Characterization of the Major Transcripts Encoded by the Regulatory *MuDR* Transposable Element of Maize

R. Jane Hershberger,* María-Inés Benito,* Kristine J. Hardeman,[†,1] Christine Warren,*,[2] Vicki L. Chandler[†] and Virginia Walbot*

**Department of Biological Sciences, Stanford University, Stanford, California 94305-5020 and*
[†]Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403-1229

Manuscript received December 6, 1994
Accepted for publication March 27, 1995

ABSTRACT

The *MuDR* element controls the transposition of the Mutator transposable element family in maize. Previous studies reported the presence of two major *MuDR*-homologous transcripts that correlate with Mutator activity. In this study, we describe the structure and processing of these two major transcripts. The transcripts are convergent, initiating from opposite ends of the element within the 220-bp terminal inverted repeats. The convergent transcripts do not overlap, and only 200 bp of internal *MuDR* sequences are not transcribed. Cloning and sequencing of multiple *MuDR* cDNAs revealed unusual intron/exon junctions, differential splicing, and multiple polyadenylation sites. RNase protection experiments indicated that some splicing failure occurs in young seedlings, and that a low level of antisense RNA exists for both transcripts. On a whole plant level, the presence of the major *MuDR* transcripts strictly correlates with Mutator activity in that no *MuDR* transcripts are observed in non-Mutator or inactive Mutator stocks. Examination of various tissues from active Mutator stocks indicates that the two transcripts are present in all organs and tissues tested, including those with no apparent transposition activity. This suggests that Mutator activity is not simply controlled by the level of the major *MuDR* transcripts.

THE *Mutator* (*Mu*) transposable elements of *Zea mays* are extremely active, causing new mutations at up to a hundred times the spontaneous rate (reviewed in WALBOT 1992). Several distinct classes of *Mu* elements have been characterized. All *Mu* elements have closely related 220-bp terminal inverted repeats, but each class has unique, unrelated internal sequences (reviewed in CHANDLER and HARDEMAN 1992). Mutator activity, as judged by the excision of *Mu* elements from reporter genes, is usually inherited as a multigenic trait; however, a few lines have been isolated in which Mutator activity segregated as a single gene, suggesting the existence of a class of *Mu* element that controls the transposition of all other *Mu* elements (ROBERTSON and STINARD 1989; CHOMET *et al.* 1991). This regulatory *Mu* element was independently cloned in several laboratories (CHOMET *et al.* 1991; HERSHBERGER *et al.* 1991; QIN *et al.* 1991) and is now designated *MuDR*. *MuDR* is 4.9 kb in length, and two major *MuDR*-homologous transcripts are present in active Mutator stocks (QIN and ELLINGBOE 1990; CHOMET *et al.* 1991; HERSHBERGER *et al.* 1991).

All active Mutator stocks contain *MuDR* elements, as shown by the presence of a diagnostic 4.7-kb *Sst*I fragment on Southern blots. Typical Mutator stocks contain between five and 30 of these elements (HERSHBERGER *et al.* 1991), although a few stocks have been generated that contain a single *MuDR* element (CHOMET *et al.* 1991; QIN *et al.* 1991). In the single copy stocks, loss of the *MuDR* element causes the loss of Mutator activity (CHOMET *et al.* 1991). Standard non-Mutator maize stocks lack the diagnostic *MuDR* fragment (C. A. WARREN, unpublished data). Inactive multi-copy Mutator stocks, which have spontaneously lost transposition activity, retain *MuDR* elements in their genomes. In some inactive stocks, the 4.7-kb fragment disappears because the *Sst*I sites are methylated (GREENE *et al.* 1994; MARTIENSSEN and BARON 1994). Despite the presence of *MuDR* elements, inactive lines do not express *MuDR* transcripts (HERSHBERGER *et al.* 1991). These correlations suggest that the *MuDR* transcripts may encode proteins necessary for *Mu* element transposition.

In this study, we have determined the structure of both major transcripts that correlate with Mutator activity. Cloning the *MuDR* cDNAs showed that the element contains two convergent transcription units that do not overlap. The 5′ ends, introns, and polyadenylation sites were determined for each transcript, and the expression of the transcripts was examined in different tissues and developmental stages of Mutator plants. We have used stocks carrying multiple copies of *MuDR* and other *Mu* elements for these studies, as most of the previous

Corresponding author: R. Jane Hershberger, Department of Biological Sciences, Stanford, CA 94305-5020.
E-mail: janieh@leland.stanford.edu

[1] *Present address:* Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902.

[2] *Present address:* Howard Hughes Medical Institute, Stanford, CA 94305-5428.

work characterizing the regulation of Mutator has been done with multi-copy lines (ROBERTSON 1981; LEVY and WALBOT 1991; BENNETZEN *et al.* 1993; MARTIENSSEN and BARON 1994). These stocks have *MuDR* elements at many genomic locations and have an undefined population of *MuDR* deletion derivatives. Despite these variables, the timing of Mutator activity appears to be very similar in independent multi-copy stocks and in the single copy stocks.

MATERIALS AND METHODS

cDNA cloning: One cDNA library was prepared in λZapII (Stratagene) using poly(A)$^+$ RNA from *bz2::mu9,* a stock with an intact *MuDR* insertion in the *bz2* gene and ~30 copies of *MuDR*-related elements (HERSHBERGER *et al.* 1991). A second library was prepared in λgt10 from poly(A)$^+$ RNA of *a1-mum2,* a stock with a single copy of *MuDR* (CHOMET *et al.* 1991). The first library was probed with three *MuDR* fragments [numbering according to HERSHBERGER *et al.* (1991)]: *Ssi*I (127) to *Dra*I (639), *Hin*dIII (1410) to *Xba*I (2476), and *Bam*HI (2865) to *Xba* I(3945). The second library was also probed with three *MuDR* fragments: *Hin*dIII (2173) to *Bam*HI (2865), *Bam*HI (2865) to *Eco*RI (4185), and a *Hae*III (3025–3380) fragment. Inserts from the hybridizing phage were either excised as pBluescriptII plasmids or subcloned into pTZ (U.S. Biochemical). These were sequenced using the Sequenase 2.0 sequencing kit (U.S. Biochemical). To reconstruct a full-length cDNA for *mudrA*, two overlapping cDNA clones were ligated together into the low copy plasmid pCL1921 (LERNER and INOUYE 1990) to overcome the difficulty of maintaining this sequence on a high copy plasmid.

Plant material: The *bz2-mu2* stock (WALBOT *et al.* 1986) carries a *Mu1* insertion in the first exon of the *bz2* gene (NASH *et al.* 1990) and has 10–25 copies of *MuDR*-related elements. It has been maintained by selfing and by outcrossing to a *bz2* tester line in the W23 background. The Mutator stocks used in the RNA blot analysis include individuals from the sibling *bz2-mu2* families L11, L12, L14, and their descendants MB2 (derived from L12–1⊗), MB10a, and MB15 (derived from L14–3⊗). The progeny of these individuals were scored for somatic revertant sectors to verify Mutator activity. The W23 *bz2* tester line was used as the non-Mutator control stock. Families of this line used for RNA blot analysis included L1 and MB12.

For the RNase protection assays, RNA was prepared from JH92.1 × 5–6, a *bz2::mu9* stock that had been maintained by outcrossing to the W23 *bz2* tester line. The inactive Mutator stock, JH90.7–6⊗, was a sibling of the grandparent of this material. The W23 *bz2* tester parent of the active stock, JH92.1⊗, was the source of the non-Mutator control RNA. Immature ear RNA from *a1-mum2* (CHOMET *et al.* 1991), which contained one active *MuDR* element, was kindly provided by PAUL CHOMET.

RNA blot analysis: Tissue was collected from field grown *bz2-mu2* sibling plants. We collected seedling leaves and roots from 3–7 leaf seedlings, adult leaves from 12–15 leaf adult plants, premeiotic tassels, postmeiotic tassels (immature tassels), mature tassels (at time of pollen shed), pollen, and immature ears from families L11, L12, L14, MB10a, and MB15. L12 *bz2-mu2* siblings were selfed, and whole kernels were isolated at 5, 11, 15, and 21 days after pollination (dap). MB2 *bz2-mu2* siblings were selfed, and embryo, endosperm, and aleurone tissues were isolated separately (when present) at 10, 12, 15, 20, and 25 dap. Tissue was immediately frozen in liquid nitrogen and stored at −80° until RNA isolation. Total RNA was isolated by grinding the samples in liquid nitrogen before extraction with Tri-Reagent (Molecular Research Center, Inc.).

