MOLECULAR AND CELLULAR BIOLOGY, May 1990, p. 2090–2095
0270-7306/90/052090-06$02.00/0
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Vol. 10, No. 5

The *Mu1* Maize Transposable Element Induces Tissue-Specific Aberrant Splicing and Polyadenylation in Two *Adh1* Mutants

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Received 25 October 1989/Accepted 9 February 1990

Insertions of the maize transposable element Robertson's Mutator (*Mu*) into intron 1 of the *Adh1* gene have produced a number of mutant alleles altered in quantitative expression. It has previously been shown that transcription and mRNA accumulation are reduced for two of these alleles, *Adh1-S3034* and *Adh1-S4477*. In this report, we describe the presence of *Mu1*-hybridizing polyadenylated transcripts in roots of anaerobically induced seedlings of these same mutants. Sequence analysis of *Mu1*-hybridizing clones from a cDNA library of *S3034* RNA indicated that these transcripts originated from the *Adh1* locus and were produced by alternative processing of *S3034* pre-mRNA. Approximately half of the cDNAs represented transcripts that had not undergone excision of the intron containing the 1.4-kilobase *Mu1* insertion but were processed in response to signals present in the transposable element. *Mu1* contains a donor splice site in the 5′-terminal inverted repeat that can be joined to the *Adh1* exon 2 acceptor, resulting in removal of most of the *Mu1* sequences from the pre-mRNA; alternatively this donor can be spliced to an acceptor within *Mu1*, removing an 89-nucleotide intron. *Mu1* also contains polyadenylation signals that are used to produce truncated transcripts. These *Mu1* transcripts produced by aberrant splicing and polyadenylation were not detected in RNA isolated from developing kernels.

Robertson's Mutator (*Mu*), a transposable element of maize, was identified by its ability to induce a 20- to 50-fold increase in the mutation rate. Many of the induced mutants exhibit instability (17). The phenotype does not segregate in a Mendelian fashion; greater than 90% of the offspring from an outcross demonstrate Mutator activity. Donald Robertson proposed that a novel transposable element was responsible for the observed phenomena, as no complementation could be observed with other families of maize mobile genetic elements (18).

The first Mutator element characterized at the molecular level was isolated as an insertion in a gene that encodes alcohol dehydrogenase (*Adh1*) (3; Fig. 1). *Mu1*, as the 1.4-kilobase (kb) insertion was named, shares a number of features with other transposable elements (1). It bears 215-base-pair (bp) inverted repeats (with 95% sequence identity) at its termini and is flanked by 9-bp direct repeats generated upon duplication of the *Adh1* sequence at the insertion site. Four open reading frames can be deduced from the *Mu1* sequence, but there are no data indicating that any are translated.

Mu1 is part of a transposable-element family and exhibits various degrees of similarity with other members. It has been suggested that *Mu1* arose from *Mu1.7* by a 380-bp deletion and assorted minor sequence changes (26). *Mu3*, on the other hand, exhibits homology only to the long terminal inverted repeats present in *Mu1* and *Mu1.7* (6). All maize lines examined contain sequences homologous to *Mu*, suggesting an ancient association with the species (4). Not all maize lines, however, exhibit the Mutator phenotype; in these cases, *Mu* inactivity has been correlated with DNA modification of the elements (2, 5). *Mu* elements are present in 15 to 50 copies in active lines, as well as in offspring produced by outcrossing to non-Mutator plants. The high copy number probably accounts for the high mutation rates.

The effect *Mu* has on gene expression can vary greatly but has been studied at relatively few loci. The insertion of a *Mu1.7* element in exon 2 of the *Bz1* gene creates a null allele, except for tissue sectors in which the transposable element has been excised from the locus (26). On the other hand, the presence of a *Mu3* insertion in the *Adh1* promoter has altered the tissue-specific pattern of expression of the gene; the mutant allele exhibits very low levels of anaerobic induction but is expressed normally in pollen grains (6). Another *Mu* insertion in a promoter region, that of the *Hcf* gene (high chlorophyll fluorescence), reduces *hcf106* RNA accumulation only when the element is hypomethylated; when the *Mu* insertion is methylated and therefore inactive, the mutant phenotype is suppressed (14).

