions, as well as pericentromeric (light grey) and nucleolar (dark grey) heterochromatin, are shown. TIR-MULE subfamilies are color-coded as in Fig. 1A.

peated genes in *Arabidopsis* (Jeddeloh et al. 1998, 1999; Paszkowski and Mittelsten Scheid 1998), which may resemble cryptic heterochromatin (Consortium 2000). For example, *DDM1* mediates silencing of the *PAI2* locus by an inverted duplication at the unlinked *PAI1* locus (Bender and Fink 1995; Jeddeloh et al. 1998). This type of allele-specific gene silencing resembles paramutation in maize (Brink et al. 1968; Kermicle 1996; Martienssen 1996), which may involve transposons and repeats because of their influence on the expression of neighboring genes (McClintock 1965; Martienssen et al. 1990; Barkan and Martienssen 1991; Martienssen 1996; Matzke and Matzke 1998).

Repeated transgenes encoding tobacco retrotransposons also undergo silencing in *Arabidopsis*, and like other silent transgenes, this silencing can be reversed in *ddm1* mutants (Hirochika et al. 2000). Importantly, however, only 1 of 20 endogenous retrotransposons (*Tar17*) was found to be transcribed in *ddm1* mutants, and it does not transpose at all (Hirochika et al. 2000). In a similar study, a truncated *Athila* transcript was induced in *ddm1* mutants, but it did not transpose and no other retrotransposons were affected (Steimer et al. 2000). Therefore, we conclude that *DDM1* has little effect on retrotransposons in *Arabidopsis*. In the mouse, IAP (Intracisternal A Particle) retroelements are transcribed in DNA methyltransferase (*dnmt1*) mutants, but transposition has not been assessed (Walsh and Bestor 1999). Nearby genes can be regulated by IAP elements (Morgan et al. 1999) suggesting that retrotransposons may mediate some of the effects of demethylation (Martienssen and Richards 1995).

We have shown that TIR-MULEs in *Arabidopsis* are quiescent and do not transpose in the *Arabidopsis* strain Columbia. Quiescence is correlated with DNA methylation and a lack of transcription, although nearly identical copies of some elements may have transposed in the recent past. In contrast, TIR-MULEs are transcribed at low levels and transpose occasionally in Landsberg *erecta*, in which they have lower levels of DNA methylation. In loss-of-function *ddm1* mutants, transposon methylation was eliminated in both strains and *AtMu1*

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Table 2. *Methylation, transcription, and transposition of selected TIR–MULEs in* Arabidopsis.

		Copy number		Methylation		Transcription			Transposition events			
TIR–MULE	chr.	Col-0	La-*er*	Col WT	Col *ddm1*	Col WT	Ler WT	Col *ddm1*	Col WT	Ler WT	Col *ddm1*	Ler *ddm1*
T3F12.12/ T11I1.3 ***AtMu1***	4, 1	2	1	+	–	–	+	+	0/88	1/122	11/85	6/36
F21A20_a	5	1	0	+	–	–	–	+	0/62	N.D.	0/50	N.D.
F14I23/K2K18.2 ***AtMu2***	5	2	2	+	–	–	+	+	N.D.	N.D.	0/35	N.D.
T22B4.180	4	1	0	+/–	–	–	–	–	N.D.	N.D.	0/26	N.D.
T13P21.20	2	1	1	+	–	–	–	–	N.D.	N.D.	0/35	N.D.
F20D23.2	1	4	2	+/–	–	–	–	–	N.D.	N.D.	0/26	N.D.
F5J5.13	1	1	1	+	–	–	–	–	N.D.	N.D.	0/26	N.D.
F15K19.3	2	1	1	+	–	–	–	–	N.D.	N.D.	0/26	N.D.
F9B22.8 ***AtMu6***	2	1	N.D.	+	+/–	+[a]	N.D.	N.D.	N.D.	N.D.	1/54	N.D.
F9D12.2	5	1	N.D.	+	–	N.D.	N.D.	N.D.	N.D.	N.D.	0/40	N.D.

