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Genetics

Mutator activity in maize correlates with the presence and expression of the *Mu* transposable element *Mu9*

(*Zea mays*/*Bronze-2*/DNA sequence)

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ABSTRACT **Mutator is a powerful system for generating new mutants in maize. Mutator activity is attributable to a family of transposable, multicopy *Mu* elements, but none of the known elements is an autonomous (regulatory) element. This paper reports the discovery of *Mu9*, a 4942-base-pair *Mu* element that was cloned after it transposed into the *Bronze-2* locus. Like other *Mu* elements, *Mu9* has ≈215-base-pair terminal inverted repeats and creates a 9-base-pair host sequence duplication upon insertion. A small gene family of elements that cross-hybridize to *Mu9* has been found in all maize lines, and one of the other known *Mu* elements, *Mu5*, probably arose as a deletion of *Mu9*. *Mu9* has several of the properties expected for the proposed regulator of Mutator activity. (*i*) The presence of *Mu9* parallels the presence of Mutator activity in individuals from a line that genetically segregates for the *Mu* regulator. (*ii*) Lines that transmit Mutator to >90% of their progeny have multiple copies of *Mu9*. (*iii*) Most maize lines that lack Mutator activity and that are not descended from Mutator lines lack the *Mu9* element. (*iv*) Transcripts that hybridize to *Mu9* are abundant in active Mutator lines, but they are absent from lines that have epigenetically lost Mutator activity. These correlations suggest that *Mu9* is a candidate for the autonomous Mutator element.**

Mutator lines of maize generate new mutants at a frequency 30- to 50-fold higher than normal lines (1, 2). Many (30–40%) of the new mutants are somatically unstable, with small, frequent revertant sectors, and the rate of germinal reversion is very low (3, 4). The mutations are attributable to a family of multicopy, transposable *Mu* elements. Transposon tagging with Mutator has been used to clone several genes, including *Bronze-2* (*Bz2*) (5), a structural gene whose product acts late in the synthetic pathway of the purple pigment anthocyanin. This mutant and the other *bz2* mutables isolated in this laboratory (6) have the characteristic Mutator phenotype: small purple (*Bz2*) spots on a bronze (*bz2*) background in the aleurone, the outermost cell layer of the endosperm.

Molecular analysis of Mutator-induced mutations has led to the discovery of a family of at least eight related transposable elements. These *Mu* elements have conserved terminal inverted repeats (TIRs) of ≈215 base pairs (bp), and their insertion sites are flanked by 9-bp direct duplications of the host sequence. The *MuA* element, recently described by Qin and Ellingboe (7), has TIRs homologous to the other *Mu* elements, but it is flanked by 8-bp rather than 9-bp repeats. The first eight *Mu* elements range in size from 1.3 to 2.2 kilobase pairs (kb); *MuA* is 5.5 kb. With the exception of *Mu1* and *Mu2*, the internal sequences of these *Mu* elements are unrelated (see ref. 8 for review).

The inheritance of Mutator activity is complex. For our purposes, maize lines can be placed in three categories: Mutator, derived from the original Robertson's Mutator plants; non-Mutator, including all the standard inbred lines; and inactive Mutator, derived from Mutator lines but no longer showing either germinal or somatic Mutator activity. The inactive lines lose activity not through the genetic segregation of the regulatory element(s) but through an epigenetic "change of phase" (9) that correlates with the methylation of *Mu* elements (10). When an active Mutator line is crossed to a non-Mutator line, >90% of the progeny inherit Mutator activity even after repeated outcrosses (1). This pattern of inheritance suggests that *Mu* element transposition is regulated by an autonomous, transposase-encoding element that is mobile and present in multiple copies (1, 8). None of the previously characterized *Mu* elements has been genetically identified as an autonomous element, and none of them is known to encode a transposase.

