Molecular mechanisms in the developmental regulation of the maize *Suppressor-mutator* transposable element

Jo Ann Banks, Patrick Masson, and Nina Fedoroff

Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210 USA

The maize *Suppressor-mutator* (*Spm*) element can exist in one of three heritable forms: (1) a stably active form, (2) a stably inactive form, termed *cryptic*, and (3) a labile form, here termed *programmable*, in which the element exhibits one of a variety of heritable developmental programs of expression. Active elements are transcribed and are hypomethylated at sites upstream of the transcription start site, whereas inactive elements are transcriptionally silent and largely methylated at the upstream sites. Active (both stable and *programmable*), inactive *programmable*, and *cryptic* elements are unmethylated, partially methylated, and fully methylated, respectively, at sites within an 0.35-kb 80% G + C region just downstream from the transcription start site. An active *Spm* element in a genome with a *cryptic* element promotes its partial demethylation but not its transcriptional activation. In contrast, a *trans*-acting *Spm* promotes extensive demethylation and transcriptional activation of an inactive *programmable* element, as well as its heritable reactivation. These observations define the molecular components of the *Spm* element's developmental regulatory mechanism. We discuss their general relevance to the developmental regulation of gene expression.

[Key Words: Maize; transposable elements; *Suppressor-mutator*; methylation, transcriptional activation, developmental regulation]

Received July 7, 1988; revised version accepted September 19, 1988.

A striking and poorly understood property of maize transposable elements is that both element expression and the timing and frequency of element transposition are developmentally regulated. The early genetic studies of McClintock on the *Suppressor-mutator* (*Spm*) element and Peterson on the cognate *Enhancer* (*En*) element established the existence of several heritable, but interconvertible, forms of the *Spm* element (McClintock 1957, 1958, 1959, 1965a,b; Peterson 1966; Fowler and Peterson 1978). These include the *cryptic* form, in which the element is genetically silent. Both McClintock and Peterson also identified elements that were capable of undergoing reversible transitions between the active and inactive phases in a characteristic and heritable pattern during development (McClintock 1957, 1958, 1962, 1965a,b, 1971; Peterson 1966; Fowler and Peterson 1978). We showed recently that the genetic mechanism responsible for the developmental control of *Spm* expression has two components, which we designated the phase *setting* and the phase *program* (Fedoroff and Banks 1988). The phase *setting* determines whether the element is genetically active or inactive. The phase *program* determines the heritability of the phase setting, as well as when, where, and how frequently it will be reversed during plant development. The underlying genetic mechanism is heritable, reversible, and capable of promoting the incremental transition of the element

from a stably active to a stably inactive (*cryptic*) form, implying the involvement of multiple epigenetic events (Fedoroff and Banks 1988). In view of the accumulating evidence that the inactivation of gene expression, in general (for reviews, see Razin et al. 1984; Adams and Burdon 1985; Holliday 1987; Cedar 1988), and maize transposable elements, in particular (Fedoroff et al. 1983; Bennetzen 1985; Chandler and Walbot 1986; Schwartz and Dennis 1986; Chomet et al. 1987; Fedoroff et al. 1988a,b), are associated with DNA modification, we have investigated the relationship between *Spm* expression and the methylation pattern of sequences within and flanking an *Spm* element inserted at the maize *a* locus.

We studied three genetically distinguishable forms of the *Spm* element. These are the stably active form and the stably inactive form, termed *cryptic*. The third form is a labile one, here designated *programmable*, in which the element can be either in the active or inactive phase and can readily be both reset and reprogrammed (Fedoroff and Banks 1988). We show that all three forms of the *Spm* element are methylated throughout most of the element's 8.3-kb sequence but the flanking *a*-gene sequences are not methylated. *Spm* elements in the three different forms can be distinguished by the pattern of cytosine methylation immediately upstream and downstream from the element's transcription start site. Be-

cause there is genetic evidence that an inactive *programmable Spm*, but not a *cryptic Spm*, can be *trans*-activated by a second, active *Spm* element present in the same genome, we investigated the effect of the *trans*-activating element on the expression and methylation of inactive elements. The abundance of element-homologous transcripts is elevated markedly by a weakly active *Spm* (*Spm-w*) in a plant containing an inactive *programmable* element but not in one with a *cryptic Spm*. Both inactive *programmable* and *cryptic* elements show reduced methylation at the 5' end in the presence of an *Spm-w* element. In addition, the *Spm-w* promotes heritable reactivation of an inactive *programmable* element.

Results

Active, cryptic, and programmable *Spm* elements

The genetic properties of the *Spm* insertion alleles used in the present study have been described elsewhere in detail and are recounted here only briefly. All are derivatives of McClintock's original *a-m2* allele, in which a standard *Spm* element (*Spm-s*) inserted just upstream of the *a* gene (McClintock 1951, 1962; Masson et al. 1987; Fedoroff and Banks 1988). As illustrated in Figure 1, the element's transcription unit, which commences within 0.2 kb of the element's left (5') terminus, is opposite in orientation to that of the *a* gene (Pereira et al. 1986;

Masson et al. 1987; Schwarz-Sommer et al. 1987). Figure 2, a and b, shows the kernel phenotypes of the active form of the two alleles used here. They are designated the *a-m2-8167B* (*reactivated*) and *a-m2-7991A1* alleles. Kernels carrying either allele exhibit a relatively uniform pale background pigmentation due to a low level of expression of the adjacent *a* gene, which encodes an enzyme in the anthocyanin pigment biosynthetic pathway (Coe and Neuffer 1977). The two alleles differ phenotypically in the density of the variegation pattern, which is a function of the frequency and timing of element excision during kernel development (for further details, see Methods). The aleurone phenotype of both alleles is colorless when the resident *Spm* element is genetically inactive or transposition defective (Fig. 2c). There are no pigmented revertant sectors because the inserted element is not excised from the *a* locus, and there is no background pigmentation because the *a* gene is not expressed. Although phenotypically indistinguishable, an inactive element differs from a mutant element by its ability to return to the original active phase (McClintock 1962; Masson et al. 1987). Reactivation of the element is marked by both the reappearance of background pigmentation due to *Spm*-dependent *a*-gene expression and variegation attributable to somatic excision of the element (Fig. 2d,e).

The difference between the *cryptic* and *programmable* forms of the element resides in the developmental and

Figure 1. The structure and sequence organization of the *Spm* element. (a) A diagrammatic representation of the *Spm* element and its relationship to the *a* gene in the *a-m2* alleles. The 8.3-kb *Spm* element is inserted at a site 0.1 kb upstream of the transcription start site at the *a* locus (Schwarz-Sommer et al. 1987). The *a* gene and element are transcribed divergently. The solid areas of the *Spm* element represent the exons of its major 2.5-kb transcript (Pereira et al. 1986; Masson et al. 1987). The arrows above the element represent the open reading frames (ORFs) within its large intron. (b) A diagrammatic representation of the location of methylatable C residues at the 5' end of the element. Each vertical bar corresponds to the location of either a CG dinucleotide or CNG trinucleotide sequence.

Figure 2. Phenotypes of the kernel aleurone layer associated with *a-m2* alleles with *Spm* elements in different forms. (a) The *a-m2-7991A1* allele, *Spm* active; (b) the *a-m2-8167B* (*reactivated*) allele, *Spm* active; (c) the *a-m2-7991A1* allele, *Spm* inactive [the *a-m2-8167B* (*cryptic*) allele gives that same phenotype]; (d) the *a-m2-7991A1* allele, inactive *Spm* undergoing reactivation; (e) the *a-m2-8167B* allele, inactive *Spm* undergoing reactivation; (f) the *a-m2-8167B* allele, *trans-activating Spm* present.

hereditary stability of the element's phase setting. The allele with the *cryptic* element that we have used is designated the *a-m2-8167B* (*cryptic*) allele (Masson et al. 1987). As shown in Table 1, the allele is extremely stable. None of the 25,435 kernels examined exhibited spontaneous excision of the resident *Spm* element. The capacity of the resident element to be excised by a *trans-acting* *Spm* element is low, as is its ability to mediate activation of the adjacent *a* gene (Fig. 2f). The *Spm* element in this allele was originally identified as transposition defective, but it was found to be structurally indistinguishable from a standard *Spm* (*Spm-s*) element (Masson et al. 1987). Recently, we have succeeded in genetically reactivating the resident element (Fig. 2a; for details, see Methods), demonstrating that the element is functionally, as well as structurally, intact. We redesignated the element as *cryptic* to denote that its state of inactivity is exceedingly stable but that the element is nonetheless capable of returning to a fully functional state.

