Somatic excision of the *Mu1* transposable element of maize

Andrea Doseff, Robert Martienssen and Venkatesan Sundaresan*
Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA

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ABSTRACT

The *Mu* transposons of the Robertsons's Mutator transposable element system in maize are unusual in many respects, when compared to the other known plant transposon systems. The excision of these elements occurs late in somatic tissues and very rarely in the germ line. Unlike the other plant transposons, there is no experimental evidence directly linking *Mu* element excision and integration. We have analyzed the excision products generated by a *Mu1* transposon inserted into the *bronze1* locus of maize. We find that the excision products or 'footprints' left by the *Mu1* element resemble those of the other plant transposable elements, rather than those of the animal transposable element systems. We also find some novel types of footprints resembling recombinational events. We suggest that the *Mu1* element can promote intrachromosomal crossovers and conversions near its site of insertion, and that this may be another mechanism by which tranposons can accelerate the evolution of genomes.

INTRODUCTION

The Robertson's Mutator (*Mu*) system of transposable elements in maize was first described by Robertson in 1978 (1). A 1.4 kb element, *Mu1*, was the first element of this system to be cloned and characterized (2, 3). Subsequently other elements of the *Mu* system (*Mu1.7*, *Mu3- Mu7*) have also been cloned (4, 5, 6). All of the *Mu* elements reported so far have 0.2 kb terminal inverted repeats (TIR) homologous to that of the *Mu1* element. However, the internal DNA sequences of the different members of the *Mu* system are non-homologous. There is as yet no evidence as to which, if any, of these *Mu* elements encodes the transposase for the *Mu* system. When *Mu* elements are active, they can excise from genes into which they are inserted, giving rise to mutable phenotypes. Also, they can transpose to new chromosomal locations to generate new mutations (reviewed in ref. 7). The *Mu* system can spontaneously become inactive, a transition characterized by loss of both excisions and forward transpositions (8, 9). This inactive state is reversible, and activity can be restored by crossing to plants with an active *Mu* system (10, 11).

Excision of transposable elements has been extensively studied in different prokaryotic and eukaryotic systems. In general, the excision of bacterial transposons such as Tn*10* appears to be independent of transposase but dependent on host-encoded functions; the excision events involve losses of DNA that is bordered by duplicated sequences at or near the ends of the element and resemble intrachromosomal recombination events (reviewed in ref. 12). On the other hand, excision of eukaryotic transposons (with the exception of the retrotransposons) is in most cases dependant on transposase function. In animal systems, the P element family of *Drosophila* and the Tc1 family of *C. elegans* have both been studied in detail with regard to excision. Excision products of the P elements are of three types (reviewed in ref. 13): 1. Large deletions within the element. 2. Deletions that remove the element and some flanking DNA. 3. 'Precise' excisions in which the progenitor sequence is restored exactly *i.e.*, the element and one of the target site duplications is lost. The three types of products are believed to be generated by the same mechanism (13). The excision products of the Tc1 elements include all three types of products generated by the P elements, and in addition one other type of excision product has been observed, *i.e.*, direct duplications of short sequences that are immediately adjacent to the site of insertion (reviewed in ref. 14). Plant transposable elements that have been studied in detail with respect to excision activity are the *Ac-Ds* and *Spm* elements of maize, and the Tam1 and Tam3 elements of *Antirrhinum*. All four of these elements generate similar types of products upon excision (reviewed in refs. 15, 16). The excision products (termed 'footprints'; ref. 17) have three general features: 1. Deletions of the target site duplication and adjacent host DNA. 2. Short inverted duplications of sequences flanking the site of insertion that can include and extend beyond the target site duplication. 3. An absence of transposon sequences remaining at the site of excision. The second and third features appear to be unique to plant transposable elements. Two models of transposon excision have been proposed to account for the types of excision products found in plant transposon systems (18, 19).