This paper reports the discovery of *Mu9*, a candidate for an autonomous Mutator element. *Mu9* is a 4.9-kb *Mu* element that was isolated after it transposed into *Bz2*. The expression of *Mu9* correlates with Mutator activity in active vs. inactive lines, and genetic and molecular evidence suggests that Mutator activity is present only in lines that contain *Mu9* elements. The sequence of the *Mu9* element has sufficient coding capacity to encode one or more large proteins.*

MATERIALS AND METHODS

Plant Material. The original purple Mutator stock used to generate the *bz2*::*mu9* allele was a gift from D. S. Robertson. D. S. Robertson also provided stable and mutable kernels from *a1-Mum2* plants 90-5004-8 and 90-5007-1 crossed to *a1 wx* testers. All of the other Mutator materials used were generated in this laboratory (11). The *bz2*::*mu9* allele was isolated from a large-scale screening for new mutants in the anthocyanin pathway following the procedure of Walbot *et al.* (11). Standard inbred lines were obtained from the Maize Genetics Stock Center.

RNA Isolation and Hybridization. RNA was isolated from pooled 10-day-old maize seedlings either by the method of Logemann *et al.* (12) or by the hot phenol method (13). For the RNA blots, 20 μg of total RNA was fractionated on formaldehyde/agarose gels and transferred to Hybond-N (Amersham). Probes were prepared by isolating the DNA fragment in a low-melting-temperature agarose gel (Seaplaque, FMC), labeling the probe in agarose by the random primer method, and purifying it on push columns (Stratagene). Prehybridization and hybridization were done according to the protocol published for GeneScreen (DuPont) using 10% dextran sulfate.

DNA Isolation and Hybridization. Maize genomic DNA was prepared from immature cobs by centrifugation on a CsCl/

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Abbreviations: *Bz2*, Bronze-2; TIR, terminal inverted repeat; ORF, open reading frame.
*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M76978).

ethidium bromide gradient as described (14) or from individual etiolated seedlings and their scutella (15). For Southern analysis, 3 μg of DNA was digested with restriction enzymes (BRL) according to the manufacturer's instructions, fractionated on agarose gels, and blotted onto Genetran (Plasco, Woburn, MA) or Hybond-N (Amersham). Probes were prepared as described above. The blots were prehybridized, hybridized, and washed as described by Walbot and Warren (15).

Library Construction and Screening. Genomic DNA from an immature ear of a *bz2::mu9/bz2* plant [I192(x)] was digested with *Eco*RI and fractionated on a 0.8% gel of low-melting-temperature agarose (Seaplaque, FMC). Using size markers visualized with ethidium bromide, DNA from 5.5 kb to 8 kb (fragment of interest, 6.7 kb) and from 2 kb to 2.5 kb (fragment of interest, 2.2 kb) was cut out of the gel, extracted with phenol, and ligated to *Eco*RI-cut, phosphorylated λZapII arms (Stratagene). The ligated DNA was packaged using GigapackII extracts (Stratagene) and plated on ER1647, an *mcrA, mcrB, mrr, hsd, recD* strain of *Escherichia coli* (New England Biolabs). Nitrocellulose filter (Schleicher & Schuell) lifts of 8×10^5 plaques were probed with random primed P420 (5′ *Bz2*) and P300 (3′ *Bz2*) (5) using standard procedures (16). Hybridizing phage were plaque-purified on *E. coli* strain XL-1 Blue (Stratagene), and plasmids were recovered from the λZapII by *in vivo* excision according to the manufacturer's directions. An *Nar* I to *Eco*RI fragment of the clone that hybridized to P420 was ligated into an *Eco*RI–*Pst* I subclone of the P300 hybridizing clone, creating the plasmid pΔMu9.

DNA Sequencing. Double-stranded, CsCl gradient-purified subclones of *Mu9* in pBluescript II SK− or KS+ (Stratagene) were sequenced using the Sequenase 2.0 kit (United States Biochemical). Mixes containing deoxyinosine provided with the kit were used to resolve band compressions in (G+C)-rich regions. *Mu9* was sequenced in its entirety in both directions using either commercially available primers or oligonucleotides prepared in the facility of the Department of Biological Sciences, Stanford University. The sequence and derived open reading frames (ORFs) were compared to the Genbank/EMBL and NBRF databases using the Genetics Computer Group software package (17).