Inactive *Spm* elements in the *programmable* form exhibit a much lower genetic stability of the phase setting than does a *cryptic* *Spm* element (Masson et al. 1987; Fedoroff and Banks 1988). Moreover, the stability of the phase setting differs markedly among different deriva-

tives and depends on the plant part and sex of the gamete that transmits the element to the progeny (Fedoroff and Banks 1988). Table 1 gives illustrative data for two inactive derivatives of the *a-m2-7991A1* allele that are in the *programmable* form. The two lineages originated from different kernels produced on a single plant and showed differences in the frequency of element reactivation that persisted through three generations of plants (Fedoroff and Banks 1988). It is evident from even the limited amount of data shown in Table 1 that there are marked differences in the heritability of the inactive state upon transmission of the element to progeny through male and female gametes, as well as the gametes produced on the plant's main stalk and its tillers.

*The *Spm* element is methylated extensively, but the *a* gene is not*

Because the results of genetic studies on both *cryptic* and *programmable* forms of the element indicate that the phase setting is both heritable and reversible, we examined the accessibility of each element form to digestion with restriction enzymes that cannot cut C-methylated cleavage sites (Kessler and Holtke 1986). The general design of the experiments is illustrated by the

Table 1. Stability of the inactive phase in *cryptic* and *programmable* *Spm* elements

A. <i>Cryptic</i> <i>Spm</i>				B. Inactive <i>programmable</i> <i>Spm</i> ^a			
Parent	Gamete source	Total no. of kernels	Fully active <i>Spm</i> (%)	lineage 1		lineage 2	
				Total no. of kernels	Fully active <i>Spm</i> (%)	Total no. of kernels	Fully active <i>Spm</i> (%)
♀	ear I	2633	0	4751	3.5	A ^b 1030	39.4
	ear II	—	—	4036	3.9	B ^b 1878	19.9
	tiller	3038	0	2920	39.0	A 529	56.0
♂	mainstalk	19,764 ^c	0	3500	1.6	B 405	43.0
	tiller			6130	21.7	A 593	54.3
						B 164	90.2
						A 866	32.2
						B 681	19.7
						A 1786	61.8
						B 2641	50.5

^a Lineages 1 and 2 in B were derived from a single parent plant but from progeny plants grown from different kernels. The numbers reported in lineages 1 and 2 are taken from the families designated 2195 and 2199, analyzed in detail in Fedoroff and Banks (1988). All of the plants in families were grown from kernels with a completely or almost completely inactive *Spm* element. The two parent ears from which the kernels were selected gave 46.5% (lineage 1) and 10% (lineage 2) of kernels with an inactive *Spm* element. Progeny of these plants were examined for the fraction of kernels showing variegation throughout the aleurone layer of the kernels, indicating that the element transmitted through the gamete in question was in the fully active state. The results are given separately for elements transmitted through female gametes produced on the main stalk (ear I, ear II) and tillers and transmitted through male gametes produced on the main stalk and tillers, except for the *cryptic* element.

^b A and B plants were grown from kernels that showed no *Spm* activity and one or two revertant sectors, respectively, indicating almost no *Spm* activity.

^c Among 19,764 gametes tested in outcrosses, 4 kernels receiving the *Spm* element showed variegation throughout the kernel. Because the *Spm* parent was heterozygous for the *a* allele containing the inactive *Spm* and an allele with a *dSpm* element [*a-m2-8167B* (*cryptic*) *Sh2/a-m1-5719A1 sh2*], each test cross onto a plant that was homozygous for the *a* and *sh2* alleles produced equal numbers of kernels that did and did not receive an inactive *Spm* element. Among those kernels that received the *dSpm* tester allele [*a-m1-5719A1*], there were also four fully variegated kernels. This observation, together with the fact that the phenotype of the *Sh2* variegated kernels was that of the original *a-m2-8167B* (*cryptic*) allele (Fig. 2f), rather than that of the fully reactivated element at the *a* locus (Fig. 2a), indicates that the variegated kernels were attributable to activation of silent *Spm* elements in the tester parent, rather than spontaneous reactivation of the *cryptic* element in the *a-m2-8167B* (*cryptic*) allele.

Figure 3. Methylation of the *PvuII* and *PstI* sites within and adjacent to the *Spm* element. (A) The location of the methylation-sensitive *PvuII*, *PstI*, and *SmaI* restriction sites and the methylation-insensitive *BamHI* sites within the *Spm* element and *a* gene. To assess the methylation state of the *PvuII* sites, genomic DNA was isolated from plants containing either *programmable* (active and inactive) or *cryptic* *Spm* elements, digested with *PvuII* and *SmaI*, and hybridized with the *a*-gene probe shown in A. Fragments with homology to the probe that can be produced by digestion with *PvuII* and *SmaI* are shown. To assess methylation of the *PstI* sites, 3.8-kb *BamHI* fragments were gel purified from *BamHI*-digested genomic DNA samples, then digested with *PstI*, and hybridized to the *a*-gene probe shown. (Bottom) The fragments homologous to the probe that will be produced, depending on the methylation state of the various *PstI* sites. (B) Results of a blot hybridization experiment to determine methylation of the *PvuII* sites. Digestion of genomic DNA with *SmaI* alone yields a 9.8-kb fragment homologous to the *a*-gene probe, indicating that the *SmaI* sites flanking the *Spm* elements are cleaved (not shown). The 2.4-kb fragment in each lane corresponds to the *a* allele present on the homolog in each plant. (C) Results of a blot hybridization experiment to assess methylation of the *PstI* sites. (●) Uncleaved, methylation-sensitive restriction site; (○) cleaved, methylation-sensitive restriction site; (∅) cleaved, methylation-insensitive restriction site. (Bm) *BamHI*; (P) *PstI*; (Pv) *PvuII*; (Sm) *SmaI*.

diagram shown in Figure 3A. The observed fragments are those expected if the methylation-sensitive sites in the *a*-locus sequence are sensitive to cleavage. If the enzyme used has cleavage sites within the element, but at a distance of more than 1 kb from the end containing its transcription initiation site, the observed fragments are those expected if all of the sites are methylated and therefore resistant to digestion. This is true both for enzymes inhibited by the methylation of the C residue in the dinucleotide CG and for those inhibited by methylation of the C residue in the trinucleotide CNG, which is commonly methylated in plant DNA (Gruenbaum et al. 1981). Representative blots illustrating the outcome of such experiments with the restriction endonucleases *PstI* and *PvuII* are shown in Figure 3B. All of the results are reported in Table 2 and represented diagrammati-

cally in Figure 6. The data show that most methylatable C residues are methylated in the element, but not in the flanking *a* locus sequence, regardless of the element's phase of activity.

Active, cryptic, and inactive programmable forms of the Spm differ in the extent of methylation at the element's 5' end

As originally noted by Pereira et al. (1986) for the cognate *Enhancer (En)* element, there is a markedly GC-rich sequence near the element's 5' end, commencing just on the 3' side of its transcription initiation site at nucleotide 209. The diagram in Figure 1b highlights the high density of methylatable CG dinucleotides and CNG trinucleotides between 245 and 565 bp from the ele-

Table 2. Restriction fragments comprising part of the *a* gene and inserted *Spm* element obtained after digestion of genomic DNA with methylation-sensitive and methylation-insensitive restriction enzymes

