Maize *Bronze 1:dSpm* Insertion Mutations That Are Not Fully Suppressed by an Active *Spm*

Gregory Bunkers,^{*,1} Oliver E. Nelson, Jr.,[†] and Victor Raboy*

^{*}USDA-ARS, Department of Plant and Soil Science, Montana State University, Bozeman, Montana 59717, and [†]Laboratory of Genetics, University of Wisconsin, Madison, Wisconsin 53706

Manuscript received April 24, 1992
Accepted for publication April 5, 1993

ABSTRACT

The *Suppressor-mutator (Spm)* family of maize transposable elements consists of autonomous *Spm* elements and nonautonomous *defective Spm (dSpm)* elements. One characteristic of this family is that the insertion of *dSpm* elements into a structural gene often permits some level of structural gene expression in the absence of *Spm* activity, and this structural gene expression is suppressed *in trans* by *Spm* activity. The *Spm*'s subterminal repetitive regions (SRRs) contain several iterations of a 12-bp repeat motif. It had been proposed that binding of an *Spm*-encoded protein to these repeat motifs blocks structural gene transcriptional readthrough, thus suppressing gene expression. The *bz-m13* allele of the *bronze 1* locus contains a 2.24-kb *dSpm* insertion in the second exon of a *Bz* allele. In the absence of *Spm* activity, *bz-m13* displays substantial *Bz* expression, and this expression is fully suppressed by *Spm*. Four intra-*dSpm* deletion derivatives are described in which this *Bz* expression is only partially suppressed by *Spm*. Each of these derivatives retains at least 12 SRR repeat motifs. Thus the presence of these repeat motifs is not sufficient to guarantee complete suppression by *Spm*. Some other property such as secondary structure or element size must play a role.

THE *Suppressor-mutator (Spm)* family of transposable elements in maize (*Zea mays* L.) consists of transposition-autonomous *Spm* elements and nonautonomous *defective Spm (dSpm)* elements (McCLINTOCK 1955; FEDOROFF 1989). The *dSpm* elements require the *trans*-acting product(s) of an active *Spm* element to be excised from a locus. *Spm* and *dSpm* elements are also referred to as *Enhancer (En)* and *Inhibitor (I)* elements, respectively (PETERSON 1953, 1960). The two systems are essentially identical (PETERSON 1965; PEREIRA *et al.* 1986). The canonical *Spm* element is 8.3 kb in length (PEREIRA *et al.* 1986; FEDOROFF 1989). Its important structural features include 13-bp inverted repeat termini, subterminal repetitive regions (SRRs) of 200–300 bp immediately internal to the termini, a "GC rich" sequence immediately internal to the 5' SRR, and internal transcriptional units which encode functions important to suppression and transposition (FEDOROFF 1989). The SRRs contain several iterations of a 12-bp repeat motif in both direct and inverted orientation, the consensus sequence of which is 5'-CCGACACTCTTA-3'. The *dSpm* elements are typically deletion derivatives of *Spm*.

McCLINTOCK (1954) found that, in the absence of *Spm* activity, *dSpm* insertions in structural genes in certain cases permit some level of structural gene expression. NELSON and KLEIN (1984) reported the

isolation of a *dSpm* insertion mutation of this type in an allele of the *bronze 1 (bz)* gene, termed *bz-m13*. Functional (*Bz*) alleles of this gene encode the enzyme UDP-glucose-flavonoid 3-O-glucosyltransferase (UFGT, EC 2.3.1.91), which catalyses a late step in anthocyanin synthesis (LARSON and COE 1977; DOONER and NELSON 1977). UFGT activity results in the accumulation of red or purple pigment in the kernel aleurone layer in genotypes with the appropriate alleles of the anthocyanin pathway regulatory loci. A nonfunctional (*bz*) allele results in a light-brown (*bronze*)-pigmented aleurone. *bz-m13* contains a 2.24-kb *dSpm* element inserted in the second exon of a *Bz* allele (SCHIEFELBEIN *et al.* 1988). The *dSpm* is inserted into the *Bz* allele in opposite orientation in terms of the *Bz* and *Spm* genes' directions of transcription. In the absence of *Spm* activity, the accumulation of anthocyanin pigment in the aleurone layer of kernels homozygous or heterozygous for *bz-m13* is indistinguishable from that of a *Bz* allele (KLEIN and NELSON 1983), and approximately 5% of the progenitor *Bz* allele's UFGT activity can be recovered in crude extracts (RABOY *et al.* 1989). An aleurone layer of the genotype *bz/bz/bz-m13*; +*Spm* (+*Spm* indicates it contains at least one active and standard, or *Spm-s*, element) displays a few large sectors of purple pigmentation superimposed on a bronze background. This illustrates the two phenomena for which McCLINTOCK (1955) named the *Spm* element following studies of *dSpm* insertion in the *a1 (anthocyaninless)* gene:

¹ Present address: Monsanto Co., M.S. GG4D, 700 Chesterfield Village Parkway, St. Louis, Missouri 63198.

suppression and mutation. *Spm*'s *trans*-acting product(s) suppresses the *Bz* expression observed in the absence of *Spm* activity, resulting in bronze cells and then excises the insertion in a few cell lineages early in development, resulting in a few, large revertant sectors. Mutations which display this type of response to *Spm* have been referred to as "*Spm*-suppressible" to distinguish them from the "*Spm*-dependent" type (MASSON *et al.* 1987). In "*Spm*-dependent" mutations structural gene expression is observed only in +*Spm* tissues, and is null in the absence of *Spm* activity.

In plants containing an active *Spm*, *bz-m13* also frequently undergoes mutation in germinal cell lineages immediately prior to or during gametogenesis (NELSON and KLEIN 1984). Typically, from 50% to 70% of the gametes produced by such plants contain *bz-m13* derivatives which can be recovered and studied in subsequent generations. The majority of these events represent excision of the *dSpm* insertion, resulting in stable functional (*Bz'*) and nonfunctional (*bz'*) derivatives (SCHIEFELBEIN *et al.* 1988). A small fraction of germinal mutations result in derivatives that contain altered *dSpm* insertions (SCHIEFELBEIN *et al.* 1985). These are recognized only if their phenotype, in the presence or absence of *Spm* activity, is altered relative to the initial insertion mutation. MCCLINTOCK (1955) termed such derivatives "changes-in-state" (CS). In the present case the initial "state" is that of *bz-m13*.

