A Modified Autonomous *En* Transposon in Maize (*Zea mays* L.) Elicits a Differential Response of Reporter Alleles

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ABSTRACT

Transposable elements in maize are composed of a defined molecular structure that includes coding sequences, determiners of functionality and ordered terminal motifs that provide binding sites for transposase proteins. Alterations in these components change the phenotypic expression of unstable genes with transposon inserts. The molecular basis for the altered timing and frequency of transposition as determined by the size and number of spots on kernels or stripes on leaves has generally been described for defective inserts in genes. Most differential patterns can be ascribed to alterations in the terminal motifs of the reporter allele structure that supplies a substrate (terminal inverted repeat motifs) for transposase activity. For autonomously functioning alleles, the explanations for changes in phenotype are not so clear. In this report, an *En*-related element identified as *F-En* is described that shares with *En* the recognition of a specific defective element *cl(mr)888104* but differs from *En* in that this *F-En* element does not recognize the canonical *cl(mr)* elements that are recognized by *En*. Evidence is provided suggesting that *F-En* does not recognize other *En*/*Spm*-related defective elements, some of whose sequences are known. This modified *En* arose from a *cl-m* autonomously mutating *En* allele.

THE timing and frequency of transposition events of transposable elements have long been observed and considered to be of interest (KNIGHT 1808; PETERSON 1987). In the early studies of mutable loci, these differential patterns were related to concepts of gene control and development (MCCLINTOCK 1951, 1961; PETERSON 1966, 1995). When, however, these transposable elements were isolated and their molecular sequences analyzed, the basis of variability of these patterns was found to be caused by changes in the component structure of the reporter alleles and especially in the terminal inverted repeat motifs (TIR) (SCHIEFELBEIN *et al.* 1985; TACKE *et al.* 1986), which have an ordered and systematic arrangement. In general, the deletion of some of the elements in these terminal motifs hampered the efficiency of binding of the transposase and thus, limited excision events (SCHIEFELBEIN *et al.* 1985; TACKE *et al.* 1986). But, these altered TIRs did not impede protein binding of the transposase (GIERL *et al.* 1988a,b, 1989), only the efficiency of element excision. When an *En*/*Spm* element was tested against a suppressible reporter allele (one with a transposon insert that does not impede transcriptional read-through of the functioning allele), the transposase binding was adequate to suppress the functioning of the allele (GIERL *et al.* 1988a,b, 1989). When the terminal motifs were genetically altered in transgenic experiments, the changes were effective in the occurrence of

excision events. The changes that were tested included the numerous alterations of the motif orientation (head to head or tail to tail) of the first 200 bases of the *En* structure. This demonstrated the significance of the precision of the ordered motif orientation in the evolution of transposon structure (GRANT *et al.* 1993; TRENTMANN *et al.* 1993).

For autonomous elements, the differences in expression have not been as clearly elucidated. Why do autonomous elements show such striking differences in phenotypic expression in timing and frequency of transposition events? In an attempt to determine the basis of the differential expression effects of two autonomous *Ac* transposons (*wx-m7* and *wx-m9*) at the *wx* locus, no clear explanation of the basis for this difference could be demonstrated by sequence analysis (MÜLLER-NEUMANN *et al.* 1984). Whether this difference in expression of two transposons with a complete structure could be ascribed to position effect phenomena (*cis* determinants) (PETERSON 1976) has yet to be determined. In an investigation of the dosage effect and functionality of the *Ac* transposon, BOEHM *et al.* (1995) have investigated the *Ac* transposase function for such *cis* determinants.

This report describes the genetic aspects of the differential expression of related autonomously functioning transposons. This type of differential activity of autonomously acting transposons is similar to those described for the *Ac* family with the distinguishable activity of *Ac* and *Uq* (CALDWELL and PETERSON 1992) and that for *Fcu-Spf* (GONELLA and PETERSON 1978). In this report, a modified *En* arose that is limited in its transposase

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FIGURE 1.—*c1-m888104* and *c1-(mr)888104*. (A) *c1-m888104*: kernels from the cross *c1-m888104* × *c1 sh wx*; similar to spotted plump kernels in Table 1B, progeny 1. (B) *c1-(mr)888104*, *F-En*: similar to spotted plump kernels, Table 1A, progeny 2.

action to a specific reporter allele *c1-(mr)888104*; however, the standard *En* (*En-std*) recognizes other reporter alleles, whereas the modified *En* only recognizes one of them (this report).

MATERIALS AND METHODS

The *c1-m888104* allele (Figure 1A) originated as an autonomous *c1*-mutable allele (PETERSON 1990) in an isolation plot with *c1 sh1 wx* as the male parent (PETERSON 1978). A derivative “reporter allele,” *c1-(mr)888104*, along with its related autonomous element (factor *En*) was isolated among segregating progenies.

