Identification of an Active New Mutator Transposable Element in Maize

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ABSTRACT Robertson’s Mutator (Mu) system has been used in large scale mutagenesis in maize, exploiting its high mutation frequency, controllability, preferential insertion in genes, and independence of donor location. Eight Mutator elements have been fully characterized (Mu1, Mu2/Mu1.7, Mu3, Mu4, Mu5, Mu6/7, Mu8, MuDR), and three are defined by TIR (Mu10, Mu11 and Mu12). The genome sequencing revealed a complex family of Mu-like-elements (MULEs) in the B73 genome. In this article, we report the identification of a new Mu element, named Mu13. Mu13 showed typical Mu characteristics by having a ~220 bp TIR, creating a 9 bp target site duplication upon insertion, yet the internal sequence is completely different from previously identified Mu elements. Mu13 is not present in the B73 genome or a Zea mays subsp. parviglumis accession, but in W22 and several inbreds that found the Robertson’s Mutator line. Analysis of mutants isolated from the UniformMu mutagenic population indicated that the Mu13 element is active in transposition. Two novel insertions were found in expressed genes. To test other unknown Mu elements, we selected six new Mu elements from the B73 genome. Southern analysis indicated that most of these elements were present in the UniformMu lines. From these results, we conclude that Mu13 is a new and active Mu element that significantly contributed to the mutagenesis in the UniformMu population. The Robertson’s Mutator line may harbor other unknown active Mu elements.

Mutator (Mu) transposable elements are a major class of class II transposons identified in maize by Donald Robertson (1978, 1981). The two-component system, one autonomous MuDR and many nonautonomous Mu elements, was exploited for efficient mutagenesis in maize. High copies of the elements offer a high forward mutation frequency, whereas limited copies of MuDR allowed turning off the transposition by removing the element through segregation (McCarty et al. 2005).

Preferential transposition into gene rich regions by Mu elements enhances mutagenesis frequency. And transposition not limited to linked loci facilitates genome wide mutagenesis. For these reasons, several mutant populations in maize were created by using the Mu system (Bensen et al. 1995; May et al. 2003; Raizada 2003; McCarty et al. 2005).

The well-characterized Mu elements (Mu1 to Mu9/MuDR) were discovered exclusively in maize. Subsequent molecular analyses and genome sequencing revealed that Mu elements are present in plants (Lisch 2002), fungi (Chalvet et al. 2003), bacteria (Eisen et al. 1994), protozoans (Pritham et al. 2005), and metazoans (Huav-Van and Capy 2008). Based on sequence similarity, these elements are classified as Mu-like elements (MULEs). MULEs belong to a superfam of transposons with complex members and diverse sequences. Typical characteristics of this family include a conserved 50–200bp terminal inverted repeat (TIR), unrelated internal sequences between the TIRs, and creating a 9bp target site duplication (TSD). In contrast, all the previously identified Mu elements from maize (Mu1-Mu9/MuDR) carry a ~220bp TIR that is highly conserved. Transposition activity of the elements is thought to be associated with the TIR sequences. Inactive elements carry mutated TIRs.

Different from Ac/Ds and Spm/dSpm transposable elements where the non-autonomous elements are deletion derivatives of
the autonomous elements, the internal sequences between TIRs among \textit{Mu} elements are often unrelated. Some \textit{Mu} internal sequences showed high similarity to host genome, suggesting a possible gene capture in the formation of these elements. This class of \textit{Mu} elements was classified as Pack-MULEs (Jiang et al. 2004). About 262 Pack-MULEs were identified in the B73 genome (Schnable et al. 2009). Because promoters are found in the TIRs, \textit{Mu} internal sequences may be transcribed in convergent orientations (Hershberger et al. 1995; Lisch 2002). Hence, it was suspected that some of the Pack-MULEs may have regulatory function, as antisense transcripts may interfere with expression of the endogenous genes (Lisch 2005; Juretic et al. 2005).