Clone Verification. Eight pairs of 20- or 21-bp oligonucleotide primers derived from the pΔMu9 sequence that span the clone between *Sst* I sites (≈95% of the total) were used to amplify 400 ng of genomic DNA or 1 ng of pΔMu9 in the polymerase chain reaction (PCR). The reactions, which used AmpliTaq polymerase (Cetus) and the standard conditions suggested by the manufacturer, underwent 30 cycles of amplification with cycle times of 94°C, 1 min, 55°C, 1 min, and 72°C, 1 min. One pair, with primer sequences 5′-CTGAGTACTTAGACCAACAC and 5′-GGCTTGTTCTTAGCAGTCTT, showed a 239-bp difference between pΔMu9 and *bz2::mu9* (see text). After restriction analysis to localize the deletion in pΔMu9, the amplified *bz2::mu9* was digested with *Bgl* II and *Xba* I, ligated into pBluescript II, and sequenced on both strands.

RESULTS

A mutable allele of *Bronze-2*, *bz2::mu9*, was isolated in the summer of 1988 by crossing Robertson's purple Mutator (*Bz2*) by a *bz2* tester line and screening for spotted progeny. This allele has the characteristic Mutator phenotype: small, frequent sectors of somatic excision and a very low rate of germinal excision. To examine the defect in *bz2::mu9* at a molecular level, we used probes from *Bz2* on Southern blots. The results indicated that the mutant allele contains an insertion of ≈5 kb in the second exon of *Bz2* (6). The restriction map derived from Southern analysis and, later, from the cloned DNA is shown in Fig. 1. This restriction map made it clear that the large insertion was in neither the *Ac* nor the *Spm* family of transposable elements; however, it was more than twice the size of the largest known *Mu* element.

We isolated genomic clones of the *bz2::mu9* allele to study it in more detail. Knowing the restriction map of the insertion and of *Bz2* (5) enabled us to use size-selected *Eco*RI fragments for the construction of a genomic library in λZapII (Stratagene). After screening with 5′ and 3′ probes from *Bz2* (shown in Fig. 1), we isolated two non-overlapping clones that together contained 4.7 kb of the insertion and about 5 kb of *Bz2* flanking sequence. The restriction map of the clones almost matched the one derived from Southern analysis of the genomic *bz2::mu9* DNA; however, certain genomic bands were slightly larger than the equivalent plasmid bands, and a *Hin*dIII site was missing. To check that no fragments were missing at the *Eco*RI cloning site, we amplified genomic *bz2::mu9* DNA in the PCR, using one primer from each clone. The sequence of the amplified DNA verified that, between them, the 5′ and 3′ clones contained the entire junction sequence (data not shown). The two clones were then ligated together, along with ≈250 bp of *bz2*, to create pΔMu9.

We subcloned and sequenced the *Mu9* insertion and the surrounding *bz2* DNA. Based on that sequence, eight pairs of primers that would span the element in blocks of 0.5–1.3 kb were selected. PCR amplification of pΔMu9 and genomic *bz2::mu9* DNA gave identical results for all but one primer pair, which gave a 1067-bp amplification product from the plasmid and a 1.3-kb product from *bz2::mu9*. A *Bgl* II to *Xba* I fragment (see Fig. 1) of the amplified genomic DNA was cloned and sequenced. There were no differences between the plasmid sequence and the amplified *bz2::mu9* DNA in the 143 bp of overlap, but pΔMu9 had a 248-bp deletion relative to the genomic sequence, with 9 bp added at the deletion point. We verified that the *Mu9* element at the *bz2* locus contained the sequence found by PCR in two ways (data not shown). First, Southern analysis using the *Bz2* probe P300 showed that *bz2::mu9* contains a second *Hin*dIII site that is present in the amplified sequence. Second, pΔMu9 and genomic DNA from *bz2::mu9* were digested with *Pst* I and *Pst* I–*Eco*RV (see Fig. 1). The band identified as *bz2::mu9* by the P300 probe also hybridized to a probe specific for the amplified genomic sequence. DNA from pΔMu9 gave bands ≈250 bp smaller than the genomic *bz2::mu9* when probed with P300. The complete sequence of the reconstructed *Mu9* is shown in Fig. 2, presented in the same orientation as the *Bz2* transcription unit.

