Excision Patterns of *Activator* (*Ac*) and *Dissociation* (*Ds*) Elements in *Zea mays* L.: Implications for the Regulation of Transposition

Manfred Heinlein

The Scripps Research Institute, La Jolla, California 92037

Manuscript received May 28, 1996
Accepted for publication September 4, 1996

ABSTRACT

The pattern of aleurone variegation of maize kernels carrying *Ac* and *bz-m2(DI)* as reporter allele for *Ac* activity depends on the dosage of both *Ac* and *Ds*. Alterations of *Ac* dosage can abolish *Ds* excision at certain times and allow it to occur at other times. *wx-m7* and *wx-m9* are different *Ac* insertions in the *Waxy* gene which have different dosage effects on *Ds* excision. Kernels, heterozygous for the two *Ac* alleles and being either *wx-m7/wx-m7/wx-m9* or *wx-m9/wx-m9/wx-m7* exhibit characteristic patterns of predominantly late excisions; this is in strong contrast to the pattern of early excisions present on *wx-m7/wx-m7/wx-m7* homozygotes. This observation supports the hypothesis that the *Ac* alleles express different amounts of transposase (TPase) during development and that above a certain level of TPase transposition is inhibited. Furthermore, experimental results suggest that the frequency of *Ac*-induced events is influenced by the dosage and composition of the transactivated *Ds* or *Ac* allele. Thus, transposition frequency seems not to be exclusively determined *in trans* by the amount of active TPase, but also by specific *cis*-acting properties of the TPase substrate.

THE maize transposable elements *Activator* (*Ac*) and *Dissociation (Ds)* were discovered by BARBARA MCCLINTOCK (1946, 1947, 1948). These elements transpose by a nonreplicative mechanism and create an 8-bp target duplication upon transposition (reviewed in FEDOROFF 1989). The *Ac* element is able to transpose autonomously and to mobilize nonautonomous *Dissociation (Ds)* elements *in trans* (MCCLINTOCK 1948; COUPLAND *et al.* 1988). Both *Ds* and *Ac* elements have been cloned (COURAGE-TEBBE *et al.* 1983; FEDOROFF *et al.* 1983; BEHRENS *et al.* 1984) and sequenced (DÖRING *et al.* 1984; MÜLLER-NEUMANN *et al.* 1984; POHLMAN *et al.* 1984) and the gene structure of *Ac* has been determined (KUNZE *et al.* 1987). The *Ac* element encodes a 3.5-kb mRNA (KUNZE *et al.* 1987; FINNEGAN *et al.* 1988) for a protein of 807 amino acids. Because expression of the corresponding cDNA is sufficient to trigger excision of *Ds* in transgenic tobacco plants (COUPLAND *et al.* 1988) as well as in *Petunia* protoplasts (HOUBA-HÉRIN *et al.* 1990; BECKER *et al.* 1992; HEINLEIN *et al.* 1994), the *Ac* protein is referred to as the transposase (TPase) of the *Ac/Ds* system. This protein is a nuclear DNA-binding protein that recognizes AAACGG sequence motifs of which multiple copies are found within the subterminal portions of both *Ac* and *Ds* elements (KUNZE and STARLINGER 1989). The DNA-binding properties (FELDMAR and KUNZE 1991) as well as the observation that certain mutant TPase derivatives act as *trans*-dominant inhibitors of transposition *in vivo* suggest that several TPase molecules participate in the formation of the transposition complex (KUNZE *et al.* 1993).

The *Ac/Ds*-system is sensitive to the copy number of *Ac* in the genome (MCCLINTOCK 1948, 1951). An increase in the number of *Ac* copies in the genome usually results in a delay of *Ds* excisions, indicating that the number of early excision events is decreased whereas the number of late excision events is increased (MCCLINTOCK 1949). The dosage effect of *Ac* is dependent on expression of the TPase protein, since deletions destroying the TPase coding region mutate *Ac* into *Ds* elements, which do not contribute to the dosage effect (DOONER *et al.* 1986), and *Ac* elements whose transcription is inactivated by transient methylation do not contribute to the dosage effect (MCCLINTOCK 1964, 1965; SCHWARTZ and DENNIS 1986; KUNZE *et al.* 1988). Therefore, it appears that the dosage effect of *Ac* may be explained by the level of its transcription and/or translation products in response to both *Ac* dosage and the development of the tissue under investigation, *e.g.*, the endosperm.

In maize endosperm, the steady state level of both TPase mRNA and protein increases with *Ac* dosage (KUNZE *et al.* 1987; FUSSWINKEL *et al.* 1991). Therefore, the low level of *Ac* activities at early times cannot be explained by negative autoregulation of TPase expression. However, experiments with heterologous plant systems provide an indication that TPase activity is post-translationally controlled. For example, it was observed in *Petunia* protoplasts that within a low range of TPase concentrations the *Ds* excision rate is proportional to

Address for correspondence: The Scripps Research Institute, Department of Cell Biology-BCC206, 10555 N. Torrey Pines Rd., La Jolla, CA 92037. E-mail: heinlein@scripps.edu

the amount of TPase; however, above a certain concentration of *Ac* TPase the *Ds* excision rate remains constant even if the amount of TPase is increased further (BECKER *et al.* 1992; KUNZE *et al.* 1993; HEINLEIN *et al.* 1994). This observation has been substantiated by the finding that the TPase tends to associate into large, rod-like aggregates. This suggests that aggregation may sequester active TPase, thereby keeping the transposition rate low even in the presence of high levels of TPase (HEINLEIN *et al.* 1994). In addition, using transgenic tobacco plants, transposition inhibition was demonstrated above a threshold TPase concentration (SCOFIELD *et al.* 1993). Therefore, increasing *Ac* dosage in maize may result in a decrease in transposition frequency because two or three copies of *Ac* lead to production of TPase levels that are above an inhibitory threshold.

The *Ac* dosage effect is dependent on the particular *Ac* insertion used to cause the excision of a constant reporter *Ds* element, as is demonstrated by the *wx-m7* and *wx-m9* alleles of the *Wx* gene. Although these alleles are identical except for the insertion site of *Ac* within the *Waxy* gene (KLÖSGEN *et al.* 1986), they are characterized by virtually opposite dosage effects. The *wx-m9* allele has a "normal" dosage effect with predominantly early excisions with one copy and predominantly late excisions with two or three copies (SCHWARTZ 1984; HEINLEIN and STARLINGER 1991). In contrast, the *wx-m7* allele has an inverse effect with predominantly late excisions in the presence of one copy and predominantly early excisions when two or three copies are present in the genome (MCCLINTOCK 1964; SCHWARTZ 1986; SCHWARTZ and DENNIS 1986; HEINLEIN and STARLINGER 1991). The genetic background of the maize lines is not responsible for these differences between *wx-m7* and *wx-m9*, and it has been proposed that the dosage-specific *trans*-activities of *wx-m7* and *wx-m9* are determined by the *Ac* alleles themselves, presumably by expression of different amounts of TPase during development (HEINLEIN 1995).

I report here on patterns of aleurone variegation that are displayed on kernels that carry different doses of *wx-m7* and *wx-m9* and the *bz-m2(DI)* allele as reporter allele for *Ac* activity. The observed patterns provide further support for the hypothesis that the two specific *Ac* alleles differ in TPase expression during development and that above a critical threshold of TPase expression transposition activity is inhibited. In addition, I describe that *Ds* or *Ac* elements may differ in transactivation patterns in the presence of the same *trans*-acting TPase source. This suggests that TPase activity is not exclusively regulated *in trans*, but also *in cis*, at the site of the TPase substrate.

MATERIALS AND METHODS

Maize stocks: The particular *Ac* alleles used in this study were *bz-m2*, *wx-m7* and *wx-m9*. *bz-m2* is a mutable allele that arose from an insertion of the 4.6-kb *Ac* element into the second exon of *Bz-McC* (MCCLINTOCK 1955; FEDOROFF *et al.* 1984; DOONER *et al.* 1985; RALSTON *et al.* 1988). *wx-m7* and *wx-m9* are unstable *Wx* alleles that arose in *bz-m2* and *a1-m4* stocks by transposition of the *Ac* element to the *Wx*-locus (MCCLINTOCK 1963, 1964). The *Ac* elements of both *Wx* alleles are identical and inserted in reverse orientation relative to the host gene. *wx-m7* carries *Ac* 46 bp upstream of the TATA box and *wx-m9* carries the element in the tenth exon of the gene (KLÖSGEN *et al.* 1986).

The alleles *sh-m5933*, *bz-m2(DI)*, *wx-m9Ds* and *c2-m4490Ds* carry *Ds* and were used as reporter alleles for *Ac trans*-activity. *sh-m5933* is a mutable allele of the *Sh* locus that contains a "double *Ds*" element, consisting of a 2-kb *Ds* element inserted in opposite orientation into the center of a second, identical 2-kb element (COURAGE-TEBBE *et al.* 1983; DÖRING and STARLINGER 1984; DÖRING *et al.* 1989). Although somatic excision events have been reported (COURAGE-TEBBE *et al.* 1983; HEINLEIN and STARLINGER 1989), the element frequently induces chromosome breakage events at the site of insertion, probably in the course of aberrant transposition attempts involving the two transposon ends that are in direct orientation (DÖRING and STARLINGER 1984; DÖRING *et al.* 1989). In this study, the *sh-m5933* is linked to the *C1-I* allele of the aleurone color gene *C1*. This allele dominantly inhibits anthocyanin synthesis in maize kernels (CHEN and COE 1977; CONE *et al.* 1986; PAZ-ARES *et al.* 1990). In the presence of *Ac*, chromosome breakage at *sh-m5933* leads to loss of the distally located chromosome fragment containing the *C1-I* allele. Kernels carrying the wild-type *C1* gene on the homologous chromosome thus display colored areas on an uncolored background. *bz-m2(DI)* is a derivative of *bz-m2* harboring a 3.3-kb *Ds* element at the locus as a consequence of a deletion mutation of *Ac* to *Ds* (MCCLINTOCK 1962; DOONER *et al.* 1985, 1986). Similarly, *wx-m9Ds* derived from *wx-m9* by a 194-bp internal deletion from *Ac* (FEDOROFF *et al.* 1983). *c2-m4490Ds* is a mutable allele of the *C2* gene that carries *Ds* in an unknown position (NEVERS *et al.* 1985). The *C2* locus encodes chalcone synthase, which catalyzes an early step in the anthocyanin biosynthesis pathway (DOONER 1983; WIENAND *et al.* 1986; FRANKEN *et al.* 1991).

In addition, maize lines were used that contain recessive alleles *sh bz* (MOTTINGER 1973), *wxB-7* (WESSLER and VARAGONA 1985) and *c2* (MCCLINTOCK 1967; FRANKEN *et al.* 1991), respectively.

New lines in which the mentioned alleles were mutually combined were developed from recombinant kernels that were phenotypically selected from F_2 ears. All lines were propagated by selfing and sibling crosses and appropriate genetic tests were carried out to verify the genetic constitution of the strains.

Phenotypic selection of kernels and quantitative analysis: The following criteria were applied to select the kernels for comparison and quantitative evaluation of variegation patterns in the aleurone: the kernels represented the predominant variegation phenotype present on the ear at hand and on other ears of the same cross; the variegation patterns on the kernels were homogeneous and, if possible, did not carry sectors indicative of "changes in state" of *Ac* or *Ds* (MCCLINTOCK 1951); and the variegation patterns in the aleurone were well expressed in the crown region of the kernels. For quantitative analysis of aleurone variegation patterns, the kernels were individually situated under a binocular microscope and the magnification was adjusted so that the magnified crown area of the kernel matched the area of a grid that was placed in the ocular. Then the number of sectors within 180 subunits of the grid (~16 mm^2 of aleurone) was determined.