[a]Sequence of this TIR–MULE is represented in the *Arabidopsis* EST database.
N.D.: Not determined.

was activated resulting in high levels (10%–20% per generation) of transposition. Given the predicted function and phenotype of *DDM1*, chromatin remodeling and DNA methylation are therefore likely required for transcriptional, as well as transpositional, repression of potentially active autonomous elements. Other TIR-MULEs appear to be defective and incapable of activation.

Interestingly, *Tar17*, *Athila*, *AtMu1*, and *AtMu2* are all located in pericentromeric heterochromatin, within a few hundred kilobase of centromeric satellite repeats, and are all transcriptionally activated in *ddm1* mutants. Therefore, some feature of heterochromatin that depends on chromatin remodeling may be responsible for transposon regulation. McClintock (1951) first noted that heterochromatin underwent the same types of rearrangement as those induced by transposons. *Dotted*, one of the first transposons to be discovered in maize by Rhoades, was mapped to the heterochromatic knob on chromosome 9S (McClintock 1951). *Dotted* and other elements were activated by heterochromatic changes during the breakage-fusion-bridge (BFB) cycle (McClintock 1951, 1984), and activation of heterochromatic transposons might account for genome instability in these plants (McClintock 1951). Recently, mutations in the human homolog of *DDM1*, the X-linked alpha-thalassemia gene *ATRX*, were shown to cause heterochromatic demethylation in a parallel manner (Gibbons et al. 2000). It will be interesting to see if human transposons are activated in these patients.

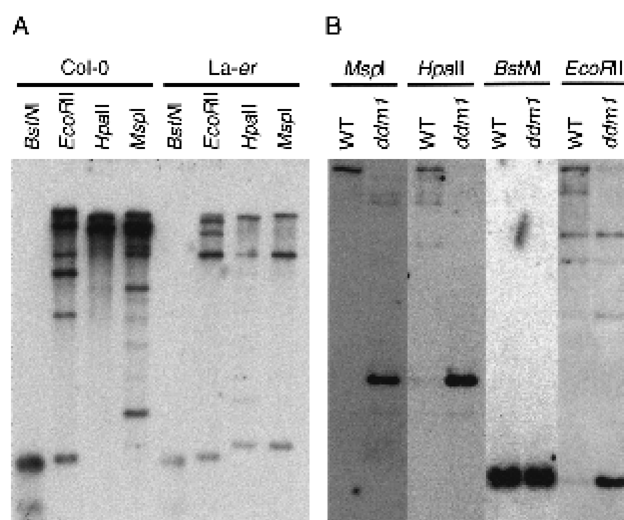

Figure 3. *Mutator* transposons are methylated in *Arabidopsis*. DNA gel blot analysis of *Arabidopsis* DNA from (*A*) wild-type Landsberg *erecta* (La-*er*) and Columbia strains (Col-0) and (*B*) wild-type and *ddm1* mutant plants (cv. Columbia) digested with *Eco*RII, *Bst*NI, *Hpa*II, and *Msp*I. The blots were hybridized with probe 2 from *AtMu1* (Fig. 4A).

The mutator phenotype observed in *ddm1* lines (Kakutani et al. 1996) may be caused in part by transposition of TIR-MULEs into or near genes, but this transposon group is unlikely to account for the high frequency with which certain mutations arise in independent *ddm1* lines. When maize *Mutator* elements are inserted into promoters and introns, methylation can promote gene expression, suppressing the original mutant phenotype (Barkan and Martienssen 1991; Bennetzen 1996; Settles et al. 2001). Pre-existing insertions of MULEs, therefore, would be expected to repress nearby genes in *ddm1*, resulting in a high frequency of epimutations at specific loci (Martienssen 1998). This model leads us to speculate on the difference between the maize and *Arabidopsis* genomes. Maize may be defective in certain components of the chromatin-remodeling complex that normally represses transposon activity (Martienssen and Henikoff 1999). For example, active *Mutator* lines may have trans-acting mutations, similar to *ddm1*, that result in the coordinate repression of multiple genes (Martienssen 1998). These mutations might influence other transposons also, consistent with the recovery of *Suppressor-Mutator* insertions in Robertson's *Mutator* strains in maize (Chandler et al. 1989; E. Vollbrecht and R.A. Martienssen, unpubl.). Insertional mutations are expected to

