Characterization of a Highly Conserved Sequence Related to Mutator Transposable Elements in Maize[1]

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Mutator stocks of maize exhibit a high mutation rate correlated with the activity of a family of transposable elements. *Mu1* and, to a lesser extent, the closely related *Mu1.7* elements are responsible for most mutator-induced mutations that have been characterized. These elements are found in 10–60 copies in mutator stocks, and zero to a few intact elements exist in nonmutator maize stocks. Additionally, the component parts of *Mu* elements exist separately in the maize genome. The *Mu* terminal inverted repeats are found in multiple copies in all maize lines and related *Zea* species tested, and *Mu* internal sequences exist unassociated with *Mu* termini. In the present paper, we describe the structure and genomic distribution of one *Mu*-homologous sequence termed *MRS-A* (for *Mu*-related *s*equence). DNA sequencing shows that *MRS-A* is closely related to the internal region of *Mu1* and *Mu1.7* elements. However, it has no *Mu* termini and does not have the structure of a transposable element. This sequence is present in one or two copies in all maize lines and is highly conserved in the genus *Zea*. A similar sequence exists in a species within the genus most closely related to *Zea, Tripsacum dactyloides,* although the *T. dactyloides* genome does not contain any *Mu* termini or intact *Mu* elements. Furthermore, an RNA transcript homologous to *MRS-A* and its flanking DNA is found in both mutator and nonmutator maize plants. These results suggest that *MRS-A* represents a stable, functional region of the maize genome, and we speculate that a similar sequence was encompassed by *Mu* termini to generate a *Mu* transposable element.

Introduction

Robertson (1978) described a heritable mutator system in maize that increased mutation rates at many loci by 20–50 fold. When a mutator stock is crossed to a standard stock of maize with a normal mutation rate, ~90% of the F_1 progeny retain the high mutation rate (Robertson 1978). This multifactor segregation, in combination with the observation that approximately one-third of new mutations isolated from a mutator stock are unstable, suggests that mutator activity results from an active transposable-element family. Consistent with this hypothesis, a 1.4-kb insertion, designated *Mu1,* with physical characteristics of a transposable element, was cloned from mutator-induced mutations at *Adh1* (Bennetzen et al. 1984), *A1* (O'Reilly et al. 1985), and *Bz2* (McLaughlin and Walbot 1987).

A second member of the mutator transposable-element family is *Mu1.7* (Barker et al. 1984; Taylor et al. 1986), which differs from *Mu1* primarily by an extra 380 bp

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of internal sequence. *Mu1* and *Mu1.7* are 97% similar within the 1.35-kb region shared by the two elements. The terminal inverted repeats of *Mu1.7* are 98% identical to the *Mu1* termini, and both elements cause a 9-bp target-site duplication on insertion (Taylor and Walbot 1987). Both elements have long internal direct repeats in addition to the terminal inverted repeats. These two classes of elements are present in ~10–60 copies in mutator stocks (Taylor and Walbot 1987). Two additional elements with *Mu* terminal inverted repeats have recently been shown to transpose and generate 9-bp target-site duplications in mutator stocks. The internal regions of these elements are not related by sequence similiarity to *Mu1* or *Mu1.7* (Chen et al. 1987; Wessler et al. 1988).

Nonmutator maize stocks (i.e., stocks whose pedigrees show no mutator ancestors) contain sequences similar to *Mu* elements (Chandler et al. 1986). These *Mu*-related sequences fall into three classes. First, some maize stocks contain one to a few intact *Mu1*- or *Mu1.7*-like elements (Chandler et al. 1988*b*). Second, all stocks examined have ~40 copies of sequences similar to the *Mu* terminal repeats but not associated with *Mu1* or *Mu1.7* internal sequences. Third, all maize stocks examined have a sequence similar to only internal regions of *Mu1* and *Mu1.7*. Thus, the component parts of *Mu* elements can exist separately in the maize genome. We are interested in the physical relationship of these component parts to each other and to active *Mu* elements. In this paper, we describe the structure and distribution of a *Mu*-related sequence that is highly conserved in maize and related species and that is 97% identical to *Mu1.7* for the 893 nucleotides common to them both. A structural comparison of this sequence, termed *MRS-A* for *m*u-*r*elated sequence, with intact *Mu* elements suggests that *Mu* termini may have transposed independently of internal *Mu* sequences. This may help explain the remarkable diversity exhibited by the mutator family of transposable elements.