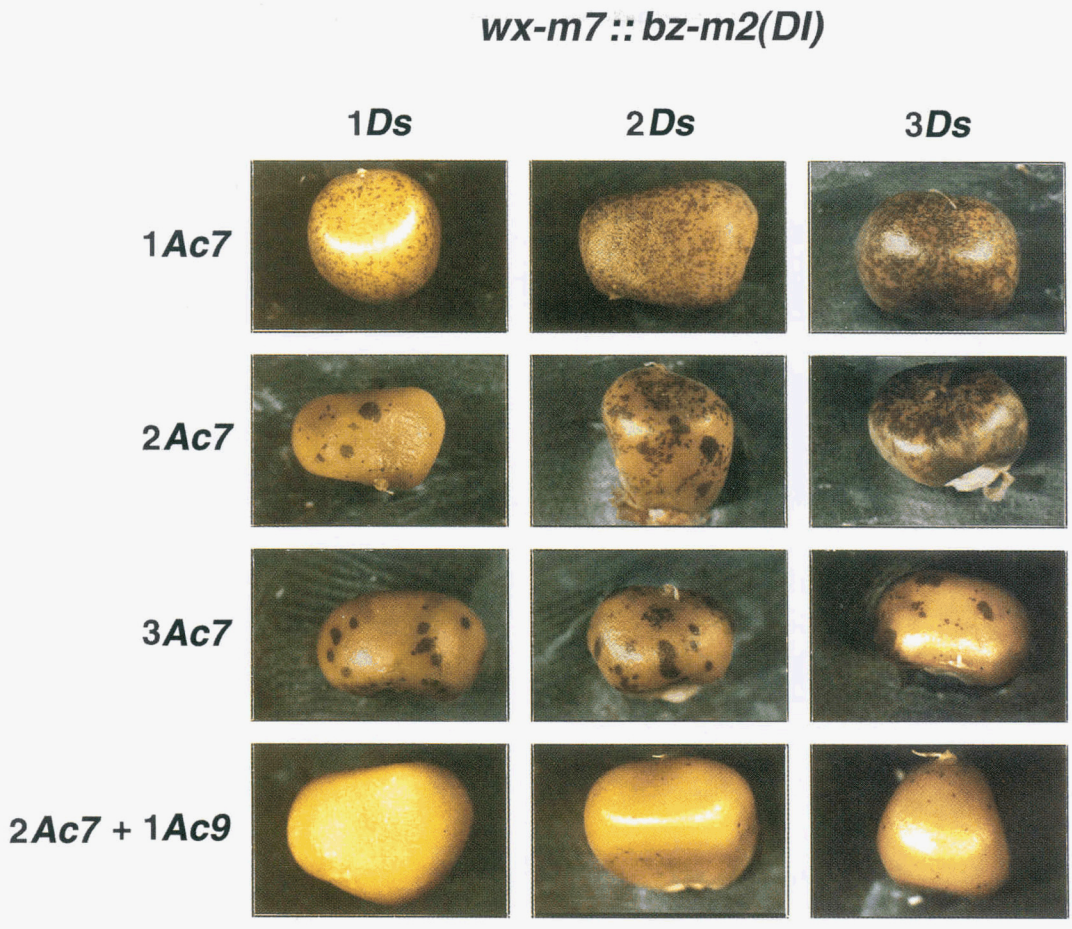

FIGURE 1.—Patterns of *Ds* excision from the *bz-m2(DI)* allele in kernels carrying one, two or three copies of *bz-m2(DI)* and one, two or three copies of *wx-m7*. An increase in *wx-m7* dosage leads to earlier events. The small sectors on kernels with two and three *wx-m7* alleles resulted from early somatic transposition events during which one copy of *Ac* has been lost (see text for detailed discussion). The effect of altering the dosage of the *bz-m2(DI)* allele is best seen on kernels carrying one copy of *wx-m7*. *Ds* excision frequency increases less than proportionally to an increase in *bz-m2(DI)* dosage from one to two copies but increases more than proportionally to an increase in the number of *Ds* alleles from two to three copies of the *Ds* allele. The phenotype of kernels in which *bz-m2(DI)* is transactivated by the action of two copies of *wx-m7* and one copy of *wx-m9* differs strongly from the phenotype of kernels which carry three doses of the *wx-m7* allele. The phenotype of the heterozygous kernels indicates that the replacement of the sperm-derived *wx-m7* allele by a *wx-m9* allele causes strong suppression of early excision events. Therefore, *Ac* activity in the cell is specified not only by the dosage of *Ac* but also by the *Ac* allele.

Histochemical methods: Paraffin embedding and sectioning of maize kernels as well as iodine staining of the inner endosperm starch was performed as described (HEINLEIN *et al.* 1994; HEINLEIN 1995).

Statistical analysis: *t*-distribution statistics was used to estimate confidence intervals for mean values μ of small samples. The *t*-statistics is defined as $t = \sqrt{n}(x - \mu)/s$, where n is the number of samples, x is the sample average and s is the sample standard deviation. The confidence interval for μ is from $x - st/\sqrt{n}$ to $x + st/\sqrt{n}$. The value of t for a given sample size can be found in published statistical tables.

RESULTS

Dosage effects of *wx-m7* and *wx-m9*: The variegated aleurone phenotype of kernels carrying the *bz-m2(DI)* allele consists of fully colored sectors on a bronze-colored background. Each revertant sector is caused by somatic excision of *Ds*, and the pattern of differently sized sectors corresponds to the frequencies of excision events that occurred during the process of kernel development. Because transposition of *Ds* requires the presence of active TPase expressed from *Ac*, the aleurone variegation pattern serves as a convenient read-out for TPase activity during development. In a previous study, the *bz-m2(DI)* variegation patterns that were displayed in the aleurone of kernels that carried one and two doses of *wx-m7* or *wx-m9* were compared and evaluated by separately counting the numbers of sectors of each sector size class. The two *Ac* alleles were determined to differ strongly in their dosage-specific *trans*-effects on *Ds* excision from *bz-m2(DI)* (HEINLEIN and STARLINGER 1991). Because these differences appeared to be genetically tightly linked to the corresponding *Ac* allele, the two *Ac* alleles were thought to express different levels of TPase during development (HEINLEIN 1995). To fur-

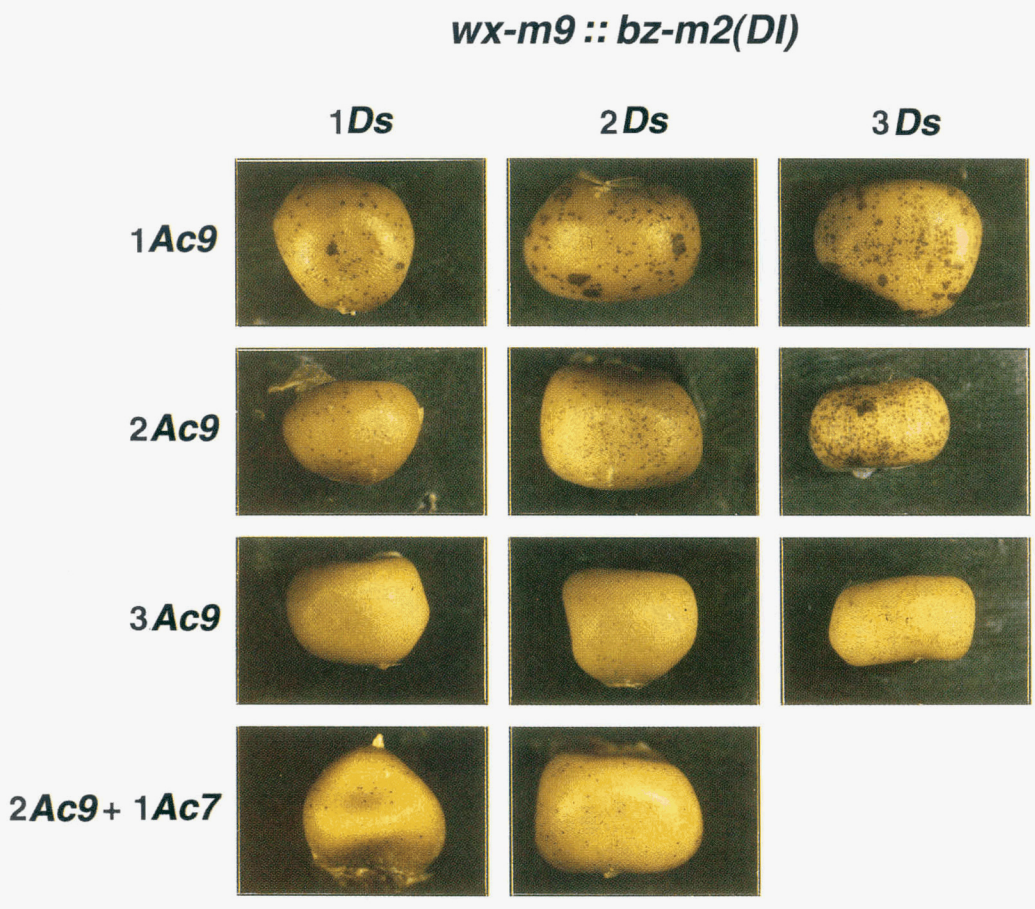

FIGURE 2.—Patterns of *Ds* excision from the *bz-m2(DI)* allele in kernels carrying one, two and three copies of *bz-m2(DI)* in the presence of either one, two or three *wx-m9* alleles. An increase in *wx-m9* dosage causes a reduction in the number of early events and thus leads to postponement of *Ds* excisions during kernel development. Upon an increase in the dosage of *wx-m9,* the number of small sectors first increases (two doses) and then decreases (three doses). Heterozygous kernels carrying two *wx-m9* alleles and one *wx-m7* allele display slightly more small sectors than the homozygous kernels with three copies of the *wx-m9* allele. The effects of altering the dosage of the *Ds* allele on the frequency of *Ds* excision are dependent on the dosage of the transactivating *wx-m9* allele (Table 2).

ther test this hypothesis, *wx-m7/wx-m7/wx-m9* and *wx-m9/wx-m9/wx-m7* heterozygotes that contain both alleles were generated and the *trans*-effects on *Ds* excision were tested again by using *bz-m2(DI)* as reporter allele. If the two *Ac* alleles encode different amounts of TPase during development, the level of TPase made by both alleles should result in new variegation patterns that are distinguishable from the patterns of *Ds* excision in *wx-m7/wx-m7/wx-m7* and *wx-m9/wx-m9/wx-m9* homozygotes. In contrast to the former study (HEINLEIN and STARLINGER 1991), the present analysis was carried out with kernels harboring constant doses of the *bz-m2(DI)* reporter allele to monitor the dosage effects of *wx-m7* and *wx-m9* in isolation from *Ds* dosage effects (described below). The dosage-specific phenotypes of *wx-m7* and *wx-m9* that were seen in this experiment are summarized in Figures 1 and 2, respectively.

As was described earlier (SCHWARTZ 1986; HEINLEIN and STARLINGER 1991) the phenotype of one copy of *wx-m7* consists of a very high number of small sectors and very rare large sectors and indicates high levels of TPase activity during late development. In contrast, kernels in which a second copy of *wx-m7* is present carry predominantly large sectors indicative of early *Ac* activity. The large sectors on kernels with two doses of *wx-m7* are of two kinds that need to be explained: the first type is represented by large colored sectors that indicate early *Ds* excision events; the second type is represented by large sectors that delineate areas of aleurone displaying a late spotting pattern similar to that found in the one dose *wx-m7* phenotype. Similar to the one-dose *wx-m7* phenotype, the density of small spots increases with increasing dosage of the *bz-m2(DI)* reporter allele. It seems likely that these areas displaying a late spotting pattern are caused by early events that, as is expected, also are induced at *Ac* itself. Mutation events in single *Ac* loci, including events that lead to increases and decreases of *Ac* copy number, are registered by changes in mutational responses of *Ds* and have been thoroughly described by MCCLINTOCK (1945,

TABLE 1

Genetic crosses to generate kernels containing different doses of *Ac* and *Ds*

Cross (female × male)	Dosage		Analyzed material		
				Kernels	
	Ac	*Ds*	Ears	Qualitative	Quantitative
		wx-m9::bz-m2(DI)			
sh bz wx × *Sh bz-m2(DI) wx-m9*	1	1	4	Whole ear	20
Sh bz-m2(DI) wx × *sh bz wx-m9*	1	2	10	Whole ear	39
Sh bz-m2(DI) wx × *Sh bz-m2(DI) wx-m9*	1	3	10	Whole ear	50
sh bz wx-m9 × *Sh bz-m2(DI) wx*	2	1	6	Whole ear	30
Sh bz-m2(DI) wx-m9 × *sh bz wx*	2	2	3	Whole ear	15
Sh bz-m2(DI) wx-m9 × *Sh bz-m2(DI) wx*	2	3	4	Whole ear	20
sh bz wx-m9 × *Sh bz-m2(DI) wx-m9*	3	1	7	Whole ear	35
Sh bz-m2(DI) wx-m9 × *sh bz wx-m9*	3	2	4	Whole ear	20
Sh bz-m2(DI) wx-m9 × *Sh bz-m2(DI) wx-m9*	3	3	7	Whole ear	35
		wx-m7::bz-m2(DI)			
sh bz wx × *Sh bz-m2(DI) wx-m7*	1	1	5	Whole ear	25
Sh bz-m2(DI) wx × *sh bz wx-m7*	1	2	11	Whole ear	55
Sh bz-m2(DI) wx × *Sh bz-m2(DI) wx-m7*	1	3	8	Whole ear	40
sh bz wx-m7 × *Sh bz-m2(DI) wx*	2	1	5	Whole ear	—
Sh bz-m2(DI) wx-m7 × *sh bz wx*	2	2	12	Whole ear	—
Sh bz-m2(DI) wx-m7 × *Sh bz-m2(DI) wx*	2	3	6	Whole ear	—
sh bz wx-m7 × *Sh bz-m2(DI) wx-m7*	3	1	7	Whole ear	—
Sh bz-m2(DI) wx-m7 × *sh bz wx-m7*	3	2	8	Whole ear	—
Sh bz-m2(DI) wx-m7 × *Sh bz-m2(DI) wx-m7*	3	3	1	Whole ear	—
		bz-m2::wx-m9Ds			
sh bz wx × *Sh bx-m2 wx-m9Ds*	1	1	7	140	3
sh bz wx-m9Ds × *Sh bx-m2 wx*	1	2	4	80	3
sh bz wx-m9Ds × *Sh bx-m2 wx-m9Ds*	1	3	4	80	3
Sh bz-m2 wx × *sh bz wx-m9Ds*	2	1	3	60	2
Sh bz-m2 wx-m9Ds × *sh bz wx*	2	2	6	120	3
Sh bz-m2 wx-m9Ds × *sh bz wx-m9Ds*	2	3	6	120	3
Sh bz-m2 wx × *Sh bx-m2 wx-m9Ds*	3	1	13	130	3
Sh bz-m2 wx-m9Ds × *Sh bx-m2 wx*	3	2	18	180	2
Sh bz-m2 wx-m9Ds × *Sh bx-m2 wx-m9Ds*	3	3	12	120	2

Genetic crosses, number of available ears and total number of kernels (qualitative analysis/quantitative analysis) analyzed to assess the effect of altering the dosage of *Ac* and *Ds* alleles on the frequency of *Ds* excision.