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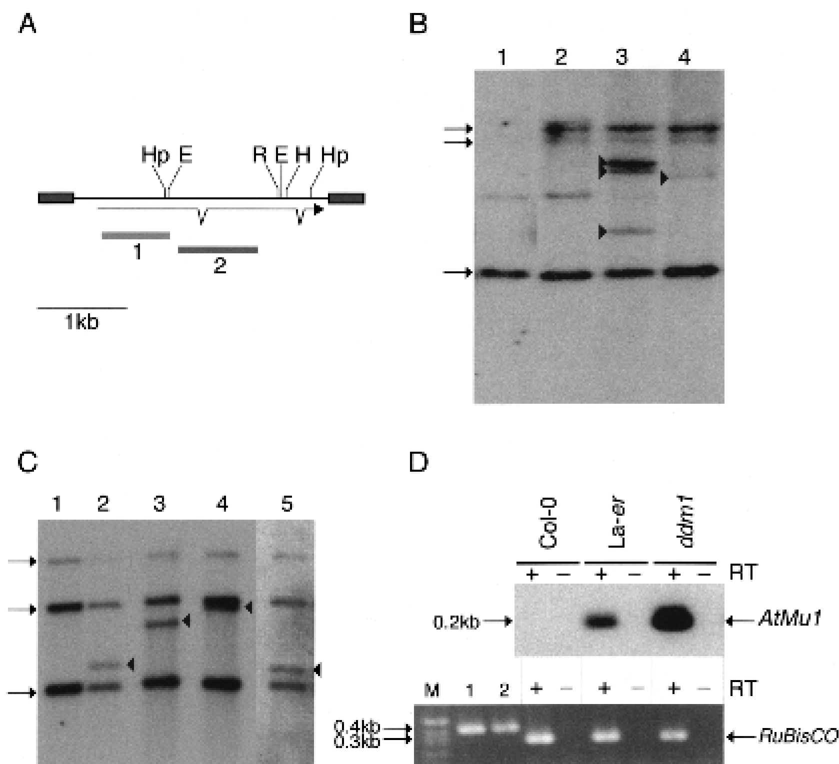

Figure 4. *Mutator* transposons are activated in *ddm1* mutants.(*A*) *AtMu1* has 295 bp TIRs (grey boxes) and encodes three exons; probes 1 and 2 are indicated below. Restriction enzyme sites for *Hpa*II (Hp), *Hin*dIII (H), *Eco*RI (R), and *Eco*RII (E) are shown. (*B*) DNA gel blot analysis of pooled wild-type and *ddm1*-mutant seedlings. DNA was digested with *Eco*RI and hybridized with probe 2. New bands (arrowheads) represent transposition of *AtMu1*. The preexisting elements T11I11.3, F21A20_a, and T3F12.12 are indicated by arrows on the *left*. The faint band in lanes *1* and *2* is partially methylated T3F12.12. Lane *1*, Landsberg *erecta* (Ler); lane *2*, Columbia wild-type (Col-0); lane *3*, Columbia *ddm1*; lane *4*, Landsberg *erecta ddm1*. (*C*) DNA gel blot analysis of individual Columbia *ddm1* plants using *Hin*dIII and probe 1. New bands (arrowheads) are transposed *AtMu1*. (*D*) RT–PCR analysis (+) of transcripts using *AtMu1* primers (top panel) and control RuBisCO primers (bottom panel). Alternate lanes (–) are mock reactions in the absence of reverse transcriptase. RuBisCO lanes *1* and *2* were amplified using Col-0 and Ler genomic DNA.

display extensive polymorphism in different genetic backgrounds, depending on the pattern of transposon insertion. Inbreeding would exacerbate these effects as observed for *ddm1* (Kakutani et al. 1996), whereas mutations in different strains would complement each other, resulting in hybrid vigor (Martienssen 1998).