For the RNA blots, 20 μg of total RNA was size-fractionated and transferred to Hybond-N (Amersham) using standard techniques (SAMBROOK *et al.* 1989). A *Bam*HI (2865) to *Xba*I (3945) fragment of *MuDR* was labeled by the random-primer method (FEINBERG and VOGELSTEIN 1983) and purified on push columns (Stratagene). Prehybridization and hybridization were done according to the protocol published for Genescreen (DuPont) using 10% dextran sulfate. Filters were washed twice in 2× SSPE, 0.1% SDS at room temperature for 10 min, once in 1× SSPE, 0.1% SDS at 65° for 15 min, and once at 0.1× SSPE, 0.1% SDS at 65° for 10 min. Autoradiography was performed for 12–24 hr at −80° using two intensifying screens.

RT/PCR analysis: Total RNA (1.5 μg) from L11–1 mature tassel and L12–1 15-dap ear was reverse transcribed with Superscript II (BRL) using 50 ng *MuDR* primer 3254B (GGCTTGTTCTTAGCAGTCTT) in a 50-μl reaction volume. (Primer numbers correspond to the position in the *MuDR* sequence, and A or B designates the strand.) The deletions in the Δ*mudrA* transcripts were localized by taking 8 μl of the cDNA and PCR amplifying it with primer sets 260A (TACTCCTCTCCTCGCCGAAT) to 1967B (GTGTTGGTCTAAGTACTCAG), 1967A (inverse of 1967B) to 3254B (above), and 260A to 3254B. Amplification was performed using AmpliTaq (Perkin Elmer Cetus) and KlenTaq-LA (Ab Peptides, Inc., St. Louis) according to the manufacturers' instructions for 30 cycles of 94°, 10 sec; 50°, 10 sec; and 70°, 4 min. The second and third reactions showed deletion products as well as full-length products. To clone the PCR products, the RT/PCR with primers 1967A and 3254B was repeated as above, except that the cycle parameters were changed to 94°, 20 sec; 60°, 20 sec; 70°, 4 min. The products of the KlenTaq PCR reaction were extracted using the Wizard PCR (Promega) purification procedure, filled in with Klenow fragment (BRL), and digested with *Bgl*II, which cuts *MuDR* at position 2091. The fragments were run on a 1% SeaPlaque GTG (FMC) agarose gel, and bands corresponding to the full-length and deleted fragments were cut out of the gel and ligated into *Bam*HI-*Eco*RV-digested pBluescriptII KS$^+$ (Stratagene). Ten deleted clones and two full-length clones were grown in 1.5 ml liquid cultures, and a standard alkaline lysis procedure was used to prepare plasmid DNA.

For each sequencing reaction, one-third of the DNA sample was denatured in 0.2 M NaOH, ethanol precipitated, and sequenced using Sequenase 2.0 (U.S. Biochemical). Each clone was sequenced with the M13-40 primer (U.S. Biochemical), the KS primer (Stratagene), and where appropriate, *MuDR* primer 2404A. This provided at least one strand of all the sequence from the deleted clones and partial sequences from the full-length clones. With the exception of the deletions, no mismatches were detected between the *mudrA* sequence and the sequences of the Δ*mudrA* clones.

RNase protection assays: Probes for the intron RNase protections were generated from the following fragments of *MuDR* subcloned into pBluescript II KS$^+$ (Stratagene): IA1, *Pst*I (−103, in the *bz2* gene) to *Bam*HI (444); IA2, *Bam*HI (444) to *Dra*I (639); IA3, *Xba*I (2476) to *Bam*HI (2865); IB1, *Stu*I (4309) to *Sal*I (4687); IB2, *Sal*I (4214) to *Hpa*I (4569); IB3, *Xba*I (3945) to *Sal*I (4214) (all enzymes from BRL). The IA1, IB1, and IB3 plasmids were digested with *Xho*I and *in vitro*-transcribed probes were generated using T7 RNA polymerase (BRL) according to the manufacturer's instructions.

The IA2, IA3, and IB2 plasmids were digested with *Xba*I and transcribed with T3 RNA polymerase (BRL).

For the antisense RNase protection shown in Figure 6A, sense probes were generated from a *Hin*dIII (1410) to *Eco*RV (2023) *MuDR* subclone and the IB3 subclone by digestion with *Sst*I and *in vitro* transcription with T3 RNA polymerase. The antisense strand of the control ubiquitin probe was transcribed with T7 RNA polymerase from an *Xba*I digest of a 95-bp fragment of the maize *ubiquitin-1* gene (CHRISTENSEN *et al.* 1992) that had been PCR-amplified from W23 *bz2* tester DNA and subcloned into pBluescript II. We have also detected antisense *MuDR* transcripts in RNase protections using sense probes generated from these *MuDR* subclones: IA1 (described above), *Sph*I (1791) to *Hin*dIII (2173), *Bam*HI (2865) to *Bst*NI (3325), *Xba*I (3945) to *Eco*RI (4185), and *Eco*RI (4185) to *Sst*I (4811).

The RNase protections were performed using the Lysate Ribonuclease Protection Kit (U.S. Biochemical) according to manufacturer's directions with 10 μg of total RNA and 4–8 $\times$ 10^4 cpm of labeled probe. For the RNase digestions shown, we used a 1:250 dilution of a stock of 5 units/μl monophoretic RNaseA (U.S. Biochemical), 10 units/μl RnaseT$_1$ (BRL) for 30 min at 50° (introns, antisense *mudrA*, and ubiquitin) or 37° (antisense *mudrB*). Each probe/RNA combination was also tested with dilutions of the RNase stock ranging from 1:100 to 1:500 and with RNase digestion temperatures ranging from 20° to 50°. Both a DNA sequencing ladder and ^{32}P-labeled RNA markers generated from the RNA Century templates (Ambion, Inc.) were electrophoresed alongside the protected fragments on a 5% Long Ranger gel (AT Biochemical).

The signal intensities for the intron protection experiments were quantified using a Molecular Dynamics PhosphoImager and accompanying software. Two separate experiments using the same RNAs and probes were scanned, one of which included an actin control in all lanes. The actin control obscured bands smaller than 180 bp, which affected the IA1, IA2 and IB3 lanes. From the two experiments, data were gathered from RNase digestions at 22° (with actin), 37°, and 50°. The relative levels of protected counts in the spliced and unspliced bands varied by <3% and are given in the text rounded to the nearest 5%.

RESULTS

Structure of the *MuDR* transcription units: Two abundant polyadenylated *MuDR* transcripts of 2.8 (*mudrA*) and 1 kb (*mudrB*) have been observed on RNA blots from active Mutator stocks (CHOMET *et al.* 1991; HERSHBERGER *et al.* 1991; JAMES *et al.* 1993). To determine their structures, cDNAs were isolated from two libraries, one made from RNA of typical Mutator seedlings with multiple copies of *MuDR*, the other from immature ear RNA from a stock with a single *MuDR* element. Sequence analysis of nine full-length or partial cDNAs from each transcript (20 kb total) indicated that the coding sequences of transcriptionally active *MuDR* elements are strongly conserved. Fifteen clones matched the *MuDR* sequence exactly, and three clones had one silent nucleotide change each: a GGG to GGA change at base 1347, a TAT to TAC change at base 1911, and a T to C change at base 3598, in the 3′ untranslated region (UTR) of *mudrB* [numbering according to Hershberger *et al.* (1991)].

Analysis of the cDNA structures showed that the two transcription units are convergent, as diagrammed in Figure 1A. Each transcript initiates in one of the 220-bp terminal inverted repeats (TIRs), and they are encoded on opposite strands. In *MuDR*, the TIRs are 99% similar to one another over the first 180 bp; thus if the promoter elements are within the TIRs, they should have very similar properties. The convergent *MuDR* transcripts terminate 200 bp apart. Both transcripts are polyadenylated, and the cDNA sequences indicate that polyadenylation occurs at multiple sites. Two *mudrA* clones had poly(A) tails separated by 27 bp, and one nonpolyadenylated *mudrB* clone extended 9 bp beyond a polyadenylated clone.

All the polyadenylation sites occurred in a region of *MuDR* that contains five distinct sets of direct repeats 11–27 bp in length, each repeated three to five times (Figure 1B). Short direct repeats are fairly common in other maize transposable elements, especially those that do not encode transpsosases (BENNETZEN and SPRINGER 1994). The repeat motifs begin at the termination codon of *mudrA* and continue through the intergenic region into the 3′ UTR of *mudrB*. There are also three copies of one inverted repeat motif that is part of a larger direct repeat (triangles in Figure 1B). The inverted repeats lie 10 to 160 bp upstream of the polyadenylation sites in both transcripts, and an internal sequence of the repeats (UUGUA) exactly matches a conserved sequence from the far upstream polyadenylation elements of other plant genes (reviewed in HUNT 1994). Because polyadenylation and possibly transcript termination of both *mudrA* and *mudrB* occur within the repeat region, we assessed the possible folding of transcripts from this region using the M-fold program (JAEGER *et al.* 1989). All of the sequences examined had considerable folding potential; as a simple example, Figure 1C shows a secondary structure for an RNA from the intergenic region on the *mudrA* strand ($\Delta G = -21$ kcal/mol).