Material and Methods

Plant Materials

Maize stocks were obtained from several sources as reported elsewhere (Chandler et al. 1986). Additionally, inbred lines B14A, B73, B85, Mo17, H95, and Va26 were from A. R. Hallauer, Iowa State University. The related species *Zea luxurians*, *Z. mexicana* (SE Balsas teosinte), and *Z. diploperennis* were from W. F. Tracy, University of Wisconsin. *Zea mexicana* (day-neutral teosinte) was from Carolina Biological. A species within the most closely related genus to *Zea, Tripsacum dactyloides,* was obtained from B. Kindiger, University of Missouri. All stocks were propagated by hand self-pollination either in the field or, with supplemental lighting in the greenhouse, in Eugene, Oregon. The tobacco species *Nicotiana tabacum* (var. Turkish Samson) was from H. Bonnett, University of Oregon.

DNA Samples

The *Mu1* plasmid, pMJ9, was obtained from J. Bennetzen (Bennetzen et al. 1984). The *Mu* internal probe A/B5 and the *Mu* terminal probe DTE1 were prepared from pMJ9 as described elsewhere (Chandler et al. 1986). The *Mu1.7* plasmid, pMu1.7, was obtained from L. Taylor, and the *Mu1.7*-specific probe was prepared as described (Taylor et al. 1986). The maize actin plasmid, pMAc1, was from R. Meagher, University of Georgia (Meagher et al. 1983).

The *MRS-A* sequence was isolated from VC391-4, a mutator stock that had been crossed for several generations to a W23/K55 hybrid nonmutator stock. A λ library

was prepared by ligating size-fractionated *Eco*RI-digested total DNA into the *Eco*RI site of λJ1 (Mullins et al. 1984) and packaging the ligation mixture into phage in vitro. The phage were plated and screened for *Mu1*-homologous sequences (Benton and Davis 1977) by using the internal *Mu1* probe. The DNA flanking *MRS-A* was identified by sequencing *Taq*I restriction fragments that had been subcloned into the *Acc*I site of M13 mp10 and M13 mp11 (Messing 1983). Fragments that represented the flanking DNA were cloned into the *Eco*RI/*Hin*dIII sites of pUC8 (Vieira and Messing 1982) and subsequently were purified for use as hybridization probes. Genomic restriction mapping of the progenitors of VC391-4 demonstrated that the cloned *MRS-A* sequence is from the 13-kbp *Eco*RI fragment of W23 (data not shown).

Maize DNA was isolated from the cobs of immature ear shoots and purified by centrifugation in CsCl/ethidium bromide (Rivin et al. 1982). DNA from other species was prepared from leaf tissue or from etiolated seedlings, as described by Dellaporta et al. (1983). *Triticum aestivum* DNA was obtained from J. Litts, Oregon State University. Southern blot hybridizations were performed as described elsewhere (Chandler et al. 1986).

RNA Samples

Total maize RNA was prepared from husk tissue by using guanidinium isothiocyanate as described by Setzer et al. (1980) and was electrophoresed through formaldehyde-agarose gels (Rave et al. 1979). Both transfer to Genetran filters (Plasco) and hybridization conditions were as described for Southern blots (Chandler et al. 1986).

DNA Sequencing

Restriction fragments of *MRS-A* and flanking DNA (385 bp of 5′ DNA and 236 bp of 3′ DNA) were subcloned into M13 mp10 and M13 mp11 (Messing 1983) and sequenced by the dideoxy chain termination method (Sanger et al. 1977) by using the 17-mer universal sequencing primer (Pharmacia). To eliminate band compression that occurred when sequencing GC-rich regions, 7-deaza-dGTP (Boehringer-Mannheim Biochemicals) was used in place of dGTP (Mizusawa et al. 1986). Sequence comparisons were made using the University of Wisconsin Genetics Computer Group software and documentation package (Devereux et al. 1984).