1948, 1949, 1951, 1955, 1956). The late spotting areas are contiguous with inner endosperm sectors that display the typical one dose of *wx-m7* wx → Wx reversion pattern (Figure 7, data not shown) or which are fully *Wx* revertant (data not shown; see Figure 1A in HEINLEIN and STARLINGER 1991) and thus may be consistent with *Ac* transposition events during which one copy of *Ac* was inactivated or lost (PETERSON 1987).

Homozygous *wx-m7/wx-m7/wx-m7* kernels carry several large colored sectors indicating that upon increasing the dosage of *wx-m7* from two to three doses early activity of *Ac* is maintained. However, in contrast to the kernels with two doses of the allele, the described large areas with late spotting patterns are absent. This is expected, because simultaneous transposition of two *Ac* copies that would lead to the formation of large sectors containing one dose of *wx-m7* is an unlikely event (HEINLEIN and STARLINGER 1991).

In contrast to the phenotype of kernels with two or three doses of *wx-m7,* the phenotype of heterozygous *wx-m7/wx-m7/wx-m9* kernels in which one *wx-m7* allele is replaced by one *wx-m9* allele exclusively consists of a very low number of unicellular revertant sectors. Large sectors are completely absent. This strongly indicates that *wx-m9* and *wx-m7* exert unique and specific *trans*-effects on *Ds* during development.

The phenotypes caused by increasing doses of the wx-m9 allele are illustrated in Figure 2. Kernels with one copy of wx-m9 carry sectors of any size indicating that the *Ac* element triggers *Ds* excisions throughout

TABLE 2

Number of *Bz* revertant sectors with increasing doses of *bz-m2(DI)*

Dosage		Revertant *Bz* sectors									
Ac	*Ds*	Ear	Counted Sectors					m	s_m	x	s_x	CI ($P = 0.95$)
								wx-m7::bz-m2(DI)				
1	1	*a*	212	205	256	230	245	230.0	22.0	223.0	39.0	223.0 ± 16.0
		b	213	321	147	276	252	242.0	66.0			
		c	240	174	264	192	222	218.0	36.0			
		d	237	198	165	243	195	208.0	32.0			
		e	237	171	264	207	216	219.0	35.0			
1	2	*a*	215	168	165	204	222	195.0	27.0	252.0	56.0	252.0 ± 56.0
		b	291	282	258	363	330	305.0	42.0			
		c	213	363	348	321	243	298.0	66.0			
		d	243	177	207	201	288	223.0	43.0			
		e	228	258	201	234	243	233.0	21.0			
		f	273	246	279	354	303	291.0	41.0			
		g	240	246	183	255	222	229.0	29.0			
		h	241	195	276	210	228	231.0	32.0			
		i	261	357	231	183	195	245.0	70.0			
		j	267	162	183	279	210	220.0	51.0			
		k	297	261	381	303	282	305.0	46.0			
1	3	*a*	486	603	459	546	726	564.0	106.0	536.0	97.0	536.0 ± 31.0
		b	531	435	513	426	507	482.0	48.0			
		c	432	531	450	429	411	451.0	47.0			
		d	591	492	594	537	543	551.0	42.0			
		e	486	747	591	594	336	551.0	152.0			
		f	477	600	702	711	456	589.0	120.0			
		g	438	543	486	675	693	567.0	163.0			
		h	471	474	555	630	534	553.0	66.0			
								wx-m9::bz-m2(DI)				
1	1	*a*	15	8	18	21	7	13.8	6.1	18.3	8.8	18.0 ± 4.1
		b	21	17	42	19	23	24.4	10.1			
		c	17	19	9	14	11	14.0	4.1			
		d	13	36	18	26	11	20.8	10.3			
1	2	*a*	65	61	57	51		58.5	6.0	75.2	18.5	75.2 ± 7.4
		b	61	40	97	63	76	67.4	21.0			
		c	55	57	69	39	65	57.0	10.4			
		d	99	70	87	54	60	74.0	18.7			
		e	104	91	65	75	105	88.0	17.7			
		f	87	132	116	48	58	88.2	36.1			
		g	86	65	76	87	49	72.6	15.9			
		h	102	128	73	81	93	95.4	21.3			
1	3	*a*	171	203	247	196	182	111.0	16.0	180.0	38.0	180.0 ± 8.0
		b	175	176	209	205	182	105.0	9.0			
		c	124	140	148	239	157	89.0	25.0			
		d	142	164	160	180	140	87.0	9.0			
		e	110	155	117	169	189	82.0	19.0			
		f	162	164	155	223	194	180.0	29.0			
		g	144	214	225	270	178	206.0	48.0			
		h	180	256	174	203	189	200.0	34.0			
		i	140	236	182	236	160	191.0	44.0			
		j	135	187	169	243	108	169.0	52.0			
2	1	*a*	35	26	18	44	39	32.4	10.4	81.6	52.7	81.6 ± 19.7
		b	105	54	80	67	56	72.4	21.0			
		c	100	103	129	91	78	100.2	18.8			
		d	135	185	145	194	220	175.8	35.3			
		e	67	105	87	54	52	73.0	22.7			
		f	37	33	23	34	51	35.6	10.1			

TABLE 2

Continued

Dosage		Revertant *Bz* sectors									
Ac	*Ds*	Ear	Counted Sectors					m	s_m	x	s_x	CI ($P = 0.95$)
						wx-m9::bz-m2(DI)						
2	2	*a*	149	173	117	229	248	183.0	55.0	186.0	53.0	186.0 ± 28.0
		b	108	176	191	274	211	192.0	60.0			
		c	108	214	254	155	185	183.0	56.0			
2	3	*a*	265	317	268	450	245	309.0	83.0	271.0	83.0	271.0 ± 39.0
		b	337	347	256	416	365	344.0	58.0			
		c	121	175	218	176	178	174.0	35.0			
		d	230	290	254	274	243	258.0	24.0			
3	1	*a*	9	3	16	8	10	9.2	4.6	24.0	19.2	24.0 ± 6.6
		b	7	17	15	28	27	18.8	8.8			
		c	56	21	27	25	30	31.8	13.9			
		d	81	51	48	49	66	59.0	14.3			
		e	4	4	6	2	13	5.8	4.3			
		f	31	35	24	21	20	26.2	6.5			
		g	16	31	3	30	6	17.2	13.1			
3	2	*a*	185	103	171	104	148	142.0	38.0	136.0	71.0	136.0 ± 33.0
		b	166	162	131	104	130	139.0	26.0			
		c	76	65	38	29	68	55.2	20.5			
		d	324	166	124	254	171	115.0	80.0			
3	3	*a*	171	122	108	130	133	133.0	23.0	171.0	58.0	171.0 ± 20.0
		b	155	191	194	297	196	207.0	53.0			
		c	155	95	122	47	92	102.0	40.0			
		d	144	223	160	178	257	192.0	47.0			
		e	196	274	202	238	295	241.0	44.0			
		f	198	124	113	142	214	158.0	45.0			
		g	171	210	173	135	133	164.0	32.0			

Number of revertant sectors seen in 1*Ac*, 2*Ac*, andd 3*Ac* aleurone of kernels carrying increasing doses of the *bz-m2(DI)* allele. Lower case letters designate individual ears generated by the same cross between different parental plants. The numbers behind each letter represent groups of total numbers of *Bz* sectors that were counted in the crown region of individual kernels taken from each ear as described in MATERIALS AND METHODS. m, group average (per ear); s_m, standard deviation of group average (per ear); x sample average; s_x, standard deviation of sample average. Confidence intervals (CI) for the "real mean" μ (95% probability) were calculated based on s_x using the t distribution statistics.

development. The phenotypes shown in Figure 2 might give rise to the impression that the sectors are either small or large and thus consist of two distinct size classes; this, however, is deceptive, as was shown by the earlier study in which the numbers of differently sized sectors were separately determined (HEINLEIN and STARLINGER, 1991).

Kernels carrying two copies of the *wx-m9* allele are characterized by a lower number of large sectors and by a much higher number of small sectors.

A third copy of *wx-m9* gives rise to exclusively small sectors. The sectors are lower in number and even appear smaller in size compared with the already small sectors present on kernels with two doses of the allele.

The phenotype of heterozygous *wx-m9/wx-m9/wx-m7* kernels (Figure 2) in which one copy of the *wx-m9* allele is replaced by one copy of the *wx-m7* allele is similar to the phenotype of homozygous *wx-m9/wx-m9/wx-m9* kernels. However, because of a slightly larger size of the sectors as well as a stochastic occurrence of large sectors (not shown) the phenotype of the heterozygotic kernels is also similar to the phenotype of kernels carrying two doses of the *wx-m9* allele.

Comparison of kernels with different doses of the *Ds* allele: The *Ac* allele- and *Ac* dosage-specific phenotypes displayed in Figures 1 and 2 carry either one, two or three genomic doses of the *bz-m2(DI)* reporter allele. This material also was suitable to assess the effect of increasing the dosage of *bz-m2(DI)* on the frequency of *bz-m2(DI)* → *Bz* reversion events. Table 2 summarizes the numbers of sectors that were present in the crown region of individual kernels that carried one, two and three doses of the *bz-m2(DI)* allele (Figures 1 and 2). Each *Ac* and *Ds* dosage combination was represented by three to 11 ears (Table 1) from each of which samples of five kernels were taken for quantitative evalua-

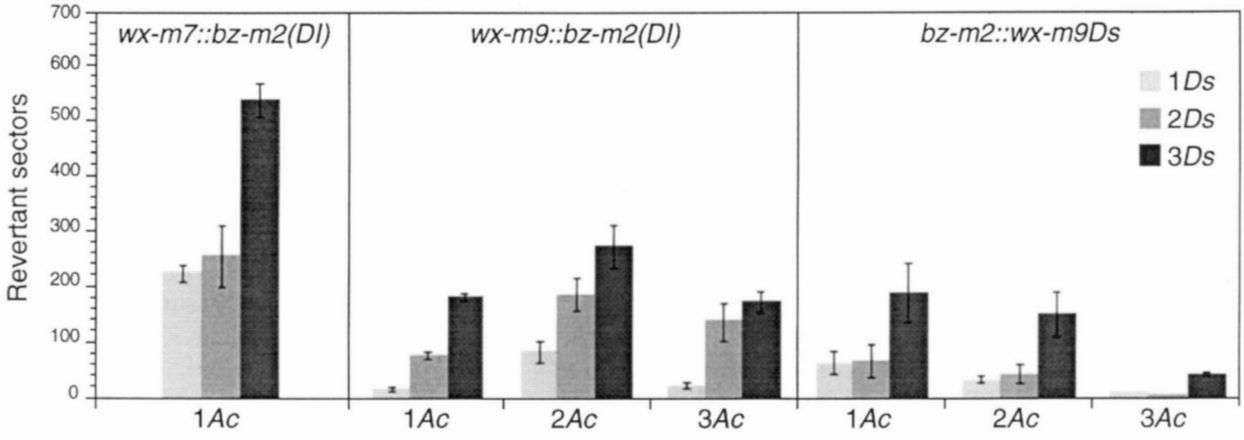

FIGURE 3.—The effect of altering the dosage of *Ds* on *Ds* excision frequency. With 95% probability, the mean value μ lies within the confidence intervals represented by the error bars. Details are explained in legend to Table 2.

tion. The numbers of total *Bz* sectors spread considerably between individual kernels of the same ear as well as between ears of the same cross and thus cause large standard deviations. Because no correlation can be drawn between the standard deviation and the size of the sample, the chance of improving the large error by increasing the number of samples seems to be low. Rather, these data may suggest that the high variability in excision frequencies is an intrinsic feature of the system that might indicate the involvement of yet unknown modifiers in the regulation of transposition.