To determine the transcription initiation sites more precisely, we used primer extension and RNase protection analysis (data not shown). The *mudrA* transcript showed a complex pattern of initiation sites; however, only two sites emerged from both types of analysis, one at nucleotide (nt) 169, the other at nt 252. As shown in Figure 2A, the first start site maps within a sequence previously identified as a protein-binding site (Site I) in *Mu1* termini from both active and inactive Mutator stocks (ZHAO and SUNDARESAN 1991). The second start site lies 3′ of the single out-of-frame AUG initiation codon in the untranslated leader. The start sites are used with approximately equal frequency. For *mudrB*, a single start site at nt 4780 (Figure 2A), which lies in the Site I box for TIR$_B$, was present in both primer extension and RNase protection experiments. There are no out-of-frame AUG codons in the *mudrB* leader.

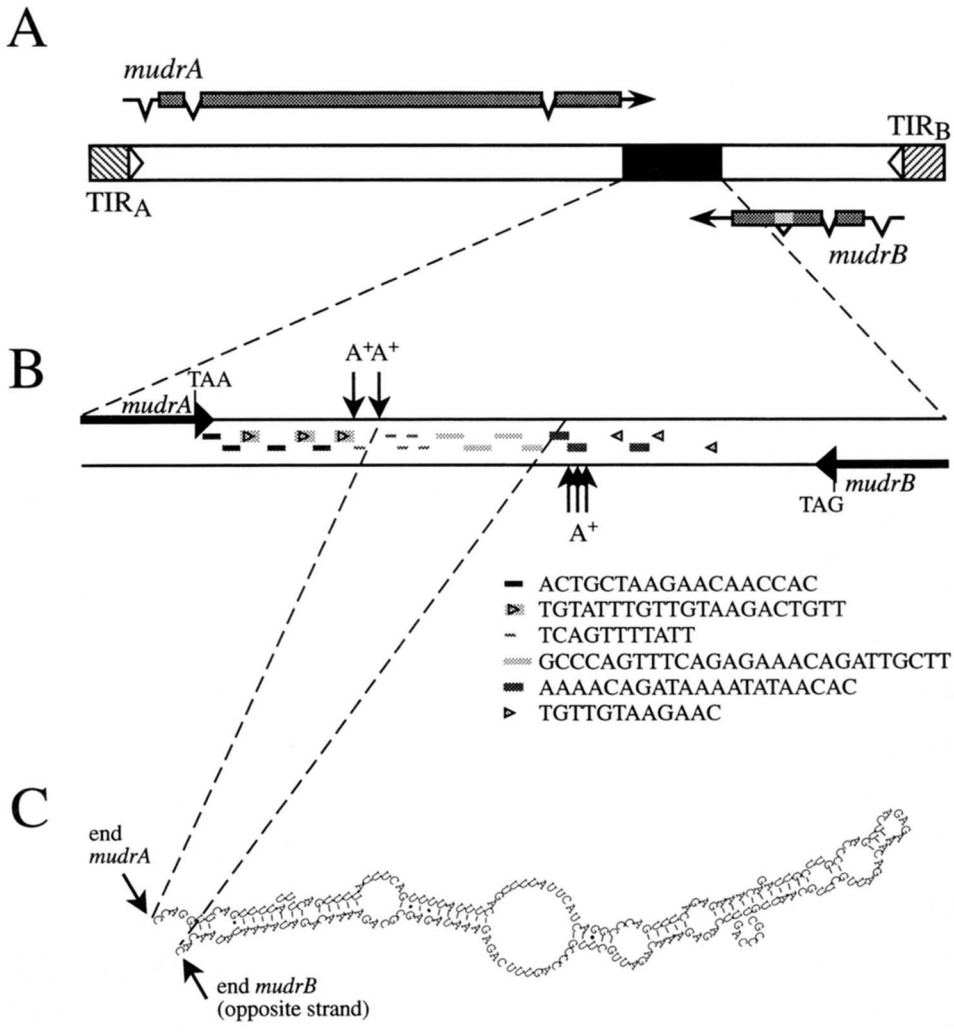

FIGURE 1.—Structure of *MuDR* and its transcripts. (A) Diagram of the *MuDR* transcripts and intergenic region. Transcription of *MuDR* initiates on opposite strands from the terminal inverted repeats (▨) and terminates in a region containing multiple direct repeats (■). □, coding regions along the transcripts. (B) Expanded view of the 3′ untranslated and intergenic regions of *MuDR* showing the 3′ ends of the cDNA clones (vertical arrows) and the repeat motifs. (C) One energetically favorable folding pattern of an RNA corresponding to the A strand of the intergenic region (nt 3325–3516).

Comparing the sequence of the *mudrA* cDNAs to the genomic sequence shows that three introns are removed, resulting in a 2.8-kb transcript. The cDNAs spanning the first *mudrA* intron used two different 5′ splice sites (at bp 333 or 337), defining introns of either 92 or 96 bp. RNase protection and primer extension analysis indicate that the two 5′ splice donors are used with approximately equal frequency (data not shown). As this intron is in the untranslated leader, the alternative splicing should not affect the sequence of the protein product. The second and third introns are 145 and 79 bp, respectively. This transcript has a single open reading frame that could encode an 823-amino acid polypeptide (MUR-A) with a predicted molecular weight of 94 kD. In seedlings, *mudrB* has two introns of 117 and 72 bp spliced out, and the 1-kb transcript could encode a 207-amino acid, 23 kD polypeptide (MUR-B). One of two *mudrB* cDNAs sequenced from an immature ear library also had a third intron of 120 bp removed (underlined in Figure 2C). The polypeptide this transcript encodes would have 40 fewer amino acids than MUR-B. The sequences of the *mudrA* (Genbank accession U14597) and *mudrB* (Genbank accession U14598) cDNAs are given in Figure 2, B and C along with their encoded proteins.

Amino acid sequence analysis of MUR-A shows that it shares a sequence motif with the putative transposases of a group of bacterial insertion sequences (EISEN *et al.* 1994). This sequence similarity suggests that the MUR-A protein may be the Mutator transposase. In rice, two partial cDNAs of unknown function have strong similarity to MUR-A (Genbank accession d15675 and d23146). The first rice cDNA has 34% identical and 60% similar residues when aligned with a 120-amino acid piece of MUR-A, and the second has 21% identical and 52% similar residues over a nonoverlapping 141-amino acid stretch [alignments given in EISEN *et al.* (1994)]. No significant similarities to MUR-B have been identified in the current databases.

RNA blot analysis of *MuDR* transcript abundance: In active Mutator plants, revertant sectors produced by excision of *Mu* elements from *Mu*-induced mutations are generated predominantly late in the development of each tissue (LEVY *et al.* 1989). To test whether transcriptional regulation of the *MuDR* genes might be a