Work in our laboratory has focused on mutant alleles of two genes, *Adh1* and *Sh1* (sucrose synthase 1). Both genes are expressed in a tissue-specific fashion during development and are inducible in seedlings subjected to anaerobic stress (22, 24). Freeling and co-workers devised a means of positive selection for impaired alcohol dehydrogenase activity. This protocol permitted isolation of a number of mutants exhibiting quantitative alterations in their expression (7); all carried *Mu1* insertions in intron 1 of *Adh1*. Thus, *Adh1-S3034* (*S3034*) and *Adh1-S4477* (*S4477*) encode a protein indistinguishable from that produced by the *1S* progenitor allele but exhibit only 20 to 40% and 50 to 70%, respectively, of the ADH1 enzymatic activity present in *1S*. It has been demonstrated that *Adh1* transcription and mRNA accumulation are comparably reduced in these mutants (20).

Recently, we discovered that in these two *Mu1* alleles, alcohol dehydrogenase activity and mRNA levels are dependent on the genetic background in which they are measured (D. F. Ortiz and J. N. Strommer, Biochemical Genetics, in press). In the course of these experiments, it was found that *S3034* anaerobic seedlings produce polyadenylated RNA species that hybridize to *Mu1*. We now report that these

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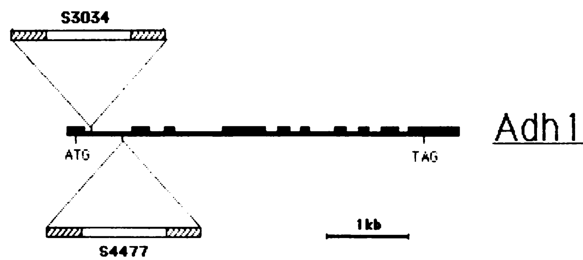

FIG. 1. Structure of the *Adh1-S3034* and *Adh1-S4477* alleles. The *Adh1* transcription unit is 3,320 bp long and contains 10 exons (raised boxes). The 1.4-kilobase-pair *Mu1* transposable-element insertions in intron 1 are indicated, and the hatched regions represent the 215-bp terminal inverted repeats. The element in *S3034* resides 71 bp downstream of the exon 1 donor splice site. The *S4477* allele contains a *Mu1* insertion, a product of an independent transposition event, 341 bp downstream of the site occupied in *S3034*; this places it 412 bp 3' from the exon 1 donor splice site and 120 upstream of the exon 2 acceptor site.

transcripts originate from the *Adh1* locus as a result of aberrant processing of the *S3034* primary transcript. Sequence analysis of a number of cDNA clones revealed that utilization of splicing and polyadenylation signals in *Mu1* generated a number of novel transcripts. Aberrant splicing of *Mu1-Adh1* transcripts is tissue or stress specific. Generation of novel transcripts from *Mu1*-mutant alleles may be a common occurrence.

MATERIALS AND METHODS

RNA isolation and Northern (RNA) analysis. Five-day-old maize seedlings were anaerobically induced by immersion in Tris-buffered tap water. Roots were harvested into liquid nitrogen and stored at −70°C until needed. Ears were collected from field-grown plants 30 days postpollination, and the immature kernels were harvested in liquid nitrogen. Frozen roots or kernels were homogenized in a coffee grinder with dry ice, and RNA was prepared by the procedure of Longemann et al. (12). Poly(A)$^+$ RNA was selected on oligo(dT) columns and subjected to Northern analysis on 1.5 to 2% denaturing formaldehyde-agarose gels as described previously (19). Prehybridization and hybridization to probes labeled with [α-^{32}P]dATP by nick translation was in 50% formamide–6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% sodium dodecyl sulfate at 42.5°C. The filters were washed in 0.2× SSC–0.1% sodium dodecyl sulfate at 68°C.

cDNA cloning and analysis. Poly(A)$^+$ RNA isolated from *S3034* seedling roots induced for 12 h was the substrate for cDNA synthesis with a kit marketed by Bethesda Research Laboratories that was used as recommended by the manufacturer. The double-stranded cDNA was treated with T4 polymerase and ligated to *Eco*RI-*Not*I adaptors (Invitrogen). Excess adaptors were separated by passage through a Sepharose CL4B (Sigma Chemical Co.) column. The cDNA was treated with T4 polynucleotide kinase and ligated to lambda gt10 arms purchased from Stratagene. In vitro packaging extracts from Stratagene were used to prepare bacteriophage particles for infection of *Escherichia coli* C-600 Hfl-150 cells. The resultant library was screened as described by Maniatis et al. (13), and phage that hybridized to *Mu1* and *Adh1* were purified. The cDNA inserts were electroeluted from agarose gels and subcloned into *pUC19* and *M13* vectors. DNA sequence analysis was by the dideoxy-chain termination method of Sanger et al. (23).