The *Mu* transposon system in maize differs from the plant transposons described above in many significant respects. The *Ac-Ds* and *Spm* elements in maize, and the Tam1 and Tam3 elements in *Antirrhinum* are all believed to transpose by a conservative transposition mechanism in which excision and

* To whom correspondence should be addressed

integration are linked (reviewed in refs. 15, 16). The fact that no transposon sequences are left behind upon excision is consistent with conservative transposition. By contrast, there is considerable evidence that *Mu* transposition is replicative. The germinal reversion rate of *Mu* insertions is very low (less than 1 in 10^4) as compared to the rate of forward transpostion (5−10 new *Mu1* insertions per progeny), and the copy number of *Mu1* elements in an active line (10−50 elements per genome) is maintained even after several outcrosses to non-*Mu* lines (20). On the other hand, the *Mu* system has similarities to some of the animal transposon systems. The P elements of *Drosophila* also do not exhibit any obvious relationship between excision and integration, and the rate of new integration events far exceeds the rate at which the complete element excises from chromosomal positions (reviewed in ref. 13). Unlike the other plant transposons, but like the Tc1 elements of *C. elegans*, the *Mu* elements can exist in a free extrachromosomal form (21). For these reasons, it seems likely that the mechanism of transposition of the *Mu* elements differs from that of the previously characterized plant transposable elements. Because germinal reversion of *Mu* insertions is very rare, there has been very little molecular information on the excision products of *Mu* elements. In a study of an insertion of the element *Mu7* (*rcy*) at the *Bronze* locus, two germinal revertants were sequenced, and were shown to be imprecise excisions with short deletions of the DNA flanking the site of insertion, in addition to the loss of the transposon sequence (6). These two excision products are consistent with those observed with other plant transposons, but they are also consistent with the types of excision products observed with the P and Tc1 elements in animal systems. In contrast to the low germinal excision frequency, *Mu* insertions exhibit high frequencies of somatic mutability leading to spotted kernels, sectored leaves etc. (7). The advent of the Polymerase Chain Reaction (PCR) makes possible the analysis of DNA from small amounts of tissue (22). Therefore, we have undertaken a more detailed study of *Mu* excision by analysing the products of *Mu1* element excision ('footprints') arising from *Mu* activity in somatic tissues.

MATERIALS AND METHODS

Genetic Stocks

The maize plants used were heterozygous for *bzMum9* /Δ(*sh bz*). The mutation *bzMum9* is a *Mu1* insertion into the *Bz1 (Bronze1)* gene, isolated by Robertson (23), and the deletion of *Sh* and *Bz* used in this study was the *sh bz* X3 allele, from J. Mottinger, U. of Rhode Island. The inactive *Mu* line used was derived from this *bzMum9* stock, and was characterized by loss of somatic excision activity (*e.g.*, absence of spots on the kernels); the excision activity could be restored by crossing back to an active *Mu* stock (24). The wild-type allele *Bz* W22 has been described in detail, and its DNA sequence has been determined by Ralston *et al.* (25).

Plant Material

Plant tissue for DNA isolation and PCR was obtained from active *bzMum9* plants as follows: 1. Revertant purple sector, and adjacent non-revertant sector from a mature leaf (Fig. 2C). This sector penetrated into the tassel, but apparently not into the germ line, as no germinal revertants were obtained from the pollen. 2. Revertant purple sector including underlying endosperm tissue from a single kernel (as shown in Fig. 2B) at 20 DAP (days after pollination). 3. Whole aleurones peeled from two sets of approximately 50 spotted kernels (as shown in Fig. 2A) at 20 DAP. In addition, as a control, DNA was isolated from aleurones of stable bronze kernels from the inactive *bzMum9* plants at 20 DAP, and also from leaf tissue of a wild-type *Bz* W22 plant.

DNA Isolation and Amplification

DNA isolations were performed essentially as described by Shure *et al.* (26). Prior to PCR amplification, the DNA was digested with *Sac* II, which cuts several times within the central region of the *Mu1* element, to select against the amplified products containing the intact *Mu1* element. PCR amplification by Taq polymerase (Perkin-Elmer Cetus) was carried out according to the manufacturer's instructions. A single reaction used approximately 50−250 ng. of DNA and 25 pmole of each primer in a 25 μl volume. The primers used on either side of the *Mu1* insertion Bz5 and BzR have the following sequences, Bz5: G-AATTCATTGCGCGCGGGTTTGATGA, BzR: TCTAGA-GGTCACGGACGCGGTAGCT. The location of these sequences on the *Bz* gene are shown in Fig. 1B. In addition to the *Bz* gene sequences, the primers contain recognition sequences at their 5′ ends for the restriction enzymes *Eco*RI (in Bz5) and *Xba*I (in BzR), to facilitate cloning. The PCR products were purified from low-melting point agarose gels using agarase (Calbiochem), cloned into pUC119, and sequenced on both strands by the dideoxy method using the Sequenase enzyme (U.S. Biochemicals).

