STABLE NON-MUTATOR STOCKS OF MAIZE HAVE SEQUENCES HOMOLOGOUS TO THE *Mu1* TRANSPOSABLE ELEMENT

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Manuscript received March 17, 1986
Revised copy accepted July 31, 1986

ABSTRACT

Mutator stocks of maize produce mutants at many loci at rates 20- to 50-fold above spontaneous levels. Current evidence suggests that this high mutation rate is mediated by an active transposable element system, *Mu*. Members of this transposable element family are found in ~10–60 copies in Mutator stocks. We report here an initial characterization of previously undetected sequences homologous to *Mu* elements in eight non-Mutator inbred lines and varieties of maize that have a normal low mutation rate. All stocks have ~40 copies of sequences homologous only to the terminal repeat and show weak homology to an internal probe. In addition, several of the stocks contain an intact *Mu* element. One intact *Mu* element and two terminal-specific clones have been isolated from one non-Mutator line, B37. The cloned sequences have been used to demonstrate that in genomic DNA the intact element, termed *Mu1.4B37*, is modified, such that restriction sites in its termini are not accessible to cleavage by the *Hin*fI restriction enzyme. This modification is similar to that observed in Mutator lines that have lost activity. We hypothesize that the DNA modification of the *Mu*-like element may contribute to the lack of Mutator activity in B37.

TRANSPOSABLE elements provide a source of genomic variation both within the lifetime of an organism and throughout evolution. They produce mutations in both prokaryotes and eukaryotes by inserting within or near genes or by causing rearrangements (reviewed in SHAPIRO 1983). Most organisms examined have repetitive sequences with the structure characteristic of transposable elements. However, it is only within a few genetically characterized species that transposons active within the lifetime of an organism have been described and their regulation analyzed. In *Zea mays*, many active transposable element systems have been described genetically, and several have been characterized at the molecular level. One of these is the highly active system Robertson's Mutator.

ROBERTSON (1978) described a genetic stock of maize with a mutation rate 20- to 50-fold above the spontaneous level. Genetic and molecular studies (reviewed in FREELING 1984) suggest that the high mutation rate is mediated

by a transposable element system. A ~1.4-kbp element, *Mu1*, was cloned from Mutator-induced mutations at *Adh1* (BENNETZEN *et al.* 1984) and *A1* (O'REILLY *et al.* 1985), and closely related elements have been identified in Mutator-induced mutations in other genes (TAYLOR, CHANDLER and WALBOT 1986; FREELING 1984).

In addition to 1.4-kbp *Mu1*-like elements (designated *Mu1.4*) a larger 1.7-kbp-size class is detected by hybridization with a *Mu* probe (BARKER *et al.* 1984). The 1.7-kbp element, termed *Mu1.7*, contains an additional 380 base pairs (bp) of unique DNA, as well as changes in a few restriction sites relative to *Mu1* (L. TAYLOR and V. WALBOT, unpublished results). This larger element is also capable of transposing (TAYLOR, CHANDLER and WALBOT 1986; ALLEMAN and FREELING 1986). The ratio of *Mu1.4* and *Mu1.7* elements varies considerably in different lines (WALBOT, CHANDLER and TAYLOR 1985; ALLEMAN and FREELING 1986), and the total copy number of *Mu* elements varies between ~10–60 copies in Mutator stocks (BENNETZEN 1984). It is not known if either element encodes all the factors required for their transposition, or if additional factors are required for mobilization.

Transposable-element-induced mutations are frequently unstable, leading to secondary mutations or reversion events after excision. While it has been postulated that the sequence divergence generated by the insertion and imprecise excision of plant transposable elements plays an important role in creating DNA sequence diversity in evolution (SCHWARZ-SOMMER *et al.* 1985), the rampant movement of a transposon family is clearly deleterious. Hybrid dysgenesis mediated by the *P* elements of Drosophila (KIDWELL, KIDWELL and SVED 1977; BINGHAM, KIDWELL and RUBIN 1982) is one example; the Mutator system in maize is another. Thus, the regulation of an active system has important implications for the viability of the organism and the maintenance of the transposon.

Recently, a correlation has been observed between an increased level of DNA modification (probably 5-methyldeoxycytosine) of *Mu* elements and loss of Mutator activity (WALBOT, CHANDLER and TAYLOR 1985; CHANDLER and WALBOT 1986; BENNETZEN 1985). Plants have been identified that have a decreased transmission of Mutator activity. A molecular characterization of the *Mu* elements within these stocks demonstrates extensive DNA modification suggesting that the elements are inactivated by this mechanism. DNA modification of transposable elements may alter their activities in a heritable but reversible manner, serving as one mechanism of regulating a potentially deleterious activity. Methylation of other transposable element systems in maize has also been reported (FEDEROFF, WESSLER and SHURE 1983; P. CHOMET and S. DELLAPORTA, personal communication), and adenosine methylation of the bacterial transposon Tn*10* decreases its transposition frequency (ROBERTS *et al.* 1985).

All maize stocks tested contain sequences homologous to the transposable elements, *Ac* and *Spm* (FEDEROFF, WESSLER and SHURE 1983; O'REILLY *et al.* 1985), even though most show no genetic activity characteristic of these elements. We investigated non-Mutator stocks of maize and determined that there

are multiple sequences homologous to *Mu* termini and at least one sequence with weak homology to internal regions. Many non-Mutator stocks also have a small number of elements with a sequence and structure highly homologous to Mutator transposons, and these elements may be modified in the same manner as *Mu* elements are in inactive Mutator stocks.