RESULTS

The *c1-m888104* allele is autonomously mutable: In tests to determine the heritability of the newly arisen mutable allele, the spotted plump kernels from the originating plant (*c1-m/c1*; *Sh1/sh1*; *Wx/wx*) were again crossed by the *c1 sh1 wx* tester. If the mutability is inherited with the *c1* allele, then this would suggest that the mutability is autonomously controlled (that the element has inserted in the *c1* locus). These and other

TABLE 1

Inheritance of the unstable *c1-m888104* allele in test crosses as females crossed by *c1 sh wx* males

A. Progeny ^a	Plump			Shrunken			Total
	C1	c1	Spotted	C1	c1	Spotted	
1	0	20	63	0	85	1	169
2 ^b	1	22	70	0	103	0	196
3	4	34	59	0	96	1	199
4	2	39	145	0	117	1	169

B. Progeny	C1	c1	Stopped	Total
1	163	8	164	335
2	152	8	144	304

C. Progeny	Plump			Shrunken			Total
	C1	c1	Spotted	C1	c1	Spotted	
1 cc	1	4	20	1	25	1	52
1 CC	118	17	86	—	—	—	221
2 cc	1	23	55	1	69	3	73
2-1CC	203	42	147	—	—	—	392
2-2CC	215	41	153	—	—	—	409
2-3CC	163	8	164	—	—	—	335

(A) Spotted, plump progeny (*c1-m Sh1 Wx*) from *c1-m/c1*; *Sh1/sh1*; *wx/wx* (the original *clm888104* ♀ crossed by *c1 sh wx* ♂ tester). (B) *bz wx* progeny selected from (*C1/C1*; *bz-rcy/bz-rcy*; *Sh1/Sh1*; *wx/wx* × *c1-m/c1*; *Sh1/sh1*; *bz/Bz*; *wx/wx*); were crossed by the *c1* tester, *c1 sh1 wx*. These progenies are presented. (C) Spotted plump from progenies 1 and 2 of A were crossed in two ways (a) by the recurrent *c1 sh wx* tester (cc) and (b) crosses to color lines (CC) and then these F₁ were crossed by the recurrent tester. Crosses *c1m/c1 sh wx* by (a) (cc) (*c1 sh wx*) and (b) on to color line (CC) and then *c1-m/C1* × *c1 sh wx/c1 sh wx*. 1 and 2 of Table C are from 1 and 2 of Table A, respectively.

^a The 88g 002 family.

^b 88g 002-8 the origin of *c1-(mr)888104*.

progenies are presented in Table 1, A–C, showing the low frequency of nonspotted (*c1*) plump kernels. This is indicative of autonomous control of mutability. The colorless kernels are expected to be newly arisen *c1m-(nr)* (nonresponding) (PETERSON 1970) and *c1m(r)* responding (PETERSON 1961) alleles. The no. 2 progeny in Table 1A, however, continued to give excess colorless kernels among successive progenies and this line became the origin of the *c1-(mr)888104* and *F-En* system.

The autonomously mutable *c1-m888104* allele is *En*-related: In genetic tests (PETERSON 1987) for the identification of the controlling transposon family, this unstable isolate was crossed with several *a1* reporter alleles [*a1-(mr)* and *a1-m1*] and with the *a2* reporter allele, *a2-(mr)* (Table 2). To demonstrate genetically that the *c1-m888104* allele is *En*-related, segregation tests were made in “blind tests” (spotted kernels *vs.* nonspotted kernels were crossed by a *c1* tester). If the mutability with the *c1* allele test corresponds with the mutability with the reporter allele for *En*, and the non-mutability

TABLE 2

The *cl-m888104* autonomous allele is crossed to two reporter alleles that report the presence of the transposon *En*

Pedigree family	$\times c1\ sh1\ wx$ progeny include			
	<i>a1</i> selections	<i>c1</i> -spotted ^a	<i>c1</i> -nonspotted ^b	Colored
A. With the <i>al(mr)/a1-m1</i> reporter alleles				
'89g 51	spotted	13	4	1
'89g 52	pale	0	2	17
'91 2437-38	spotted	9	5	7
'91 2439	pale	0	2	11
Progeny ears from <i>c1 sh wx</i> cross				
<i>a2-(mr)</i> selection	Colored	Spotted	Colorless	
B. With the <i>a2-(mr)</i> reporter allele				
1991g				
Sibs				
280	Spotted	0	8	3
281	Colorless	13	0	0
Sibs				
282	Spotted	5	4	2
283	Colorless	14	1	1
1992				
Sibs				
92 2651	Spotted	12	0	0
92 2652	Colorless	0	0	10

(A) (*Cl/Cl*; *a1-m1/a1-m1* $\times$ *c1-m/c1*; *Sh1/sh1*; *wx/wx*; *A1/A1*); the colored progeny (*Cl/c1m/c1 Sh1/Sh1/sh1 Wx/wx A1/a1-m1*) from this cross were then crossed by *a1/a1* and this yields two classes of non-full-colored kernels. Pedigree numbers in column 1 and *a1*-related selections shown in column 2 (spotted) and *a1* (pale) (*a1-m1*). These progeny are then crossed by a *c1* tester (*c1 sh1 wx*) to reveal the progeny phenotypes shown in columns 3–5. (B) The *c1-m* allele originated from the spotted progenies from crosses of the *c1-m* allele by the *c1* tester. Plants from spotted kernel types were crossed by *a2-(mr)*, and the resulting colored progeny were again crossed by *a2-(mr)*, yielding progeny that were separated into spotted (*i.e.*, with *En* present) and colorless (*i.e.*, no *En* present) (column 3). These separated seed were then crossed by *c1 sh1 wx* to reveal the *c1* allelic condition (columns 4–6).