Enzymes	Possible fragment lengths (kb) ^a	Observed fragment lengths (kb)					
		Active	Active programmable	Inactive programmable	Inactive programmable + <i>Spm</i> -weak	Cryptic	Cryptic + <i>Spm</i> -weak
<i>Pvu</i> II + <i>Sma</i> I	6.6 <u>S</u> m1, <u>P</u> v1 6.6 <u>S</u> m1, <u>P</u> v1,2 6.6 <u>S</u> m1, <u>P</u> v1,2,3 8.1 <u>S</u> m1, <u>P</u> v1,2,3,4 9.7 <u>S</u> m1, <u>P</u> v1,2,3,4, <u>S</u> m2	9.7	9.7	9.7	9.7	9.7	9.7
<i>Pst</i> I + <u><i>Bam</i></u> HI	0.5 <u>P</u> 1,2/3 1.4 <u>P</u> 2/3,4 2.2 <u>P</u> 2/3,4,5 2.4 <u>P</u> 2/3,4,5,6 2.9 <u>P</u> 2/3,4,5,6, <u>B</u> m2	0.5	0.5	0.5	0.5	0.5	ND
<i>Pst</i> I + <u><i>Sca</i></u> I	0.5 <u>P</u> 1,2/3 1.4 <u>P</u> 2/3,4 1.5 <u>P</u> 2/3,4, <u>S</u> c1	ND	0.5	0.5	0.5	0.5	ND
<i>Pst</i> I + <u><i>Eco</i></u> RI	0.5 <u>P</u> 1,2/3 1.4 <u>P</u> 2/3,4 1.9 <u>P</u> 2/3,4, <u>R</u> 1	ND	0.5	0.5	0.5	0.5	ND
<i>Pst</i> I + <i>Sma</i> I	0.5 <u>P</u> 1,2/3 1.4 <u>P</u> 2/3,4 2.2 <u>P</u> 2/3,4,5 2.4 <u>P</u> 2/3,4,5,6 8.7 <u>P</u> 2/3,4,5,6, <u>S</u> m2	0.5	0.5	0.5	0.5	0.5	0.5
<i>Ava</i> I + <u><i>Eco</i></u> RI	1.8 <u>A</u> 1,2 2.8 <u>A</u> 1,2, <u>R</u> I	ND	1.8	1.8	1.8		ND
<i>Ava</i> I + <u><i>Bam</i></u> HI	1.8 <u>B</u> m1/ <u>A</u> 1,2 3.8 <u>A</u> 1,2, <u>B</u> m2	1.8	1.8	1.8	1.8	3.8	ND
<i>Sal</i> I + <u><i>Bam</i></u> HI	1.1 <u>S</u> 1,2 3.2 <u>S</u> 1,2,3 3.4 <u>S</u> 1,2,3, <u>B</u> m2	1.1	1.1	1.1	1.1		1.1
<i>Sal</i> I + <i>Sma</i> I	1.1 <u>S</u> 1,2 3.2 <u>S</u> 1,2,3 9.2 <u>S</u> 1,2,3, <u>S</u> m2	ND	1.1	1.1	1.1	3.4	ND
<i>Sal</i> I + <u><i>Kpn</i></u> I	1.1 <u>S</u> 1,2 2.0 <u>S</u> 1,2, <u>S</u> c1	ND	1.1	1.1	ND	2.0	ND
<i>Ava</i> I + <i>Sal</i> I	1.1 <u>S</u> 1, <u>S</u> 2 1.3 <u>S</u> 1,2, <u>A</u> 2	1.1	1.1	1.1	1.1		ND
+ <i>Sma</i> I	9.2 <u>S</u> 1,2,3, <u>S</u> m2 0.9 <u>B</u> 1,2 1.0 <u>B</u> 1,2,3 3.0 <u>B</u> 1,2,3, <u>B</u> m2			9.2		9.2	ND
<i>Bgl</i> I ^c + <u><i>Bam</i></u> HI	0.9 <u>B</u> 1,2 1.0 <u>B</u> 1,2,3 3.0 <u>B</u> 1,2,3, <u>B</u> m2	0.9	0.9	0.9	0.9		ND
<i>Eco</i> 0109	3.4 <u>E</u> 2, <u>H</u> d4 6.2 <u>E</u> 2,3, <u>H</u> d4 8.0 <u>H</u> d2/3, <u>E</u> 2,3, <u>H</u> d4			3.4	3.4	3.4	ND
+ <u><i>Hind</i></u> III ^b	1.4 <u>B</u> m1, <u>E</u> 1 3.8 <u>B</u> m1, <u>E</u> 1, <u>B</u> m2	8.0	8.0	8.0	8.0	8.0	ND
<i>Eco</i> 0109 + <u><i>Bam</i></u> HI	0.5 <u>H</u> 1, <u>H</u> 2 0.2 <u>H</u> 2, <u>H</u> 3 0.6 <u>H</u> 2, <u>H</u> 3, <u>H</u> 4	1.4	1.4	1.4 ^f	1.4	1.4 ^f	1.4
<i>Hae</i> III ^{c,d}		0.5	0.5	0.5	0.5	0.5	ND
		0.2	0.2	0.2 ^e	0.2	0.2 ^e	ND
				0.6 ^e		0.6 ^e	ND

Genomic DNA was isolated from plants with different forms of the *Spm* element, digested with the enzymes listed in the first column, fractionated on agarose gels, transferred to nylon membranes, and hybridized with a ³²P-labeled cloned fragment of the *a*-gene sequence immediately adjacent to the *Spm* insertion site. The *a*-gene probe extends from the left end of the element to the *Bgl*I site in the *a* gene (see Fig. 6), unless otherwise indicated. Restriction enzymes that are insensitive to methylation are underlined. All of the other enzymes are sensitive to methylation at C residues and contain either a CG dinucleotide or a CNG trinucleotide, except *Hae*III (see footnote d). (ND) Not determined.

^a Fragment lengths to which the *a*-gene probe will hybridize if the underlined sites shown next to the fragment size are not cleaved and all *a*-gene sites are cleaved. The location of each restriction site is shown in Fig. 6. The length of each fragment detected by the *a*-gene probe therefore depends on whether or not a particular restriction site is methylated. The remaining columns list the sizes of

(Continued on p. 1370.)

ment's end. The GC-rich sequence corresponds to most of the first, untranslated exon of the element's major transcript. It has a G + C content of almost 80% and contains >70 methylatable C residues (Pereira et al. 1986; Masson et al. 1987). The GC-rich region also contains 11 directly repeated copies of a sequence with strong homology to the 17-bp consensus sequence CGGGCGGGCGGCCTCGC.

There are striking differences among the active, *cryptic*, and *programmable* forms of the *Spm* element in the methylation of the GC-rich region. The *Sall* and the *AvaI* sites within the GC-rich region are almost fully methylated in *cryptic* elements and almost unmethylated in active ones (Fig. 4B,C). Identical results are obtained with stably active *Spm* elements (Table 2) and *programmable* *Spm* elements in the active phase (Fig. 4B,C). *Programmable* elements in the inactive phase give a mixture of the fragments expected if the *Sall* and *AvaI* sites within the GC-rich region are and are not methylated. The *Sall* site outside of the GC-rich region and near the element's internal *Bam*HI site is methylated in all forms of the element. Comparable results have been obtained with *Bgl*II, for which there are two cleavage sites within the GC-rich region of the element (Table 2). The different forms of the element are therefore distinguishable in genomic DNA by their sensitivity to cleavage with methylation-sensitive restriction enzymes within the GC-rich region of the element.

Active and inactive elements can also be distinguished based on their sensitivity to two methylation-sensitive enzymes, *Eco*0109 and *Hae*III, which cleave at sites in the 0.2-kb sequence upstream of the element's transcription start site (Fig. 1). The design and outcome of an experiment using *Eco*0109 are shown in Figure 5. The *cryptic* and the *programmable* elements in the inactive phase are indistinguishable and largely resistant to digestion with *Eco*0109, whereas the active element is almost fully cleaved. Similar results have been obtained using *Hae*III, which cleaves a site within the *Spm* element 50 bp from its 5' end, except that the *Hae*III site is methylated less extensively in inactive *programmable* and *cryptic* elements (Table 2). The element's activity phase is therefore correlated with the extent of methylation of upstream sequences, irrespective of the genetic stability of the phase setting. The results of all of the

experiments on element methylation appearing in Table 2 are represented in the diagram shown in Figure 6.

Inactive programmable elements can be methylated partially at more than one site

An experiment was done to determine whether the genomic DNA isolated from a plant with a *programmable* element in an inactive form comprises a mixture of completely methylated and unmethylated element copies or whether each element copy is methylated at a subset of sensitive C residues. Genomic DNA digested with both *Bam*HI and *Ava*I was fractionated on a gel, and both the *Ava*I-resistant 3.8-kb fragment and the *Ava*I-digested 1.3-kb fragments containing the 5' end of the element and the adjacent *a*-gene sequence were recovered and digested with *Sall*, as diagramed in the lower part of Figure 4A. Both fragment populations proved to contain a mixture of sensitive and resistant *Sall* sites (Fig. 4D). Hence, there are elements in the plant that are methylated at some sites but not at others, although there may also be elements that are methylated either at all possible sites or none of them.

*The *Spm* element's genetic activity is correlated with transcript levels*

To determine whether the genetic activity phase of an element is correlated with element transcription, poly(A)⁺ RNAs from plants with active and inactive elements were analyzed for the presence of transcripts homologous to the *Spm* element. We reported previously that a 2.5-kb transcript was unique to plant tissues with an active *Spm* element (Pereira et al. 1986; Masson et al. 1987). This corresponds to the major transcript whose structure is shown in Figure 1. Consistently, we observe three to four transcripts that are smaller than the element's major transcript but are not unique to plants containing an active *Spm* element (Fig. 7). These may represent read-through transcripts from external promoters into defective elements or defective element transcripts initiated from the *Spm* promoter. Although they are not visible in the relatively brief autoradiographic exposure shown in Figure 7, there are also small amounts of two large transcripts of ~6 kb that are

(Continued from p. 1369.)

the fragments detected by hybridization to the *a*-gene probe in digests of DNA isolated from plants in which the *Spm* element had been identified genetically as being in the form indicated at the top of the column.

^b An *a*-gene probe extending ~900 bp from the 3' end of the *Spm* element was used for this analysis.