Transposition of all \textit{Mu} elements required the presence of an active \textit{MuDR} element. The \textit{MuDR} element contains two genes, \textit{mudrA} encoding a transposase (MURA) and \textit{mudrB} whose product (MURB) is of unknown function. MURA showed high similarity to bacterial transposase and the virus integrase (Walbot and Rudenko 2002); hence, it is essential for transposition. Transposable elements containing only \textit{mudrA}-like genes were found in both monocots and eudicots (Saccaro et al. 2007). The \textit{mudrB} gene is only present in the genus \textit{Zea} (Lisch et al. 2001). Jittery, an autonomous transposon identified in maize, contains a \textit{mudrA}-like gene, but with TIR sequences distinct from \textit{Mu} elements (Xu et al. 2004). Jittery exhibited high frequency of excision, causing somatic and germinal reversion, but apparently lost its activity for new insertions. Transposition of \textit{Mu} elements employs two distinct mechanisms. In somatic cells, transposition mostly uses a "cut-and-paste" mechanism. The element cuts itself and reinserts it in a new locus elsewhere in the genome. High-frequency excision of \textit{Mu} elements is restricted to the late stage of cells in development during organogenesis. In germinal cells, \textit{Mu} transposition uses a "replicate-and-insert" mechanism where the element replicates just before meiosis or in the gametophytes and inserts in a new locus in the genome. Consequently, "cut and paste" transposition does not increase the copy number, whereas "duplicate-and-insert" transposition does. Excision of a \textit{Mu} element left a footprint of the 9bp TSD, which sometimes restored the function of the donor gene such as in \textit{bz1-mum9} (McCarty et al. 2005).

Prior to the sequencing of the maize genome, eleven \textit{Mu} elements were reported in maize, of which eight were characterized by full sequences of \textit{Mu1} to \textit{Mu9}/\textit{MuDR} (Bennetzen et al. 1984; Taylor and Walbot 1987; Talbert et al. 1989; Fleenor et al. 1990), and three were indicated by TIRs (Dietrich et al. 2002). The sequencing of the B73 genome revealed a surprisingly complex view of the Mutator family, which accounts for approximately 1% of the 2.3 gbp genome (Schnable et al. 2009). These include MULEs, Pack-MULEs, and SOLOs that contain only one TIR. Many of these elements contain a shorter TIR, suggesting that these elements may have lost the capacity for transposition. In this study, we report a new \textit{Mu} element, \textit{Mu13}, which was identified from the UniformMu population, a derivative \textit{Mu} active line from the Robertson’s \textit{Mu} line. \textit{Mu13} exhibits typical \textit{Mu} characteristics and is active in transposition. It contributes significantly to mutagenesis. The finding of \textit{Mu13} adds to the active \textit{Mu} reservoir and facilitates cloning of causative insertions in the \textit{Mu} tagged mutants in phenotype-driven forward genetics in maize.

MATERIALS AND METHODS

Genetic stocks

The maize lines used in this study were derived from the UniformMu population, a Mutator line with the mutable \textit{bz1-mum9} anthocyanin biosynthetic gene introgressed into the W22 genetic background (McCarty et al. 2005). The teosinte lines \textit{Zea mays} subsp. \textit{parviglumis} (Accession: PI 384061) and \textit{Zea mays} subsp. \textit{mexicana} (Accession: PI 566684) were provided by the Maize Genetic Stock Center. Other inbred lines (W22, B73, Mo17, M14, Q66, Q67, B77, and B79) were generously provided by Donald R. McCarty (University of Florida).

Cloning of \textit{Mu13} from \textit{UniformMu} population

The \textit{Mu13} transposable element was amplified by a pair of primers (5'–CTGGTCCTGGTCTATCCTGGC-3' and 5'-ACCAAAACACAAAGAGGCGTC-3') flanking a \textit{Mu13} insertion in a gene coding for a putative plastid Sigma factor3 (ZmSig3). Template DNA was isolated from line 03S-4081-01, homozygous for the insertion. As \textit{Mu} elements carry a long terminal inverted repeat (~220bp), it interferes with PCR amplification. We tested different conditions with DNA polymerses of various sources. ExTaq (TaKaRa, Japan) and ThermalAce DNA polymerases (Invitrogen, USA) yielded successful amplification. The PCR reaction was composed of 20mu Tris-HCl pH 8.4, 50 mM KCl, 2.5mu MgCl2, 200 μM of each dNTP, 100 nM each primer, 5% DMSO, and 1 U of DNA polymerase. PCR conditions were 96°C/3min for initial denaturation, 8 cycles (95°C/30 sec, 62°C/30 sec, 72°C/2min) followed by 30 cycles (95°C/30 sec, 58°C/30sec, 72°C/2min), with final extension at 72°C/10min. The PCR fragment was purified from gel by gel extraction kit (Zymo Research, USA), ligated into pCR4-TOPO (Invitrogen, USA), and sequenced.

