Origination of *Ds* Elements From *Ac* Elements in Maize: Evidence for Rare Repair Synthesis at the Site of *Ac* Excision

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ABSTRACT

Although it has been known for some time that the maize transposon *Ac* can mutate to *Ds* by undergoing internal deletions, the mechanism by which these mutations arise has remained conjectural. To gain further insight into this mechanism in maize we have studied a series of *Ds* elements that originated *de novo* from *Ac* elements at known locations in the genome. We present evidence that new, internally deleted *Ds* elements can arise at the *Ac* donor site when *Ac* transposes to another site in the genome. However, internal deletions are rare relative to *Ac* excision footprints, the predominant products of *Ac* transposition. We have characterized the deletion junctions in five new *Ds* elements. Short direct repeats of variable length occur adjacent to the deletion junction in three of the five *Ds* derivatives. In the remaining two, extra sequences or filler DNA is inserted at the junction. The filler DNAs are identical to sequences found close to the junction in the *Ac* DNA, where they are flanked by the same sequences that flank the filler DNA in the deletion. These findings are explained most simply by a mechanism involving error-prone DNA replication as an occasional alternative to end-joining in the repair of *Ac*-generated double-strand breaks.

THE maize transposon *Activator* (*Ac*) was the first autonomous element described by McClintock (1949). Autonomous elements, such as *Ac*, *Spm*, and *MuDR*, can transpose on their own, whereas their counterpart nonautonomous elements (respectively, *Ds*, *dSpm*, and *Mu1*) cannot and require the presence of the autonomous element for transposition (McClintock 1956a; Chomet *et al.* 1991). McClintock (1956b, 1962, 1963) reported several instances in which the *Ac* element at a locus appeared to mutate to *Ds* and referred to this change as the origination of a two-element system from a one-element system. Subsequent molecular characterization of three such *Ds* elements revealed that they had arisen by the deletion of internal sequences from *Ac* (reviewed in Fedoroff 1989).

In other transposons, like the *P* element from Drosophila (O'Hare and Rubin 1983; Takasu-Ishikawa *et al.* 1992) and the *MuDR* element from maize (Hsia and Schnable 1996), internal deletions tend to occur between short direct repeats of a few base pairs. These findings have led to the proposal that defective *P* and *Mu* elements arise by some type of repair synthesis of the double-strand break (DSB) generated upon transposon excision (Engels *et al.* 1990; Nassif *et al.* 1994; Lisch *et al.* 1995; Hsia and Schnable 1996). Somatic rearrangements of an *Ac* element in transgenic tobacco, isolated by PCR, also consisted mostly of internal deletions with breakpoints occurring at short repeats (Rubin and Levy 1997). Abortive gap repair was likewise postulated as the underlying mechanism for the origin of those rearrangements, as well as of new *Ds* elements. Yet, the deletion junctions in the two *de novo* arisen *Ds* elements that have been sequenced in maize, *wx-m9(Ds)* and *bz-m2(DI)* (Pohlman *et al.* 1984; Dooner *et al.* 1986), provide little clue as to how new *Ds* elements may originate from *Ac*. There is no direct repeat adjacent to the deletion junction in the former, and in the latter the direct repeat is only 3 bp long. Ample additional evidence supports a role for repair synthesis of *P*-element-induced DSBs in Drosophila: the frequency of *P*-element excision is homology dependent and excision of the element promotes a form of efficient premeiotic gene conversion (Engels *et al.* 1990; Gloor *et al.* 1991; Lankenau *et al.* 1996). *Ac*, on the other hand, does not display either of these properties: the frequency of *Ac* excision at meiosis does not depend on the makeup of the homolog, and excision of *Ac* is repaired almost exclusively by end-joining (Dooner and Martinez-Ferez 1997). Furthermore, *Ds* deletion derivatives arise much more rarely than nonautonomous defective *P* elements. Therefore, based on the available evidence, it is not clear that the mechanism generally proposed for the origin of defective transposable elements, interrupted or abortive gap repair, also operates in the generation of new *Ds* elements from *Ac*.

To gain further insight into the possible mechanism of origin of *Ds* from Ac, we set up genetic screens to isolate new *Ds* derivatives from *Ac* elements located in

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two different loci in the maize genome. One of the screens enabled us to recover a new *Ds* element at the former *Ac* locus as one of the two products of an *Ac* transposition event, thereby confirming the long-held belief that *Ds* elements arise *de novo* in the genome as a consequence of *Ac* transpositions. These *Ds* derivatives are rare relative to *Ac* excision footprints. We have characterized the new *Ds* elements and confirm that, as expected, they have suffered internal deletions. We find not only that short direct repeats of variable length occur adjacent to the deletion junction in most, but also that extra sequences or filler DNA (Roth and Wilson 1985; Roth *et al.* 1989) can be inserted at the junction. The inserted nucleotides are identical to sequences found close to the junction in the *Ac* DNA. These findings support the role of repair synthesis in the generation of *Ds* elements after *Ac* excision. However, the low frequency with which new *Ds* elements arise relative to excision footprints suggests that repair synthesis makes a much more limited contribution than end-joining to the genetic diversity that is created from repair of the *Ac*-initiated DSBs.

