Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2219–2223, April 1989
Botany

Mobility of the maize Suppressor–mutator element in transgenic tobacco cells

(transposition/*Agrobacterium* transformation/β-glucuronidase/hygromycin phosphotransferase)

PATRICK MASSON AND NINA V. FEDOROFF

Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, MD 21210

Communicated by Oliver E. Nelson, December 19, 1988

ABSTRACT **Maize Suppressor–mutator (*Spm*) transposable elements have been introduced into tobacco cells and a visual assay for *Spm* activity has been developed using a bacterial β-glucuronidase gene. The *Spm* element is mobile in tobacco and can trans-activate excision of a transposition-defective *Spm* (*dSpm*) element either from a different site on the same transforming Ti plasmid or from a second plasmid. An *Spm* element expressed from the stronger cauliflower mosaic virus 35S promoter trans-activates transposition of a *dSpm* element earlier after its introduction into tobacco cells than an element expressed from its own promoter.**

The Suppressor–mutator family consists of a transposition-competent *Spm* element and mutant elements that are either altered or defective in their ability to transpose (1, 2). The *Spm* element was independently isolated by McClintock (3) and by Peterson (4), who designated it the Enhancer (*En*) element. The standard *Spm* (*Spm-s*) and *En* elements are virtually identical, 8.3-kilobase (kb) elements with 13-base-pair (bp) terminal inverted repetitions. The element encodes one moderately abundant 2.5-kb transcript, as well as one or two less abundant large transcripts (5–7). Studies on *Spm* mutations in maize have provided evidence that *Spm* encodes a gene product required for transposition (transposase; originally designated the "mutator" function) and a positive autoregulatory gene product (6–14).

Further progress in understanding the *Spm* element depends on the development of a system in which its functions can be altered by *in vitro* mutagenesis. Using a strategy devised for the Activator (*Ac*) element (15–17), we have constructed *Agrobacterium* Ti plasmids that contain an *Spm* element between the cauliflower mosaic virus (CaMV) 35S promoter and a bacterial reporter gene. We have developed a visual assay for *Spm* by using a bacterial β-glucuronidase (GUS) reporter gene. We show that the *Spm* element transposes and trans-activates *dSpm* excision in tobacco.

MATERIALS AND METHODS

Construction of Test Plasmids. The plasmids used to monitor the mobility of *Spm* in tobacco contain either the bacterial hygromycin phosphotransferase (hph, ref. 18) or GUS (19) reporter genes expressed from the CaMV 35S promoter (20). Expression of the reporter gene was disrupted by inserting an *Spm-s* or *dSpm* element between the promoter and the open reading frame (ORF) at a unique *Xho* I site, designated the excision assay site (Fig. 1*a*). The test plasmids were derived from the *Escherichia coli–Agrobacterium* shuttle vector pMON530 (S. Rogers, Monsanto), which contains the Tn*5* neomycin phosphotransferase II gene as the plant cell transformation marker (20). The test plasmids are designated p\hph and p\gus. The symbol "\" designates the excision assay site (Fig. 1).

The p\hph plasmid was constructed by inserting a *Sma* I fragment containing the hph ORF, excised from the pMAP1004 plasmid (Monsanto), into an *Eco*RI site of pMON530 downstream of a CaMV 35S promoter (18). A *Sma* I site (site 1; Fig. 1) was substituted for the plasmid's *Stu* I site. The p\gus plasmid was derived from the p\hph plasmid by replacing the 5′ terminal portion of the hph gene with the GUS ORF of the pBI121 plasmid (Clontech).

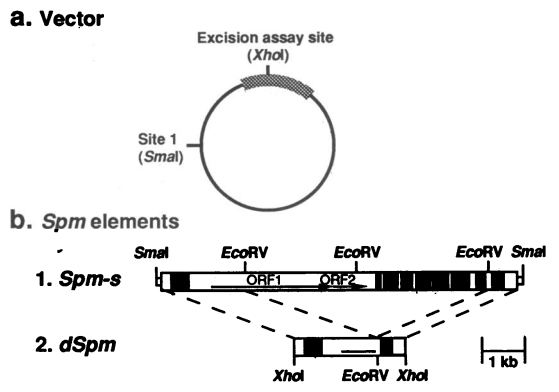
The sequences flanking the *Spm* element cloned from the *a-m2-7991A1* allele of the *a* gene (6) were reduced to 42 and 68 bp on the 5′ and 3′ sides of the element, respectively, by subcloning the 5′ end of the element in the pEMBL19 plasmid (6) and removing the flanking *a* locus sequence by exonuclease III digestion (21). A *Bam*HI fragment containing the 3′ end of the element and 68 bp of flanking *a* locus sequence extending to the adjacent *Sma* I site was reinserted into this plasmid, reassembling the complete element with *Sma* I sites on both sides. An *Spm* element in which the promoter region was replaced by a CaMV promoter (*35S-Spm*) was constructed by replacing the 5′ end of the element to the first *Sal* I site by the CaMV 35S promoter derived from a plasmid obtained from M. Fromm (22). The *dSpm* element was derived from the *Spm* element by removing the two internal *Eco*RV fragments (Fig. 1) and further trimming the flanking *a* gene sequences, as described above, so that the *dSpm* element was flanked by an *Xho* I site on the 3′ side and only 3 bp of the flanking gene sequence remained between the element and an *Xho* I site on the 5′ side.