Due to this high variability, the mean average x of the whole sample provides only an estimate for the *real* mean. To know how precise this estimate is, *t*-distribution statistics (Student's distribution) was applied. On the basis of the known sample average, its (imprecise) standard deviation and the (limited) size of the sample this statistical method allows to predict confidence intervals for the *real* mean value μ. The confidence intervals for μ (Table 2) for each of the corresponding *Ac* and *Ds* dosage combinations were calculated on the basis of a 95% probability that the real mean value μ lies within the shown confidence interval. The confidence intervals of μ in this experiment are graphically displayed in Figure 3 and can be cautiously described as follows.

In the presence of one copy of *wx-m7,* the estimated total number of revertant sectors displayed in the crown region of the kernels was in the range of 200–250 for one *Ds* copy, in the range of 250–300 for two *Ds* copies, and in the range of 500–570 for three *Ds* copies. Thus, the number of sectors did not increase in proportion to the increase in the number of *bz-m2(DI)* alleles. The number of sectors on kernels with two copies of the *bz-m2(DI)* allele was clearly less than two times larger than the number of sectors counted on kernels with only one copy of the allele. The value for three doses of the reporter allele only approached the value expected from the number of sectors that was seen with one dose (*ca.* 600–720 expected) and was (with some likelihood) higher than the expected 1.5 times the value of sectors seen with two doses (*ca.* 300–450 expected). Unfortunately, because of the inherent somatic instability of *Ac* dosage described above, it was not possible to gain reliable *Ds* dosage-specific data with higher doses of *wx-m7.*

When the *Ds* element was transactivated by *wx-m9,* the effect of increasing the dosage of the *bz-m2(DI)* reporter allele on the number of *bz-m2(DI)* → *Bz* revertant sectors was different. Even more surprisingly, the numbers of revertant sectors showed different *Ds* dosage-specific increments when the reporter allele was transactivated by different doses of *wx-m9* (Table 2, Figure 3). With one *wx-m9* allele, the reversion frequencies were in the ranges of 16–22 with one dose, of 67–83 with two doses, and of 170–190 with three doses of *bz-m2(DI).* Thus, the reversion frequencies increased more strongly than was expected from the factor of increase in *Ds* dosage. The number of sectors increased by a factor of three when *bz-m2(DI)* dosage was only increased by a factor of two (from one to two copies) and increased by a factor of two (at least) when *bz-m2(DI)* dosage was increased by a factor of only 1.5 (from two to three copies). With two copies of *wx-m9,* the frequencies were in the range of 60–100 with one dose, of 150–220 with two doses, and of 230–410 with three doses of *bz-m2(DI).* Thus, in this case the frequency of *Ds* excision appeared to be proportional to the number of alleles. With three *wx-m9* copies, the *bz-m2(DI)* reversion frequencies were in the range of 17–31 with one dose, of 100–170 with two doses, and of 150–190 with three doses of the *Ds* allele. Accordingly, reversion frequencies increased by a factor between three and nine when *Ds* dosage was increased by only a factor of two (from one to two doses) and by a factor between 0.9 and 1.9 when the dosage was increased by a factor of 1.5 (from two to three doses). The value for three *Ds* was at least 4.7 times higher than the value for one *Ds.* Therefore, whereas a direct proportionality between *bz-m2(DI)* dos-

TABLE 3

Number of revertant *Wx* sectors with increasing doses of *wx-m9Ds*

Dosage		Revertant *Wx* sectors												
Ac	*Ds*	Ear	Counted sectors										x	s_x	CI ($P = 0.95$)
			bz-m2::wxm9Ds												
1	1	*a*	41	39									56.0	21.0	56.0 ± 22.0
		b	50	42											
		c	82	84											
1	2	*a*	103	106									64.0[a]	36.0	64.0 ± 30.0
		b	52	51											
		c	39	37	34	32									
1	3	*a*	227	186									184.0	51.0	184.0 ± 53.0
		b	107	141											
		c	202	239											
2	1	*a*	24	31	39	35							29.4	6.2	29.4 ± 5.2
		b	23	24	34	25									
2	2	*a*	73	71	74								37.9[a]	17.3	37.9 ± 17.3
		b	17	13	13	14	12								
		c	43	44	43										
2	3	*a*	119	109	106								145.0	41.0	145.0 ± 41.0
		b	101	114	107										
		c	209	231	206										
3	1	*a*	0	2	0	0	0	2	3	1	1	1	1.5	1.5	1.5 ± 0.6
		b	1	1	2	0	0	0	0	0	1	0			
		c	3	1	3	3	4	4	2	4	4	4			
3	2	*a*	2	2	2	0	0	0	0	1	0		2.1	1.8	1.5 ± 0.9
		b	1	2	3	4	2	3	5	4					
3	3	*a*	32	29	30	29	35	43	41	44	40	44	38.3	5.8	38.3 ± 2.2
		b	36	36	30	33	29	47	47	47	47	40			
		c	43	35	39	36	40	44	38	34	40	40			

Number of revertant sectors seen in 1*Ac*, 2*Ac*, and 3*Ac* inner endosperm of kernels carrying increasing doses of *wx-m9Ds*. Lower case letters designate individual kernels. The numbers behind each letter represent the number of total *Wx* sectors counted in individual sectors made from the same kernel. Sample averages x were calculated on the bases of the whole sample, except in cases marked by *a*. Other details are described in the legend for Table 2. CI, confidence intervals for μ.

[a] Corresponding groups (of individual sections) varied in size and thus the sample average was calculated from group averages.

age and *bz-m2(DI)* reversion could be measured in the presence of two doses of *wx-m9*, considerable deviations from proportional values were found for one and three doses of the allele.

In another set of genetic experiments, the effect of *Ds* dosage on *Ds* excision frequency was analyzed by monitoring the variegated *Waxy* phenotypes of kernels that carried increasing doses of *wx-m9Ds* allele in the presence of constant doses of *bz-m2* as the transactivating allele. The revertant *Wx* sectors were visualized at 30 DAP by staining endosperm sections with iodine. As is shown in Figure 4, the number of iodine-stained sectors increased only weakly when the number of *wx-m9Ds* alleles was increased from two to three copies. In contrast, a much stronger increase was seen when *Ds* dosage was increased from two to three copies. This remarkable behavior of *Ds* was seen with either one, two, or three doses of *bz-m2*. The number of kernels that could be analyzed by embedding, sectioning and staining with iodine was limited. However, similar differences in reversion frequencies were also observed when ≥10 kernels of each available ear (Table 1) were filed and directly observed after iodine staining of the endosperm starch. To quantify *wx-m9Ds* reversion frequencies in response to *wx-m9Ds* dosage, the number of sectors present in 2–10 endosperm sections that were prepared from two to three kernels per genotype (Table 1) were counted separately for each sector size and the averaged numbers are displayed in Figure 5. The graphs show that the low and strong increases in reversion frequencies that were seen by an increase in *wx-m9Ds* dosage from one to two and from two to three, respectively, can be measured over all sector sizes and, thus, are independent from developmental state of the kernel.

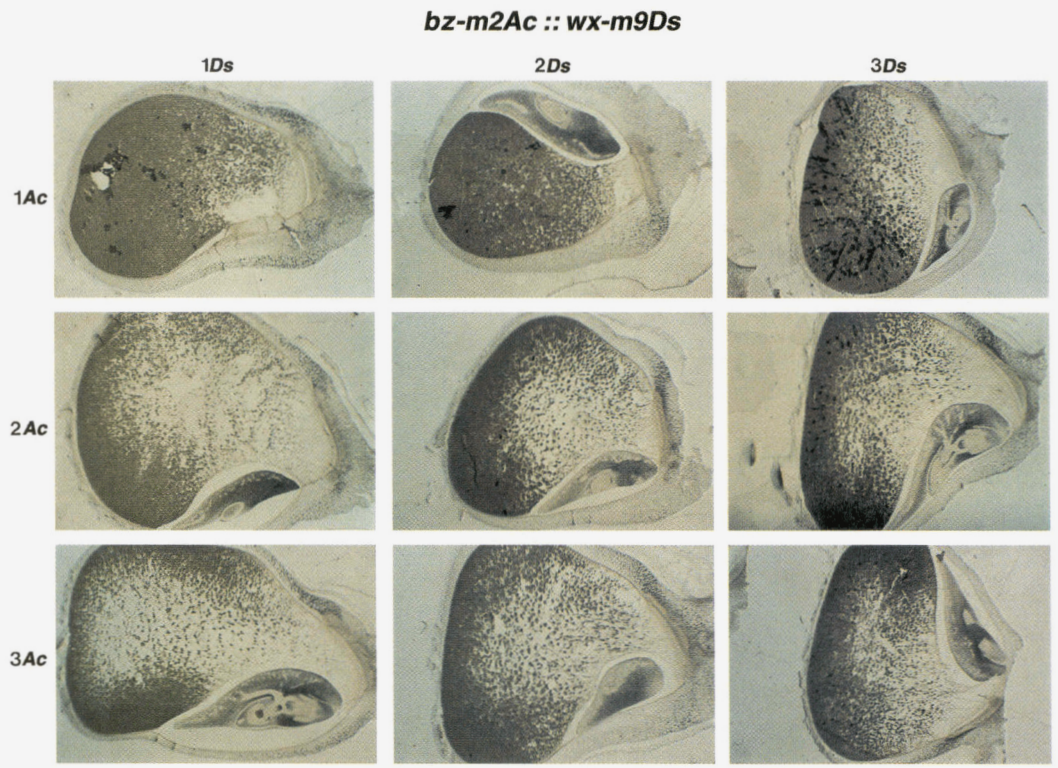

FIGURE 4.—Dosage-specific mobilization of *Ds* from the *wx-m9Ds* allele by one, two or three doses of *bz-m2* harboring *Ac*. The number of *Wx* revertant sectors did not increase in proportion to the increase in the genomic dosage of the *wx-m9Ds* allele. Sections of kernels were stained with iodine to visualize wild-type starch containing cell clones. The number of *Wx* revertant sectors increased only slightly if the number of *wx-m9Ds* alleles was increased from one to two, but showed a dramatic increase if the dosage of this allele was further increased to three copies.

Table 3 displays the total numbers of sectors that were counted in the paraffin sections. The confidence intervals for μ calculated for one, two and three doses of *wx-m9Ds* in kernels with one dose of *Ac* are in the ranges of 30–80, of 30–90, and of 130–230, respectively. The intervals with two doses of *bz-m2* are ~24–34, 20–55, and 100–180, and with three doses of *Ac* ~1–2, 1–3, and 36–40, respectively. These numbers confirm the phenotypic observation described above that at all *Ac* dosage levels, the number of *wx-m9Ds* → *Wx* reversion events did not or only weakly increase by an increase in *wx-m9Ds* dosage from one copy to two copies, but increased strongly when the dosage of the reporter allele was increased from two to three copies. With two doses of *Ds*, the number of sectors was less than twice as high as the number of sectors seen with one dose of the allele. In contrast, the number of iodine stained sectors in kernels with three *Ds* copies was more than proportional higher than in kernels with two *Ds* copies. With three copies, the number of sectors was even higher than the total number of sectors obtained with one and two copies.