^a *c1*, colorless, kernels with spots.

^b *c1*-nonspotted, colorless kernels lacking spots.

of the *c1* allele test corresponds with the non-mutability of the reporter allele tester, then this is evidence that the mutability is *En* related. This showed a positive relationship to *En* (meaning the new mutable responds to reporter alleles of the *En* family) (crosses 1–3) and (Table 2, A and B). The *En*-related reporter allele elicited a positive mutability response. No other autonomous transposon was present. With the *a2-(mr)* crosses, spotted and colorless kernels were selected (as in the progeny of cross 2) and these were tested in crosses by *c1 sh1 wx* (Table 2B). The tests with the 280 and 281 progeny (Table 2B) indicate that the only *En* was associated with the *c1-m* allele. With the 282 and 283 progeny,

a free *En* (segregating independently of the *c1m* autonomous allele) is present. With the 283 progeny, the appearance of one individual progeny is not explainable unless the original colorless selection was an *a2-(nr)*.

Cross 1: *A1/A1*; *c1-m888104/_* $\times$ *a1-m1/a1-m1*; CC $\rightarrow$ colored kernels.

Cross 2: Colored kernels (progeny from cross 1) $\times$ *a1/a1* $\rightarrow$ *a1*-locus genotype; spotted and pale kernels were selected.

Cross 3: *a1*-locus; spotted and pale (progeny from cross 2) $\times$ *c1 sh wx/c1 sh wx* $\rightarrow$ results shown in Table 2A.

When a significant number of colorless (*c1*) segregants are observed in the progeny of a cross as in Table 1A, there is a suspicion that these could be responsive alleles and are responding to more than one element that is segregating and giving 3:1 or more ratios. This would be opposed to the locus itself with an autonomous element insert giving rise to colorless derivatives. If it were an autonomously mutable allele, one would expect all the colorless (*c1*) alleles to be mutable. In lieu of a molecular confirmation, the putative autonomous element can be crossed to a color line and then backcrossed by a recessive tester. One can then predict that if two elements are segregating and giving rise to the excess colorless kernels such as in Table 1A, the cross will yield some with one element, fewer with two, and a few with none. In addition, crosses could be made with the colored sibs of the cross and the same predictions can be made.

Spotted plump kernels from progenies 1 and 2 of Table 1A were crossed in two ways; the *c1-m/c1 sh wx* plant (a) was crossed as a female by the recurrent recessive tester line *c1 sh wx* and (b) the same plant was crossed as a male on to a color line *Cl/Cl* and the *F*₁ was crossed by the recessive tester line *c1 sh wx*. These progenies are shown in Table 1C. Again, colorless derivatives appear in the crosses. This shows that the colorless kernels are arising in approximately the same frequency as shown in Table 1A and thus not due to one or more segregating elements as predicted.

A further test is to cross the colorless derivatives by *c1*-reporters. Over 90% of them were carrying *En* indicating that these are colorless derivatives arising from imperfect excisions of *En* yielding *c1(nr)* nonresponsive derivatives. This is similar to the values obtained with the *a1-m(papu)* allele (PETERSON 1970). This test is supportive that the *c1-m888104* allele is *En*-related.

The correlation of *c1*-spotted with the *a1*-reporter spotted selection (Table 2A) and with the *a2* selections (Table 2B) is evidence for a relationship between the *c1-m888104* allele and *En/Spm*. The nine *c1*-nonspotted individuals in the *a1*-spotted selections (Table 2A) could be *En*-containing nonresponding types as was found in numerous instances with the *a1-m(papu)* study (PETERSON 1970). The colored kernel selections (Table 2A) contain only one individual (in '89g51) with a segregating *En*, which could have arisen from a *c1-m* to *Cl*

TABLE 3
Origin of the *F-En* line

Cross	Spotted $\times$ <i>cI</i> tester ^a		Plump			Shrunken			Total
			<i>C1</i> ^a	<i>c1</i> ^b	<i>sp</i> ^c	<i>C1</i> ^a	<i>c1</i> ^b	<i>sp</i> ^c	
1	'89	2605-4/2734	18	55	95	0	128	4	300
2	'89	2605-6/2734	6	46	176	0	216	9	453
3	'89	2605-12/2734	13	85	187	0	238	2	525

Spotted kernels from progeny no. 2 (Table 1A) were crossed by *cI shl wx* tester.