The final test plasmids were constructed by inserting the *Spm* and *dSpm* elements described above in the excision assay site and site 1 of the vector (Fig. 1*c*). The first element in the plasmid designation is the trans-acting element in site 1, while the element immediately preceding the "\" symbol is in the excision assay site. In some cases, the *Spm-s* element was on a separate plasmid (pMON754, Monsanto) that carried the hph gene as the transformation marker. Either the *Spm-s* or *35S-Spm* element was inserted at its *Stu* I site to obtain the pSpm-s and p35S-Spm plasmids, respectively.

Tobacco Transformation. The test plasmids were transferred to *Agrobacterium* strains containing a second plasmid carrying the *vir* genes as described by Rogers *et al.* (23). The structure of each test plasmid was verified by blot hybridization analysis. Leaf fragments from axenically grown *Nicotiana tabacum* SR1 were infected with *Agrobacterium* strains carrying the test plasmids as described by Rogers *et al.* (23), then transferred to a "shooting" medium containing carbenicillin at 500 μg/ml and the selective drug 4–6 days after transformation (23). Cells were cotransformed with plasmids carrying the neomycin phosphotransferase II and hph genes by co-infecting leaf fragments with two *Agrobac-*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CaMV, cauliflower mosaic virus; ORF, open reading frame; GUS, β-glucuronidase; hph, hygromycin phosphotransferase.



c. Plasmids

Plasmid	Reporter gene	Site 1	Excision assay site	Transformation marker
p\hph	hph	-	-	NPT II
pdSpm\hph	hph	-	*dSpm*	NPT II
pSpm-s:dSpm\hph	hph	*Spm-s*	*dSpm*	NPT II
p\gus	GUS	-	-	NPT II
pdSpm\gus	GUS	-	*dSpm*	NPT II
pSpm-s\gus	GUS	-	*Spm-s*	NPT II
pSpm-s	-	*Spm-s*	-	hph
p35S-Spm	-	35S-*Spm*	-	hph

FIG. 1. Structure of the test plasmids and *Spm* elements used to assess the mobility of *Spm* in tobacco. (*a*) *Spm* elements (*b*) were inserted either in site 1 or in the excision assay site of the vector. The vector is a derivative of the pMON530 plasmid (thin line) and contains either the bacterial GUS or hph reporter gene expressed from a CaMV 35S promoter (thick line). The excision assay site is a single *Xho* I site located in the 5′ untranslated region of the reporter gene. Site 1 is a unique *Sma* I site. (*b*) *Spm-s* and *dSpm* elements are represented by open boxes; the exons of the major transcript (5) are represented by solid boxes. The ORFs in the largest intron are represented by arrows. The maize *a* gene sequences flanking the *Spm-s* element are represented by small open boxes. (*c*) Designations of the plasmids used in the present experiments.

terium strains, each carrying one of the plasmids, then selecting on medium containing both kanamycin (250 μg/ml) and hygromycin (20 μg/ml). When the hph gene was used to test excision, infected leaf fragments were transferred to shooting medium containing both kanamycin and hygromycin. When the GUS gene was used to test for *Spm* excision, transformed calli were grown on kanamycin-containing medium. Pieces of calli or regenerating shoots were treated with 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid (X-glu; Clontech), which is converted to an insoluble blue product by GUS (19).

Transient Gene Expression Assay. A derivative was made of the GUS plasmid pBI121 in which the CaMV 35S promoter was replaced by a fragment of the 5′ end of the *Spm* element extending to the first *Dde* I site at nucleotide 573 (5, 6). Both plasmids were further modified by introducing a bacterial chloramphenicol transacetylase (CAT) gene expressed from a CaMV 35S promoter as an internal marker for transformation efficiency. The resulting plasmids are designated p35S/gus-cat and pSpmp/gus-cat. SR1 tobacco protoplasts were prepared from axenically grown leaves and transformed with the plasmids either as described in ref. 24 or by electroporation (BRL Cellporator, as recommended by the manufacturer). Cell homogenates were assayed 2 days after transformation as described in ref. 19 for GUS and in ref. 25 for CAT. The level of GUS activity was normalized to the CAT activity in each sample.