Comparison of the activity of different mutable alleles in the presence of the same TPase source: It was shown earlier that two different elements usually are not mobilized simultaneously in the same cell (HEINLEIN and STARLINGER 1991). One idea to explain this observation is to propose a low availability of *trans*-acting factors (including TPase), which manifests a low probability of setting up active transposition complexes. But, could the TPase substrates play a role by themselves? Could they differentially act *in cis* and differ in their responsiveness to specific TPase levels in the cells? As one approach to answer this question, the activity patterns of different TPase substrate elements were compared. The kernel shown in Figure 6 has the genotype *C sh bz-m2/C sh bz-m2/C-I sh-m5933 bz-m2*. The TPase encoded by the *Ac* element in *bz-m2* is required for excision of *Ac* itself as well as for transactivation of the "double-*Ds*" structure present in the *sh-m5933* allele. In case of *sh-m5933*, *Ac* causes chromosome breakage at the site of the element resulting in the loss of the chromosome fragment carrying the distally located *C1-I* allele. Sectors generated by this loss are colored since the wild-type *C1* gene on the homologous chromosome is expressed (DÖRING *et al.* 1989). In the kernel shown in Figure 6, the breakage events did not result in wild-type colored sectors, which demonstrates that the breakage events were not accompanied by simultaneous transposition of *Ac*. Instead, with the *Ac* element remaining in the *Bz* gene, the formation of wild-type pigment was precluded and

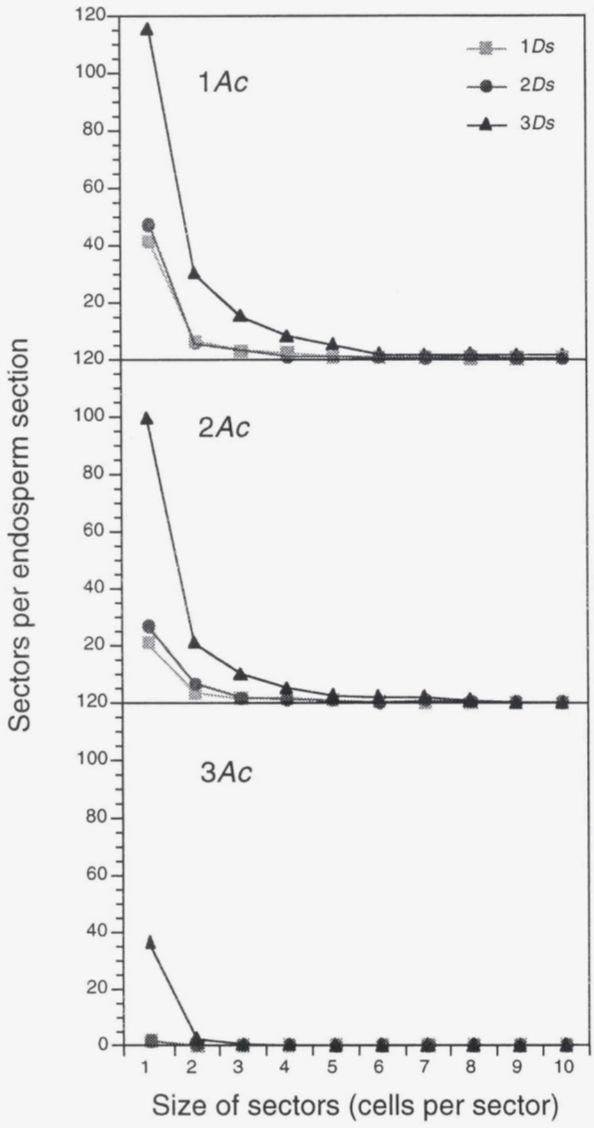

FIGURE 5.—In the presence of the *bz-m2* allele the number of *Ds* excisions from the *wx-m9Ds* allele did not increase in proportion to an increase in the number of *Ds* alleles. A small increase in the number of *Ds* excisions that was seen upon an increase in the dosage of *Ds* from one to two copies was followed by a dramatic increase upon further increase in *Ds* dosage to three copies. The number of revertant *Wx* sectors seen in iodine-stained kernel sections were counted for each sector size separately. The effect of increasing *Ds* dosage on *Ds* excision frequency was the same with all sector sizes and thus appears to be independent on the developmental state of the kernel. The effect was the same also regardless of whether one, two or three doses of the transactivating *bz-m2* allele were present.

FIGURE 6.—Different timing of mobilization of two different elements in the presence of the same TPase source. The genotype of the kernel is *C sh bz-m2/ C sh bz-m2/ C-I sh-m5933 bz-m2*. The *bz-m2* allele contains *Ac* and the *sh-m5933* allele harbors a composite double-*Ds* element. The kernel is colorless over most areas since the *sh-m5933* allele is linked to the distally located *C1-I* allele; this prevents aleurone color formation. The large bronze colored sectors originated from chromosome breakage events that were induced during early development at the site of insertion of the double-*Ds* element by the action of *Ac*. These events led to loss of the distally located inhibitory *C1-I* allele and thus subsequently allowed the formation of pigments. Wild-type anthocyanin accumulation was precluded, however, as long as the *Ac* element resided in the *Bronze* gene. Wild-type sectors are consistently small indicating that excision of *Ac* from *bz-m2* occurred much later during development than chromosome breakage at *sh-m5933*. The early events at the *sh-m5933* locus prove that during early development TPase was expressed. Apparently, this early activity was not sufficient to induce excision of *Ac* from *bz-m2*. As indicated by small wild-type colored sectors, excision of *Ac* ocurred much later during development at a time when chromosome breakage no longer occured. Neither small bronze-colored nor small wild-type-colored sectors were seen on the kernels.

resulted in bronze-colored breakage sectors. The small size of the wild-type pigmented sectors shows that *Ac* excision occurred much later during development at a time when *Ds* transactivation must have ceased, because bronze-colored sectors of equal size are not present. This phenotype confirms that *Ac*-dependent events do not occur simultaneously in the same cells (HEINLEIN and STARLINGER 1991). In addition, this phenotype also displays a situation in which during early development the TPase expressed from *Ac* was able to transactivate *Ds* but apparently was not sufficient to induce excision of *Ac* itself. The high frequency of late *Ac* excision events and also the lack of intermediate-sized sectors seems to exclude the possibility that the lack of early *Ac* excision is caused by an overall low excision frequency. The high amount of revertant *Bz* spots rather suggests that despite of early *Ac* transactivity, the frequency of *Ac* excision was low during early development. Along the same line, breakage events occurred early in development when there was only a low number of cells; this strongly indicates that the overall probability of breakage events is quite high. However, despite the fact that during late development the endosperm consists of many more cells in which breakage events potentially can occur, late breakage sectors are missing. The absence of breakage events during late development cannot be due to the lack of active *Ac* TPase at this

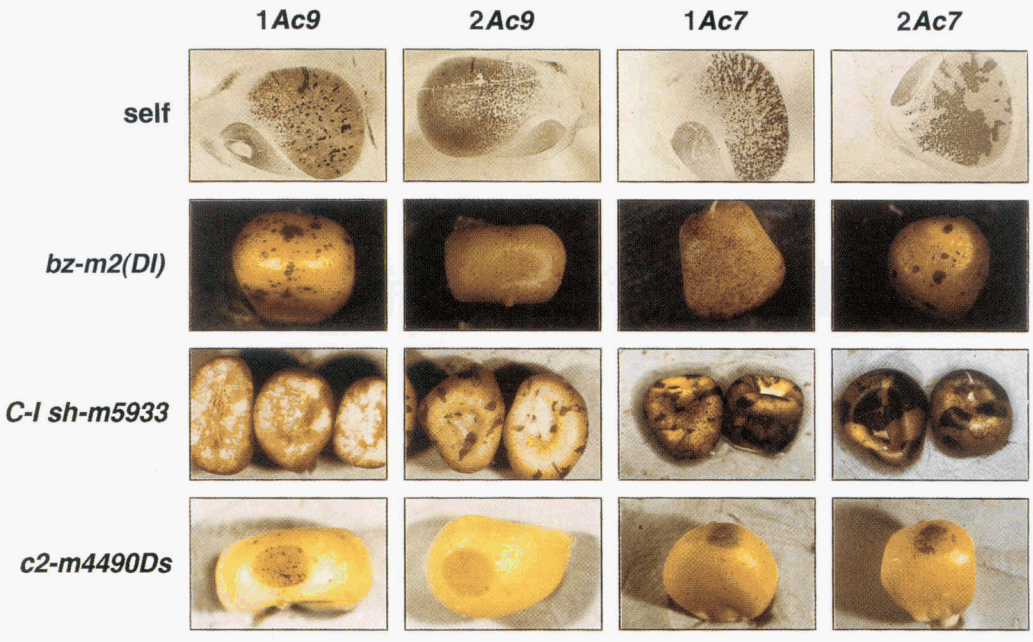

FIGURE 7.—Patterns of mobilization of Ac ("self") and *Ds* elements from different alleles in the presence of one or two doses of *wx-m7* and *wx-m9*, respectively. Except for "self" phenotypes, crosses were performed between *Ac* strains and *Ds* strains. Accordingly, the "*bz-m2(DI)*", the "*C-I sh-m5933*" and the "*c2-m4490Ds*" kernels with one and two copies of *Ac* carried two and one copies of the *Ds* allele, respectively.

"self": Patterns of *Ac* excision from *wx-m7* and *wx-m9*; self/1Ac9: *wx/wx/wx-m9*; self/2Ac9: *wx-m9/wx-m9/wx*; self/1Ac7: *wx/wx/wx-m7*; and self/2Ac7: *wx-m7/wx-m7/wx*. Paraffin-embedded kernels were sectioned and stained with iodine.

"*bz-m2(DI)*": *trans*-activation of *Ds* in the *bz-m2(DI)* allele; *bz-m2(DI)*/1Ac9: *bz-m2(DI) wx/bz-m2(DI) wx/bz wx-m9*; *bz-m2(DI)*/2Ac9: *bz wx-m9/bz wx-m9/bz-m2(DI) wx*; *bz-m2(DI)*/1Ac7: *bz-m2(DI) wx/bz-m2(DI) wx/bz wx-m7*; and *bz-m2(DI)*/2Ac7: *bz wx-m7/bz wx-m7/bz-m2(DI) wx*. When filed and stained with iodine, the kernels showed the *Ac* allele- and *Ac* dosage-specific wx → Wx variegation patterns (seen in line "self") in the inner endosperm (data not shown). "*C-I sh-m5933*": *trans*-induction of chromosome breakage events at the site of the *Ds* insertion in *sh-m5933* (see legend to Figure 6).

C-I sh-m5933/1Ac9: *C1-I sh-m5933 Bz Wx/C1-I sh-m5933 Bz Wx/C sh bz wxm9*; *C-I sh-m5933*/2Ac9: *C sh bz wx-m9/C sh bz wx-m9/C1-I sh-m5933 Bz Wx*; *C-I sh-m5933*/1Ac7: *C1-I sh-m5933 Bz Wx/C1-I sh-m5933 Bz Wx/C sh bz wx-m7*; and *C-I sh-m5933*/2Ac7: *C sh bz wx-m7/C sh bz wx-m7/C1-I sh-m5933 Bz Wx*. The red pigment present in the aleurone of kernels carrying one copy of *wx-m9* was caused by a stable *pr* allele segregating in the material. The colored/bronze twinned character of the larger sectors originated from the breakage-fusion-bridge cycle that was induced following the initial chromosome breakage event (MCCLINTOCK 1953, 1956; PETERSON 1987). Somatic changes in *Ac* dosage are responsible for larger colorless sectors on kernels with one dose of *Ac* as well as for areas displaying the one dose of *Ac* chromosome breakage pattern on kernels with two copies of *Ac*.

"*c2-m4490Ds*": mobilization of *Ds* from the *c2-m4490* allele. Kernels were filed and stained with iodine to expose the variegation patterns of *wx-m9* and *wx-m7* in the inner endosperm. These were the same as those seen in the stained kernel sections displayed in the upper row ("self"). The excision activity of the *Ds* element does not correlate with *Ac* excision activity. *c2-m4490Ds*/1Ac9: *c2-m4490Ds, wx/c2-m4490Ds, wx/c2, wx-m9*; *c2-m4490Ds*/2Ac9: *c2, wx-m9/c2, wx-m9/c2-m4490Ds, wx*; *c2-m4490Ds*/1Ac7: *c2-m4490Ds, wx/c2-m4490Ds, wx/c2, wx-m7*; and *c2-m4490Ds*/2Ac7: *c2, wx-m7/c2, wx-m7/c2-m4490Ds, wx*.

time, as *Ac* transposition occurred. The absence of small *bz* sectors rather suggests that late breakage events did not occur during late development when the TPase was highly active.

Differences in transactivation patterns of *Ds* elements were also seen among *bz-m2(DI), C1-I sh-m5933* and *c2-m4490Ds* in the presence of one or two doses of *wx-m7* and *wx-m9*, respectively (Figure 7). Whereas the patterns of excision of *Ds* from the *bz-m2(DI)* allele were quite similar to the self-excision patterns of *Ac*, deviations from the *Ac* dosage-specific phenotype were seen with *C-I sh-m5933* and *c2-m4490Ds*. The *Ac* dosage-specific chromosome breakage patterns of *sh-m5933* were reminiscent of the *Ac* dosage-specific excision patterns of *bz-m2(DI)*. However, the number of early events induced at *sh-m5933* clearly exceeded the number of early events that occurred at *bz-m2(DI)*; even much larger sectors were seen. While these differences might be due to the overall higher frequency of events at *sh-m5933* that lead to a visible phenotype, these differences might also suggest that during early development the double *Ds* element present in *sh-m5933* is more efficiently transactivated than the *Ds* element in *bz-m2(DI)*.

More specific *Ds* reversion patterns were exhibited by the *c2-m4490Ds* allele. In the presence of either *wx-m7* or *wx-m9*, the patterns of revertant *C2* sectors in the aleurone consisted of very rare and small spots and thus were completely different from the aforementioned *Ds* transactivation patterns. This was not due to a change in activity of *wx-m7* and *wx-m9* as was verified by iodine

staining of the inner endosperm of the kernels. To the contrary, marked differences in sizes between *C2* and *Wx* sectors were visualized in these kernels. Moreover, unlike the other *Ds* reporter alleles, the *c2-m4490Ds* allele showed almost identical transactivation patterns regardless whether *wx-m7* or *wx-m9* was the transactivating allele. The nature of the unusual low frequency and late timing of reversion events of *c2-m4490Ds* is unknown. The lack of large sectors could be explained by a low frequency of *Ds* excision events that lead to restoration of a functional *C2* allele. However, it seems remarkable that late during development, when the probability of reversions is increased by a higher cell number and revertant sectors are actually being formed, the number of *c2-m4490Ds* → *C2* reversions does not correspond to the rate of *Ac* activity at this time. On kernels with one dose of *wx-m7*, which is highly active during late development, the number of small *C2* sectors is expected to be higher than on kernels with one dose of *wx-m9*, which has a low activity during late development. However, no such correlation between *Ac* activity and *Ds* reversion frequency was observed.