^a *C1*, colored kernels.

^b *c1*, colorless kernels.

^c *sp*, spotted kernels.

^d *c1-m* spotted/*c1 sh wx* $\times$ *c1 sh wx/c1 sh wx* (*c1* tester).

event after transposition. This result is indicative that there is no other *En* segregating in the *c1-m* progeny. The key feature of this test is that none of the pale kernels selected have a *c1-m* allele, and this is indicative of their not containing an *En/Spm* element.

Origin and definition of an independently controlled derivative: isolating a reporter allele and an autonomous element: In examining the progenies arising from the original tests to confirm the heritability of the *c1-m888104* allele, the no. 2 progeny in Table 1A seemed to have a different pattern expression (Figure 1B) from the other progeny and an excess number of colorless kernels in the resulting progeny of a testcross. The spotted progeny (from cross no. 2, Table 1A) were again crossed by the *c1 sh wx* tester, and a larger number of colorless kernels again appeared among the progeny (Table 3). The colored progeny among the plump kernels were most likely from *c1-m* $\rightarrow$ *C1* events.

In genetic tests to characterize further this interaction of mutability controlled by an independently segregating factor (the autonomously acting transposon and the newly uncovered reporter allele), crosses were initiated to isolate each of the two units [the *c1-(mr)* reporter allele and the functioning autonomous element] of this interaction. To avoid misrepresentation, this new reporter allele will be called *c1-(mr)888104* and the standard *c1-(mr)* reporter allele called *c1-(mr)std*. From the no. 3 progeny of Table 3, an experiment was initiated to reconstitute the purported new *c1(mr)888104* allele with the newly derived autonomous elements to verify that they interact to induce mutability. On the assumption that a *c1* reporter allele with an insert was linked to *Sh1* and was responding to a segregating autonomous element, colorless-plump and colorless-shrunken kernels from the no. 3 progeny (Table 3) were intercrossed. It is anticipated that the colorless-plump kernels or most of them [crossovers ($\pm 3\%$) between the *c1* and *sh1* alleles would alter the linkage relations of some of the gametes] represent the reporter allele, and the colorless-shrunken kernels represent the *c1 sh wx/c1 sh wx* recurrent tester parent but include an expected segregating autonomous element in approximately

one-half of these kernels. If the assumption is correct that an independently segregating factor is inducing mutability in the *c1* allele with a responsive insert, then the intercrosses of the expected autonomous element containing kernels and the kernels with the responsive insert should cause mutability to appear among the progeny kernels. This cross, between plants from the colorless plump *vs.* shrunken kernels, produced spotted kernel progeny, providing evidence that a *c1* allele with an insert was responding to a segregating autonomous element. From this line, a *c1 sh1 wx* line with a segregating autonomous element was isolated (Table 4A) by retesting on the responding *c1* allele and selfing to isolate a *c1 sh1 wx* line with the autonomous factor. This became the element-containing line identified later as *F-En* in Table 5.

With this positive reconstitution test, colorless plump kernels were collected to isolate this new reporter allele for *En*. To verify this line, these plump kernels were used in tests illustrated in Table 5.

An independently segregating autonomous element activates a newly uncovered reporter allele, *c1-(mr)888104*: In an effort to isolate the *c1-(mr)888104* reporter allele, numbered plants containing this allele were crossed (Table 5, 214 ♂ on 215 ♀) both to the segregating autonomous element used in cross 4 and to a *c-En* line (cross 6 and Table 5, 214 ♂ on 216 ♀). If the reporter *c1(mr)888104* responds in crosses to the sib colorless, shrunken, waxy sib kernels, then the self of these plants would establish a new *En* reporter line. To verify further the *En* relationship, crosses were made also to an *En* line with this same colorless line (numbered plants, Table 5) (214 ♂ on 216) where the appearance of mutability would clearly establish a relationship of the new reporter to *En*. Spots appearing in the progenies of both crosses indicated a positive response of this allele to both the segregating autonomous element of cross 4 and *En*. The results from these crosses are indicative that the colorless shrunken kernels (Table 5, 215) do carry a factor that activates the reporter allele (produces colored spots). This result confirms existence of a transposon system that includes a re-

TABLE 4

Origin of a two-factor control (autonomous element and defective element allele) of mutability arising from *cl-m888104*

		Plump			Total
		Cl	cl	Spotted ^a	
A. Confirmation of a two-factor control of mutability arising from <i>cl-m888104</i>					
'89	5425-3/5426-1	11	195	119 (36)	325
'89	5426/5425-3 ^b	11	105	56 (32)	172

B. Cross of colorless plump from the '89 5425 (Table 4A) progeny with *En*

'89g 349-1 × 387-3

↓

Colorless plump × colorless, shrunken, waxy

[*cl(mr) Sh1 Wx* × *cl sh1 wx En*]

↓

Spotted progeny; no count was made but indicative responds to *En*.^b

From a self of a plant arising from a plump mutable kernel, plump round and colorless shrunken progeny were intercrossed. The cross was as follows: colorless shrunken (5425) × colorless plump (5426); therefore, 5426 = *cl(mr)*.