^c Methylation of *Bgl*II and *Hae*III sites could not be determined by analysis of total genomic DNA due to the presence of comigrating fragments that hybridize to the *a*-gene probe. The comigrating fragments originate from the *a* allele on the homolog and other genomic sequences homologous to the *a* locus. To analyze these sites, 3.8-kb fragments were first gel purified from *Bam*HI-digested genomic DNA samples, digested with *Bgl*II or *Hae*III, refractionated, then hybridized to an *a*-gene probe. Rehybridization of the same filters with an internal *Spm* probe gave results consistent with those obtained using the *a*-gene probe (not shown).

^d The *Hae*III restriction enzyme will not cleave its recognition sequence (5'GGCC) if the internal C residue of the sequence is methylated. Because CNG trinucleotide sequences are commonly methylated in plants, the *Hae*III recognition site can be methylated if followed by a G residue. There is a G residue adjacent to the H1, 2, and 3 sites, but not the H4 site. The H4 site is therefore methylation insensitive, whereas the H1, H2, and H3 sites are potential methylation sites.

^e The 0.2- and 0.6-kb fragments hybridize to the *a*-gene probe with equal intensity.

^f This fragment hybridizes faintly to the *a*-gene probe, compared to other fragments.

Figure 4. Methylation of the *SalI* and *AvaI* sites within the 5' GC-rich region of *Spm*. (A) The location of the methylation-sensitive *SalI* and *AvaI* sites and the methylation-insensitive *BamHI* sites within the *Spm* element and *a* gene. The solid box represents the first exon of the major *Spm*-encoded transcript. To determine the methylation state of the *AvaI* and *SalI* sites, 3.8-kb fragments were gel purified from *BamHI*-digested genomic DNAs, then digested with either *AvaI* or *SalI*, and hybridized with the *a*-gene probe shown. Digestion with *SalI* or *AvaI* can give a number of fragments detectable by the *a*-gene probe, depending on whether or not the sites are methylated. (A) The possible fragments and their sizes, assuming complete cleavage of the *a*-gene sites, are shown. To determine the methylation state of the *SalI* site in fragments either digested or undigested by *AvaI*, DNA isolated from a plant with an inactive *programmable* element was digested with both *AvaI* and *BamHI*. DNA fragments in the 3.8-kb size range (uncleaved at the *AvaI* site) and 1.8-kb size range (cleaved at the *AvaI* site) were gel purified, digested with *SalI*, and hybridized to the *a*-gene probe. The sizes of the fragments detectable by the *a*-gene probe (bottom) will depend on whether or not the *SalI* sites are cleaved. (B,C) The results of *a*-gene probe hybridization to the *SalI*-digested (B) and *AvaI*-digested (C) 3.8-kb *BamHI* fragments. (D) Results of *a*-gene probe hybridization to the gel purified and *SalI*-digested 1.3- and 3.8-kb *AvaI*-*BamHI* fragments. (●) Uncleaved, methylation-sensitive restriction site; (○) cleaved, methylation-sensitive restriction site; (Ø) cleaved, methylation-insensitive restriction site. (A) *AvaI*; (Bm) *BamHI*; (S) *SalI*.

unique to *Spm*-containing plants. One of these has also been noted in *En*-containing plants (Pereira et al. 1986), and they may be splicing intermediates or alternatively spliced mRNAs.

Plants with active and inactive *Spm* elements differ in the abundance of the major 2.5-kb element-encoded transcript (Fig. 7). The transcript is relatively abundant

in plants containing either a stably active or active *programmable Spm* element, although there is considerable variation between plants in the abundance of the transcripts (Fig. 7, lanes 1 and 2). The 2.5-kb *Spm* transcript is not detectable in plants containing either an inactive *programmable* or a *cryptic* element. The genetic observation that an inactive *programmable* element can be

Figure 5. Methylation of the *Eco0109* site at the 5' end of the *Spm* element. (A) The locations of the methylation-sensitive *Eco0109* site and the methylation-insensitive *Bam*HI sites within the *Spm* element and a gene. The solid box represents the first exon of the *Spm*-encoded transcript. To determine the methylation state of the *Eco0109* site, genomic DNAs isolated from plants having either *cryptic* or *programmable* (active and inactive) *Spm* elements were digested with *Bam*HI and fractionated on agarose gels. (A) DNA fragments in the 3.8-kb size range were gel purified, digested with *Eco0109*, and then hybridized to the *a*-gene probe. (Bottom) The probe will hybridize to a 1.4-kb fragment if the *Eco0109* site is cleaved and to a 3.8-kb fragment if the site is not cleaved. (B) The results of a blot hybridization experiment described in A. (●) Uncleaved, methylation-sensitive restriction site; (○) cleaved, methylation-sensitive restriction site; (○) cleaved, methylation-insensitive restriction site. (Bm) *Bam*HI; (E) *Eco0109*.

reactivated by an active element (McClintock 1957, 1958, 1959, 1971; Fedoroff and Banks 1988) was investigated by examining the effect of a *trans*-acting *Spm*-w element on the abundance of element-encoded transcripts in the presence of either an inactive *programmable* element or a *cryptic* element. The results show that element-homologous transcripts are markedly more abundant in plants containing both an *Spm*-w and an inactive *programmable* element than in plants containing the *Spm*-w and a *cryptic* element or either an inactive *programmable* or an *Spm*-w element alone. We conclude that genetic reactivation of an inactive *programmable*

Spm element by an active one is correlated with an increase in the abundance of element-encoded transcripts and probably occurs by the transcriptional reactivation of the inactive element.

*Changes in methylation of inactive programmable and cryptic elements occur in the presence of a trans-activating *Spm*-w element*

The effect on element methylation of a *trans*-activating element was investigated in genomic DNA of plants containing an *Spm*-w element and either an inactive *programmable* element or a *cryptic* element. The *Sall*, *Ava*I, and *Eco0109* sites of inactive *programmable* elements are partially and variably methylated in the absence of a *trans*-activating *Spm*-w element (Fig. 8B,C; Table 2). These sites are not methylated when the plant contains a *trans*-activating *Spm*-w element (Fig. 8B; Table 2). The *Pst*I and *Pvu*II sites within the element remain methylated in the presence of the *Spm*-w element (Table 2). The methylation state of the *cryptic* element can also change in the presence of the *trans*-activating element. As shown in Figure 8D, the methylation of the *Sall* site within the GC-rich region of the element is reduced in a majority of the plants examined, although the site remains at least partially methylated in all cases.

A trans-activating element promotes the heritable reactivation of an inactive programmable element

If changes in the element's methylation pattern are related causally to changes in the genetic transmission of its phase setting, then *trans*-activation of an inactive element by the *Spm*-w element should promote its conversion to a heritable active form. This was determined by comparing the transmission of an inactive *programmable* element from each of several parental plants, through an intermediate generation in which it was or was not exposed to a *trans*-activating *Spm*-w, to the following generation.

Because sibling plants exhibit large variations in the heritability of *programmable* *Spm* element's state of activity, the ability of a *trans*-activating element to reprogram an inactive element was evaluated by comparing the inheritance of the element's state of activity through the male and female gametes produced on the main stalks of individual plants. We reported previously that an inactive *programmable* element has a higher probability of being transmitted in an inactive state through male than female gametes (Fedoroff and Banks 1988). Moreover, an active element in the *programmable* form has a higher probability of being inactivated upon transmission through male than female gametes. This tendency is evident when the fraction of progeny kernels on ears receiving an element from a plant through the female gametes is compared with the fraction of kernels that receives an inactive element through the male gametes produced on the main stalk of the same plant (Fig. 9, left panel).

Figure 6. The location of methylated restriction enzyme sites in the *Spm* element and adjacent sequences. The solid boxes in the *Spm* element represent the exons of the major *Spm*-encoded transcript. The arrows correspond to the two open reading frames within the first intron of the transcription unit. Restriction sites for enzymes that are insensitive to methylation and used in these experiments (Table 2) are shown above the element. Each of the several restriction sites for a given enzyme has been numbered from left to right in the diagram. The number above each restriction site is the distance (bp) of that site from the 5' end of the element. (A) *AvaI*; (B) *BglII*; (Bm) *BamHI*; (E) *EcoRI*; (H) *HaeIII*; (Hd) *HindIII*; (K) *KpnI*; (P) *PstI*; (Pv) *PvuII*; (R) *EcoRI*; (S) *SalI*; (Sc) *ScaI*; (Sm) *SmaI*. (Solid circle) Methylation-sensitive restriction site almost completely methylated; (shaded circle) methylation-sensitive restriction site partially methylated (the density of shading reflects the extent of methylation); (open circle) methylation-sensitive restriction site almost or completely unmethylated.