Table 3. *Summary of transposed* AtMul *elements and insertion sites in* ddm1 *mutant plants.*

ddm1-line	Ecotype	Generation	chr.	Gene nearest to insertion	Accession	Orientation of TIR–MULE	Comments	Target site duplication
4853/7	Col-0	F_3	1	F25P12.97	AC009323.4	5′-3′	hypothetical protein, 222bp upstream	ttaaatctt (9bp)
4853/155	Col-0	F_3	2	T20F21.11	AC006068.3	3′-5′	AP2 domain transcription factor 317bp downstream	aagtttttc (9bp)
4853/48	Col-0	F_3	2	F12K2.19	AC006233.3	3′-5′	unknown protein, 774bp upstream	ttatttaaa (9bp)
4855/4	Col-0	F_5	4	T3H13.5	AF128396.1	3′-5′	retrotransposon 2524bp from HY4	aatttatta (9bp)
4853/6	Col-0	F_3	5	MBM17.4	AB019227.1	3′-5′	protein kinase, 414bp upstream	aagattcct (9bp)
4853/154[b]	Col-0	F_3	5	MRO11.11	AB005244.2	3′-5′	unknown protein 290bp upstream	aagtatcaa (9bp)
4220/11[b]	Col-0	F_6	5	MCA23.3	AB016886.1	N.D.	GTPase activating protein	N.D.[a]
4574/21	La-*er*	F_3	1	F8K4.9	AC004392.1	5′-3′	hypothetical protein 800bp upstream	agtattatt (9bp)
4575/21	La-*er*	F_4	3	MBK21.21	AB024033.1	5′-3′	putative auxin-regulated protein 1423bp upstream	ttttttttt (9bp)
204/7[b]	La-*er*	F_5	3	MFE16.2	AB028611.1	N.D.	Unknown protein, downstream	N.D.[a]

[a]Exact insertion site and orientation of element could not be determined.
[b]Line extinct.

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Material and methods

Plant material

The *ddm1-2* mutation which was used in this study was identified in the Columbia ecotype (Vongs et al. 1993). Homozygous seed (var. Columbia) were obtained by self-pollinating heterozygotes that had been backcrossed for at least eight generations (E. Richards, Washington University). Individual progeny were genotyped by DNA blot analysis (Vongs et al. 1993) and a homozygous mutant selected for self-pollination. Heterozygotes were backcrossed for six generations into Landsberg *erecta* and genotyped by progeny testing before selecting the next backcross. The plants were self-pollinated to obtain homozygotes, and individual F3 to F6 progeny were examined for DNA methylation and transposition.

Informatics

GenBank and TAIR (http://www.arabidopsis.org/blast) databases were searched, most recently in December 1999, using the key words *Mutator*, *MuDR*, and MuRA. The predicted *AtMu1 mudrA*-like transposase T3F12.12 was used to perform TBLASTN (BLOSUM62) and PSI-BLAST (BLOSUM45) searches of plant sequences with two rounds of iteration (Altschul et al. 1997). Gene models of unannotated *Mutator*-like elements were predicted with Genscan (http://CCR-081.mit.edu/GENSCAN.html). TIRs were located by aligning 4 kb of either side of the presumed coding sequence with BLAST2 (Tatusova and Madden 1999). Conserved 100-bp regions from the TIRs of each element were aligned using PILEUP (GCG 10.0, Madison, WI). Distance trees (GrowTree) used the UPGMA and the Tamura algorithms for correction. Parsimony trees were constructed heuristically using PAUP*4.0.0d55 and the maize *MuDR* element at *waxy* (Accession no. M76978) as the outgroup. TreeViewPPC Version 1.6.2 (Page 1996) was obtained at http://taxonomy.zoology.gla.ac.uk/rod/rod.html. CLUSTALW analysis of a conserved 98-aa domain from the MURA transposase (Fig. 1B) was performed using MacVector6.5.1 (Oxford Molecular Group) and the BLOSUM30 matrix.