Selection and cloning of new \textit{Mu} elements in \textit{B73}: A conserved 200bp \textit{Mu} TIR sequence based on known \textit{Mu} elements (\textit{Mu1} to \textit{Mu9}/\textit{MuDR}) was used in a BLAST search of the GenBank maize sequences, with a cut-off E value of < e-10. Within this collection, the known \textit{Mu} elements were identified by a BLAST search with the internal sequence of each \textit{Mu} element. Identical sequences were clustered using BLASTCLUST (ftp://ftp.ncbi.nih.gov/blast). The resulting collection was analyzed for left- and right-TIR in terms of orientation and homology, as well as the presence/absence of a 9bp host sequence direct duplication.

We amplified the internal sequences of six new \textit{Mu} elements that carry highly conserved TIR at both ends. The primers were listed in Table I, and the PCR conditions were similar to those present in the amplification of \textit{Mu13}. The internal sequences were cloned in pCR4-TOPO and sequenced.

Selection of \textit{UniformMu} mutants for Southern blot analyses: UniformMu mutants segregating for visible mutant phenotype of embryo defective (emb), small kernel (smk), empty pericarp (emp), shrunk (sh), and defective kernel (dek) were randomly chosen. The 18 mutants were 06S-6001 (smk), 06S-6002 (emp); 06S-6004 (defective kernel, dek); 06S-6005 (emp); 06S-6016 (smk); 06S-6018 (dek); 06S-6019 (smk); 06S-6020 (emb); 06S-6023 (emp); 06S-6026 (smk/ dek); 06S-6029 (smk); 06S-6032 (smk); 06S-6033 (emb); 06S-6034 (dek); 06S-6044 (dek); 06S-6045 (emp); and 06S-6055 (sh/sm). Each DNA was extracted from seedlings of three individual ears that were genotyped based on the seed phenotype. All these ears did not exhibit active \textit{MuDR} activity, as indicated by the mutable \textit{bz1-mum9} anthocyanin biosynthetic marker. All these lines were back-crossed with W22 twice.

DNA extraction and Southern analysis: Genomic DNA was isolated by a urea–phenol–chloroform-based method. 1g fresh weight of leaf tissues was ground in liquid nitrogen and extracted with 5 ml of DNA
extraction buffer (7 M urea, 0.3 M NaCl, 50 mM Tris-HCl, 24 mM EDTA, and 1% Sarkosine, pH 8.0). After mixing with 4 ml phenol-chloroform-isomyl alcohol (25:24:1), the extraction was carried out with gentle shaking for 30 min at room temperature. The mixture was separated by centrifugation at 4800 × g for 15 min. The aqueous phase was transferred to a new tube and mixed with 0.1 volume of 3M sodium acetate (pH 5.2) and 3.8 ml isopropanol. DNA was pelleted at 4800 × g for 5 min, washed with 70% ethanol, and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). Approximately 10 µg genomic DNA was digested with appropriate restriction enzymes at 37°C for 6 hr. The DNA was resolved on a 0.7% agarose gel, denatured, and blotted onto a Hybond-N membrane (GE Healthcare). The membrane was cross-linked and hybridized. The probe was labeled with Ready-To-Go DNA labeling beads and purified with ProbeQuant G-50 micro column (GE Healthcare).

The probes used for Southern analyses for Mu14-Mu19 were amplified from the B73 genome by PCR with primers listed in Table 1. The primer anchor positions with respect to TIR and probe sequences are listed in supporting information, File S1. For Mu13, it was derived from UniformMu by PCR with a single primer (5'-ATCAATGTCTCTGTCACCGGTTTACCGT-3') that was anchored in the TIR region.