Results

MRS-A Is a Stable Sequence in the Maize Genome

We cloned a copy of a sequence, termed *MRS-A,* that previous studies identified as a sequence in all maize stocks that hybridized weakly to internal *Mu1* probes (Chandler et al. 1986) and that also hybridized to probes specific for *Mu1.7.* We were interested in the relationship of *MRS-A* to intact *Mu* elements and to the multiple copies of *Mu* termini present in all maize lines. Southern blot analysis was performed using *Mu* probes and a probe made from DNA flanking *MRS-A,* to determine the distribution of *MRS-A* in maize lines and related species. A Southern blot utilizing *Hinf*I-digested genomic DNA from several species is shown in figure 1. *Hinf*I fragments of 1.65 and 1.80 kb homologous to the *Mu1* probe (fig. 1, panel A), to the *Mu1.7*-specific probe (fig. 1, panel B), and to the *MRS-A* flanking probe (fig. 1, panel C) are seen in two *Zea mexicana* samples (fig. 1, lanes e and g), *Z. luxurians* (fig. 1, lanes d), *Z. diploperennis* (fig. 1, lanes f), and the *Z. mays* inbred line B37 (fig. 1, lanes i). The 1.65-kb fragment comigrates with the *Hinf*I-digested clones *MRS-A* (fig. 1, lanes

