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Mutator transposons

Damon Lisch

***Mutator* (*Mu*) element insertion has become the main way of mutating and cloning maize genes, but we are only beginning to understand how this transposon system is regulated. *Mu* elements are under tight developmental control and are subject to a form of epigenetic regulation that shares some features with the regulation of paramutable maize genes. *Mu*-like elements (MULEs) are widespread among angiosperms, and multiple diverged functional variants appear to have coexisted in genomes for long periods. In addition to its utility, the means by which this widespread and highly mutagenic system is held in check should help us to address fundamental issues concerning the stability of genomes.**

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Mutator (*Mu*) is the most active and mutagenic plant transposon discovered to date. In lines of maize with active *Mu* elements, transposition frequency can reach 100% (an average of one transposition event per element per generation) [1]. Transposition preferentially occurs to low-copy-number sequences [2,3], resulting in a mutation rate 50 times that of background [4]. Because of these properties, *Mu* is being used to clone, sequence and mutate most maize genes [5]. In spite of its usefulness, relatively little was known until recently about *Mu*-element regulation and behavior because of the same properties that make it so useful: high copy number and high transposition frequency. The isolation and cloning of the regulatory element of the system (*MuDR* [6,7]), the creation of an engineered *Mu* element [3] and the availability of low-copy-number or minimal *Mutator* lines [8] have made it possible to begin systematically to dissect this remarkably active plant transposon system.

All maize *Mu* elements contain conserved ~220 bp terminal inverted repeats (TIRs), but each class of element contains unique, apparently unrelated internal sequences (Fig. 1) [9]. The system is regulated by autonomous (self-replicating) *MuDR* elements, whose presence is required for transposition of non-autonomous classes of *Mu* elements [7,10]. *MuDR* elements contain two genes, *mudrA* and *mudrB*, each of which is transcribed inwards from promoters located within the terminal inverted repeats, which are 97% identical to each other (Fig. 1) [11]. As a result of alternative splicing (primarily intron retention), each reading frame is predicted to encode several proteins. The best-characterized *mudrA* transcript encodes a 120 kDa transposase (MURA); it and the other predicted products contain a domain with similarity to a several bacterial transposases [12]. The major transcript of *mudrB* encodes the 23 kDa protein MURB. The *mudrB* gene is not similar to any sequences outside of maize in any public database and its precise function remains enigmatic. Although only a few maize lines contain active *MuDR* elements, all maize lines carry homologous *MuDR* sequences (*hMuDRs*), whose coding regions are 80–99% identical to those of *MuDR* [13]. Although they are not associated with *Mutator* activity, they can be expressed and might play an as-yet-undetermined role in *Mutator* regulation.

Most new mutations in *Mutator* lines are caused by non-autonomous elements, which outnumber the *MuDR* elements (>100 versus ~10) [9]. The non-autonomous elements appear to represent host

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(non-transposon) sequences that have been captured by *Mu* element TIRs (Fig. 1). The same pattern of gene capture has also been observed among *Mu*-like elements (MULEs) in *Arabidopsis* [14] and rice [15], suggesting that this process is a generic feature of the creation of non-autonomous MULEs.

A new class of *Mu* elements was recently constructed by combining parts of both *Mu1* and the pBluescript plasmid [3]. This element, *RescueMu*, has an *Escherichia coli* origin of replication (*ori*), a selectable marker and a unique 400 bp DNA tag composed of *Rhizobium meliloti* DNA (Fig. 1). This element behaves similarly to other classes of *Mu* element in its timing and insertion preferences. DNA flanking individual insertions of this element can be excised, circularized, transformed into *E. coli* and sequenced. A major effort is now under way to use this element to clone and sequence the genic space in maize, thus avoiding sequencing the part of the maize genome that is composed of retrotransposons (>65%) [16].