Bioinformatics analysis: Sequence alignments were carried out using the CLUSTALW algorithm available online (http://workbench.sdsc.edu/). For phylogenetic tree construction, the phylogenetic tree files from CLUSTALW analysis were imported into a TreeView program (http://taxonomy.zoology.gla.ac.uk/rod/treewiew.html).

RESULTS

Identification of Mu13 element

In a large scale extraction of Mu flanking sequences from mutants isolated from the UniformMu population (McCarty et al. 2005), a Mu element was found in a gene coding for a putative plastid Sigma factor 3 (ZmSig3). The element was inserted in the third exon of the gene (refer to Figure 4, Accession no. CG893004). We cloned the Mu element and found that the element is 1494bp long, containing a 223bp TIR with an 88% identity to the consensus of previously known Mu TIRs (Figure 1). The left and right TIR showed a higher identity (92%), which is a general feature of the Mu elements. A 9bp direct target site duplication was found at the insertion site. The internal sequence of this element is completely different from any previously identified Mu elements (Bennetzen 1984; Taylor & Walbot 1987; Chen et al. 1987; Talbert et al. 1989; Fleemor et al. 1990; Hersberger et al. 1991). Searching the GenBank and the nearly completely B73 genome did not find the presence of this element. In light of the partially characterized Mu elements, Mu10, Mu11, and Mu12 (Dietrich et al. 2002), we designated this element as Mu13 (Accession: HQ698272).

Bioinformatic analysis revealed that the Mu13 internal sequences contain two open reading frames (ORF). The conceptually translated protein sequences of these two ORFs showed high similarity to a maize protein that was annotated as nucleotide binding protein (accession: ACG25371, GRMZM2G317614). Further analysis revealed that it encodes a WD40 protein, containing seven WD repeats. As indicated in Figure 1D, the first highly similar ORF started from the first methionine and covered 73 amino acid (aa) residues in length. This region shared an 88% identity with the maize WD40 protein, and a similar identity with apparent orthologs in sorghum (Sb01g008680) and rice (Os03g0738700, also annotated as trans-dicin family protein). The second highly similar region (95% identity) was about 42 aa long and coincided with the first repeat of the WD40 protein. In the maize WD40 protein, these two regions were separated by 26 amino acid residues, which were not found in the Mu13. This maize WD40 gene was expressed as indicated by ESTs, suggesting that it may be a functional gene. Another WD40 gene on maize chromosome 5 (GRMZM5G852097) is apparently a syntactic paralogous duplicate of GRMZM2G317614, which is also probably functional.

Mu13 is active in the UniformMu population

The insertion of Mu13 in a functional gene in the UniformMu population suggested that it was active in transposition. This insertion was not present in the parental lines that gave rise to the mutant. It is known that Mu elements are not equally active. Mu4, Mu5, and Mu7 were less active than the other known ones (Talbert et al. 1989), and so far most genes cloned by transposon tagging were inserted by Mu1/2, Mu3, Mu8, and MuDR. To assess the Mu13 transposition activity, we analyzed 18 UniformMu seed phenotype mutants randomly selected from a large set of available seed phenotype mutants. For each mutant, seeds showing no MuDr activity (lack of somatic transposition indicated by the bz1-mum9 marker gene) were selfed to produce an F2 mutant segregating family. The genotype of each F2 individual was scored by examining the ear. DNAs from three F2 individuals of either wild type (not segregating mutant phenotypes, N) or segregating mutants (S) were analyzed with ProbeQuant G-50 micro column (GE Healthcare). The probe used in this analysis contained 80bp TIR sequences, it cross-hybridized with related Mu elements and produced weak signals. The Mu13 signals were strong. Three Mu13 containing fragments (4.5kb, 5.8kb, >12kb) showed uniform presence in all the members, suggesting that they are apparently parental. When the same blot was hybridized with a Mu1/Mu2 specific probe, comparable numbers of Mu1/Mu2 insertions were detected (Figure 2B). Some of these insertions were unique to individual lines, suggesting new transposition by Mu1/Mu2 as well. This result indicated that Mu13 is active in the UniformMu population.