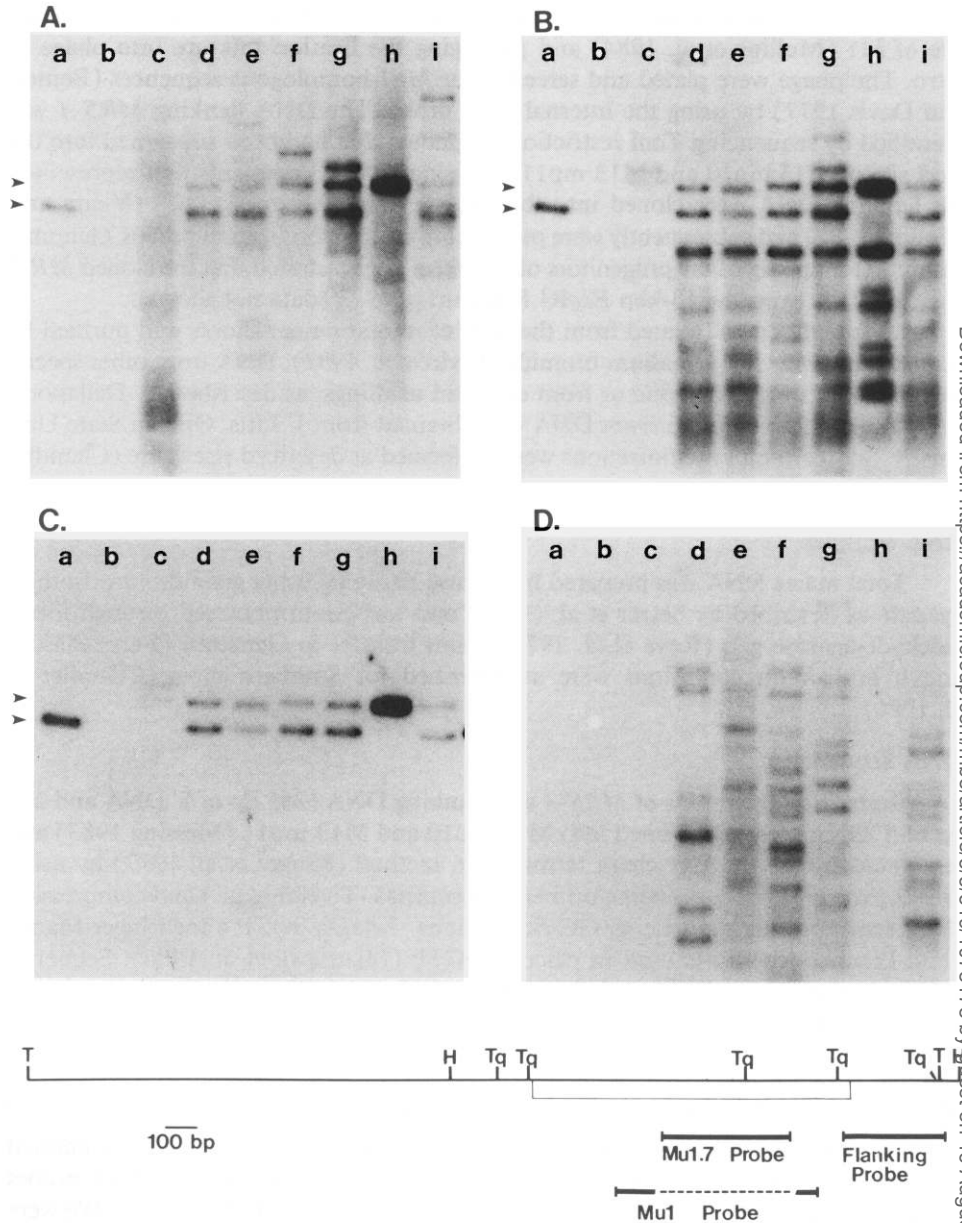

FIG. 1.—Genomic distribution of *MRS-A* in several plant species. *Hinf* I digests of DNA from several plant species was electrophoresed through agarose and blotted onto Genetran. Amount of DNA digested was 4 μg for *Zea* species and 6 μg for *Nicotiana tabacum, Triticum aestivum,* and *Tripsacum dactyloides.* Hybridization was at 16 degrees C below T_m for each probe. Panel A: Blot hybridized to the *Mu1* internal probe. Lane a, Single-copy reconstruction with the cloned 1.65-kb *Hinf* I fragment containing *MRS-A* and flanking DNA; b, *Nicotiana tabacum* (var. Turkish Samson); c, *Triticum aestivum* (var. Cheyenne); d, *Zea luxurians;* e, *Zea mexicana* (SE Balsas teosinte); f, *Zea diploperennis;* g, *Zea mexicana* (day-neutral teosinte); h, *Tripsacum dactyloides;* i, *Zea mays* (inbred B37). Panel B: Same blot hybridized to the *Mu1.7*-specific probe. Panel C: Same blot hybridized to the *MRS-A* flanking probe. The arrowheads in Panels A–C denote the 1.65-kb fragment characteristic of the cloned *MRS-A* and the 1.80-kb fragment which also hybridizes to all three probes in the *Zea* species and *Tripsacum.* Panel D: Same blot hybridized to the *Mu* terminal probe. A partial restriction map of *MRS-A* and flanking DNA, showing regions of homology to the probes, is shown. The restriction sites indicated are as follows: H = *Hinf* I; T = *Tth*III-I; Tq = *Taq*I.

a). Comparison of signal intensities to a single-copy reconstruction with the cloned *MRS-A* (fig. 1, lanes a) suggests that both the 1.65- and 1.80-kb fragments are present at approximately one copy per haploid genome in the *Zea* species. Additional fragments hybridize to both the *Mu1*- and *Mu1.7*-specific probes, although only the 1.65- and 1.80-kb *Hinf* I fragments also hybridize to the *MRS-A* flanking probe. Both the 1.65- and 1.80-kb *MRS-A*-like fragments have been observed in a total of 15 North-American stocks of maize. These include the inbred lines B37, A188, B14A, W23, Mo17, H95, B85, Va26, B73, W22, P39, and C30, in addition to individuals from the varieties Black Mexican Sweet and Wilbur's Knobless Flint. The larger *Hinf* I fragments in B37 DNA (fig. 1, panel A, lane i) have been shown to represent a unique-copy *Mu1* element with differentially modified DNA (Chandler et al. 1986). This modification prevents digestion of the *Hinf* I sites in the *Mu* termini (Chandler et al. 1986).