When the plant transmitting the inactive *programmable* element also contains a *trans*-activating *Spm-w* element, the element is more likely to be transmitted to the next generation in an active form than if the plant does not contain a *trans*-activating element. In the plant family for which data appear in Figure 9, the difference is most striking when the tendency of the element to be reactivated is compared between male and female gametes of the same plant (Fig. 9, right panel). The presence of a segregating, *trans*-activating *Spm* element reverses the tendency toward element inactivation in the male germ line. Although the structure of the crosses used in the present experiment precludes a rigorous evaluation of the effect of the *trans*-acting element within the family, a recently completed study has provided extensive documentation of the *Spm-w* element's ability to promote the heritable reactivation of inactive elements (Fedoroff 1989). Thus, a *trans*-activating *Spm*

Figure 7. Northern blot analysis of poly(A)⁺ RNA isolated from individual plants with various *Spm* elements. Approximately 5 μ g of poly(A)⁺ RNA isolated from whole plant tissue was fractionated on a formaldehyde-agarose gel, transferred to membrane filters, and hybridized with a ³²P-labeled *Spm* cDNA fragment (see Methods).

Figure 8. Methylation of sites at the 5' end of inactive *programmable Spm* elements and *cryptic* elements in the presence and absence of an *Spm-w* element. (A) The location of the methylation-sensitive *Sal*I and *Ava*I sites and the methylation-insensitive *Bam*HI sites in the *Spm* element and adjacent *a*-gene sequences. The solid box represents the first exon of the *Spm*-encoded transcript. The lines below the diagram represent the fragments detectable by hybridization to the *a*-gene probe after digestion of DNA with different combinations of *Bam*HI, *Sal*I, and *Ava*I. (B) To show the effect of an *Spm-w* element on the methylation of an inactive *programmable* element, fragments in the 3.8-kb size range were gel purified from *Bam*HI-digested genomic DNAs, digested with *Ava*I and *Sal*I, refractonated, and hybridized with the *a*-gene probe shown in A. Results of blot hybridization to DNA isolated from plants with a *cryptic* element but no *Spm-w* element (lane 1), both an inactive *programmable* and an *Spm-w* element (lane 2), and an inactive *programmable* element but no *Spm-w* element (lane 3). (C) Variation in the extent of methylation of sites at the 5' end of the *Spm* element among plants with an inactive *programmable* element. Genomic DNA was isolated from two sibling plants (lanes 1 and 2) with an inactive *programmable* element, digested with *Ava*I and *Sal*I, and hybridized with the *a*-gene probe shown in A. The ratio of 1.3- to 1.1-kb fragments corresponds to the proportion of DNA molecules that are not cleaved to those that are cleaved by *Sal*I in the population of DNA molecules that are cleaved at the *Ava*I site. The 0.9-kb fragment in each lane corresponds to the *a* allele present on the homolog in both plants. (D) The effect of an *Spm-w* element on the methylation of a *cryptic Spm* element. Genomic DNA was isolated from plants with only a *cryptic Spm* element (lane 1) or both a *cryptic Spm* and an *Spm-w* element (lanes 2–4), digested with *Bam*HI and *Sal*I, and hybridized with the *a*-gene probe shown in A. The 1.2-kb fragment corresponds to the *a-m5-Spm-w* allele present on the homolog in all plants. The additional fragments that hybridize weakly to the probe correspond to other genomic sequences with homology to the *a* gene and are observed in control plant DNAs as well. (●) Uncleaved, methylation-sensitive restriction site; (○) cleaved, methylation-sensitive restriction site; (Ø) cleaved, methylation-insensitive site. (A) *Ava*I; (Bm) *Bam*HI; (S) *Sal*I.

element not only promotes the transient activation of an inactive element but which also promotes its heritable reactivation (Fig. 9; Fedoroff 1989).

Similar genetic experiments have also been done with the *a-m2-8167B* (*cryptic*) element, which shows variably reduced methylation of sites within the GC-rich region (Fig. 8d). The *cryptic* element almost always segregates from the *trans*-activating *Spm-w* element in its original form. However, very rare instances of genetic reactivation have been observed in the presence of a *trans*-acting element (N. Fedoroff, unpubl.) and were the source of the *a-m2-8167B* (*reactivated*) allele shown in Figure 2b

(for details, see Methods). It appears, therefore, that there is a correlation between the extent to which element methylation is reduced by a *trans*-activating element and the probability that it will be heritably reactivated. Although the precise magnitude of the increase in the frequency of reactivation attributable to the *trans*-activating *Spm-w* element has not been determined for the *a-m2-8167B* allele, it has been for other *cryptic Spm* elements and is between 2 and 3 orders of magnitude (N. Fedoroff, in prep.). Thus, the *trans*-acting element can both *reset* the element's phase of activity and heritably reprogram the element.

Figure 9. Heritable reactivation of an inactive *programmable Spm* following exposure to a *trans*-activating *Spm-w* element. In the previous generation, plants carrying an inactive *programmable Spm* element [*a-m2-7991A1* (*Spm-i*) allele] were used as pollen parents in crosses to plants that were homozygous either for the *a* or the *a-m5-Spm-w* alleles. Kernels that were heterozygous for the *Spm* insertion allele and either the *a* or *a-m5-Spm-w* allele were selected, grown, and the first ear on the main stalk was backcrossed to a tester strain that was homozygous for the *a* allele. All of the selected kernels had the variegated phenotype expected if the *Spm* element at the *a* locus is active. The tassel on the main stalk of each plant was used as the pollen parent in the same type of backcross. Progeny ears were examined to determine the fraction of kernels with an inactive *Spm*. The frequency with which the *Spm* element is transmitted in an inactive form through the male and female gametes is compared for one family of plants that received the *Spm* element from the same original plant through a plant in the following generation that had no active *Spm* (left) or was homozygous for the *a-m5-Spm-w* allele (right). Each pair of dots connected by a line represents the percentage of progeny kernels that received an inactive *Spm* element through the female and male gametes, respectively, produced on the main stalk of a single plant.

Discussion

Control of Spm expression is exerted at the level of element transcription

We have presented evidence that a genetically inactive *Spm* element is transcriptionally silent, whereas an ac-

tive element is transcribed, suggesting that the phase setting mechanism controls element activity at the transcriptional level. Regardless of whether the element is in the stable or *programmable* form, active elements are hypomethylated at sites upstream of the element's transcription start site, whereas inactive elements are almost completely methylated. The extent of methylation at the element's upstream *Eco*0109 and *Hae*III sites is the same in a *cryptic* element and in an inactive element in the *programmable* form, suggesting that the methylation level of sites in the element sequence upstream of its transcription start site determines its phase of activity.

Molecular differences between cryptic and programmable elements in the inactive phase

A *cryptic* element differs from a *programmable* element in the inactive phase by the developmental and hereditary stability of the inactive phase setting (Table 1). *Programmable* elements can display different patterns of phase reversal during development (McClintock 1957, 1962, 1965a; Peterson 1966; Fowler and Peterson 1978; Fedoroff and Banks 1988). Such patterns are heritable but relatively unstable and subject to frequent further changes. In the present experiments, we have found that *cryptic* and *programmable* elements differ in the methylation pattern of several sites within a GC-rich region of the element located just downstream of its transcription start site. A *cryptic* element is fully methylated at all of the sites that we have examined, whereas the inactive *programmable* elements in a given plant are partially methylated at each site, implying that a given element is methylated at a subset of sites. Moreover, different plants in a population of sibling plants grown from kernels of the same phenotype vary in the extent of methylation at a given site. Reactivation of an inactive element is accompanied by a reduction in the level of methylation of sites within the GC-rich region of the element but not elsewhere in the element.

An Spm element-encoded gene product promotes both the transient and heritable activation of an inactive element

Cryptic and *programmable* elements differ in their response to the presence of a *trans*-activating element. A *cryptic* element is not transcriptionally activated in the presence of a *trans*-acting *Spm* element. In contrast, an inactive element in the *programmable* form is fully active genetically, as well as transcriptionally, in the presence of a *trans*-activating element. The first implication of these observations is that an element-encoded gene product can override the genetic mechanism that sets the element's phase, effecting its transcriptional activation. Their second implication is that the ability of the element-encoded gene product to override the phase-setting mechanism depends on the stability of the element's inactive state, which we have ascribed to the mechanism that is responsible for programming the element. Although the elements we have compared in the

trans-activation tests reported here exhibit extreme differences in the stability of the inactive state, genetic tests conducted on plants with elements in the *programmable* form reveal these to be endpoints in a continuum. That is, plants in which the resident element has been reprogrammed to increase the heritability of the inactive state exhibit a concomitant decrease in the ability of an incoming *trans*-activating element to reactivate the resident, inactive element (N. Fedoroff, unpubl.).