Six previously described CS derivatives of *bz-m13* have been described; *bz-m13 CS1* (CS1), CS3, CS5, CS6, CS9, and CS12 (SCHIEFELBEIN *et al.* 1985). These were selected for study because their *dSpm* insertions were excised in +*Spm* tissues relatively later during development than in the initial state of *bz-m13*, resulting in small rather than large somatic sectors. Like *bz-m13*, pigmentation in the absence of *Spm* activity was indistinguishable from that of a *Bz* allele. However, *Bz* expression (mRNA production and UFGT activity) conditioned by a given CS in the absence of an active *Spm* element varied from a low of approximately 1% (CS12) to 40–60% (CS9) of that conditioned by the progenitor *Bz* allele (*Bz-McC2*). Regardless of the level of *Bz* expression conditioned by a given CS in the absence of an active *Spm*, suppression of this *Bz* expression by *Spm* was complete. Here we describe the isolation and characterization of four novel CS derivatives of *bz-m13* in which *Bz* pigmentation in the absence of *Spm* activity remains indistinguishable from that of a *Bz* allele, and in which UFGT activity range from 17% to 48% of *Bz*. However, unlike the previously studied CS alleles, this *Bz* expression is not fully suppressed by *Spm* activity. Instead, suppression by *Spm* is incomplete, resulting in a "diffuse" aleurone phenotype. In kernels displaying the "diffuse" phenotype, aleurone cells are pigmented to a variable

extent intermediate between that observed with *bz* and *Bz* alleles.

MATERIALS AND METHODS

Plant materials: The isolation of *bz-m13* was described previously (NELSON and KLEIN 1984). The five previously described (SCHIEFELBEIN *et al.* 1985) CS alleles included in this study for comparative purposes were *bz-m13 CS3* (CS3), CS5, CS6, CS9 and CS12. The four novel CS alleles are designated CS13, CS14, CS15 and CS16. The additional alleles included as controls were: *bz-R*, the null reference allele; *Bz-McC2*, the *Bz* allele that served as the progenitor to *bz-m13*; *Bz'3*, a functional derivative of *bz-m13* in which the *dSpm* insert and the 3-bp host-sequence duplication have been excised, restoring the progenitor sequence.

All alleles in this study originated in or were incorporated into the common background of the inbred W22. These stocks carry functional alleles at complementary loci necessary for anthocyanin synthesis in the aleurone layer (*A1*, *A2*, *Bz2*, *C1*, *C2*, *R*, *Vp1*). A stock containing an active *Spm* element was provided by B. MCCLINTOCK and was used to backcross an *Spm* into the W22 background. Continued *Spm* activity in the stocks used in this study was demonstrated by test-crosses onto stocks containing either *bz-m13* or CS5.

UFGT extraction and analysis: Ears were harvested from field-grown plants at maturity, shelled, and stored at room temperature. The pedicel, germ and pericarp were removed from kernels selected for analysis. The endosperm samples thus prepared were homogenized at 4° in 0.75 ml extraction buffer per endosperm as previously described (RABOY *et al.* 1989). UFGT activity in crude extracts was determined using a modification (RABOY *et al.* 1989) of the method of KLEIN and NELSON (1983). Unless otherwise indicated, all extracts were assayed in duplicate.

Southern blot analyses: Genomic DNAs were isolated from the leaf tissue of plants homozygous for the alleles in this study using the method of MURRAY and THOMPSON (1980). Five micrograms of genomic DNA were digested with *Bst*EII and *Sst*I, fractionated in a 1% agarose gel, transferred to nitrocellulose, and hybridized with the ³²P-labeled plasmid pD3MS9, which contains a 650-bp *Sst*I/*Mlu*I *Bz* fragment that spans the *dSpm* insertion site (SCHIEFELBEIN *et al.* 1985). Autoradiography was for 9 days with an intensifying screen.

Sequence analysis: Sequence data were obtained from polymerase chain reaction (PCR)-amplified genomic DNA. Genomic DNAs were those used for the Southern blot analysis. The oligonucleotide primers used were: (1) 5'-TCGCTCAGGGAGGACGTCGG-3', an upper strand primer, the 5' base being 174 bp 5' of the *dSpm* insertion site; (2) 5'-TGCGCGCGGGTTTGATGAGC-3', an upper strand primer, the 5' base being 87 bp 5' of the *dSpm* insertion site; (3) 5'-ACGTGTTGAGTGCCACGGCG-3', a lower strand primer, the 5' base being 136 bp 3' of the *dSpm* insertion site; (4) 5'-TGGGCAGGATCTCGCGAGC-3', a lower strand primer, the 5' base being 190 bp 3' of the *dSpm* insertion site. The PCR reagents were as described by KIM and SMITHIES (1988), using a Perkin-Elmer Cetus DNA Thermal Cycler. Reactions contained 50 ng of a given primer and 80 ng of genomic DNA.

To obtain abundant and specific sequence amplification, a "nested-PCR" approach was used. First, amplification with the "outside" primers 1 and 4 and 80 ng genomic DNA as template was performed with the following Cycler program: 1 min, 92°, denaturation; 2 min, 68°, annealing; 4 min, 72°, elongation; 20 cycles. Next, a second round of ampli-

fication using the "inside" primers 2 and 3 and 1 μ l of a 1:50 dilution of first round products was performed using the following Cycler program: 1 min, 92°, denaturation; 2 min, 68°, annealing; 4 min, 72°, elongation; 30 cycles. Total reaction volume was 50 μ l, and 10- μ l aliquots were fractionated in 0.8% agarose gels containing ethidium bromide and photographed. Amplified products were digested with *Mbo*I and *Hpa*II and cloned into the *Bam*HI and *Acc*I sites of the phage vectors M13mp18 and M13mp19. Alternatively, amplification products were digested with *Hpa*II, and cloned into the *Acc*I site of M13mp18 and M13mp19. A minimum of three randomly picked clones with each strand represented at least once was sequenced with the dideoxy chain-termination method (SANGER, NICKLEN and COULSON 1977).

