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Genetic and Molecular Analysis of the *Spm-dependent a-m2* Alleles of the Maize *a* Locus

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ABSTRACT

The *Suppressor-mutator* (*Spm*) transposable element family of maize consists of the fully functional standard *Spm* (*Spm-s*) and many mutant elements. Insertion of an *Spm* element in or near a gene can markedly alter its expression, in some cases bringing the gene under the control of the mechanisms that regulate expression of the element. To gain insight into such mechanisms, as well as to enlarge our understanding of the *Spm* element's genetic organization, we have analyzed derivatives of a unique *Spm* insertion at the maize *a* locus in which the gene is co-expressed and co-regulated with the element. We describe the genetic properties and the structure of the *a* locus and *Spm* element in 9 strains (collectively designated the *a-m2* alleles) selected by McClintock from the original *a-m2* allele for heritable changes affecting either the *Spm* element or expression of the *a* gene. Most of the mutations are intra-element deletions within the 8.3-kb *Spm* element; many alter both *Spm* function and expression of the gene. *Spm* controls *a* gene expression in alleles with internally deleted, transposition-defective *Spm* elements and element ends contain the target sequences that mediate *Spm's* ability to activate expression of the gene. We argue that the properties of the *a-m2* alleles reflect the operation of an element-encoded positive regulatory mechanism, as well as a negative regulatory mechanism that affects expression of the element, but appears not to be mediated by an element-encoded gene product.

TRANSPOSABLE elements, retroviruses and retrotransposon insertions often alter the way in which expression of the target gene into which they insert is regulated (Flavell *et al.* 1980; Errede *et al.* 1980; Roeder and Fink 1983; Varmus 1983; Campuzano *et al.* 1986; Zachar *et al.* 1985). From molecular analyses of several retrotransposon insertions in Drosophila and yeast, it has become increasingly apparent that the inserted elements impose their own mechanism of transcription regulation, often effecting changes in the target gene's developmental pattern of expression (Errede *et al.* 1985; Roeder, Rose and Pearlman 1985; Zachar *et al.* 1985; Campuzano *et al.* 1986). Some of the earliest descriptions of transposable element-mediated changes in gene expression were those of McClintock in maize. The best-studied examples are provided by insertions of *Suppressor-mutator* (*Spm*) elements (McClintock 1951, 1954, 1955, 1957–1959, 1961–1965, 1968, 1971). McClintock has described several *Spm* mutations that bring the target gene under the negative control of the element, as well as one that brings the gene under its positive control.

The *Spm* family comprises a group of structurally related elements, some of which can transpose autonomously and others of which are transposition defective (*dSpm* element), capable of transposing only in the presence of a nondefective *Spm* element (McClintock 1965b; Fedoroff 1983). In the most common type of insertion allele for which the element is named, a mutant gene with a *dSpm* insertion is expressed at a reduced level relative to the wild-type allele of the gene, but only in the absence of a nondefective *Spm*. When both mutant allele and *Spm* are present in the same genome, expression of the mutant gene is inhibited and the *dSpm* insertion is excised in some cell lineages (McClintock 1954). Hence, the *Spm* acts in *trans* both to prevent expression of the mutant gene and to excise the insertion. We have designated such alleles *Spm-suppressible* (Figure 1a). Several have been analyzed at the molecular level, revealing that they contain short, internally deleted *Spm* elements, which mediate the *Spm's* inhibitory effect on gene expression (Schiefelbein *et al.* 1985; Schwarz-Sommer *et al.* 1985b; Tacke *et al.* 1986).

McClintock isolated a very different type of *Spm* insertion allele of the *a* locus[1] in anthocyanin biosynthesis in which the gene was co-expressed and co-regulated with the inserted element (McClintock 1951, 1961a, 1962–1965a, 1968). She reported that reversible genetic changes that inactivated the element concomitantly inactivated the gene (McClintock 1962). McClintock (1962) selected a number of spontaneous derivatives of the original allele,

[1] We follow the revised genetic nomenclature recommendations in Coe and Neuffer (1971); the *a* locus was previously designated the *A1* locus.

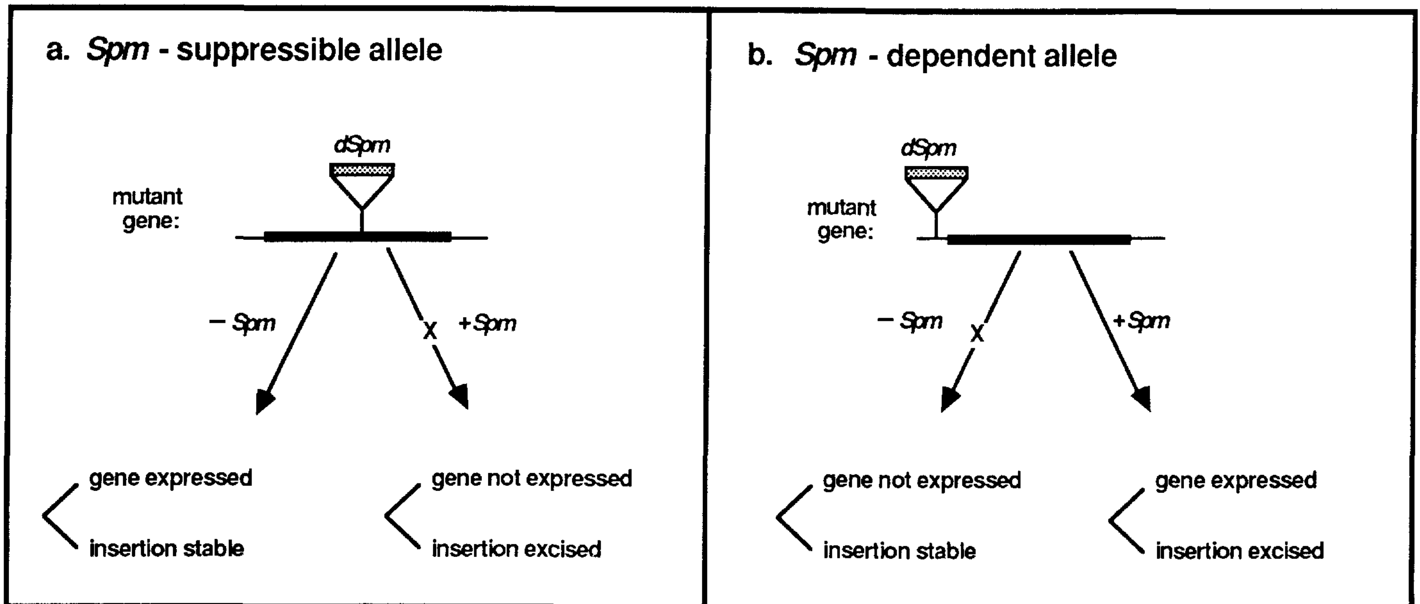

FIGURE 1.—Effect of the *Spm* element on genes with *dSpm* insertion mutations. Two different types of interactions between the *trans*-acting *Spm* element and a gene with a *dSpm* insertion mutation are illustrated. a, The response of an *Spm-suppressible* allele. In the absence of an *Spm* element, the mutant gene (*filled box*) is expressed at an intermediate level and the insertion is not excised. In the presence of an *Spm*, the gene is not expressed, and the insertion is excised in some cell lineages to give revertant sectors. b, The response of an *Spm-dependent* allele. The mutant gene is not expressed in the absence of an *Spm* and the insertion is stable. In the presence of an *Spm*, the mutant gene is expressed and the insertion is excised to give revertant sectors.

designated the *a-m2* allele, and found that some had sustained a mutation that rendered the resident *Spm* element transposition defective. Expression of the *a* gene in such *dSpm* derivatives remained under the control of the element. We have designated such derivative alleles *Spm-dependent* because the gene is expressed in the presence, but not in the absence of a *trans*-acting nondefective *Spm* (Figure 1b).

MCCLINTOCK isolated a number of derivatives of the original *a-m2* allele with heritable changes affecting either the functions of the element, the *a* gene, or both. Although the original allele has been lost, we have extended the genetic analysis and undertaken the structural study of the *a* locus in nine of the derivatives, which we refer to collectively as the *a-m2* alleles. Because the expression of the *a* gene is regulated with and by the element, the analysis of the *a-m2* alleles promises to offer insight into the molecular mechanisms that regulate expression of the element itself.

Several elements belonging to the *Spm* element family have recently been cloned and some sequenced (SCHWARZ-SOMMER *et al.* 1984; FEDOROFF *et al.* 1984; PEREIRA *et al.* 1985; BANKS *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985b; GIERL *et al.* 1985; PEREIRA *et al.* 1986; TACKE *et al.* 1986). Among these is the *Enhancer* (*En*) element, first identified genetically by RHOADES and DEMPSEY (1950) and later studied and shown to be genetically equivalent to *Spm* by PETERSON (1953, 1960, 1961, 1965). The *En* element is 8.3 kb in length and encodes a major transcript whose structure has been deduced from the sequence of a cDNA clone (PEREIRA *et al.* 1986), but whose function is not known.

The *a-m2* alleles have the potential of extending our understanding of *Spm* functions. They contain related *Spm* elements, each with a different mutation affecting the element. There are allelic differences affecting 4 different properties of the *Spm* element. These are (1) the ability of the resident *Spm* to *trans*-activate transposition of *dSpm* elements located elsewhere, (2) the ability of a *dSpm* to respond to a *trans*-activating element, (3) the ability of the resident element to affect expression of *Spm-suppressible* and *Spm-dependent* alleles, and (4) the capacity of a resident *Spm* element to mediate expression of the *a* locus. Analysis of the *a-m2* alleles was therefore undertaken to define the element sequences that encode the *Spm's* *trans*-active functions, as well as those that mediate its response to the *trans*-active functions. The results of studies on the genetic properties, structure, expression and sequence of the *a-m2* alleles are reported here. A preliminary account of the present study has been published (BANKS *et al.* 1985). The results of our analyses have led us to formulate a model for the regulation of the *Spm* element.

MATERIALS AND METHODS

Maize strains: Genetic stocks containing the *a-m2* alleles, as well as tester strains, were received from B. MCCLINTOCK. The *a-m2* alleles analyzed here are designated *a-m2-7991A1, a-m2-7995, a-m2-7977B, a-m2-8004, a-m2-8010A, a-m2-8011, a-m2-8167B, a-m2-8417* and *a-m2-8745*. Each allele

number corresponds to the number of the plant in McClintock's cultures that gave rise to the allele. Commonly used tester alleles were the *a-m1-5719A1* allele, the *wx-m8* allele of the *wx* locus, and a recessive *a* strain with an *Spm-s* at the locus (McClintock 1951, 1954, 1961a, 1965b). The *a-m1-5719A1* allele shows *a* gene expression at a level intermediate between the colorless null phenotype and the deeply pigmented wild-type in the absence of an *Spm* element. The no-*Spm* phenotype of the *a-m1-5719A1* allele is referred to as "pale" in the text. In the presence of an *Spm* element, the allele has a colorless null phenotype with deeply pigmented revertant sectors in the kernel aleurone layer (McClintock 1965b). This is referred to as "variegated" in the text. The *wx-m8* allele gives a null *wx* phenotype in the absence of an *Spm* element (McClintock 1961a) and shows frequent somatic and germinal reversion to the wild-type *Wx* allele in the presence of an *Spm-s* element. Other tester strains employed in the present study contained *Spm-s* and *Spm-w* elements unlinked to the *a* locus and alleles of the *a* and closely linked (0.1-0.2% recombination) *shrunken-2* (*sh2*) locus that gave stable null phenotypes in the present tests (*e.g., a sh2,* with or without *Spm-s* or *Spm-w* unlinked).

Genetic tests: The *a-m2* alleles were tested for the presence and type of insertion at the *a* locus, expression of the gene in the presence and absence of *Spm-w* and *Spm-s* elements elsewhere in the genome, as well as the ability of the *Spm* elements of the *a-m2* alleles to inhibit and activate *Spm-suppressible* and *Spm-dependent* alleles, respectively, of the *a* locus. Alleles which gave *a* gene expression were tested for the presence of an *Spm-s* or *Spm-w* element at the locus by co-segregation of the unstable allele with the closely linked *sh2* locus and their ability to *trans*-activate excision of the *dSpm* element of the *wx-m8* allele. Alleles with a colorless phenotype were also tested for their ability to suppress expression of the *Spm-suppressible a-m1-5719A1* allele and to *trans*-activate expression of the *a* gene of an *a-m2* allele with a *dSpm* element. *dSpm* insertions were identified by their stability in the absence and their instability in the presence of an *Spm-s* element located elsewhere in the genome. Reciprocal crosses were done in all cases to assess the response of each allele in one and two copies and to one and two copies of an *Spm* element. Certain alleles with *dSpm* insertions were also tested for their ability to suppress the *a-m1-5719A1* allele.