Examination of the methylation pattern of inactive elements in plants containing a *trans*-activating *Spm* revealed a marked reduction in methylation of the GC-rich region, as well as of the upstream *Eco*0109 and *Hae*III sites. The results of genetic tests showed that exposure to a *trans*-activating element also promotes the heritable conversion of a genetically inactive element to an active one. Moreover, there is a good correlation between the initial extent of methylation, the reduction in methylation during *trans*-activation, and the heritable reversal of the inactive state. These observations imply that the *Spm* element encodes a gene product that not only activates element transcription but also promotes the heritable reprogramming of an inactive element by reducing its level of methylation.

A simple molecular model for the developmental regulation of the *Spm* element

A rather simple molecular mechanism can explain the full range of the observed modes of *Spm* expression in development (Fig. 10). Based on the present results, we propose that the element's phase setting is determined by the methylation state of sequences on the 5' side of its transcription start site (upstream control region, or UCR), whereas its developmental program is determined by the methylation pattern within the GC-rich sequence of its first exon (downstream control region, or DCR). We postulate that the element's promoter is constitutively active when both UCR and DCR are hypomethylated but that its expression becomes dependent on the presence of the element's positive autoregulatory gene product (circled A in Fig. 10) when the UCR is methylated and the DCR is partially methylated. We postulate further that whether and to what extent the element's positive autoregulatory gene product can activate element expression depends on the methylation state of the GC-rich DCR (Fig. 10). When both UCR and DCR are fully methylated, the element's promoter is inactive and uninducible.

Figure 10. A molecular model of *Spm* regulation. The diagrams represent the *Spm* element's 5' end. The transcription start site at nucleotide 209 (Pereira et al. 1986) is indicated by the base of the arrow above the diagram of each element. Extended and truncated arrows designate transcriptionally active and inactive elements, respectively. The 0.2-kb UCR contains 11 methylatable C residues; these are represented by four circles. The DCR commences at the transcription start site and includes the 0.35-kb GC-rich sequence within the element's first exon. There are 75 methylatable C residues in the DCR, and these are represented by 16 circles in the DCR. Open circles represent unmethylated sites, whereas shaded and solid circles represent partially and fully methylated sites. The circled A represents the postulated *Spm*-encoded positive autoregulatory gene product (Masson et al. 1987). (A) The stably active *Spm* element is represented as unmethylated. The looped arrow indicates that the active element remains in an active form as a consequence of producing the circled A, the postulated autoregulatory gene product that functions as a transcriptional activator and interferes with methylation of the UCR and DCR. (B) The *programmable Spm* (top) is depicted as methylated within the UCR, partially methylated in the DCR, and transcriptionally silent. (Bottom) The transcriptionally activated, hypomethylated form of the *programmable* element observed in the presence of a *trans*-activating *Spm*, which provides the element with the circled A, thereby promoting its transcriptional activation and decreasing its level of methylation. (C) The *cryptic* element (top) is represented as almost fully methylated and transcriptionally inactive. (Bottom) The reduced methylation of the *cryptic* element observed in the presence of the circled A provided by the *trans*-activating *Spm* element at the left. The density of methylation in the presence of the circled A is depicted as sufficiently high to prevent its transcriptional activation.

We suggest that when the element is in the stably active form, its autoregulatory gene product serves to maintain it continuously in a transcriptionally active form, both by overriding the inhibitory effect of methylation and by continuously promoting the heritable 'erasure' of methylation, possibly by binding directly to UCR and DCR sequences to activate transcription and interfere with remethylation during DNA replication (Fig. 10). In contrast, an element in the extremely *cryptic* state is genetically and transcriptionally silent because both its UCR and DCR are fully methylated. Although its methylation level decreases in the presence of a *trans*-activating element, the reduction is partial. Such an element can neither be expressed constitutively nor *trans*-activated by the positive autoregulatory gene product supplied by an active element in the same genome. Finally, we postulate that a *programmable* element is in one of many possible intermediate states of methylation, within each of which the density and, perhaps, the pattern of methylation of the GC-rich DCR determines when, where, and how frequently in development the element will undergo a change in its phase setting, mediated by a change in the methylation state of the UCR. As discussed below, developmental differences in the plant's ability to methylate the UCR and DCR comprise the final component of the *Spm*'s regulatory scheme.

There are indications that the DCR also plays a role in determining the timing and frequency of element transposition, in addition to participating in the regulation of element transcription. We have reported that a mutant element from which the DCR sequence is deleted, but which retains both the element's terminal inverted repeats and the subterminal repetitive regions adjacent to them, excises very infrequently in the presence of a *trans*-activating *Spm* element (Masson et al. 1987). The excisability of the highly methylated element of the *a-m2-8167B* (*cryptic*) allele is also low (Fig. 2f) and comparable to that of a defective element lacking the DCR (Masson et al. 1987). When the resident *Spm* element is reactivated fully, it shows a high frequency of somatic excision (Fig. 2b). Moreover, we have recovered derivatives of the *a-m2-8167B* (*cryptic*) allele that exhibit a high frequency of excision in the presence of a *trans*-activating element (N. Fedoroff and J. Banks, unpubl.). These observations not only suggest that the interaction between the *Spm*'s autoregulatory protein and the DCR is involved in element transposition but also that the methylation state of sequences within the element and the plant's ability to remethylate are important determinants of transposition frequency.

It is not yet clear whether the element's autoregulatory gene product and the element-encoded transposition function reside in the same or different gene products (Pereira et al. 1986; Masson et al. 1987). There is a single major 2.5-kb transcript unique to *Spm*-containing plants (Pereira et al. 1986; Masson et al. 1987), as well as two minor larger transcripts (Pereira et al. 1986; J. Banks and N. Fedoroff, unpubl.). Data obtained from the molecular analysis of the structure of mutant *Spm*

elements are consistent with the interpretation that both genetic activities of the element are encoded by the element's major transcript but do not eliminate the possibility that one of the activities resides in a protein encoded by a minor transcript obtained by an alternative splicing pattern from the element-encoded primary transcript (Pereira et al. 1986; Masson et al. 1987). Our recent demonstration that the *Spm* element transposes in transformed tobacco cells will facilitate the resolution of these questions (Masson et al. 1988).

Methylation is also correlated with the inactivation of other maize transposable elements

Although the inactivation and reactivation of the maize *Activator* (*Ac*) element have been studied in less detail than that of the *Spm* element, the *Ac* element provided the first genetic, as well as the first molecular, evidence that an epigenetic mechanism associated with element sequence methylation was responsible for its inactivation (McClintock 1965a,b; Fedoroff et al. 1983; Schwartz and Dennis 1986; Chomet et al. 1987; Schwartz 1988). Changes in the methylation pattern of sequences around the transcription start site accompany changes in the genetic activity of the *Ac* element as they do in the *Spm* element. However, partially methylated *Ac* elements appear to retain their genetic activity (Schwartz and Dennis 1986). A correlation has also been reported between the extent of methylation and the mobility of *Mu* element sequences in maize (Bennetzen 1985; Chandler and Walbot 1986; Walbot 1988). However, in the case of the *Mu* elements, there is insufficient genetic information to permit a distinction to be made between effects on expression of a mobilizing element and on the mobility of a transposition-defective element.

*The *Spm* element may be under the control of a basic plant developmental regulatory mechanism*

The ability of different parts of the maize plant to differentially reset and reprogram *Spm* elements in the *programmable* form appears to be universal. Both McClintock (1958) and Fowler and Peterson (1978) noted marked differences in element expression between ears borne on tillers and on the plant's main stalk. Our studies on *programmable* derivatives of two different *Spm* insertion alleles of the *a* locus amply confirmed these early observations and further established the existence of differences between male and female germ lines in the capacity to maintain or impose an inactive phase setting (Fedoroff and Banks 1988).

The results of our genetic studies on the *Spm* element revealed a consistent relationship between the fate of the element's *phase program* and the position on the plant of the meristem that gives rise to the plant part or reproductive structure transmitting the element to the succeeding generation (Fedoroff and Banks 1988). The plant's ability to inactivate an active element increases with distance from the base of the plant of the meristem producing each reproductive structure: tiller, second ear, first ear, and tassel (Fedoroff and Banks 1988). Moreover,

there is a parallel between the mode of operation of the *Spm* element's developmental regulatory system and that which determines the developmental fate of a given meristem. The lowermost nodes can give rise to uncommitted lateral buds that produce fertile tillers. Those produced at successively higher nodes first undergo an incremental determination that commonly results in the production, both on field-grown plants and in culture-derived plants, of shoots with shortened internodes that terminate in an inflorescence producing both male and female flowers (Raman et al. 1980). Higher nodes are fully determined to produce ears, which bear only female flowers. Finally, bud development above the ear-bearing nodes is suppressed completely. The observation that the developmental fate of a given meristem is determined early and maintained in culture suggests the involvement of a stable genetic change. Based on these evident parallels, we have formulated the hypothesis that the *Spm* element has come under the control of a mechanism that is involved in the plant's transition from the juvenile to adult phase and participates in determining the developmental fate of the apical and lateral meristems.