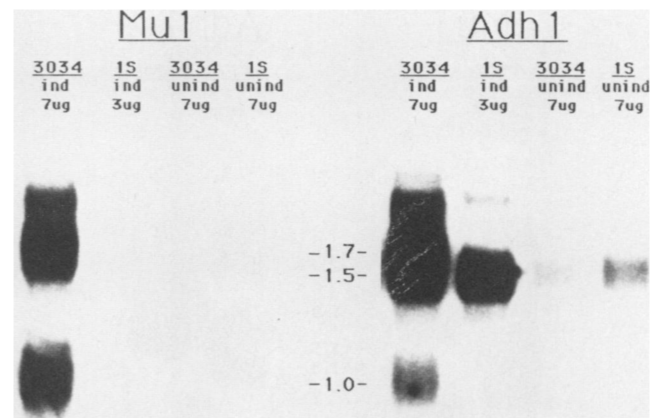

FIG. 2. Autoradiograms of a Northern blot containing poly(A)$^+$ RNA isolated from *1S*-uninduced (1S unind, *S3034*-uninduced (3034 unind), *1S* (12 h)-induced (1S ind), and *S3034* (12 h)-induced (3034 ind) 5-day-old maize seedling roots. The amount of RNA loaded onto each lane is indicated above it. The radioactively labeled probes hybridized to the filters were nick-translated pUC19 plasmids containing inserts with the following sequences: *Mu1*, a 1-kb *Taq*I fragment including the *Mu1* 5'-terminal inverted repeat and most of the central region, and *Adh1*, a 2.2-kb *Pst*I fragment containing the first five exons and introns of *Adh1*. The lengths of hybridizing transcripts are indicated in thousands of nucleotides between the autoradiograms.

RESULTS

The 1.4-kb *Mu1* insertion in the *Adh1-S3034* allele more than triples the length of intron 1. The drastic alteration in gene structure suggested to us that processing of the primary transcript might be altered such that a Northern blot would detect a pattern of splicing intermediates different from that seen for the *1S* allele. To test this hypothesis, up to 10 μg of *S3034* poly(A)$^+$ RNA from anerobically induced seedlings was subjected to Northern analysis. Previous work in this laboratory had indicated that transcription of *S3034* is reduced to 40% of that exhibited by the *1S* progenitor allele (19), so the amount of *1S* RNA loaded was adjusted accordingly.

Hybridization of the filter to an *Adh1* genomic probe demonstrated a number of differences between *S3034* and the *1S* progenitor (Fig. 2). There was strong hybridization to a 1,500-nucleotide (nt) species in lanes containing RNA from induced seedlings, corresponding to the length of the mature *Adh1* message; however, the *S3034* sample included higher-molecular-weight transcripts whose hybridization overlapped with the *Adh1* mRNA, giving rise to a band that extended up to 1,900 nt. Additionally, a faint, diffuse signal was detected at approximately 1,000 nt only in the *S3034* lane. When the same blot was probed with *Mu1*, we detected strongly hybridizing bands of 1,600 to 1,900 nt, as well as an additional set of transcripts 900 to 1,100 nt long. The two lanes to the right of the induced *1S* control in Fig. 2 contained poly(A)$^+$ RNAs extracted from aerobic *1S* and mutant seedlings. The *S3034* lane exhibited low levels of hybridization to *Adh1* and no appreciable *Mu1* homology, indicating that accumulation of the *Mu1* transcripts, like *Adh1* mRNA, responds to anaerobic induction. The 900- to 1,100-nt RNA species exhibited strong hybridization to *Mu1*. No hybridization in this region was detected with a probe containing sequences 3' to exon 2 of *Adh1* (data not shown), indicating that these transcripts were probably polyadeny-

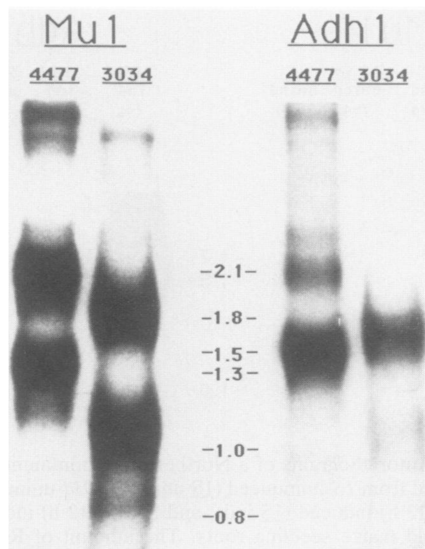

FIG. 3. Autoradiogram of a Northern blot containing 7 μg of *S4477* and *S3034* poly(A)$^+$ RNAs isolated from roots of seedlings induced for 12 h. The filter was initially hybridized to *Mu1*, stripped, and then reprobed with *Adh1* (the probes were the same as those described in the Fig. 2 legend). The approximate sizes of the transcripts that hybridized to these sequences are indicated in thousands of nucleotides between the autoradiograms.

lated in the *Mu1* element or at a cryptic polyadenylation site a short distance downstream from the *Mu1* insertion.