MATERIALS AND METHODS

Genetic stocks: All the stocks used in this study shared the common genetic background of the inbred W22. The *bronze* alleles and the aleurone phenotypes of the various stocks are described below.

Bz-McC (purple): the normal progenitor allele of the *bz-m2(Ac)* mutation.

bz-m2(Ac) (purple spots on a bronze background): an allele that arose from the insertion of the 4.6-kb *Activator* (*Ac*) element at position 755-762 in the second exon of Bz-McC (McClintock 1955; Ralston *et al.* 1988).

bz-m2(DI) (bronze in the absence of *Ac*; spotted, in its presence): the first derivative from *bz-m2(Ac)*, harboring a 3.3-kb internally deleted *Dissociation* (*Ds*) element at the same position as *Ac* in *bz-m2(Ac)* (McClintock 1962; Dooner *et al.* 1986).

bz-m2(DII) (bronze in the absence of *Ac*; spotted, in its presence): the second derivative from *bz-m2(Ac)*, harboring a 3.6-kb internally deleted *Dissociation* (*Ds*) element at the same position as *Ac* in *bz-m2(Ac)* (McClintock 1962; Schiefelbein *et al.* 1985).

Bz Ac2094 (purple): a derivative of *bz-m2(Ac)* harboring a *trAc* (transposed *Ac* element) 0.05 cM proximal to *bz* (Dooner and Belachew 1989). The *Ac* element at that location, which has been cloned, is referred to as *Ac2094* and the insertion site as *tac2094* (Ralston *et al.* 1989).

bz-R (bronze): the *bz* reference allele, associated with a 340-bp deletion that extends from within the single intron to the second exon of *bz* and includes the *Ac* insertion site in *bz-m2* (Rhoades 1952; Ralston *et al.* 1987, 1989).

Selection and analysis of new *Ds* derivatives: The mutations *sh* (shrunken endosperm) and *wx* (waxy endosperm) were used as markers flanking *bz*. They map, respectively, ~2–3 cM distal and 25 cM proximal to *bz* in *9S*. The *sh-wx* region exhibits high chiasma interference (Dooner 1986), so double crossovers in the region are rare.

Ds derivatives of the *Ac* elements at *bz* and *tac2094* were recovered in separate screens as described below. Figure 1 summarizes diagrammatically the outcome of the screens and identifies the genetic makeup of the immediate *Ac* progenitors.

New *Ds* derivatives at the *bz* locus were identified as follows. Stable bronze derivatives having the *Sh* and *Wx* flanking markers of the *bz-m2(Ac)* chromosome (Figure 1) were selected as single-kernel events from crosses of *Sh bz-m2(Ac) Wx/sh bz-R wx* heterozygotes to *sh bz-R wx* pollen parents. The derivatives were crossed to a *Ds* reporter stock, *sh bz-m2(DI) wx*, to score for the presence of *Ac* and to an *Ac* stock, *sh bz-R wx-m9(Ac)*, to determine whether a new *Ds* element had originated at the *bz* locus. The recovery of ~50% spotted seeds constitutes a positive outcome in either test. Leaf DNA was made from all individuals and analyzed by Southern blots for the presence of the diagnostic 2.6-kb *Pvu*II fragment of *Ac* (Fedoroff *et al.* 1983). The DNA of individuals lacking *Ac*, including those individuals that carried new *Ds* elements by the above genetic criteria, was then analyzed to determine the size of the fragment hybridizing to a *bz* probe (Ralston *et al.* 1988) and, thus, the size of the insertion. Three new *Ds* derivatives [Figure 1: *bz-m2(D3)*, *bz-m2(D4)*, and *bz-m2(D5)*] were identified and sequenced to characterize the deletion junctions.

Ds derivatives at *tac2094* were obtained as follows. Numbered *Bz Ac2094/bz-R* + plants (Figure 1) were crossed as male and female parents to a *sh bz-R wx* stock and new unstable *bz-m* alleles were selected as rare spotted seeds from ears segregating purple and bronze seeds. The resulting plants were selfed to test for heritability of the spotted kernel phenotype. Leaf DNA was made from all selections and the sizes of the insertions at *tac2094*, the *Ac* donor locus, and *bz*, the putative target locus, were determined by genomic Southern blots. Two *bz-m* derivatives of interest were identified (Figure 1: *bz-m41* and *bz-m43*) and subsequently sequenced to determine the location and makeup of the insertions at *bz* and *tac2094*.