^a Values in parentheses are percentages.

^b Cross reversed: the female parent 5425-3, became the male parent in the second cross.

^c This shows that the new colorless plump kernels (from Table 4A) contain an insert that responds to *En*.

porter allele controlled by an independently segregating factor like *En/Spm* (because of the origin of *cl-m888104*) but not necessarily *En/Spm*.

TABLE 5

Original detection of distinction between *cl(mr)std* and *cl(mr)888104*

1990 <i>cl sh wx</i>	<i>cl(mr)888104</i> (214)	215 ♀ on <i>cl(mr)std</i> (126)	214 ♀ on 216
215-1 ^a	NA	—	+
215-4 El ^b	+	—	+
215-7 El	+	NA	+
215-9 El	+	NA	+
215-9 El	+	NA	+
215-11 El	+	—	+

General procedure in crosses: 214 ♂ on 215 ♀ and 216 ♀: +, spots present; 215 ♂ on 126 ♀: —, no spots; NA, data not available. The crosses included the following: '90g 214: colorless, plump kernels originating from Table 4; '90g 215: colorless, shrunken waxy kernels from Table 3. In cross, all plants had a element (El) segregating. Crossed on 214; '90g 216: colorless, shrunken; expected to have *En* segregating; a known *En*-line—*cl sh1 wx En*. Crossed by 214; '90g 126: colorless, plump [*cl(mr)std*]. Crossed by 215.

^a Not available.

^b El, element present, eventually called *F-En*.

TABLE 6

The *cl(mr)std* allele reporter responds to *En*

Progeny ^a	Cross	Spots ^b
1	Colorless plump (Table 5) × <i>c-En</i>	+
2	Colorless plump (Table 5) × <i>c-En</i>	+
3	Colorless plump (Table 5) × <i>c-En</i>	+

^a '91 2509-2512 series. [Colorless plump kernels from the cross *cl(mr)std* by the colorless shrunken plants known to carry *F-En* that did not show spots (from 215-1, -4, -11 from Table 5) were crossed by a *c-En* line.] Therefore, this shows the 126 *cl(mr)* line is responsive to *En*. There are 41 other crosses that tested the *cl(mr)std* from Table 5 with *c-En* and all these show that *cl(mr)std* is an allele responsive with *En*.

^b +, spots on colorless background.

Cross 4: 1990 215 — with autonomous element (Table 5) × *cl(mr)888104* = spots.

Cross 5: *cl(mr)std* × 1990 215 — with autonomous element (Table 5) = no spots.

The next step to test an *En*-relationship further is to cross this same plant (1990 215, Table 5) carrying the presumed autonomous acting transposon [that was also crossed by the *cl(mr)888104* plants] to a standard *En* reporter allele, namely *cl(mr)* (126 plants in Table 5 and cross 5) (PETERSON 1963, 1978). Usually, this cross is not necessary if the previous crosses (215 with 214 and 216 in Table 5) were positive. However, in this case, the crosses were made between the 215 line (Table 5) and the *cl(mr)std* reporter allele. In these tests no spots occurred on these kernels (Table 5, 215 ♂ on 126 ♀). This is surprising because the same cross of this reporter in a number of previous tests with the standard autonomous *En* produced spots. To confirm further that this particular line of the *cl(mr)std* allele does indeed respond to *En*, confirmatory crosses of the same *cl(mr)std* plants (colorless progeny of cross 5) with a standard *En* resulted in spots (Table 6) supporting the responding aspect of the *cl(mr)std*. Thus, results from the crosses in Table 6 cannot be an anomaly due to a particular altered *cl(mr)* reporter allele.

Cross 6: *cl(mr)888104*/— × *cl sh wx/cl sh wx; EnEn* → spots.

Cross 7: *cl(mr)std* × *cl sh wx/cl sh wx; EnEn* → spots.

Crosses 4 and 5 illustrate the Table 5 crosses. The 1990 215 numbered plants were crossed as females by the *cl(mr)888104* plants (214) and these same plants were crossed as males on to the *cl(mr)std* plants (126). The progeny kernel results are indicated.

The testcrosses with the *cl(mr)888104* responding allele with *En* showed a positive response (i.e., spots) indicating that *En* activates this allele. These crosses (crosses 6 and 7) include a standard *En* with each plant crossed to both the standard *cl(mr)* and the *cl(mr)888104* alleles (Table 5).

These results illustrate that the standard *En/Spm* can activate both *cl(mr)std* and *cl(mr)888104*, but the *cl-*

	<i>c1-(mr)</i>	<i>c1-(mr)888104</i>
<i>En</i>	+	+
<i>F-En</i>	–	+

FIGURE 2.—*F-En* interactions with reporter alleles. Diagram illustrating the distinction of the activity of two autonomous transposons on two reporter alleles.