Transposable elements in the Mutator family are defined by conserved TIRs of ≈215 bp and by flanking 9-bp direct repeats of the host sequence (8). The sequence of *Mu9* shows that its insertion site is in the second exon of *Bronze-2*, 97 bp downstream of the 3′ splice site of the single intron (6). It is flanked by 9-bp direct duplications of the sequence TCCTGGAGG. The sequence has 215-bp TIRs (Fig. 2, italicized) that contain only two differences in the first 180 bp; overall they

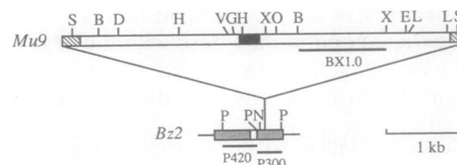

FIG. 1. Restriction map of *Mu9* showing its insertion site in *Bz2*. The hatched regions in the *Mu9* diagram denote the 215-bp TIRs, and the two *Bz2* exons are shaded. The *Bz2* probes used to clone *bz2::mu9* and the *Mu9* probe used for Figs. 4 and 5 are displayed as dark lines beneath the corresponding restriction fragments. S, *Sst* I; B, *Bam*HI; D, *Dra* I; H, *Hin*dIII; V, *Eco*RV; G, *Bgl* II; X, *Xba* I; O, *Xho* I; E, *Eco*RI; L, *Sal* I; P, *Pst* I; N, *Nar* I.

GAGATAATTGCCATTATAGACGAAGAGCGGAAGGGATTCGACGAAATGGAGGCCATGGCGTTGGCTTCTATGATCTGGAGACGCAGAGGACAGCCAATCGCCAAAACAGA 110

*AAGGTGACAGCGCTTGGAGCTCCTTAAACAGGTATTACTCTCCTGTCGGCGTTTACCGTTCGCCCGCGCACACGCCGTCTGGCATACTCCTCTTGTCACCGTCTC*TCCTC 220

TAAATGCTCTCTGGTTCGGCCTGCTCGCGGCAGTTGGCGTACTCCTCTCCTCGCCGAATTGGAGTGTTCTCGGGAGCTGGCGTCTTCCTACTGCGGCTGCTTCCGGTTTC 330

CTGTTCGTGAGTTCTCCTCCTATCCTTTGTCTCCCATGGCTATCGTATGTGAACCATGGCTATCGTGTTCCCCTCACCGAACCCGGTTGTGAAATTAGGTTTTCTCTGAT 440

TTTGGATCCATGGACTTGACGCCCAGTTTCAATTCGCTAGACTCCAACGGCATTCCCAACTCCCCCGATGTAGATCCGGCATTGGGCGAAACAGGTGGCAGTGAGGTGAG 550

TTTAATTTAGGTACATTGTCTTCAATTTCTAAAATAGTGTTGGAAGACTGCTCAGTTTTAGTTGTAAGACTGCCCATGGTCTTCAATTTTTAAAACAGTCTCGCTTATGC 660

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GGTTGTTTGTTTCCTAACATGAAAGAATTTAGGATTGCTATGCGACAGTATGCAATAAAACATGAGTTCGAGCTTGGAATTGAAGTTACTTCGACAACAAGATACGTTGG 1100

ATACTGTAAGGGTGGTGATTGCCCGTGGAGGATCTATGCACGTGAAGAGAAGAAAGGATTGCCTACTATTGTGGTAGCTGTACTAGATGATGTTCACACTTGCACATCTA 1210

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GTCGTGAATGCAGAACTGAAACAGTGTTCTTGTGAGGAATGGCAACACACTGGGAAACCGTGTCAACATGGTCTAGCCCTAATAATAGCCCAAGATTCCAGAGATGTAGG 2420

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CCAGTGGAGTGTTTGCACCAATAGCTAGAAGAGGTCTTGGAAGACAACGAAAAAATAGAATTAAAAGCTGTCTCGAGGGTGGAGTGCTAGAAATAAAAGTACCAACGAA 2640