Mu13 presence in maize inbred lines and teosinte

The UniformMu mutagenic population was derived from introgressing Robertson’s Mu-active line into W22 genetic background (McCarty et al. 2005). Hence, the Mu13 element can be derived from either W22 or Robertson’s Mu-active line. To determine the presence

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Table 1: Primers used in amplification of selected Mu elements in B73

| Primer    | Sequence                           |
|-----------|------------------------------------|
| Mu14-F1   | 5'-CTCTTCCCAACACCTATTGC-3'         |
| Mu14-R1   | 5'-GAGATGCCTCGGATTACAT-3'          |
| Mu15-F1   | 5'-TAAAGGATTTGCTCCGGGGTCT-3'       |
| Mu15-R1   | 5'-TCTCTTGGTCCTCGGTCTCC-3'         |
| Mu16-F1   | 5'-CACCCTAGGCTTAAACAAG-3'          |
| Mu16-R1   | 5'-CGGTTAGGCTTCCCTTCCTTG-3'        |
| Mu17-F1   | 5'-CTACGCAGACCTCTTGACAC-3'         |
| Mu17-R1   | 5'-CCTCCCTCCTCGGTCTCC-3'           |
| Mu18-F1   | 5'-TTGAGGTCTGCTGAAGTGACTG-3'       |
| Mu18-R1   | 5'-ACAGCTTCTCCTCGGTCTCTTG-3'       |
| Mu19-F1   | 5'-ATGGAGTGCTCTCGGGGT-3'           |
| Mu19-R1   | 5'-AGACGTCGGTCTCAGGATT-3'          |
of Mu13, nine inbred lines of maize were analyzed by Southern blot analysis using the Mu13 internal sequence as a probe. Six inbred lines (W22, M14, Q66, Q67, Q77, and Q79) that founded the Robertson’s Mutator population were included. To ensure a complete digestion, EcoRI was used, because it is methylation insensitive and does not cut Mu13 internally (Figure 1B). As shown in Figure 3A, Mu13 was detected in W22, Q67, Q77, and Q79, and was not detected in B73, Mo17, or A188. Mu13 was probably not present in M14 and Q66 because the hybridized bands were substantially weak in comparison to other lines. PCR analysis by using Mu13 specific primers did not detect Mu13 in B73, Mo17, or A188. Mu13 was probably not present in M14 and Q66 because the hybridized bands were substantially weak in comparison to other lines. PCR analysis by using Mu13 specific primers did not detect Mu13 in B73, Mo17, A188, M14, and Q66, suggesting that the weak signal may have arisen from non-specific hybridization with the probe. W22 appeared to contain two copies of Mu13, whereas other inbred lines contained one to two copies. In B73, a ~4.4kb fragment was weakly hybridized. This fragment is consistent with a WD40 gene (Accession no. ACG25371), which predicts a 4382 kb EcoRI fragment. The fragment contained a 258bp region that has 95% identity and a 126bp region that has 98% identity to the Mu13 probe. It was expected to weakly hybridize with the Mu13 probe. In Mo17, the corresponding fragment is predicted to be 4302bp which was cross-hybridized as well. The Mu13 probe carried an 80bp sequence of the TIR (refer to File S1), which predictably would weakly hybridize with related Mu elements. This might explain the background and weak signals.

To test whether Mu13 is present in the ancestor of maize, we analyzed Zea mays subsp. parviglumis (Accession no. PI 384061).
and *Zea mays* subsp. *mexicana* (Accession no. PI 566684). The former is believed to be the ancestor of maize from a single domestication process (Matsuoka et al. 2002). To reduce the chance that Mu13 may have resulted from a large fragment that escaped from Southern detection, five restriction enzymes (EcoRI, EcoRV, HindIII, KpnI and SalI) that did not digest inside Mu13 were used to restrict the genomic DNA. *Zea mays* subsp. *parviglumis* did not contain any Mu13 element, as indicated by the absence of a hybridized signal (Figure 3B). A 5kb HindIII fragment was detected in *Zea mays* subsp. *mexicana*, but the signal intensity was much weaker than the Mu13 signals in W22 or Q79. Because this hybridization was carried out under the same conditions at which the inbred DNAs were hybridized (and the loading was comparable with samples such as W22 or Q79), the signal produced in *Zea mays* subsp. *mexicana* was more likely from the WD40 fragment or an unknown homologous fragment than the real Mu13 element. Although the primers were proven robust, subsequent PCR detection by Mu13-specific primers failed to amplify the Mu13 element from *Zea mays* subsp. *mexicana*, indicating that Mu13 was not present in *Zea mays* subsp. *mexicana* either. This result indicated that Mu13 is not present in the sample of two teosinte accessions tested, but as substantial genetic diversity exists among teosinte accessions, we cannot infer its absence among all teosintes.