(*mr*)888104-related autonomous transposon (called *F-En*) (in cross 4) can only activate *c1-(mr)888104* and not the *c1-(mr)std* (cross 5 and Figures 2 and 3). Because of this relationship (Figures 3 and 4B), the following designations are given: *En1*, *c1-(mr)std*; factor-*En*(*F-En*);

c1-(mr)888104. The designation of *F-En* is given because this autonomous element shares with a standard *En*, this ability to activate *c1-(mr)888104* in genetic tests.

Tests were conducted on other isolated colorless derivatives that originated from the original *c1-m888104* allele to determine their responding status. These colorless kernels from spotted parents were crossed by a *c1-En* line. Results are presented in Table 7. Among the 21 colorless derivatives tested, two gave spots in this test, indicating ~10% of the progeny are alleles with inserts responsive to *En*. These two lines responding to *En* have not been tested with *F-En*.

In maize transposon biology, there is a predicted and anticipated consequence in crosses of various reporter alleles and their autonomous elements. It follows that reporter alleles respond to their autonomous elements

FIGURE 3.—Spotted kernels [*c1-(mr)888104*, *En*] were crossed with both *a2-(mr)* and *a1-m1*. The F_1 was then simultaneously crossed with the reporter alleles and tested on *c1* for the *c1-(mr)888104* + *F-En* interaction. (A) *a2-(mr)*. (B) *a1-m1*. (C) Colorless kernels from the cross *c1-(mr)std* x *F-En*. (Top) Colorless kernels crossed with the *c1-(mr)888104*. Bottom: Colorless kernels crossed with *c-En*.

TABLE 7

Test of colorless derivatives arising from *c1-m888104/CI*

<i>c En</i> line	No spots	Spots
'91 2543	8	1
'91 2544	11	1
Total	19	2

Therefore, $1/10$ of colorless derivatives are *c1-(mr)* types that respond to *En*. [Origin of colorless derivatives from the *c1-m* allele crossed by a color line (*c1-m888104/CI*) and then crossed by *c1 sh wx* tester: the original *c1-m* allele from spotted kernels of progeny no. 3 and 4 of Table 1) crossed by *c En*.

and this response allies newly discovered mutable alleles with the specificity of a particular element system. This (*F-En*)-[*(c1-(mr)888104)*] interaction and the failure of *F-En* to activate *c1-(mr)std* is similar to the exceptions of system quasi-relationship that would include the *Uq-DsII*, *Uq-Ds Breaker*, and *Spf-rcu* (Figure 4B). In view of this consideration, tests were conducted with an *F-En* and several other reporters of the *En* system. In the *En* system, this includes the reporter alleles *a2-(mr)*, *a1-(mr)*, *c2-(mr)*, *a1-m1 5719*, *bz1-(mr)* and several others. Could the *c1(mr)-std* be unique in this anomolous interaction result and are the other standard reporter alleles

equally nonresponsive to *F-En*? Thus if *F-En* is crossed to other reporter alleles and no mutability results, then the *c1(mr)888104* is the unique allele. With the available *F-En* line, tests were made with some of these reporter alleles. These are presented in Table 8, A and B, and Figure 3, A and B. In these tests, the reporters *a2-(mr)* (Table 8A) and *a1-(mr)/a1-m* (Table 8B) were crossed with the *c1-(mr)888104*, *F-En* line and the *F₁* was then recrossed by the same reporter, and simultaneously to a *c1* tester to verify *F-En* in the crosses. In tests with *a1-(mr)* and *a2-(mr)*, all the tests were negative (*i.e.*, no spots) and the *c1* tester cross verified the presence of *F-En* in these same crosses. As with *c1-(mr)std*, *F-En* cannot activate these other standard *En*-reporter alleles. Table 8, A and B, and Figure 3, A and B, illustrate the lack of activation of *F-En* containing genotypes on both the *a2-(mr)* and *a1-m1* standard reporters alleles.

In Figure 3C, the *c1-(mr)std* and *F-En* genotype is illustrated. The reporter, *c1-(mr)std* was crossed with an *F-En* line and the resulting kernels were colorless-non-spotted. Plants from the colorless plump progeny from this cross were simultaneously crossed as a male parent to the reporter, *c1-(mr)888104* to reveal the *F-En* presence (Figure 3C, top ear) and to a *c-En* line to verify the *c1-(mr)std* allele (Figure 3C, bottom ear). The spotted