Molecular assays of somatic excision: Genomic DNAs were extracted from the fourth or fifth subapical leaf at pollination, using the method of DELLAPORTA, WOOD and HICKS (1983). Individuals tested were of the genotype *bz-m13* allele/*bz-R*; +*Spm*. For the Southern Blot approach, 5 μ g of genomic DNA was digested with *Bst*EII and *Sst*I, and fractionated in 0.8% agarose gels. Following transfer to nitrocellulose, blots were probed with ³²P-labeled pD3MS9, and autoradiographed for 7 to 14 days. For the PCR approach, the same genomic DNAs (80 ng) were amplified using primers 2 and 3 and the following Cycler program: 1 min, 92°, denaturation; 2 min, 65°, annealing; 4 min, 72°, elongation; 30 cycles. Total reaction volume was 50 μ l. Following amplification, 15 μ l were fractionated in 0.8% agarose gels containing ethidium bromide, photographed, transferred to nitrocellulose, and probed with ³²P-labeled pD3MS9.

RESULTS

The diffuse phenotype: During the inspection of the progeny of *bz-m13*; +*Spm* plants, several kernels were observed to display a "diffuse" aleurone phenotype (Figure 1, A and B). This phenotype is characterized by what appears to be incomplete suppression of *Bz* expression by *Spm*; nearly every aleurone cell is pigmented to a variable extent intermediate between bronze (Figure 1D) and red (Figure 1E). Well defined revertant (red) sectors superimposed on a bronze background, as observed in the previously described and fully suppressible states of *bz-m13* when in the presence of *Spm* (Figure 1C), were typically not observed. The appropriate cross-pollinations were made to obtain kernels containing each putative novel *bz-m13* derivative in the absence of *Spm*. Subsequent reintroduction of an active *Spm* confirmed the isolation of four independent events representing some change in *bz-m13*, which in the presence of an active *Spm*, results in the diffuse aleurone phenotype. These four derivatives were designated *CS13* through *CS16*. Figure 1, A and B, illustrates the +*Spm* phenotype of an aleurone containing one copy of *CS14*, which is typical of the four novel alleles. When aleurones contain two or three copies of *CS13* through *CS16* in the presence of *Spm*, the diffuse pigmentations conditioned independently by each copy are superimposed, resulting in what appears to be a fully pigmented

aleurone indistinguishable from that conditioned by a typical *Bz* allele (data not shown). Also, the kernels shown in Figure 1 are homozygous *pr*, a recessive allele of the *red aleurone* locus, which conditions a red pigmentation, *vs.* the darker purple pigmentation conditioned by kernels of the *Pr/-* genotype. In a *Pr/-* background, the diffuse pigmentation conditioned by one or more copies of *CS13* through *CS16* in the presence of an active *Spm* is nearly indistinguishable from that of a typical *Bz* allele.

Like *bz-m13* (Figure 1E), aleurone pigmentation conditioned by *CS13* through *CS16* in the absence of *Spm* was observed to be indistinguishable from a *Bz* allele. In a previous report (RABOY *et al.* 1989), mature endosperms homozygous for *bz-m13* and *CS9* were found to contain 5% and 67% of the UFGT activity conditioned by *Bz'3*, respectively. Crude extracts of mature endosperms homozygous for *CS13* through *CS16* in the absence of *Spm* were found to contain approximately 40%, 17%, 36% and 48% (respectively) of the UFGT activity found in crude extracts of mature *Bz'3*, as compared with 7% for *bz-m13* and 50% for *CS9*, which were included as controls. Thus these alleles, in the absence of *Spm*, are capable of conditioning sufficient UFGT activity to produce full pigmentation.

The diffuse pigmentation observed in the presence of *Spm* activity appears to be the result of incomplete, or leaky, suppression. This incomplete suppression is not simply due to the increased *Bz* expression conditioned by these alleles relative to *bz-m13* since two previously characterized CSs, *CS3* and *CS9*, condition similarly increased levels of *Bz* expression and are fully suppressible. To quantify suppression we compared the UFGT activity observed in aleurones containing two doses of a given *bz-m13* allele and two doses of an active *Spm* with that observed in aleurones containing two doses of the same *bz-m13* allele and no active *Spm* (Table 1). Plants of the genotype *bz-m13* allele/*bz-R*; +*Spm*/*-Spm* were pollinated by tester plants of the genotype *bz-R/bz-R*; *-Spm/-Spm*. Resulting ears contained three phenotypic classes representing four aleurone genotypes: (1) 50% bronze (25% *bz-R/bz-R/bz-R*; +*Spm*/+*Spm*/*-Spm* and 25% *bz-R/bz-R/bz-R*; *-Spm/-Spm/-Spm*); (2) 25% sectorial or diffuse, depending on the allele (*bz-m13* allele/*bz-m13* allele/*bz-R*; +*Spm*/+*Spm*/*-Spm*); (3) 25% purple (*bz-m13* allele/*bz-m13* allele/*bz-R*; *-Spm/-Spm/-Spm*). "Percent Suppression" was expressed as the UFGT activity observed in *-Spm* kernels (class 3), minus the UFGT activity observed in +*Spm* kernels (class 2) sampled from the same ear, as a percent of that observed in the *-Spm* kernels (class 3). This approach minimized both environmental and genetic background sources of variation. UFGT activity was as-

FIGURE 1.—Aleurone phenotypes of incompletely suppressed (*CS14*) and fully suppressed (*CS5*) *bz-m13* change-in-state derivatives. (A and B) Aleurones of the genotype *bz-R/bz-R/bz-m13 CS14; -Spm/-Spm/+Spm*. (C) Aleurone of the genotype *bz-R/bz-R/bz-m13 CS5; -Spm/-Spm/+Spm*. (D) Aleurone homozygous for *bz-R*. (E) Aleurone homozygous for *bz-m13* in the absence of *Spm*. Close ups of the boxed areas of each aleurone are given immediately below each aleurone photograph.

TABLE 1

Quantification of the suppression by *Spm* of the *Bronze 1* expression conditioned by *bz-m13 CS13* through *bz-m13 CS16* in the absence of *Spm*

Allele ^b	Spm ^b	Single-kernel assay ^a				5-Kernel assay ^a			
		Exp. 1		Exp. 2		Exp. 1		Exp. 2	
		UFGT Act. ^c	% Supp ^d	UFGT Act. ^c	% Supp ^d	UFGT Act. ^c	% Supp ^d	UFGT Act. ^c	% Supp ^d
<i>CS6</i>	—	1,369	—	262	—	664	—	50	—
	+	0 (99)	100	0 (55)	100	0 (41)	100	0 (44)	100
<i>CS13</i>	—	9,289	—	6,616	—	11,368	—	2,106	—
	+	976	89	178	97	3,058	73	84	96
<i>CS14</i>	—	976	—	1,369	—	1,350	—	788	—
	+	625	36	80	94	159	88	34	96
<i>CS15</i>	—	7,724	—	10,292	—	5,988	—	2,005	—
	+	579	93	774	92	100	98	165	92
<i>CS16</i>	—	775	—	1,276	—	1,203	—	1,618	—
	+	200	74	48	96	399	67	230	86

^a Kernels were prepared for UFGT assay as described in the MATERIALS AND METHODS. Either single kernels or 5-kernel samples representing a given genotype (see footnote ^b) were extracted. Single-kernel extracts were assayed in duplicate while 5-kernel extracts were assayed in triplicate, and two experiments were conducted using each approach.