A different *Adh1* allele, *S4477*, was also analyzed. In this mutant, *Mu1* resides in intron 1 of *Adh1*, 341 bp downstream from the *S3034* insertion site and in the same orientation (data not shown; K. Oishi, personal communication). Two bands that hybridized to a *Mu1* probe were detected in a Northern blot containing poly(A)$^+$ RNA isolated from *S4477*-induced seedlings (Fig. 3). The transcripts are approximately 2,100 and 1,400 nt long, e.g., 350 nt longer than the two most prevalent *Mu1* RNA species observed in *S3034*. This is the distance separating the *Mu1* insertions in *S3034* and *S4477*. The 2,100-nt species present in the *S4477* lane also hybridized to *Adh1*; it was difficult to ascertain whether the 1,400-nt *Mu1* transcript hybridized to *Adh1*, as it is similar in length to the correctly spliced *Adh1* message. The bands in *S4477* displayed the same intensity of hybridization to *Mu1* as the *S3034* transcripts, although *S4477* exhibited approximately twice the amount of *Adh1*-hybridizing RNA, suggesting that a higher proportion of *S4477* transcripts was correctly spliced.

Transposable elements in general, and *Mu1* in particular, can cause rearrangements of flanking host sequences (25). The *Mu1* transcripts could originate from a rearranged *Adh1* gene rather than by aberrant RNA processing. To rule out this possibility, DNAs isolated from anaerobically induced *S3034* and *S4477* seedlings were digested with a number of restriction endonucleases and subjected to Southern analysis. No DNA fragments of unexpected length were detected following hybridization with various *Adh1* probes (data not shown).

To characterize the aberrant RNA species, we constructed a cDNA library. The *S3034* allele was chosen for this purpose, as it exhibits a higher ratio of *Mu1* transcripts to mature *Adh1* message. Northern analysis of RNA isolated from seedlings subjected to anaerobiosis for 7, 12, and 24 h indicated that hybridization to *Mu1* was strongest after 12 h of induction (data not shown), suggesting that the aberrant transcripts follow the same accumulation kinetics as *Adh1* mRNA (20). A cDNA library in lambda gt10 was generated from poly(A)$^+$ RNA of roots induced for 12 h and subsequently screened with a probe derived from an *S3034* genomic clone containing both *Adh1* and *Mu1* sequences. Fifteen recombinant phage were purified and analyzed by restriction mapping and Southern blotting. Two clones were identified as cross-hybridizing *Adh2* cDNAs. It was shown that six phage that hybridized exclusively to *Adh1* contained inserts representing normally spliced *Adh1* messages by sequence analysis of two clones and restriction mapping of the remainder.

The remaining seven clones hybridized to both *Adh1* and *Mu1* probes. The 5′ termini of all of these cDNAs are located upstream of the *Mu1* insert and downstream of the *Adh1* transcriptional start site; in three clones, the 5′ end resides within 10 nt of the *Adh1* cap site. This demonstrates that at least a large proportion of the *Mu1* transcripts detected by Northern analysis originated from *S3034* and not from any of the other 30 to 50 *Mu* elements in the genome. It also strongly suggests that they were the result of transcription that was controlled by the *Adh1* promoter and initiated at the same transcriptional start site used in the *1S* allele.

The exon 1 donor splice site was bypassed in all of the cDNAs containing *Mu1* sequences; thus, 71 nt of the intron 1 sequence upstream of the *Mu1* insert, as well as various portions of the element itself, were not excised from the primary transcript. Use of novel splicing and polyadenylation signals present in *Mu1* has given rise to a variety of aberrantly processed transcripts. Their structures, as determined by the cDNA sequences, are diagrammed in Fig. 4.