DNA extraction, Southern blotting, PCR amplification, and sequencing: Leaf DNA was isolated by the urea extraction procedure of Greene *et al.* (1994). Restriction enzyme digestion and genomic blotting were carried out as described (Dooner *et al.* 1985). Genomic DNA was amplified in the presence of 10% DMSO by the polymerase chain reaction (Saiki 1990) in a GeneAmp System [Perkin-Elmer (Norwalk, CT) model 2400] using a variety of primers based on the sequences of *Bz-W22* (Ralston *et al.* 1988), *tac2094* (Ralston *et al.* 1989; Z. Zheng and H. K. Dooner, unpublished results), and *Ac* (Muller-Neumann *et al.* 1984; Pohlman *et al.* 1984; English *et al.* 1987).

The PCR amplification essentially followed the instructions of the PE GeneAmp XL PCR kit, which includes the 40 and 60 μl of the lower and upper layer mixture, respectively, and holds the genomic DNA at 95° for 4 min. The DNA corresponding to the different *Ds* and *Ac* elements was amplified with 20 cycles of 20-sec denaturation at 95° and 5 min of combined annealing-extension at 65°, followed by 15 cycles under the same conditions, but with a 15-sec auto-increment time per cycle in the anneal-extend steps. The PCR reactions were terminated with a 12-min incubation at 72° and held at 4°.

The amplified PCR product was purified on a 0.8% agarose gel, and treated with 2 units of AmpliTaq DNA polymerase (Perkin-Elmer) and 1 μl of a 10 mm dATP solution in a 50-μl reaction at 72° for 20 min. The PCR product was then purified on a Sephadex G-50 column (Pharmacia Biotech, Piscataway, NJ), cloned into a pGEM-T vector (Promega, Madison, WI), and sequenced on an ALF automatic DNA sequencing system (Pharmacia Biotech) using the labeled universal and reverse primers. New fragments were subcloned and sequenced if the deletion junction could not be located in the first sequencing attempt.

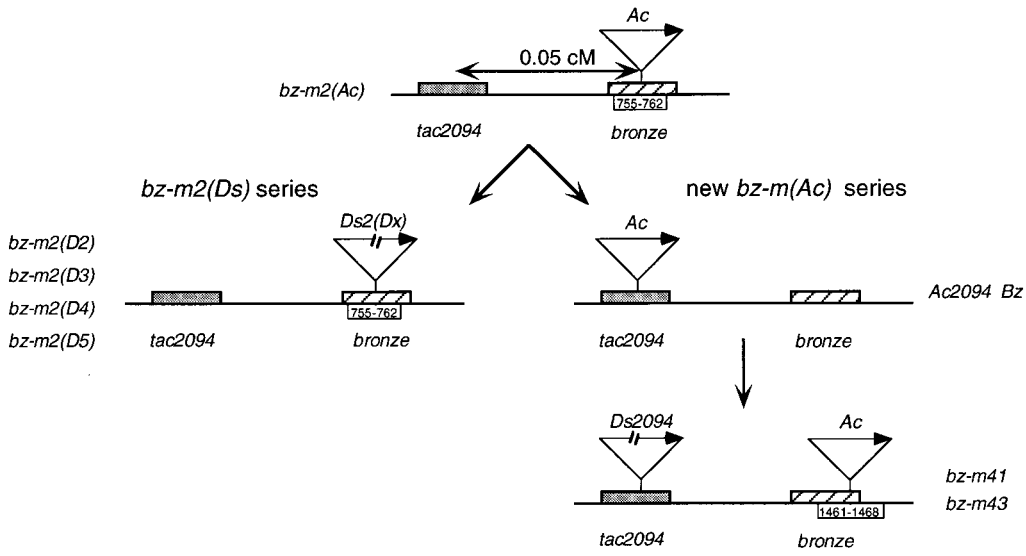

Figure 1.—Origin and analysis of new *Ds* derivatives. Two series of derivatives from the *Ac* element in the progenitor allele *bz-m2(Ac)* were studied. (Left) Series of *bz-m2(Ds)* derivatives resulting from internal deletions of the *Ac* element at position 755-762 in *bz-m2(Ac)*. (Right) Series of *bz-m(Ac)* derivatives produced by reinsertion of *Ac* into *bz*. The *Ac* element in *bz-m2(Ac)* transposed to the nearby *tac2094* locus to give *Ac2094 Bz*. Subsequently, *Ac* reinserted at position 1461-1468 of the *bz* locus to produce *bz-m41 and bz-m43*. These derivatives carry an internally deleted *Ds2094* at the *tac2094* locus. The boxes labeled *tac2094* and *bronze* represent unique sequences in the maize genome into which *Ac* has transposed.

The four *bz* primers used in this study were the following: *bz*C, CTCAACACGTTCCCAGGC; *bz*599, CGAATGGCTGTTGCATT TCCATCG; *bz*F, CGACAGACTATCTCCACGA; and *bz*863r, AC GGGACGCAGTTGGGCAGGAT. The two *tac2094* primers were *tac2094*#3, TCGGCGGTGCGGAGGAT; and *tac2094*#4; AGGA AGGCACGTAGGAGGACC. The four *Ac* primers were *Ac* 132r, TCTACCGTTTCCGTTTCCGTTTAC; *Ac*1297, GCACATCACC ATCATCATCAACAG; *Ac*4372, ACCGAACAAAAATACCGGTT CCCG; and *Ac*4552R, GTCGGTAACGGTCGGTAAAATACC.