^b The genotype of the kernels assayed was either *bz-m13* allele/*bz-m13* allele/*bz-R*; +*Spm*/+*Spm*/—*Spm* (indicated as + in the column labeled “*Spm*”) or *bz-m13* allele/*bz-m13* allele/*bz-R*; —*Spm*/—*Spm*/—*Spm* (indicated as — in the column labeled “*Spm*”). In each experiment, +*Spm* and —*Spm* kernels were sampled from a single ear segregating for the indicated *bz-m13* allele and *Spm*. *CS6* was included as the fully suppressed control.

^c UFGT activity is expressed as the counts/min incorporated into isoquercitrin per 25 μ l of extract assayed minus that observed in *CS6* + *Spm* (which is given in parentheses for reference).

^d % Supp: percent suppression, the UFGT activity observed in kernels containing a given allele-*Spm*, minus the UFGT activity observed in kernels containing the same allele +*Spm*, as a percent of the UFGT activity observed in kernels containing that allele-*Spm*.

sayed in single kernels or in 5-kernel samples representing a given class.

The *CS6* allele was used as a “fully suppressed” control since: the *Bz* expression conditioned by this

allele in the absence of *Spm*, which is similar to that of *bz-m13* and represents approximately 5% of the progenitor *Bz* allele, is fully suppressed in the presence of an active *Spm* and; it undergoes rare and late

somatic excisions in the presence of *Spm*, resulting in few, small revertant sectors. Thus revertant tissue contributes less than 1% to the total aleurone tissue (SCHIEFELBEIN *et al.* 1985). In *bz-m13* and the other five previously described CSs, with the exception of *CS12*, revertant tissue represents a much larger proportion of aleurone tissue, complicating direct assay of suppressed tissue. In the case of *CS12*, UFGT activity in *-Spm* tissues is too low to reliably assay its suppression. Table 1 presents the results of four experiments. Aleurones containing *CS13* through *CS16* in the presence of *Spm* typically had substantially more UFGT activity than did those containing *CS6* in the presence of *Spm*, which represents the background. "Percent Suppression" varied substantially between individuals containing a given allele, and between alleles, and ranged from a low of 36% to a high of 98% in individual assays. The mean value for each allele ranged from 79% in *CS14*, to 94% in *CS15*. Thus the leaky suppression observed with these alleles in the presence of *Spm* activity conditioned approximately 6–21% of the UFGT activity observed in the absence of *Spm* activity. It is not surprising that these levels of UFGT activity result in a pigmented aleurone since a previous study (RABOY *et al.* 1989) has shown that less than 1% of the UFGT activity conditioned by *Bz'3* is sufficient to result in a fully pigmented aleurone.

Molecular characterization: Preliminary restriction mapping indicated that *CS13* through *CS16* each contained a single *dSpm* insertion in approximately the same position as that in *bz-m13* (data not shown). To estimate the size of each *dSpm* insert genomic DNAs were digested with *Bst*EII and *Sst*I, whose sites are 288 bp 5' and 354 bp 3' of the *dSpm* insertion site, respectively. Digestion of *Bz-McC2* genomic DNA produces a 0.64-kb fragment which hybridizes to pD3MS9 (Figure 2A, lane 1). The four novel CS alleles yielded the following hybridizing fragments: *CS13*, 0.95 kb; *CS14*, 0.98 kb; *CS15*, 1.35 kb; *CS16*, 1.2 kb (Figure 2A, lanes 2 through 5, respectively). This indicates that *CS13* through *CS16* contain *dSpm* insertions of approximately 0.31, 0.34, 0.71, and 0.56 kb, respectively. Nested-PCR (see MATERIALS AND METHODS) of *Bz-McC2* DNA using primers 1 through 4 yields a fragment of 224 bp (Figure 2B, lane 1). Based on the above results, nested-PCR of *CS13* through *CS16* should yield fragments of approximately 0.53, 0.56, 0.93 and 0.78 kb, respectively. The observed results (Figure 2B, lanes 2 through 5) closely approximated this prediction.

Sequencing of these nested-PCR products confirmed that the insertion site, orientation of insertion and flanking *Bz* sequence in *CS13* through *CS16* was identical to that of *bz-m13*. In Figure 3A, the molecular structures of the four CS alleles are compared

FIGURE 2.—Characterization of *bz-m13* *CS13* through *bz-m13* *CS16*'s *dSpm* insertions. (A) Southern Blot analysis. Genomic DNAs were isolated from leaf tissue of plants homozygous for a given allele and were digested with *Bst*EII and *Sst*I. Following agarose gel fractionation and transfer to nitrocellulose, these were hybridized with the 32 P-labeled plasmid pD3MS9 which contains a 650-bp *Bz-McC2* fragment that spans the *dSpm* insertion site. Lane 1, *Bz-McC2*; lanes 2 through 5, *CS13* through *CS16*. (B) Nested PCR amplification of the *dSpm* insertions. Lane 1, nested PCR using primers 1 through 4 (see MATERIALS AND METHODS for details) produces a 0.22-kb *Bz-McC2* fragment which spans the *dSpm* insertion site. Lanes 2 through 5, nested PCR of *CS13* through *CS16*, respectively. Amplified products were fractionated in an agarose gel containing ethidium bromide and photographed.

with that of *bz-m13*. The 5' to 3' orientation is that of *Spm*'s direction of transcription, to facilitate comparisons with other published *Spm* element sequences. Each was the result of a single intra-element deletion. The sizes of the remnant *dSpm* insertions in *CS13* through *CS16* are 258, 275, 689 and 512 bp in length, respectively. These results are consistent with the restriction mapping results. In the case of *CS13* and *CS14*, the 5' and 3' deletion endpoints are within the 5' and 3' SRRs, respectively. In *CS15*, the 5' deletion endpoint is immediately 3' of the GC-rich sequence and the 3' endpoint is within the 3' SRR. In *CS16*, the 5' deletion endpoint is 2 bases 3' of the 13-bp terminal inverted repeat and the 3' endpoint is 200 bp 5' of the 3' SRR. In this derivative the 5' SRR and GC rich sequence are entirely deleted.