DISCUSSION

This study investigated the variegation patterns on maize kernels to gain information about the *trans*- and *cis*-acting determinants by which the *Ac/Ds* system is controlled.

While several independent transposable element systems in Drosophila as well as in bacteria keep control of transposition by exploiting either two promoters, translational frameshifts, or a splicing anomaly to synthesize an inhibitory molecule along with the TPase from the same gene (SIMONS and KLECKNER 1988; ROBERTSON and ENGELS 1989; SEKINE and OHTSUBO 1989; MISRA and RIO 1990; ESCOUBAS *et al.* 1991), the *Ac/Ds* system appears to use only one protein, which is the *Ac*-encoded TPase (KUNZE *et al.* 1987; COUPLAND *et al.* 1988; HOUBA-HÉRIN *et al.* 1990; FUSSWINKEL *et al.* 1991). Therefore, the *Ac* allele- and *Ac* dosage-specific *trans*-effects of *Ac* are probably caused by specific levels of active TPase in the cells. However, my observations suggest that *Ac* or *Ds* elements may react in different ways in the presence of given TPase levels providing evidence for the possibility that transposition is not exclusively controlled by the availability of active *trans*-acting TPase molecules. Rather, I surmise that the *trans*-activity of *Ac* in a cell is interpreted *in cis* at the site of the element to be excised, namely by effects, which in the presence of a given amount of active TPase, determine (or influence) the capability of a particular *Ac* or *Ds* element to serve as an excision substrate.

***wx-m9* and *wx-m7* differ in *trans*-effects:** A previous analysis showed that the different patterns of *Ds* excision from the *bz-m2(DI)* allele that are seen in the presence of *wx-m7* and *wx-m9* are caused by the presence of the *Ac* alleles themselves and not by the products of *trans*-acting modifier genes (HEINLEIN 1995). The specific variegation phenotypes that are displayed by heterozygous *wx-m7/wx-m7/wx-m9* and *wx-m9/wx-m9/wx-m7* kernels established that *wx-m7* and *wx-m9* indeed exert unique *trans*-effects during development. I propose that the *Ac* alleles express different amounts of active TPase during kernel development and that the phenotypes of the heterozygotes result from the total level of active TPase made by both *Ac* alleles. This hypothesis is depicted in the model shown in Figure 8, which describes one possibility of how *Ac* allele- and *Ac* dosage-specific TPase levels in the cells might determine the specific TPase activities during development that are registered as specific patterns of large and small *bz-m2(DI)* → *Bz* aleurone sectors. Basically, this model consists of three components: *wx-m7* and *wx-m9* express different TPase levels during development, the level of TPase produced in the cells is proportional to *Ac* dosage, and high levels of TPase reduce TPase activity and inhibit transposition.

The unique phenotype of heterozygous *wx-m7/wx-m7/wx-m9* and *wx-m9/wx-m9/wx-m7* kernels established that *wx-m7* and *wx-m9* confer unique *Ac* activities during development, thus providing support for the hypothesis that the two *Ac* alleles express specific levels of TPase. The pattern observed on *wx-m7/wx-m7/wx-m9* heterozygotes consisted of only a few unicellular revertant sectors and therefore differed strongly from the pattern of predominant large spots seen on *wx-m7/wx-m7/wx* and *wx-m7/wx-m7/wx-m7* kernels. The *wx-m9/wx-m9/wx-m7* heterozygous kernels differed from homozygous *wx-m9* kernels by the presence of a low number of larger sectors; however they differed strongly from homozygous *wx-m7* kernels by a considerable high amount of small sectors and a much lower number of large sectors. The position of *Ac* insertion in the *waxy* gene may provide an explanation for the proposed differences in TPase expression of the two *Ac* insertions. In the *wx-m7* allele, *Ac* is inserted 46 bp upstream of the TATA box of *waxy*, whereas in *wx-m9*, the *Ac* is inserted in the tenth exon, ~2.5 kb downstream of the transcriptional start site (KLÖSGEN *et al.* 1986). Because of the close proximity between the *Ac* promoter and the *waxy* promoter in the *wx-m7* allele, the *Ac* element might to some extent be controlled by *waxy* gene activity. In *wx-m9*, this interaction between *Ac* and *waxy* might be much weaker because the *Ac* promoter and the 5′ region of the waxy gene are much more distant from each other.

The *Ac* allele- and *Ac* dosage-specific *bz-m2(DI)* reversion patterns suggest that TPase expression from both *Ac* alleles is under developmental control. For example, the absence of large sectors but the presence of a very high number of small revertant *Bz* sectors on kernels

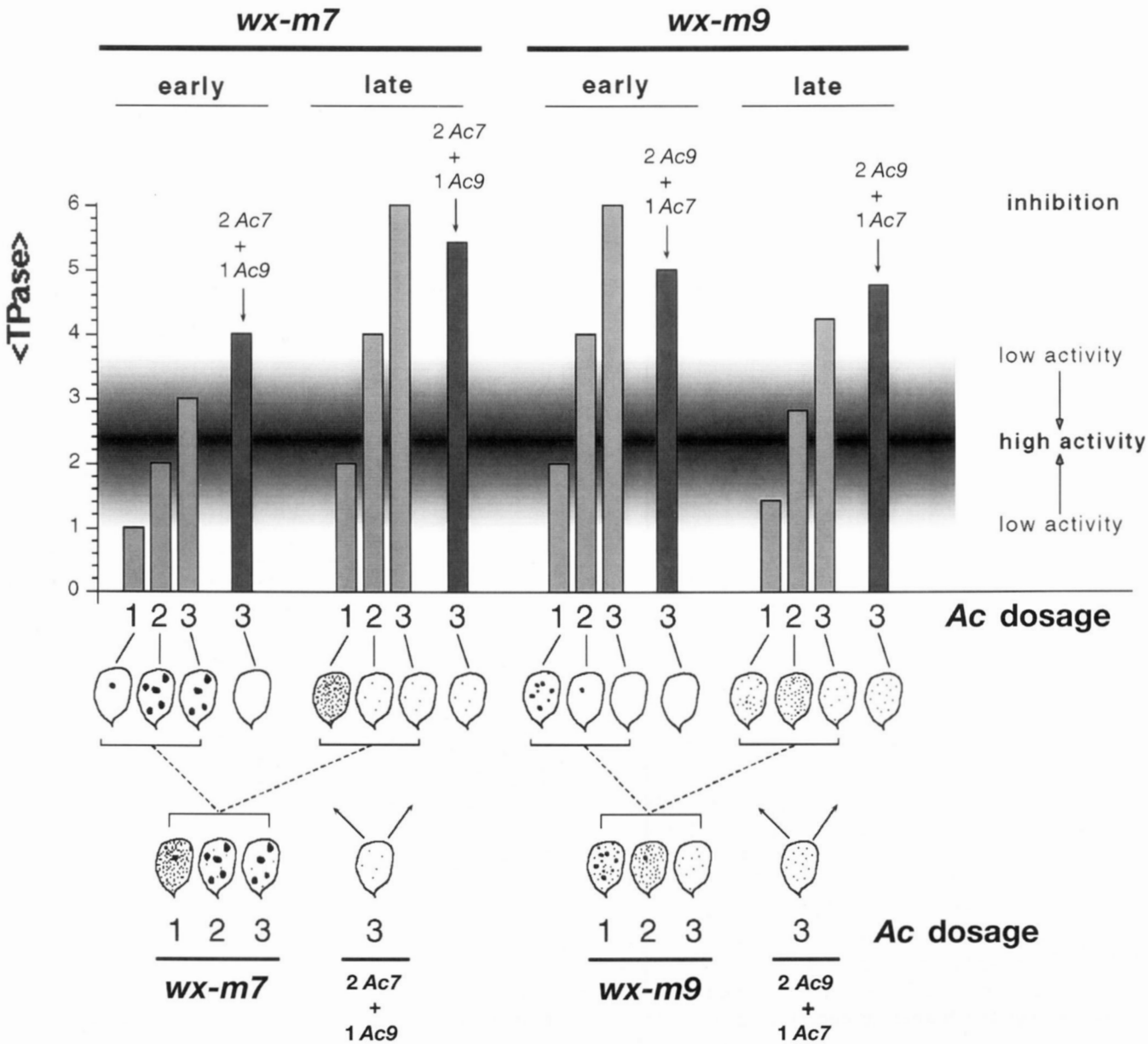

FIGURE 8.—Model to explain the different *bz-m2(DI)* variegation phenotypes seen with one, two and three doses of *Ac* alleles *wx-m7* and *wx-m9*. Consideration is confined to large and small sectors that are indicative of early and late excision events, respectively. Very small sectors sectors indicating very late events are not considered. The generalized phenotypes of kernels with different doses of *wx-m7* and *wx-m9* and also of kernels that are heterozygous for the two alleles ("2Ac7 + 1Ac9" = *wx-m7/wx-m7/wx-m9*; "1Ac9 + 2Ac7" = *wx-m9/wx-m9/wx-m7*) are shown at the bottom of the figure (A). According to the occurrence of small- and large-sized sectors, these phenotypes are dissected into their component "early" and "late" phe,notypes (B) which are separately considered further (C). The "early" and "late" *Ac* allele- and *Ac* dosage-specific variegation patterns are consistent with the following assumptions (with one exception—for *wx-m9/wx-m9/wx-m7*—see DISCUSSION): the amount of TPase expressed from *Ac* is positively correlated with *Ac* dosage; the level of TPase expressed from *Ac* changes during development; during development, the amount of TPase expressed from *wx-m7* does increase, whereas TPase expression from *wx-m9* does decrease; and finally, transposition activity is inhibited at higher TPase expression levels; thus, with increasing levels of TPase expression the activity of the protein first increases and then decreases (as is illustrated by the gradient in the figure).

carrying one *wx-m7* allele suggest that *wx-m7* expresses a low TPase activity during early development and an increased TPase activity during late development. It has to be borne in mind, however, that an increasing number of endosperm cells during development increases the probability of revertant cells during late development. Thus, the number of small sectors is expected to exceed the number of larger sectors even if the trans activity of *Ac* remains constant. However, a constant TPase level can not account for the heavily variegated phenotype of one dose of *wx-m7* that almost exclusively consists of small sectors that are homogeneous in size.

In contrast to kernels carrying one dose of *wx-m7*, kernels carrying one copy of *wx-m9* exhibit a higher number of larger sectors and a lower number of small sectors, suggesting that during early development *wx-m9* encodes a higher TPase activity than *wx-m7*, whereas during late development, the TPase activity provided by *wx-m9* is lower than the TPase activity provided by *wx-m7*.

The exact *in vivo* levels of TPase mRNA and protein expressed by *wx-m7* and *wx-m9* are not known and still need to be determined. However, it has been shown that at 20 days after pollination the amount of *Ac* protein present in the developing *wx-m7* endosperm depends roughly proportionally on the *Ac* copy number (FUSSWINKEL *et al.* 1991). It is assumed in the model shown in Figure 8 that both *Ac* alleles produce specific TPase levels that linearly increase with increasing dosage of the allele.

The variegation patterns are consistent with the hypothesis that high levels of TPase expression inhibit transposition as was suggested by SCOFIELD *et al.* (1993). The patterns indicate that at times when the activity of one copy of *Ac* is low, an increase in *Ac* dosage causes a positive effect on *Ds* excision frequency. At the times during development at which an *Ac* allele is highly active with one dose, however, an increase in *Ac* dosage has a negative effect on *Ds* excisions. For example, during early development when the TPase activity provided by one dose of *wx-m7* is relatively low TPase activity is increased by increasing the dosage of the allele. In contrast, during late development, when the TPase activity expressed by one dose of *wx-m7* is relatively high, an increase in the dosage of the allele leads to inhibition of TPase activity. The model illustrates that during early development the TPase level remains below the inhibitory threshold even if two and three doses of the allele are present and TPase activity increases rather than decreases with higher dosage. During late development, however, an increase in the dosage of the allele leads to TPase levels above the threshold and transpositions are inhibited. The existence of an inhibition threshold is also supported by the *bz-m2(DI)* variegation patterns seen in the presence of *wx-m9*. During early development, when several *Ds* exision events occur with one dose, an increase in *wx-m9* dosage leads to inhibition of *Ds* excision events. This allele has a positive dosage effect during late development, however, when TPase activity of one dose of *wx-m9* is relatively low. The phenotype of the *wx-m7/wx-m7/wx-m9* heterozygote provides further support. As described above, one copy of *wx-m7* is not very active during early development and presumably expresses low TPase levels. Thus, TPase activity is increased with two copies of the allele and transpositions are not inhibited even in the presence of three doses of *wx-m7*. However, replacement of the third dose of *wx-m7* with one dose of the more highly active *wx-m9* allele causes complete inhibition, presumably because a higher level of TPase produced by *wx-m9* causes the total TPase level to exceed the critical threshold.