Approximately two to four copies of the 1.80-kb fragment homologous to all three probes are seen in *Tripsacum dactyloides* (fig. 1, lanes h), although the 1.65-kb fragment characteristic of the cloned *MRS-A* is absent. Similar estimates of *MRS-A* copy number in the *Zea* species and *Tripsacum* were obtained with other restriction enzymes (*Hin*dIII, *Eco*RI, and *Tth*111-I). *Triticum aestivum* DNA (fig. 1, lanes c) shows fragments hybridizing to every probe, although no fragment hybridizes to all three probes, a result suggesting that a complete *MRS-A* sequence may not be in wheat. *Nicotiana tabacum* DNA (fig. 1, lanes b) does not hybridize to any of the probes at this stringency (16 degrees below T_m for each of the three probes).

A probe specific for the *Mu* termini (fig. 1, panel D) hybridizes to multiple fragments in all the *Zea* species (fig. 1, lanes d–g and i). The *Mu* terminal probe does not hybridize to the cloned MRS-A (fig. 1, lanes a) and does not hybridize to a 1.65- or 1.80-kb *Hinf* I fragment in most of the *Zea* species. This probe does hybridize with an ~1.65-kb *Hinf* I fragment in *Z. mexicana* (fig. 1, lanes e); however, results with other enzymes demonstrate that another restriction fragment that hybridizes to *Mu* termini comigrates with the *MRS-A Hinf* I fragment. *Tripsacum dactyloides* (fig. 1, lanes h) contains no sequences that hybridize to the *Mu* terminal probe, yet it contains an *MRS-A*-like sequence. In all experiments, hybridization with the terminal probe was at 16 degrees below T_m; thus, sequences with >84% identity should have been detected (Bonner et al. 1973). Empirically, plasmid reconstructions with cloned *Mu* elements indicate that single-copy sequences that are 88%–90% identical with the *Mu* termini are easily detectable by our conditions (data not shown). These results show that *MRS-A* is highly conserved in the genus *Zea* and is not associated with *Mu* termini. Furthermore, a sequence similar to *MRS-A* is found in *T. dactyloides*, although *Tripsacum* contains no sequences that hybridize to *Mu* termini—and thus no intact *Mu* elements.