The sequences flanking the deletion endpoints are illustrated in Figure 3B. Those deletion endpoints which fall within either SRR are either within an SRR repeat motif, or as in the case of the 3' deletion endpoint of *CS13*, immediately flank a repeat motif. The deletion endpoints of *CS13*, *CS14* and *CS16* are also delineated by direct repeats of 3 (ACA), 7 (AA-GAGTG) and 3 (AAC) bp, respectively. The *CS15*

FIGURE 3.—Structure of the *dSpm* insertions in *bz-m13* CS13 through *bz-m13* CS16. (A) Location of deletion endpoints. The schematic of *bz-m13*'s *dSpm* insertion is given at the top for comparative purposes. IR, the 13-bp terminal inverted repeat. SRR, the subterminal repetitive regions. The arrows in the SRRs represent each 12-bp repeat motif and indicate relative orientation. GC indicates the GC-rich sequence. The vertical arrows indicate the position of each deletion endpoint. (B) Sequences flanking the deletion endpoints. Uppercase letters represent retained sequence, lowercase letters represents deleted sequence. The numbered bases indicate the deletion breakpoints relative to the 2.24-kb *bz-m13* *dSpm*. Boxed sequences indicate the repeats delineating each deletion endpoint. When deletion breakpoints are delineated by direct repeats there is ambiguity regarding which of the corresponding phosphodiester bonds within the repeats are involved. For consistency, we have assumed that the corresponding 3'-most phosphodiester bonds were involved. Solid underlines indicate an iteration of the 12-bp SRR repeat motif. Dashed underline indicates the 13-bp terminal inverted repeat.

deletion endpoint is delineated by a single base (A) repeat.

Molecular assays of somatic excision: The diffuse aleurone pigmentation conditioned by these alleles in the presence of *Spm* reflects both incomplete suppression and an apparent absence or near absence of excision. To test this latter observation, we assayed for the presence or absence of somatic excision prod-

ucts using two approaches. Genomic DNAs were prepared from leaf tissue of individual plants of the genotype *bz-m13* allele/*bz-R*; +*Spm*. Previous work (SCHIEFELBEIN *et al.* 1988; unpublished data) has shown that the relative differences in excision rates of the various CS alleles observed in leaf tissue parallel closely those observed in aleurone tissue. Three individuals representing each allele were tested, and typical results are given in Figure 4, A, B and C. We first assayed for excision products using a Southern Blot approach (Figure 4A). DNAs were digested with *Bst*EII and *Sst*I, fractionated in agarose gels, transferred to nitrocellulose and probed with pD3MS9. Plants homozygous for *bz-R* yield a 1.9-kb hybridizing fragment (lane 1), *Bz-McC2* homozygotes yield a 0.64-kb hybridizing fragment (lane 2), and plants homozygous for *bz-m13* in the absence of *Spm* yield a 2.88-kb fragment (reflecting 0.64-kb of flanking *Bz-McC2* sequence plus the 2.24-kb *dSpm* insertion, lane 3).

Spm-induced excision of a given *dSpm* insertion should restore the 0.64-kb band observed with *Bz-McC2*. Lack of excision should for a given allele yield a band representing the 0.64-kb flanking *Bz-McC2* sequence plus the size of the given allele's *dSpm* insert. DNAs isolated from leaves of *bz-m13/bz-R*; +*Spm* plants yield the 1.9-kb *bz-R* fragment as well as 0.64- and 2.88-kb fragments in approximately equal amounts (Figure 4A, lane 4). Thus the *dSpm* insert was excised in approximately half the cells of the leaf tissue. At this level of sensitivity, excision products were not observed in tissues heterozygous for the five previously described fully suppressible CS alleles (lanes 5 through 9) or the diffuse CS alleles (lanes 10 through 13). The only bands observed in each lane were those representing the *bz-R* allele and those of variable size corresponding to that expected if excision had not occurred. The similar level of the 1.90-kb *bz-R* band detected in each lane serves as an internal control, and indicates that approximately equal amounts of genomic DNA were tested in each case.

A previous and similar Southern blot assay of somatic excision in leaf tissues of +*Spm* plants heterozygous for CS6 and CS9 also failed to detect excision products (SCHIEFELBEIN *et al.* 1988). However, genetic tests demonstrated that the small revertant sectors observed in the kernels of these CS alleles, as well as CS3, CS5 and CS12, were attributable to *Spm*-induced excision rather than inactivation of *Spm* in some cell lineages, which would also result in pigmented sectors (SCHIEFELBEIN *et al.* 1988). Since a Southern blot approach could not detect a difference in somatic excision rates between the fully suppressible CS alleles (CS3 through CS12) *vs.* the diffuse CS alleles (CS13 through CS16), we next determined whether a PCR-based approach would provide the necessary sensitivity.

FIGURE 4.—Molecular assays of somatic excision. (A) Southern Blot approach. Genomic DNAs were digested with *Bst*EII and *Sst*I, fractionated in an agarose gel, transferred to nitrocellulose and probed with 32 P-labeled pD3MS9. This yields a 1.90-kb hybridizing *bz-R* fragment (lane 1), a 0.64-kb hybridizing *Bz-McC2* fragment (lane 2), and a 2.88-kb hybridizing *bz-m13* fragment (lane 3). Lanes 4 through 13, genomic DNAs were isolated from leaf tissue of the genotype *bz-m13* allele/*bz-R*; +*Spm*/–*Spm*. Lane 4, *bz-m13*; lane 5, *bz-m13* *CS3* (*CS3*); lane 6, *CS5*; lane 7, *CS6*; lane 8, *CS9*; lane 9, *CS12*; lane 10, *CS13*; lane 11, *CS14*; lane 12, *CS15*; lane 13, *CS16*. (B and C) PCR approach. (B) The genomic DNAs used for the Southern blot approach were amplified using primers 2 and 3 (see MATERIALS AND METHODS for details). Reaction products were fractionated in an agarose gel containing ethidium bromide. Since the deletion giving rise to *bz-R* deleted the sequence corresponding to primer 2, no amplification product is observed (lane 1). Amplification of *Bz-McC2* yields a 0.22-kb fragment (lane 2). Lanes 3 through 12, genomic DNAs were isolated from tissue of the genotype *bz-m13* allele/*bz-R*; +*Spm*/–*Spm*. Lane 3, *bz-m13*; lane 4, *bz-m13* *CS3* (*CS3*); lane 5, *CS5*; lane 6, *CS6*; lane 7, *CS9*; lane 8, *CS12*; lane 9, *CS13*; lane 10, *CS14*; lane 11, *CS15*; lane 12, *CS16*. (C) The gel photographed in (B) was transferred to nitrocellulose, and hybridized with 32 P-labeled pD3MS9.