In summary, the model predicts that an increase in *Ac* dosage may lead either to an increase or to a decrease in excision frequency, dependent on whether the *Ac* allele- and *Ac* dosage-specific level of TPase expression remains below or exceeds the proposed inhibitory threshold. The model proposes that with increasing TPase concentrations the transposition activity goes through a maximum: a certain optimal TPase concentration causes the highest possible excision frequency whereas any other TPase concentration is suboptimal for transposition and causes a lower excision frequency. Several possible mechanisms have been suggested to explain negative dosage effects of *Ac*, including TPase titration by a limiting host factor (HEINLEIN and STARLINGER 1991) and TPase aggregation (HEINLEIN *et al.* 1994). Positive effects of increasing *Ac* dosage may occur with TPase concentrations remaining below the threshold at which the inhibitory mechanism becomes effective.

The model as shown in Figure 8, however, shows inconsistencies. For example, in the heterozygotic kernels as well as in kernels with three doses of the *wx-m9* allele, late excision events occur even though the depicted level of TPase in the cells is above the critical threshold. This might be explained by an additional assumption that the inhibitory mechanism is leaky and only decreases the probability of transpositions. Thus, a low probability may prevent reversion events during early development when cell number is low but may result in the formation of revertant sectors during late development, when cell number is high. However, in the case of late sectors seen with three doses of *wx-m9*, one might also consider the following: the sectors on kernels carrying three doses of *wx-m9* are smaller in size than the already small sectors that are present on kernels carrying two doses of the allele. This indicates that an increase in *wx-m9* dosage from two to three copies postpones the timing of excision events even beyond the timing of the already late events that occur with two doses of the allele as was already noted by MCCLINTOCK (1948, 1951). The model as shown in Figure 8 is limited to only one "late" stage of development. Consideration is limited to the time of events with two doses of *wx-m9*, and events occurring at even later stages with three doses of the allele are not considered. The model predicts, however, that the TPase level produced by *wx-m9* decreases during development. Thus, the three doses of *wx-m9* phenotype may be explained by stating that the events that occur at "late" times with two doses of *wx-m9* are inhibited with three doses of the allele as is indicated by the model. However, at an even later time (not shown in Figure 8, but consistent

with the model), the TPase level produced by three doses of *wx-m9* might drop below the inhibition threshold, thus permitting *Ds* excision events at very late stages of endosperm development. The developmental decrease in TPase levels encoded by *wx-m9* might also explain the lack or low number of very small sectors on kernels with only two doses of *wx-m9:* during the "late" stage of kernel development, the TPase level causes high TPase activity in the cells. During very late stages, however, the TPase level has declined to a low level, which is not sufficient to promote transposition events.

Nevertheless, the variegation phenotype of heterozygous *wx-m9/wx-m9/wx-m7* kernels appears to be inconsistent with the model. The model predicts that TPase levels exceed the inhibitory threshold throughout development (Figure 8). Despite that, revertant spots are observed that are comparable in size with the spots present on kernels with two doses of *wx-m9*. The discrepancy between the occurrence of late events in *wx-m9/wx-m9/wx-m7* kernels and the inhibition of late TPase activity presumed by the model indicates that the regulation of transposition involves more complex mechanisms than the model considers.

The frequency of excision events is not exclusively controlled by the availability of active TPase molecules: I observed that *sh-m5933* and *bz-m2* give rise to differently sized sectors in the same kernels. The phenotype of the kernel shown in Figure 6 indicates that the breakage activity of the *sh-m5933* allele was high during early development and then ceased (no small *bz* sectors are visible although the probability of events increased with the number of cells during development). In contrast, excision of the *Ac* element that provides the TPase required for early *sh-m5933* activity did not occur during early development and was limited to late stages of development when it happened with high frequency (control kernels that also carried three doses of *bz-m2* but not *C-I sh-m5933* displayed no large sectors despite the high frequency of small sectors). This case is intriguing, because in terms of TPase production, the *bz-m2* allele was apparently highly active during early development as evidenced by the chromosome breakage events that occurred. The phenotype also suggests that chromosome breakage events did not occur during late development, even though active TPase was present at this stage as evidenced by the small *Bz* spots. Thus, in this phenotype the lack of *Ac* activity cannot be explained by a lack or inactivated TPase. To the contrary, this phenotype provides a case that strongly suggests that the presence of TPase is required but not necessarily sufficient to induce *Ac*-dependent events. Hence, elements that differ in structure and composition may be transactivated at different times during development in the presence of the same active TPase source. Possibly, TPase reactions are not exclusively controlled *in trans., e.g.,* by the availability of active TPase molecules, but also *in cis,* at the site of the excision substrate. This opens the interesting prospect that even the *Ac* dosage effect may be not exclusively determined *in trans* by the amount of active TPase molecules but also *in cis* at the site of the TPase substrate element. Thus, TPase substrate elements may vary with regard to their probability to support the setting up of active transposition complexes in the presence of given TPase concentrations. However, more phenotypes have to be found which underscore that mutable alleles may show different reactions in response to changes in Ac dosage. Also, it has to be noted that chromosome breakage and transposition are not identical events and might *per se* require different levels of TPase. Thus, the difference in transactivation patterns between *sh-m5933* and *bz-m2* might not be representative for combinations of mutable alleles where mutability is exclusively the result of transposition.

The apparently higher number of large sectors on *sh-m5933* kernels *vs.* the lower number of large sectors on *bz-m2(DI)* kernels (Figure 7) may suggest that during early development, *sh-m5933* is more strongly active than *bz-m2(DI)* in the presence of the same TPase source. This observation has to be interpreted with great caution, however. The high number of large breakage sectors may be due to an overall higher frequency of events that lead to a visible phenotype. According to the genetic constitution of the *sh-m5933* kernels, each breakage event may lead to exposure of the *C1* gene and restoration of color, whereas in the *bz-m2(DI)* kernels, many of the *Ds* excisions may rather lead to stable mutation of the *Bz* gene because of the footprints left behind in the second exon of the *Bz* gene after transposition. In contrast to *bz-m2(DI),* the probability that *sh-m5933* events lead to a visible phenotype may be sufficiently high to regularly produce colored sectors even early during development when the number of cells in the endosperm is low.

Along the same line, the low frequency of *c2-m4490Ds* reversion events may be the result of a low frequency of *Ds* excisions that restore the function of the *C2* gene. In this case the lack of large revertant sectors may be as expected because, even if excision events may occur during early development, the probability of reversion events may be not sufficiently high to give rise to revertant cells. Thus, the lack of large sectors on *c2-m4490* kernels does not necessarily indicate a low or absent *Ds* excision activity during early development. However, a certain degree of autonomy of the *c2-m4490* allele might be suggested by the observed low correspondence between the late reversion pattern of this allele and the different dosage-specific TPase activities of the transactivating *wx-m7* and *wx-m9* alleles.

Cis effects may also be indicated by the changes in *Ds* excision frequencies in response to changes in *Ds* dosage. If *Ds* would simply act as substrate for the TPase

encoded by *Ac*, an increase in the dosage of the *Ds* allele should cause a proportional increase in the number of revertant sectors, as the number of TPase substrate molecules is increased. However, such a proportional increase was observed only in one out of seven tested genetic situations [*bz-m2(DI)* transactivated by two doses of *wx-m9*]. In the other six genetic situations, the number of revertant sectors did not increase in proportion to *Ds* dosage.

Distinct dosage effects were observed with the *wx-m9Ds* allele. Regardless of whether one, two or three copies of the transactivating *bz-m2* allele were present, an increase in the dosage of the *wx-m9Ds* allele from one to two doses gave rise to much less than twice as many excision events than were seen with one dose. Although this observation is based on very limited amounts of data, this may suggest the possibility that the two *Ds* elements derived from the central cell are worse substrates for excision than the element derived from the sperm. While it is too early to draw any conclusions, one might speculate that the TPase target sites of *Ds* elements are subject to epigenetic modification during or after meiosis. This assumption is supported by several instances of parental imprinting reported for the maize endosperm (KERMICLE 1970, 1978; KERMICLE and ALLEMAN 1990; CHAUDHURI and MESSING 1994; LUND *et al.* 1995). The efficiency of the protein/DNA binding reaction between the TPase and its target sites on the DNA is affected by methylation *in vitro* (KUNZE and STARLINGER 1989; WANG *et al.* 1996). Thus, parental imprinting may produce distinct patterns of TPase target site methylation and render the female- and male-derived elements—irrespective of their identity in sequence and sequence context—physically distinct substrates for the TPase.

The sum of *Ds* excision events for either one and two doses of *wx-m9Ds* was found to be smaller than the value found for three doses. Possibly, the female- and male-derived *Ds* elements are better substrates for excision if they are combined in one nucleus than if they are separate. As a possible explanation one might consider the idea that the differentially imprinted elements in some way complement each other *in trans* by a direct interaction, *e.g.*, by somatic pairing of homologous chromosomes (MATZKE and MATZKE 1993).

The hypothesis that the higher excision rate may result from a physical interaction of allelic *Ds* elements implies the possibility that the allelic interaction involves, or even is mediated by, the TPase. This could provide an explanation for the differences in *bz-m2(DI)* dosage effects that are seen between kernels carrying different doses of *wx-m7* or *wx-m9*. Such a role for TPase would be consistent with other reported examples of TPase mediated interactions between distantly located as well as unlinked *Ds* and Ac elements. For instance, the composite *Ds* elements present in the *sh-m5933* and *sh-m6258* alleles are assumed to have originated from TPase-mediated interactions between *Ds* elements located on two sister chromatids (DÖRING *et al.* 1989, 1990). The mobilization of "macrotransposons" consisting of two closely spaced elements or parts thereof (RALSTON *et al.* 1989; DOONER and BELACHEW 1991; WEIL and BELACHEW 1993) also demonstrates that the protein can mediate between distantly located elements.

In summary, the observed differences in transactivation patterns between TPase substrate elements that differ in structure or dosage suggest the possibility that transposition is influenced by *cis*-acting properties of the elements and that these properties are involved in determining TPase activity in cis at the site of the substrate in the presence of given cellular levels of TPase encoded by *Ac*.

I thank HUGO DOONER for providing *bz-m2* and *bz-m2(DI)wx*, HANS-PETER DÖRING for providing *C1-I sh-m5933* and UDO WIENAND for providing *wxB-7*, *c2* and *c2-m4490Ds*. This work was accomplished in the laboratory of PETER STARLINGER (University of Cologne, Germany), whom I thank for supplies, many discussions and helpful comments. I am also very grateful to FRANCESCO SALAMINI and MARIO MOTTO for propagation of maize lines at the Istituto Sperimentale per la Cerealicoltura in Bergamo, Italy. This work was supported by the Deutsche Forschungsgemeinschaft through grant SFB 274 to P. STARLINGER and by a fellowship provided by the German National Scholarship Foundation to M.H.

LITERATURE CITED

BECKER, D., R. LÜTTICKE, M.-G. LI and P. STARLINGER, 1992 Control of excision frequency of maize transposable element *Ds* in *Petunia* protoplasts. Proc. Natl. Acad. Sci. USA **89:** 5552–5556.

BEHRENS, U., N. FEDOROFF, A. LAIRD, M. MUELLER-NEUMANN, P. STARLINGER, *et al.*, 1984 Cloning of the *Zea mays* controlling element *Ac* from the *wx-m7* allele. Mol. Gen. Genet. **194:** 346–347.

CHAUDHURI, S., and J. MESSING, 1994 Allele-specific parental imprinting of *dzr1*, a posttranscriptional regulator of zein accumulation. Proc. Natl. Acad. Sci. USA **91:** 4867–4871.

CHEN, S. M., and E. H. COE JR., 1977 Control of anthocyanin synthesis by the *C* locus in maize. Biochem. Genet. **15:** 333–346.

CONE, K. C., F. A. BURR and B. BURR, 1986 Molecular analysis of the maize anthocyanin regulatory locus *C1*. Proc. Natl. Acad. Sci. USA **83:** 9631–9635.

COUPLAND, G., B. BAKER, J. SCHELL and P. STARLINGER, 1988 Characterization of the maize transposable element *Ac* by internal deletions. EMBO J. **7:** 3653–3659.