Four of seven *Mu1* clones represent transcripts produced from splicing a donor site 142 nt from the upstream *Mu1* terminus (Mu-DS) to the *Adh1* exon 2 acceptor site (WT-AS) (Fig. 4B). Partial sequence analysis and restriction mapping suggest that processing of the remainder of the transcript proceeds normally, generating a transcript containing the 1,450 nt present in the *Adh1* mRNA, as well as 71 nt of intron 1 and 141 nt of the upstream *Mu1* terminal inverted repeat. This 1,692-nt species, overlapping with *Adh1* mRNA on Northern blots, would correspond to the 1,600- to 1,900-nt *S3034* band that hybridized to *Adh1* and *Mu1*. Sequence analysis of the 3′ termini of cDNA clones representing the wild-type *Adh1* message and the *Mu1* transcript mentioned above identified three polyadenylation sites in exon 10 (Fig. 5B and C; see also the Discussion).

Mu1 contains a variety of processing signals. The donor splice site described above is spliced to a downstream acceptor, removing an 89-nt intron. Three of the cDNA clones that hybridized to *Mu1* terminate in the transposable element, with 15- to 30-nt poly(A) tracts at their 3′ ends, indicating that polyadenylation occurs in response to signals present in *Mu1*. One of the transcripts is polyadenylated 110 nt upstream of the 3′ *Mu1* terminus; the 89-nt *Mu1* intron has been excised from this RNA species, producing a 1,486-nt transcript containing virtually all of the *Mu1* element (Fig. 4C).

The two remaining cDNA clones exhibited polyadenylation sites in the central portion of *Mu1*, 678 and 696 nt from the 5′ terminus of the element. In the first, the 89-nt *Mu1* intron was removed, producing a transcript 790 nt long (Fig. 4D); in the latter, the intron was retained (Fig. 4E). If these transcripts have 100- to 200-nt poly(A) tails, they should be 900–1,100 nt long, the same size as the lower band seen on a Northern blot hybridized to a *Mu* probe (Fig. 2). These

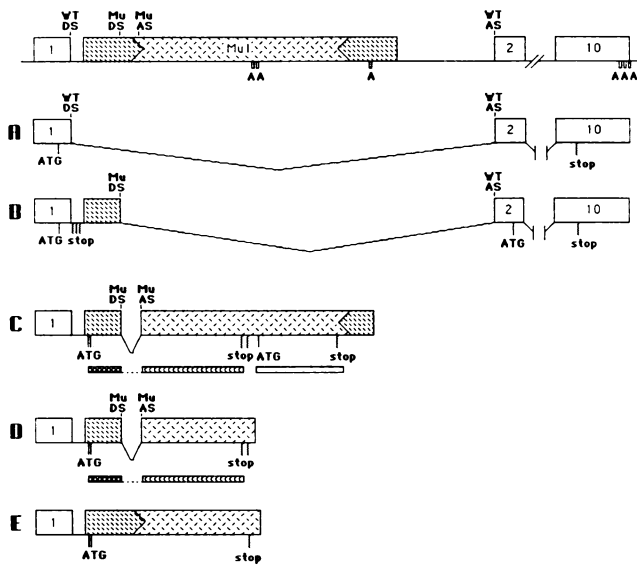

FIG. 4. Structures of the *S3034* pre-mRNA and cDNAs. Pertinent *Adh1* exons are indicated by open boxes and identified by number. The *Mu1* insertion is represented by a hatched box, and terminal inverted repeats are indicated (▦). Panels A to E diagram the structures of cDNA clones that hybridized to *Adh1* and *Mu1* probes, determined by sequence analysis. The splice sites used to generate the cDNAs isolated are indicated above the *S3034* pre-mRNA and the respective cDNA clones. WT-DS and Mu-DS refer to the *Adh1* exon 1 and *Mu1* terminal inverted repeat donor splice sites, respectively. WT-AS and Mu-AS indicate the *Adh1* exon 2 and *Mu1* acceptor splice sites, respectively. The cleavage and polyadenylation sites identified at the 3′ termini of the cDNAs are indicated beneath the *S3034* pre-mRNA by the letter A. Translation start and stop codons are represented by ATG or stop under cDNAs containing open reading frames. These open reading frames are represented by the boxes beneath the *Mu1* cDNAs (C to E). The change in reading frame of the upstream *Mu1* open reading frame caused by excision of the 89-nt intron is indicated by a change in the hatching pattern in the open reading frame boxes in panels C and D. The approximate sizes of the transcripts as deduced from the cDNA sequences are as follows: A, 1,450 nt; B, 1,692 nt; C, 1,486 nt; D, 781 nt; E, 898 nt.

shorter *Mu* transcripts exhibited much-reduced hybridization to a probe containing only *Mu1* sequences downstream of the central polyadenylation sites (data not shown), indicating that the shorter cDNAs were not the product of reverse transcription initiated by oligo(dT) primers hybridized to internal transcript sequences.