RESULTS

Selection and analysis of new *Ds* derivatives at the *bz* locus: New *Ds* elements were isolated from the *Ac* element at *bz-m2(Ac)* using the strategy detailed in materials and methods. Individuals with the flanking markers of the *bz-m2(Ac)* allele but which had lost the mutable (*i.e.*, spotted) phenotype specified by that allele were selected as single plump, nonwaxy, bronze seeds from testcross ears of Sh *bz-m2(Ac) Wx/sh bz-R wx* heterozygotes. Most of them were expected to carry a stable *bz-s* allele with a transposon footprint at position 755-762 of the *bz* second exon as a consequence of *Ac* excision from that location (McClintock 1956a,b; Dooner and Belachew 1989; Dooner and Martinez-Ferez 1997). By appropriate crosses, the selections were sorted out into different genetic classes, as summarized in Table 1. Crosses to a *Ds* reporter reveal that ~40% of them also carry a *trAc* element somewhere in the genome (Dooner and Belachew 1989). To identify *bz-s* individuals with new *Ds* elements, the selections were crossed to the *Ac* source *sh bz-R wx-m9(Ac)*. Two of the selections produced ~50% spotted kernels in the cross, indicating that they carried a new *Ds* element. McClintock (1956a) had earlier identified two *Ds* derivatives of *bz-m2(Ac)*, which she termed *bz-m2(DI)* and *bz-m2(DII)* for derivatives I and II of *bz-m2*. Following her lead, but simplifying the Roman numerals to Arabic numerals, we have designated the two new derivatives *bz-m2(D3)* and *bz-m2(D4)* (Figure 1). Southern blots established that the *Ds* elements in these derivatives were ~2.2 and 4 kb, respectively (data not shown).

Though the above scheme fails to detect *Ds* derivatives that might retain *Ac* in the genome (they would still produce a spotted kernel phenotype), it is clear from Table 1 that new *Ds* elements represent just a minor fraction (2/37) of those derivatives lacking *Ac*. Because of the small number of *Ds* derivatives recovered and the bias just mentioned, it is not possible to obtain an accurate estimate of the mutation of *Ac* to *Ds*. Neverthe-

TABLE 1

Analysis of bz-s (stable bronze) selections from *bz-m2(Ac)*

bz-m2(Ac) gametes	bz-s selections	*trAc* present[a]	*trAc* absent[a]	
			bz-s[b]	*bz-m2(Ds)*[b]
3867	61	24 (0.39)	35 (0.57)	2 (0.04)

Sh bz Wx seed selected from the cross *Sh bz-m2(Ac) Wx/sh bz-R wx* × *sh bz-R wx*.

[a] Based on crosses to the *Ds* reporter stock *sh bz-m2(DI) wx* and Southern blots to detect the 2.6-kb *Pvu*II band characteristic of *Ac*.

[b] Based on crosses to the *Ac* stock *sh bz-R wx-m9(Ac)* and Southern blots to detect a *Bgl*II band larger than wild type.

less, the observed frequency of *bz-m2(Ds)* derivatives in this experiment (2/3867 gametes) is remarkably similar to the frequency with which McClintock (1963) recovered *Ds* derivatives from *wx-m9(Ac)* in a comparable experiment (2/4613 gametes).

A fifth derivative of *bz-m2(Ac)* was recovered in the self-progeny of a homozygous plant. Seven plants in this family segregated about equal numbers of spotted and bronze seeds in crosses to *bz-R*. Upon subsequent testing, all turned out to carry a *Ds* element of roughly the same size (>4 kb) at the *bz* locus. Sequencing of the deletion junction in two of them (see below) confirmed that they carried the same *Ds* element, which we have designated *bz-m2(D5)* (Figure 1). Unlike *bz-m2(D3)* and *bz-m2(D4)*, which most likely have a meiotic origin, *bz-m2(D5)* clearly originated in a mitotic division preceding sporogenesis.

Sequence of *Ds* insertions at *bz*: The *Ds* insertions in the new derivatives *bz-m2(D3)*, *bz-m2(D4)*, and *bz-m2(D5)* are 2.2 kb, 4.0 kb, and 4.2 kb, respectively. The *Ds* insertions in *bz-m2(D1)* and *bz-m2(D2)*, the two derivatives isolated by McClintock (1956b), are 3.3 kb and 3.7 kb, respectively (Schiefelbein *et al.* 1985; Dooner *et al.* 1986). Thus, an *Ac* element at a particular location can undergo deletions of various sizes when it mutates to *Ds*. To determine if, as with other transposons, deletions occurred preferentially between short direct repeats, the deletion junctions in a series of *bz-m2(Ds)* derivatives were sequenced. The series included the new derivatives described in this article and *bz-m2(D2)*, which had been characterized previously by restriction digests only. The location of the deletions relative to the sequence of the *Ac* progenitor is shown in Figure 2 and the sequences of all the deletion junctions are presented in Figure 3.