Analysis of Genomic DNA. DNA was extracted from transformed calli or plants as described in ref. 26, with the addition of a CsCl gradient purification step in some cases. DNA was digested with restriction enzymes as described in ref. 27, fractionated on 0.7% agarose gels, transferred to GeneScreen*Plus* membranes (DuPont), and hybridized to the gel-purified probe fragments labeled with ^{32}P by the random-primer method, as described by the supplier (Amersham).

To determine the sequence of the empty donor site, genomic DNA fragments of the appropriate length to contain an empty donor site were gel-purified from a *Bam*HI digest of genomic DNA. Using appropriate oligonucleotides flanking the putative empty donor site, the fragments were subjected to 30 cycles of polymerase chain reaction amplification, using the GeneAmp kit (Perkin–Elmer/Cetus) under the conditions specified by the supplier (28). The oligonucleotides used to prime the polymerase chain reaction were complementary to 25-bp and 24-bp sequences, respectively, of the p\hph plasmid and located about 130 bp apart, flanking the original *Spm-s* insertion site on the pSpm:dSpm\hph plasmid. The amplified fragments were cloned in a Bluescript plasmid (Stratagene) and sequenced using the Sequenase kit (United States Biochemical), as recommended by the supplier.

RESULTS

The *Spm* Element Is Mobile in Tobacco and Trans-Activates Excision of a *dSpm* Element. Two Ti plasmids were used in initial experiments to assess the mobility of the maize *Spm* element in tobacco. Both had an hph gene whose expression had been disrupted by the insertion of a *dSpm* element at the excision assay site (pdSpm\hph). One plasmid had a second, mobilizing *Spm-s* element (pSpm-s:dSpm\hph), while the second, control plasmid did not. Both were constructed in such a way that the excision of the *dSpm* element would restore expression of the hph gene and confer hygromycin resistance on the tobacco transformants (Fig. 1). However, few calli were obtained with either plasmid if leaf fragments were subjected to hygromycin selection immediately after transformation. When selection commenced 1 month after transformation, 57% and 71% of the calli transformed with the pdSpm\hph and pSpm-s:dSpm\hph plasmids, respectively, grew on hygromycin (data not shown).

Nonetheless, evidence that the *Spm-s* element is mobile in tobacco was obtained by analyzing DNA isolated from transformed tobacco tissue. Genomic DNA digests were probed with a fragment of the Ti plasmid sequence adjacent to the site at which the *Spm-s* element was inserted, as illustrated in Fig. 2*a*. DNA samples isolated from cells transformed with the plasmid that contained no *Spm-s* element exhibited only the expected 1.8-kb fragment homologous to the probe (Fig. 2*b*, lane 1). All but one of the DNAs extracted from plant tissue transformed with the pSpm-s:dSpm\hph plasmid showed the 7.1-kb fragment expected if the *Spm-s* element is at its original insertion site on the plasmid (Fig. 2*b*). In addition, five of the six DNA samples contained a 1.9-kb *Bam*HI–*Bst*EII fragment homologous to the probe. This is the length expected if the *Spm-s* element has excised leaving the original 0.11-kb *a* gene sequence that flanked the element on the input Ti plasmid (Fig. 2*b*, lanes 2, 3, 4, 6, and 7).

To prove that the 1.9-kb fragment was that anticipated if the *Spm-s* element transposed, DNA fragments of the appropriate size were gel-purified from the genomic DNA sample whose analysis is shown in Fig. 2, lane 7. The expected empty donor site sequences were amplified by the polymerase chain reaction, using oligonucleotides homologous to sequences flanking the insertion site, and cloned (28). All but 1 of the 16 empty donor site clones analyzed had the nucleotide sequence expected for the corresponding T-DNA fragment from which the *Spm-s* element had excised, including the expected Ti plasmid sequence and the 0.11 kb of flanking maize *a* gene sequence. Although the *Spm* element is known to generate a 3-bp duplication upon insertion, the *a*