Stable revertants of the *a-m2-7991A1* allele were selected as a single palely or deeply pigmented kernels. Plants grown from these kernels were tested for the presence of *Spm* at the *a* locus and the response of the allele to an *Spm* element located elsewhere in the genome as described above.

Cloning of the *a-m2* alleles: DNA was extracted from 4-week-old plants as described by Shure, Wessler and Fedoroff (1983), partially digested with *Sau*3a and fractionated on a 5–20% potassium acetate gradient. Fragments in the 15–20-kb range were recovered by ethanol precipitation and cloned into the *Bam*HI site of the EMBL4 phage (Frischauf *et al.* 1983) as previously described (Shure, Wessler and Fedoroff 1983). Alternatively, DNA was either partially or completely digested with *Bam*HI and ligated directly to purified phage arms, followed by *in vitro* packaging in extracts obtained from Amersham. The initial phage library was screened with plasmid subclones of a 2.2-kb *dSpm* element previously cloned from the *wx-m8* allele (Fedoroff *et al.* 1984). Clones with homology to the *dSpm* element were rescreened with a *Pst*I fragment of the *a* locus, kindly supplied by Zs. Schwarz-Sommer (O'Reilly *et al.* 1985). A *Bam*HI fragment containing the *Spm* insertion site of the *a-m2* alleles was cloned from a stable *A* revertant and used as a source of further *a* locus subclones for subsequent screening.

Analysis of the *a-m2* alleles in genomic DNA and cloned fragments: Cloned DNA fragments were analyzed by restriction endonuclease digestion and blot hybridization (Southern 1975; Fedoroff, Mauvais and Chaleff 1983). Plasmid subclones of the *a* locus cloned from an *A* revertant of the *a-m2-7991A1* allele, the 2.2-kb *dSpm* element of the *wx-m8* allele, and plasmid subclones of internal *Eco*RI fragments of the *Spm-s* element of the *a-m2-7991A1* allele were used to map the inserts in fragments cloned from other *a-m2* alleles. Restriction endonuclease data derived from cloned fragments were compared with data obtained from genomic DNA blots probed with *a*-locus fragments, since the genomic redundancy of sequences homologous to the *Spm* element precluded direct analysis of the element at the *a* locus with *Spm*-derived probes. All data derived from cloned fragments were in agreement with data derived from analysis of the mutant *a* locus in genomic DNA, indicating the absence of cloning artifacts.

Transcript analysis: Poly(A)$^+$ RNA was prepared from 20–25 g of frozen plant tissue as described by Fedoroff (1985). The plants used were either homozygous for an allele of the *a* locus having an *Spm-s* insertion or an *Spm-w* insertion or were heterozygous for such an allele and either the *a-m1-5719A1* allele or a stable recessive allele. To compare *Spm* mRNA levels, RNA was extracted from plant, endosperm and cob tissue of plants homozygous for one of 4 different *Spm-s* and 4 different *Spm-w* alleles, including the *a-m2-7991A1, a-m2-8011,* and *a-m2-8745* alleles. RNAs from 1–2 plants were analyzed for each allele. Ten micrograms of poly(A)$^+$ RNA was fractionated on a 1.0% formaldehyde agarose gel and transferred to Nytran membrane filters (Maniatis, Fritsch and Sambrook 1982). Six internal fragments spanning most of the *Spm-s* element were subcloned into the SP64 or SP65 plasmids (Melton *et al.* 1984) to generate probes for detection of transcripts homologous to the *Spm* element. From the left end of the element, the fragments were A, *Sal*I to *Eco*RI; B, *Eco*RI to *Eco*RI; C, *Eco*RI to *Pvu*II; D, *Pvu*II to *Eco*RI; E, *Eco*RI to *Pst*I; and F, *Pst*I to *Hinc*II (0.1 kb from the element's right end). Plasmids having these fragments in both orientations with respect to the promoter were used to probe RNA filters. The SP6 transcripts were labeled as described by Green, Maniatis and Melton (1983) using ^{32}P-UTP. Hybridization was carried out under conditions described by Zinn, DiMaio and Maniatis (1983), except that the filters were hybridized at 42°. To quantify the amount of transcript, either the band was cut out of the filter and counted in a liquid scintillation counter or the autoradiogram was scanned with a densitometer. For an internal standard, filters were washed to remove the hybridized probe and reprobed with labeled, cloned fragments of the maize *shrunken* or *alcohol dehydrogenase* loci. The levels of these transcripts were used to normalize the *Spm* transcript levels.

DNA sequencing: The *Spm-s* element cloned from the *a-m2-7991A1* allele was subcloned in pEMBL18 (+ and −) and pEMBL19 vectors (Dente, Cesareni and Costese 1983; S. Lazarowitz, unpublished data). Progressive deletions were generated as described by Yanish-Perron, Vieira and Messing (1985) on the left 1.1-kb and the 5-kb *Pst*I fragments of *Spm* subcloned in pEMBL19 for upper strand sequencing, the 1.3-kb *Eco*RI fragment and 2.4-kb *Pst*I-*Eco*RI fragments of *Spm* were subcloned in pEMBL18 and the 3.0-kb *Eco*RV fragment was subcloned in pEMBL19 for lower strand sequencing. Finally, several subclones were constructed by shotgun cloning *Sau*3A digests of the 5-kb

*Pst*I, the 2.1-kb *Sal*I and the 3.0-kb *Eco*RV fragments of *Spm* in the pEMBL vectors.

Subclones for sequencing the deletion breakpoints of the *Spm-w-8011* derivative were obtained by progressively deleting the 3.2-kb internal *Pst*I fragment of *Spm* subcloned in pEMBL19, as described above. Similarly, sequences surrounding the deletion breakpoints of the *dSpm-7995, dSpm-7977B* and *dSpm-8004* elements were obtained by subcloning, respectively, a 3.1-kb *Ava*I fragment (*dSpm-7995*), a 2.9-kb *Pst*I fragment (*dSpm-7977B*) in pEMBL19, and both a 7.7-kb *Bam*HI-*Sal*I and a 2.5-kb *Eco*RI-*Eco*RV fragment (*dSpm-8004*) in pEMBL18. Finally the sequences of the left ends of the *a-m2* derivatives, as well as the *dSpm-8167B* element were obtained by subcloning the left 1.1-kb *Sal*I fragment, while the right border *Spm* sequences were obtained from either the deletion breakpoint subcloning (*dSpm-7995* and *dSpm-7977B*), the subcloning of an *Ava*I-*Pst*I fragment in pEMBL18 (*Spm-w-8011*) or the cloning of an 0.9-kb *Sma*I-*Eco*RV fragment in pEMBL18 (*dSpm-8004* and *dSpm-8167B*).

Phage were prepared from exponentially growing cells as described by DENTE, CESARENI and CORTESE (1983). Single-stranded DNA was extracted and sequenced using the dideoxynucleotide chain termination procedure (SANGER, NICKLEN and COULSON 1977) as described in BIGGIN, GIBSON and HONG (1983). Ambiguities were resolved using either the MAXAM and GILBERT (1980) procedure or the method described by BENCINI, O'DONOVAN and WILD (1984).

RESULTS

Genetic analysis of the *Spm* elements of the *a-m2* alleles: The *a-m2* alleles analyzed in the present study are derivatives of the original *a-m2* allele isolated by MCCLINTOCK in 1950 (B. MCCLINTOCK, personal communication). The first *a-m2* allele had a nondefective *Spm* element inserted at the *a* locus (MCCLINTOCK 1962). Each derivative originated from a single kernel with a phenotype that differed from that of the parental allele and is designated by the number of the plant in MCCLINTOCK'S cultures from which it was derived. The phenotypes of the *a-m2* alleles are described in Table I. Most of the alleles in the present group arose directly from the original one, although two are secondary derivatives (Figure 2). MCCLINTOCK identified three general categories of *Spm* elements [reviewed in FEDOROFF (1983)], all of which are represented among the nine *a-m2* alleles in the present group. These are (1) the fully functional standard *Spm (Spm-s)* element; (2) the weak *Spm* (*Spm-w*) element, so designated for its propensity to transpose less frequently and later in development than the standard element; and (3) the transposition-defective *Spm* (*dSpm*) element, capable of transposing only in the presence of a nondefective element. Although some of the *a-m2* alleles investigated here had been studied extensively by MCCLINTOCK (1963–1965, 1968) (*a-m2-7995, a-m2-7977B, a-m2-8004* and *a-m2-8011*), others had received only preliminary analysis (*a-m2-7991A1, a-m2-8010A, a-m2-8167B, a-m2-8417* and *a-m2-8745*). The latter were first subjected to genetic tests to verify the identity of the element at the locus. Since the tests are not novel, they will be described only briefly, accompanied by examples of the kinds of results obtained (MCCLINTOCK 1954, 1955, 1961a, 1962, 1968).

To test whether the resident *Spm* element can *trans*-activate transposition, plants having the genetic constitution *a-m2-Sh2/a sh2, wx* or *wx-m8* were intercrossed with tester plants homozygous for the *Spm-suppressible a-m1-5719A1* allele and the *Spm*-responsive *wx-m8* allele. The *a-m1-5719A1* and *wx-m8* alleles contain *dSpm* insertions of 0.8 kb and 2.2 kb, respectively, which can be *trans*-activated to excise by an *Spm* element located elsewhere in the genome (MCCLINTOCK 1951, 1961a; FEDOROFF *et al.* 1984; SCHWARZ-SOMMER *et al.* 1984, 1985b). Both alleles are useful in distinguishing between an *Spm-s* and an *Spm-w* element based on the frequency and developmental timing of somatic excision events (MCCLINTOCK 1957). In tests of pigmented *a-m2* alleles, the *wx-m8* allele was used to assess the ability of the resident element to *trans*-activate transposition; both tester alleles were used for analysis of colorless *a-m2* alleles. An example of the results obtained in such a cross is given in Table 2 for the *a-m2-8745* and *a-m2-8167B* alleles with the *a-m1-5719A1* tester allele. The variegated kernels on the ears resulting from the first cross reveal the presence of a functional *Spm* element in the *a-m2-8745* strain (Table 2A). The observation that variegation is confined to the *Sh2* class of kernels indicates close linkage of the *Spm* element to the *sh2* and *a* loci, themselves closely linked (0.1–0.2 cM). The absence of variegated kernels on the ears resulting from the second cross indicates that there is no active *Spm* present (Table 2B). Tests of this type show that the *a-m2-7991A1, a-m2-8010A, a-m2-8011* and *a-m2-8745* alleles have a non-defective *Spm* element at the *a* locus (Figure 2 and Table 1).

To determine whether an allele had a *dSpm* element, plants carrying the allele were intercrossed with plants containing an active *Spm* element and homozygous for a stable recessive allele of the *a* locus. In addition, plants with the allele and an *Spm* element were backcrossed to plants homozygous for the *a* and *sh2* alleles (Table 3). Ears were scored for the appearance of variegated kernels, indicating the presence of an allele of the *a* locus with an *Spm*-responsive *dSpm* insertion. Such tests confirmed that there are *dSpm* insertions at the *a* locus in the *a-m2-7995, a-m2-7977B, a-m2-8004, a-m2-8167B* and *a-m2-8417* alleles (Figure 2 and Table 1).