It is clear from Figures 2 and 3 that deletions can arise at multiple locations within *Ac* and that there is no sequence preference for deletion formation. The deletion junctions in all five *Ds* elements occur adjacent to short direct repeats of 2–5 bp. Interestingly, five extra nucleotides of filler DNA, shown in lowercase letters in Figure 3, were inserted at the *Ds2(D5)* junction. The

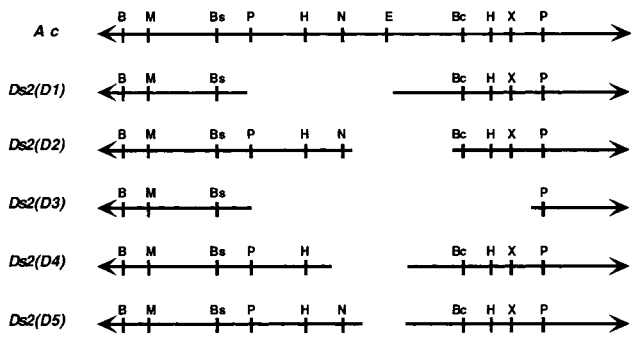

Figure 2.—Structure of five *Ds* elements produced from the *Ac* element at *bz-m2(Ac)* by internal deletions. The deletions are represented as the loss of various restriction sites in the *Ac* progenitor. B, *Bam*HI; M, *Mlu*I; Bs, *Bst*XI; P, *Pvu*II; H, *Hin*dIII; N, *Nar*I; E, *Eco*RI; Bc, *Bcl*I; X, *Xho*I.

pentanucleotide sequence TTTTA also occurs very close to the deletion junction in the *Ac* progenitor, 23 bp downstream relative to the direction of transcription of the *Ac* transposase (Kunze *et al.* 1987), where it is flanked by the same sequences (TCT and AGTG) that flank the filler DNA in the deletion. Filler DNA has been found at the junction of other genetic rearrangements in animals, plants, and fungi (Roth and Wilson 1985; Roth *et al.* 1989; Sainsard-Chanet and Begel 1990; Wessler *et al.* 1990), including *P* and *Mu1* element excision sites (O'Hare and Rubin 1983; Doseff *et al.* 1991; Takasu-Ishikawa *et al.* 1992). Its homology to nearby sequences and, particularly, the homology of the sequences flanking both the filler DNA and the deletion junction have led to models that explain its origin in terms of slipped mispairing during DNA synthesis (Roth and Wilson 1985; Wessler *et al.* 1990).

Recovery of both elements in the generation of a two-element system: McClintock described four instances of change from an *Ac* or one-element system of mutability to an *Ac-Ds* or two-element system: two at the *bz* locus (McClintock 1955, 1962) and two at the *wx* locus (McClintock 1963). In all four cases, *Ac* was lost from the genome initially and it was only the subsequent crossing of the stable *bz* and *wx* derivatives to an *Ac* source that revealed the presence of *Ds* at *bz* and *wx*. Similarly, the scheme we used to isolate new *Ds* derivatives at *bz* precludes the recovery of a potential *trAc*.

To show that, in fact, new *Ds* elements arise following *Ac* transposition one would have to recover both elements from the same transposition event. One can take advantage of the strong tendency of *Ac* to transpose to closely linked sites (Van Schaik and Brink 1959; Greenblatt 1984; Dooner and Belachew 1989) to perform a different type of selection. Instead of selecting for changes of *Ac* to *Ds* by the loss of mutability of *bz-m2(Ac)*, and consequently against a possible *trAc*, one could first select *Ac* transpositions into *Bz* from a closely linked donor site and then examine the donor locus for potential changes of *Ac* to *Ds*. *Ac2094* is a *trAc* from *bz-m2(Ac)* that maps only 0.05 cM proximal to *bz* (Dooner and Belachew 1989). Its site of insertion, identified as *tac2094*, has been cloned and sequenced and shown to be unique DNA (Ralston *et al.* 1989). Hence, *tac2094* constitutes a suitable donor site from which to select for *Ac* transpositions into the *bz* locus that might have resulted in the generation of *Ds* at the donor locus.