MATERIALS AND METHODS

Maize stocks: The maize stocks were obtained from various sources. The inbred stocks W23 and Ky21 were obtained from E. COE (University of Missouri), W22 from J. KERMICLE (University of Wisconsin), A188 from Pfizer Inc., and B37 and B73 from Pioneer Hi-Bred International. Wilbur's Knobless Flint was obtained from M. G. NEUFFER (University of Missouri), and Black Mexican Sweet was from the Maize Genetic Cooperation Stock Center (Champaign-Urbana, Illinois). All stocks were propagated in our laboratories by sib or self-hand pollinations. The Mutator stock used in these experiments is a bulked purple aleurone stock obtained from D. S. ROBERTSON (Iowa State University), maintained by backcrossing into a W23/K55 hybrid background.

DNA samples: The *Mu1* plasmid, pMJ9, was obtained from J. BENNETZEN (BENNETZEN *et al.* 1984). The plasmid pA/B5 was prepared by subcloning the 650-bp *Ava*I/*Bst*NI internal fragment of *Mu1*; it was isolated and the termini were treated with T4 DNA polymerase in the presence of all four deoxyribonucleotides to remove the overhanging bases (ENGLUND 1971). The insert was then subcloned in the *Sma*I site of pUC8 (VIEIRA and MESSING 1982). The 650-bp insert can be removed from the vector sequences by digesting with *Eco*RI and *Bam*HI. The plasmid pDTE1 was prepared by subcloning the 202-bp *Hin*dIII/*Mlu*I fragment of *Mu1*. This fragment contains most of one terminal repeat and 15 bp of *Adh1* sequence. The *Mlu*I terminus was treated with T4 DNA polymerase as described above, and the *Hin*dIII/*Mlu*I fragment was subcloned in the *Hin*dIII/*Sma*I site of pUC8. The insert can be removed from the vector sequences by digesting with *Hin*dIII and *Eco*RI. The maize ars sequence, pZmA3111 (described in RIVIN 1986), was provided by R. BERLANI.

Maize DNA was isolated from the cobs of immature ear shoots and purified by centrifugation in CsCl/ethidium bromide (RIVIN, ZIMMER and WALBOT 1982).

Southern blot hybridizations: Maize DNA was digested for 2 hr with a fourfold excess of restriction enzyme (Pharmacia, BRL, New England Biolabs, IBI; buffers and temperatures according to suppliers' instructions), electrophoresed through agarose gels and transferred to Genetran (Plasco) by the method of SOUTHERN (1975). After baking, the filters were washed for 2 hr at 65° in Wash buffer (15 mM sodium chloride, 1.5 mM sodium citrate and 0.1% $NaDodSO_4$). Filters were prehybridized overnight in Hybridization solution [50% formamide (EM Science), 450 mM sodium chloride, 45 mM sodium citrate, 0.05% Ficoll 400, 0.05% polyvinylpyrrolidone, 0.05% Bovine serum albumin, 1% $NaDodSO_4$, 100 μg/ml salmon sperm DNA (Sigma)] at 42°. Hybridizations were then carried out in the same buffer with the addition of 5% dextran sulfate (Pharmacia) and 2×10^6 cpm/ml radioactively labeled fragments for 24–36 hr at 42°. DNA fragments used as probes were isolated from the appropriate plasmid by restriction endonuclease digestion and electrophoresis in low melting-temperature agarose (BRL), extracted with phenol and precipitated with ethanol. Fragments were labeled with [α-^{32}P]dCTP (New England Nuclear) to a specific activity of 2×10^8–10^9 cpm/μg by a random hexamer (Pharmacia) primer reaction (FEINBERG and VOGELSTEIN 1983). Unreacted nucleotide triphosphates were removed by spin dialysis (NEAL and FLORINI 1973) through Bio-Gel P-10 resin (Bio-Rad).

After hybridization, Genetran filters were washed once in 50% formamide, 600 mM sodium chloride, 60 mM sodium citrate, 0.2% $NaDodSO_4$ at 42° for 30 min, followed by three washes in Wash buffer at 65° for 45 min each. Filters were exposed to XAR film (Kodak) at −80°, using Lightning Plus Intensifying screens (Dupont). For sequen-

tial hybridizations the probe was removed by washing the filter at 95° in Wash buffer for three 15-min washes.

Restriction digests of plasmid and phage DNA were electrophoresed on agarose gels, transferred to nitrocellulose (SOUTHERN 1975), baked and prehybridized for 6 hr in Hybridization solution. Hybridizations were carried out in the same buffer with 10^6 cpm/ml nick-translated (RIGBY *et al.* 1977) plasmid sequences for 6–12 hr. The filters were washed twice in Wash buffer at 65° for 30 min each, and autoradiography was performed as described above.

Slot blots: Denatured genomic DNA samples and a plasmid DNA dilution series were loaded onto nitrocellulose filters through a slotted template (RIVIN 1986). The filters were baked and hybridized as described above for the phage DNA Southern blots, and autoradiography was performed on preflashed film. The hybridization signal was measured on several exposures using a laser densitometer, taking care that the signals were within the linear range. Rehybridization of filters with a maize repeated sequence, pZmA3111, that does not vary in copy number among maize varieties was used to control for loading errors (RIVIN, CULLIS and WALBOT 1986). The plasmid dilution series was used to calibrate the densitometric signal expected from a specific quantity of *Mu1* homologous sequences in the nuclear DNA samples. The fraction of the genome homologous to the probe can be expressed as genomic signal/plasmid signal × plasmid amount/genomic amount × fraction of probe homologous to the plasmid (CULLIS, RIVIN and WALBOT 1984; RIVIN 1986). The number of copies of *Mu* internal and terminal sequences were then estimated as genome size (kbp) × fraction homologous DNA/sequence size (kbp). For calculating *Mu* sequence copy numbers, a genome size measurement of 5.5×10^6 kbp was used (GALBRAITH *et al.* 1983). This technique is described in detail in RIVIN (1986).