Methods

Maize strains and genetic methods

All of the experiments were done with two derivatives of the original *a-m2* allele (McClintock 1962; Masson et al. 1987). These are the *a-m2-7991A1* and the *a-m2-8167B* (*cryptic*) alleles. Both contain structurally identical, full-length *Spm-s* elements at precisely the same insertion site, and both *Spm-s* elements have been cloned (Masson et al. 1987). The element in the *a-m2-7991A1* allele has been completely sequenced, whereas the *Spm-s* element of the *a-m2-8167B* (*cryptic*) allele has been extensively analyzed by restriction endonuclease mapping, and its termini have been sequenced (Masson et al. 1987). No differences were detected between the two elements, but a trinucleotide insertion was found adjacent to the 3' terminus of the element in the *a-m2-7991A1* allele that was not present in either the *a-m2-8167B* (*cryptic*) allele or any of the other *a-m2* derivatives for which insertion site sequences were determined (Masson et al. 1987).

The original *a-m2-8167B* allele received from McClintock was phenotypically similar to other derivatives of the original *a-m2* allele in which the *Spm* element had sustained an internal deletion, rendering it transposition defective. That is, the element does not excise or transpose, except in the presence of a *trans*-acting *Spm* located elsewhere in the same genome. In our original molecular description of this allele, we raised the possibility that its insertion was not genetically defective but actually represented a *cryptic* element (Masson et al. 1987). Using genetic conditions that promote activation of *cryptic* and inactive elements (Fedoroff 1986; Fedoroff and Banks 1988), we recently recovered a derivative of the original *a-m2-8167B* allele with a reactivated *Spm-s* element that exhibits the phenotype of the original *a-m2* allele, itself no longer in existence. This was done by selecting and growing kernels exhibiting a high frequency of somatic reversion, either in sectors or throughout the aleurone layer, on ears produced by plants containing a *trans*-acting second *Spm* element. We have reclassified the *a-m2-8167B* allele as having an insertion of a *cryptic*, and not a genetically defective, *Spm* element, redesignating it

the *a-m2-8167B* (*cryptic*) allele. The derivative with a reactivated element is designated *a-m2-8167B* (*reactivated*).

The second allele used in the present studies, the *a-m2-7991A1* allele, was selected as a starting point for the isolation of a derivative with an element capable of undergoing the types of programmed changes in activity that had been described previously for *Spm* elements at other (and unidentified) chromosomal locations (McClintock 1957; Masson et al. 1987). Our purpose was to isolate such a derivative at a locus for which cloned probes were available to simplify the analysis of the element in genomic DNA, a task that is complicated by the abundance of genomic sequences with homology to the *Spm* element. The derivation and genetic analysis of such alleles, here termed *programmable*, has recently been described (Fedoroff and Banks 1988).

Genetic methods for the analysis of the *a-m2* derivatives are those previously described (Fedoroff and Banks 1988). In all cases except that of the *cryptic* element, the chromosome carrying the *a-m2* derivative with an *Spm* element in either the active or inactive form was maintained on a chromosome in which it was closely linked to the *Sh2* allele of the *sh2* locus and which was heterozygous with a chromosome carrying either stable null alleles of both loci (*a*, *sh2*) or the *sh2* allele and an allele of the *a* locus containing a *dSpm* element (*a-m1-5719A1*). Table 1 gives the percentage of progeny kernels with an inactive element on ears, either of plants that had the heterozygous constitution just described or on tester plants crossed by such *Spm*-carrying heterozygotes (Fedoroff and Banks 1988). In all cases, only one of the parents contained an *Spm* element, whereas the other parent was homozygous for either a stable *a* or the *a-m1-5719A1 dSpm* allele of the *a* locus, as well as homozygous for a stable *sh2* allele. In crosses to introduce a *trans*-activating *Spm-w* element, one parent had the homozygous constitution *a-m5-Spm-w sh2*, whereas the other parent was an *Spm*-carrying heterozygote of the constitution described above (Fedoroff and Banks 1988). The *a-m5-Spm-w* allele has an *Spm-w* insertion at a site within the coding region of the *a* gene, and the element's restriction pattern is similar to that of an *Spm-s* element (McClintock 1962, 1963; Fedoroff and Banks 1988; J.A. Banks, unpubl.).

DNA extraction and blot hybridization

DNA was extracted from 4- to 6-week-old plants by the method of Shure et al. (1983) or Dellaporta et al. (1985), modified by the addition of a CsCl banding step. Genomic DNA was digested with restriction endonucleases, as specified by the supplier (Boehringer-Mannheim, Bethesda Research Laboratories, IBI, and New England Biolabs). Between 30 and 50 units of enzyme were used to digest 6 μ g of DNA. Initially, DNA was isolated and analyzed from 5 to 10 plants with an inactive *programmable* element, 4 plants with an active element, and 4 plants with a *cryptic* element. If a methylation-sensitive restriction enzyme had more than one site in the *Spm* element and adjacent *a* gene, several digests were performed to determine the methylation status of each individual restriction site. Each sample was digested with two or three restriction enzymes, one or two of which were insensitive to methylation and the other of which was sensitive to methylation. Interpretation of the methylation state of a given restriction site was based on the outcome of blot hybridization analysis of all digests. Ambiguities in interpretation arose due to the fact that most of the plants used in the present studies were heterozygous at the *a* locus and that there are several other sequences in the maize genome with substantial homology to the *a* locus (T. Helentjaris, pers. comm.). To identify other fragments with homology

to the probe used in a given experiment, DNA from plants that were homozygous for the *a* allele on the homolog of the plant carrying the *Spm* element under study were included in all experiments to ensure the correct identification of DNA fragments derived from the homolog.

When it was essential to completely eliminate cross-hybridizing sequences, fragments of the appropriate size range to contain an *Spm* fragment were gel purified from genomic DNAs digested with a methylation-insensitive enzyme. The gel-purified fragments were then digested with a methylation-sensitive enzyme, refractionated on an agarose gel, transferred to a nylon membrane, and hybridized to an *a*-gene or *Spm* probe. Once the overall pattern of methylation of restriction sites was established, DNA samples from additional plants were digested with appropriate pairs of methylation-insensitive and methylation-sensitive enzymes. Analyses were done on DNAs from 4 plants with an active element, 4 plants with an active *programmable* element, 12 plants with an inactive *programmable* element, 4 plants with an inactive *programmable* and an *Spm-w* element, 5 plants with a *cryptic* element, and 15 plants with a *cryptic* element and an *Spm-w* element.

Because certain restriction sites in genomic DNA from many of the plants were either partially or completely resistant to cleavage, internal controls for completeness of digestion were included in all experiments. These were done by incubating one tenth of each genomic DNA digest concurrently in a separate tube with a minute amount of ^{32}P end-labeled bacteriophage λ or linearized pEMBL18 plasmid DNA. After a 2-hr incubation, the control samples were fractionated on a 0.5% agarose gel, which was then dried and exposed to X-ray film. The autoradiogram was examined for completeness of digestion of the labeled DNA prior to fractionation of the genomic DNA digest. If the labeled DNA in the control samples was completely digested, the genomic DNA digests were fractionated on agarose gels (0.7–0.9%) and transferred to nylon membranes (GeneScreen Plus, NEN Research Products) by the alkaline transfer method (Reed and Mann 1985). The membrane was then hybridized with a cloned fragment of either the *a* gene or the *Spm* element labeled to a sp. act. of at least 2×10^8 cpm/ μg with [^{32}P]dCTP by the random primer labeling method, as specified by the supplier (Amersham). The *a*-gene probe was 552 bp in length and extended 72 bp beyond the 3' side of the element and 480 bp beyond the 5' side of the element. The *Spm* probe was 1.7 kb in length and extended from nucleotide 267 to nucleotide 1963 of the element's 5' end. Hybridization was carried out as specified by NEN Research Products for nylon membrane filters.

DNA fragments were purified from agarose gels by mixing an equal volume of phenol with the macerated agarose gel slice, freezing the sample, extracting the resulting aqueous phase first with phenol, then with a 1:1 mixture of phenol and chloroform, and finally with chloroform alone, followed by ethanol precipitation of the DNA.