Molecular analysis of the *a* locus in the *a-m2* alleles: The structure of the *a* locus was analyzed in genomic DNA of plants homozygous for various *a-m2* alleles using probes specific for the locus (see MATERIALS AND METHODS). All but one of the alleles have a single insertion in the *a* locus; one allele has two

TABLE 1

Phenotypes of *a-m2* alleles

Allele	Phenotype	
A. Alleles with *Spm* at *a* locus		
1. Original *a-m2*	Pigmented background, many small and some large, pigmented sectors	
2. *a-m2-7991A1*	Pigmented background, moderate number of small to large, deeply pigmented sectors	
3. *a-m2-8010A*	Colorless background, many small to large palely pigmented sectors	
4. *a-m2-8011*	Pigmented background, few small deeply pigmented sectors	
5. *a-m2-8745*	Colorless	
B. Alleles with *dSpm* at *a* locus		
	−Spm	*+Spm*
1. *a-m2-7995*	Colorless	Pigmented background, fairly many small and some large, deeply pigmented sectors
2. *a-m2-7977B*	Colorless	Pigmented background, fairly many small and some large, deeply pigmented sectors
3. *a-m2-8004*	Colorless	Palely pigmented background, few small, deeply pigmented sectors
4. *a-m2-8167B*	Colorless	Palely pigmented background, few small, deeply pigmented sectors
5. *a-m2-8417*	Colorless	Colorless, many small, palely pigmented sectors

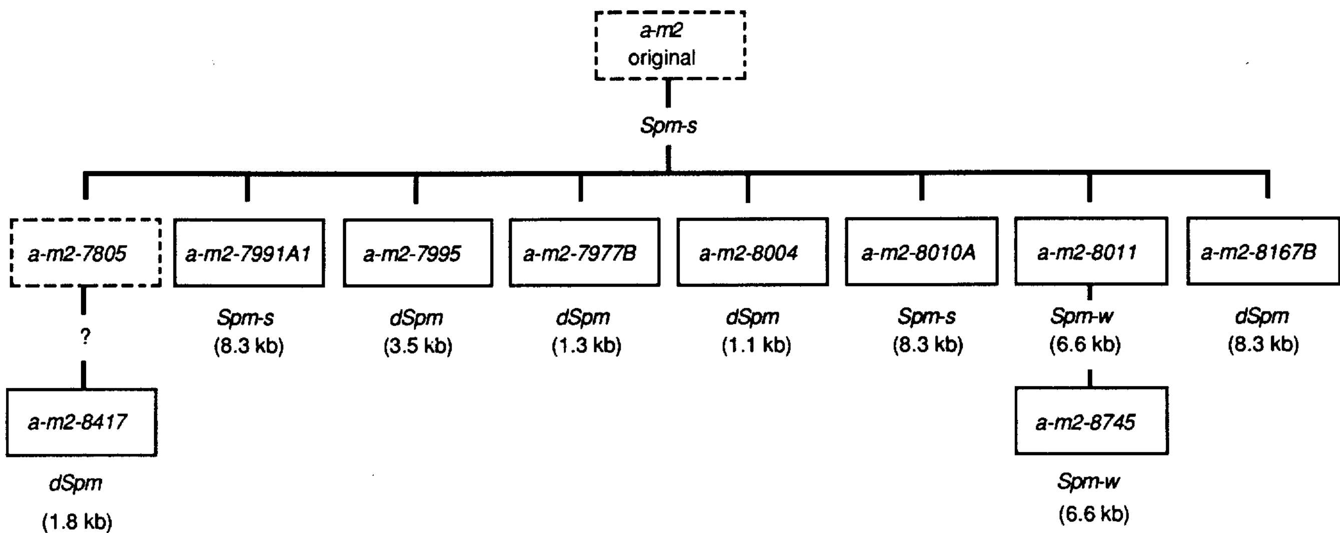

FIGURE 2.—Derivation of the *a-m2* alleles. The alleles analyzed here are designated by numbers enclosed in boxes; those in the lineage, but not analyzed are indicated by discontinuous boxes. The type and size of the *Spm* insertion is shown for each allele.

insertions (Figure 3). The insertion common to all of the *a-m2* alleles is missing from the two stable revertant alleles that were analyzed, identifying it as the *Spm* element. Within the resolution of genomic mapping, the *Spm* insertion site was found to be the same in all of the alleles, an observation that is consistent with their derivation from the original *a-m2* allele by mutations in or very near the element. The *Spm* insertions differ in length from 1.1 to 8.3 kb. The internal structure of several elements was further investigated by genomic mapping, as well as restriction endonuclease analysis and sequencing of DNA fragments cloned from genomic DNA of plants carrying the allele (see MATERIALS AND METHODS).

The *Spm-s* alleles

The *Spm-s* element is an 8.3-kb insertion: Two of the alleles in the present group contain elements that show the genetic characteristics of *Spm-s* elements. These are the *a-m2-7991A1* and the *a-m2-8010A* alleles. Both have an 8.3-kb insertion at the *a* locus (Figure 3). The *Spm* element of the *a-m2-7991A1* allele has been cloned, mapped and sequenced (Figure 4). It strongly resembles the independently identified *En* element shown by PETERSON (1965) to be functionally interchangeable with the *Spm* element (PEREIRA *et al.* 1986). The *Spm* element of the *a-m2-7991A1* allele differs from the *En* element by six single bases and is shorter by 4 nucleotides (Table 4). Most of the differences reside outside of the sequence encoding the *En* element's major transcript, reproduced in Figure 4(PEREIRA *et al.* 1986). Both elements contain two large open reading frames (ORFs; Figure 4), the corresponding sequences of which are identical in the two elements. There are three single base differences in the protein coding region of the major element-encoded transcript, but only one of the three alters the encoded amino acid. Thus the 8.3-kb element of the *a-m2-7991A1* allele, and probably that of the less

TABLE 2

Analysis of *a-m2* alleles for the presence of *Spm* at the *a* locus

Cross	*a-m2* parent	Tester parent		Kernel phenotypes: Colorless, variegated *Sh2*	Colorless, variegated *sh2*	Pale, nonvariegated *Sh2*	Pale, nonvariegated *sh2*
A. 1	♂ *a-m2-8745 Sh2/a sh2*	♀ *a-m1-5719A1 sh2*		183	0	0	184
2	♀ Same	♂ Same		91	0	1	97
3	♀ *a-m2-8745 Sh2/a-m1-5719A1 sh2*	♂ Same		176	0	0	153
4	♀ Same	♂ Same		125	0	0	118
5	♀ Same	♂ Same		172	0	0	187
6	♀ Same	♂ Same		161	0	0	149
			TOTAL:	908	0	1	888
B. 1	♀ *a-m2-8167B Sh2/a sh2*	♂ *a-m1-5719A1 sh2*		0	0	201	180
2	♀ Same	♂ Same		0	0	201	211
3	♀ Same	♂ Same		0	0	164	158
4	♀ Same	♂ Same		0	0	165	75
5	♂ Same	♀ Same		0	0	167	189
			TOTAL:	0	0	898	913

TABLE 3

Analysis of *a-m2-8167B* allele

Cross	*a-m2* parent	Tester parent	Kernel phenotypes: Pale, variegated *sh2*	Pale, variegated *Sh2*	Excision frequency	Colorless, nonvariegated *sh2*	Colorless, nonvariegated *Sh2*
1	♂ *a-m2-8167B Sh2/a sh2, Spm* present	♀ *a sh2,* no *Spm*	0	101	Moderate	193	87
2	♀ Same	♂ *Same*	0	77	Moderate	168	68
3	♀ Same	♂ *Same*	0	68	Moderate	154	70
4	♀ *a-m2-8167B Sh2/a sh2*	♂ *a Spm-s Sh2*	0	96	Moderate	0	97
5	♀ Same	♂ *a Spm-s Sh2*	0	192	Moderate	0	215
6	♂ Same	♀ Same	0	175	Moderate	0	160
7	♂ Same	♀ *a Spm-w Sh2* (*a-m2-8745* allele)	0	121	Low	0	133

well-studied *a-m2-8010A* allele, appear to correspond to the standard, fully functional *Spm* element.

Genetic and phenotypic differences among *Spm-s* alleles: Although the original *a-m2* allele has been lost, its phenotype resembled that of the *a-m2-8011* (*Spm-w*) allele in the presence of an *Spm-s* (Figure 5a) (B. McCLINTOCK, personal communication). The kernel aleurone layer is pigmented, indicating that the gene is expressed in mutant tissue. The original *a-m2* allele showed a high frequency of late somatic excision events, evidenced by the dense pattern of small somatic sectors exhibiting the deeply pigmented *A* phenotype. The *a-m2-7991A1* derivative differs from the original *a-m2* allele primarily by its lower frequency of deeply pigmented somatic sectors (Figure 5b). The *a-m2-8010A* allele differs from both by the absence of background pigmentation (Figure 5c). It exhibits a moderate somatic excision frequency and the revertant sectors are less intensely pigmented than those of the other two alleles.

Despite the phenotypic differences among the alleles, the resident *Spm* elements have the genetic properties of the standard *Spm* element and are capable of excising premeiotically to give germinally stable alleles lacking the *Spm* insertion. There are, however, differences in the types of stable alleles produced. The *Spm* element is known to generate a 3-bp direct duplication on insertion and to undergo excision by a mechanism that often leaves small sequence alterations at the former insertion site (SCHWARZ-SOMMER *et al.* 1985a). Among these are the original 3-bp duplication, with or without a minor sequence change, and small deletions. Consistent with such a mechanism, it is observed that excision of the element from the *Spm-s* alleles gives rise to stable alleles with different phenotypes, ranging from null to fully pigmented. The two surviving *Spm-s* alleles differ from each other and from the original *a-m2* allele in the relative frequency of stable derivatives with different phenotypes. McCLINTOCK (1961a, 1968) reported that the original *a-m2* allele most commonly gave stable alleles with an irregularly pigmented, "mottled" phenotype. Table 5 lists the

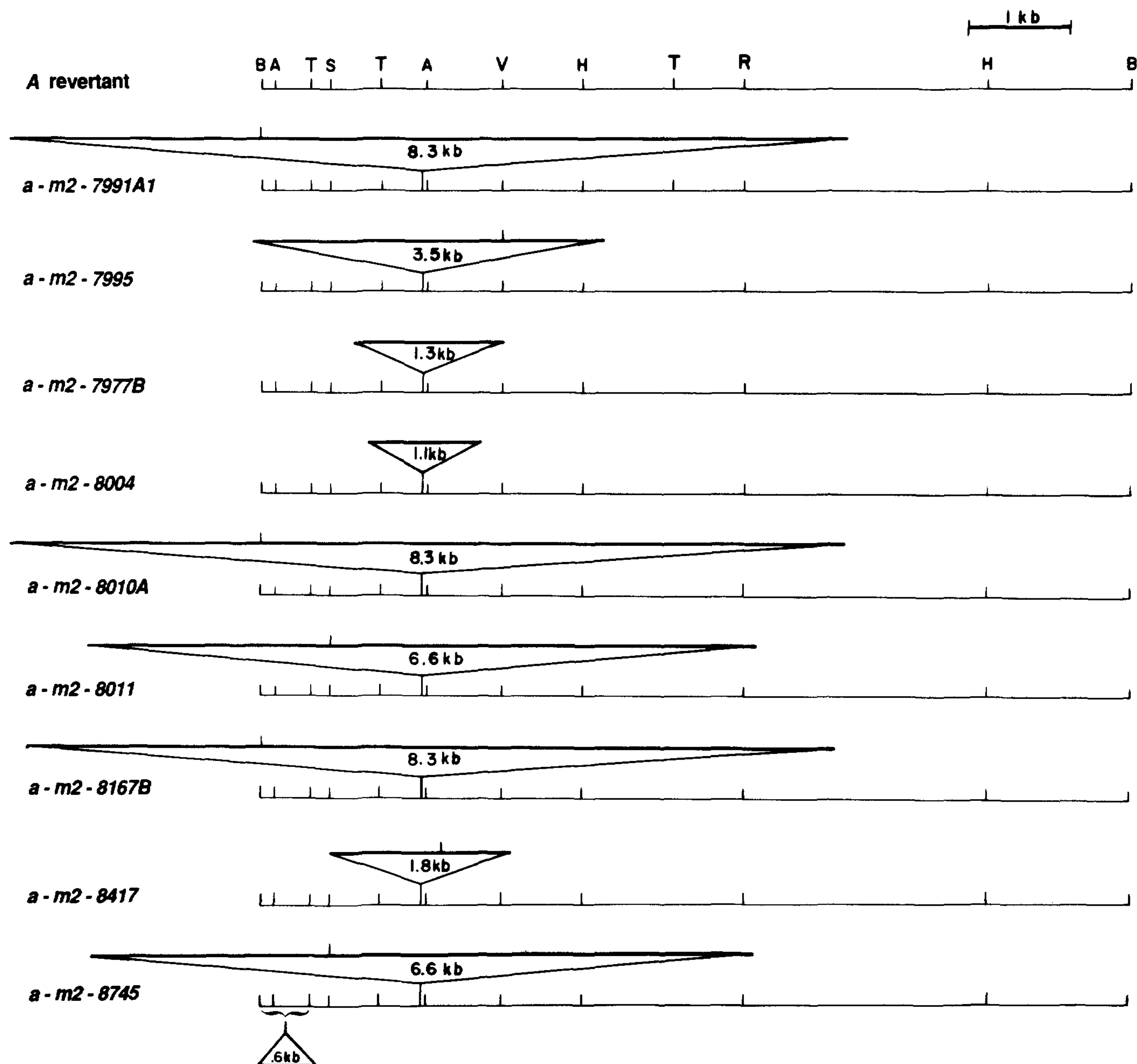

FIGURE 3.—Insertions at the *a* locus of the *a-m2* alleles. Restriction endonuclease cleavage site maps of the *a* locus were derived from analysis of genomic DNA fragments cloned from the indicated *a-m2* derivatives, except for the *a-m2-8010A*, *a-m2-8417*, and *a-m2-8745* alleles, which were mapped only in genomic DNA. The *a* allele map is that derived from fragments cloned from 2 stable revertants (see MATERIALS AND METHODS). All of the *a-m2* derivatives contained insertions at the same site and the lengths of the insertions are indicated. Restriction abbreviations: A = *Ava*I; B = *Bam*HI; H = *Hin*dIII; R = *Eco*RI; S = *Sal*I; T = *Pst*I; V = *Eco*RV. The *vertical bars* on successive lines refer to the restriction sites on the top line, except for the single vertical bar on the insertion, which corresponds to its internal *Bam*HI site and is the only site whose location within the element is represented. A restriction map of the *Spm* element appears in Figure 4.