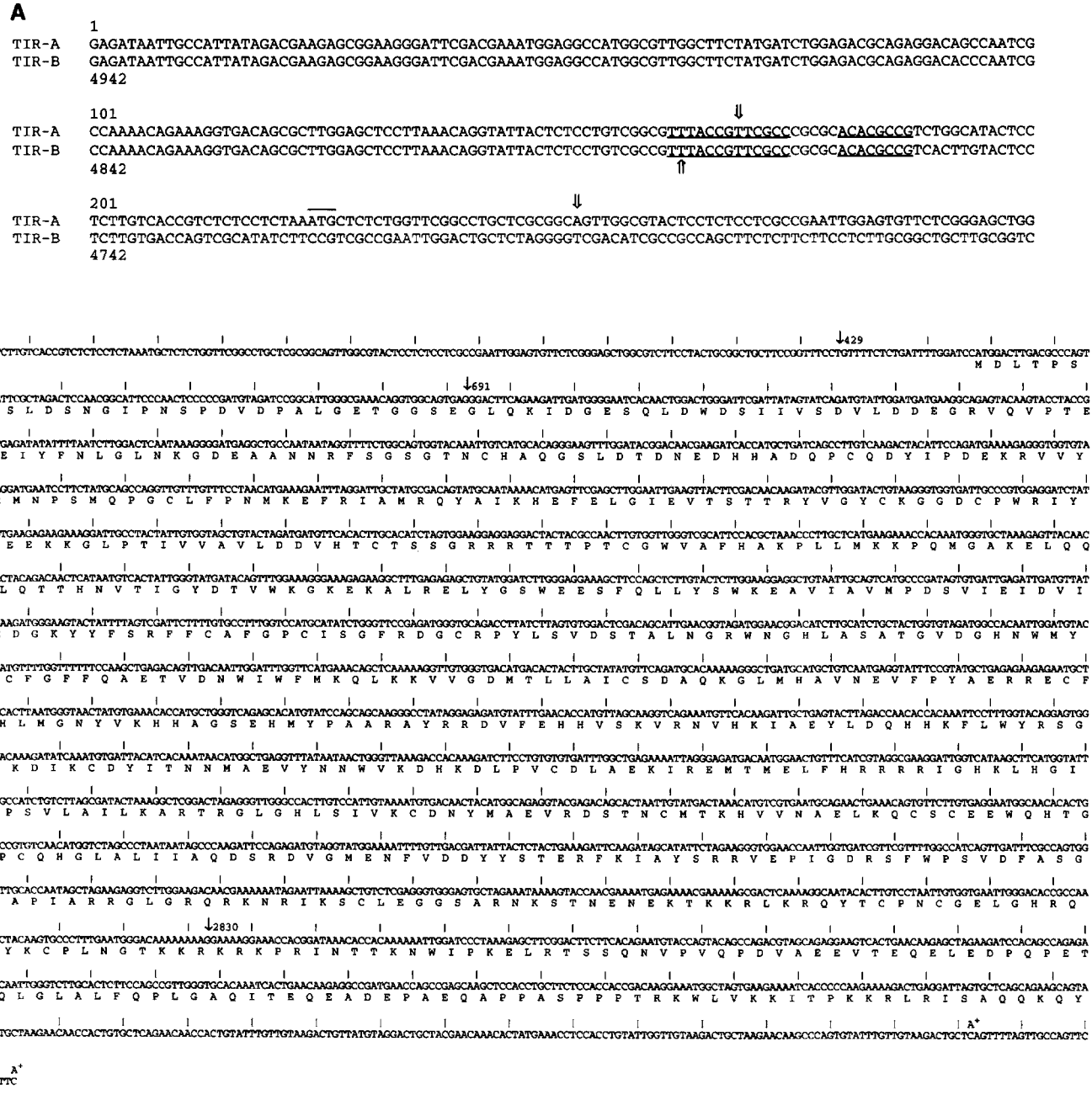

FIGURE 2.—Sequences of the major *MuDR* transcripts. The numbering indicates the position in the genomic *MuDR* sequence (HERSHBERGER *et al.* 1991). (A) Major transcription start sites, indicated by ⇓ for *mudrA* (top line) and ⇑ for *mudrB* (bottom line). The sequence of the *MuDR* terminal inverted repeats is given as the sense strand of each message. The protein-binding sites identified in ZHAO and SUNDARESAN (1991) for the *Mu1* terminal inverted repeats are underlined, and the out-of-frame ATG in *mudrA* is marked with an overline. (B) Sequence of the cloned *mudrA* cDNA and the inferred MUR-A polypeptide. (C). Sequence of the cloned *mudrB* cDNA, including the infrequently spliced third intron (underlined), and the inferred MUR-B polypeptide sequence. In B and C, the sites of intron removal are indicated by arrows, and the number after the arrow indicates the position of the first base of the downstream exon in the genomic *MuDR* sequence. *, denotes the stop codons terminating the polypeptides, and A^+ indicates a known polyadenylation site.

molecular mechanism underlying this behavior, we prepared a series of RNA blots to investigate the presence and relative abundance of *mudrA* and *mudrB* during development. In addition, we wanted to search for any additional *MuDR*-homologous transcripts that might be present and developmentally regulated in our stocks.

Total RNA from various maize organs and tissues isolated at different developmental stages was extracted from an active Mutator stock (*bz2-mu2*) with multiple copies of *MuDR*. The RNA blots in Figures 3 and 4 were probed with BX1.0 (Figure 3B), a double-stranded DNA probe that hybridizes to the 3′ ends of *mudrA* and *mudrB*

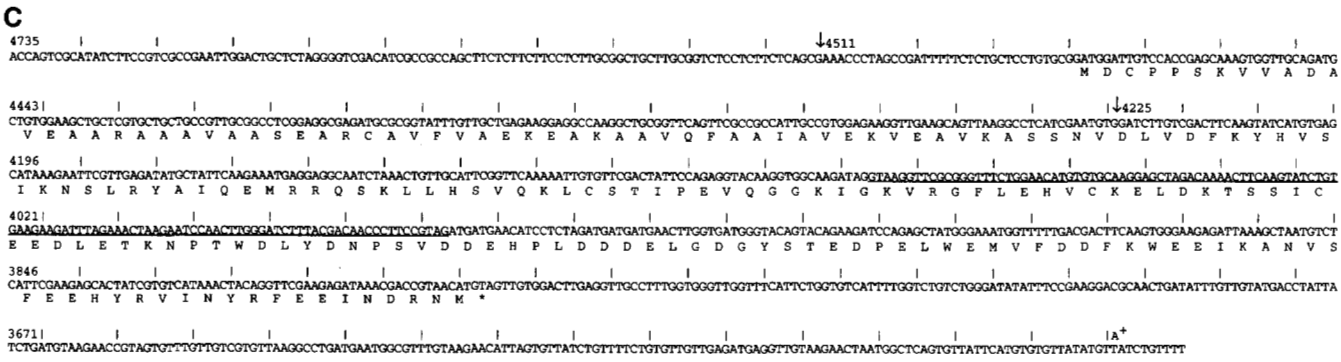

FIGURE 2.—*Continued*

and the intergenic region. Additional RNA probes, single-stranded DNA probes, and other double-stranded DNA probes that hybridize to different parts of *MuDR* (see Figure 3B for schematic summary of the probes used) were also tested on these RNA blots and gave similar results (data not shown). To assess the quantity and quality of the RNA, the blots were probed with maize ubiquitin (bottom, Figures 3 and 4).

The RNA blot shown in Figure 3A indicates that all the organs tested from active Mutator plants express both *mudrA* (2.8 kb) and *mudrB* (1 kb). In contrast, neither inactive plants (Figure 4) (M.-I. BENITO, unpublished data) nor non-Mutator plants (Figures 3 and 4) (M.-I. BENITO, unpublished data) have detectable levels of *MuDR* transcripts in any tissue. The *MuDR* transcripts appear to be moderately abundant in active Mutator plants, based on a comparison to the level of actin transcripts in the same samples (data not shown). On some samples, the *mudrB* signal was weak due to the diffuse nature of the band and slight RNA degradation. We have confirmed the presence of *mudrB* in these cases by using *mudrB*-specific probes on these samples and by checking the same tissues in other individuals (data not shown). Figure 3A also shows that although *mudrA* and *mudrB* are present in all the active Mutator samples, the ratio of the two transcripts can vary widely. For example, the sample derived from an unpollinated ear has a much higher ratio of *mudrB* to *mudrA* than the samples derived from tassel and leaf tissues.

Figure 4 shows an RNA blot prepared from developing embryo and endosperm samples. Previous work has indicated that Mutator excision activity in the endosperm, as measured by reversion of *Mu*-containing reporter genes, is essentially absent before ~15–20 dap and then rises to high levels (LEVY and WALBOT 1990). Little or no reversion takes place in the early embryo, and reversion in the scutellum has not been tested. Our youngest sample of embryo (including scutellum) and endosperm was harvested at 10 dap. Endosperm samples were collected at 12, 15, and 20 dap, and aleurone was collected at 20 and 25 dap. If transcription of *mudrA* and *mudrB* paralleled Mutator excision activity, we would not expect transcripts to be observed before 15 dap. This was not observed; all of these samples contain detectable levels of *mudrA* and *mudrB*. These results demonstrate that *mudrA* and *mudrB* are transcribed even in early developmental stages of the embryo and endosperm when no excision activity is apparent. The lack of correlation between excision activity and transcript level is supported by GREENE *et al.*, who found that meristem-enriched samples had about the same level of *MuDR* transcripts as leaf samples from the same individuals (GREENE *et al.* 1994).