Mu-element behavior

The most visible manifestation of *Mu*-element activity is somatic excision of *Mu* elements from visible reporter genes, resulting in small revertant sectors. Most *Mu*-induced reversions occur within the last two or three cell divisions of a given lineage [17]. Germinally transmitted revertants are extremely rare ($<10^{-4}$), suggesting that excisions (at least, those that result in reversion) are prevented in cells that give rise to the gametes [9]. Germinally transmitted insertions (the source of new mutations in *Mutator* lines) are common and are not associated with the loss of the donor element [8]. Duplications can occur throughout development, particularly in minimal lines carrying a single *MuDR* element. For instance, ~25% (8/31) of reciprocally crossed plants carrying a single *MuDR* element had evidence of *MuDR* duplications in one direction of crossing and not the other, consistent with duplications of *MuDR* early enough to skew genetic rations from the expected 1:1 [8]. However, in more complex lines, most insertions (like somatic excisions) occur late in development. In a typical *Mutator* line, only 20% of all new mutations are premeiotic; 80% occur in only a single seed and, of those, 25% are postmeiotic [18–20].

Data from *RescueMu* and several other projects surveying sequences next to standard *Mu* elements have demonstrated that *Mu* elements generally transpose into low-copy-number regions of the genome [3,21]. In one study, 69% of 88 *RescueMu* flanking sequences were genes and only 4% were exclusively associated with retrotransposons. Interestingly, different classes of *Mu* elements appear to have different affinities for different genes [22]. One target of *Mu* insertions that has been examined extensively (*gl8*) also showed a strong preference for the 5′ region of the gene (62/75 insertions examined) [23]. These data are similar to those obtained for *P* elements [24], suggesting that 5′ targeting might be a generic feature of at least some classes of class-II transposons (transposons that transpose via a DNA intermediate). *Mutator* also exhibits some degree of target-site sequence preference [3,23], but the observed sequence consensus might be a reflection of overall structural characteristics of target regions rather than specific sequences.

5′-Methylation of cytosines at CG and CNG sites within TIRs of non-autonomous *Mu* element is a diagnostic feature of *Mu*-element inactivation [9]. Methylation of these elements occurs after *MuDR* elements are lost by genetic segregation in minimal lines [8]. In this case, if *MuDR* is restored genetically then the methylation is lost, suggesting that TIR methylation represents a default state that occurs in the absence of the transposase. In more typical *Mutator* lines, *MuDR* elements themselves can also be methylated, a process that is associated with transcriptional silencing [7,13]. When this occurs, activity is lost even in the presence of potentially active *MuDR* elements [25]. Once silenced, these elements are rarely reactivated ($<10^{-4}$), except when exposed to agents such as ultraviolet radiation [26].

Functions of *mudrA*

The *mudrA* gene is required for all aspects of *Mutator* activity. Some of the best evidence for this comes from analysing deletion derivatives of *MuDR*. These deletions can occur at various stages of development, including germinal cells [27]. A *MuDR* element that showed a position effect in its duplication frequency [8] (half that observed at a second position) exhibited a particularly high frequency of deletions: ten of 30 elements examined in one family contained deletions (D. Lisch, PhD thesis, University of California at Berkeley, 1995).

In plants that carry deletion derivatives of *MuDR* that lack full-length *mudrA*, *Mu* TIRs acquire methylation and no new excisions or insertions occur [27]. Truncated nonfunctional versions of *mudrA* and intact *mudrB* are expressed at near-normal levels in lines carrying only these derivatives, suggesting that transcriptional activity of *MuDR* genes is not dependent on the presence of a functional transposase. A similar result was obtained using *mudrB* TIR sequences driving production of firefly luciferase and glucuronidase [28]; this construct did not depend on *Mutator* activity for expression. By contrast, the levels of *hMuDR* expression drops precipitously when *Mutator* activity is lost, and only a subset of elements continue to express constitutively [13].