The *Adh1* gene is expressed during kernel and anther development, as well as in response to anaerobic stress. To determine whether the aberrant transcripts present in anaerobically induced roots are also produced when *Adh1* is developmentally induced, poly(A)$^+$ RNAs were isolated from *S3034*, *S4477*, and *1S* developing kernels and subjected to Northern analysis (Fig. 6). An *Adh1* probe hybridized with comparable intensity to the *S3034* kernel and induced-root lanes. Induced-root RNA exhibited the expected wide band at 1,600 to 1,900 nt and a faint signal detectable at 1,000 nt in the original autoradiogram. Kernel RNA, on the other hand, gave rise to a band that was distinguishable from the *1S* control only by its intensity, and no hybridization to the shorter transcripts was detected. Hybridization to a *Mu1* probe revealed intense 1,700- and 1,000-nt bands in anaerobic roots; these transcripts were absent or present at very low levels in kernel RNA. The other bands present in the

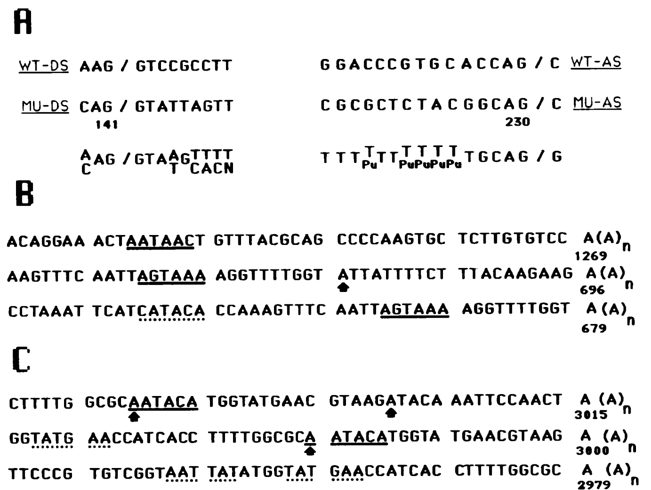

FIG. 5. Processing signals present in *Mu1* and *Adh1*. (A) Sequence of splice junctions present in the cDNAs diagrammed in Fig. 4. WT-DS and Mu-DS indicate donor splice signals and WT-AS and Mu-AS refer to acceptor splice signals in *Adh1* and *Mu1*, respectively. Both *Mu1* and *Adh1* sequences have been aligned with the monocot consensus (10); the number beneath each *Mu* signal refers to the distance from the 5′ terminus of the element. The sequence upstream of the polyadenylation sites in *Mu1* and *Adh1* are shown in panels B and C, respectively. The number beneath each 3′ terminus indicates its position with respect to the *Mu1* 5′ end (B) or the transcription start site in the *Adh1S* pre-mRNA (C). No exact match to the animal AATAAA consensus was identified; hexamers containing a single mismatch are boldly underlined, and those that exhibit two mismatches and conserve the central T residue are indicated by a dotted underline. The arrows mark polyadenylation sites present in the sequence upstream of the 3′ terminus depicted.

kernel lanes when *Mu1* was used as a probe were probably due to hybridization to splicing intermediates, as well as to RNA species from other loci containing *Mu* insertions (the stronger bands were not present in all *S3034* lines).

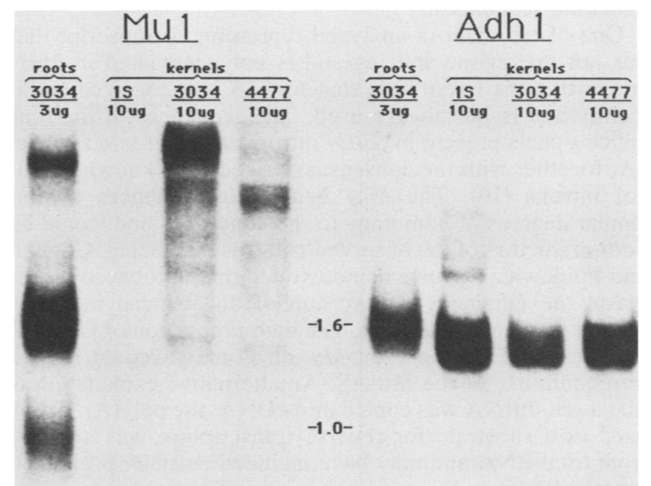

FIG. 6. Autoradiogram of a Northern blot containing poly(A)$^+$ RNA isolated from developing kernels harvested 30 days after pollination. The filter was hybridized to the probes described in the legend to Fig. 2. The amount of RNA loaded is indicated above each lane; to obtain equivalent levels of *Adh1* hybridization, 3 μg of root RNA induced for 12 h was used. Lengths of hybridizing transcripts are indicated in thousands of nucleotides between the autoradiograms.