RESULTS

Every maize stock tested has sequences homologous to *Mu1*: We have used the cloned *Mu1* element (BENNETZEN *et al.* 1984) as a hybridization probe to look for cross-hybridizing sequences in eight non-Mutator stocks of maize. All materials used came from pedigreed stocks propagated by hand pollination, and the non-Mutator stocks have no pedigree record of ever having been crossed by a Mutator stock.

Two subclones of *Mu1* containing either the terminal repeated sequence or a 650-bp internal fragment (see map, Figure 1A) were hybridized to Southern blots and to slot blots of DNA from maize inbred lines and varieties. For each inbred line and variety we have examined at least three different DNA preparations from several individuals or from pooled samples. All samples of each genotype show a restriction pattern and copy number of *Mu* sequences characteristic of that line.

DNA samples from Mutator and non-Mutator stocks were digested with *Eco*RI, an enzyme that does not cleave within *Mu1*, and Southern blots were prepared, hybridizing with the internal and terminal *Mu1* probes. Figure 1B shows the results of hybridization with the internal (*Ava*I/*Bst*NI) probe. The Mutator stock (lane b) contains approximately 20 restriction fragments homologous to the internal portion of *Mu1*, whereas the non-Mutator stocks (lanes c–i) contain only 1–3 cross-hybridizing fragments. Since the amounts of DNA in each lane are not equal, we cannot accurately determine the copy number of each homologous sequence from this experiment. We estimate that the stocks shown in lanes c(WKF), d(B37), h(W22) and i(W23) each contain a

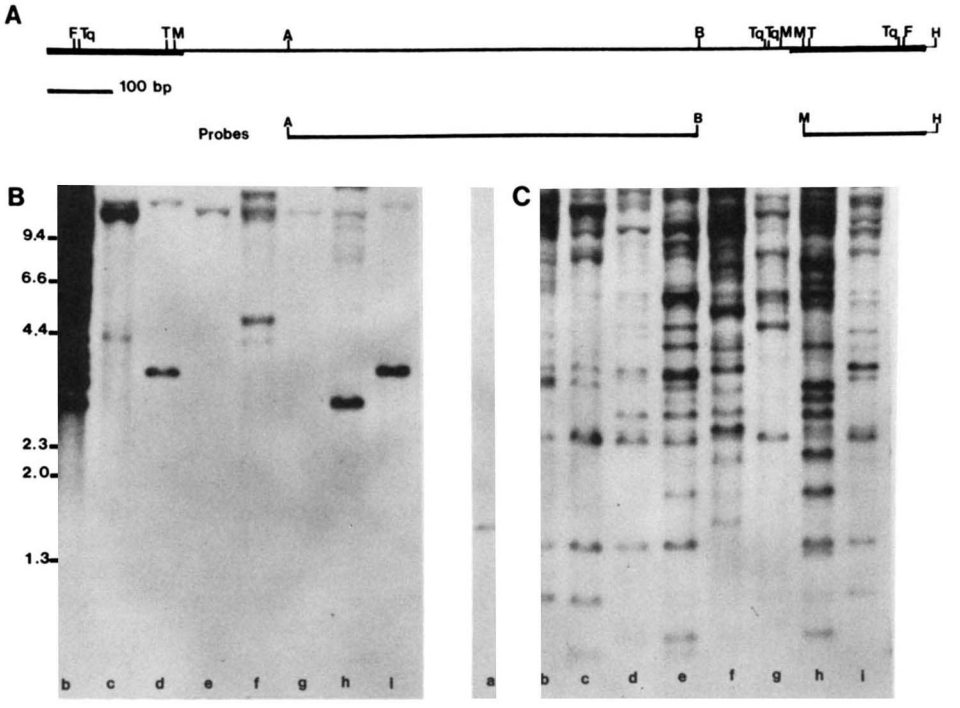

FIGURE 1.—*Eco*RI digests on DNA from non-Mutator maize plants. A, Simplified map of *Mu1*. The restriction enzymes used in this study are F, *Hin*fI; H, *Hin*dIII; T, *Tth*111-I; Tq, *Taq*I; A, *Ava*I; B, *Bst*NI; M, *Mlu*I. The internal probe is an *Ava*I/*Bst*NI fragment, and the terminal probe is a *Hin*dIII/*Mlu*I fragment, each subcloned as described in MATERIALS AND METHODS. B, DNA samples (ranging in concentration from 4 to 10 μg were digested to completion with *Eco*RI, electrophoresed through a 0.5% agarose gel, transferred to Genetran and hybridized with the internal probe, as described in MATERIALS AND METHODS. Lane a: a three-copy reconstruction with *Mu1* plasmid DNA, assuming 8 μg of genomic DNA per lane. Lane b: Mutator stock. Lanes c–i: non-Mutator inbred lines and varieties of maize; c, Wilbur's Knobless Flint (WKF); d, B37; e, B73; f, A188; g, Ky21; h, W22; i, W23. Size standards are marked in kilobase pairs (kbp). C, Same blot as in B; the internal probe was removed, and the DNA on the blot was rehybridized with the terminal probe.

strongly cross-hybridizing sequence at approximately one copy per haploid genome, whereas the other DNA samples from non-Mutator stocks contain one or more weakly hybridizing fragments. The blot was washed to remove the internal probe, and the DNA was rehybridized with the terminal-specific probe (Figure 1C). This probe hybridized to multiple restriction fragments in the Mutator and non-Mutator stocks. Comparison of the two hybridizations shows that some of the fragments homologous to the internal portion of the *Mu1* element also hybridize to the terminal probe, whereas there are many more fragments with homology only to the terminal probe in both Mutator and non-Mutator stocks.