RESULTS

The mutation chosen for this study is a *Mu1* insertion into the *Bronze1 (Bz1)* locus isolated by Robertson called *bzMum9* (23, 24). The site of the *Mu1* insertion has been mapped (23) and sequenced (Colasanti and Sundaresan, unpublished results), and has been found to lie at the intron-exon boundary of the *Bz1* gene (Fig.1A). The insertion creates a 9 bp duplication of the sequence GCCGCAAAC (the target site duplication or TSD; Fig.1A). Plants carrying this mutation exhibit somatic mutability when the *Mu* system is active, *i.e.*, purple revertant spots on bronze coloured kernels, purple revertant sectors on leaves, *etc.* (Fig.2). When the *Mu* system is inactive, no somatic mutability is observed (*e.g.*, the kernels are stable bronze without purple spots). We have analyzed two sectors of revertant somatic tissue, the first is a revertant purple sector from a leaf (Fig.2C), and the second is a rare large revertant sector from a kernel (Fig.2B). In addition, to get a more detailed picture of *Mu* excision, we have analyzed the DNA from whole aleurone tissue of spotted kernels, without specifically isolating revertant tissue. In this way, we hoped to get a representation of all the products of *Mu* excision, and not just those that restored gene function. Two independant preparations of aleurone DNA from two different sets of spotted *bzMum9* kernels were analyzed. As a control, DNA was isolated from the stable bronze kernels of an inactive *Mu* plant carrying the *bzMum9* allele. For the revertant sector on the leaf, we also isolated DNA from adjacent non-revertant tissue as another control. In all cases, the DNA was digested with a restriction enzyme that cuts within the *Mu1* element, and then amplified by PCR using the primers Bz5 and BzR shown in Fig.1B (and as detailed in Materials and Methods).

As shown in Fig. 3, amplified products of the expected size (about 120 bp) were obtained in all cases except from the stable bronze kernels of the inactive *Mu* line. Not surprisingly, the amount of PCR product obtained from competely revertant tissue (Fig. 3, lanes 3 and 6) was significantly higher than that from whole aleurone tissue with small revertant sectors (Fig. 3, lanes

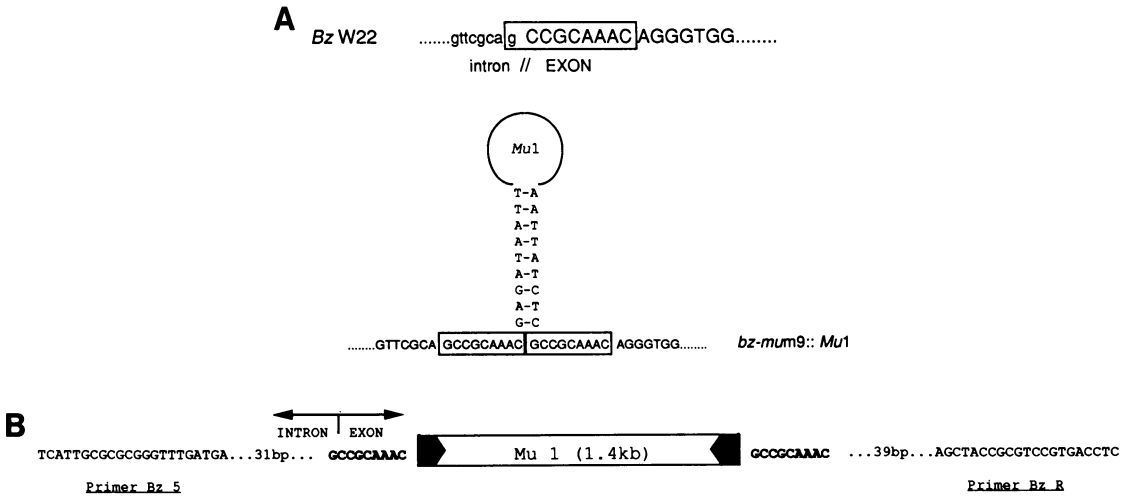

Fig.1 A. Site of insertion of the *Mu1* element in the *bzMum9* mutant allele and comparison with wild-type progenitor *Bz* W22. Lower case letters indicate intron sequences; the target site duplication is boxed. **B.** Positions of the Bz5 and BzR primers used for PCR amplification; see Materials and Methods for actual primer sequences.