DNA and RNA analysis

Seedling, leaf, or inflorescence DNA was purified and subjected to DNA gel blot analysis as described by Vongs et al. (1993). Hybridization probes specific for *AtMu1* elements were obtained by amplifying parts of the *MuRA* gene using the following primers: probe 1, 5′-GTCGAGTACAATGGGGGTAAC-3′ and 5′- CAACAGACCCTGGGTTTTGAG-3′; probe 2, 5′-CCGAGAACTGGTTGTGGTTT-3′ and 5′-TGGTGGCTGTCTCATAGCTG-3′.

RNA was isolated from pooled F_4 or F_5 20-d seedlings using Trizol reagent (Life Technologies) and DNAse treated with RQ1 DNAse according to the manufacturer. RT–PCR was performed using the OneStep RT–PCR Kit (Qiagen). RNA quality and concentration were determined by gel analysis and absorbance; 2µg of total RNA was reverse transcribed and then dilutions of the resulting cDNA were amplified using control primers for the *RuBisCO* gene to ensure even loading. RT–PCR was performed using primers from each of the *AtMu* exons. For *AtMu1* these primers were T3F12.12: 5′-CCGAGAACTGGTTGTGGTTT-3′, 5′-GCTCTTGCTTTGGTGATGGT-3′ (spanning intron 1), 5′-CAAGAGCTGTGGTGAAGCTG-3′, 5′-TGCTTGAGAAGGTTGTGTGATG-3′ (spanning intron 2); T11I11.3: 5′-CGCACCACCAGAACCTATTT-3′, 5′-CTTGAGAAGGTTGTGTGATA-3′; T11I11.3 nested: 5′-GAAGCTGGGCATAACGCATTAG-3′, 5′-TCCTTCTTAGAGTTCTTCTCATC-3′. Other primer sequences are available on request. PCR products were directly sequenced using dye terminators on ABI Prism 377 sequencers. The sequences of the RT–PCR products are shown below. Exon borders correspond to the predicted splice sites of *AtMu1*.

Exon borders 1 and 2: Ler WT, 5′-TCTTTCAGACCGCTCAAAG/GGTCTTCTGAGTGCTGTT-3′; *ddm1*(Col), 5′-TCTATCAGATCGCTCAAAG/GGTCTTCTGAGTGCTGTT-3′. Exon borders 2 and 3: Ler WT, 5′-GAATGATGGCAATGATGAG/ATTGAAAAGAAGGCTAAG-3′; *ddm1*(Col), 5′-GAATGATGGCAATGATGAG/ATTGAAAAGAAGGCTAAG-3′. (The splice-site junction is marked with a slash. Exon borders are underlined.)

RT–PCR products were transferred to Nylon N+-membranes (Amersham) and hybridized with genomic probes obtained with the same primer pairs as used for RT–PCR.

Amplification of flanking sequences

Insertion sites were amplified using a modification of the AIMS technique (Frey et al. 1998) and using the following adapter oligonucleotides and primers: Adapter-upper, 5′-GACTCATGCTTACCTAGTCCAGTTGACAGTACCATATG-3′; Adapter-lower, 5′-ATTGGAGTCTGGTATACAT-3′ (phosphorylated at the 5′ end). Adapter primer 1 (AD-AIMS1), 5′-GACTCATGCTTACCTAGTCCAG-3′; Adapter primer 2 (AD-AIMS2), 5′-GACTCATGCTTACCTAGTCCAGTTG-3′. *AtMu1* specific primers: *AtMu1*_TIR150, 5′-GCTTGATTAATGTTGGTTAATTAC-3′ (primer binds both TIRs); *AtMu1*_TIRA1, 5′-GGGTGGAACCCAGTTGAAACAATATAC-3′; *AtMu1*_TIRA2, 5′-TTGAAACAATATACTTGAGGGGGG-3′; *AtMu1*_TIRB1, 5′-GGGTGGAACCCAGTTGAAACAATATAT-3′; *AtMu1*_TIRB2, 5′-TTGAAACAATATATTTGAGGGGGC-3′, (primers bind either TIRA or TIRB). Upper and lower adapters were denatured and annealed in equimolar amounts at room temperature for >4 h. Genomic DNA (100–300 ng) was cut with *Bfa*I and ligated with 50 pmole reconstituted adapter for 1 h at room temperature and 2 h at 37°C. The ligation was purified with spin columns (Qiagen) and one-tenth of the purified reaction was subjected to the PCR, using AD-AIMS1 and the specific *AtMu* primers and using the same cycling conditions as for secondary TAIL PCR (Liu et al. 1995), except that the buffer was supplemented with 3% DMSO. Annealing temperature was varied according to the primer 64°C (*AtMu1*_TIRA1/*AtMu1*_TIRB1) or 55°C (*AtMu1*_TIR150). Products were gel purified or diluted 1/100 before reamplification with the second primer pair, using 35 cycles and a 60°C annealing temperature.