The *bz2-mu2* stocks used for our RNA blots are typical Mutator stocks containing multiple copies of *MuDR*. In addition to the major *MuDR* transcripts, these stocks produce a 2.4-kb *mudrA*-homologous transcript designated Δ*mudrA* in Figures 3A and 4. To determine the structure of this transcript, we did RT/PCR analysis on two RNA samples with high levels of Δ*mudrA* (mature tassel and 15 dap ear; see MATERIALS AND METHODS for details). Cloning and sequencing the RT/PCR products and PCR analysis of DNA from these plants suggests that the Δ*mudrA* transcripts are heterogeneous. From six Δ*mudrA*-derived clones sequenced, there were four different deletions of 600–700 bp from near the 3′ end of *mudrA*. Three clones had identical deletions, from 2627 to the end of the PCR fragment at 3254, and the remaining three had deletions from 2211 to 2854, 2457 to 3069, and 2484 to 3117. None of the deletions occurred at sequences matching splice consensus sites; thus Δ*mudrA* transcripts are unlikely to be generated by alternative splicing. We conclude that deleted *MuDR* elements, which can frequently be detected in Mutator stocks (HARDEMAN and CHANDLER 1993), may be producing these transcripts.

***MuDR* splicing:** Table 1 shows that several of the *MuDR* introns lack one or more of the consensus properties for maize introns. The most obvious divergence from the intron consensus is in the third intron of *mudrA*, which has a GC dinucleotide at the 5′ splice junction instead of the invariant GU (WHITE *et al.* 1992; BROWN *et al.* 1993). The presence of a GC dinucleotide at the 5′ splice junction has been documented for ≥20 animal

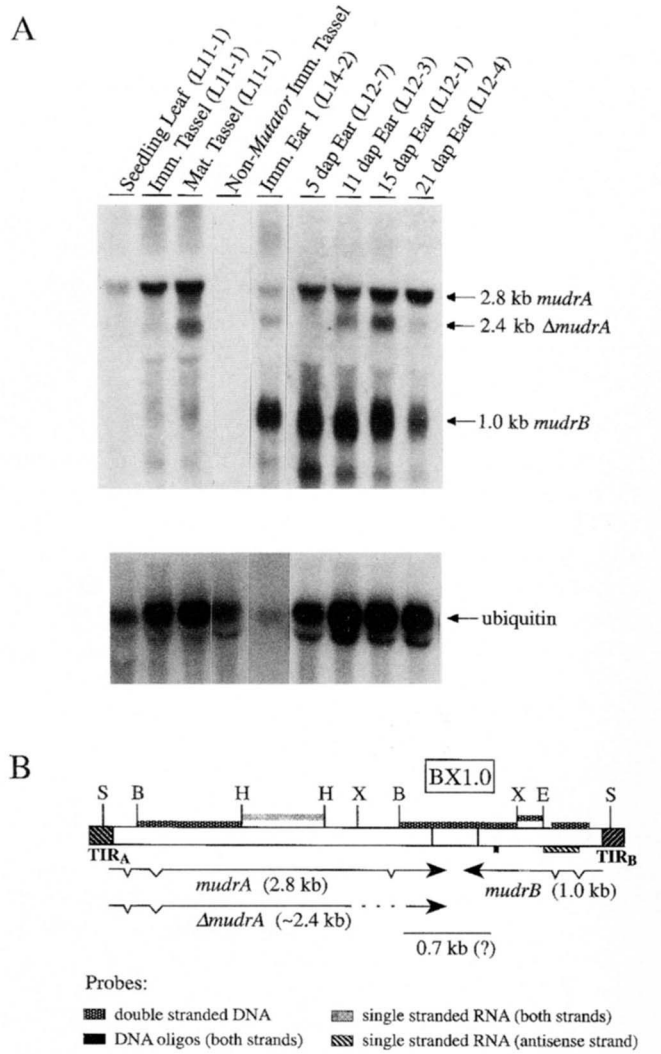

FIGURE 3.—*MuDR* transcript expression in maize organs and tisues. (A) RNA blot analysis of a panel of tissues from active Mutator plants and a non-Mutator control. Twenty micrograms of total RNA was probed with BX1.0, a probe that hybridizes to the 3′ ends of both *MuDR* transcripts (top). The same blot was probed with maize ubiquitin as a control for loading (bottom). L11–L14 are active *bz2-mu2* stocks from sibling ears; the number after the dash indicates the specific individual within the family. (B) Diagram of all the *MuDR* probes used on the RNA blots and the transcripts they detect. S, *Sst*I; B, *Bam*HI; H, *Hin*dIII; X, *Xba*I; E, *Eco*RI.

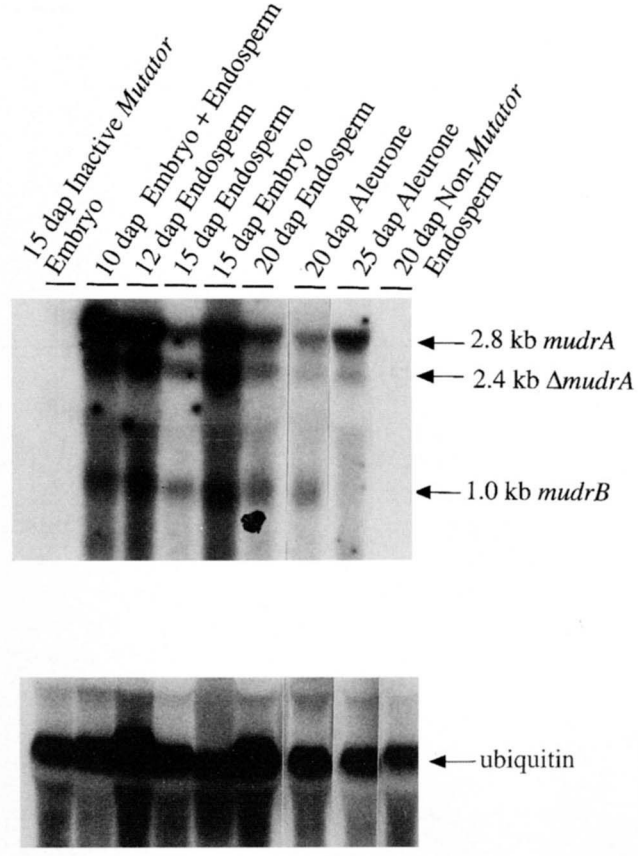

FIGURE 4.—RNA blot analysis of *MuDR* transcript expression during kernel development. Each sample contains 20 μg of total RNA probed with BX1.0 (top) and ubiquitin (bottom).

introns and six plant introns (reviewed in JACKSON 1991; LUEHRSEN *et al.* 1994). The second *mudrB* intron (Table 1), at 72 bp, is unusually short for maize, and it is below the size that is spliced efficiently in transient assays (GOODALL and FILIPOWICZ 1990). Interestingly, the other well-known maize transposases *Ac* and *Spm/En* also contain introns of 72 bp or less (KUNZE *et al.* 1987; MASSON *et al.* 1989). The third *mudrB* intron is not AU-rich relative to the surrounding exons, and high AU-content in introns relative to exons is thought to be important for intron recognition in plants (GOODALL and FILIPOWICZ 1991; LUEHRSEN and WALBOT 1994). Given these exceptions to the common rules, we wanted to determine whether these introns were spliced efficiently.

Figure 5A shows RNase protection results for the splicing of each *MuDR* intron in an active Mutator stock containing multiple copies of *MuDR* (*bz2::mu9*). The RNA used for these studies comes from the above-ground parts of seedlings, and Mutator is active in at least some seedling tissues (LEVY *et al.* 1989). Figure 5B diagrams the probes used in the RNase protection assay. To control for transcripts resulting from read-through of adjacent genes into the deleted or diverged *MuDR*-like elements present in all maize lines (HERSHBERGER *et al.* 1991; HARDEMAN and CHANDLER 1993), RNAs from the non-Mutator parent of the analyzed stock and from an inactive relative of the active Mutator stock were also examined. No protected bands were present in the non-Mutator and inactive samples (data not shown). To quantify the levels of spliced and unspliced products, the intensities of the protected bands were measured directly using an image analyzer (see MATERIALS AND METHODS). For each probe, identification of the spliced and unspliced bands was based on the sizes predicted from the *MuDR* sequence.