DISCUSSION

The *Adh1-S3034* and *Adh1-4477* alleles have suffered *Mu1* transposable-element insertions in intron 1, and as a result, they exhibit quantitative reductions in ADH1 activity (7), *Adh1* mRNA levels, and *Adh1* transcription (19). We now show that anaerobically induced *S3034* and *S4477* seedlings contain chimeric RNA species that hybridized to *Adh1* and *Mu1* sequences (Fig. 2 and 3). Alternative processing of the primary *S3034* transcript yields at least five different transcripts due to utilization of splicing and polyadenylation signals in the *Mu1* element. *Adh1* sequences are always present upstream of *Mu1*, indicating that these cDNAs represent transcripts that originate from the *Adh1* locus, probably as a result of transcription directed by the *Adh1* promoter and initiating at the normal transcriptional start site.

The most prevalent cDNA isolated represents the wild-type *Adh1* mRNA; splicing of the exon 1 donor site (WT-DS) to the exon 2 acceptor (WT-AS) resulted in excision of *Mu1* along with the intron 1 sequences. Alternatively, exon 2 is joined to the *Mu1* element upstream terminal inverted repeat via a novel donor splice site (Mu-DS). Thus, 71 nt of the *Adh1* intron and 141 nt of *Mu1* are included in a transcript otherwise processed identically to the wild-type pre-mRNA. If translation of this 1,700-nt species initiated at the normal *Adh1* ATG start codon, the ribosomes would encounter three stop codons in the intron 1 sequence. The next in-frame ATG resides in exon 2; its use would produce a truncated peptide lacking 34 amino acids from the N terminus. Analysis of alcohol dehydrogenase activity by native starch gel electrophoresis of *S3034* extracts does not reveal the presence of this aberrant polypeptide (7, 19), indicating that if this truncated protein is translated, it is inactive or unstable.

The Mu-DS can also be spliced to an acceptor site downstream in *Mu1* (Mu-AS), thus excising an 89-nt intron. The novel *Mu1* open reading frame generated by this splice represents a new candidate for the elusive *Mu1* transposase-encoding sequences.

One of the cDNAs analyzed represents a transcript that has not undergone splicing and is polyadenylated in *Mu1*, supporting the hypothesis that Mu-AS is recognized inefficiently and is not always used. The sequences of the four splice signals present in *S3034* intron 1 are presented in Fig. 5A, together with the consensus derived from known monocot introns (10). The *Mu1* and *Adh1* sequences exhibit similar degrees of homology to the consensus and would be poor predictors of the observed patterns of splicing. Goodall and Filipowicz (9) have demonstrated that in tobacco protoplasts the efficiency of excision of an artificial intron is related to its A+U content. The high proportion of G and C residues (61%) in the 89-nt *Mu* intron may account for the infrequent use of the Mu-AS. An alternative explanation is that a pre-mRNA was copied into cDNA; the poly(A)$^+$ RNA used as a substrate for reverse transcriptase was selected from total RNA and may have included partially processed nuclear RNA.

Transcripts containing *Mu1* sequences in addition to the 141 nt of the terminal inverted repeat have all been polyadenylated in response to signals present in the transposable element. Polyadenylation was detected in two regions: the 3′-terminal inverted repeat, producing a transcript containing most of *Mu1* (Fig. 4C), and two sites at the midpoint of the element (Fig. 4D and E). Premature polyadenylation of transcripts containing transposable-element sequences has also been reported for the *Spm* family (8).

The sequences upstream of the polyadenylation sites are presented in Fig. 5B. The signals that direct cleavage and polyadenylation of plant transcripts are less obvious, or more complex, than their animal counterparts. Although a sequence similar to the AATAAA animal signal is found upstream of most of these sites, the degree of homology to the consensus varies greatly (11); furthermore, many plant genes exhibit multiple 3′ termini. A case in point is the *Adh1* gene: Sachs et al. (21), by using S1 nuclease analysis, mapped four polyadenylation sites for the *Adh1S* allele and seven for *Adh1F*. We sequenced 3′ ends of four cDNA clones terminating in *Adh1* exon 10 (two representing wild-type *Adh1* mRNA and two containing the *Mu1* Mu-DS spliced to the WT-AS) and uncovered three different termini (Fig. 5C). Two of these 3′ ends matched the mapped cleavage sites, and the third represented a novel site located within 30 nt of the other two. The sequences upstream of these sites can be seen in Fig. 5C. As with the *Mu1* 3′ termini, the homology 20 to 30 nt upstream of the AATAAA motif can be so degenerate as to be unrecognizable.