MURA has been produced in yeast and shown to bind to a specific region within *Mu* termini [29]. Interestingly, this region is not the same as those previously identified as being bound using gel-shift assays using maize protein extracts; presumably host proteins recognize motifs within the TIRs (Fig. 1). Plants that carry deletion derivatives expressing only *mudrA* or transgenic plants containing only the *mudrA* cDNA driven by the 35S promoter can cause characteristically late somatic excisions and

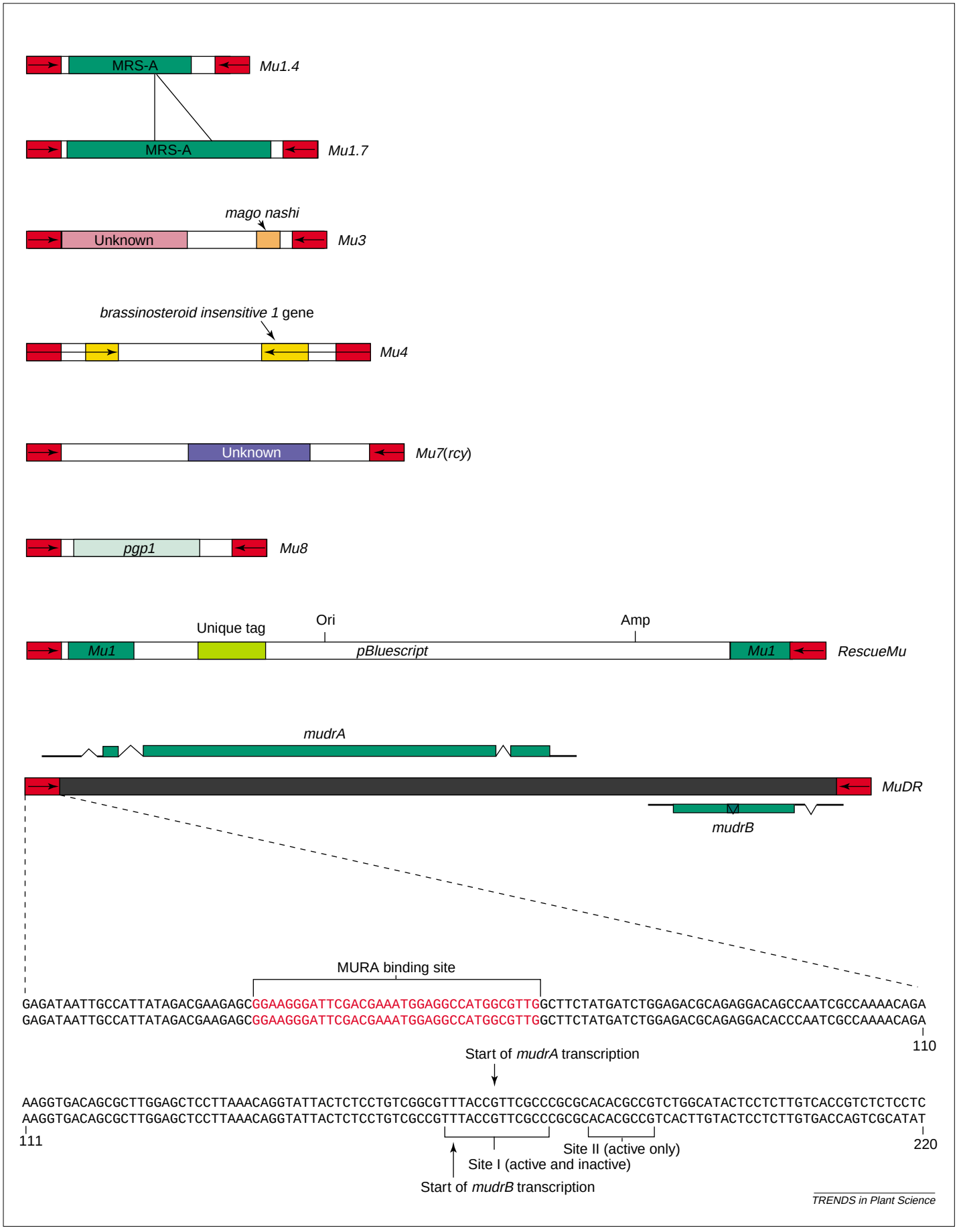
MRS-A
Mu1.4
MRS-A
Mu1.7
mago nashi
Unknown
Mu3
brassinosteroid insensitive 1 gene
Mu4
Unknown
Mu7(rcy)
pgp1
Mu8
Unique tag
Ori
Amp
Mu1
pBluescript
Mu1
RescueMu
mudrA
MuDR
mudrB
MURA binding site
GAGATAATTGCCATTATAGACGAAGAGCGGAAGGGATTCGACGAAATGGAGGCCATGGCGTTGGCTTCTATGATCTGGAGACGCAGAGGACAGCCAATCGCCAAAACAGA
GAGATAATTGCCATTATAGACGAAGAGCGGAAGGGATTCGACGAAATGGAGGCCATGGCGTTGGCTTCTATGATCTGGAGACGCAGAGGACACCCAATCGCCAAAACAGA
110
Start of mudrA transcription
AAGGTGACAGCGCTTGGAGCTCCTTAAACAGGTATTACTCTCCTGTCGGCGTTTACCGTTCGCCCGCGCACACGCCGTCTGGCATACTCCTCTTGTCACCGTCTCTCCTC
AAGGTGACAGCGCTTGGAGCTCCTTAAACAGGTATTACTCTCCTGTCGCCGTTTACCGTTCGCCCGCGCACACGCCGTCACTTGTACTCCTCTTGTGACCAGTCGCATAT
111
220
Site II (active only)
Site I (active and inactive)
Start of mudrB transcription
TRENDS in Plant Science