![Figure 2](image1.png) **Figure 2** Detection of new transpositions of the Mu13 element in the UniformMu population. Genomic DNAs from eighteen randomly selected lines that segregate different seed mutant phenotypes were digested with EcoRI and hybridized with a Mu13 (A) and a Mu1 specific probe (B). A pooled WT (non-segregant, N) and a segregant (segregating each mutant phenotype, S) sample were used from each line (refer to Materials and Methods). Arrows indicate Mu13 insertions that were not found in the progenitors.

![Figure 3](image2.png) **Figure 3** Southern analysis of Mu13 element presence in teosinte and maize lines. (A) Genomic DNAs of selected maize inbreds were digested with EcoRI and hybridized with a Mu13 probe. The arrow indicates the 4382bp EcoRI fragment of the WD40 gene (Accession no. ACG25371). (B) Genomic DNAs from *Zea mays* subsp. *parviglumis* (Accession no. PI 384061) and *Zea mays* subsp. *mexicana* (Accession no. PI 566684) were digested with five different restriction enzymes (RV: EcoRV, H3: HindIII, KpnI, SalI, RI: EcoRI) and hybridized with a Mu13 probe.
Insertions of Mu13 in functional genes

A Mu13 insertion was first identified in molecular characterization of the ZmSig3 gene (Accession no. CG893004, GRMZM5G830932). The ZmSig3 gene consists of six exons, and the Mu13 was inserted in the third exon (Figure 4B). Analysis of the progenitor lines and a population segregating zmsig3 mutants by PCR using Mu-TIR primer (TIR8) and the ZmSig3 specific primer (ZmSig3-R) indicated that this insertion was not present in WT and the progenitor lines, suggesting that it was a new transposition event.

The insertion in ZmSig3 suggested that the Mu13 element may contribute significantly to mutagenesis in the UniformMu population. In a previous study on seed mutants isolated from the UniformMu mutagenesis, Mu-flanking sequences were extracted by the Mu-TAIL method (deposited in GenBank, McCarty et al. 2005). The identity of the Mu element, however, is unknown. To search for insertions by using Mu13, we chose two Mu insertions in known functional genes for analysis. Analysis of the Mu flanking sequences indicated that one Mu element was inserted in a paralog of Vp14 gene on chromosome 5S, named as Vp14b (GRMZM5G838285), and that the other was inserted in a gene coding for an NAC (NAM, ATAF1.2, CUC2) transcription factor domain containing protein, named ZmNAC1 (GRMZM2G312201). Both insertions were novel, as they were not present in the progenitors and were segregated specifically in individual lines. We cloned and sequenced the inserted Mu elements. The insertion in Vp14b was a Mu7 element (data not shown), and the one in ZmNAC1 was a Mu13 element (Figure 4D). We analyzed a population of 12 individual plants derived from a selfed heterozygote of the Mu13 insertion by using Mu TIR specific primer TIR8 and ZmNAC1 specific primers. The 12 individual plants were genotyped (Figure 4C). Plants homozygous for the Mu13 insertion showed a dwarf phenotype (#5, #8, and #10 in Figure 4E). Although the insertion was not confirmed as the cause of the dwarf phenotype, it indicated at least a linkage between this Mu13 insertion and the dwarf phenotype. The ZmNAC1 is likely a functional gene, as multiple ESTs were found in GenBank.