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Note added in proof

While this manuscript was under review, a computational analysis of some of the *Mutator*-like elements in the *Arabidopsis* genome was published (Yu et al. 2000).

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* **25:** 3389–3402.

Banks, J.A., Masson, P., and Fedoroff, N. 1988. Molecular mechanisms in the developmental regulation of the maize *Suppressor-mutator* transposable element. *Genes & Dev.* **2:** 1364–1380.

Barkan, A. and Martienssen, R.A. 1991. Inactivation of maize transposon *Mu* suppresses a mutant phenotype by activating an outward-reading promoter near the end of *Mu1*. *Proc. Natl. Acad. Sci.* **88:** 3502–3506.

Bender, J. and Fink, G.R. 1995. Epigenetic control of an endogenous gene family is revealed by a novel blue fluorescent mutant of Arabidopsis. *Cell* **83:** 725–734.

Benito, M.I. and Walbot, V. 1997. Characterization of the maize *Mutator* transposable element MURA transposase as a DNA-binding protein. *Mol. Cell. Biol.* **17:** 5165–5175.

Bennetzen, J. 1996. The *Mutator* transposable element system of maize. *Curr. Top. Microbiol. Immunol.* **204:** 195–229.

Brink, R.A., Styles, E.D., and Axtell, J.D. 1968. Paramutation: Directed genetic change. Paramutation occurs in somatic cells and heritably alters the functional state of a locus. *Science* **159:** 161–170.

Chandler, V.L. and Walbot, V. 1986. DNA modification of a maize transposable element correlates with loss of activity. *Proc. Natl. Acad. Sci.* **83:** 1767–1771.

Chandler, V.L., Radicella, J.P., Robbins, T.P., Chen, J., and Turks, D. 1989. Two regulatory genes of the maize anthocyanin pathway are homologous: Isolation of *B* utilizing *R* genomic sequences. *Plant Cell.* **1:** 1175–1183.

Chomet, P., Lisch, D., Hardeman, K.J., Chandler, V.L., and Freeling, M. 1991. Identification of a regulatory transposon that controls the *Mutator* transposable element system in maize. *Genetics* **129:** 261–270.

Consortium, C.W.P.A.S. (The Cold Spring Harbor Laboratory, Washington University Genome Sequencing Center, and PE Biosystems *Arabidopsis* Sequencing Consortium). 2000. The complete sequence of a heterochromatic island from a higher eukaryote. *Cell* **100:** 377–386.

Das, L. and Martienssen, R. 1995. Site-selected transposon mutagenesis at the *hcf106* locus in maize. *Plant Cell* **7:** 287–294.

Eisen, J.A., Benito, M.I., and Walbot, V. 1994. Sequence similarity of putative transposases links the maize *Mutator* autonomous element and a group of bacterial insertion sequences. *Nucleic Acids Res.* **22:** 2634–2636.

Fedoroff, N.V. 1999. The *suppressor-mutator* element and the evolutionary riddle of transposons. *Genes Cells* **4:** 11–19.

Fransz, P.F., Armstrong, S., de Jong, J.H., Parnell, L.D., van Drunen, C., Dean, C., Zabel, P., Bisseling, T., and Jones, G.H. 2000. Integrated cytogenetic map of chromosome arm 4S of *A. thaliana*: Structural organization of heterochromatic knob and centromere region. *Cell* **100:** 367–376.