Ac transpositions from *tac2094* into *Bz* were selected as spotted kernels in testcrosses of *Bz Ac2094 /bz-R* + heterozygotes to *sh bz-R wx*. Twenty-one new *bz-m* alleles were recovered and confirmed by Southern blots and DNA sequencing to carry *Ac* reinsertions in the *bz* locus. Two of them, *bz-m41* and *bz-m43*, are uniquely interesting and will be discussed here. These two *bz-m* derivatives arose in the progeny of a single *Bz Ac2094/bz-R* + plant crossed as male to the *sh bz-R wx* tester (Figure 1). By

Transposon	5' deletion junction	3' deletion junction
Ac *Ds2(D1)*	v1250 v1260 v1270 AGTCGGATTCAGAA**TGT**ACGTGCACG AGTCGGATTCAGAA---------- ^1250 ^1260	v2570 v2580 CGCAGGTATGTT**TGT**CTCAATTGT ----------**TGT**CTCAATTGT ^1270
Ac *Ds2(D2)*	v2180 v2190 v2200 TTGCCTTGTCTTTG**GA**TAATGCTAGT TTGCCTTGTCTTTG---------- ^2180 ^2190	v3060 v3070 TCCAAAGACAAA**GA**CAACTACTAA ----------**GA**CAACTACTAA ^2200
Ac *Ds2(D3)*	v1310 v1320 ACCATCATCATC**AACAG**CTGAGGC ACCATCATCATC---------- ^1310	v3760 v3770 ATGGCTGGCATT**AACAG**ATTTTTG ----------**AACAG**ATTTTTG ^1320
Ac *Ds2(D4)*	v1990 v2000 v2010 AACTATGGATAT**GTGGA**CATCTTG AACTATGGATAT---------- ^1990	v2660 v2670 TAAAGCCGAGGA**GTGGA**AGATGGC ----------**GTGGA**AGATGGC ^2000 ^2010
Ac *Ds2(D5)* *Ac* *Ds2(D5)*	v2260 v2270 TGATTCAAA**TCT**AGTTTGTGATGG TGATTCAAA**TCT**----tttta---- ^2260	v2660 v2670 v2680 CTAAAGCCGAGG**AGTG**GAAGATGGCATT ----------**AGTG**GAAGATGGCATT ^2270 ^2280 v2690 v2700 AAC**TCT**TTTTA**AGTG**TTTGAAGAA AAC**TCT**TTTTA**AGTG**TTTGAAGAA ^2290 ^2300
Ac *Ds2094* *Ac* *Ds2094*	v1770 v1780 v1790 AAGTG**GTT**AGCCTAAAGAAGC**T**TCATTT AAGTG**GTT**AGCCTAAAGAAGC**T**TCATTT ^1770 ^1780 ^1790 v1840 v1850 v1860 GAGTACTTT**GTT**GAGTTTGTTAAGTC GAGTACTTT**GTT**--agcctaaagaagc- ^1840	v3770 v3780 ACAGATTTTTGA**T**CTCACTGCATG ----------**T**CTCACTGCATG ^1870

Figure 3.—Sequence of the deletion junctions in the *Ds* elements at *bz* and *tac2094*. The sequence of the 5′ and 3′ ends of the deletion and the corresponding sequence in *Ac* are shown for each *Ds* element. The sequence of the deletion junction in *Ds2(D1)* is from Dooner *et al.* (1986); the other four sequences are from this work. The deletion junctions in all five *Ds* elements occur adjacent to short direct repeats of 2–5 bp in the *Ac* progenitor (shown in boldface type). The filler DNAs at the deletion junctions of *bz-m2(D5)* and *Ds2094* are shown in lowercase letters and the nearby homologous sequences in the *Ac* parental DNA are underlined. The *Ac* sequence is shown in the same orientation as its transposase transcript and the numbered carats refer to positions in that sequence (1–4565). The *Ds* sequences are in the same orientation as *Ac* and the numbers refer to the corresponding positions in the shorter *Ds* sequences.

Southern blots (data not shown) it was established that several of the new *bz-m* alleles retained an *Ac*-sized insertion at the *tac2094* locus, but that *bz-m41 and bz-m43* appeared to carry smaller insertions at that locus, suggesting a possible change of *Ac* to *Ds* at the donor locus following transposition.

Sequence of *Ac* and *Ds* in *bz-m41* and *bz-m43*: Sequence analysis of the *Ac-bz* junctions in *bz-m41* and *bz-m43* revealed that *Ac* was inserted in the same location within *bz* (1461-1468) and in the same orientation (data summarized in Figure 1). These observations, coupled to the fact that the two mutants occurred in the progeny of a single plant, strongly indicate that they originated from a common premeiotic transposition event. Analysis of the genetic make-up of the *tac2094* locus confirmed this. The *bz-m41 and bz-m43* derivatives have the same *tac2094*-transposon junctions as the *Ac2094* progenitor, but they carry a smaller (2.7-kb) insertion at the *tac2094* locus. This suggests that the *Ac* element at *tac2094* did not move but suffered an internal deletion to become a *Ds* element. To confirm this, the 2.7-kb insertions in *bz-m41* and *bz-m43* were sequenced and found to be the same; hence this *Ds* insertion has been designated *Ds2094*. Like *Ds2(D5)*, *Ds2094* has filler DNA at the deletion junction (Figure 3). The filler in *Ds2094* is a 13-bp-long sequence with the same properties as the filler in *Ds2(D5)*. The identical sequence also occurs close to the deletion junction in the *Ac* progenitor, 61 bp upstream, and is flanked at this location by the same sequences (GTT and T) that flank the filler DNA in the deletion junction, suggesting that both *Ds2(D5)* and *Ds2094* may have arisen by the same mechanism.