phenotypes of *Sh2* kernels produced on backcrossed plants of the genetic constitution *a-m2-7991A1 Sh2/a sh2* and *a-m2-8010A Sh2/a sh2*. Nonvariegated *Sh2* kernels are common in both alleles, but differ in phenotype. The most frequent class is palely pigmented for the *a-m2-7991A1* allele and unpigmented for the *a-m2-8010A* allele. Plants were grown from nonvariegated kernels and tested for the presence of the element at the locus, as well as the capacity of the locus to respond to an *Spm* element located elsewhere in the genome. All of the tested plants were found to have stable alleles of the *a* locus with no genetically detectable *Spm* element (data not shown).

The observation that the different *Spm-s* alleles produce stable derivatives of a given phenotype at different frequencies suggests that they differ in the sequence of the locus in the immediate vicinity of the insertion site, rather than in the element. There is evidence to support such an interpretation for the *a-m2-7991A1* allele. The ends of several of the cloned elements have been sequenced, including those of the *dSpm-7995*, *dSpm-7977B*, *dSpm-8004*, *dSpm-8167B*,

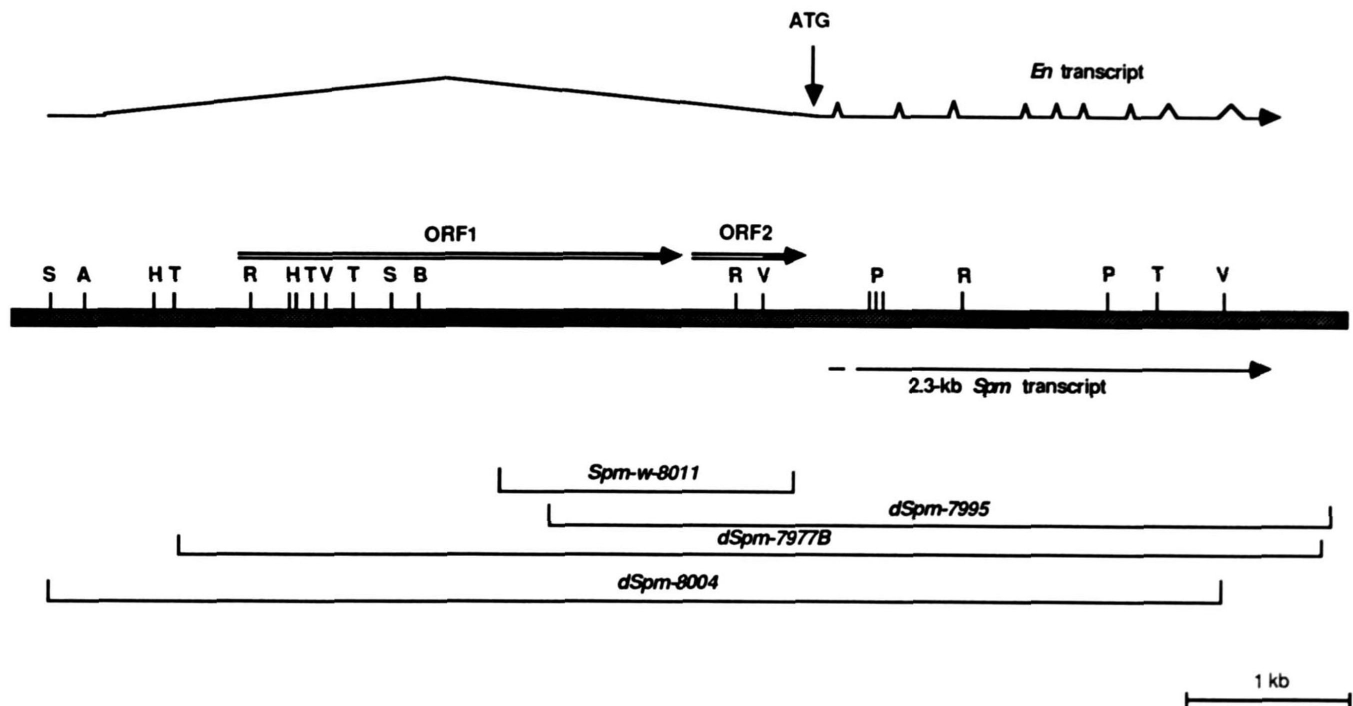

FIGURE 4.—A diagrammatic representation of the *Spm* sequence. The size, extent and orientations of ORFs 1 and 2 are represented by *open arrows* over the diagram. The approximate extent of the *Spm*'s major 2.3-kb transcript is indicated below the diagram. Also represented by the lines below the diagram are the regions of the element deleted in each of the mutant elements analyzed. The extended arrow over the diagram represents the sequence of the mRNA of the closely related *En* element (PEREIRA *et al.* 1986), with horizontal portions of the arrow corresponding to exons. Several restriction sites within the element are indicated by the letters designating the following enzymes: A = *Ava*I; B = *Bam*HI; H = *Hind*III; S = *Sal*I; R = *Eco*RI; P = *Pvu*II; T = *Pst*I; and V = *Eco*RV.

TABLE 4

Differences in nucleotide sequence between the *En* and *Spm* elements

Nucleotide[a]	*Spm*[b]	*En*[a]	Mutation[c]
65	G	C	P
960	G	GGG	D
1125	T	A	P
6139	C	T	P
6694	T	TAT	D
7633	G	A	P
7659	A	C	P
8270	G	A	P

[a] From PEREIRA *et al.* (1986) for the *En* sequence.
[b] Nucleotide sequence of the *Spm* element cloned from the *a-m2-7991A1* allele.
[c] P = point mutation; D = deletion in *spm* sequence (or insertion in *En* sequence).

FIGURE 5.—Phenotypes of the *Spm-s* alleles. a, *a-m2-8011* allele with an *Spm-s* (illustrates original *a-m2* phenotype; see text for explanation); b, *a-m2-7991A1* allele; c, *a-m2-8010A* allele.

TABLE 5

Stable alleles produced by excision of *Spm* from *a-m2* alleles

		Percent Sh2 kernels with each phenotype				
a-m2 parent	Backcross parent	Varie-gated	Color-less	Pale	Mottled	A
a-m2-7991A1 Sh2/a sh2	*a sh2*	88	2	9	0.5	<0.1
a-m2-8010A Sh2/a sh2	*a sh2*	66	31	2.4	0	0.3

and the *Spm-s-7991A1* elements. All but the *Spm-s-7991A1* element are flanked at each end by the trinucleotide ATT, which represents the 3-bp duplication generated upon insertion of the *Spm* element in the original *a-m2* allele. The *a-m2-7991A1* allele has the additional trinucleotide AAT inserted immediately adjacent to the right end of the element. Since it is outside of the element, all or part of the inserted trinucleotide will remain upon excision of the element, yielding a range of revertants with a slightly different sequence than expected for the other elements. This may account for the phenotypic differences observed among the stable revertant alleles (Table 5). It should be noted that the high germinal excision frequency of the *Spm-s* element of the *a-m2-7991A1* allele indicates that the trinucleotide sequences flanking the element need not be identical for efficient excision. Although the *a-m2-8010A* element has not been cloned and sequenced, it appears

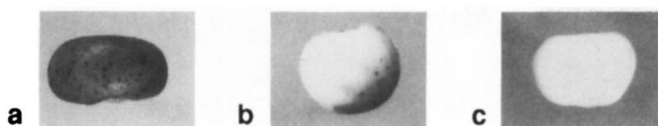

FIGURE 6.—Inactivation and reactivation of the *Spm* element. Phenotypes of *a-m2-7991A1-i* kernels in which the *Spm-s* element is a, active; b, inactive (colorless sector) and active (pigmented sector) containing deeply pigmented spots) in different areas of the kernel; and c, inactive.

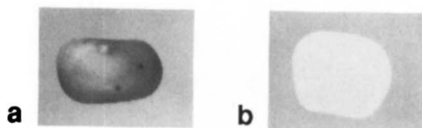

FIGURE 7.—Phenotypes of the *Spm-w* alleles. a, The *a-m2-8011* allele and b, the *a-m2-8745* allele.

to contain a standard *Spm* element and the genetic change that distinguishes this allele phenotypically may also lie outside of the element.

The *a* gene of the *Spm-s* alleles is co-expressed with the *Spm* element: *Spm* elements are subject to inactivation by a reversible mechanism (MCCLINTOCK, 1957–1959, 1961b, 1965b, 1971). An inactive *Spm* (*Spm-i*) differs from a *dSpm* element by its ability to return spontaneously to a genetically active state. MCCLINTOCK (1962) reported that when the *Spm-s* element of the original *a-m2* allele became inactive, the *a* gene was no longer expressed. We have isolated an analogous derivative of the *a-m2-7991A1* allele, in which the *Spm-s* element has become inactive as judged by its inability to excise itself and *trans*-activate excision of the *dSpm* insertion in the *wx-m8* allele. The *Spm-i* element of the inactive derivative (*a-m2-7991A1-i*) undergoes spontaneous reactivation relatively often and both early and late in plant development. Figure 6 shows the phenotypes of kernels in which the *Spm* is active (a), inactive (c), or has undergone reactivation during kernel development (b). The pale background pigmentation characteristic of the parent allele is evident only in kernels or kernel sectors in which the element is active, as indicated by the appearance of deeply pigmented somatic revertant sectors. Hence the *a* gene is inactivated and reactivated simultaneously with the element. Expression of the gene is therefore influenced by the same negative mechanism that affects the activity of the *Spm* element.

The *Spm-w* alleles

The phenotypes of the *Spm-w* alleles: We have analyzed two phenotypically different *Spm-w* alleles. The *a-m2-8011* allele was derived from the original *a-m2* allele and exhibits the intermediate background pigmentation of the parent allele (Figure 7a). The resident *Spm* element excises at a low frequency. The *a-m2-8745* allele is a derivative of the *a-m2-8011* allele and has a completely null phenotype (Figure 7b). Both alleles *trans*-activate excision of the *dSpm* element of the *wx-m8* allele less frequently and later in kernel development than is characteristic of the *Spm-s* element.

Structure of the *a* locus and *Spm* elements in the *Spm-w* alleles: Both the *a-m2-8011* and the *a-m2-8745* alleles contain 6.6-kb *Spm* insertions. The colorless *a-m2-8745* allele contains an additional insertion (or duplication) at a distance of about 1.5 kb from the *Spm* insertion site (Figure 3). The two alleles therefore appear to have the same *Spm-w* element and the phenotypic difference between them is probably attributable to the second mutation in the *a-m2-8745* allele. The *Spm* element has been cloned from the *a-m2-8011* allele and its structure is indicated in Figure 4. The *Spm-w* element differs from the *Spm-s* element by a 1.7-kb deletion commencing 0.5 kb to the right of the internal *Bam*HI site and terminating approximately 0.1 kb to the right of the central *Eco*RV site. Sequence analysis of the *Spm-w-8011* element reveals that the deletion eliminates parts of both ORF1 and ORF2 (Figure 4).