Fig.2 A. Typical kernel from active *bzMum9* plant showing a high frequency of small revertant sectors. **B.** Example of rare *bzMum9* kernel with a large revertant sector. **C.** Revertant leaf sector used in the study. Arrows indicate purple sector in edge of the leaf, and where the same sector has penetrated the stem.

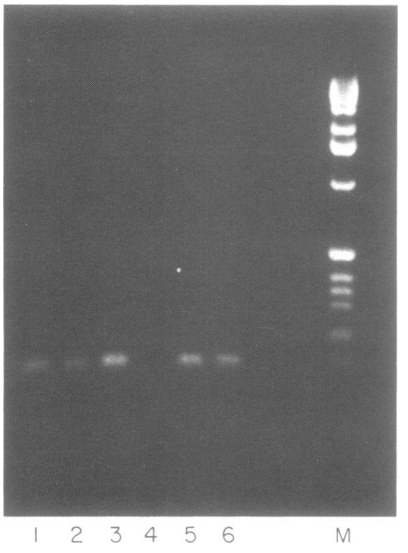

Fig.3. Gel electrophoresis of PCR amplification products of DNA from different sources, on a 1.8% agarose gel stained with Ethidium bromide. Lanes 1 and 2, Total aleurone DNA from two different sets of active *bzMum9* kernels; lane 3, DNA from large revertant sector of a *bzMum9* kernel; lane 4, DNA from aleurone of inactive *bzMum9* kernels; lane 5, DNA from leaf of *Bz* W22 plant; lane 6, DNA from revertant sector of a *bzMum9* leaf; lane M, DNA size markers-kb ladder and *Hin*f1 digested pBR322 (B.R.L.).

1 and 2); in the former case, a visible band was evident after 40 cycles, while the latter required 50 cycles to see a visible product. Probably this difference is due to a much higher fraction of excision product in the DNA from revertant sectors vs. DNA from whole aleurones. The amplified DNA was eluted from the gel and cloned into the vector pUC119 for sequencing. The sample from the inactive *Mu* line was also carried through this procedure even though no amplified DNA was visible in the gel (Fig. 3, lane 4). As expected, we did not obtain any recombinant clones from this sample. The other control that we attempted, DNA from the non-revertant leaf tissue, also did not give any PCR product (not shown). In this case, however, we found that the DNA preparation inhibited the activity of the Taq polymerase altogether. It is known that in a *bronze1* mutant background, adult leaf tissue shows considerable necrosis because of the accumulation of toxic anthocyanin precursors (27). Presumably the DNA from this necrotic tissue contained inhibitors of PCR, and therefore PCR amplification of this DNA cannot be used as a control. The revertant tissue, having no necrosis, yielded DNA that could be amplified by PCR.

The DNA sequences of the excision products are shown in Fig. 4. Both of the revertant sectors showed small deletions of the TSD (target site duplication) and single nucleotide sequence changes. The leaf sector (R1) had a deletion of one TSD (9 bp) and a A→C transversion. Thus the reading frame is restored,