MRS-A Is Closely Related to the Transposable Element *Mu1.7*

The conservation of *MRS-A* in the genus *Zea* and in *T. dactyloides*, as well as the absence of *Mu* termini in *Tripsacum*, suggested to us that this DNA may predate *Mu* elements in evolutionary history—and that it might provide insights into the origin of the mutator family of transposable elements. Thus, we sequenced the cloned *MRS-A* as described in Material and Methods. Figure 2 contains a map of *MRS-A* in comparison with a map of the transposable element *Mu1.7* (Taylor and Walbot 1987), and figure 3 contains the DNA sequence of *MRS-A*. There are two major structural differences between *MRS-A* and *Mu1.7* (fig. 2). First, *MRS-A* does not have *Mu* termini or any other terminal repeats. The similarity between *MRS-A* and *Mu1.7*

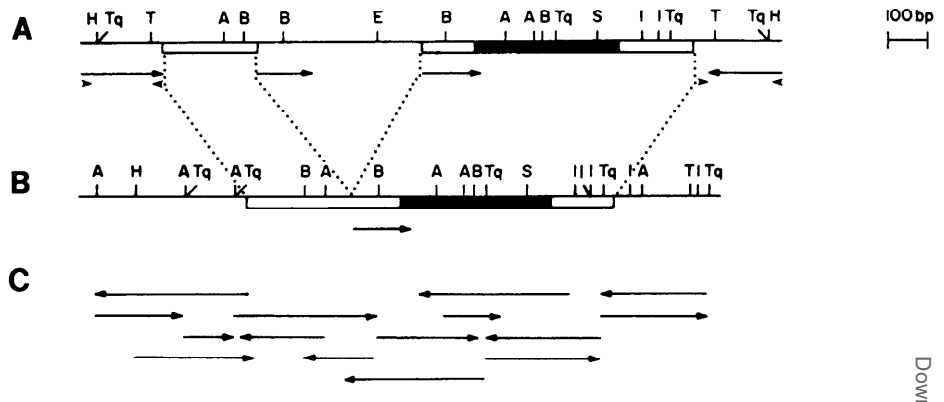

FIG. 2.—Structure of *MRS-A* relative to *Mu1.7*. A: Restriction map of *Mu1.7*. The regions conserved between *Mu1.7* and *MRS-A* are indicated by the open and darkened bars. The darkened bar represents *Mu1.7*-specific sequence; the open bar represents regions shared by *Mu1.7* and *Mu1*. The terminal inverted repeats and the internal direct repeats are indicated by arrows. The location of small (8-bp) terminal inverted repeats of the *Mu1.7* termini are indicated by arrowheads. B: Restriction map of *MRS-A*. The dotted lines represent the junctions of sequence similarity. Note that *MRS-A* has no *Mu* termini and contains an internal deletion relative to *Mu1.7*. The restriction sites indicated are as follows: H = *Hinf* I; Tq = *Taq*I; T = *Tth*111-I; A = *Ava*I; B = *Bgl*I; E = *Bst*EII; S = *Sst*I; I = *Bst*NI. Within the region of similarity, not all restriction sites are conserved. C: Arrows indicate the regions of *MRS-A* that were subcloned and sequenced.

begins 1 bp from the most internal nucleotide of the left terminal repeat of *Mu1.7* and ends 1 bp within the most internal nucleotide of the right terminal repeat, as shown in figure 3. A second structural difference is that, relative to Mu1.7, *MRS-A* has a large (401-bp) segment of DNA missing (fig. 2). *Mu1.7* has an internal direct repeat of 138 bp separated by 263 bp of DNA (fig. 2), and *MRS-A* is missing one of the direct repeats and the 263-bp intervening sequence, suggesting that the deletion occurred by paralogous recombination between the direct repeats. In addition to the major structural differences, *MRS-A* has, relative to *Mu1.7*, three segments of extra DNA of 8, 27, and 9 bp, respectively (fig. 3). Interestingly, the 8- and 9-bp segments are also found in *Mu1* (Barker et al. 1984), while the 27-bp segment is unique to *MRS-A*. The remaining 893-bp region is 97% conserved between *MRS-A* and *Mu1.7*. In this region, *MRS-A* differs from *Mu1.7* by 24 single-base-pair substitutions, four insertions of single nucleotides, two missing nucleotides, and one missing three-nucleotide segment. A 523-bp region is 96% conserved between *MRS-A* and *Mu1*.