The structure of the deletion breakpoint in the *Spm-w* element is complex. In addition to the extensive deletion of internal element sequences, there is a short insertion of sequences duplicated from nearby (Figure 8a). The first half of the insertion is identical to a sequence that commences 36 nucleotides to the right of the right breakpoint, while the second half is a copy of a sequence located 56 nucleotides to the left of the left breakpoint. In addition, there is a single base change from an A residue in the *Spm-s* element to a T residue 3 nucleotides to the right of the leftmost sequence duplicated at the breakpoint.

Expression of the *Spm-w* element: To determine whether the *Spm-w* phenotype is attributable to reduced expression of the *Spm* element, we analyzed transcripts of the element in poly(A)$^+$ RNA prepared from plants with *Spm-s* and *Spm-w* elements. Because there are many copies of sequences homologous to *Spm* in the genome (N. FEDOROFF, unpublished data), we compared RNAs from plants grown from sibling kernels from the same ears, having and lacking the *Spm* element, to identify *Spm*-specific transcripts (data not shown). The most abundant *Spm*-specific transcript is about 2.3 kb in length and has so far been detected only with probes from the right half of the element (see MATERIALS AND METHODS). Its position and approximate extent are shown in Figure 4. The same transcript is present in both *Spm-s* and *Spm-w* strains, but is much less abundant in the latter (Figure 9). Similar results have been obtained with several different *Spm-s* and *Spm-w* elements, with plant, endosperm and cob tissue, and with tissue of different ages (see MATERIALS AND METHODS). The only other *Spm*-specific transcript that has been detected is a minor 1.1-kb transcript homologous to the *Sal*I-*Eco*RI

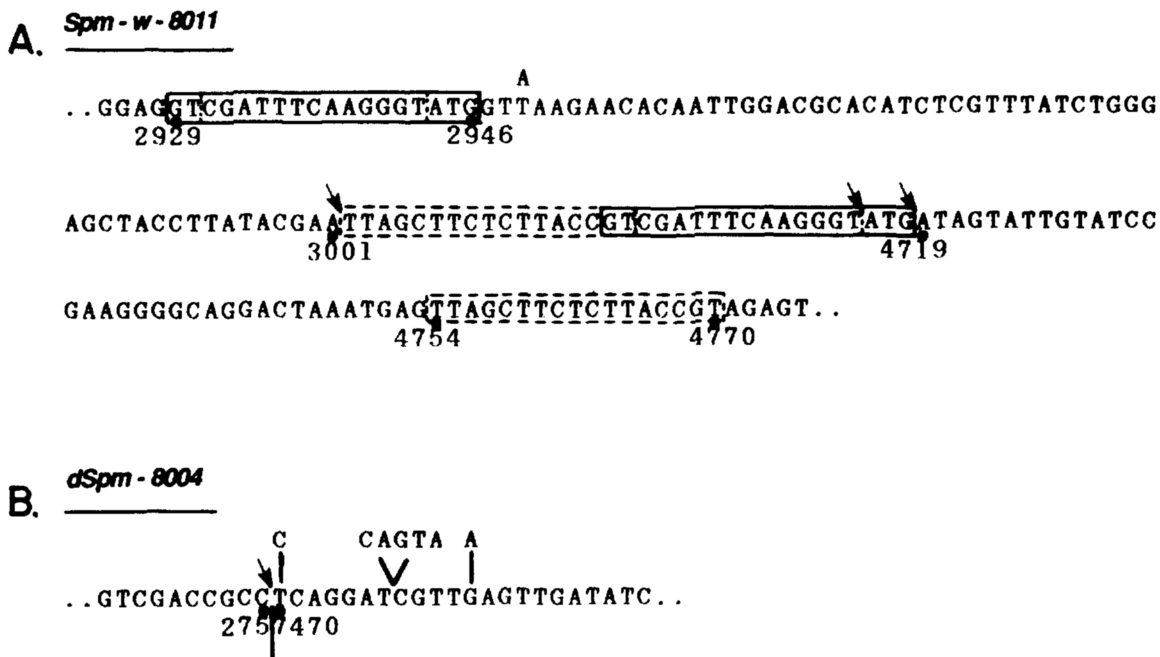

FIGURE 8.—The nucleotide sequence at the rearrangement breakpoint in the *Spm-w-8011* and *dSpm-8004* elements. The part of the *Spm-w-8011* sequence represented in A extends from nucleotide 2925 to nucleotide 4775 of the corresponding *Spm* element; the deletion eliminates the 1.7-kb sequence extending from nucleotide 3002 to nucleotide 4715-18 of the *Spm* element. Between the deletion endpoints, which are marked by diagonal arrows above the sequence, there is a 30-33 bp insertion (the 3-bp ambiguity in the lengths of the insertion and deletion is due to the repetition of an ATG in the original and inserted sequences). The sequence inserted at the deletion breakpoint consists of two shorter sequences that are identical to nearby sequences within the element. The left half of the insertion is identical to a sequence commencing 36 nucleotides to the right of the right breakpoint (both enclosed in interrupted boxes). The right half of the insertion is identical to a sequence that commences 56 nucleotides to the left of the left breakpoint (both enclosed in solid boxes). The *Spm-w* element also differs from the *Spm-s* element by the indicated A → T base change. The rearrangement breakpoint of the *dSpm-8004* element is shown in B. The site of the element's 7.2-kb deletion is indicated by an arrow and the several additional nucleotides that appear in the *Spm-s* element, but not the *dSpm-8004* element, or differ between the elements, are shown over the sequence.

fragment at the extreme left end of the element and its abundance is the same in *Spm-s* and *Spm-w* strains (data not shown). The minor transcripts evident in Figure 9 are often detected in RNA from plants lacking an active *Spm* element and have not yet been further investigated. Thus the major effect of the *Spm-w* mutation is a three- to ninefold reduction in the abundance of the 2.3-kb *Spm* transcript.

The *Spm-w* element retains the ability to affect expression of *dSpm* alleles: To determine whether the internal deletion of the *Spm-w* element interferes with the element's ability to affect the expression of *Spm-suppressible* and *Spm-dependent* alleles of the *a* locus, plants carrying the colorless *a-m2-8745* allele were intercrossed with plants that were homozygous for the *Spm-suppressible a-m1-5719A1* allele, as well as plants carrying one of several different *Spm-dependent a-m2* alleles. The results of such crosses (Tables 2 and 3) indicate that the *Spm-w* element of the *a-m2-8745* allele can affect the expression of both types of alleles and differs from the *Spm-s* element only in the frequency with which it *trans*-activates somatic excision, as illustrated in Figure 10. Thus the *Spm-w* mutant retains all of the element's *trans*-active functions.

The *dSpm* alleles

Phenotypes of the *dSpm* alleles: The remaining 5 alleles in the present series, *a-m2-7995, a-m2-7977B, a-m2-8004, a-m2-8167B* and *a-m2-8417*, contain *dSpm* insertions at the *a* locus. All exhibit a stable null phenotype in the absence of a nondefective *Spm* element and a variegated phenotype in the presence of either an *Spm-s* or an *Spm-w* (Figure 11). In all but the colorless *a-m2-8417* allele, the *a* gene is expressed in the presence, but not the absence, of a non-defective *Spm* element (Figure 11). Thus the *dSpm* insertions mediate the *Spm-dependent* expression of the *a* gene. The *dSpm* alleles differ both in the level of *a* gene expression and in the somatic excision frequency. The *a-m2-7995* and *a-m2-7977B* alleles resemble each other; they both show a rather high level of *a* gene expression and a somewhat lower somatic excision frequency than the original *a-m2* allele. The *a-m2-8167B* and *a-m2-8004* alleles have low levels of *a* gene expression, as well as low somatic excision frequencies. Somatic excision is more frequent in the former allele than in the latter, but often occurs extremely late in development to give very small revertant sectors. The *a-m2-8417* allele has a colorless background and a high frequency of pale somatic sectors, its phenotype

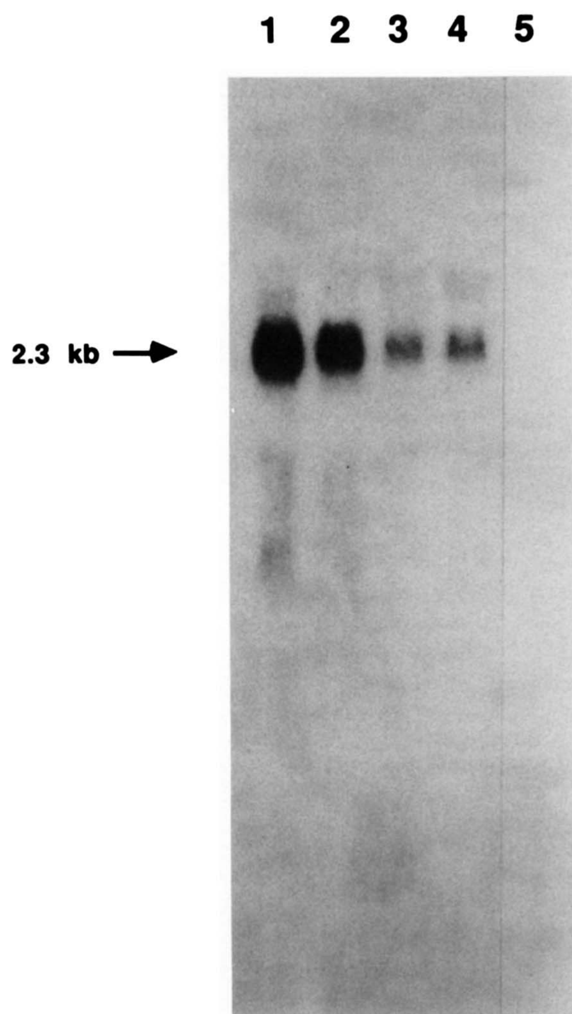

FIGURE 9.—Poly(A)$^+$ RNA homologous to the *Spm* element in endosperm tissue of plants homozygous for an *Spm-s* or an *Spm-w* element. Ten micrograms of poly(A)$^+$ RNA from endosperm tissue of plants that were homozygous for the *Spm* element was fractionated in a 1.0% formaldehyde-agarose gel, transferred to a Nytran membrane filter and hybridized with a labeled SP6 containing fragment E of the *Spm* element (see MATERIALS AND METHODS). Lane 1, *Spm-s* (*a-m2-7991A1* allele); lane 2, another *Spm-s* allele; lane 3, *Spm-w* (*a-m2-8011* allele); lane 4, *Spm-w* (*a-m2-8745* allele); lane 5, no *Spm* element.

resembling that of the *a-m2-8010A* allele.

Structure of the *dSpm* elements: Analysis of the *dSpm* insertions in genomic DNA shows that the *dSpm* elements range in size from 1.1 to 8.3 kb. All of the *dSpm* elements except the 8.3-kb *dSpm-8167B* element were derived from the original *Spm-s* element by internal deletions ranging in size from 1.4 kb to more than 7 kb. The *dSpm* elements in alleles derived directly from the original mutant have a single large deletion (Figure 4), while the *a-m2-8417* allele, which was derived in two steps, has two large deletions (1.4 and 5.1 kb; data not shown). The ends and deletion breakpoints of the cloned *dSpm-7995*, *dSpm-7977B* and *dSpm-8004* elements have been sequenced. The *dSpm-7995* and *dSpm-7977B* elements have simple deletions whose approximate extent is shown in Figure 4. The *dSpm-8004* element, which has also been sequenced by SCHWARZ-SOMMER *et al.* (1987), has a large deletion as well as a small deletion and a single base change immediately adjacent to the right deletion breakpoint (Figures 4 and 8b). The *dSpm-8167B* element has been subjected to extensive restriction endonuclease analysis and its termini have been sequenced. The structure of the *dSpm-8167B* element is the same as that of the *Spm-s-7991A1* element, although the extra trinucleotide at the *Spm-s-7991A1* insertion site is not present at the *dSpm-8167B* insertion site.

Genetic tests of the *dSpm* alleles: Although several of the *dSpm* elements have sustained such large deletions that they are unlikely to encode any *trans*-active functions, the alleles with the two longest elements (*a-m2-7995* and *a-m2-8167B*) were tested for their ability to affect expression of an *Spm-suppressible* allele. The full-length *a-m2-8167B* allele was also tested for its ability to complement the genetic defect in the *Spm-w* element to give an *Spm-s* phenotype. Plants that were heterozygous for one of the two alleles and a stable recessive *a* allele, but had no active *Spm* element, were intercrossed with plants homozygous for the *Spm-suppressible a-m1-5719A1* allele. The results are given in Table 6. Neither allele is able to inhibit expression of the *Spm-suppressible* allele. The results of complementation tests between the *a-m2-8745* (*Spm-w*) allele and *a-m2-8167B* alleles appear in Table 3, line 7, and are illustrated by the kernel in Figure 10c. The variegation pattern obtained is that expected for the *Spm-w* element, hence the *dSpm* element cannot complement the *Spm-w* defect and is devoid of all known *trans*-active functions of the element.