The same genomic DNAs used for the Southern blot test were amplified using primers 2 and 3. First, reaction products were fractionated in agarose gels containing ethidium bromide, and photographed (Figure 4B). Since the deletion giving rise to the *bz-R* allele removed the sequence corresponding to primer 2, genomic DNA isolated from plants homozygous for

this allele were used as a negative control, and PCR amplification yielded no detectable bands (Figure 4B, lane 1). Amplification of *Bz-McC2* genomic DNA yields a 0.22-kb fragment (Lane 2), which should also be produced following *Spm*-induced excision of a given *dSpm* insertion. The predicted 0.22-kb band was readily observed in genomic DNAs isolated from *bz-m13/bz-R*; +*Spm* plants (lane 3), but not with either the fully suppressible *CS* alleles or the diffuse *CS* alleles (lanes 4 through 12).

The fractionated PCR products were then transferred to nitrocellulose and probed with pD3MS9 (Figure 4C). No bands were detected with *bz-R* (lane 1). The 0.22-kb band produced by *Bz-McC2* (lane 2) which is also that produced by *dSpm* excision was again readily detected in *bz-m13/bz-R*; +*Spm* tissues (lane 3). It was also consistently observed although at reduced levels in tissues heterozygous for *CS3* through *CS12* in the presence of *Spm* (lanes 4 through 8). The excision product was consistently not observed in tissues heterozygous for *CS13*, *CS14*, and *CS16* in the presence of *Spm* (lanes 9, 10 and 12). In the case of *CS15*, the excision product was either not observed, or observed at trace levels (as in Figure 4C, lane 11).

DISCUSSION

Element structure: Here we describe four intra-*dSpm* deletion derivatives of *bz-m13* whose *Bz* expression is not fully suppressible in *trans* by an active *Spm*. The small size of *CS13* through *CS16*'s *dSpm* insertions, when compared with those found in the six previously described *bz-m13* *CS* alleles and most other published *dSpm* elements, was the result of deletions which typically extended from within or near the 5' SRR to within or near the 3' SRR. Previously described intra-*Spm* deletions typically removed internal sequences, extended from one of the SRRs to internal sequences, or as in the case of *bz-m13* *CS6*, removed 2 bp of a terminal inverted repeat (FEDOROFF 1989; SCHIEFELBEIN *et al.* 1988). Recently, AUKERMAN and SCHMIDT (1992) described a 168-bp *dSpm* insertion in an allele of the maize *opaque-2* (*o2*) gene, termed *o2-23*, which represents the smallest known *Spm* remnant. As in the case of *CS16*, the 5' endpoint of the deletion giving rise to the 168-bp *dSpm* in *o2-23* was immediately 3' of the 5' 13-bp terminal inverted repeat, removing the entire 5' SRR, but also extended further 3', into the 3' SRR, resulting in its small size. The deletion giving rise to the *dSpm* insertion in the *anthocyaninless2-m1* (*a2-m1*) "class II" state (MENSSEN *et al.* 1990) also removed the entire 5' SRR (see below for further discussion).

Thus *CS13* through *CS16*, along with other similar elements, represent a class of elements distinct in terms of their size or extent of deleted sequence. However, the sequences involved in the deletion end-

points (Figure 3B) are similar to those associated with previously described intra-element deletions (MASSON *et al.* 1987; SCHIEFELBEIN *et al.* 1988). That is: (1) those deletion endpoints falling within an SRR typically occurred within or at the end of an SRR repeat motif and (2) the deletion endpoints typically were associated with short direct repeats, with the sequence between these repeats, and one of the repeats, comprising the deletion.

Incomplete suppression in *a2-m1* and *bz-m13* alleles: MCCLINTOCK (1958, 1971) described two insertion mutations of the maize *a2* gene, termed *a2-m1* (the initial state) and its derivative *a2-m1* "class II" state, which were subsequently shown by MENSSEN *et al.* (1990) to contain *dSpm* insertions nearly identical in sequence and orientation of insertion to those found in the original state of *bz-m13* and its derivative *CS16*, respectively. The relative differences in gene expression in the absence of *Spm*, and in response to *Spm*, of the two *a2-m1* states also parallel very closely the relative differences between *bz-m13* and *CS16*. In the absence of *Spm*, the original state of *a2-m1* conditions a reduced level of A2 expression resulting in a palely pigmented aleurone (MCCLINTOCK 1958). The intra-*dSpm* deletion giving rise to *a2-m1* "class II" state restored expression to a nearly wild-type level, resulting in a nearly fully pigmented aleurone (MCCLINTOCK 1958). The aleurone expression conditioned by either the initial state of *a2-m1* or the "class II" state appeared completely suppressed by the introduction of *Spm*. However, MCCLINTOCK (1958) reported that in plant tissue (presumably leaf sheaf and husk tissue) "that is *a2-m1* (class II)/*a2* in constitution and carries a single active *Spm* element . . . , suppression of the effects of *a2-m1* gene action is not complete." Thus an allele of the *a2* locus that contains a *dSpm* insertion nearly identical in structure to the *dSpm* in *CS16* also displays incomplete suppression by *Spm*. Therefore the phenomenon of incomplete suppression is not restricted to *dSpm* insertions of the *bz* gene. The comparison of *a2* and *bz-m13* alleles also suggests that the observation of incomplete suppression may be dependent upon a given host-gene and the tissue in which its expression is observed.

MCCLINTOCK (1958, 1971) termed the derivative of *a2-m1* a "class II" state since aleurone sectoring attributable to excision was not observed. Thus the *a2-m1* "class II" state appeared responsive to *Spm*'s first function (suppression), albeit only completely so in aleurone tissue, but not to the second function (mutation). In the present study, both phenotypic and molecular assays detected excision in all CS alleles which are completely suppressed by *Spm*. As with the *a2-m1* "class II" state, only with those CS alleles which are not completely suppressed by *Spm* is excision not readily observed. These results provide additional

evidence to support the observation of MCCLINTOCK (1957) that suppression of a *dSpm*-containing gene by *Spm* is a prerequisite for excision (mutation).