The expected unspliced RNAs account for <5% of

TABLE 1

Intron properties

	Position[a]	Splice junctions	Features
Intron A1	333–428	CT|GUUCGU . . . UAG	Alternative 5′ splice sites
	337–428	TC|GUGAGU . . . UAG	
Intron A2	546–690	AG|GUGAGU . . . UAG	—
Intron A3	2751–2829	AG|GCUAGU . . . UAG	Nonconsensus 5′ splice site
Intron B1	4628–4512	CG|GUGAGU . . . CAG	—
Intron B2	4297–4226	UG|GUAUGG . . . CAG	Unusually short
Intron B3	4083–3964	UG|GUAAGG . . . UAG	No U rich relative to exons

[a] Numbering as in HERSHBERGER *et al.* (1991).

the protected counts when a probe for the untranslated leader intron of *mudrA* is used (IA1). Few of the many fragments protected by the IA1 probe, with the exception of the marked unspliced product, are large enough to contain both the intron and more than a few bases of exon sequence; therefore, it appears that multiple *mudrA* start sites and the alternative splicing of the intron (92 *vs.* 96 bp) probably account for the large number of protected fragments. In contrast to the low level of unspliced RNA for the leader intron, ~20% of *mudrA* transcripts fail to remove the second *mudrA* intron (IA2). The third intron of *mudrA*, which contains the GC dinucleotide at the 5′ splice junction, also fails to splice ~20% of the time (IA3). The second intron occurs very early in the coding region for the MUR-A polypeptide, and failure to splice it would result in a peptide no longer than 36 amino acids. The third intron, however, is near the 3′ end of the transcript, and a message retaining this intron would encode a polypeptide of 736 amino acids that terminates at a stop codon within the intron.

In *mudrB*, the leader intron (IB1) is very efficiently spliced (>90%). The second intron (IB2) was also efficiently spliced in this experiment (~90%) despite its small size. If the 72-bp second *mudrB* intron were not spliced, it would add 24 amino acids to the encoded polypeptide. A third band whose size is not accounted for by either spliced or unspliced message is present in both the IB1 and IB2 lanes (circles in Figure 5A); it represents ~15% of the total protected counts in both samples under all RNase digestion conditions tested. The size of this band using the IB1 probe suggests that it is a *mudrB*-like message that retains the first intron and is cleaved at a polymorphism in the untranslated leader exon 5′ of the first intron. Using the IB2 probe, which overlaps extensively with IB1 and also contains part of the first intron, the anomalous protected band is exactly the size expected for a message with the first intron retained and the second intron removed. Because this band has the same intensity relative to the correct bands as the anomalous message detected by IB1, it suggests that most of the normal transcripts splice both introns, while the anomalous transcript retains the first and removes the second intron. If there were no other sequence changes, this transcript would contain two upstream AUG codons out of frame with MUR-B.

The third *mudrB* intron (IB3) is spliced at a very low level (<10%) in seedling RNA. This intron was originally found in a cDNA clone that came from an immature ear RNA library. RNase protection of the immature ear RNA with the IB3 probe gave a pattern very similar to the results shown here for seedling RNA (data not shown), suggesting that this intron is rarely spliced from *mudrB* transcripts. Splicing this intron would remove 40 amino acids from the MUR-B polypeptide.

Antisense *MuDR* RNA: The convergent transcription units in *MuDR* suggest that transcription of either *mudrA* or *mudrB* could produce long sense/antisense hybrid messages by failing to terminate transcription in the intergenic region. On RNA blots, Mutator seedlings contain the expected sense *MuDR* messages as two discrete bands without detectable longer transcripts. This implies that polyadenylation and/or transcript termination in the intergenic region is very efficient. However, we have detected low levels of antisense transcripts for both *mudrA* and *mudrB* using RNase protection of seedling RNA from active Mutator plants.

Figure 6A shows RNase protection results using representative probes (dark lines, Figure 6B) for antisense *mudrA* and *mudrB* on total seedling RNA from active, inactive, and non-Mutator stocks. The protected bands in the active Mutator stock are the full length predicted for those probes, which implies that the antisense RNA matches the *MuDR* sequence exactly in the probe regions. We have used probes spanning multiple regions of both genes (Figure 6B), and we have been able to detect a small amount of fully protected antisense RNA in all the tested regions (data not shown). In RNase protection experiments using probes for both sense and antisense in the same reaction, the fully protected antisense fragments appear to be at ~5–10% of the level of a protected sense *mudrA* fragment in seedlings (data not shown). Because the RNAs were prepared from all the above ground parts of the seedling, we do not know if the low level of antisense represents a small subset of cells with moderate levels of transcripts or low levels of transcripts in all the seedling tissues.

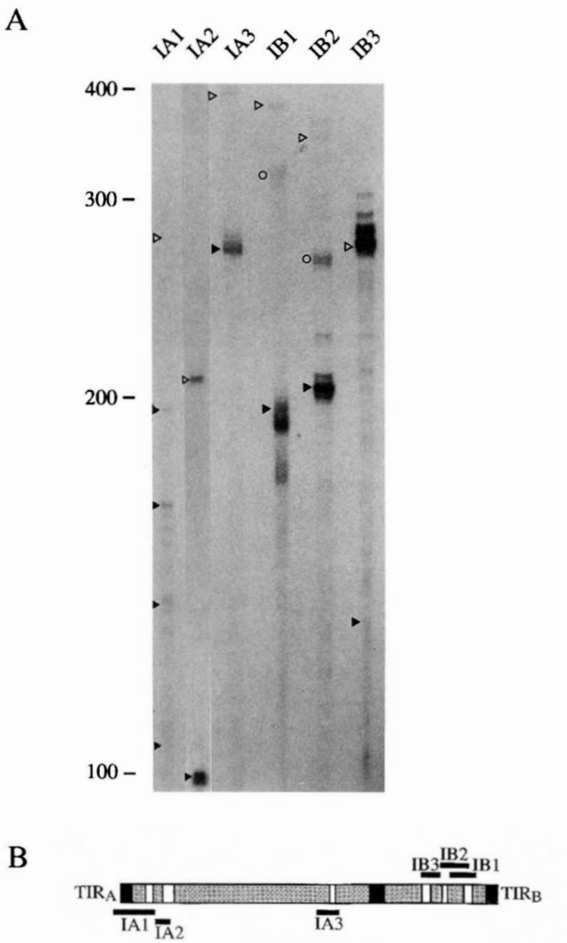

FIGURE 5.—Splicing efficiency of the *MuDR* introns. (A) RNase protection analysis of splicing efficiency in total RNA from active *bz2::mu9* seedlings. ▶, fragment sizes expected for the spliced bands; ▷, fragment sizes for the unspliced bands; ○, major bands that do not match the expected sizes for spliced or unspliced *MuDR* transcripts. The positions of the RNA size markers are indicated on the left. (B) Diagram of the *in vitro*-transcribed probes used in this RNase protection assay. In the *MuDR* depiction, ■ represents untranscribed regions, □ represents introns, and ▣ represents the regions retained in fully processed transcripts.

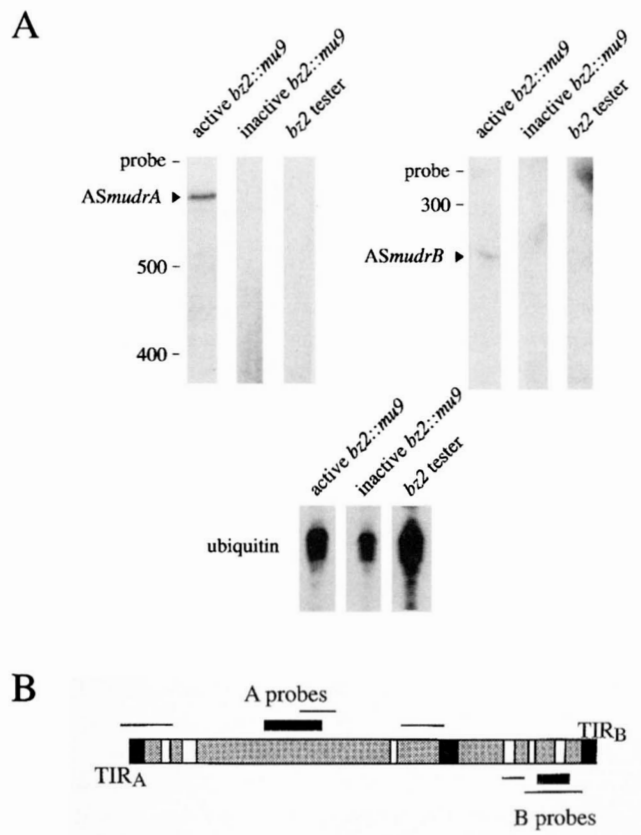

FIGURE 6.—Active Mutator seedlings contain antisense *MuDR* transcripts. (A) RNase protection assays using probes to detect antisense *MuDR* transcripts in total seedling RNA (top). The fully protected fragments are marked with an arrowhead. An RNase protection assay with a ubiquitin probe that detects sense transcripts was also run to control for the quality and amount of RNA in the three samples (bottom). (B) Diagram of the *in vitro*-transcribed probes used for the RNase protections shown in A (thick lines) and probes used in other RNase protections that detected fully protected antisense fragments (thin lines).