The *dSpm-8167B* element has so far proved structurally indistinguishable from the *Spm-s* element of the *a-m2-7991A1* allele. There are two possible explanations of its properties. It either has a lesion sufficiently small to have escaped detection so far or it is an inactive element. We have made an extensive search for progeny of the *a-m2-8167B* allele that show genetic reactivation and have found none (data not shown). We have also observed that the inactive *Spm-s* element of the *a-m2-7991A1-i* allele can be readily reactivated by the introduction of an *Spm-w* element, while the *dSpm-8167B* element cannot be (N. FEDOROFF, unpublished data). Thus the *dSpm-8167B* element is probably defective by virtue of a small, as yet undetected genetic lesion, although the possibility that it is cryptic element cannot be completely eliminated.

Deletion endpoints often occur in or adjacent to sequences with homology to subterminal consensus repeats: MCCLINTOCK (1955) reported that mutations that affect the genetic properties of *dSpm* elements occur in the presence, but not in the absence, of a

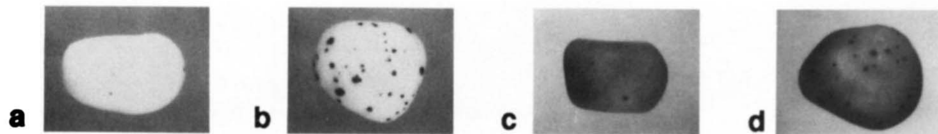

FIGURE 10.—Phenotypes of *Spm-dependent* and *Spm-suppressible* alleles with *Spm-w* and *Spm-s* elements. a, The *Spm-suppressible a-m1-5719A1* allele with *Spm-w* (*a-m2-8745* allele); b, *a-m1-5719A1* allele with *Spm-s*; c, the *Spm-dependent a-m2-8167B* allele with *Spm-w* (*a-m2-8745* allele); and d, *a-m2-8167B* with *Spm-s*. The *a-m1-5719A1* allele gives a palely pigmented phenotype in the absence of *Spm*, while the *a-m2-8167B* allele is colorless in its absence (see Figure 11a).

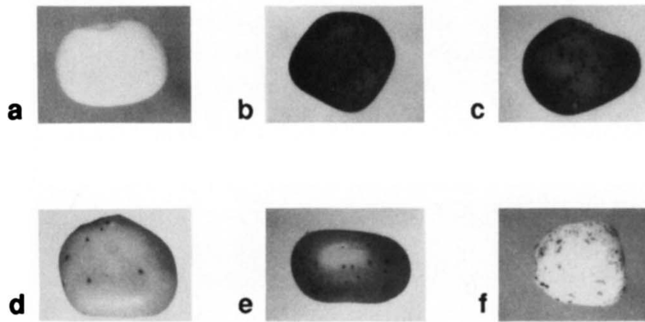

FIGURE 11.—Phenotypes of the *dSpm* alleles. a, Colorless phenotype observed with all *dSpm* alleles in the absence of an *Spm* element; b, *a-m2-7995*, with *Spm-s*; c, *a-m2-7977B*, with *Spm-s*; d, *a-m2-8004*, with *Spm-s*; e, *a-m2-8167B*, with *Spm-s*; f, *a-m2-8417*, with *Spm-s*.

TABLE 6

Tests of *a-m2 dSpm* alleles for suppression of *a-m1-5719A1* allele

		Percent kernels of each phenotype		
a-m2 parent	Backcross parent	Pale, *sh2*	Pale, *Sh2*	Colorless
a-m2-7995 Sh2/a sh2	*a-m1-5719A1 sh2*	49.4	50.6	0
a-m2-8167B Sh2/a sh2	*a-m1-5719A1 sh2*	49.6	50.4	0

trans-acting *Spm* element. It has been shown in several studies, including the present one, that such mutations are frequently the result of intra-element deletions (SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985b; TACKE *et al.* 1986). The implication of these observations is that an *Spm*-encoded protein is involved in the production of intra-element deletions. The results of our present analyses show that the endpoints of intra-element deletions frequently coincide with sequences within the element that are homologous to a short sequence present in several copies near each end of the element. As first noted by SCHWARZ-SOMMER *et al.* (1984), there is a repetitive region commencing within 6 bp of the element's 13-bp terminal inverted repetition (CACTACAAGAAAA) at each element end. The repetitive sequences, which we refer to as the "subterminal repetitive regions," extends to a distance of 180 bp from the element's left end and 299 bp from its right end. The short repetitive elements that comprise the subterminal repetitive regions are compiled in Table 7, revealing that there is a 12-bp consensus repeat sequence (CCGACACTCTTA), which tends to have adjacent A residues at one end. There are 9 copies of the 12-bp repeat at the element's left end and 15 copies at its right end; they occur about equally frequently in each orientation. The 12-bp repetitive motif is highly conserved. All 24 copies are at least 75% homologous to the consensus repeat and 17 of the 24 copies (70%) are either identical to the consensus sequence or differ by only 1 nucleotide. The remain-

TABLE 7

Subterminal repeats at *Spm* ends

	Sequence	First nucleotide[a]
Left end:		
1.	AACTGACACTCCTT i[b]	19
2.	CCCCGACACTCTTT i	37
3.	GGCCGACACTCTTA d	51
4.	AACCCACACTTTTA i	70
5.	CACCGACACTCTTA d	92
6.	CCCCGACACTCTTA i	107
7.	AACCGACGCTTTTA d	132
8.	AACCCACACTCTTA i	147
9.	CACCGACACTCTTA d	169
Right end:		
1.	GGCCGACACTCTTA i	7985
2.	CCCCCACACTCTTA d	7999
3.	GGCCGTCACTCTTA i	8045
4.	GGCCGACACTTTTA d	8069
5.	AACCGACACTCTTA i	8084
6.	AACCGACACTTTTA d	8108
7.	GACCGACGCTCTTA i	8123
8.	TCCCCACACTTCTA d	8137
9.	AATGGACGCTCTTA i	8155
10.	AGCCGTCACTCTAA i	8169
11.	GACCGACACTCTTA i	8182
12.	AACCGACACTCTTA d	8198
13.	AGCCGACACTCTAA i	8213
14.	ACCCCACACTCTTA i	8235
15.	GGCCGACACTCCTT d	8254

Consensus:

A	11	12	0	0	0	22	0	21	0	0	0	0	2	21
C	5	5	23	22	5	0	24	0	24	0	19	3	0	0
G	7	7	0	1	19	0	0	3	0	0	0	0	0	0
T	1	0	1	1	0	2	0	0	0	24	5	21	22	3
	a	a	C	C	G	A	C	A	C	T	C	T	T	A

[a] The number corresponds to the position of the first nucleotide of the sequence homologous to the 12-bp consensus sequence. Nucleotide 1 corresponds to the left end of the element, as depicted in Figure 4.

[b] To facilitate comparison of sequences, all have been listed in the same order with respect to homologous nucleotides; i indicates inverted order and d indicates direct order (see above).

A. ***dSpm - 7977B***

```
Left breakpoint:

                    1074 •
..GAGACTAGGCATATATCTTGATATGAGCAAAGTAGTCAGAT..
      :     ::::  :: :::      :
 5\12      CCGACACTCTTA TAAGAGTGTCGG       6\12
           ----------->  <-----------

Right breakpoint:

                         8082
..CGGTACTGGCCGACACTTTTAACATAAGAGTGTCGGTTGCT..
          :::::::: :::   ::::::::::::
11\12     CCGACACTCTTA   TAAGAGTGTCGG  12\12
          ----------->   <-----------
```

B. ***dSpm - 7995***

```
Left breakpoint:

                         3315 •
..GGCAGTTAACCTCGACACTGGTAAACTCGTTGGGTTGAAGAGTCATGACTATCACAT..
             :::::::  ::
 9\12        CCGACACTCTTA
             ----------->

Right breakpoint:

                                    •8109
..ATAAGAGTGTCGGTTGCTTGTTGAACCGACACTTTTAACATAAGAGCGTCGGTCCCC..
                             :::::::: :::
                             CCGACACTCTTA   11\12
                             ----------->
```

FIGURE 12.—The sequences surrounding the left and right deletion breakpoints of the *dSpm-7995* and *dSpm-7977B* elements. The possible deletion breakpoints are indicated by the diagonal arrows over each breakpoint. The 12-bp consensus repeat is reproduced under the sequences that are immediately adjacent to the breakpoint and underlined by an arrow showing the orientation. The extent of homology with the consensus sequence is indicated.

der of the element has no sequences identical to the consensus repeat, but there are several internal sequences with substantial homology (>70%).

Of the 8 deletion breakpoints that we have sequenced, 6 occur within or at the end of a sequence with homology to the 12-bp consensus repeat. The most striking examples are provided by the *dSpm-7995* and *dSpm-7977B* elements. The sequences surrounding the deletion breakpoints in these elements are shown in Figure 12. The consensus 12-bp repeat of the subterminal repetitive region has been reproduced under the sequence adjacent to each breakpoint. Examination of the sequences reveals that there is homology to the 12-bp repeat at all 4 breakpoints and that each breakpoint coincides with one end of the sequence homologous to the 12-bp repeat. Although the *Spm-w* and *dSpm-8004* elements have somewhat more complicated rearrangements, including insertions and additional deletions, at least one breakpoint in each is located within a sequence that is homologous to the consensus 12-bp repeat. The implication is that the element-encoded protein involved in deletion-formation recognizes and promotes the occasional cleavage and religation of element se-

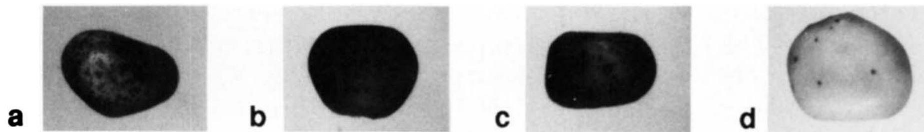

FIGURE 13. Phenotypes of mutant *a-m2* alleles with different excision frequencies in the presence of an *Spm-s*. a, *a-m2-8011*; b, *a-m2-7995*; c, *a-m2-7977B*; d, *a-m2-8004*.

quences resembling the subterminal 12-bp repeats, both at element ends and elsewhere in the element's sequence.

***Cis*-acting determinants of *Spm* excision frequency:** Mutations that affect the ability of a *dSpm* element to excise occur at a high frequency and several elements with mutations of this type have been subjected to molecular analysis (SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985b; TACKE *et al.* 1986). Mutant *dSpm* elements with a reduced frequency of excision often have deletions extending into the subterminal repetitive regions near element ends. The results of the present study show that there is a *cis*-determinant of transposition frequency at the element's left end, adjacent to, but not within the subterminal repetitive region.

The somatic excision patterns of several *a-m2* alleles are shown in Figure 13. The *Spm-w* element of the *a-m2-8011* allele has a high somatic excision frequency in the presence of an *Spm-s*, the *dSpm-7995* and *dSpm-7977B* elements show similar, intermediate frequencies, while the *dSpm-8004* allele shows a low frequency of somatic excision. All of the elements have identical terminal IR and insertion site sequences. The phenotypically similar *dSpm-7977B* and *dSpm-7995* elements contain 1.07 and 3.32 kb, respectively, of sequence from the element's left end and 203 bp and 178 bp, respectively, of sequence from the right end. Both deletions extend into the subterminal repetitive region at the right end, eliminating 4 and 5 copies of the 12-bp repeats from the *dSpm-7977B* and *dSpm-7995* elements, respectively. Both elements excise at a lower frequency in the presence of a *trans*-acting *Spm* element than does the longer *Spm-w-8011* element (Figure 13). This observation is consistent with the results of previous studies, which have been interpreted as suggesting the involvement of the element's subterminal repetitive regions in transposition (SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985b; TACKE *et al.* 1986).