These results were confirmed and additional stocks tested by slot blot hybridization. For the experiment shown in Figure 2, 1-μg samples of genomic

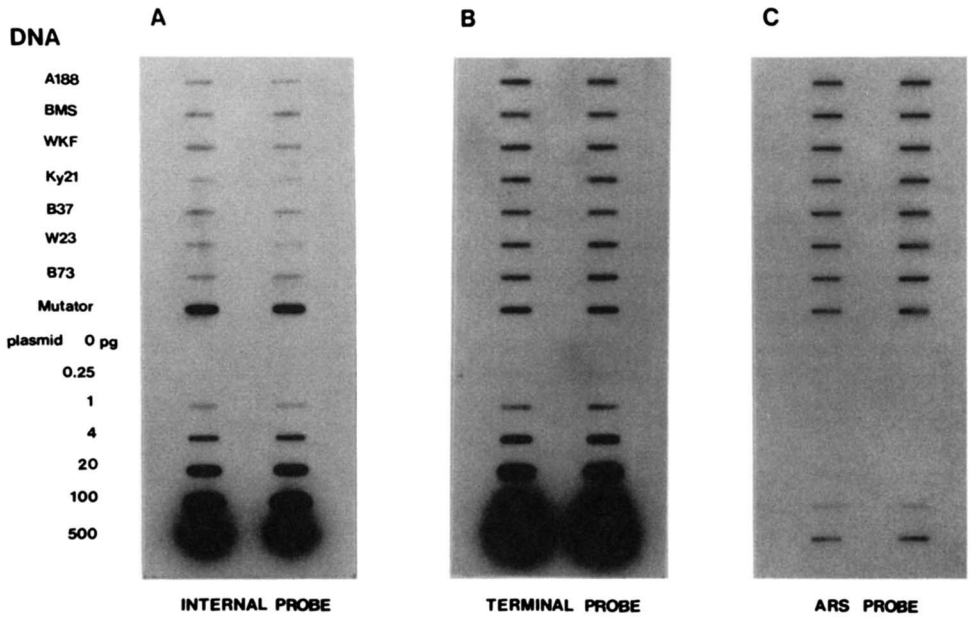

FIGURE 2.—Slot blot analysis on DNA samples from non-Mutator maize plants. Each filter contains duplicate DNA samples (1 μg) as labeled. Abbreviations: BMS, Black Mexican Sweet; WKF, Wilbur's Knobless Flint. The concentration of each DNA sample was determined spectrophotometrically, and aliquots were denatured and applied to nitrocellulose strips through a slotted template. After blotting, the filters were baked and hybridized, as described for Southern blots in MATERIALS AND METHODS. Several autoradiographic exposures were prepared on each filter in order to perform the densitometric scans in the linear range. Results were normalized to the ars signal (a repeated sequence that does not vary in copy number among inbred lines and varieties) to compensate for slight loading differences. The signal from the Mutator stock was 20 times that of the non-Mutator stocks when hybridized with the internal probe and about 1.5 times that of the non-Mutator signal when probed with the terminal sequence. The hybridization signal from the plasmid dilution series was compared with the genomic DNA samples to calculate the approximate copy number of each sequence. A signal equivalent to 1 pg of *Mu1* plasmid corresponds to about two copies of the *Mu1* internal sequence per haploid genome (panel A) and to about 15 copies of the terminal sequence per haploid genome (panel B). The calculations are described in MATERIALS AND METHODS.

DNA from various maize stocks were denatured and applied to nitrocellulose strips. Samples of *Mu1* plasmid DNA (0–500 pg) were mixed with 1 μg of salmon sperm DNA, denatured and loaded on the nitrocellulose strips to provide copy number standards. Replicate filters were hybridized with the terminal and internal *Mu* probes, and the hybridization signals were measured by densitometry. Loading errors were controlled for by rehybridizing the filters with the ars probe, pZmA3111, a highly repeated maize sequence that does not vary in copy number among individual plants or inbred lines (RIVIN, CULLIS and WALBOT, 1986). Since the amount of homologous DNA in the bound sample controls the rate of hybridization, the differences in hybridization, intensity between genomic samples reflect differences in the copy numbers of *Mu*-homologous sequences in various genetic stocks. An estimation of the copy

numbers was made by comparing the genomic samples with the plasmid dilution series (see MATERIALS AND METHODS).

The results show that the internal portion of the *Mu1* element is represented in about one to three copies per haploid genome in each non-Mutator stock and ~20 times in the Mutator stock. The terminal sequence is more highly represented with ~40 copies in all the non-Mutator stocks and ~60–70 copies in the Mutator genome. (For details on the calculations, see MATERIALS AND METHODS). Taken together, the slot bot and Southern blot experiments suggest that all the stocks contain multiple copies of sequences homologous to *Mu1* termini, as well as at least one sequence with partial homology to the internal sequence. In addition, several stocks such as B37, W23, WKF and W22 have one sequence per haploid genome that is highly homologous to both *Mu1* internal and terminal sequences.

The observation that the terminal sequences of *Mu1* are found more frequently in the genome than are internal sequences is one that has been made for many transposable element systems. In maize, the internal portion of the *Ac* transposable element hybridizes to four to ten distinct homologous sequences in all maize stocks tested, whereas terminal *Ac* probes recognize many more sequences (DÖRING, TILLMAN and STARLINGER 1984; FEDEROFF, WESSLER and SHURE 1983). Similar results have been reported for yeast, Drosophila and bacterial transposons (SHAPIRO 1983). In these systems, pairs of termini that lack the internal transposon sequences can be shown to transpose in the presence of complete elements. We do not know if this is also the case for *Mu1* terminal sequences.