The preponderance of one splice product may depend on the relative strengths of the alternative processing signals involved; it may also depend on their relative positions in the transcript. The *Mu1* element in the *S4477* allele resides 341 bp downstream from the *S3034* insertion (Fig. 1). Although *S4477* exhibited nearly double the amount of alcohol dehydrodenase activity and *Adh1* RNA levels present in *S3034*, hybridization to the *Mu1* RNA species was equivalent for both alleles (Fig. 3). Thus, a lesser proportion of *S4477* transcripts underwent faulty processing. Shortening the distance between the *Mu1* insertion and exon 2, therefore, appears to favor the wild-type splice.

A higher frequency of premature polyadenylation of *S3034* transcripts relative to *S4477* could also explain the pattern of transcription exhibited by the mutant alleles. Runoff transcripts produced in *S3034* (and *S3034*b, which we have shown is identical to *S3034* [unpublished data]) demonstrate decreased hybridization to sequences downstream from the *Mu1* insertion (19, 27). Moreover, the transcriptional attenuation described for *S3034* was markedly less severe in *S4477*, as expected if relatively fewer *S4477* transcripts are polyadenylated in response to *Mu1* signals, and termination occurs 1 to 2 kb 3′ to the cleavage site.

The *Adh1* gene, in addition to its induction during anaerobiosis, is also expressed in developing kernels and anthers. Northern analysis of RNAs isolated from *S3034* and *S4477* kernels failed to detect the *Mu1* transcripts present in anaerobically induced seedlings of these mutants (Fig. 6). Thus, the aberrant processing events that generate the *Adh1-Mu1* transcripts occur in a tissue-specific or stress-dependent manner. The maize anaerobic genes are not intron deficient as are the *Drosophila* heat shock genes, and splicing is not blocked during anaerobiosis. However, it is possible that the splicing apparatus of anaerobic seedlings is less tolerant of alterations in intron structure. Thus, the *Mu1* transcripts observed in anaerobic seedlings may be the result of anaerobically impaired splicing of the *Adh1* intron containing *Mu1*.

Experiments similar to those reported here are being performed with the *sh9026* allele, a mutation of the *Sh1* gene containing a *Mu1* insertion in untranslated exon 1 (15). Preliminary data from cDNA and Northern analyses indicate that the *sh9026* primary transcript is prematurely cleaved and polyadenylated in the transposable element in both

anaerobically induced roots and maturing kernels (unpublished data). Moreover, Northern analysis of a *bz* gene containing a *Mu1.7* insertion has suggested that aberrant transcripts are also produced from that allele (26). Therefore, the factors responsible for recognition of *Mu1* polyadenylation signals and aberrant cleavage of the *S3034* transcript may be present in both induced roots and developing kernels but utilized differently.

Maize exhibits a bewildering array of transposable elements. The *Ac-Ds* and *Spm-dSpm* systems, originally discovered and described by Barbara McClintock, are the best characterized. Recently, it has been shown that a number of nonautonomous elements from both of these families possess splicing signals in or immediately adjacent to their terminal inverted repeats (reviewed in reference 28). Because the terminal repeats of both *Ac* and *Spm* are much shorter than those of *Mu1*, the insertion can be almost completely excised from the pre-mRNA. As a consequence, an allele containing a transposable element in an exon can give rise to spliced transcripts that exhibit only minor alterations and encode proteins with various levels of enzymatic activity. This behavior has led to the suggestion that transposable elements play a role in the evolutionary origin of some introns (16, 29). If *Mu1* were to be spliced from an exon, on the other hand, 141 nt from the element would remain in the transcript; although the reading frame would be conserved, the additional 47 amino acids would be likely to preclude protein activity. However, the capability for tissue-specific splicing displayed by *Mu1* leaves open interesting possibilities regarding the role transposable elements may play in the evolution of developmental patterns of gene expression.

ACKNOWLEDGMENTS

We gratefully acknowledge the advice and criticism offered by Susan Wessler, Robert Ivarie, Claiborne Glover III, and Richard Meagher.

D.O. was supported by Public Health Service training grant GM07103 from the National Institutes of Health to the Genetics Department, University of Georgia. This research was supported by Public Health Service grant GM38616 from the National Institutes of Health to J.S.

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