The *dSpm-8004* element exhibits a very low excision frequency in the presence of a *trans*-acting *Spm-s*, but contains all of the element's subterminal repetitive regions. The *dSpm-8004* element comprises 275 bp of the element's left end and 800 bp of the element's right end. As indicated above, the left deletion breakpoints in the *dSpm-7995* and *dSpm-7977B* alleles are located 1.07 and 3.3 kb from the end of the element. Since the two alleles containing these elements are similar in phenotype, the sequence that distinguishes them from the *dSpm-8004* element is located between 0.28 and 1.07 kb from the element's left end. The *dSpm-8004* deletion eliminates a GC-rich sequence that corresponds to the first exon, as well as part of the first intron (PEREIRA *et al.* 1986). These observations identify a nonrepetitive *cis*-acting determinant of transposition frequency within the element's transcription unit.

DISCUSSION

Effect of mutations on the *Spm* element's *trans*-active functions: There are two types of mutant *Spm* elements in the present group of alleles: *Spm-w* and *dSpm* elements. *Spm-w* elements comprise a common class of mutations and the genetic behavior of the *Spm-w* element we have analyzed is representative (MCCLINTOCK 1957, 1962). The *Spm-w* element *trans*-activates transposition less frequently and later in development than the *Spm-s* element, but retains the element's ability to affect expression of *Spm-dependent* and *Spm-suppressible* alleles. The *Spm-w* element's internal 1.7-kb deletion eliminates parts of both of the element's major ORFs, indicating that neither ORF need be intact for expression of either of the element's known genetic functions. The *Spm-w* mutation is associated with a reduction in the amount of the element's major transcript. Moreover, the deletion in the *dSpm-7995* element, which lacks all of the *Spm*'s *trans*-active functions, partially overlaps that in the *Spm-w* element and eliminates sequences encoding most of the transcript. These observations suggest that the element's major transcript encodes its transposition function. The full-length *dSpm-8167B* element is also defective for all of the element's known genetic functions and is unable to complement the defect in the *Spm-w* element. Taken together, these results suggest that the *Spm* element's *trans*-active functions are not encoded by separate, complementing genes.

***Cis*-determinants of excision frequency:** Mutations that reduce the ability of a *dSpm* element to excise in response to a *trans*-acting *Spm* element were first identified and studied by MCCLINTOCK (1955), who designated them "changes of state" of a mutant locus. The results of molecular studies have revealed that such mutations are commonly attributable to intra-element deletions that extend into the element's subterminal repetitive regions (SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985b; TACKE *et al.* 1986). SCHWARZ-SOMMER *et al.* (1984) first drew attention to the possibility that the subterminal repetitive sequences form an intra-molecular duplex and SCHWARZ-SOMMER *et al.* (1985b) suggested that the

stability of the proposed intra-element duplex was important in determining excision frequency. This proposal was based on the observation that a *dSpm* element with a deletion extending into the subterminal repetitive region at the element's left end exhibits a markedly lower excision frequency than the parent *dSpm* element containing 0.86 kb of sequence from the element's left end (SCHWARZ-SOMMER *et al.* 1985b). Our finding that the *dSpm-8004* element retains the complete subterminal repetitive regions from both element ends, yet excises at a low frequency, indicates that excision frequency is determined by a sequence adjacent to, but not in, the subterminal repetitive region and located between 0.28 and 1.07 kb from the element's left end. The observation that a 2.2-kb *dSpm* element containing 0.86 kb from the element's left end and 1.3 kb of its right end excises at a high frequency from all three loci into which it is known to have inserted (FEDOROFF *et al.* 1984; SCHWARZ-SOMMER *et al.* 1984, 1985b; SCHIEFELBEIN *et al.* 1985) suggests that the *cis*-determinant of transposition frequency can be further localized between 0.27 and 0.86 kb from the element's left end. The implicated sequence comprises most of the element's first GC-rich exon and part of its first intron (PEREIRA *et al.* 1986).

Although the deletions in the *dSpm-7995* and *dSpm-7977B* elements extend into the subterminal repetitive region at the element's right end and reduce the element's excision frequency, we cannot confidently attribute the reduction to the loss of part of the repetitive sequence, because there are no deletions with an endpoint just outside of the right subterminal repetitive region. Perhaps the strongest indication that an element-encoded protein recognizes and interacts with the subterminal repetitive regions is provided by the observation that element-promoted intra-element deletions often occur in or immediately adjacent to either one of the 12-bp repeats near an element end or a homologous internal element sequence (Figure 12). But whether this is relevant to transposition is unclear. It could, for example, be incidental to a different function of the element's transposition protein, mediated by binding to the 12-bp repeat.

***Spm-dependent* and *Spm-suppressible* alleles.** An *Spm* element can have markedly different effects on genes with *dSpm* insertions, either activating or inhibiting their expression. Several suppressible alleles of the *bronze* and *a* loci have been examined (NELSON and KLEIN 1984; SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985b; TACKE *et al.* 1986). Whether an allele is *Spm-dependent* or *Spm-suppressible* is not determined either by the particular target gene or by internal element sequences. The same 2.2-kb element has given suppressible alleles of two different genes and derivatives with *dSpm* elements as short as 0.8 kb retain *Spm*-suppressibility (SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985b; TACKE *et al.* 1986). Similarly, *Spm*-dependent expression among the *a-m2* alleles is observed with insertions ranging from a full-length element to the 1.3-kb *dSpm-7977B* and 1.1-kb *dSpm-8004* elements. Thus element ends appear to suffice to confer either *Spm*-suppressibility or *Spm*-dependence. The explanation for the opposite effects of *Spm* on gene expression in the two types of alleles must therefore lie either in the orientation of the *dSpm* insertion or in the position of the insertion site. Since the orientation of the *dSpm* insertions in the *Spm-suppressible a-m1-5719A1* and the *a-m2* alleles is the same, the difference is most probably attributable to the location of the insertion site in the locus (SCHWARZ-SOMMER *et al.* 1985b; TACKE *et al.* 1986). The *dSpm* insertion site in the *a-m1* alleles has been identified at an exon/intron junction within the gene, while the *a-m2* insertion site is located upstream of the gene's proposed transcription start site (SCHWARZ-SOMMER *et al.* 1987).

Expression of the *a* gene of the *a-m2* alleles: The most striking property of the *a-m2* alleles is that expression of the gene is dependent on the *Spm* element. When the resident element is an *Spm-s,* the gene is continuously expressed at an intermediate level, except in the intensely pigmented revertant somatic sectors. If the *Spm-s* element becomes inactive, so does the gene. If the element returns to an active state, the gene is re-expressed. Intra-element deletions that inactivate the element also inactivate the gene, yet leave its expression under the control of the element, even when most of the element is deleted. Implicit in the physical separation of the activating *Spm* element from the *dSpm* sequence at the locus is that expression of the gene is mediated by an interaction between a diffusible element-encoded gene product and the *dSpm* sequence at the locus.

Element sequences that mediate expression of the *a* gene: The *a* locus transcription unit has recently been characterized, and the *Spm* insertion site in the *a-m2* alleles is 99 bp upstream of the putative 5′ end of the gene (SCHWARZ-SOMMER *et al.* 1987). The gene and the *Spm* element of the *a-m2* alleles are transcribed divergently. Hence the *Spm* element's transcription start site, identified at nucleotide 209 by PEREIRA *et al.* (1986), is located 0.3 kb from the *a* gene's transcription initiation site. Although nothing is known about the sequences that regulate expression of the gene, the *Spm* insertion site is probably within its upstream control region. It appears likely that the element sequences that mediate *a* gene expression in the *a-m2* alleles are near the element's left end. Neither internal sequences nor sequences at the right end are likely to be important, since similar levels of *a*

gene expression are associated with the *a-m2-8011* allele, in which the element's right end is at a distance of more than 6 kb from the *a* gene, and with the *a-m2-7995* and *a-m2-7977B* alleles, whose *dSpm* elements are missing 60 and 85% of the internal sequence and all but 178 and 203 bp, respectively, of the element's right end. It appears more likely that sequences near the element's left end mediate *Spm*-dependent expression of the *a* gene, since they are present in immediate proximity to the gene in all of the *dSpm* alleles examined. Because *Spm*-dependent *a* gene expression is observed with the *a-m2-8004* allele, the minimal requisite sequence probably lies within the 275 bp of the left end present in the *dSpm-8004* element (Figures 4 and 8). This region of the element comprises the leftmost 13-bp terminal IR, the 180-bp left subterminal repetitive region, the transcription start site and 66 bp of the element's first exon (PEREIRA *et al.* 1986). However, the level of *a* gene expression exhibited by the *a-m2-8004* allele is substantially less than that exhibited by the *a-m2-7977B* allele (Figure 13), whose *dSpm* element contains an additional 0.8 kb of the *Spm* element's left end, including the GC-rich first exon and part of the first intron. Thus sequences in the immediate vicinity of the element's own transcription start site and near the 5′ end of the transcription unit may serve as promoter or enhancer elements (or both) for expression of the adjacent *a* gene in the presence of an element-encoded gene product.

Control of gene expression by transposable elements and retroviruses: There are examples in other organisms of transposable element and retroviral insertions that substantially alter regulation of the target gene. Insertion of a yeast Ty retrotransposon often enhances expression of the gene and brings its expression under mating type control (ERREDE *et al.* 1980; ROEDER and FINK 1983). The results of recent studies suggest that the regulatory effects of the Ty insertions are attributable to sequences within the element that are homologous to regulatory sequences of genes under mating type control (ROEDER, ROSE and PEARLMAN 1985; ERREDE *et al.* 1985). Retroviral and retrotransposon insertions that alter gene regulation have also been studied in both mammals and Drosophila. In some cases, a retroviral insertion inactivates a nearby gene by a mechanism that involves DNA methylation and resembles the reversible inactivation of the *Spm* element (JAHNER and JAENISCH, 1984, 1985). In other cases, genes come under the direct or indirect influence of retroviral promoters, enhancer elements and other developmental controls (FLAVELL *et al.* 1980; JAENISH *et al.* 1981; VARMUS 1983; ZACHAR *et al.* 1985; CAMPUZANO *et al.* 1986). Whatever the origin of the transposon's regulatory system, the immediate source of the mutant gene's new pattern of regulation is the insertion itself.

Control of gene expression by the *Spm* element: The way in which *Spm* insertions influence gene expression is unique. Retrotransposon insertions in yeast and Drosophila can either enhance or inhibit gene expression, and second site suppressors of the insertion mutations have been identified (MODOLELL, BENDER and MESELSON 1983; SIMCHEN *et al.* 1984; WINSTON *et al.* 1984). However, the suppressors do not map to sites of homologous transposable element insertions, are recessive, and their ability to suppress (reverse the mutant phenotype) appears to be attributable to reduced transcription of the transposon (ZACHAR *et al.* 1985; CAMPUZANO *et al.* 1986). In the case of both *Spm-suppressible* and *Spm-dependent* alleles, the source of the gene product that influences expression of the mutant gene with a *dSpm* insertion is the *Spm* element itself. An effect on gene expression resembling that of *Spm* on an *Spm-suppressible* allele has recently been identified in *Drosophila* and is believed to result from the interaction of a *P* element-encoded repressor with a defective *P* element at the *sn* locus (H. ROBERTSON and W. ENGELS, personal communication). SCHWARZ-SOMMER *et al.* (1985b) have suggested that the *Spm*'s inhibitory effect on *Spm-suppressible* alleles results from the ability of the element's transposase to bind to the *dSpm* element ends and block transcription from the gene's promoter. We believe it more likely to be attributable to an element-encoded regulatory mechanism, as detailed below.

***Spm* has a positive autoregulatory mechanism:** Although it has been postulated that *Spm* encodes both positive and negative regulators (NEVERS and SAEDLER 1977), most of the existing evidence on the interactions between *Spm* elements indicates that the *Spm*-encoded regulatory mechanism is a positive one. Unlike the *Activator* element of maize, which shows a decrease in the frequency and a delay in the developmental timing of transposition with increasing element copy number, the *Spm* element does not show a dosage effect (MCCLINTOCK 1948, 1955, 1957). Evidence that there is an element-encoded positive regulatory gene product comes from MCCLINTOCK's (1957–1959, 1971) observation that an inactive element can be transiently activated by the introduction of an active one. This includes elements that have undergone inactivation recently, as described for the *a-m2-7991A1-i* element (Fig. 6) and for an element MCCLINTOCK (1957) designated *Modifier*. The *Modifier* element belongs to the *Spm* family by the criterion that *Spm trans*-activates its transposition, but its characteristic ability to enhance excision frequency is dependent on the simultaneous presence of either an *Spm-s* or an *Spm-w* element. The ability of the *Spm* element to activate expression of the *a* gene in the

dSpm a-m2 alleles parallels the interactions between elements, suggesting that the *a* gene is directly under the control of the *Spm* element's positive autoregulatory mechanism. It also suggests that the element's positive regulatory gene product normally interacts with an element sequence or sequences to activate expression of the element. We further suggest that the same interactions that mediate *a* gene activation in *Spm-dependent* alleles underlie the *Spm*'s ability to inhibit expression of *Spm-suppressible* alleles, the difference in the phenotype depending on both the effect of the original insertion on gene expression and on the insertion site within the gene.