Presence and transposition of six new Mu elements in UniformMu lines

The sequencing of the maize genome revealed a surprising view of the Mutator family, which accounts for 1% of the B73 genome (Schnable et al. 2009). We used the conserved 200bp Mu TIR sequences and performed a BLAST search of the maize genomic sequences in GenBank. A high stringency search (E value < e^{-10}) resulted in a low return of Mu elements. It appears that four types of Mu elements with distinct TIRs are present in the B73 genome. One class of Mu elements possesses TIRs with high similarity to known Mu elements in sequence and length (left and right TIR ~210 bp). A second class contains a left TIR of ~215bp and a right TIR of ~90bp. A third class contains both short TIRs (~100bp), and a fourth class is called SOLOs, which contain only one TIR. The previously known Mu elements only account for a very small fraction of this family. Mu1, Mu2, Mu8, and Mu13 do not exist in the B73 genome. B73, however, does contain truncated and apparently non-functional derivatives of the autonomous MuDR, as well as one copy of Mu3 and Mu7, two copies of Mu4 and Mu5, and four copies of Mu7 derivatives that have insertions or deletions in their internal sequences. Because of the absence of MuDR, these elements are dormant, and some may have lost their transposition activity due to accumulated mutations. To analyze the presence and possible activity of the unknown Mu elements in the UniformMu population, we identified a subset of MULEs from the B73 genome. The criteria are that the element 1) contains a highly conserved ~220bp TIR on both ends (>85% identity to consensus Mu TIR sequence); 2) contains perfect TIR ends

![Figure 4](https://example.com/figure4.png)
(GAGATA at the 5' and TATCTC at 3'); and 3) possesses perfect TSD in the insertion site, which is indicative for recent transposition. The known active Mu elements all contain these features. We chose six MULEs that showed the highest similarity to Mu TIR consensus and with unrelated internal sequences. These elements were named as Mu14 to Mu19 (Accessions no. HQ698273–HQ698278, refer to File S1).

A phylogenetic analysis performed by using the internal sequences indicated that these elements are not related, except for Mu1 and Mu2 (also known as Mu1.7), Mu5, and MuDR (Figure 5A). Sequence analysis strongly supports the notion that Mu1 is a deletion derivative of Mu2, and that Mu5 a deletion derivative of MuDR. Mu2 contains a 140bp direct repeat in the internal sequence (Figure 5B, box arrows). Its 3’ region (from 893 to 1330bp) showed a 91% identity to maize and rice genomic sequences, suggesting possible gene fragment capture. The conceptual translation product of this region showed high similarity (80% identity) to Os05g0128200, which was annotated as zinc finger CCCH domain-containing protein 33 in rice. In sequence alignment with Mu2, Mu1 lacks most of this region, but still retains a residual 41 bp of the likely captured fragment. Similarly, Mu5 contains two segments of the mudrA gene that codes for MURA transposase (Figure 5B). Mu15 showed slight similarity to Mu19, in which three short segments of the internal sequences shared some similarity, suggesting that the two elements are likely of the same origin. The divergent sequences indicated that deletion and insertion also occurred fairly long ago. It has been known that the internal sequences of Mu elements are likely captured gene fragments. The captured gene fragments were analyzed in the known Mu elements (Lisch 2002). Our analysis indicated that Mu3 and Mu4 can be classified as Pack-MULEs. A fragment from maize chromosome 6 accounted for most of the internal sequence of Mu3. Additionally, two fragments fused from maize chromosome 1 and 3 accounted for the internal sequence of Mu4.

We analyzed the internal sequences of new Mu elements identified in this work. As indicated in Figure 5B, the Mu13 element contains two regions that showed high similarity to a WD40 protein. Mu14 contains a fragment highly similar (89% identity) to a putative cucumisin-like serine protease on chromosome 1. Mu15 contains

![Figure 5 Sequence and structure of new Mu elements (Mu13–Mu19) and previous known Mu elements. (A) Phylogenetic tree derived by CLUSTALW by using the internal sequences of each Mu element. (B) Schematic structure of each Mu element. Arrows indicate terminal inverted repeats. Internal captured gene fragments are labeled based on similarity to host genome. Refer to text for captured gene fragments.](image-url)
a fragment that is similar to xylem serine proteinase 1 on chromosome 1 (LOC100281759). *Mu16* contains a fragment similar to a receptor protein kinase TMK precursor (95% identity, Accession no. BT054484) on chromosome 3. *Mu17* contains fragments from different chromosomes. *Mu18* contains a fragment of an auxin response factor 15 (ARF15) gene (Accession no. HM004530, 97% identity at nt level) and a calmodulin (LOC100286292, 98% identity at nt level). *Mu19* contains a fragment of a putative xylem serine proteinase 1 (Accession no. NM_001154679). All of these elements except *MuDR* are between 1.4 and 2.5 kb in length.