Cis-acting determinants of suppression by *Spm*-s: SCHWARZ-SOMMER *et al.* (1985) first proposed a model for the molecular mechanism by which *Spm* activity suppresses expression of genes containing *dSpm* insertions. They hypothesized that binding of an *Spm* product to the *dSpm* ends physically blocks host-gene transcriptional readthrough, thus suppressing host-gene expression. The integrity of a single end (presumably consisting of the terminal inverted repeat and an SRR) was proposed to be sufficient for suppression. Studies of *Spm* transcription have identified a major, 2.5-kb transcript, termed tnpA, which encodes a DNA binding protein (GIERL, LUTTICKE and SAEDLER 1988). *In vitro* assays revealed that tnpA binds to the 12-bp SRR repeat motif, and it is this interaction which is thought to result in suppression of host-gene suppression.

To test this model GRANT, GIERL and SAEDLER (1990) studied the *cis*-acting sequences necessary for suppression using a transient expression assay in transgenic tobacco cells. They found that tnpA expressed from a cloned cDNA will suppress transient expression, assayed at the level of enzymatic activity, of *Escherichia coli* β -glucuronidase (GUS) gene constructs each containing one of a variety of SRR repeat motif structures positioned in the transcriptional unit downstream of the GUS promoter. They observed that a construct containing the entire 3' SRR and the terminal inverted repeat, a construct analogous to *CS16*, was completely suppressed by tnpA. Furthermore, they found that at least two SRR repeat motifs placed in inverted orientation were necessary for suppression. In constructs containing at least two repeat motifs in inverted orientation, GUS expression in the presence of tnpA ranged from 1.5% to 30% of that observed in the absence of tnpA. Thus suppression by tnpA in *trans* ranged from 70% to 99.5%. Also, one of the two possible inverted orientations, referred to as the "tail to tail" orientation, was most efficient as a *cis*-acting determinant of suppression. In the presence of tnpA, GUS expression in constructs containing the "tail-to-tail" motif ranged from 1.5% to 15% of that observed in the absence of tnpA. Thus in this case suppression ranged from 85% to 98.5%.

The results reported here parallel and support the results of GRANT, GIERL and SAEDLER (1990). *CS13* through *CS16* each retain a number of SRR repeat motifs (the minimum being 12 in *CS13* and *CS15*) in both orientations with at least one "tail to tail" pair. Suppression by *Spm* in *trans*, as assayed at the level of UFGT activity in a whole maize tissue, typically ranged from approximately 80% to 95% of that observed in a fully suppressible CS, *CS6*. Thus these alleles are suppressible, just not completely so. How-

ever, the *Bz* expression resulting from this incomplete suppression is sufficient to produce a phenotype that can in certain cases be indistinguishable from that of the fully functional *Bz* precursor.

The presence *per se* of the SRR repeat motifs is not sufficient, in this case, to guarantee complete suppression by *Spm* in a whole maize tissue. The structures of *CS15* and *CS16* indicate that the presence of either a complete 3' or 5' SRR (respectively) is also not sufficient to guarantee complete suppression by *Spm*. In addition to the terminal inverted repeat and the SRR repeat motifs, the GC-rich sequence immediately 3' of the 5' SRR has been identified as an important nonrepetitive *cis*-acting determinant of a *dSpm*'s response to *Spm* activity (MASSON *et al.* 1987). *CS15* retains the entire GC-rich sequence. Thus the presence of repeat motifs and the GC-rich sequence are not sufficient for complete suppression. Other properties such as secondary structure, stability or size must be involved.

Evolutionary relationships between transposable elements and introns: In the *a2-m1* and *bz-m13* cases, structural gene expression in the absence of *Spm* is possible because the *dSpm* sequence is spliced out of the pre-mRNA as either part of a novel intron (*bz-m13*; KIM *et al.* 1987; RABOY *et al.* 1989) or simply as an intron (*a2-m1*; MENSSEN *et al.* 1990). In both cases the splicing event removes nearly all of the *dSpm* sequence, maintains the reading frame, and produces an mRNA encoding an altered but functional protein. Similar phenomena have been observed with maize *Ds* (*Dissociation*) transposable elements (reviewed in WESSLER 1989) and with a *D. melanogaster* retrotransposon (FRIDELL, PRET and SEARLES 1990). Thus this phenomenon is not restricted to *dSpm* elements or to maize. These studies demonstrate that transposable element insertions can either alter intron structure or simply function as novel introns. The ability of transposable elements to be spliced from pre-mRNAs may provide a selective advantage by mitigating the negative impact of insertion on structural gene expression (reviewed by WESSLER 1989).

In a study of the fully suppressible *bz-m13* CS derivatives, RABOY *et al.* (1989) found that certain intra-*dSpm* deletions increased splicing efficiency in the absence of *Spm*. A multistep sequence of events was described which began with the insertion, in the presence of an active *Spm*, of a *dSpm* element into a *Bz* allele, and led to an allele containing a novel intron which was in part a *dSpm* element. However, since the particular CS deletion derivatives in question were fully suppressible by *Spm*, the *dSpm* element functions as an intron only in the absence of *Spm* activity. MENSSEN *et al.* (1990) observed that in the case of *dSpm* elements, intra-*dSpm* deletions which remove the SRR repeat motifs would rid the element of the

cis-acting sequences necessary for suppression by *Spm* in *trans*. This would lead "to the genesis of a perfect intron" (MENSSEN *et al.*): a *dSpm* remnant that permits structural gene expression both in the absence and presence of *Spm*. The intra-*dSpm* deletions which gave rise to *CS13* through *CS16* each represent such a case. *Bz* gene expression, albeit at a reduced level, is observed both in the absence and presence of *Spm*. In some instances this expression can represent a substantial fraction of that observed with a wild-type *Bz* allele, and result in a phenotype which is indistinguishable from that of a wild-type *Bz* allele.

The authors would like to thank NINA FEDOROFF, THOMAS SULLIVAN and RONALD OKAGAKI for constructive criticism of the manuscript, and SUEWIYA PICKETT for assistance in the laboratory. The authors would also like to thank RUSSEL HUSETH for assistance in the field work conducted in Wisconsin, and GIL STALLKNECHT and his staff at the Southern Agricultural Research Center of the Montana Agricultural Extension Service, for their assistance in the field work conducted in Montana. This work was supported in part by the University of Wisconsin College of Agriculture and Life Sciences, by grant DCB-8507895 from the National Science Foundation (to O.E.N.), and by the Montana Agricultural Experiment Station. Montana Agricultural Experiment Station Journal Series No. J-2839.