AATGAGAAAACGAAAAAGCGACTCAAAAGGCAATACACTTGTCCTAATTGTGGTGAATTGGGACACCGCCAATCTAGCTACAAGTGCCCTTTGAATGGGACAAAAAAAAG 2750

GCTAGTTCTCAACTTACTTCTATATGTTCAATTTATATTAGTACTCGTGAACTAAATGTTTGAGTATTTTTTTGAGTAGGAAAAGGAAACCACGGATAAACACCACAAAA 2860

AATTGGATCCCTAAAGAGCTTCGGACTTCTTCACAGAATGTACCAGTACAGCCAGACGTAGCAGAGGAAGTCACTGAACAAGAGCTAGAAGATCCACAGCCAGAGACAGA 2970

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GGCTAGTGAAGAAAATCACCCCCAAGAAAAGACTGAGGATTAGTGCTCAGCAGAAGCAGTATTAACTGCTAAGAACAACCACTGTGCTCAGAACAACCACTGTATTTGTT 3190

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GGCCTTAACACGACAACAAACACTACGGTTCTTACATCAGATAATAGGTCATACAACAAATATCAGTTGCGTCCTTCGGAAATATATCCCAGACAGACCAAAATGACACC 3740

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CTGGTCA*CAAGAGGAGTACAAGTGACGGCGTGTGCGCGGGCGAACGGTAAACGGCGACAGGAGAGTAATACCTGTTTAAGGAGCTCCAAGCGCTGTCACCTTTCTGTTTT* 4840

GGCGATTGGGTGTCCTCTGCGTCTCCAGATCATAGAAGCCAACGCCATGGCCTCCATTTCGTCGAATCCCTTCCGCTCTTCGTCTATAATGGCAATTATCTC 4942

FIG. 2. DNA sequence of the complete *Mu9* element. The sequence is given in the orientation of the *Bz2* transcription unit. The TIR sequences are italicized, the first and last bases of the sequence solely derived from the PCR-amplified product are in bold type, and the restriction sites used to generate probes and sequencing subclones are underlined. (*Bam*HI, GGATCC; *Hin*dIII, AAGCTT; *Xba* I, TCTAGA; *Eco*RI, GAATTC; *Sal* I, GTCGAC.)

have a 96% sequence similarity. These TIRs are ≈80–90% similar to other Mutator TIRs (Table 1), indicating that *Mu9* is another member of the *Mu* element family.

Searching the computer data base with the *Mu9* sequence revealed the expected homology to the *Mu* element TIRs but no significant similarities to any non-Mutator sequence. Only one of the *Mu* elements, *Mu5*, has any homology to the internal sequence of *Mu9*. *Mu5* is a 1.3-kb element isolated from a non-Mutator line of maize (18). It has extended 358-bp TIRs, the first 220 bp of which are homologous to other *Mu* TIRs. The 3′ end of *Mu9* is homologous to these extended TIRs (84% sequence similarity), but, on the 5′ end, there is little similarity to the *Mu5* extended TIR; thus, *Mu9* does not have *Mu5*-like TIRs. Overall, however, the 5′ end of *Mu9* (about 950 bp) is homologous to the *Mu5* element, with an average sequence similarity, allowing for gaps, of ≈70%. From these results, we conclude that *Mu5* is a deletion derivative of a *Mu9*-like element.

Table 1. Sequence similarities (in %) between *Mu9* and other *Mu* TIRs

	1L	1R	2L	2R	3L	3R	4L	4R	5L	5R	6L	7L	7R	8L
9L	83	78	82	80	79	82	81	82	83	82	84	86	83	89
9R	83	77	82	80	78	79	79	80	81	81	84	83	81	87

Numbers along the top indicate the *Mu* element TIR being compared to *Mu9*. L, left end (5′ end for *Mu9*); R, right end (3′ end for *Mu9*).

The 4.9-kb *Mu9* element has a total of 10 ORFs of >300 bp (Fig. 3). The largest of these could encode a 704-amino acid polypeptide if a methionine were provided by an upstream exon or a 611-amino acid peptide if not. The second largest

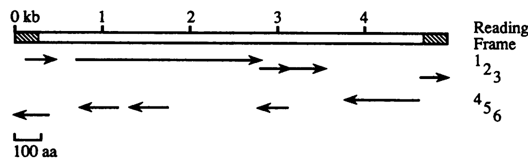

FIG. 3. *Mu9* open reading frames. Each ORF of 300 bp or longer is indicated by an arrow proportional to its length under the region of *Mu9* that encodes it. The TIRs are shown as hatched boxes.