We cloned the internal sequences of these *Mu* elements and used them as probes to test their presence in inbred lines Mo17, W22, and six randomly selected UniformMu mutant lines. As shown in a Southern blot analysis (Figure 6), *Mu14* to *Mu18* elements were found in W22 and the UniformMu lines. The identical sizes of the fragments between W22 and the UniformMu lines strongly suggested that these elements were likely derived from W22. *Mu19* was not found in Mo17, W22, or the UniformMu lines, but was found in B73. The analysis revealed that these elements represent part of the non-collinear genome fraction of the three inbred lines. B73, Mo17, and W22 were all variable for these six elements in terms of copy numbers and RFLP size. *Mu19* was not present in either Mo17 or W22. It was also not detected in the six UniformMu lines. Some elements showed identical size among the three inbred lines, indicating likely early transposition events prior the separation of these inbreds. These *Mu* elements in the UniformMu population were derived from W22. Within the limited number of the UniformMu samples, new transposition events were not detected.

**DISCUSSION**

**Mu13 is a new Mu transposable element**

*Muator* elements share a highly conserved ~220 bp TIR sequence and create a 9bp TSD upon insertion (Walbot and Rudenko 2002). Different *Mu* elements are defined by the internal sequences between the TIRs. *Mu13* has a TIR of 223bp that is highly similar to the conserved TIR sequences of known *Mu* elements (Figure 1), and yet the internal sequence is completely different from known *Mu* elements. *Mu13* element was not found in the sequenced B73 genome, nor was it detected by Southern hybridization analysis (Figure 3). Of the two *Mu13* insertions identified in this study, each created a 9bp TSD. Hence, we concluded that *Mu13* is a new *Mu* element.

**Mu13 contributes significantly to mutagenesis in the UniformMu population**

Among the previously identified *Mu* elements, not all are equally active in transposition. *Mu* element transposition was driven by the autonomous element *MuDR* (Hershberger et al. 1991). *Mu4* and *Mu5* were found inactive (Talbert et al. 1989), which may likely be due to the absence of the *MuDR* element. However, in a large scale tagging of...
unidentified Mu elements in the maize genome and their activity

The identification of active Mu13 in the maize genome suggested that there are many more unknown Mu elements in the genome. The sequencing of the B73 genome recovered many of these elements (Schnable et al. 2009), but evidence suggested that there are more. We have analyzed six Mu elements identified in B73, and Mu19 was not detected in the UniformMu population, which is largely W22 introgressed with the Robertson’s Mu line. Mu13 was not found in the B73 genome, and in the tagging of 80 alleles of g8, Mu13 was not detected in the population that derived from the Robertson’s Mu-active line. Mu13 apparently was present in W22 and was activated during introgressing with Mu-active lines. The W22 line did not contain any active MuDR element (B. C. Tan and D. R. McCarty, unpublished data), hence Mu13 was inactive. Because different maize inbred lines harbor different spectrums of Mu elements, more unknown Mu elements are expected. It is highly likely that most of the maize inbred lines did not contain any active MuDR elements, hence all the MuDR driven Mu elements are dormant. Upon introducing the MuDR element, Mu element activity may be restored. If this is the case, Southern blot based cosegregation analysis using known Mu internal sequences as probes may encounter some problems. But this will not affect analysis based on the TIR sequences such as AIMS (Frey et al., 1998), Mu-TAIL. PCR (Settles et al. 2004), AIMS and Mu-TAIL-PCR combined (Yi et al. 2009), and the use of PCR-coupled pyrosequencing (Williams-Carrier et al. 2010). In addition, if the creation of new Mu elements is associated with the MuDR activity, it will be expected that there will be many new Mu elements in Robertson’s Mu-active line. It will be interesting to know the Mu landscape in the Robertson Mu active line.

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