Structure of *Mu1* homologous sequences: To determine if *Mu1* structure, as well as homology, was conserved among these different non-Mutator stocks, DNA samples were digested with *Tth*111-I and *Taq*I, enzymes that cleave within the terminal repeats of the *Mu1* element (see map, Figure 1A). Fragments hybridizing with the internal probe were compared with *Tth*111-I and *Taq*I digests of *Mu1* DNA. Five of the seven stocks examined yielded *Tth*111-I fragments characteristic of *Mu1* (Figure 3A). Additional fragments were also seen in every stock. Reconstruction experiments using the cloned *Mu1* fragment were consistent with one to two copies of sequences homologous to the internal portion of *Mu1* in each line (data not shown). Similar results were observed with *Taq*I digestions (Figure 3B). Three of the seven lines contain a fragment characteristic of *Mu1*. These results suggest that the lines B37, W23 and A188 contain a structure much like *Mu1*, with both the *Tth*111-I and *Taq*I sites conserved. The Northern flint-type varieties, Wilbur Knobless Flint and Black Mexican Sweet, have retained the *Tth*111-I sites, but have an additional *Taq*I site in the *Mu1*-like element producing a smaller restriction fragment. The W22 inbred has a strong homology to *Mu1*, but contains *Tth*111-I and *Taq*I hybridizing fragments larger than *Mu1*. Digests with *Mlu*I and *Bst*NI also indicate that this line carries a larger element that is similar to *Mu1* but may be more closely related to the larger element *Mu1.7*, frequently observed in Mutator stocks (L. TAYLOR and V. WALBOT, unpublished results). For sim-

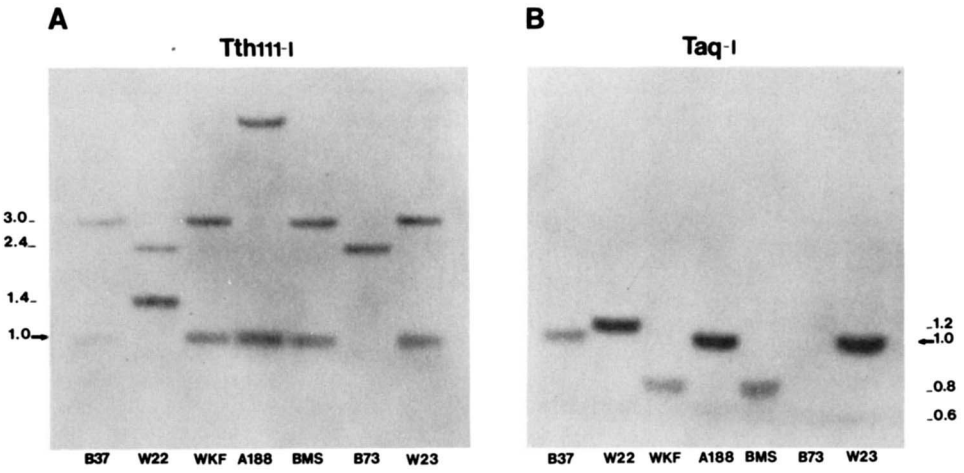

FIGURE 3.—*Tth*111-I and *Taq*I digests on DNA samples from non-Mutator stocks. DNA samples were prepared from the indicated maize stocks, digested with *Tth*111-I (Panel A) or *Taq*I (Panel B), electrophoresed through a 1% agarose gel, transferred to Genetran and hybridized with the internal probe. The arrows point to the size fragment characteristic of *Mu1*; all sizes are in kilobase pairs.

plicity of discussion, we shall refer to these *Mu1*-homologous sequences in non-Mutator stocks as endogenous *Mu* sequences.

Isolation of endogenous *Mu* sequences from B37: To characterize the endogenous *Mu* sequences in more detail, *Mu1*-homologous sequences were isolated from a genomic λ phage library of nuclear DNA from the stable inbred line B37. The library was prepared by partially digesting B37 DNA with *Sau*3A; isolating 15- to 20-kbp fragments from NaCl gradients, ligating with *Bam*HI-digested λJ1 arms (MULLINS *et al.* 1984) and packaging into phage *in vitro* (HOHN 1979). The resulting phage were plated on *E. coli* (strain CES201) and screened for *Mu1*-homologous sequences (BENTON and DAVIS 1977) using the entire *Mu1* element. Three separate phage with unique *Mu1* homologous sequences were isolated and further analyzed. DNA was isolated from each phage and digested with *Hin*fI, and a Southern blot was prepared hybridizing sequentially with the internal and the terminal-specific probes (Figure 4). One phage contained a 1.3-kbp *Hin*fI fragment that hybridized with both the internal- and terminal-specific probes and is the size characteristic of *Mu1*. We have termed this endogenous *Mu* element *Mu1.4B37*. The other two phage each contained small *Hin*fI fragments that hybridized only to the terminal probe.

A series of restriction digestions was done to compare the cloned *Mu1.4B37* and *Mu1* elements. Of 28 restriction sites examined, only one difference was found (see map, Figure 5). *Mu1.4B37* is missing a *Cla*II (*Ava*II) site near its right terminal repeat; otherwise *Mu1* and *Mu1.4B37* appear to be identical in structure.