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Bz        ..GAATGGCTGTTGCATTTCCATCGTTCGCAGCCGCAAACAGGGTGGACGAGCCACTGATCTCCCACCCGGGCCTCGCCAGCTACCGCGTCCGTGACCTACCTC
bz Mum9           ..ATCGTTCGCAGCCGCAAAC▼GCCGCAAACAGGGTGG...
R1(2)             ..ATCGTTCGCAGCCGCAAAC _________CGGGTGG...
R2(3)             ..ATCGTTCGCAGCC_____ ACCGCAAACAGGGTGG...
F1(1*,2**)        ..ATCGTTCGC _________ __CGCAAACAGGGTGG...
F2(1**)           ..ATCGTTCGCAGCCGCAA__ _________CAGGGTGG...
F3(5*,6**)        ..ATCGTTCGCAGCCGCAAAC _________AGGGTGG...
F4(1*)            ..ATCGTTCGCAGCCGGC___ ___GCAAACAGGGTGG...
F5(1**)  ..GAATG    Δ24 bp  _________  GCCGCAAACAGGGTGG...
F6(1**)           ..ATCGTTCGCA_________ _________          Δ26 bp      CCCGGG...
F7(1**)           ..ATCGTTCGCAGCCGCAAACGAGATAATTGCCATTATGGACGAGCCACTGA...
F8(4*)            ..ATCGTTCGCA_________GCTACCGCGTCCGTGACCT _______Δ22bp CCCACCCGGGCCTCGCCAGCTACCGCGTCCGTGACCTACCTC
F9(1**)           ..AT        _________GAGTTGCAGCAGCAGT_____AACAGGGTGG...
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Fig.4. DNA sequences of the excision products observed with the *Mu1* insertion in *bzMum9*. The sequence of the *Bz* W22 gene from 28 bp upstream of the insertion site to the end of the BzR primer (Fig.1B), is shown on top for reference. The *Mu1* insertion in *bzMum9* allele is shown by a solid triangle, and the 9 bp TSD (target site duplication) is underlined. R1, Revertant leaf sector; R2, Revertant kernel sector; F1- F9, Different excision products ('footprints') from aleurones isolated from two different sets of kernels, distinguished by one (*) or two (**) asterisks. The number of sequenced clones is shown in parantheses. In each case the region between the two primers shown in Fig.1B was sequenced, but only the region in which there were sequence changes is shown. Dots indicate sequence identity with *Bz* W22; bold letters indicate new sequences; blank spaces or open triangles indicate deletions; italicized letters in F8 indicate the sequence in the *bronze* gene that is found at the excision site.

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A  ...GCCGCAAACgagataattgccatta [tggacga] agaggg..Mu 1..ctcGCCGCAAACAGGG [TGGACGA] GCCACTGA...
B  ...CG [CAGC] CGCAAAC..Mu 1..GCCGCAAAC..18 bp..AT [CTCCCA] CCCGGGCCTCGC [CAGC] TACCGCGTCCGTGAC [CTCCCA] GAC...
C  ...TC [CAT] CGTTCGCAGCCGCAAAC..Mu 1..GCCGCA [AACAG] GG..934 bp..TA [CAT] GAGTTGCAGCAGCAGT [AACAG] CCTCCTGT...
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Fig.5. Possible origin of three footprints that resemble recombinational events. The sequences shown are of the progenitor *bzMum9* allele. **A.** F7 progenitor; **B.** F8 progenitor; **C.** F9 progenitor. Regions of homology are boxed. *Mu1* sequences are in lower case; the TSD (target site duplication) is underlined; and sequences in the *bronze* gene that are found at the excision site are italicized.

and the transversion can be considered to be a one base pair inverted repeat. Short inverted repeats generated by excision are a common feature of other plant transposons (see Introduction). The kernel sector (R2) had a deletion of 6 bp of one TSD and a G→A transition. In this case also, the reading frame was restored upon excision. The G→A change is unusual, but could be explained by error-prone repair following excision. The DNA sequences of excision products from the two preparations of whole aleurone tissue were obtained by sequencing several random clones. These sequences are shown in Fig. 4 as footprints F1-F9. When two or more clones have the same DNA sequence, they are shown as the same footprint, even if they were obtained from different aleurone preparations. The footprints can be classified into 4 classes as follows:

1. Precise excisions.

These are events where the wild-type sequence is restored exactly (F3 in Fig. 4). This was the largest single 'footprint' (11 clones out of 24 clones sequenced), and is presumably a frequent event. However, we cannot rule out contamination from wild-type DNA as a source of some or all of these clones, despite the fact that the control performed with DNA from the stable allele did not yield any clones.

2. Imprecise excisions.

These are events where deletions and/or small inverted duplications of the DNA flanking the insertion site are left behind after excision. The two revertant sectors (R1 and R2) fall into this category, as do footprints F1, F2, F4, F5 and F6 (Fig. 4). Short inverted duplications were seen in two cases, F4 and R1 (see above). Two of the deletions (F5 and F6) extended beyond the TSD. It should be noted that deletions that extended beyond the sequences of the primers would not be observed by us, as they would not be amplified.