LITERATURE CITED

- AUKERMAN, M. J., and R. J. SCHMIDT, 1992 A 168 bp deletion derivative of *Suppressor-mutator/Enhancer* is responsible for the maize *o2-23* mutation. *Plant Mol. Biol.* **21**: 315-362.
- DELLAPORTA, S. L., J. WOOD and J. B. HICKS, 1983 A plant DNA mini-preparation: version II. *Plant Mol. Biol. Rep.* **1**: 19-21.
- DOONER, H. K., and O. E. NELSON, 1977 Genetic control of UDPglucose:flavonoid 3-O-glucosyltransferase in the endosperm of maize. *Biochem. Genet.* **15**: 509-519.
- FEDOROFF, N., 1989 Maize transposable elements, pp. 375-411 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington D.C.
- FRIDELL, R. A., A.-M. PRET and L. L. SEARLES, 1990 A retrotransposon 412 insertion within an exon of the *Drosophila melanogaster* *vermilion* gene is spliced from the precursor RNA. *Genes Dev.* **4**: 559-566.
- GIERL, A., S. LUTTICKE and H. SAEDLER, 1988 *TnpA* product encoded by the transposable element *En-1* of *Zea mays* is a DNA binding protein. *EMBO J.* **7**: 4045-4053.
- GRANT, S. R., A. GIERL and H. SAEDLER, 1990 *En/Spm* encoded *tnpA* protein requires a specific target sequence for suppression. *EMBO J.* **9**: 2029-2035.
- KIM, H.-S., and O. SMITHIES, 1988 Recombinant fragment assay for gene targeting based on the polymerase chain reaction. *Nucleic Acids Res.* **16**: 8887-8903.
- KIM, H.-Y., J. W. SCHIEFELBEIN, V. RABOY, D. B. FURTEK and O. E. NELSON, 1987 RNA splicing permits expression of a maize gene with a defective *Suppressor-mutator* transposable element insertion in an exon. *Proc. Natl. Acad. Sci. USA* **84**: 5863-5867.
- KLEIN, A. S., and O. E. NELSON, 1983 Biochemical consequences of the insertion of a *suppressor-mutator (Spm)* receptor at the *bronze-1* locus in maize. *Proc. Natl. Acad. Sci. USA* **80**: 7591-7595.
- LARSON, R. L., and E. H. COE, 1977 Gene dependent flavonoid glucosyltransferase in maize. *Biochem. Genet.* **15**: 153-156.
- MASSON, P., R. SUROSKY, J. A. KINGSBURY and N. V. FEDOROFF, 1987 Genetic and molecular analysis of the *Spm*-dependent

- a-m2* alleles of the maize *a* locus. *Genetics* **117**: 117–137.
- MCCLINTOCK, B., 1954 Mutations in maize and chromosomal aberrations in *Neurospora*. *Carnegie Inst. Wash. Year Book* **53**: 254–260.
- MCCLINTOCK, B., 1955 Controlled mutation in maize. *Carnegie Inst. Wash. Year Book* **54**: 245–255.
- MCCLINTOCK, B., 1957 Genetic and cytological studies of maize. *Carnegie Inst. Wash. Year Book* **56**: 393–401.
- MCCLINTOCK, B., 1958 The suppressor-mutator system of control of gene action in maize. *Carnegie Inst. Wash. Year Book* **57**: 415–429.
- MCCLINTOCK, B., 1971 The contribution of one component of a control system to versatility of gene expression. *Carnegie Inst. Wash. Year Book* **70**: 5–17.
- MENSSEN, A., S. HOHMANN, W. MARTIN, P. S. SCHNABLE, P. A. PETERSON, H. SAEDLER and A. GIERL, 1990 The *En/Spm* transposable element of *Zea mays* contains splice sites at the termini generating a novel intron from a *dSpm* element in the *A2* gene. *EMBO J.* **9**: 3051–3057.
- MURRAY, M. G., and W. F. THOMPSON, 1980 Rapid isolation of high molecular weight plant DNA. *Nucleic Acid Research* **8**: 4321–4325.
- NELSON, O. E., and A. S. KLEIN, 1984 Characterization of an *Spm*-controlled bronze-mutable allele in maize. *Genetics* **106**: 769–779.
- PEREIRA, A., H. CUYPERS, A. GIERL, Z. SCHWARZ-SOMMER and H. SAEDLER, 1986 Molecular analysis of the *En/Spm* transposable element system of *Zea mays*. *EMBO J.* **5**: 835–841.
- PETERSON, P. A., 1953 A mutable pale green locus in maize. *Genetics* **38**: 682.
- PETERSON, P. A., 1960 The pale green mutable system in maize. *Genetics* **45**: 115–133.
- PETERSON, P. A., 1965 The relationship between the *Spm* and *En* control systems in maize. *Am. Nat.* **99**: 391–398.
- RABOY, V., H.-Y. KIM, J. W. SCHIEFELBEIN and O. E. NELSON, JR., 1989 Deletions in a *dSpm* insert in a maize *bronze-1* allele alter RNA processing and gene expression. *Genetics* **122**: 694–703.
- SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463–5467.
- SCHIEFELBEIN, J. W., V. RABOY, N. V. FEDOROFF and O. E. NELSON, 1985 Deletions within a defective Suppressor-mutator in maize affect the frequency and developmental timing of its excision from the bronze locus. *Proc. Natl. Acad. Sci. USA* **82**: 4783–4787.
- SCHIEFELBEIN, J. W., V. RABOY, H.-Y. KIM and O. E. NELSON, 1988 Molecular characterization of *Suppressor-mutator (Spm)*-induced mutations at the *bronze-1* locus in maize: the *bz-m13* alleles, pp. 261–278 in *Plant Transposable Elements*, edited by O. E. NELSON. Plenum Press, New York.
- SCHWARZ-SOMMER, Z., A. GIERL, R. BERNDTGEN and H. SAEDLER, 1985 Sequence comparison of 'states' of *a1-m1* suggests a model of *Spm(En)* action. *EMBO J.* **4**: 2439–2443.
- WESSLER, S. R., 1989 The splicing of maize transposable elements from pre-mRNA—a minireview. *Gene* **82**: 127–133.

Communicating editor: B. BURR