A Sequence Hybridizing to *MRS-A* Is Transcribed in Both Mutator and Nonmutator Maize Plants

Taylor and Walbot (1987) reported a poly-A^+ transcript in both mutator and nonmutator maize plants, one that hybridized to the *Mu1.7*-specific probe. Northern blot analysis with total RNA from nine nonmutator plants representing four different genetic backgrounds (W23, P39, Black Mexican Sweet, and Wilbur's Knobless Flint) and from nine mutator maize plants reveals a transcript of ~2.3 kb that hybridizes to both *Mu* internal probes and to the *MRS-A* flanking probe. Four individuals are shown in figure 4, panels A–C. Variation is observed in the amount of transcript per lane, but this does not correlate with mutator activity. On the basis of hybridization to the maize actin gene (fig. 4, panel D), it is apparent that loading differences do not account for the plant-to-plant variation observed for the *MRS-A* transcript. These

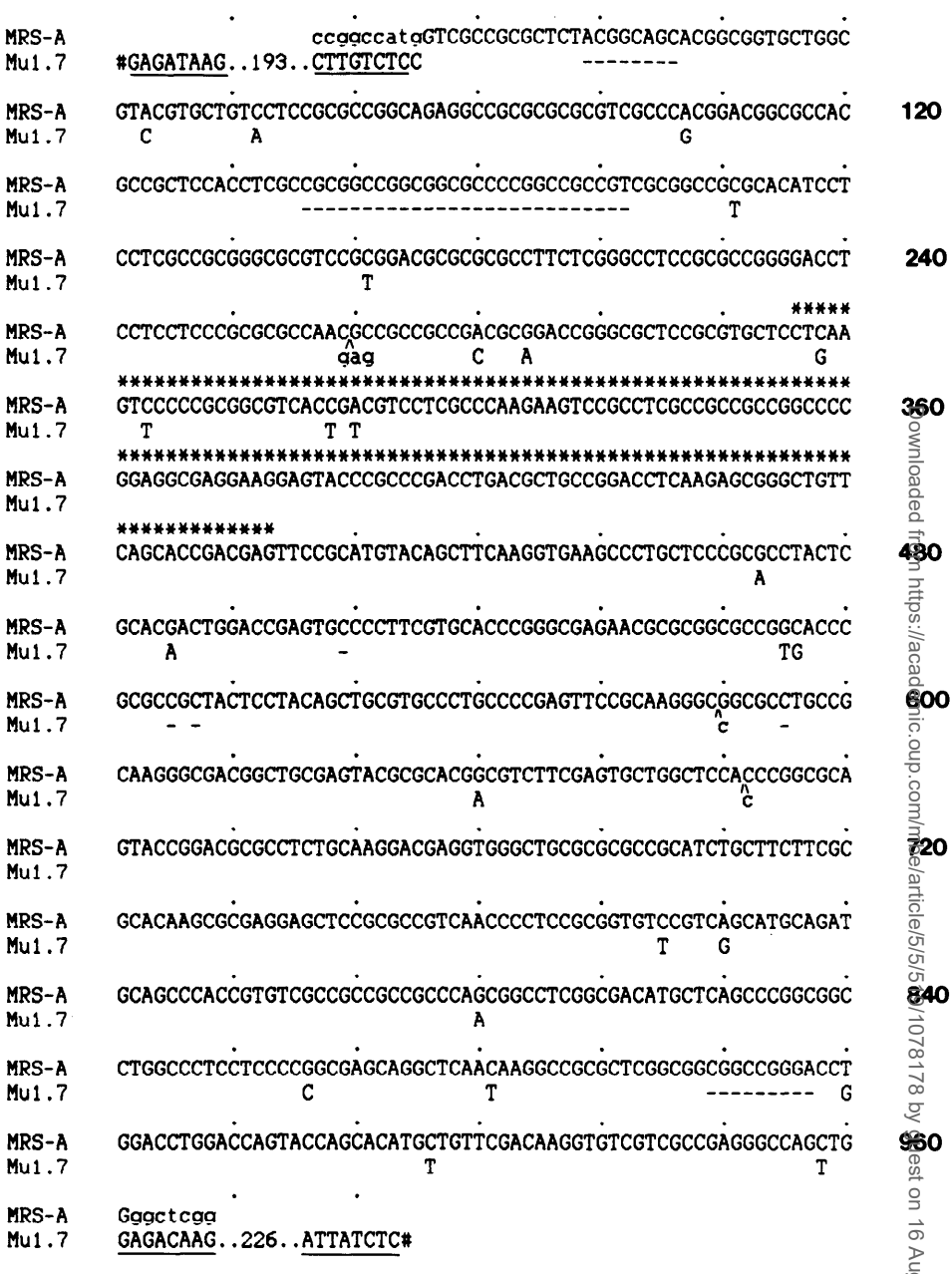

FIG. 3.—DNA sequence of *MRS-A*. DNA flanking the *Mu*-homologous DNA is in lowercase. The asterisks above *MRS-A* represent the sequence that corresponds to the internal direct repeats of *Mu1.7*. The sequence of *Mu1.7* is indicated below the *MRS-A* sequence; sites of conserved nucleotides are left blank, and base substitutions are indicated by capital letters. Regions underlined by dashes represent DNA missing in *Mu1.7* relative to *MRS-A*. Small segments of *Mu1.7* sequence not found in *MRS-A* are indicated by lowercase, and their sites of insertion are indicated by arrows. The sequence of the 8-bp terminal inverted repeats in the *Mu1.7* termini are underlined and shown at their correct positions relative to the internal *Mu1.7* sequence. The number of base pairs that separate each pair of the 8-bp inverted repeats are indicated. # = Ends of the *Mu1.7* element.

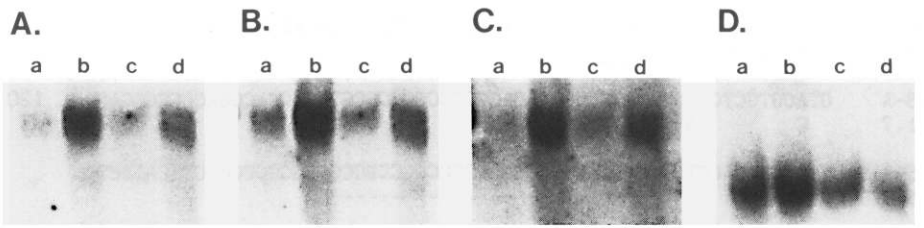

FIG. 4.—RNA sequence hybridizing to *MRS-A* in nonmutator and mutator maize plants. Approximately 10 μg of total leaf RNA from two nonmutator and two mutator maize plants was electrophoresed through agarose-formaldehyde and blotted onto Genetran. Panel A: Blot hybridized to the *Mu1* internal probe. Lanes a and b, nonmutator maize plants; lanes c and d, mutator maize plants. Panel B: Same blot hybridized to the *Mu1.7*-specific probe. Panel C: Same blot hybridized to the *MRS-A* flanking probe. Panel D: Same blot hybridized to a probe specific for the maize actin gene. The *Mu* and *MRS-A* probes used are shown in fig. 1.

results show that the steady-state level of transcript varies among plants; however, a transcript hybridizing to *MRS-A* and its flanking DNA is found in both mutator and nonmutator maize plants. This transcript has also been observed in *Z. luxurians, Z. diploperennis,* and *T. dactyloides* (data not shown).

Discussion

MRS-A does not have the structure and distribution of a transposable element. It lacks the large terminal inverted repeats characteristic of *Mu* elements and is not flanked by direct repeats indicative of a target-site duplication. *MRS-A* is found in all maize stocks and related *Zea* species tested and is flanked by the same DNA in all stocks, suggesting it is a stable sequence in the maize genome. Additionally, in the inbred lines W22 and W23, *MRS-A* maps to the same genomic location, segregating with markers on the short arm of chromosome 2 (V. L. Chandler, unpublished data). Although *MRS-A* does not appear to be a transposable element, 97% of the 893 nucleotides aligned to *Mu1.7* are conserved. Relative to *Mu1.7,* structural differences in *MRS-A* include (1) an apparent 401-bp deletion that may have arisen by paralogous recombination between the direct repeats and (2) three small segments of extra DNA. Two of these extra segments are also found in *Mu1,* and a 523-bp region is 96% conserved between *MRS-A* and *Mu1.* Thus, *Mu1, Mu1.7,* and *MRS-A* are homologous.