Frey, M., Stettner, C., and Gierl, A. 1998. A general method for gene isolation in tagging approaches: Amplification of insertion mutagenised sites (AIMS). *Plant J.* **13:** 717–721.

Gibbons, R.J., McDowell, T.L., Raman, S., O'Rourke, D.M., Garrick, D., Ayyub, H., and Higgs, D.R. 2000. Mutations in *ATRX*, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation. *Nat. Genet.* **24:** 368–371.

Hershberger, R.J., Benito, M.I., Hardeman, K.J., Warren, C., Chandler, V.L., and Walbot, V. 1995. Characterization of the major transcripts encoded by the regulatory *MuDR* transposable element of maize. *Genetics* **140:** 1087–1098.

Hirochika, H., Okamoto, H., and Kakutani, T. 2000. Silencing of retrotransposons in *Arabidopsis* and reactivation by the ddm1 mutation. *Plant Cell* **12:** 357–369.

Jeddeloh, J.A., Bender, J., and Richards, E.J. 1998. The DNA methylation locus DDM1 is required for maintenance of gene silencing in *Arabidopsis*. *Genes & Dev.* **12:** 1714–1725.

Jeddeloh, J.A., Stokes, T.L., and Richards, E.J. 1999. Maintenance of genomic methylation requires a SWI2/SNF2-like protein. *Nat. Genet.* **22:** 94–97.

Kakutani, T., Jeddeloh, J.A., Flowers, S.K., Munakata, K., and Richards, E.J. 1996. Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. *Proc. Natl. Acad. Sci.* **93:** 12406–12411.

Kakutani, T., Munakata, K., Richards, E.J., and Hirochika, H. 1999. Meiotically and mitotically stable inheritance of DNA hypomethylation induced by *ddm1* mutation of *Arabidopsis thaliana*. *Genetics* **151:** 831–838.

Kermicle, J.L. 1996. Epigenetic silencing and activation of a maize *r* gene. In *Epigenetic mechanisms of gene regulation* (ed. V.E.A. Russo et al.), pp. 267–289. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kunze, R. and Starlinger, P. 1989. The putative transposase of transposable element Ac from *Zea mays* L. interacts with subterminal sequences of *Ac. EMBO J.* **8:** 3177–3185.

Le, Q.H., Wright, S., Yu, Z., and Bureau, T. 2000. Transposon diversity in *Arabidopsis thaliana. Proc. Natl. Acad. Sci.* **97:** 7376–7381.

Lin, X., Kaul, S., Rounsley, S., Shea, T.P., Benito, M.I., Town, C.D., Fujii, C.Y., Mason, T., Bowman, C.L., Barnstead, M., et al. 1999. Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana. Nature* **402:** 761–768.

Lisch, D., Chomet, P., and Freeling, M. 1995. Genetic characterization of the *Mutator* system in maize: Behavior and regulation of *Mu* transposons in a minimal line. *Genetics* **139:** 1777–1796.

Lisch, D., Girard, L., Donlin, M., and Freeling, M. 1999. Functional analysis of deletion derivatives of the maize transposon *MuDR* delineates roles for the MURA and MURB proteins. *Genetics* **151:** 331–341.

Liu, Y.G., Mitsukawa, N., Oosumi, T., and Whittier, R.F. 1995. Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR. *Plant J.* **8:** 457–463.

Martienssen, R. 1996. Epigenetic phenomena: Paramutation and gene silencing in plants. *Curr. Biol.* **6:** 810–813.

———. 1998. Chromosomal imprinting in plants. *Curr. Opin. Genet. Dev.* **8:** 240–244.

Martienssen, R. and Baron, A. 1994. Coordinate suppression of mutations caused by *Robertson's Mutator* transposons in maize. *Genetics* **136:** 1157–1170.

Martienssen, R.A. and Richards, E.J. 1995. DNA methylation in eukaryotes. *Curr. Opin. Genet. Dev.* **5:** 234–242.