DISCUSSION

McClintock recognized that *Ac* could undergo various types of modifications, one of which was mutation to *Ds*. She described four such cases, two at the *bz* locus (McClintock 1956b, 1962) and two at the *wx* locus (McClintock 1963) as examples of the "origin of a two-element system of control of gene action from an apparently one-element system." Though subsequent work has established that three of these *Ds* derivatives are internal deletions (Fedoroff *et al.* 1983; Schiefelbein *et al.* 1985; Dooner *et al.* 1986), the mechanism by which these deletions arise has remained conjectural. It has been generally believed that changes of *Ac* to *Ds* are transposase mediated, but that is difficult to prove and other mechanisms, including recombination between elements at different chromosomal locations, have been considered (Fedoroff 1983). In somatic tissues of transgenic tobacco, internal deletions were found to occur within *Ac*, but not within an almost-identical *Ds* element, arguing that they are excision, rather than sequence, dependent (Rubin and Levy 1997). In this work we present evidence that a *Ds* ele-

ment can arise at a locus where *Ac* resided as a consequence of an *Ac* transposition event and, based on the sequence of the deletion junctions of several new *Ds* elements, we propose a mechanism for this change.

Using genetic screens designed to identify mutations of *Ac* to *Ds*, we isolated three new *Ds* elements at *bz* and one at the tightly linked *tac2094* locus (Dooner and Belachew 1989; Ralston *et al.* 1989), all of which turned out to carry internal deletions. The deletion junctions in these four *Ds* elements and in a fifth one that had been previously characterized as a deletion (Schiefelbein *et al.* 1985) were located and sequenced. As shown in Figures 2 and 3, there is no obvious site or sequence preference for deletion formation in *Ac* [though it should be pointed out that the 3′ deletion end points in *Ds2(D4)* and *Ds2(D5)* are only 1 bp apart].

The deletion junctions have two interesting features: they occur adjacent to short direct repeats of a few base pairs in most cases, and in two cases, *Ds2(D5)* and *Ds2094*, they contain filler DNA. Filler DNA refers to the extra nucleotides that are frequently found at the junction of genetic rearrangements in animals, plants, and fungi (Roth and Wilson 1985; Roth *et al.* 1989; Sainsard-Chanet and Begel 1990; Wessler *et al.* 1990; Doseff *et al.* 1991). In maize, it often occurs at the junction of spontaneous deletions, *i.e.*, of deletions of unknown origin that have been collected by geneticists throughout the years (Wessler *et al.* 1990). Filler DNA can vary in size and composition from one to a few base pairs of random sequence to a short oligonucleotide of as many as 20 bp that is homologous to a sequence found close to the deletion junction in the parental DNA. In *Ds2(D5)* and *Ds2094*, the filler DNAs are 5 bp and 13 bp long, respectively, and in both cases the extra sequences are found close to the deletion junction in the *Ac* DNA, where they are flanked by the same sequences that flank the filler DNA in the deletion (Figure 3).

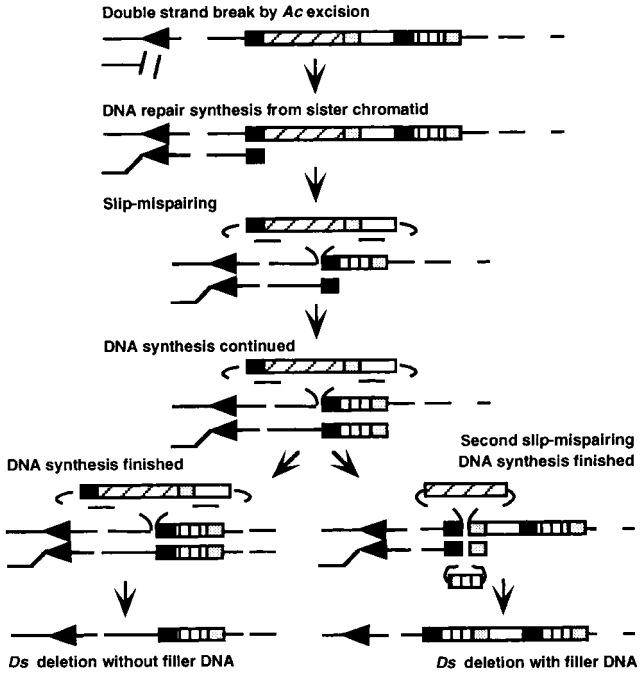

Figure 4.—Model for the origin of *Ds* internal deletion derivatives from *Ac* (adapted from Roth and Wilson 1985 and Wessler *et al.* 1990). Two sister chromatids are shown after *Ac* has excised from the lower one, generating a transient DSB. For illustration purposes, only the left terminal inverted repeat of *Ac* is shown (arrow). Repair of the DSB is initiated by strand invasion and DNA synthesis using the *Ac* element in the upper chromatid as template. Slip-mispairing occurs between direct repeats (black boxes) in the newly synthesized strand and in the *Ac* template, after which DNA synthesis continues. Completion of DNA synthesis (left) results in a *Ds* element deleted for the sequence (diagonal bars) between the direct repeats and for one copy of the repeat. A second slip-mispairing between different direct repeats (hatched boxes) followed by completion of DNA synthesis results in an internally deleted *Ds* element with filler DNA (vertical bars) in place of the deleted sequence (diagonal bars).