The endogenous *Mu1.4B37* element is modified in B37 DNA: To test for DNA modification, the restriction map of the cloned *Mu1.4B37* element and

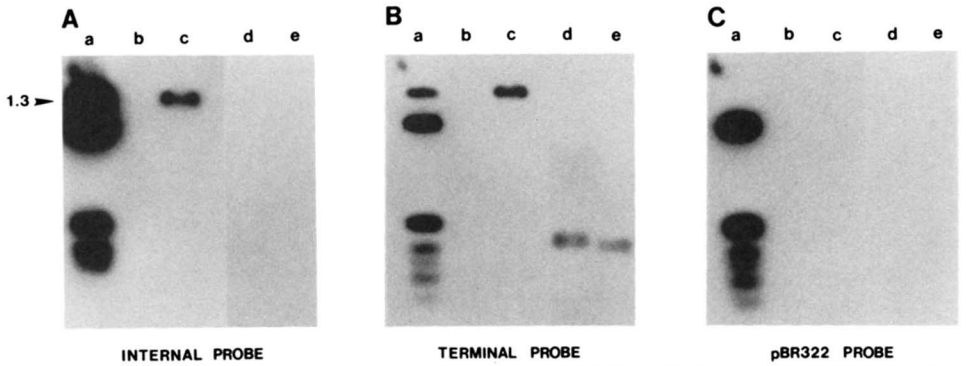

FIGURE 4.—*Mu1*-homologous sequences cloned from B37 DNA. DNA was prepared from plate lysates of recombinant phage. Approximately 0.5 μg of each DNA sample was digested with *Hin*fI, electrophoresed on a 0.8% agarose gel and transferred to two nitrocellulose filters (MANIATIS, FRITSCH and SAMBROOK 1982). One blot was hybridized with a plasmid containing the internal probe (panel A), the other with pBR322 (as a control to confirm that the maize-λ DNA clones contained no plasmid DNA (panel C). After autoradiography, the pBR322 probe was removed and the blot was rehybridized with the terminal probe (panel B). Lane a: the *Mu1* plasmid digested with *Hin*fI. The top restriction fragment is the 1.3-kbp *Mu1* fragment; the other fragments are pBR322 vector sequences. Lane b: λJ1 vector DNA. Lanes c–e: DNA samples from the three unique maize-λJ1 recombinant phage.

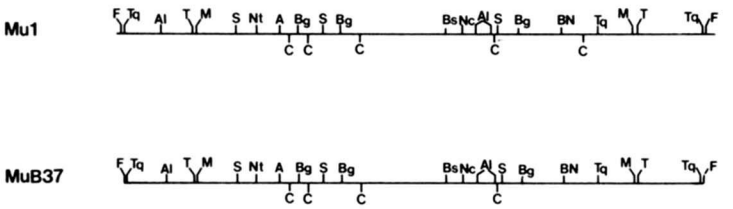

FIGURE 5.—Restriction maps of *Mu1.4B37* and *Mu1*. Subcloned DNA samples containing either the *Mu1* or *Mu1.4B37* elements were digested with the indicated enzymes and were electrophoresed on agarose or acrylamide gels in side-by-side comparisons. The sequence of *Mu1* (BARKER *et al.* 1984) indicates additional sites for several enzymes that produce small fragments that were not detected by gel electrophoresis in these experiments. Only the sites directly mapped and compared in *Mu1* and *MuB37* were indicated on the map. F: *Hin*fI, Tq: *Taq*I, T: *Tth*111-I, Al: *Alu*I, M: *Mlu*I, A: *Ava*I, C: *Cla*II (AvaII), Bg: *Bgl*I, S: *Sst*II, Bs: *Bst*EII, BN: *Bst*NI, Nt: *Not*I, Nc: *Nco*I.

flanking sequences was compared with the restriction map generated from digestions of genomic DNA, examined on Southern blots. The genomic Southern blots yielded the size fragments predicted from the cloned sequences for most enzymes tested; however, *Hin*fI was an exception. Digestion of B37 DNA with *Hin*fI yielded fragments larger than the 1.3 kbp predicted from the cloned sequences. The inhibition of *Hin*fI digestion of *Mu* elements, and the complete digestion by *Tth*111-I and *Taq*I, is identical to the DNA modification we observed in *Mu* elements from inactive Mutator lines (CHANDLER and WALBOT 1986). We hypothesize that the *Mu1.4B37* element is modified *in vivo* inhibiting *Hin*fI digestion. This was confirmed by using a DNA sequence flanking the *Mu1.4B37* element as a probe on genomic DNA Southern blots. *Eco*RI, *Tth*111-I and *Hin*fI digestions of B37 DNA are shown in Figure 6. The 850-bp *Hin*fI/*Eco*RI fragment adjacent to *Mu1.4B37* (on the right of the map as