COURAGE-TEBBE, U., H.-P. DÖRING, N. V. FEDOROFF and P. STARLINGER, 1983 The controlling element *Ds* at the *Shrunken* locus in *Zea mays*: Structure of the unstable *sh-m5933* allele and several revertants. Cell **34:** 383–393.

DOONER, H. K., 1983 Coordinate genetic regulation of flavonoid biosynthetic enzymes in maize. Mol. Gen. Genet. **189:** 136–141.

DOONER, H. K., and A. BELACHEW, 1991 Chromosome breakage by pairs of closely linked transposable elements of the *Ac-Ds* family in maize. Genetics **129:** 855–862.

DOONER, H. K., E. WECK, S. ADAMS, E. RALSTON, M. FAVREAU, *et al.*, 1985 A molecular genetic analysis of insertions in the *bronze* locus of maize. Mol. Gen. Genet. **200:** 240–246.

DOONER, H. K., J. ENGLISH, E. J. RALSTON and E. WECK, 1986 A single genetic unit specifies two transposition functions in the maize element *Activator*. Science **234:** 210–211.

DÖRING, H.-P. and P. STARLINGER, 1984 Barbara McClintock's controlling elements: now at the DNA level. Cell **39:** 253–259.

DÖRING, H. P., E. TILLMANN and P. STARLINGER, 1984 DNA se-

quence of the maize transposable element *Dissociation*. Nature **307:** 127–130.

DÖRING, H.-P., B. NELSEN-SALZ, R. GARBER and E. TILLMANN, 1989 Double *Ds* elements are involved in specific chromosome breakage. Mol. Gen. Genet. **219:** 299–305.

DÖRING, H.-P., I. PAHL and M. DURANY, 1990 Chromosomal rearrangements caused by the aberrant transposition of double *Ds* elements are formed by *Ds*-sequences and adjacent non-*Ds*-sequences. Mol. Gen. Genet. **224:** 40–48.

ESCOUBAS, J. M., M. F. PRERE, O. FAYET, I. SALVIGNOL, D. GALAS, *et al.*, 1991 Translational control of transposition activity of the bacterial insertion sequence *IS1*. EMBO J. **10:** 705–712.

FEDOROFF, N., S. WESSLER and M. SHURE, 1983 Isolation of the transposable maize controlling elements *Ac* and *Ds*. Cell **35:** 235–242.

FEDOROFF, N. V., D. B. FURTEK and O. E. NELSON, 1984 Cloning of the *bronze* locus in maize by a simple and generizable procedure using the transposable controlling element *Activator* (*Ac*). Proc. Natl. Acad. Sci. USA **81:** 3825–3829.

FEDOROFF, N., 1989 Maize transposable elements, pp. 375–411 in *Mobile DNA*, edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, DC.

FELDMAR, S., and R. KUNZE, 1991 The ORFa protein, the putative transposase of maize transposable element *Ac*, has a basic DNA binding domain. EMBO J. **10:** 4003–4010.

FINNEGAN, E. J., B. H. TAYLOR, E. S. DENNIS and W. J. PEACOCK, 1988 Transcription of the maize transposable element *Ac* in maize seedlings and in transgenic tobacco. Mol. Gen. Genet. **212:** 505–509.

FRANKEN, P., U. NIESBACH-KLÖSGEN, U. WEYDEMANN, L. MARÉCHAL-DROUARD, H. SAEDLER, *et al.*, 1991 The duplicated chalcone synthase genes *C2* and *Whp* (white pollen) of *Zea mays* are independently regulated; evidence for translational control of *Whp* expression by the anthocyanin intensifying gene *in*. EMBO J. **10:** 2605–2612.

FUSSWINKEL, H., S. SCHEIN, U. COURAGE, P. STARLINGER and R. KUNZE, 1991 Detection and abundance of mRNA and protein encoded by transposable element *Activator* (*Ac*) in maize. Mol. Gen. Genet. **225:** 186–192.

HEINLEIN, M., 1995 Variegation patterns caused by excision of the maize transposable element *Dissociation* (*Ds*) are autonomously regulated by allele-specific *Activator* (*Ac*) elements and are not due to *trans*-acting modifier genes. Mol. Gen. Genet. **246:** 1–9.

HEINLEIN, M., and P. STARLINGER, 1989 Tissue- and cell-specific expression of the two sucrose synthase isoenzymes in developing maize kernels. Mol. Gen. Genet. **215:** 441–446.

HEINLEIN, M., and P. STARLINGER, 1991 Variegation patterns caused by transposable element *Ac*. Maydica **36:** 309–316.

HEINLEIN, M., T. BRATTIG and R. KUNZE, 1994 *In vivo* aggregation of maize *Activator* (*Ac*) transposase in nuclei of maize endosperm and *Petunia* protoplasts. Plant J. **5:** 705–714.

HOUBA-HÉRIN, N., D. BECKER, A. POST, Y. LARONDELLE and P. STARLINGER, 1990 Excision of a *Ds*-like maize transposable element (*Ac*Δ) in a transient assay in *Petunia* is enhanced by a truncated coding region of the transposable element *Ac*. Mol. Gen. Genet. **224:** 17–23.

KERMICLE, J. L., 1970 Dependence of the R-mottled phenotype in maize on mode of sexual transmission. Genetics **66:** 69–85.

KERMICLE, J. L., 1978 Imprinting of gene action in maize endosperm, pp. 357–371 in *Maize Breeding and Genetics*, edited by D. B. WALDEN. John Wiley, New York.

KERMICLE, J. L., M. ALLEMAN and S. L. DELLAPORTA, 1989 Sequential mutagenesis of a maize gene, using the transposable element *Dissociation*. Genome **31:** 712–716.

KERMICLE, J. L. and M. ALLEMAN, 1990 Gametic imprinting in maize in relation to the angiosperm life cycle, pp. 9–14 in *Genomic Imprinting Development Supplement*, edited by M. MONK and A. SURANI. Company of Biologists, Cambridge, UK.

KLÖSGEN, R. B., A. GIERL, Z. SCHWARZ-SOMMER and H. SAEDLER, 1986 Molecular analysis of the *waxy* locus of *Zea mays*. Mol. Gen. Genet. **203:** 237–244.

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

KUNZE, R., U. STOCHAJ, J. LAUFS and P. STARLINGER, 1987 Transcription of transposable element *Activator* (*Ac*) of *Zea mays* L. EMBO J. **6:** 1555–1563.

KUNZE, R., P. STARLINGER and D. SCHWARTZ, 1988 DNA methylation of the maize transposable element *Ac* interferes with its transcription. Mol. Gen. Genet. **214:** 325–327.

KUNZE, R., U. BEHRENS, U. COURAGE-FRANZKOWIAK, S. FELDMAR, S. KÜHN, *et al.*, 1993 Dominant transposition-deficient mutants of maize *Activator* (*Ac*) transposase. Proc. Natl. Acad. Sci. USA **90:** 7094–7098.

LUND, G., P. CICERI and A. VIOTTI, 1995 Maternal-specific demethylation and expression of specific alleles of zein genes in the endosperm of *Zea mays* L. Plant J. **8:** 571–581.

MATZKE, M., and J. M. MATZKE, 1993 Genomic imprinting in plants: parental effects and *trans*-inactivation phenomena. Annu. Rev. Plant Physiol. Plant Mol. Biol. **44:** 53–76.

MCCLINTOCK, B., 1945 Cytogenetic studies of maize and *Neurospora*. Carnegie Inst. Wash. Year Book **44:** 108–112.

MCCLINTOCK, B., 1946 Maize genetics. Carnegie Inst. Wash. Year Book **45:** 176–186.

MCCLINTOCK, B., 1947 Cytogenetic studies of maize and neurospora. Carnegie Inst. Wash. Year Book **46:** 146–152.

MCCLINTOCK, B., 1948 Mutable loci in maize. Carnegie Inst. Wash. Year Book **47:** 155–169.

MCCLINTOCK, B., 1949 Mutable loci in maize. Carnegie Inst. Wash. Year Book **48:** 142–154.

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

MCCLINTOCK, B., 1953 Mutation in maize. Carnegie Inst. Wash. Yearbook **52:** 227–237.

MCCLINTOCK, B., 1955 Controlled mutation in maize. Carnegie Inst. Wash. Yearbook **54:** 245–255.

MCCLINTOCK, B., 1956 Intranuclear systems controlling gene action and mutation. Brookhaven Symp. Biol. **8:** 58–74.

MCCLINTOCK, B., 1962 Topographical relations between elements of control systems in maize. Carnegie Inst. Wash. Yearbook **61:** 448–461.

MCCLINTOCK, B., 1963 Further studies of gene-control systems in maize. Carnegie Inst. Wash. Year Book **62:** 486–493.

MCCLINTOCK, B., 1964 Aspects of gene regulation in maize. Carnegie Inst. Wash. Year Book **63:** 592–602.

MCCLINTOCK, B., 1965 Components of action of the regulators *Spm* and *Ac*. Carnegie Inst. Wash. Year Book **64:** 527–534.

MCCLINTOCK, B., 1967 Regulation of pattern of gene expression by controlling elements in maize. Carnegie Inst. Wash. Yearbook **65:** 568–578.

MISRA, S., and D. C. RIO, 1990 Cytotype control of *Drosophila P* element transposition: the 66 kd protein is a repressor of transposase activity. Cell **62:** 269–284.

MOTTINGER, J. P., 1973 Unstable mutants of *Bronze* induced by premeiotic X-ray treatment in maize. Theor. Appl. Genet. **43:** 190–195.

MÜLLER-NEUMANN, M., J. I. YODER and P. STARLINGER, 1984 The DNA sequence of the transposable element *Ac* of *Zea mays L.* Mol. Gen. Genet. **198:** 19–24.

NEVERS, P., N. SHEPHERD and H. SAEDLER, 1985 Plant transposable elements. Adv. Bot. Res. **12:** 102–203.

PAZ-ARES, J., D. GHOSAL and H. SAEDLER, 1990 Molecular analysis of the *C1-I* allele from *Zea mays*: a dominant mutant of the regulatory *C1* locus. EMBO J. **9:** 315–321.

PETERSON, P. A., 1987 Mobile elements in plants. CRC Crit. Rev. Plant Sci. **6:** 105–228.

POHLMAN, R. F., N. V. FEDOROFF and J. MESSING, 1984 The nucleotide sequence of the maize controlling element *Activator*. Cell **37:** 635–643.

RALSTON, E. J., J. J. ENGLISH and H. K. DOONER, 1988 Sequence of three *bronze* alleles of maize and correlation with the genetic fine structure. Genetics **119:** 185–197.

RALSTON, E. J., J. ENGLISH and H. K. DOONER, 1989 Chromosome-breaking structure in maize involving a fractured *Ac* element. Proc. Natl. Acad. Sci. USA **86:** 9451–9455.

ROBERTSON, H. M., and W. R. ENGELS, 1989 Modified *P* elements that mimic the *P* cytotype in *Drosophila melanogaster*. Genetics **123:** 815–824.

SCHWARTZ, D., 1984 Analysis of the *Ac* transposable element dosage effect. Mol. Gen. Genet. **196:** 81–84.

SCHWARTZ, D., 1986 Analysis of the autonomous *wx-m7* transposable element mutant of maize. Maydica **31:** 123–129.

SCHWARTZ, D., and E. S. DENNIS, 1986 Transposase activity of the *Ac* controlling element in maize is regulated by its degree of methylation. Mol. Gen. Genet. **205:** 476–482.

SCOFIELD, S. R., J. J. ENGLISH and J. JONES, 1993 High level expression of the *Activator* (*Ac*) transposase gene inhibits the excision of *Dissociation* (*Ds*) in tobacco cotyledons. Cell **75:** 507–517.

SEKINE, Y., and E. OHTSUBO, 1989 Frameshifting is required for production of the transposase encoded by insertion sequence 1. Proc. Natl. Acad. Sci. USA **86:** 4609–4613.

SIMONS, R. W., and N. KLECKNER, 1988 Biological regulation by antisense RNA in prokaryotes. Annu. Rev. Genet. **22:** 567–600.

WANG, L., HEINLEIN, M. and R. KUNZE, 1996 Methylation pattern of *Activator* transposase binding sites in maize endosperm. Plant Cell **8:** 747–758

WEIL, C. F., and S. R. WESSLER, 1993 Molecular evidence that chromosome breakage by *Ds* elements is caused by aberrant transposition. Plant Cell **5:** 515–522

WESSLER, S. R., and M. J. VARAGONA, 1985 Molecular basis of mutations at the *waxy* locus of maize: correlation with the fine structure genetic map. Proc. Natl. Acad. Sci. USA **82:** 4177–4181.

WIENAND, U., U. WEYDEMANN, U. NIESBACH-KLÖSGEN, P. A. PETERSON and H. SAEDLER, 1986 Molecular cloning of the *c2* locus of *Zea mays*, the gene coding for chalcone synthase. Mol. Gen. Genet. **203:** 202–207.

Communicating editor: V. SUNDARESAN