These structural features of filler DNA have been explained by mechanisms that involve slipped mispairing of repeat sequences during DNA synthesis (Roth and Wilson 1985; Roth *et al.* 1989; Wessler *et al.* 1990). We propose a mechanism for the origin of internal deletions from *Ac* (Figure 4) similar to the one proposed by Wessler *et al.* (1990) for the origin of spontaneous deletions in maize. We have modified it to incorporate the production of a DSB by *Ac* excision as the event that triggers repair DNA synthesis. As in the earlier models, slipped mispairing between nearby repeats during DNA replication would result in a deletion of the sequences between the repeats and of one repeat (Figure 4, left). The more common type of *Ds* deletions—*Ds2(D1)*, *Ds2(D2)*, *Ds2(D3)*, and *Ds2(D4)*—would be produced that way. A second slip mispairing during replication is required to explain the origin of the filler DNA in *Ds2(D5)* and *Ds2094* (Figure 4, right). An alternative model for the origin of the simple *Ds* deletions is interrupted or abortive gap repair by a synthesis-dependent strand annealing (SDSA) pathway (Nassif *et al.* 1994; Rubin and Levy 1997), but this model would still have to be modified to include slip mispairing to account for the presence of filler DNA at a deletion junction. Besides, intrachromosomal deletions between direct repeats in yeast, thought to occur by the mechanistically related single strand annealing (SSA) mechanism, require a minimum of 60–90 bp of homology (Sugawara and Haber 1992), whereas the deletions that we observe are flanked by direct repeats of only a few base pairs. In either case, the DNA replication process would appear to be error prone. The possibility that the DNA replication process involved in the repair of transposition-generated DSBs may be more prone to error than normal chromosome replication was originally suggested by O'Hare and Rubin (1983) to explain the unusual structure of deletion end points within the *P* element of Drosophila. Other *Ds* elements of undetermined origin that carry sequences unrelated to *Ac* have been described in the maize genome (*e.g.*, Merckel-

bach *et al.* 1986; Klein *et al.* 1988; Varagona and Wessler 1990). In one instance, the non*Ac* sequence is also found within 1 kb of the *Ds* element (Klein *et al.* 1988). Because the origin of these elements is not known, it is conceivable that they arose in multiple steps. However, whether these elements arose in one or multiple steps, ectopic sequence capture can be readily accommodated by models that postulate repair synthesis at the site of a DSB.

How frequently are the DSBs produced by *Ac* excision repaired by DNA repair synthesis? Available evidence indicates that the homologous chromosome rarely, if ever, serves as DNA repair template at meiosis and that *Ac*-generated DSBs are most frequently repaired by end-joining, *i.e.*, by direct fusion of the broken ends (Dooner and Martinez-Ferez 1997). The findings reported here would support some role of repair synthesis using the sister chromatid as template, but this type of repair may not be very common. One could argue that repair synthesis using the sister chromatid as template is frequent and is simply not detected because it results in the synthesis of a complete *Ac* element at the *Ac* excision site. However, the study of pericarp sectors in ears carrying the *Ac*-mutable allele *P-vv* would suggest that that is not so. If it were, the frequency of untwinned light-variegated pericarp sectors would be higher than the frequency of untwinned red sectors, and it is not (Greenblatt 1974; Fedoroff 1983; Chen *et al.* 1992). Therefore, *Ac*-induced DSBs would appear to be only rarely repaired by repair synthesis. Consequently, the end-joining events that result in excision footprints contribute the bulk of the genetic diversity that is generated by *Ac* movement in maize (Sutton *et al.* 1984; Moreno *et al.* 1992; Scott *et al.* 1996; Dooner and Martinez-Ferez 1997). Still, the apparently error-prone DNA replication mechanism that occasionally repairs the DSBs produced by *Ac* excision may account for the observation that mutation of *Ac* to *Ds* (Table 1 and McClintock 1963) is two orders of magnitude higher than the spontaneous mutation frequency in maize. Of the eight *Ds* derivatives from *Ac* that have been described to date, two—*Ds2(D5)* and *Ds2094*—clearly had a premeiotic origin. Peculiarly, these are the two *Ds* derivatives with filler DNA at the deletion junction. Because of the small number of cases studied, this finding may not be significant. Alternatively, it could suggest that the DNA repair mechanism is more error prone at mitosis than meiosis.

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