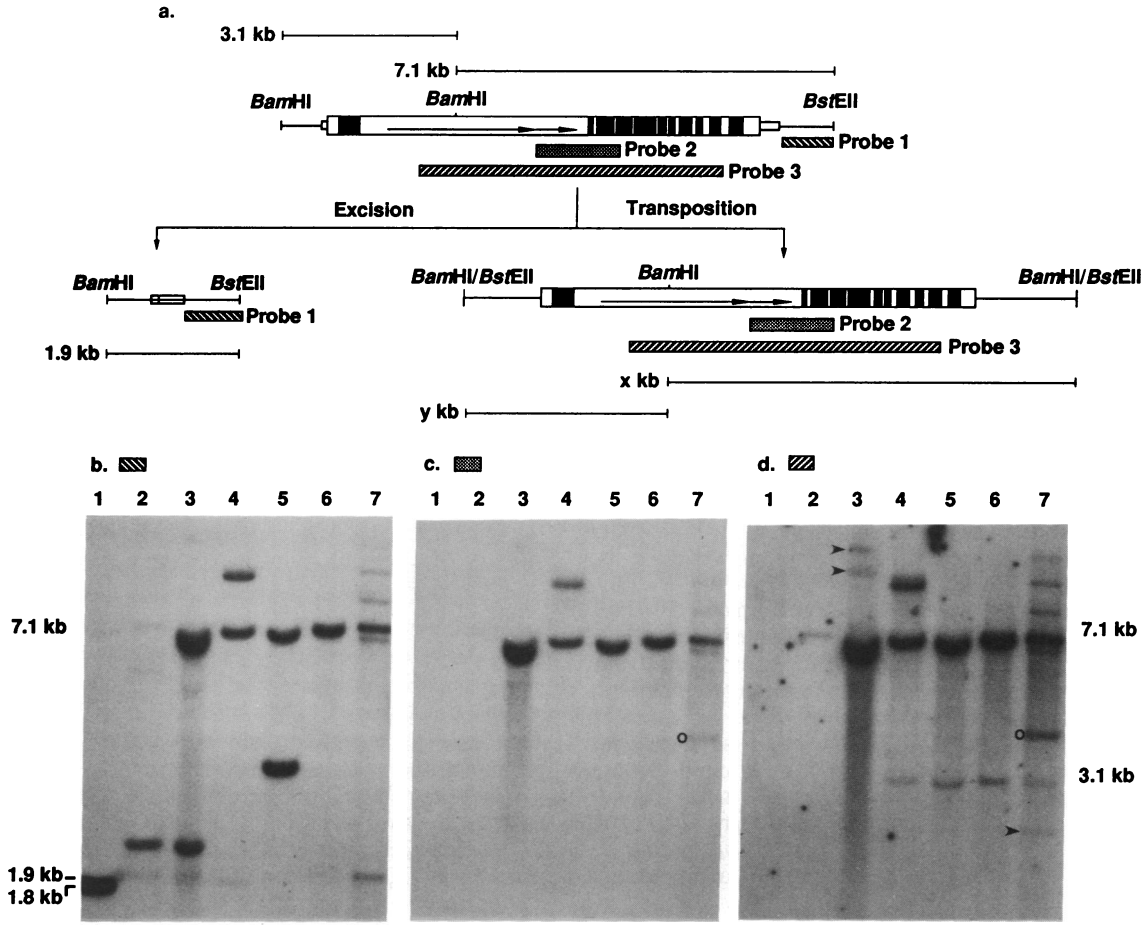


FIG. 2. Blot hybridization analysis of DNAs extracted from transgenic plants transformed with either the pSpm-s:dSpm\hph or the pdSpm\hph plasmid. (*a*) (*Upper*) Structure of the *Spm-s* element and its flanking sequences located at site 1 of the pSpm-s:dSpm\hph plasmid. (*Lower left*) Structures of the empty donor fragment expected if the *Spm-s* element has excised. It consists of the 1.8-kb *Bam*HI–*Bst*EII fragment into which the element was inserted and 0.11 kb of *a* gene sequence that originally flanked the element and remains upon excision. The control pdSpm\hph plasmid has no insertion at site 1 and is, therefore, expected to yield a 1.8-kb *Bam*HI–*Bst*EII fragment. (*Lower right*) Expected structure of a transposed copy of *Spm*. The flanking Ti plasmid (*Upper* and *lower left*) or the *Spm-s* elements are represented as described in the legend to Fig. 1. The sequences used as probes are represented by various patterned boxes. In each part of the diagram, the *Bam*HI and *Bst*EII cleavage sites are shown, as are the sizes of the *Bam*HI or *Bam*HI–*Bst*EII fragments expected to hybridize with the probes. (*b–d*) Results of the blot hybridization analyses of genomic DNA from transformed tobacco. The same genomic blot of *Bam*HI- and *Bst*EII-digested DNAs extracted from plants transformed with pdSpm\hph (lane 1) or with pSpm-s:dSpm\hph (lanes 2–7) was successively hybridized to probes 1 (*b*), 2 (*c*), and 3 (*d*). DNA fragments hybridizing to probe 3, but not to probes 1 and 2, are indicated by arrowheads, whereas the fragments hybridizing to probes 2 and 3, but not probe 1, are indicated by a circle.