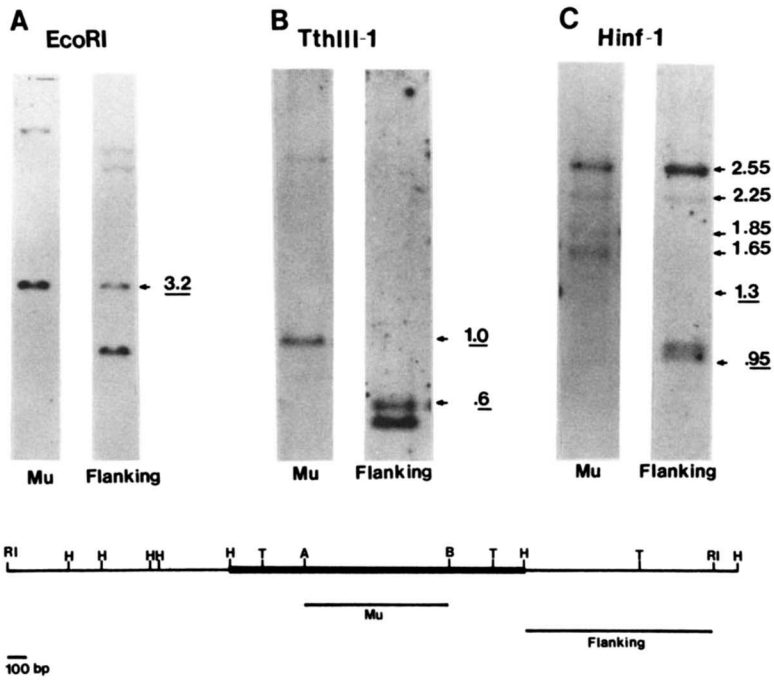

FIGURE 6.—Modification of *Mu1.4B37* in genomic DNA. DNA from a B37 plant was digested with *Eco*RI, *Tth*111-I or *Hin*fI, electrophoresed through agarose gels, transferred to Genetran and hybridized with the internal *Mu* probe. After autoradiography, the *Mu* probe was removed and the blot was rehybridized with the 850-bp *Hin*fI/*Eco*RI DNA fragment that flanks the *Mu* sequences (see map of the cloned MuB37 element and flanking DNA). The left lane of each panel shows the *Mu* hybridization signal, and the right lane shows the hybridization signal from the flanking DNA probe; the fragments (sizes in kilobase pairs) expected from the restriction map of the cloned DNA are underlined. A, *Eco*RI digests; B, *Tth*111-I digests; C, *Hin*fI digests. The restriction map was prepared by digesting the subcloned 3.2-kbp *Eco*RI fragment with a series of restriction enzymes. The thickened portion is the region homologous to *Mu1*. There are four *Hin*fI sites in the flanking DNA on the left side of the *Mu* element. These have not been accurately mapped; thus, the *Hin*fI sites drawn on the left side of the map are only approximate. H: *Hin*fI, T: *Tth*111-I, RI: *Eco*RI, A: *Ava*I, B: *Bst*NI.

drawn in Figure 6) and the internal 650-bp fragment of *Mu1* were used as hybridization probes. As shown in panel A, *Eco*RI digestion results in the expected 3.2-kbp fragment that hybridizes to both the *Mu* and flanking sequence probes. (Note that both the *Mu* and flanking sequence probes hybridize to other sequences as well.) Digestion with *Tth*111-I (panel B) produces the expected 1.0-kbp internal *Mu*-hybridizing fragment; as predicted this fragment does not cross-hybridize with the flanking sequence probe. A similar result would be expected after digestion if the *Hin*fI site on the right end of the element is recognized. However, both the *Mu* and flanking sequence probes hybridize strongly to the same 2.55-kbp fragment and, to a lesser extent, to a 2.25-kbp fragment (Figure 6, panel C). This suggests that the *Hin*fI site in the *Mu* element is not cleaved, so that the *Mu* and flanking sequences remain contiguous.

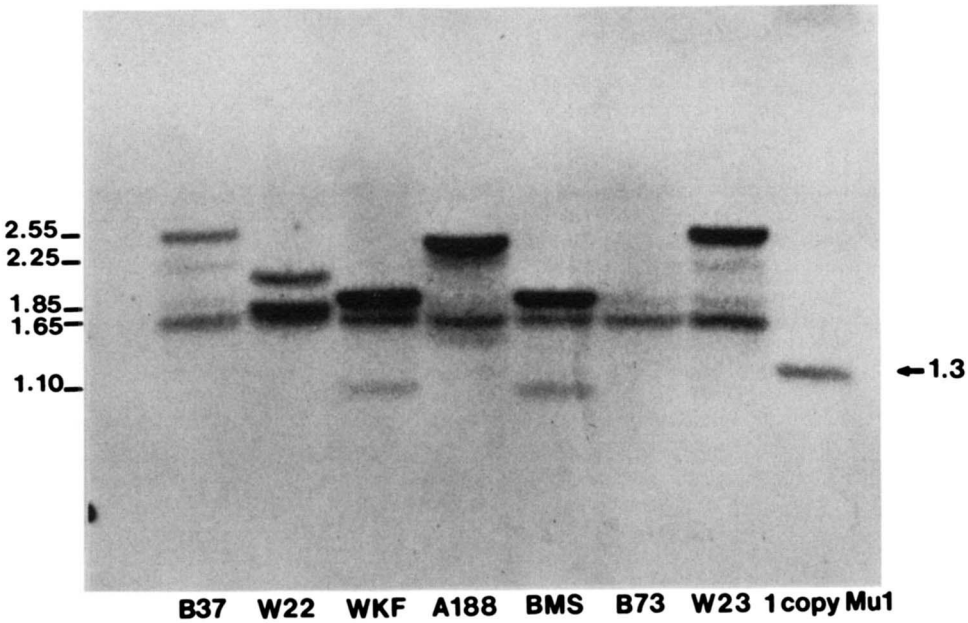

FIGURE 7.—*Hin*fI digestions on DNA samples from non-Mutator stocks. DNA samples were prepared from the maize stocks, digested with *Hin*fI, electrophoresed through a 1% agarose gel, transferred to Genetran and hybridized with the internal probe. The arrow points to the 1.3-kbp *Hin*fI fragment of *Mu1*; all size standards are in kilobase pairs.

The 2.55-kbp fragment corresponds to inhibition at both of the *Hin*fI sites in the *Mu1.4B37* element, the 2.25 kbp to cleavage at the left site and inhibition at the right site as drawn on the map (Figure 6). The 2.25-kbp fragment is much less than one copy per genome, suggesting that in most cells neither site within the *Mu* element is cleaved. The additional 1.65- and 1.85-kbp *Hin*fI fragments homologous to the *Mu1* probe that are visible in Figure 6 probably result from the other *Mu*-homologous sequences in the genome, as these fragments are observed in other non-Mutator lines (see Figure 7).