Fig. 1. The *Mutator* family of transposons in maize. All classes share similar 220 bp terminal inverted repeats (TIRs; red boxes), but each class has a unique internal sequence. Colored boxes within each element indicate regions with homology to various proteins. *Mu1*/*Mu1.7* (accession nos X13019 and Y00603) is similar to *MRS-A* (*Mu*-related sequence A) [50]. *Mu3* (accession no. U19613) is similar to an unknown *Arabidopsis* protein (Accession no. NP_187190) and *mago nashi* protein (Accession no. NG_000085). *Mu4* (Accession no. X14224) is similar to *brassinosteroid insensitive 1* from *Arabidopsis* (Accession no. AAC49810). Notice the extended TIRs of *Mu4*, which include a sequence 95% identical to a maize expressed sequence tag (Accession no. BG266445). *Mu7* (Accession no. X15872) is similar to an unknown *Arabidopsis* protein (Accession no. NP_192120). *Mu8* (Accession no. X53604) is similar to *pgp1* from *Arabidopsis* (Accession no. A42150). *Pgp1* is similar to multiple-drug-resistance proteins in a wide range of organisms. *RescueMu* is a *Mu1* element into which *pBluescript* has been inserted, including the origin of replication (*ori*), a selectable marker and a unique 'marker' sequence from *Rhizobium meliloti* [3]. For *MuDR*, the two transcripts are indicated above and below [11]. Exons are depicted as green boxes. Introns are depicted as thin black lines. The second intron of *mudrB* is only infrequently spliced out. The TIR sequence shows where the transposase binds [29] (in *Mu1*), where gel-shift experiments identified binding sites [51] and where *mudrA* and *mudrB* transcription is initiated. In addition to the indicated site, half of *mudrA* transcripts initiate at position +252, just inside the TIR.

hypomethylation of *Mu1* elements, but they do not cause germinal duplications [27,30].