Neither the non-Mutator parent (*bz2* tester) of the active stock nor an inactive related stock contain any antisense transcripts that complement the full length of the sense *MuDR* probes (Figure 6A). This suggests that the antisense transcripts seen in the active stock are not the result of read-through into *MuDR* from external promoters. With some probes, protected bands that are less than full length are visible in all samples (data not shown). These may result from incomplete digestion by the RNases, or they may represent antisense transcripts from smaller or diverged *MuDR*-like elements.

DISCUSSION

Genetic analysis has shown that a single controlling element is sufficient to direct Mutator activity in maize (SCHNABLE and PETERSON 1988; ROBERTSON and STINARD 1989), and that *MuDR* is this controlling element (CHOMET *et al.* 1991; QIN *et al.* 1991). There is an excellent correlation between the presence of the major *MuDR* transcripts and Mutator activity when active, inactive and non-Mutator stocks are compared, suggesting that they may encode gene products required for element activity. Consistent with this hypothesis, *mudrA* encodes a putative polypeptide with homology to a family of bacterial transposons (EISEN *et al.* 1994).

The structure of the *MuDR* element is unusual relative to other characterized inverted repeat elements. For example, the maize transposons *Ac* and *Spm* both contain single promoters that initiate transcription near one end of the element, and most of the element sequences are transcribed. In contrast, *MuDR* has two major transcripts that are convergent but nonoverlapping. The nearly identical terminal inverted repeats, which are required for transposition (LEVY and WALBOT

1991), also contain the transcription start sites for the major *MuDR* transcripts. Only 200 bp of internal *MuDR* sequences are not represented in these major transcripts. This intergenic region contains multiple sets of direct repeats, with polyadenylation for each transcript occurring at several sites within this region.

Failure to terminate or polyadenylate the convergent transcripts could lead to antisense mRNA production. A low level of antisense message to both transcripts has been detected. For most genes, antisense regulation depends on independent control over the promoters of the sense and antisense transcripts. The convergent structure of the *MuDR* transcription units suggests that for these genes, the antisense transcripts could be produced either by transcription from specific antisense promoters or by failure to terminate the normal sense transcripts. Determining the structure of the antisense transcripts will help us understand which mechanism is responsible for antisense production. We will also be analyzing various tissues at different developmental stages to determine whether antisense *MuDR* transcription correlates with the observed regulation of Mutator activity.

Antisense transcripts have been found in two transposon systems in addition to Mutator. An antisense transcript from the prokaryotic transposable element IS*10* binds to the sense transposase RNA and blocks ribosome binding, inhibiting transposition when IS*10* is present in multiple copies (KLECKNER 1989). The eukaryotic *micropia* retrotransposon from *Drosophila hydei* makes an antisense RNA complementary to the reverse transcriptase and RNase H coding regions (LANKENAU *et al.* 1994). Production of this antisense transcript is under the control of a testis-specific promoter and accumulates only during spermatogenesis in males. The function of this transcript is not known, but its expression pattern is conserved among six Drosophila species.

RNA blots, performed on RNA from a variety of tissues and developmental stages, indicate that the major *MuDR* transcripts may be ubiquitously expressed in active Mutator stocks. This includes a high level of expression early in embryo and endosperm development, stages during which *Mu* excision is rarely seen. This result strongly suggests that Mutator excision activity is not simply controlled by *MuDR* transcript levels. Unlike *MuDR*, the maize elements *Spm* and *Ac* produce very low steady-state levels of the large transposase-encoding transcripts (KUNZE *et al.* 1987; FUSSWINKEL *et al.* 1991), and element excision can occur throughout development (LEVY and WALBOT 1990). In transgenic plants, transcript levels for these elements fail to show simple correlations with activity during development (SCOFIELD *et al.* 1993; SCHLÄPPI *et al.* 1994).

High copy Mutator stocks can produce a complex set of *MuDR* transcripts in addition to the predominant 2.8-kb *mudrA* and 1-kb *mudrB*. We detected a 2.4-kb population, Δ*mudrA*, that is found in many tissues and is likely to be produced from *MuDR* deletion derivatives. In addition to *mudrA*, *mudrB*, and Δ*mudrA*, we have observed *MuDR*-hybridizing transcripts of other sizes in one or a few individuals (data not shown). Some of these may come from deleted *MuDR* elements, some may be transcripts initiated from external promoters, while others may represent failure to terminate the normal *mudrA* and *mudrB* transcripts. These transcripts are only observed in active Mutator stocks, suggesting *MuDR* is somehow required for their expression. Several studies have shown that the P cytotype in *D. melanogaster* can be established by deletion derivatives of the *P* element (LEMAITRE *et al.* 1993; MISRA *et al.* 1993; RASMUSSON *et al.* 1993); thus, it will be interesting to determine whether the aberrant *MuDR* transcripts contribute to Mutator regulation.

Tissue-specific splicing is an important regulatory mechanism in both plants and animals (MCKEOWN 1992; RIO 1993; LUEHRSEN *et al.* 1994). In *D. melanogaster*, transposition of *P* elements is limited to the germline because the third intron of the transposase gene fails to splice in somatic tissues, and the unspliced message encodes a truncated protein that acts as a repressor of transposition (RIO 1991). The third *mudrA* intron, which has a nonconsensus GC dinucleotide at the 5′ splice junction, fails to splice 15–20% of the time in seedlings, and translation of the unspliced product would result in a truncated protein product. Surprisingly, the second *mudrA* intron, which has all the consensus features for maize introns, also fails to splice ~20% of the time.

In other cases, successful splicing can create an inactive molecule. The maize *Spm/En* transposable element encodes at least four alternatively spliced transcripts, and of these, only the shortest (*tnpA*) and the longest (*tnpD*) are required for transposition in heterologous tobacco plants (FREY *et al.* 1990; Masson *et al.* 1991). The *tnpC* transcript is very similar to *tnpD*, but it has a 90-bp in-frame intron spliced out. The absence of those 30 amino acids may prevent the *tnpC* product from participating in transposition. Splicing out the second or third *mudrB* introns would remove amino acids from the polypeptide; thus, regulating the splicing of either of these *MuDR* introns could potentially regulate Mutator transposition.

Overall, the *Mu* element family differs in several respects from the other well-characterized maize elements *Spm* and *Ac*. These include Mutator's ability to cause mutations at high frequency (ROBERTSON and MASCIA 1981), the large number and diversity of *Mu* elements, potential differences in transposition mechanism (reviewed in CHANDLER and HARDEMAN 1992), and the very different structure of the regulatory element reported here. A common theme for all three element systems is that the activity of the regulatory elements,

MuDR, Spm, and *Ac,* can vary dramatically from generation to generation and that these changes frequently correlate with methylation and demethylation of the elements. Another common theme is that their developmental regulation does not correlate simply with transcript levels. In no case is the complex regulation understood. Future experiments will explore whether the unique structure of *MuDR* allows it to employ distinct mechanisms of regulation.

The authors acknowledge JONATHON EISEN for his help with the computer analysis of RNA structures and PAUL CHOMET for the gift of *a1-mum2* RNA. Thanks are also due to the many members of the Walbot lab, past and present, who contributed ideas and editing to this manuscript. This work was supported by an National Science Foundation Postdoctoral Fellowship in Plant Molecular Biology to R.J.H., a Howard Hughes graduate fellowship to M.-I.B., National Science Foundation grant DMB9024307 to V.L.C., and National Institutes of Health grant GM49681 to V.W.

LITERATURE CITED

BENNETZEN, J. L., and P. S. SPRINGER, 1994 The generation of *Mutator* transposable element subfamilies in maize. Theor. Appl. Genet. **87:** 657–667.

BENNETZEN, J. L., P. S. SPRINGER, A. D. CRESSE and M. HENDRICKX, 1993 Specificity and regulation of the *Mutator* transposable element system in maize. Crit. Rev. Plant Sci. **12:** 57–95.

BROWN, J. W., C. G. SIMPSON, G. G. SIMPSON, A. D. TURNBULL-ROSS and G. P. CLARK, 1993 Plant pre-mRNA splicing and splicing components. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **342:** 217–224.

CHANDLER, V. L., and K. J. HARDEMAN, 1992 The *Mu* elements of *Zea mays.* Adv. Genet. **30:** 77–122.

CHOMET, P., D. LISCH, K. J. HARDEMAN, V. L. CHANDLER and M. FREELING, 1991 Identification of a regulatory transposon that controls the *Mutator* transposable element system in maize. Genetics **129:** 261–270.

CHRISTENSEN, A. H., R. A. SHARROCK and P. H. QUAIL, 1992 Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. Plant Mol. Biol. **18:** 675–689.