ORF is at the opposite end of the element and on the other strand; it could encode a 280- or 231-amino acid polypeptide, respectively. Neither polypeptide shows significant similarity to any sequence in the protein data base. A thorough analysis of the peptides encoded by *Mu9* will depend on the isolation of cDNAs.

We examined the expression of *Mu9* in seedling or husk tissue from various Mutator, inactive Mutator, and non-Mutator stocks on RNA blots (Fig. 4*A*). Transcripts that hybridize to *Mu9* probes are found exclusively in the RNA isolated from active Mutator plants (lanes 1–4), and they are very abundant. In contrast, the inactive Mutator plants (lanes 5–7) have no detectable message. We did detect a low level of hybridizing transcripts in one plant grown from a somatically inactive (unspotted) kernel that had active (spotted) siblings (data not shown); this observation indicates that in this plant, either the phenotype of the triploid endosperm did not match that of the diploid embryo or the inactivation of the Mutator system is a gradual process, as is also suggested by phenotypic observations (19, 20). Non-Mutator stocks (lanes 8–11) have no detectable *Mu9*-hybridizing transcripts. Fig. 4*B* is the same blot stripped and hybridized to a maize actin probe (21) as a control for the amount of mRNA loaded.

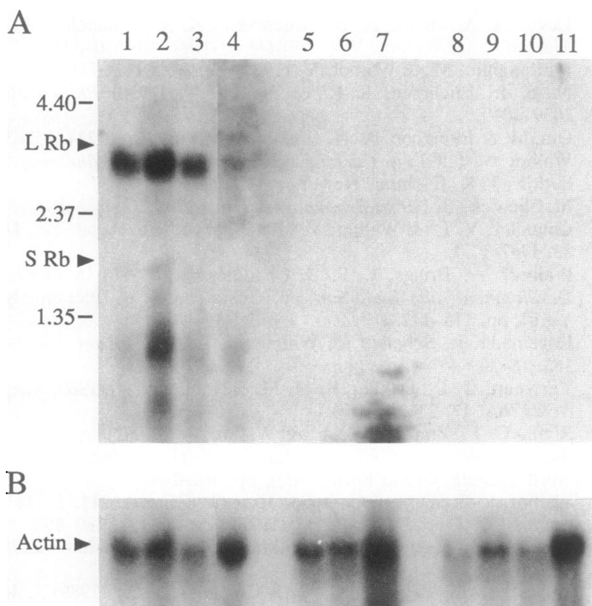

FIG. 4. Northern analysis of *Mu9* expression. (*A*) Total RNA hybridized to the *Mu9* BX1.0 probe (see Fig. 1). (*B*) The same blot stripped and reprobed with a maize actin clone. The positions of the rRNAs (L Rb and S Rb) and the RNA molecular weight markers are shown. The tissue source is 10-day-old seedlings unless otherwise noted. Lanes: 1, active *bz2::mu9*; 2, Robertson's purple Mutator; 3, active *bz1-mu5*; 4, active *bz2::mu1* (scutellum); 5, inactive *bz2::mu9*; 6, inactive *bz2::mu1*; 7, inactive *bz2::mu1* (aleurone); 8, W23 *bz2* tester (husk); 9, A188; 10, B73; 11, Δ*an bz2* (husk). The BX1.0 and the actin probes were of similar specific activity, and the exposure times for the two blots were approximately equal.

The *Mu9* probe BX1.0, used on the blot in Fig. 4*A*, hybridizes to transcripts of 2.9 kb, 1.1 kb, and 0.7 kb in active Mutator lines. This probe, from *Bam*HI 2865 to *Xba* I 3945 (Fig. 2), contains the DNA from just 3′ of the largest ORF to 150 bp into the second largest ORF. Interestingly, a probe from the largest ORF (*Hin*dIII 1410 to *Xba* I 2476) detects only the 2.9-kb transcript, whereas a probe from the other large ORF (*Sal* I 4214 to *Sal* I 4687) detects only the 1.1-kb transcript (data not shown). Three mechanisms could account for the multiple transcripts. (*i*) Alternative splicing could convert a single primary transcript into mRNAs lacking portions of the 3′ end (2.9-kb transcript), 5′ end (1.1 kb), or both (0.7 kb). (*ii*) Multiple transcription initiation sites could result from promoters in both of the highly conserved TIRs, thus producing at least two of the three messages. (*iii*) The *Mu9* probes could hybridize to transcripts from deleted or partially homologous *Mu* elements, as *Mu9* is a member of a small gene family (Fig. 5 and C.A.W., unpublished data). The P420 probe from *Bz2* (Fig. 1) does not hybridize strongly to the *Mu9* transcripts in *bz2::mu9*, indicating that transcript initiation from the external *Bz2* promoter does not account for the high message level in this line. Cloning and sequencing cDNAs will enable us to determine exactly how the three *Mu9* transcripts have been produced and to verify that they come from the *Mu9* element itself, rather than a related element.