3. Crossovers.

We observed one event of this type (F7 in Fig.4). In this event, a deletion of 1346 bp of *bzMum9* DNA has occurred between the sequence TGGACGA which is present in *Mu1* at 16 bp downstream of the left TSD and is repeated in the *Bronze* gene at a position 4 bp downstream of the right TSD (Fig.5A). This event resembles an intrachromosomal recombination event, and generates an insertion of 16 bp of *Mu1* DNA and a deletion of 4 bp of *Bz* DNA relative to the wild-type allele.

4. Sequence replacements.

Two events (F8 and F9; Fig.4) resulted in removal of the *Mu1* element and some flanking DNA, and its replacement by a different sequence of 19 bp (F8) or 16 bp (F9). The substituted sequences appear to be part of the *Bz* gene. In the case of the substituted sequence in F8, the same sequence occurs in the *Bz* gene 40 bp 3′ of the TSD (italicized in Fig.4 and Fig.5B). We note that the substituted sequence in F8 also includes part of the sequence of the BzR primer, and therefore it is necessary to consider the possibility that the sequence of F8 was artefactually generated during PCR amplification by internal hybridization of the BzR primer to the sequence being amplified. This possibility is highly unlikely since the BzR primer also contained an *Xba*I site at its 5′end, which is absent in the substituted sequence in F8. In the case of the substituted sequence in F9, the same sequence occurs in the *Bz* gene 940 bp 3′ of the TSD (italicized in Fig.5C). Here the substituted sequence has no significant homology to either primer, and the possibility of a PCR artefact by internal hybridization does not arise. The position of the sequence in the progenitor allele lies well outside the region sequenced in F9, so that we cannot be certain that it was retained in its original position following the event as in F8. If we assume that it was, then both F8 and F9 resemble gene-conversion events (see Discussion).

DISCUSSION

The extremely low rate of *Mu* excision in the germline combined with the small size of somatic revertant sectors, has prevented a detailed study of *Mu* element excision. By utilizing PCR to analyze a *Mu1* insertion in the *Bronze* gene, we have been able to overcome this difficulty. Four types of problems could occur with a PCR based approach: 1. DNA contamination. 2. Generation of artifacts by mispriming. 3. Errors by the Taq polymerase. 4. Selective amplification of certain products over others. We have controlled for the first two problems by demonstrating that no PCR product or clones were obtained from the inactive *bzMum9* stock, although all procedures from DNA isolation to cloning into pUC119 were carried out in parallel. We do not think that errors by the Taq polymerase significantly affected our results, because the entire region between the two primers was sequenced in each clone, and the only sequence changes that we observed were in the DNA immediately adjacent to the site of insertion of the *Mu1* element. It seems unlikely that any errors of the Taq polymerase were confined solely to this region of the sequence, because previous studies have shown that such errors appear to be random (22). Regarding the last point, *i.e.* selective amplication, we have found that sequences containing large stretches of both *Mu1* inverted repeats are resistant to amplification by PCR, presumably because of their capacity to form stable hairpin structures. We have not previously encountered any difficulty in amplifying segments of the wild-type *Bz* sequence flanking the site of *Mu1* insertion, nor in amplifying single *Mu1* terminii or the internal sequences of the *Mu1* element. Further, the fact that each PCR amplification yielded a different set of products, and the fact that different types of products were obtained, suggest that this could not be a major problem; however, we cannot rule out the possibility that some excision products have structures that are resistant to PCR amplification. Despite this possibility, we believe that the data presented above represent a large enough number of different excision events to arrive at some general conclusions regarding the mechanisms of *Mu* element excision.