***Spm* expression is also controlled by a negative mechanism:** There is genetic evidence that maize transposable elements can be reversibly inactivated (McClintock 1957–1959, 1971; Peterson 1966) and growing molecular evidence that DNA modification, most probably methylation, is the molecular mechanism underlying the inactivation of elements belonging to several different families (Fedoroff, Wessler and Shure 1983; Dellaporta and Chomet 1985; Bennetzen 1985; Chandler and Walbot 1986; Schwartz and Dennis 1986; Chomet, Wessler and Dellaporta 1987), including *Spm* (Cone, Burr and Burr 1986). The mechanism that inactivates the *Spm* element is probably not element-encoded since there is no evidence that it co-segregates with the element, although the involvement of defective elements cannot be excluded. The observation that the *a* gene and inserted *Spm-s* elements in *a-m2* alleles undergo simultaneous inactivation suggests that the locus has come under the control of the same negative mechanism as the element (McClintock 1962).

The inactivity of the *a* gene in *a-m2* derivatives with either an inactive or an internally deleted *dSpm* can be explained in either of two ways. First, the lack of *a* gene expression may be due simply to the absence of an *Spm*-encoded positive regulatory gene product that promotes both *Spm* and *a* gene expression. In the *a-m2* alleles, the regulatory sequences that normally control *a* gene expression are displaced 1–8 kb upstream by the inserted element. Transcription of the *a* gene may therefore have become dependent on *cis*-acting sequences in the element and *trans*-acting factors encoded by the *Spm* element. The *a* gene is therefore not expressed in the absence of a functional *Spm* element. The second and alternative explanation for the lack of *a* gene expression is that the gene has come directly under the control of the same negative regulatory mechanism that inactivates the *Spm* element. This possibility is suggested by the long-standing genetic observation that the *a* gene in several *a-m2* alleles can be expressed in the absence of an active *Spm* element under certain circumstances.

Presetting and the mechanism of *Spm* regulation: McClintock (1963–1965a) reported that the *a-m2-7995* and *a-m2-7977B* alleles can be *preset* by an *Spm* element for subsequent expression in the absence of the element. If either allele is present in a plant with an *Spm* and the element subsequently segregates away from the allele at meiosis, many of the kernels receiving the allele exhibit an unexpected and distinctive pigmentation pattern (Figure 14). Such kernels are not colorless throughout, as anticipated from the phenotype of the allele when propagated in the absence of *Spm* (Figure 14d), nor are they palely pigmented with deeply pigmented sectors resulting from somatic excision of the element, as observed in the presence of an *Spm* element (Figure 14a). Instead, they show areas of irregular pigmentation, as if the gene had commenced development able to be expressed and had undergone inactivation in some cell lineages during kernel development (Figure 14, b and c). Preset patterns are generally not heritable (McClintock 1965a). Continued expression of the *a* gene is also observed with several *a-m2* alleles when the *Spm* element present in the plant undergoes somatic inactivation (McClintock 1968). Somatic sectors within which the element has become inactive can be identified in *a-m2-7995* and *a-m2-7977B* kernels as areas of the aleurone exhibiting a different pigmentation intensity than the surrounding tissue and devoid of deeply pigmented somatic revertant sectors (not shown). Although the *a-m2-8004* allele does not exhibit presetting, it responds to inactivation of the *Spm* element in the same way as the *a-m2-7995* and *a-m2-7977B* alleles, except that kernel sectors with an *Spm-i* exhibit deeply pigmented rims (McClintock 1965b, 1968). Hence expression of the *a* gene of the *a-m2* alleles can persist after the removal or inactivation of the *trans*-acting *Spm* element.

Continued expression of the *a* gene in the absence of the *Spm* is not likely to be explained by the persistence and gradual dilution of an *Spm*-encoded gene product, since most *dSpm* alleles respond immediately to the loss or inactivation of the *trans*-acting *Spm* by exhibiting the phenotype characteristic of the allele in the absence of the element (McClintock 1957–1958, 1965b). Instead, the ability of *Spm* to *preset* the *a* gene suggests that an *Spm* gene product effects a semistable change in the ability of the gene to be expressed. The *preset* gene is not permanently activated by the presence of the *Spm* element, yet its capacity for *Spm*-independent expression is propagated through a number of cell divisions after removal or inactivation of the element. Thus, *preset* expression reveals that the *a* gene with a *dSpm* insertion is not inherently incapable of being expressed in the absence of the *Spm* regulatory gene product. It further suggests that an *Spm*-encoded gene product can heritably affect the

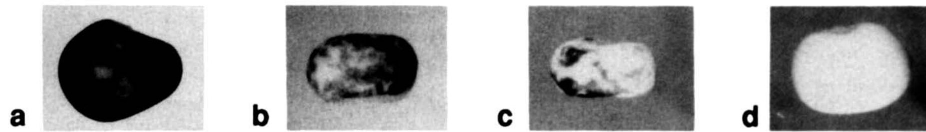

FIGURE 14.—Presetting of the *a* locus of the *a-m2-7995* allele by *Spm*. a, The phenotype of the *a-m2-7995* allele in the presence of an *Spm-s;* b and c, preset patterns of *a* gene expression observed after meiotic segregation of the *a-m2-7995* allele away from the *Spm* element; d, the *a-m2-7995* allele without an *Spm* element.

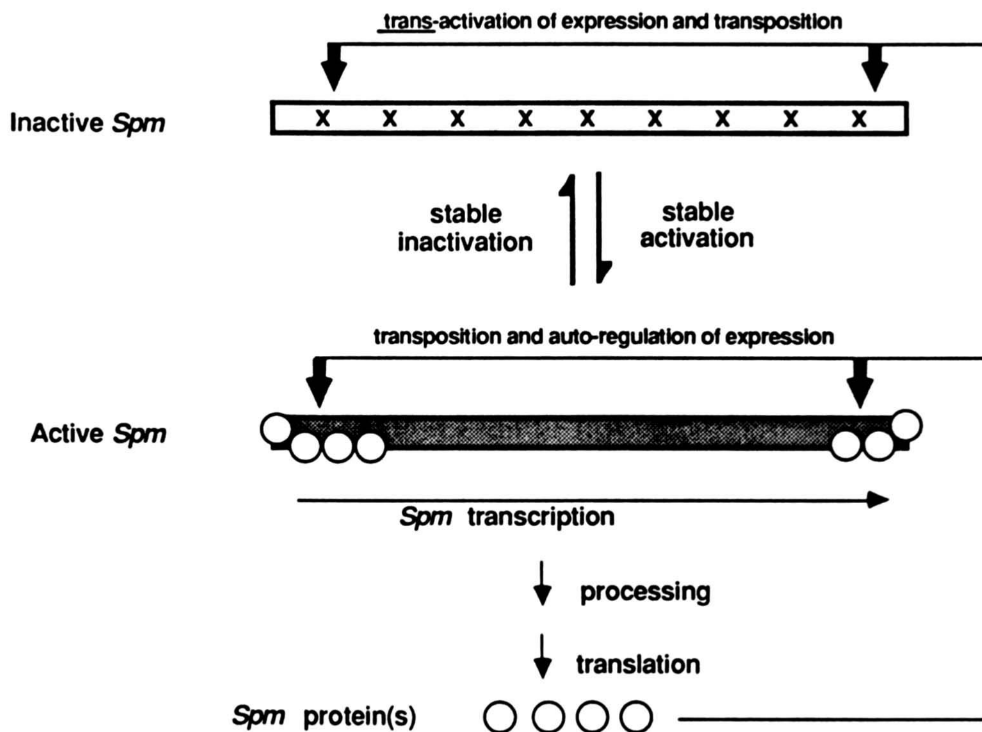

FIGURE 15. A model of *Spm* regulation. The *Spm* element is postulated to exist in two forms, active and inactive, which differ from each other in the extent of sequence modification. The active element is expressed to yield a protein or proteins, represented by open circles. The element-encoded protein(s) are postulated to interact with the element to promote transposition, as well as to promote transcription of the element, as represented by their location at and near element ends. The *x*'s in the diagram of the inactive element symbolize sequence modification; conversion of an inactive to an active element is postulated to occur by a change in the modification pattern. The ability of an active *Spm* element to interact with an inactive one is represented by the *arrows* above the diagram of the inactive element.

control of *a* gene expression. Because *a* gene expression in the *a-m2* alleles is mediated by the *Spm* insertion, the inactivity of the *a* gene, both in *dSpm* alleles as well as in alleles with an inactive *Spm-s* element, may be a direct consequence of the mechanism that inactivates the element. We suggest, therefore, that the significance of *presetting* is that it reveals a second function of the *Spm*'s positive regulatory mechanism, which is to maintain the element in a genetically active state by preventing or interfering with its inactivation.

A simple diagrammatic representation of our current concept of *Spm* regulation appears in Figure 15. We postulate that the *Spm* element encodes both a protein that participates in transposition and a positive regulatory protein. Although the results of our studies on mutant *Spm* elements suggest that the element's transposition and regulatory functions are not encoded by separate, complementing genes, they do not offer definitive proof that the functions reside in a single protein. However, the observations that intra-element deletions are element-promoted and tend to terminate in sequences homologous to the subterminal 12-nucleotide repeats implies that an element-encoded protein that can promote DNA cleavage and rejoining recognizes and binds to internal element sequences. The further observation that 9 copies of the 12-nucleotide subterminal repeat occupy more than half of the element sequence upstream of the transcription initiation site at nucleotide 209 (PEREIRA *et al.* 1986) raises the possibility that the same protein or protein complex is involved in the regulation of element transcription and the site-specific DNA cleavage and religation reactions involved in excision and transposition. We have inferred from the properties of the *a-m2* alleles that the *Spm*'s regulatory protein can not only *trans*-activate expression of an inactive element, but can also interfere heritably with the inactivating mechanism. The first inference is consistent both with MCCLINTOCK's (1957–1959, 1971) early genetic observations that an active *Spm* element can transiently activate an inactive one and our recent observation that the inactive *Spm-s* element of the *a-m2-7991A1-i* (Figure 6) allele is fully active in the presence of an *Spm-w* element (N. FEDOROFF, unpublished data). The further inference drawn from the *presetting* phenomenon that an *Spm* gene product can interfere heritably with maintenance of the inactive state is consistent with the recent finding that an *Spm-w* element promotes the genetic activation of cryptic *Spm* and *Modifier* elements (FEDOROFF 1986).

Evolutionary implications: The existence of a mechanism that stably inactivates the *Spm* element may be critical for its long term survival. There is ample evidence that stresses that induce chromosome breakage result in the activation of transposable elements (McCLINTOCK 1945–1947, 1950; NEUFFER 1966; DOERSCHUG 1968, 1973; BIANCHI, SALAMINI and RESTAINO 1969). Active elements are unstable by virtue of their tendency to sustain self-inflicted intra-element deletions (SCHIEFELBEIN *et al.* 1985; SCHWARZ-SOMMER *et al.* 1985b; TACKE *et al.* 1986). Our model of *Spm* regulation implies that once an element is activated, it maintains itself in an active state until it either undergoes an inactivation event or sustains an internal deletion. The implication is that an *Spm* element may have a higher probability of surviving genetically intact as an inactive, than as an active element. Intra-element deletions leave behind stable insertions with termini that provide target sequences for subsequent genetic rearrangements. Intra- and interchromosomal rearrangements involving transposable elements have been especially well-documented in Drosophila, yeast and maize (McCLINTOCK 1978; ROEDER and FINK 1980; BERG, ENGELS and KREBER 1980). Plant transposable elements excise by a slightly imprecise mechanism, leaving small sequence changes at the former insertion site (SACHS *et al.* 1983; FEDOROFF, WESSLER and SHURE 1983; SCHWARZ-SOMMER *et al.* 1985a). A newly activated element can, therefore, not only cause mutations by and consequent on insertion, but can promote chromosomal rearrangements between defective elements at different chromosomal sites. Thus from an evolutionary standpoint, the element constitutes a heritable, stress-inducible, self-limiting genetic mechanism for the production of mutations and chromosomal rearrangements at high frequency.

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