Autonomous (regulatory) elements of the Mutator family of transposable elements have proven difficult to isolate genetically; however, in 1989, Robertson and Stinard (19) reported the isolation of two lines carrying *Mu*-induced *a1* mutables that also have independently segregating regulators of somatic instability. The presence of an active regulatory element can be followed in the kernels: the *A1* gene is in the anthocyanin pathway; thus, if the *Mu* regulatory element is present, somatic excision creates purple (*A1*) spots on the kernels, and kernels without the regulator are colorless. Material from the *a1-Mum2* line that contains zero, one, or two genetically defined regulatory elements was kindly provided by D. S. Robertson. Fig. 5*A* shows Southern blots of DNA from individual seedlings with two regulatory elements (lanes 1–4) and their siblings with no regulatory elements (lanes 5–8). The DNAs were digested with *Sst* I, which cuts

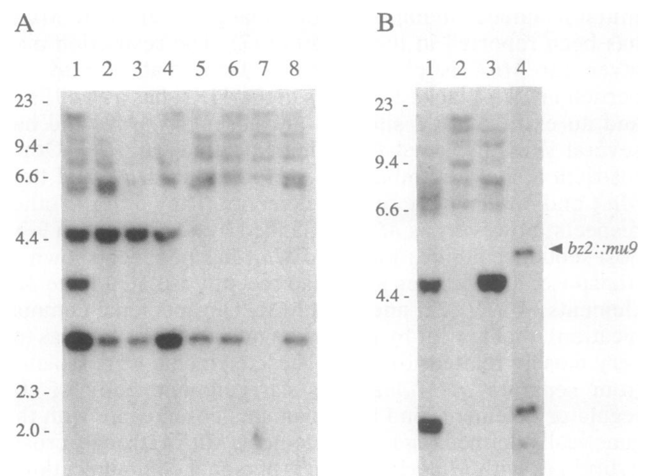

FIG. 5. Southern analysis of *a1-Mum2* individuals segregating for the *Mu* regulatory element. (*A*) DNA of individuals grown from spotted kernels that were genetically determined to have two copies of the *Mu* regulatory element (lanes 1–4) and from sibling colorless kernels with no regulatory elements (lanes 5–8) was digested with *Sst* I and probed with BX1.0 (Fig. 1). (*B*) DNA from *a1-Mum2* (lane 1), W23 *bz2* tester (lane 2), and *bz2::mu9* (lane 3) was digested with *Sst* I and probed with BX1.0. Lane 4 contains *bz2::mu9* DNA digested with *Pst* I and probed with P300. Sizes are indicated in kb.

in the *Mu9* TIRs. A 4.7-kb hybridizing fragment is present in the spotted (Mutator) individuals and absent from their colorless siblings; it is also present in individuals with one regulatory element (data not shown). Although other sizes of hybridizing fragments are present in all samples, those do not correlate with the presence or absence of the genetically defined regulatory element.