gene allele from which the present element was cloned has a 3-bp insertion adjacent to one end of the element (6). Of the 15 empty donor site sequences obtained, 2 resulted from precise excision of the *Spm* element, 6 had a 1-bp deletion in the left flanking sequence, and 7 had a 1-bp insertion at the same position (Table 1). The last sequence may also have been derived by an inverted duplication of two nucleotides of the right flanking sequence and a 1-bp deletion in the left flanking sequence.

To determine whether excised *Spm* elements transposed to new sites, genomic DNA digests were examined for novel restriction fragments homologous to internal *Spm* sequences, but not to the Ti plasmid sequences that initially flanked the element. The internal *Spm* probe fragments used for this purpose were not represented in the internally deleted *dSpm* element on the same Ti plasmid and were, therefore, specific for the *Spm-s* element (Fig. 2*a*). Both Spm probes detect novel fragments of the expected type (Fig. 2 *c* and *d*). However, there are fragments that can be detected with probe 3, but not with the central probe 2 (Fig. 2*d*, lanes 3 and 7, arrowheads), and the novel fragment detected with both probes 2 and 3 is shorter than anticipated for an intact *Spm* element (Fig. 2 *c* and *d*, lane 7, circle). These observations suggest that the transposed *Spm* elements have deletions.

Similar experiments were done to determine whether the *Spm-s* element is capable of trans-activating excision of the *dSpm* element on the same input plasmid and yielded similar results. That is, empty excision sites were detected only in DNA from plants transformed with the pSpm-s:dSpm\hph plasmid (data not shown).

A Visual Assay for *Spm* Activity. To determine whether the inability to detect *dSpm* excision by the acquisition of a hygromycin-resistant phenotype is due to late or infrequent excision, a visual assay capable of detecting expression of the reporter gene in small numbers of cells was developed using the bacterial GUS gene (Fig. 1). Calli and plantlets transformed with GUS test plasmids were assayed for cells expressing the GUS gene. About 10% of calli containing a Ti plasmid with an *Spm-s* element at the excision assay site (pSpm-s\gus) exhibited blue GUS-positive sectors (Table 2). About half of the calli from cells cotransformed with the pdSpm\gus plasmid and an pSpm-s plasmid had GUS-positive sectors. The GUS-positive sectors were almost always small and few, in both calli and plantlets (Fig. 3 *c* and

Table 1. Nucleotide sequences of empty donor sites

Source	Sequence	Sites, no.*
Original sequence	...TTTGAATATT–*Spm*-AATGGA...	
Empty donor fragments	...TTTGAATATT AATGGA...	2
	...TTTGAATATT ATGGA...	6
	...TTTGAATAT TTAATGGA...	7

*Number of cloned empty donor sites having the sequence.

e). By contrast, 100% of the calli obtained from cells transformed with an undisrupted GUS gene expressed from the CaMV 35S promoter were GUS-positive (Fig. 3*a*).

A plant transformed with the pdSpm\gus plasmid and showing no evidence of GUS activity (Fig. 3*b*) was retransformed with a Ti plasmid carrying the *Spm-s* element (Fig. 1). Seven of eight calli obtained upon retransformation with the pSpm-s plasmid gave GUS-positive sectors, whereas none of the six calli retransformed with the same plasmid lacking the *Spm-s* element showed evidence of GUS activity (data not shown). An empty excision site fragment was detected in DNA extracted from a callus with GUS-positive sectors, but not in DNA extracted from calli retransformed with the control Ti plasmid lacking the *Spm-s* element (data not shown). We conclude that GUS-positive sectors are attributable to the trans-activation of *dSpm* excision promoted by an *Spm*-encoded gene product.