These results suggest that, in B37 genomic DNA, the termini of this *Mu*-like element are modified in a manner similar to that found in inactive Mutator stocks (CHANDLER and WALBOT 1986). In those stocks the DNA modification caused inhibition of *Hin*fI cleavage of *Mu* elements, whereas digestion by *Tth*111-I and *Taq*I was unaffected. This modification was correlated with the sudden loss of Mutator activity.

We were interested in determining if other non-Mutator stocks contained modified *Mu* elements. Therefore, DNA from several non-Mutator stocks of maize was digested with *Hin*fI, a Southern blot was prepared and the DNA was probed with the internal *Mu* sequence. As shown in Figure 7, the *Hin*fI digestion pattern suggests that these sites may also be blocked in other non-Mutator stocks. None of the stocks contain a 1.3-kbp *Hin*fI fragment characteristic of *Mu1* and the cloned form of *Mu1.4B37*. However, several of these stocks do contain the expected *Tth*111-I and *Taq*I fragments (Figure 3). The fact that the *Taq*I sites are only 4 bp from the *Hin*fI sites and that most of the

*Hin*fI fragments are larger than 1.3 kbp suggests that many of the non-Mutator stocks may contain endogenous *Mu*-like elements with modified *Hin*fI sites.

DISCUSSION

We describe here the presence of sequences homologous to the transposable element *Mu1* in stable non-Mutator stocks of maize. Every stock we have tested contains sequences homologous to both the internal and terminal portions of *Mu1*. The internal portion is found in low copy number, ranging from less than one to three copies per haploid genome. The low copy number in non-Mutator stocks compared with Mutator stocks may explain why these endogenous sequences were previously undetected (BENNETZEN 1984; ALLEMAN and FREELING 1986). The terminal repeat sequences are present in approximately 40 copies per genome. Further studies will be required to determine the extent of homology and conservation of structure between the partially homologous endogenous sequences and *Mu* elements. In many of the lines, at least one of the internal sequences is flanked by the terminal repeats. Restriction enzyme mapping suggests that some of these endogenous sequences are very similar to *Mu* elements. However, there is no genetic evidence of a high mutation rate in these lines, suggesting the endogenous elements are not transposing at any detectable frequency.

An intact *Mu1*-like element has been cloned from the non-Mutator inbred line, B37. This element, designated *Mu1.4B37*, contains 27 of the 28 restriction sites examined relative to *Mu1*, demonstrating that the two elements are very similar. We have shown that the *Hin*fI sites in *Mu1.4B37* are modified, such that they are not accessible to digestion in genomic DNA. This modification is much like that observed in Mutator stocks that have lost activity. The endogenous Mutator elements in normal stocks may be inactive as a result of this DNA modification, although there are other hypotheses for the lack of detectable Mutator activity. Small deletions (<20 bp) or point mutations may have rendered the elements inactive. Alternatively, the low copy number in non-Mutator stocks may prevent detection of their activity. There are only one to two intact elements in most stable stocks, whereas in active Mutator stocks the copy number ranges from 10–60. Further studies will be required to distinguish between these hypotheses.

A common source of genomic instability may be the liberation of previously silent transposable element systems. Environmental and genetic stresses have been correlated with increased mutability in plants and have been hypothesized to be responsible for activating transposable elements (MCCLINTOCK 1984). Thus, a question of great interest is whether the modified endogenous *Mu* elements are capable of giving rise to active *Mu* elements. The correlation between the DNA modification, which is likely to be 5-deoxycytosine methylation, of *Mu* elements and their inactivity (CHANDLER and WALBOT 1986) is exciting in this respect. Treatments that evoke DNA repair pathways are thought to activate transposable elements, and it is possible that the cryptic elements may become transiently demethylated and activated. Experiments are

in progress utilizing both genetic and chemical treatments to try to activate the endogenous *Mu* elements.

We are also interested in learning about the biological history of the endogenous *Mu* sequences. One hypothesis for their origin is that they represent relics of a previously active system. Creation of stable inbred lines by maize breeders by generations of self- and sibcrossing may have selected for loss of transposable element activity as a result of element modification, dilution of copy number, or segregation from regulatory factors. This could have yielded the patterns we observe in which a few intact *Mu* elements are inherited in a simple Mendelian fashion within many stable non-Mutator lines. A more thorough survey of different maize stocks might reveal inbred lines that have a higher copy number of endogenous *Mu* sequences or that lack them altogether.

We have observed the modification and subsequent dilution of *Mu* elements in Mutator stocks that have become inactive. Experimentally, dilution of *Mu* copy number has been detected by comparing active Mutator stocks with progeny in which Mutator activities have been lost. Active Mutator stocks tend to maintain the copy number of *Mu* elements between 20–40 copies per genome when outcrossed to non-Mutator stocks (ALLEMAN and FREELING 1986; V. CHANDLER, unpublished data). However, when stocks containing completely modified elements are outcrossed, the copy number decreases each generation (V. CHANDLER and V. WALBOT, unpublished data). Further studies of the inheritance of these modified *Mu* elements from recently inactivated stocks and a more thorough survey of non-Mutator stocks may contribute to our understanding of the origin of the endogenous *Mu* sequences.

This research was supported by a National Science Foundation Plant Biology Postdoctoral Fellowship to V.C. while at Stanford University, and by grants to V.C. from the National Science Foundation (DCE-8451656), to C.R. from the Department of Energy (DE-FG06-85ER13377) and to V.W. from the National Institutes of Health (GM 32422). We gratefully recognize and thank all the maize geneticists and breeders who provided the maize stocks used in these experiments, L. TALBERT and T. DIETTERICH for critical reading of this manuscript, and E. COOKSEY for help in its preparation.

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Communicating editor: W. F. SHERIDAN