RNA extraction and transcript analysis

Total RNA was isolated from several individual plants of each genetic constitution, using ~20 g tissue per sample, as described by Fedoroff (1985). Poly(A)⁺ RNA was isolated by oligo(dT)-cellulose chromatography and fractionated on formaldehyde-agarose gels as described by Ausubel et al. (1987). The amount of RNA applied was standardized using a labeled cDNA fragment encoding the small subunit of RuBP carboxylase, kindly provided by N.-H. Chua of the Rockefeller University. RNA was transferred to GeneScreen (NEN), and the membrane filter was stained with methylene blue according to Herrin and Schmidt (1988) to detect the RNA. Prehybridization

and hybridization was done according to Maniatis et al. (1982). Each blot was probed with a cDNA corresponding to the major *Spm* transcript, washed, and exposed to X-ray film. Radioactive probe was removed from the membrane by incubating the membrane four times in 0.01% SDS and $0.01 \times \text{SSC}$. The membrane was then reprobed with the RuBP carboxylase cDNA to confirm that all of the RNA samples showed roughly equivalent hybridization intensities to the RuBP carboxylase probe. All probes were labeled to a sp. act. of 1×10^9 cpm/ μg by the random-primer labeling method as described by the supplier (Amersham). Autoradiographic exposure to X-ray film was generally 16 hr.

Acknowledgments

We thank Jeff Kingsbury and George Rutherford for technical assistance and Bob Hoover of the Beltsville Agricultural Research Center of the U.S. Department of Agriculture for providing field space, preparation, and maintenance. This work was supported by fellowships to J.B. from the National Institutes of Health (NIH), P.M. from the Fonds National Belge de la Recherche Scientifique of Belgium, and by NIH grant 5-R01-GM34296-02.

References

- Adams, R.L.P. and R.H. Burdon 1985. *Molecular biology of DNA methylation*. Springer-Verlag, New York.
- Ausubel, F., R. Brent, R. Kingston, D. Moore, J. Seidman, J. Smith, and K. Struhl, eds. 1987. *Current protocols in molecular biology*. pp. 4.9.1–4.9.8. John Wiley and Sons, New York.
- Bennetzen, J.L. 1985. The regulation of *Mutator* function and *Mu* transposition. *UCLA Symp. Mol. Cell. Biol.* **85**: 343–354.
- Cedar, H. 1988. DNA methylation and gene activity. *Cell* **53**: 3–4.
- Chandler, V.L. and V. Walbot. 1986. DNA modification of a maize transposable element correlates with loss of activity. *Proc. Natl. Acad. Sci.* **83**: 1767–1771.
- Chomet, P.S., S. Wessler, and S.L. Dellaporta. 1987. Inactivation of the maize transposable element *Activator* (Ac) associated with DNA modification. *EMBO J.* **6**: 295–302.
- Coe, E.H., Jr. and M.G. Neuffer 1977. The genetics of corn. In *Corn and corn improvement* (ed. G.F. Sprague), pp. 111–223. American Society of Agronomy, Madison, Wisconsin.
- Dellaporta, S., J. Wood, and J. Hicks. 1985. Maize DNA miniprep. In *Molecular biology of plants. A laboratory course manual* (instructors, R. Malmberg, J. Messing, and I. Sussex), pp. 36–37. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Fedoroff, N.V. 1985. Biochemical and molecular techniques in maize research. In *Genetic engineering* (ed. J.K. Setlow and A. Hollaender), vol. 7, pp. 115–133. Plenum Press, New York.
- . 1986. Activation of *Spm* and *Modifier* elements. *Maize Genet. Coop. Newslett.* **60**: 18–20.
- . 1989. The heritable activation of *cryptic Suppressor-mutator* elements by an active element. *Genetics*, (in press).
- Fedoroff, N.V. and J.A. Banks. 1988. Is the *Suppressor-mutator* element controlled by a basic developmental regulatory mechanism? *Genetics* (in press).
- Fedoroff, N.V., J.A. Banks, and P. Masson. 1988a. Developmental determination of *Spm* expression. In *Molecular basis of plant development* (ed. R. Goldberg), Alan R. Liss, New York, (in press.)

Banks et al.

- Fedoroff, N., P. Masson, J. Banks, and J. Kingsbury 1988b. Positive and negative regulation of the *Suppressor-mutator* element. In *Plant Transposable Elements* (ed. D.E. Nelson). Plenum Press, New York, (in press.)
- Fedoroff, N., S. Wessler, and M. Shure. 1983. Isolation of the transposable maize controlling elements *Ac* and *Ds*. *Cell* **35**: 243–251.
- Fowler, R.G. and P.A. Peterson. 1978. An altered state of a specific *En* regulatory element induced in a maize tiller. *Genetics* **90**: 761–782.
- Gruenbaum, Y., T. Naveh-Man, H. Cedar, and A. Razin. 1981. Sequence specificity of methylation in higher plants. *Nature* **292**: 860–862.
- Herrin, D. and G. Schmidt. 1988. Rapid, reversible staining of Northern blots prior to hybridization. *BioTechniques* **292**: 196–200.
- Holliday, R. 1987. The inheritance of epigenetic defects. *Science* **238**: 163–170.
- Kessler, C. and H.-J. Holtke. 1986. Specificity of restriction endonucleases and methylases—A review (edition 2). *Gene* **47**: 1–153.
- Maniatis, T., E. Fritsch, and J. Sambrook. 1982. *Molecular cloning. A laboratory manual*, pp. 326–327. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- 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* **177**: 117–137.
- Masson, P., K. Toohey, and N. Fedoroff. 1988. Excision of *Spm* in tobacco. *Maize Genet. Coop. Newslett.* **62**: 26–27.
- McClintock, B. 1951. Mutable loci in maize. *Carnegie Inst. Wash. Yearb.* **50**: 174–181.
- . 1957. Genetic and cytological studies of maize. *Carnegie Inst. Wash. Yearb.* **56**: 393–401.
- . 1958. The Suppressor-mutator system of control of gene action. *Carnegie Inst. Wash. Yearb.* **57**: 415–429.
- . 1959. Genetic and cytological studies of maize. *Carnegie Inst. Wash. Yearb.* **58**: 452–456.
- . 1962. Topographical relations between elements of control systems in maize. *Carnegie Inst. Wash. Yearb.* **61**: 486–493.
- . 1963. Further studies of gene-control systems in maize. *Carnegie Inst. Wash. Yearb.* **62**: 486–493.
- . 1965a. The control of gene action in maize. *Brookhaven Symp. Biol.* **18**: 162–184.
- . 1965b. Components of action of the regulators *Spm* and *Ac*. *Carnegie Inst. Wash. Yearb.* **64**: 527–536.
- . 1967. Genetic systems regulating gene expression during development. *Dev. Biol.* (suppl.) **1**: 84–112.
- . 1971. The contribution of one component of a control system to versatility of gene expression. *Carnegie Inst. Wash. Yearb.* **70**: 5–17.
- Pereira, A., H. Cuypers, A. Gierl, Zs. 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. 1966. Phase variation of regulatory elements in maize. *Genetics* **54**: 249–266.
- Raman, K., D.B. Walden, and R.I. Greyson. 1980. Propagation of *Zea mays* L. by shoot tip culture: A feasibility study. *Ann. Bot.* **45**: 183–189.
- Razin, A., H. Cedar, and A.D. Riggs. 1984. *DNA methylation*. Springer-Verlag, New York.
- Reed, K. and D. Mann. 1985. Rapid transfer of DNA from agarose gel to nylon membranes. *Nucleic Acids Res.* **13**: 7207–7221.
- Schwartz, D. 1988. Comparison of methylation of the male and female derived *wx-m9 Ds-cy* allele in endosperm and sporophyte. In *Plant Transposable Elements* (ed. D.E. Nelson). Plenum Press, New York, (in press.)
- Schwartz, D. and E. Dennis. 1986. Transposase activity of the *Ac* controlling element is regulated by its degree of methylation. *Mol. Gen. Genet.* **205**: 476–482.
- Schwarz-Sommer, Zs., N. Shepherd, E. Tacke, A. Gierl, W. Rhode, L. Leclercq, M. Mattes, R. Berndtgen, P.A. Peterson, and H. Saedler. 1987. Influence of transposable elements on the structure and function of the *A1* gene of *Zea mays*. *EMBO J.* **6**: 287–294.
- Shure, M., S. Wessler, and N. Fedoroff. 1983. Molecular identification and isolation of the *Waxy* locus in maize. *Cell* **35**: 235–242.
- Walbot, V. 1988. Reactivation of the *Mutator* transposable element system following gamma irradiation of seed. *Mol. Gen. Genet.* **212**: 259–264.

Molecular mechanisms in the developmental regulation of the maize Suppressor-mutator transposable element.

J A Banks, P Masson and N Fedoroff

Genes Dev. 1988, **2**:

Access the most recent version at doi:[10.1101/gad.2.11.1364](https://doi.org/10.1101/gad.2.11.1364)

References

This article cites 31 articles, 4 of which can be accessed free at:
<http://genesdev.cshlp.org/content/2/11/1364.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).