The Timing of *Spm-s*-Activated *dSpm* Excision Is Determined by an Element-Encoded Gene Product. The strength of the *Spm-s* element promoter (5, 6) was compared in a transient protoplast assay with that of the CaMV 35S promoter and found to be 7–10 times weaker (Fig. 4). The *Spm* promoter was replaced by the CaMV 35S promoter and a Ti plasmid carrying the resulting p35S-Spm element was cotransformed into tobacco cells with the pdSpm\gus test plasmid. The percentage of calli showing GUS-positive sectors was similar for the transformants containing the *35S-Spm* promoter and transformants containing the unaltered *Spm-s* (Table 2). However, calli and plantlets containing *35S-Spm* gave large GUS-positive sectors more frequently than did those containing an unaltered *Spm-s* element (Fig. 3 *d* and *f*). Expression of the element from the stronger promoter, therefore, permitted excision of the *dSpm* element earlier in development of the callus and plant.

Table 2. Percentage of calli showing evidence of *Spm-s* and *dSpm* excision

Test plasmid	Plasmid with trans-acting *Spm*	Time after transformation, month(s)	Excision frequency, %	Calli analyzed, no.
pSpm-s\gus	—	1	7	71
pdSpm\gus	pMON754	1	0*	57
		2	0	25
pdSpm\gus	pSpm-s	1	52	31
		2	56	15
pdSpm\gus	p35S-Spm	1	39	65
		2	55	39

The percentage of calli showing GUS-positive sectors was determined 1 or 2 months after transformation.
*Seven calli cotransformed with both pdSpm\gus and pMON754 (negative controls) showed a single blue sector. Calli showing *dSpm* excision (Fig. 3) have many blue sectors on each tissue fragment.

DISCUSSION

***Spm* Element Can Both Excise and Trans-Activate Excision in Transgenic Tobacco Cells.** We have presented molecular evidence that the *Spm* element can promote its own excision, as well as that of a *dSpm* element, either on the same Ti plasmid or a different one. The detection of 3 empty donor site sequences among 15 analyzed in DNA from a single transformant implies that the element had been excised independently in several cell lineages. All three of the empty donor site sequences are among the types observed after excision of the element in maize (29). The element therefore appears to use the same transposition mechanism in both maize and tobacco. The finding that an internally deleted, transposition-defective element cannot move in tobacco, except in the presence of a trans-acting *Spm-s* element,