With the exception of footprints F7, F8 and F9 which are discussed separately below, the *Mu1* excision products have the two distinguishing features of the other plant transposable elements that have been studied: 1. The absence of any transposon sequences left behind following the excision event. 2. The presence of short inverted duplications of DNA flanking the excision site. Neither of these is true for the animal transposon systems that have been studied. Recently, similar footprints left by *Mu1* excision have been seen with a different *Mu1* insertion in the *Bronze* gene (A. Britt and V. Walbot, personal communication, *Maize Newsletter*, 1990), and also with a *Mu1* insertion in the *Hcf106* gene (R. Martienssen and A. Jahrsdoerfer, unpublished results). Therefore, despite the fact that the *Mu* system appears to share some properties with animal transposon systems, *i.e.*, absence of any direct correlation between excision and integration events like the P elements (and unlike the other plant transposons cited above), and extrachromosomal circles like the Tc1 elements (but again, unlike the other plant transposons), it is likely that the reactions involved in excision of the *Mu* elements and repair of the site of excision are similar to those utilized in excision by the other plant transposon systems. If all plant transposons excise by a common mechanism which is

different from that of the animal transposon systems, it would suggest that they utilize a common set of host factors and host repair activities that are particular to plant cells. This study examines only the excision of *Mu*; it is possible that excision and forward transposition of *Mu* elements occur by different mechanisms, even though both types of events may be catalyzed by the same transposase.

Three of the excision products observed (F7, F8 and F9 in Fig.4) differ from the footprints typical of plant transposable elements. The F7 event results in a short sequence (16 bp) of the *Mu1* element remaining after excision, and in this regard resembles some of the excision products observed with the P and Tc1 transposable element systems. However, as discussed earlier (see Results), this excision product can be interpreted as the result of an intrachromosomal recombination event, *i.e.*, a crossover event between repeats of the sequence TGGACGA that occurs in both the *Mu1* element and the flanking DNA (Fig. 5A). The two other excision products, F8 and F9, can also be interpreted as the result of recombinational events. As discussed earlier (see Results), these excision products involve replacements of the *Mu1* element and some flanking DNA by short sequences of the *Bz* gene, that in the case of F8 and F9 are 40 bp and 940 bp downstream of the TSD respectively (italicized in Fig.5B and 5C). The same sequence is repeated in its original location in the case of F8 (Fig.4); for F9, this information is not available because the sequence lies beyond the amplified region. Assuming that this is also true for F9, both events resemble gene-conversion events, as seen from Fig.5. In both cases, there are short regions of homology between the ends of the sequence that is removed and the sequence that is substituted (shown by boxes in Fig.5B and 5C). The fact that no such products were obtained with DNA from kernels of the inactive *Mu* line suggests that these recombinational events are related to *Mu* transposition activity. It is possible that double strand breaks made by the transposase at the ends of the element result in initiation of recombinational processes by the host recombination machinery, which then generates these types of events, *i.e.*, crossovers or conversions. The conversion-type events that we observe resemble the DNA rearrangements known to occur during mating type switching in yeast; in the latter case, a gene-conversion event is initiated by a double-strand break at the mating-type locus, and the sequences being switched share homologies at their ends (reviewed in ref. 28).

Although such events have not been reported for the other plant transposon systems, it has recently been proposed that transposase dependent excision of the P elements of *Drosophila* occurs through a mechanism involving gene-conversion (29). In the case of the P element system, the sequence of the wild-type gene on the homologous chromosome is believed to act as the template in the conversion event. The conversion type events seen in our study however, were not due to the wild-type allele, as the plants used here were heterozygous for *bzMum9* and a *sh bz* deletion; instead, sequences elsewhere in the mutant gene acted as the template. Events of this type most likely lead to stable null phenotypes, and could conceivably have been overlooked in the studies with the other plant transposon systems. Most other studies have focussed on revertant or partial revertant alleles of transposon induced mutations, and derivative null alleles of these mutations may not have been studied as intensively. While the excision events seen with the *Mu* system are almost all somatic, with the other plant transposon systems a significant fraction of excisions are germinal and the footprints left by the transposons are heritable. If such conversion type events are a general feature of plant transposon activity, they provide another mechanism by which transposons could participate in the evolution of plant genomes, *i.e.*, in addition to deletions and inversions, segments of DNA from elsewhere in the chromosome can be duplicated at the site of excision, leading to further reshuffling of the genome. Finally, it has been proposed that gene-conversion promoted by the P element system could be utilized in technologies for directed mutagenesis and gene-replacement in *Drosophila* (29); the *Mu* element system might be similarly useful in maize.

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