Fig. 5*B* shows that the 4.7-kb *Sst* I fragment that is segregating in the *al-Mum2* line (lane 1) is present in multiple copies in our *bz2*::*mu9* line (lane 3), whereas the *bz2* tester (lane 2), which is a non-Mutator stock, does not have that fragment. A similar analysis of other Mutator stocks that transmit Mutator activity to most or all of their progeny indicates that they contain roughly 5–30 copies of the 4.7-kb *Sst* I fragment, whereas 19 of 21 non-Mutator stocks we have tested do not contain this fragment and thus do not contain *Mu9 per se* (C.A.W., unpublished data). Fig. 5*B*, lane 4, shows that the copy of *Mu9* at the *bz2* locus is also the same size as the *al-Mum2* segregating fragment. DNA from *bz2*::*mu9* digested with *Pst* I and probed with P300 (Fig. 1) gives an ≈5.3-kb band, which is the expected size for the 4.7-kb fragment with the addition of 553 bp of *bz2* and *Mu9* TIR DNA. PCR amplification using eight primer pairs that span *Mu9* also indicates that *al-Mum2* (two regulators), *bz2*::*mu9*, and Robertson's purple Mutator all contain *Mu9* elements similar or identical to each other and to the cloned *Mu9* (data not shown).

Mutator and non-Mutator stocks have multiple fragments that are not *Mu9* but that hybridize to it (Fig. 5). These elements are of different sizes and appear to be part of a small *Mu9* gene family. Like other *Mu* elements (18), different regions of *Mu9* hybridize to different numbers of fragments, indicating that the set of hybridizing fragments may include deletion derivatives of *Mu9* as well as more distantly related elements (C.A.W., unpublished data).

DISCUSSION

Mu9 is a member of the Mutator transposable element family; it contains the conserved TIRs and flanking 9-bp host sequence duplications. *Mu9* is a mobile element, based on its transposition into *Bz2*, and somatic excision of *Mu9* gives the *bz2*::*mu9* allele the phenotype characteristic of an unstable Mutator-induced mutant. One other large *Mu* element, *MuA*, has been reported in the literature (7). The restriction map given for *MuA* is clearly distinct from that of *Mu9*. In particular, *MuA* lacks *Sst* I sites in the TIRs, has a *Pvu* II site and an extra *Eco*RV site that are absent in *Mu9*, and has several sites displaced from their locations in *Mu9*. Other restriction sites resemble the ones found in *Mu9*, and thus *MuA* and *Mu9* may be related elements. They differ in other respects, however, as *MuA* is flanked by 8- rather than 9-bp host sequence duplications, and *MuA* has not been shown to transpose. Two groups have also recently isolated large *Mu* elements, *MuR1* (22) and *MuA2* (M. Qin, personal communication), that appear by restriction map to be the same as (or very closely related to) *Mu9*. These elements were isolated from separate *al-Mum2* lines carrying one copy of the regulatory element, and both elements cosegregate with the genetically defined regulatory element. *MuR1* strongly cross-hybridizes with *Mu9* (P. Chomet, personal communication), but since neither *MuR1* nor *MuA2* has been sequenced yet, we do not know if the three elements are identical.

The correlations between the presence and expression of *Mu9* with Mutator activity suggest that *Mu9* is either the autonomous Mutator element or a close relative. In the *al-Mum2* lines, the presence of the *Mu9* fragment on Southern blots parallels the genetically determined segregation of the *Mu* regulatory element (Fig. 5*A*; additional examples, data not shown). All other active Mutator lines have multiple copies of *Mu9*, as has been predicted for the *Mu* regulator (8, 19), and most non-Mutator lines lack the *Mu9* element. Inactive Mutator lines, which are derived from active lines, contain *Mu9* elements as expected; however, like other *Mu* element TIRs, certain sites in the *Mu9* TIRs that are unmethylated in active lines are methylated in inactive lines (C.A.W., unpublished data). Active and inactive Mutator lines can be distinguished by the presence and absence, respectively, of *Mu9*-hybridizing transcripts.

The function of the proteins encoded by the multiple *Mu9* transcripts is not yet known. One current model for the excision of DNA transposons invokes two activities: a subterminal DNA-binding protein and a separate transposase protein. The maize *Spm* element apparently encodes both products (23, 24), and although the initial characterization of *Spm* identified four transcripts produced by alternative splicing (25), transferring the cDNAs of these transcripts into tobacco revealed that only the shortest (*tnpA*) and the longest (*tnpD*) are required to excise a *dSpm* element from a reporter gene construct (23, 24). The full *Mu9* element can also be transferred to see if it can transpose in a heterologous host. These experiments should define the functions of the *Mu9* proteins and prove whether or not *Mu9* is an autonomous Mutator element.

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