Martienssen, R. and Henikoff, S. 1999. The House & Garden guide to chromatin remodelling. *Nat. Genet.* **22:** 6–7.

Martienssen, R., Barkan, A., Taylor, W.C., and Freeling, M. 1990. Somatically heritable switches in the DNA modification of *Mu* transposable elements monitored with a suppressible mutant in maize. *Genes & Dev.* **4:** 331–343.

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Matzke, A.J. and Matzke, M.A. 1998. Position effects and epigenetic silencing of plant transgenes. *Curr. Opin. Plant Biol.* **1:** 142–148.

Mayer, K., Schuller, C., Wambutt, R., Murphy, G., Volckaert, G., Pohl, T., Dusterhoft, A., Stiekema, W., Entian, K.D., Terryn, N., et al. 1999. Sequence and analysis of chromosome 4 of the plant *Arabidopsis thaliana. Nature* **402:** 769–777.

McClintock, B. 1951. Chromosome organization and genic expression. *Cold Spring Harbor Symp. Quant. Biol.* **16:** 13–47.

———. 1965. The control of gene action in maize. *Brookhaven Symp. Biol.* **18:** 162–184.

———. 1984. The significance of responses of the genome to challenge. *Science* **226:** 792–801.

Morgan, H.D., Sutherland, H.G., Martin, D.I., and Whitelaw, E. 1999. Epigenetic inheritance at the *agouti* locus in the mouse. *Nat. Genet.* **23:** 314–318.

Page, R.D. 1996. TreeView: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* **12:** 357–358.

Paszkowski, J. and Mittelsten Scheid, O. 1998. Plant genes: The genetics of epigenetics. *Curr. Biol.* **8:** 206–208.

Rabinowicz, P., Schutz, K., Dedhia, N., Yordan, C., Parnell, L., Stein, L., McCombie, W., and Martienssen, R. 1999. Differential methylation of genes and retrotransposons allows shotgun sequencing of the maize genome. *Nat. Genet.* **23:** 305–308.

Raizada, M.N. and Walbot, V. 2000. The late developmental pattern of *Mu* transposon excision is conferred by a cauliflower mosaic virus 35S-driven *MURA* cDNA in transgenic maize. *Plant Cell* **12:** 5–21.

SanMiguel, P., Gaut, B.S., Tikhonov, A., Nakajima, Y., and Bennetzen, J.L. 1998. The paleontology of intergene retrotransposons of maize. *Nat. Genet.* **20:** 43–45.

Settles, A.M., Baron, A., Barkan, A., and Martienssen, R.A. 2001. Duplication and suppression of chloroplast protein translocation genes in maize. *Genetics* **157:** 349–360.

Steimer, A., Amedeo, P., Afsar, K., Fransz, P., Scheid, O.M., and Paszkowski, J. 2000. Endogenous targets of transcriptional gene silencing in *Arabidopsis. Plant Cell* **12:** 1165–1178.

Sundaresan, V. and Freeling, M. 1987. An extrachromosomal form of the *Mu* transposons of maize. *Proc. Natl. Acad. Sci.* **84:** 4924–4928.

Tatusova, T.A. and Madden, T.L. 1999. BLAST 2 Sequences, a new tool for comparing protein and nucleotide sequences. *FEMS Microbiol. Lett.* **174:** 247–250.

Taylor, L.P. and Walbot, V. 1985. A deletion adjacent to the maize transposable element *Mu-1* accompanies loss of *Adh1* expression. *EMBO J.* **4:** 869–876.

Vongs, A., Kakutani, T., Martienssen, R.A., and Richards, E.J. 1993. *Arabidopsis thaliana* DNA methylation mutants. *Science* **260:** 1926–1928.

Walsh, C.P. and Bestor, T.H. 1999. Cytosine methylation and mammalian development. *Genes & Dev.* **13:** 26–34.

Yu, Z., Wright, S.I., and Bureau, T.E. 2000. *Mutator*-like elements in *Arabidopsis thaliana*: Structure, diversity and evolution. *Genetics* **156:** 2019–2031.