Regulator-Receptor Interactions

		Receptor		
		1	2	
A	Reg 1	+	-	Case A Reg 1 = <i>Ac</i> ; Reg 2 = <i>En</i> . Rec 1 = <i>Ds</i> allele such as <i>wx-m7</i> ; Rec 2 = an <i>l</i> -containing allele such as <i>a1-m(r)</i> . (Basis for specificity of systems.)
	Reg 2	-	+	
<i>Uq</i> case (others)				
B	Reg 1	+	+	Case B Reg 1 = <i>Ac</i> ; Reg2 = <i>Uq</i> . Rec 1 = a standard <i>Ds</i> allele. Rec 2 = a <i>Ds1</i> allele (<i>wx-m1</i>). (Distinguishes <i>Ac</i> from <i>Uq</i> .)
	Reg 2	-	+	
Helper case (<i>Med</i>)				
C	Reg 1 alone	+	-	Case C Reg 1 = <i>En</i> . Rec 1 = <i>wx-m8</i> . Rec 2 = <i>c2-m881058Y</i> . (Distinguishes the two different receptors and uncovers Mediator.)
	<i>Med</i> alone	-	-	
	Reg 1 + <i>Med</i>	+	+	

FIGURE 4.—Diagram illustrating the kinds of interactions indicating relationships between an autonomous transposon and reporter alleles. The receptor columns 1 and 2 represent the specificity of interaction between a reporter and the autonomous element. +, response (*e.g.*, spots as in Figure 2B); -, no response [no spots as in A and B (left ears)]. (A) The typical type of specificity ascribed to a transposon family. Autonomous element (*e.g.*, *En*) recognizes reporter allele 1 [*e.g.*, *a1-(mr)*] and not reporter allele 2 [*e.g.*, *a-rug(Ds1)*]. (B) Instance in which one transposon recognizes two reporter alleles but the second autonomous transposon only recognizes one of the reporter alleles. For example, autonomous transposons *Ac* (1) *vs.* *Uq* (2); *Ac* (+) on all reporter alleles (all *Ds*'s) (1 and 2) but *Uq* only on *DsI* (2); *F-En* *vs.* *En* is similar. (C) Helper instance. *En* recognizes a common reporter allele for *En* (*wx-m8*), but not the *IRMA* allele (2). *Med* (2) does not recognize either reporter allele but when included with *En* in the genotype recognizes the *IRMA* allele (2).

TABLE 8
Test of *F-En* on *a2-(mr)*, and *a1-(mr)/a1-m1*

Colored progeny	$\times a2-(mr)^a$	On <i>c1 sh wx</i>
A. The <i>a2-(mr)</i> tests		
'92 2646 - 7	-	+
'92 2646 - 13	-	+
'92 2647 - 11	-	+
'92 2647 - 13	-	+
'92 2648 - 6	-	+ ^b
Colored progeny	$\times a1-(mr)/a1-m1^c$	On <i>c1-(mr)888104</i>
B. The <i>a1-(mr)/a1-m1</i> tests		
'92 1450 - 4	-	+
'92 1459 - 5	-	+
'92 1450 - 9	-	+
'92 1450 - 12	-	+

For activation of mutability, spotted progeny [*c1-(mr)888104*, *F-En*] were crossed with *a2-(mr)* and *a1-(mr)/a1-m1* reporter alleles. The colored progeny were then crossed by the respective reporter alleles and simultaneously on a *c1* tester.

^a There were seven other *a2-(mr)* tests with no spotting evident. Crosses illustrated are those in which both tests were available.

^b In the 2648 family there were four others of the same combinations (data not shown).

^c There were four other tests that did not show mutability.

progeny from both these crosses verify that though these colorless kernels were nonspotted, they did contain both *F-En* and *c1-(mr)std*. These crosses illustrate that although potentially active [*F-En* and *c1-(mr)std*], they were not interactive and thus, this illustrates the distinction in activity between *En* and *F-En* in their transposase activity.

DISCUSSION

Nonreciprocal transposon interactions: Molecular studies have demonstrated that the changes in the terminal motifs of reporter alleles will cause differential mutability patterns (SCHIEFELBEIN *et al.* 1985; TACKE *et al.* 1986). These phenotypic differences are ascribed to altered transposase binding capacity. This was verified via engineering terminal transposase binding motifs whereby the orientation and spacing of the canonical sequences maximized element excision efficiency (GRANT *et al.* 1993; TRENTMANN *et al.* 1993). The genetic basis for differences elicited by autonomously functioning transposons with a functional transposase is not immediately obvious and was investigated here.

In an attempt to determine the basis of differences displayed by autonomous elements, MÜLLER-NEUMANN *et al.* (1984) sequenced the *wx-m9* and *wx-m7* alleles. This analysis showed two autonomous acting alleles at the same locus (*wx*) could express distinguishable phenotypes. Because no differences could be found in their

DNA sequences, flanking the two elements or in the two alleles themselves, a position effect was considered responsible for their differences in expression (MÜLLER-NEUMANN *et al.* 1984). This suggests that the flanking sequences of inserted elements are significant in the expression of these inserts and also on the expression of the genes themselves with inserts. Whether there was a latent methylation effect is not known as this was not tested.