An RNA transcript hybridizing to *MRS-A* and its flanking DNA is found in both mutator and nonmutator plants. The transcript must derive from either the 1.65- or 1.80-kb *Hinf* I fragment observed on Southern blots, since these are the only DNA fragments hybridizing to all three probes (i.e., *Mu1, Mu1.7*-specific, and *MRS-A* flanking probes). Two hypotheses are apparent for explaining the existence and conservation of an *MRS-A* homologous transcript. First, *MRS-A* may represent an *Mu1.7* element that inserted into a gene, suffered rearrangements, and is now being transcribed from that gene's promotor. Second, *MRS-A* may be related to a part of a gene that was encompassed by *Mu* termini to generate a *Mu1.7* element. The first hypothesis requires that the *Mu* termini must have excised from a *Mu1.7* element after the original insertion. However, our sequencing data are not consistent with this hypothesis in that there are no direct repeats flanking *MRS-A,* as would be expected for a transposable-element insertion. Furthermore, Southern blot analysis suggests that *MRS-A* may predate *Mu* elements in evolutionary history in that a sequence similar to *MRS-A* is found in *Tripsacum dactyloides,* although this species does not contain intact *Mu*

elements. For these reasons, we favor the second hypothesis; that is, a sequence closely related to *MRS-A* was encompassed by *Mu* termini to generate an *Mu1.7* transposable element. Deletion of a portion of *Mu1.7* might then generate an *Mu1* element, as proposed by Taylor and Walbot (1987). Thus, we suggest that *MRS-A, Mu1.7,* and *Mu1* all derived from a common ancestor.

Both of these hypotheses require that *Mu* termini were once able to function independently of the *Mu1.7* or *Mu1* internal regions. The sequence of *Mu1.7* lends credence to this hypothesis in that its large terminal inverted repeats also contain small imperfect inverted repeats (figs. 2, 3). This is similar to IS sequences in bacteria (Iida et al. 1983) and may have allowed for transposition of the termini alone, as is the case for IS sequences. Consistent with this idea, the large *Mu* terminal inverted repeats have been found flanking a wide array of internal sequences. An element termed *Mu3* that was cloned owing to its presence in the *Adh1* gene shares sequence similarity with *Mu1* and *Mu1.7* only at its termini (Chen et al. 1987), and an element cloned from the *wx-mum5* allele isolated by Don Robertson also contains the large *Mu* termini flank, but the termini are an internal sequence unrelated to *Mu1* or *Mu1.7* (Wessler et al. 1988). In addition, multiple restriction fragments that hybridize to *Mu* termini but are not associated with *Mu1, Mu1.7,* or *Mu3* internal regions are found in both mutator and nonmutator maize lines (Chandler et al. 1986). Several cloned representatives of this class have been shown to have the structure and distribution expected of *Mu* transposable elements (Talbert et al. 1987; Chandler et al. 1988*a*). We speculate that a progenitor sequence similar to *MRS-A* was encompassed by the large *Mu* termini to generate an ancestral *Mu1.7* transposable element. Thus, *Mu1.7* may be analogous to the class of bacterial transposons that is flanked by IS elements in inverted orientation (Iida et al. 1983). The other *Mu* elements with internal sequence different from *Mu1* and *Mu1.7* may have been generated via a similar mechanism, since there are sequences related to their internal regions not associated with *Mu* termini (V. L. Chandler, unpublished data). Studies are in progress to determine whether these *Mu*-related sequences bear the same relationship to intact elements as does *MRS-A* to *Mu1* and to *Mu1.7.*

The regulation of *Mu* element transposition is not understood. The *Ac/Ds* and *Spm* families of maize transposable elements are better characterized, and internal regions of autonomous *Ac* and *Spm* elements are known to encode factors required for transposition (Fedoroff 1983; Döring and Starlinger 1986). The hypothesis that independently transposing *Mu* termini may have encompassed genomic DNA to generate new transposable elements suggests that the internal regions of *Mu* elements may not encode factors necessary for transposition. Additionally, the 220-bp *Mu* termini may not be large enough to encode a transposase. For instance, the transposase gene encoded by the *IS10* component of the bacterial transposon *Tn10* is 1.2 kb (Halling et al. 1982). Thus, one hypothesis is that genomic sequences physically separated from *Mu* elements may encode the enzymes necessary for transposition.

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