EISEN, J. A., M.-I. BENITO and V. WALBOT, 1994 Sequence similarity of putative transposases links the maize *Mutator* autonomous element and a group of bacterial insertion sequences. Nucleic Acids Res. **22:** 2634–2636.

FEINBERG, A. P., and B. VOGELSTEIN, 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:** 6–13.

FREY, M., J. REINECKE, S. GRANT, H. SAEDLER and A. GIERL, 1990 Excision of the *En/Spm* transposable element of *Zea mays* requires two element-encoded proteins. EMBO J. **9:** 4037–4044.

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

GOODALL, G. J., and W. FILIPOWICZ, 1990 The minimum functional length of pre-mRNA introns in monocots and dicots. Plant Mol. Biol. **14:** 727–733.

GOODALL, G. J., and W. FILIPOWICZ, 1991 Different effects of intron nucleotide composition and secondary structure on pre-mRNA splicing in monocot and dicot plants. EMBO J. **10:** 2635–2644.

GREENE, B., R. WALKO and S. HAKE, 1994 *Mutator* insertions in an intron of the maize *knotted1* gene result in dominant suppressible mutations. Genetics **138:** 1275–1285.

HARDEMAN, K. J., and V. L. CHANDLER, 1993 Two maize genes are each targeted predominantly by distinct classes of *Mu* elements. Genetics **135:** 1141–1150.

HERSHBERGER, R. J., C. A. WARREN and V. WALBOT, 1991 Mutator activity in maize correlates with the presence and expression of the *Mu* transposable element *Mu9.* Proc. Natl. Acad. Sci. USA **88:** 10198–10202.

HUNT, A. G., 1994 Messenger RNA 3′ end formation in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **45:** 47–60.

JACKSON, I. J., 1991 A reappraisal of non-consensus mRNA splice sites. Nucleic Acids Res. **19:** 3795–3798.

JAEGER, J. A., D. H. TURNER and M. ZUCKER, 1989 Predicting optimal and suboptimal secondary structures for RNA, pp. 281–306 in *Molecular Evolution: Computer Analysis of Protein and Nucleic Acid Sequences, Methods in Enzymology.* Vol. 183, edited by R. F. DOOLITTLE. Academic, San Diego.

JAMES, M. G., M. J. SCANLON, M. M. QIN, D. S. ROBERTSON and A. M. MYERS, 1993 DNA sequence and transcript analysis of transposon *MuA2,* a regulator of Mutator transposable element activity in maize. Plant Mol. Biol. **21:** 1181–1185.

KLECKNER, N., 1989 Transposon Tn*10,* pp. 227–268 in *Mobile DNA,* edited by D. E. BERG and M. M. HOWE. American Society for Microbiology, Washington, D.C.

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

LANKENAU, S., V. G. CORCES and D.-H. LANKENAU, 1994 The *Drosophila micropia* retrotransposon encodes a testis-specific antisense RNA complementary to reverse transcriptase. Mol. Cell. Biol. **14:** 1764–1775.

LEMAITRE, B., S. RONSSERAY and D. COEN, 1993 Maternal repression of the *P* element promoter in the germline of *Drosophila melanogaster:* a model for the P cytotype. Genetics **135:** 149–160.

LERNER, C. G., and M. INOUYE, 1990 Low copy number plasmids for regulated low-level expression of cloned genes in *Escherichia coli* with blue/white insert screening capability. Nucleic Acids Res. **18:** 4631.

LEVY, A. A., and V. WALBOT, 1990 Regulation of the timing of transposable element excision during maize development. Science **248:** 1534–1537.

LEVY, A. A., and V. WALBOT, 1991 Molecular analysis of the loss of somatic instability in the *bz2::mu1* allele of maize. Mol. Gen. Genet. **229:** 147–151.

LEVY, A. A., A. B. BRITT, K. R. LUEHRSEN, V. L. CHANDLER, C. WARREN *et al.,* 1989 Developmental and genetic aspects of *Mutator* excision in maize. Dev. Genetics **10:** 520–531.

LUEHRSEN, K. R., and V. WALBOT, 1994 Addition of A- and U-rich sequence increases the splicing efficiency of a deleted form of a maize intron. Plant Mol. Biol. **24:** 449–463.

LUEHRSEN, K. R., S. TAHA and V. WALBOT, 1994 Nuclear pre-mRNA splicing in higher plants. Prog. Nucleic Acids Res. Mol. Biol. **47:** 149–193.

MARTIENSSEN, R., and A. BARON, 1994 Coordinate suppression of mutations caused by Robertson's *Mutator* in maize. Genetics **136:** 1157–1170.

MASSON, P., G. RUTHERFORD, J. A. BANKS and N. FEDOROFF, 1989 Essential large transcripts of the maize *Spm* transposable element are generated by alternative splicing. Cell **58:** 755–765.

MASSON, P., M. STREM and N. FEDOROFF, 1991 The *tnpA* and *tnpD* gene products of the *Spm* element are required for transposition in tobacco. Plant Cell **3:** 73–85.

MCKEOWN, M., 1992 Alternative mRNA splicing. Annu. Rev. Cell Biol. **8:** 133–155.

MISRA, S., R. M. BURATOWSKI, T. OHKAWA and D. C. RIO, 1993 Cytotype control of *Drosophila melanogaster P* element transposition: genomic position determines maternal repression. Genetics **135:** 785–800.

NASH, J., K. R. LUEHRSEN and V. WALBOT, 1990 *Bronze-2* gene of maize: reconstruction of a wild-type allele and analysis of transcription and splicing. Plant Cell **2:** 1039–1049.

QIN, M., and A. H. ELLINGBOE, 1990 A transcript identified by *MuA* of maize is associated with *Mutator* activity. Mol. Gen. Genet. **224:** 357–363.

QIN, M., D. S. ROBERTSON and A. H. ELLINGBOE, 1991 Cloning of the *Mutator* transposable element *MuA2,* a putative regulator of somatic mutability of the *a1-Mum2* allele in maize. Genetics **129:** 845–854.

RASMUSSON, K. E., J. D. RAYMOND and M. J. SIMMONS, 1993 Repres-

sion of hybrid dysgenesis in *Drosophila melanogaster* by individual naturally occurring *P* elements. Genetics **133:** 605–622.

RIO, D. C., 1991 Regulation of Drosophila *P* element transposition. Trends Genet. **7:** 282–287.

RIO, D. C., 1993 Splicing of pre-mRNA: mechanism, regulation and role in development. Curr. Opinion Genet. Devel. **3:** 574–584.

ROBERTSON, D. S., 1981 Mutator activity in maize: Timing of its activation in ontogeny. Science **213:** 1515–1517.

ROBERTSON, D. S., and P. N. MASCIA, 1981 Tests of 4 controlling-element systems of maize for *Mutator* activity and their interaction with *Mu Mutator*. Mutat. Res. **84:** 283–289.

ROBERTSON, D. S., and P. S. STINARD, 1989 Genetic analyses of putative two-element systems regulating somatic mutability in *Mutator* induced aleurone mutants of maize. Devel. Genet. **10:** 482–506.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, NY.

SCHLÄPPI, M., R. RAINA and N. FEDOROFF, 1994 Epigenetic regulation of the maize *Spm* transposable element: novel activation of a methylated promoter by *TnpA*. Cell **77:** 427–437.

SCHNABLE, P. S., and P. A. PETERSON, 1988 The *Mutator*-related *Cy* transposable element of *Zea mays* L. behaves as a near-Mendelian factor. Genetics **120:** 587–596.

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

WALBOT, V., 1992 Strategies for mutagenesis and gene cloning using transposon tagging and T-DNA insertional mutagenesis. Annu. Rev. Plant Phys. Plant Mol. Biol **43:** 49–82.

WALBOT, V., C. P. BRIGGS and V. CHANDLER, 1986 Properties of mutable alleles recovered from *mutator* stocks of *Zea mays*, pp. 115–142 in *Genetics, Development, and Evolution*, edited by J. P. GUSTAFSON, G. L. STEBBINGS and F. J. AYALA. Plenum Publishing, NY.

WHITE, O., C. SODERLUND, P. SHANMUGAN and C. FIELDS, 1992 Information contents and dinucleotide compositions of plant intron sequences vary with evolutionary origin. Plant Mol. Biol. **19:** 1057–1064.

ZHAO, Z. Y., and V. SUNDARESAN, 1991 Binding sites for maize nuclear proteins in the terminal inverted repeats of the *Mu1* transposable element. Mol. Gen. Genet. **229:** 17–26.

Communicating editor: J. A. BIRCHLER