***F-En*:** The (*F-En*)-(*c1-(mr)888104*) interaction is a new addition to the unexplained nonreciprocal interactions (Figure 4B) that have thus far been uncovered. In previous studies, the *Fcu-Spf* (GONELLA and PETERSON 1978) and the *Ac-Uq* relationships (CALDWELL and PETERSON 1989, 1992) were described as nonreciprocal interactions. In the *Ac-Uq* instance, the *Uq* can only activate the *Ds1* element whereby *Ac* can activate all known *Ds* alleles. By sharing only the terminal motif, *Ds1* is comparable to the other *Ds*'s; however, the remaining internal part of the *Ds1* element is unrelated to other *Ds* elements but is related among other *Ds1* elements themselves (PISABARRO *et al.* 1991). The *Ac-Uq* difference is amenable to experimental resolution if the *Uq* containing *Mn-Uq* (PAN and PETERSON 1989) can be isolated and tested. The *Fcu-Spf* nonreciprocal case (GONELLA and PETERSON 1978) could be studied by analyzing the two reporter alleles *r-cu* and *r-Spf* because both alleles are at the *r* locus. Such a comparison of alleles would provide a clue to the changes in the reporter alleles that might be responsible for this altered behavior.

A three-element system: The IRMA element (MUSZYNSKI *et al.* 1993) is the only instance of a nonreciprocal relationship in which two components of the interaction have been analyzed molecularly. This is a three-element system that includes a reporter allele, IRMA, requiring *En* and *Mediator* (*Med*) (Figure 4C). Here, the difference between a standard reporter allele and the IRMA reporter allele is the presence of an additional unrelated insert within the IRMA allele that requires interaction of *Med* and *En* for transposition. Furthermore, some of the flanking sequences of the IRMA reporter show nucleotide alterations of the terminal motifs. The additional *Med* element has been hypothesized to act as a "helper" and thus activates excision of the insertion in *c2-(mr)*, whereas *En* alone cannot (MUSZYNSKI *et al.* 1993).

The (*F-En*)-(*c1-(mr)888104*) case, a consideration: There are several possibilities that could explain the (*F-En*)-(*c1-(mr)888104*) case. These include a weakened transposase signal and/or a complementary interaction between the autonomously functional *F-En* and the reporter *c1-(mr)888104*.

Weakened transposase signal: This differential strength of transposase of *Uq* vs. *Ac* was proposed by PISABARRO *et al.* (1991). In the *F-En* case, the appearance of numerous colored revertants are observed,

which is not the usual case with *Uq*. This would suggest earliness and strong transposase binding to the TIR.

A complementary *F-En c1(mr)888104* interaction: This would involve the complementary interaction of the M and S functions of *En/Spm* such as was proposed for the TNPR case (CUYPERS *et al.* 1988) or for the *in vitro* demonstration of TNPA and TNPD (FREY *et al.* 1990). This interaction is not applicable in the *F-En* case. In the crosses illustrated in Table 8 and Figure 3, spotted kernels were used in plants crossed to the standard reporter alleles *a2(mr)* and *a1m-1*. The cross following with recurrent *c1 sh wx* tester uncovered spotted kernels in the progeny and nonspotted in backcrosses to the standard reporter alleles. This indicates that the colorless kernels of the standard reporter alleles harbored both *F-En* and *c1(mr)888104*, and thus a complementary interaction of the M and S functions of *En/Spm* are not applicable here.

One could consider that the *F-En* harbors additional nucleotides that occlude this autonomous transposon from fully recognizing the standard reporter alleles such as *c1(mr)std*, *a2(mr)std* and *a1m1(std)* (only sequences of the TIR of *a1m1* are known of these three *En/Spm* reporters). This is the case proposed for the IRMA three-element system (MUSZYNSKI *et al.* 1993).

The S and M functions of *En/Spm*: The crosses illustrated in Table 8B with *a1m1* (a suppressible *En/Spm* reporter) indicate that the binding efficiency of the S component of the transposase of *F-En* to the TIR is fully functional. The spotted kernels (Figure 1B) provide support for the effectiveness of the M component of *F-En*. Thus the two components of *F-En* are active units as proposed for the *En/Spm* functions (FREY *et al.* 1990).

These three instances of nonreciprocal cross relationships (Figure 4B) are significant in the determination of transposon "control relationship." In most published work on genetic tests of mutable gene relationships to a known transposon family, data on numbers of segregating progeny are often not given, or genotypes involved in the cross are not given [for example, *Ac* and *Dt* (MCCLINTOCK 1950), *Mrh* and *Mut* (RHOADES and DEMPSEY 1982, 1983), *Uq* (FRIEDEMANN and PETERSON 1982)]. Usually, the report indicates that it is a positive response with a standard reporter allele, and, if a negative response, a lack of relationship is reported. Only rarely are reciprocal crosses made with all the parents identified as in the *Ac-Uq* case (CALDWELL and PETERSON 1992), thus allowing novel element relationships to be uncovered as this article has demonstrated for the *F-En* case.

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