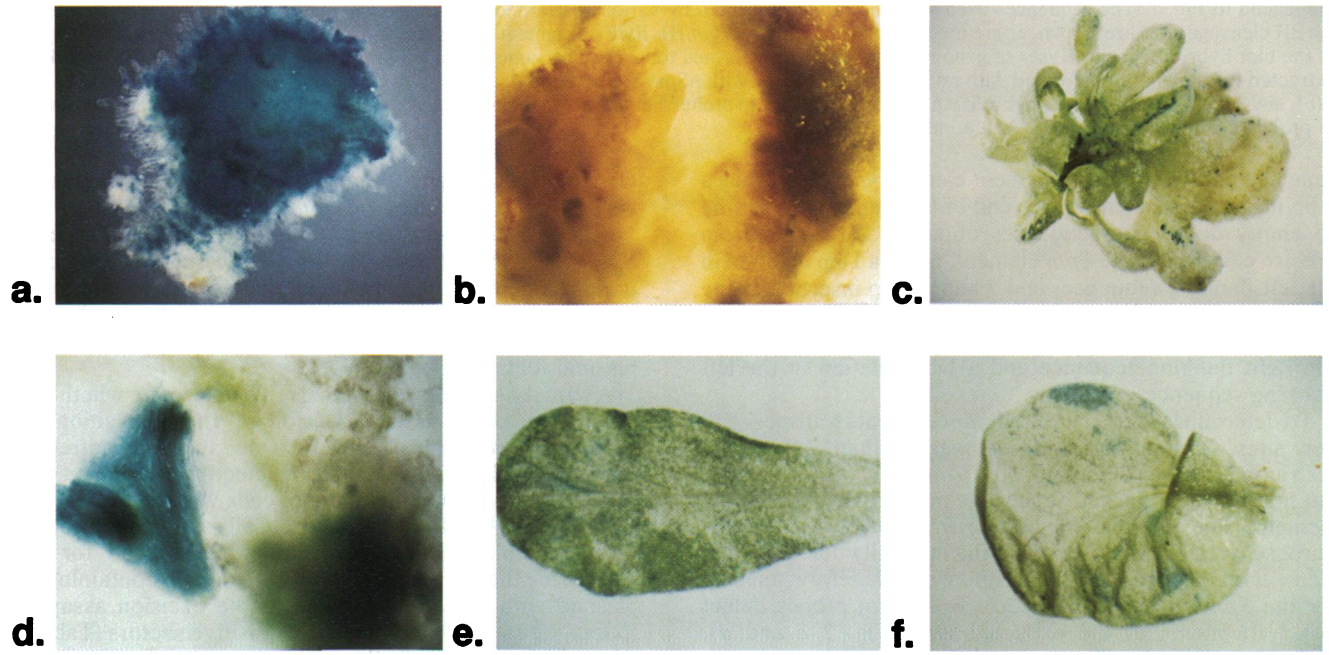


FIG. 3. Detection of sectors of cells expressing the GUS gene in transgenic plant tissues. Tobacco calli or regenerating tissues, transformed with p\gus (*a*), pdSpm\gus (*b*), pdSpm\gus + pSpm-s (*c* and *e*), or pdSpm\gus + p35S-Spm (*d* and *f*) after incubation with 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid to detect cells expressing the GUS gene.

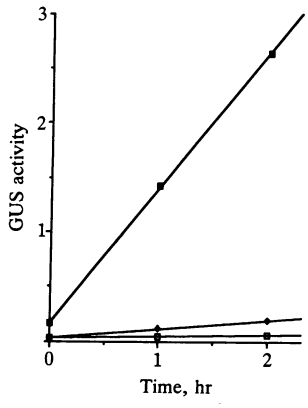


FIG. 4. Expression of the GUS gene from the *Spm* and CaMV 35S promoters. The level of GUS activity was determined in protein extracts prepared from protoplasts 2 days after transformation with p35S/gus-cat (■), pSpmp/gus-cat (◆), or no plasmid DNA (□). One unit of GUS-activity corresponds to the production of 4-methyl-umbelliferone to a concentration of 3.8 μM in extracts prepared from 5 × 10^4 transformed protoplasts under the conditions described in ref. 19.

demonstrates that the mobility of the element requires an element-encoded gene product. It follows that the element's transposase is expressed and active in tobacco, as is that of the maize *Ac* element (15, 17).

We have consistently observed that the *Spm-s* element excises less frequently from the excision site of a test Ti plasmid than from site 1 (Table 2; unpublished data). We do not understand the reason for the element's low mobility in the excision assay site, especially in view of the high excision frequency of the *Ac* element from comparable Ti plasmids (17). The feature that distinguishes the excision site from site 1 is the proximity of the CaMV 35S promoter. It may be that transcription from a strong outside promoter interferes with correct element transcription or produces transcripts that are processed incorrectly (30–32).

The small size of GUS-positive sectors in calli and plantlets transformed with the GUS test plasmids and the low frequency of hygromycin-resistant calli after transformation with hph test plasmids suggest that excision does not commence immediately after the *Spm* element is introduced into tobacco cells. The observation that early excision events that give rise to large GUS-positive cell clones are more frequent when the *Spm* promoter is replaced with the CaMV 35S promoter implies that transposition timing is determined by the concentration of an element-encoded gene product.

***Spm-s* Transposition in Tobacco may be Accompanied by the Production of Intraelement Deletions.** Although we have not isolated transposed *Spm* elements from tobacco, the appearance of new genomic restriction endonuclease fragments with homology to the element, but not the original sequences flanking the element in the transforming Ti plasmid, makes it likely that the element is not only excising, but also transposing in the transgenic tobacco cells. However, there are indications that at least some newly transposed elements are internally deleted (Fig. 2). Since it is evident from the GUS-based excision assay that there are multiple late excision events, it is difficult to assess the relative frequency of transposition events that result in the production of defective elements. However, it is well known that the *Spm* elements frequently mutate by self-induced internal deletions in maize (6, 9–13, 33).

The GUS-Based Visual Excision Assay Permits Rapid Assessment of *Spm* Mobility in Transgenic Tobacco. Our assay for *dSpm* excision, based on the cytochemical detection of cell clones expressing the GUS gene after excision of a *dSpm* element from its promoter region, provides a rapid means of assessing *Spm* function. GUS-positive clones can be reliably detected in small fragments of transformed callus tissue and the assays can be performed within weeks of introducing the trans-activating *Spm* element. Moreover, when the recipient tobacco plants used for transformation already contain the GUS excision assay plasmid, the frequency of calli exhibiting GUS-positive sectors is commonly >50%, making the assay a reliable one for analyzing *Spm* elements altered by *in vitro* mutagenesis.

We thank Dr. J. Banks for fruitful discussions, K. Toohey and G. Rutherford for excellent technical assistance, and Drs. H. Kazazian and L. Dawling for assistance with the polymerase chain reaction amplification of genomic DNA fragments. This work was supported by Grant 5-RO1-GM34296-02 from the National Institutes of Health. P.M. was supported by fellowships from the Fonds National de la Recherche Scientifique of Belgium and the Lucille P. Markey Charitable Trust.

1. McClintock, B. (1965) *Brookhaven Symp. Biol.* **18,** 162–184.
2. Fedoroff, N. (1983) in *Mobile Genetic Elements*, ed. Shapiro, J. (Academic, New York), pp. 1–54.
3. McClintock, B. (1954) *Carnegie Inst. Washington Yearb.* **53,** 254–260.
4. Peterson, P. (1953) *Genetics* **38,** 682–683.
5. Pereira, A., Cuypers, H., Gierl, A., Schwarz-Sommer, Zs. & Saedler, H. (1986) *EMBO J.* **5,** 835–841.
6. Masson, P., Surosky, R., Kingsbury, J. & Fedoroff, N. (1987) *Genetics* **177,** 117–137.
7. Banks, J., Masson, P. & Fedoroff, N. (1988) *Genes Dev.* **2,** 1364–1380.
8. Fedoroff, N. & Banks, J. (1988) *Genetics* **120,** 559–577.
9. Schwarz-Sommer, Zs., Gierl, A., Klosgen, R. B., Wienand, U., Peterson, P. & Saedler, H. (1984) *EMBO J.* **3,** 1021–1028.
10. Schwarz-Sommer, Zs., Gierl, A., Berndtgen, R. & Saedler, H. (1985) *EMBO J.* **4,** 2439–2443.
11. Gierl, A., Schwarz-Sommer, Zs. & Saedler, H. (1985) *EMBO J.* **4,** 579–583.
12. Schiefelbein, J. W., Raboy, V., Fedoroff, N. & Nelson, O. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4783–4787.
13. Tacke, E., Schwarz-Sommer, Zs., Peterson, P. A. & Saedler, H. (1986) *Maydica* **31,** 83–91.
14. McClintock, B. (1957) *Carnegie Inst. Washington Yearb.* **56,** 393–401.
15. Baker, B., Schell, J., Lorz, H. & Fedoroff, N. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4844–4848.
16. Van Sluys, M., Tempé, J. & Fedoroff, N. (1987) *EMBO J.* **6,** 3881–3889.
17. Baker, B., Coupland, G., Fedoroff, N., Starlinger, P. & Schell, J. (1987) *EMBO J.* **6,** 1547–1554.
18. Lloyd, A. M., Barnason, A. R., Rogers, S. G., Byrne, M. C., Fraley, R. T. & Horsch, R. B. (1986) *Science* **234,** 464–466.
19. Jefferson, R. A., Kavanagh, T. A. & Bevan, M. W. (1987) *EMBO J.* **6,** 3901–3907.
20. Shah, D. M., Horsch, R. B., Klee, H. J., Kishore, G. M., Winter, J. A., Tumer, N. E., Hironaka, C. M., Sanders, P. R., Gasser, C. S., Aykent, S., Siegel, N. R., Rogers, S. G. & Fraley, R. T. (1986) *Science* **233,** 478–481.
21. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33,** 103–119.
22. Fromm, M., Taylor, L. & Walbot, V. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5824–5828.
23. Rogers, S. G., Klee, H. G., Horsch, R. B. & Fraley, R. T. (1987) *Methods Enzymol.* **153,** 253–277.
24. Negrutiu, J., Schillito, I., Potrykus, I., Biasini, G. & Sala, F. (1987) *Plant Mol. Biol.* **8,** 363–373.
25. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
26. Dellaporta, S. L., Wood, J. & Hicks, J. B. (1985) in *Molecular Biology of Plants: A Laboratory Manual*, eds. Malmberg, R., Messing, J. & Sussex, I. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 36–37.
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
28. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230,** 1350–1354.
29. Schwarz-Sommer, Zs., Gierl, A., Cuypers, H., Peterson, P. & Saedler, H. (1985) *EMBO J.* **4,** 591–597.
30. Rothstein, S. J., DiMaio, J., Strand, M. & Rice, D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8439–8443.
31. Van der Krol, A. R., Lenting, P. E., Feenstra, J., Van der Meer, I. M., Koes, R. E., Gerats, A. G. M., Mol, J. N. M. & Stuitje, A. R. (1988) *Nature (London)* **333,** 866–869.
32. Smith, C. J. S., Watson, C. F., Ray, J., Bird, C. R., Morris, P. C., Schuch, W. & Grierson, D. (1988) *Nature (London)* **334,** 724–726.
33. McClintock, B. (1955) *Carnegie Inst. Washington Yearb.* **54,** 245–255.