Functions of *mudrB*

The evolution and function of *mudrB* are somewhat mysterious. Nearly all *mudrA*-like genes carry a motif generally associated with integrase function (a D–34–E motif) (Fig. 2), but the available data suggest that integration requires MURB as well. *MuDR* derivatives that both express *mudrB* and produce MURB do not cause excisions, duplications or *Mu*-element hypomethylation [27]. Interestingly, *mudrB* appears to be missing from all other MULEs discovered to date, including those that are known to transpose in *Arabidopsis* [31]. It is possible that low levels of transposition are possible even in the absence of *mudrB*, and that the acquisition of a *mudrB* sequence in an ancestor of maize resulted in the extremely high insertion frequency observed in this species.

Developmental regulation of *Mu* activity

The promoters of the convergently transcribed *mudrA* and *mudrB* genes are within the TIRs [32], which are 97% identical to each other [7], suggesting that these two genes are coordinately regulated. Both genes are expressed at high levels in the actively dividing tissues of the embryo, young leaves and floral organs [33]. Promoter analysis using *MuDR* TIRs linked to a glucuronidase-encoding gene shows similar patterns of expression, along with a 20-fold increase in expression in pollen. This analysis also revealed that the *Mu* TIRs contain sequences similar to enhancers specific for plant pollen expression, as well as those that are important for the regulation of cyclin, kinesin and H3 and H4 histone genes in dividing cells of both monocots and dicots [32].

Protein abundance and distribution monitored with antibodies to the predicted proteins gives conflicting results. One antibody to MURB localizes to the same tissues that express the *mudrB* gene, with the strongest signal coming from actively dividing tissue, particularly floral tissues [34]. This antibody does not recognize protein from plants that lack the single functional *MuDR* element in the minimal line [27]. Interestingly, MURB protein was not detected in cells that have finished dividing, nor in the prepollen mother cells, which are those cells about to undergo meiosis [34], although it is present in pollen (D. Lisch, unpublished).

Additional antibodies raised to both MURA and MURB have given different results. These antibodies recognize protein in all maize lines (with either active or inactive *Mu*) at equivalent levels [13]. This implies that, even though *mudrA* and *mudrB* expression is tightly regulated at the level of transcription, the overall quantity of protein is maintained regardless of activity level. The presence of expressed *hMuDR* sequences might explain the discrepancy. These elements are expressed in both active and inactive maize lines, albeit at much lower levels in inactive lines [13]. The differences between antibodies raised in different laboratories might be related to variations in the ability to recognize the *hMuDR* products.

Any model for developmental regulation of *Mu* activity must take into account a series of observations. The *mudrA* and *mudrB* genes are transcribed and translated in tissues in which excisions are not observed. Furthermore, duplications, deletions and changes in methylation at *Mu* TIRs can all occur in tissues without excisions resulting in reversion [8]. Two models have been proposed (Fig. 3). One model (the gap-repair model) suggests that repair of the gaps left by *Mu*-element excision varies depending on the cell lineage in which the gap is introduced [34]. In germinally transmitted cell lineages, gap repair using the sister chromatid is hypothesized to be highly efficient, resulting in the transmission of duplications rather than reversions. Late during development of somatic tissues, gap repair is much less efficient or is replaced by end joining and ligation, resulting in the observed spectrum of footprints [3], many of which restore gene function, resulting in visible reversions.

A second model (the replicative/duplicative model) [3] suggests that the mode of transposition, rather than the repair of gaps, varies between tissues. According to this model, as in the gap-repair model, inefficient gap repair occurs late during somatic development. However, in germinally transmitted lineages, gap repair is replaced by a semiconservative duplicative transposition mechanism similar to that used by the bacterial transposon Tn7, which is known to be competent to make a similar switch between cut-and-paste and duplicative transposition [35].

Because there is good evidence for gap repair (based on footprint data and deletion derivatives of *MuDR* [36]) and it is known that gap repair using the sister chromatid can result in duplication of transposons [37], the gap-repair model is more parsimonious. However, it makes assumptions about variations in gap repair for which there are currently no evidence. Furthermore, the gap-repair model demands that there be no excisions in pollen following the last S phase, in spite of the observation that up to

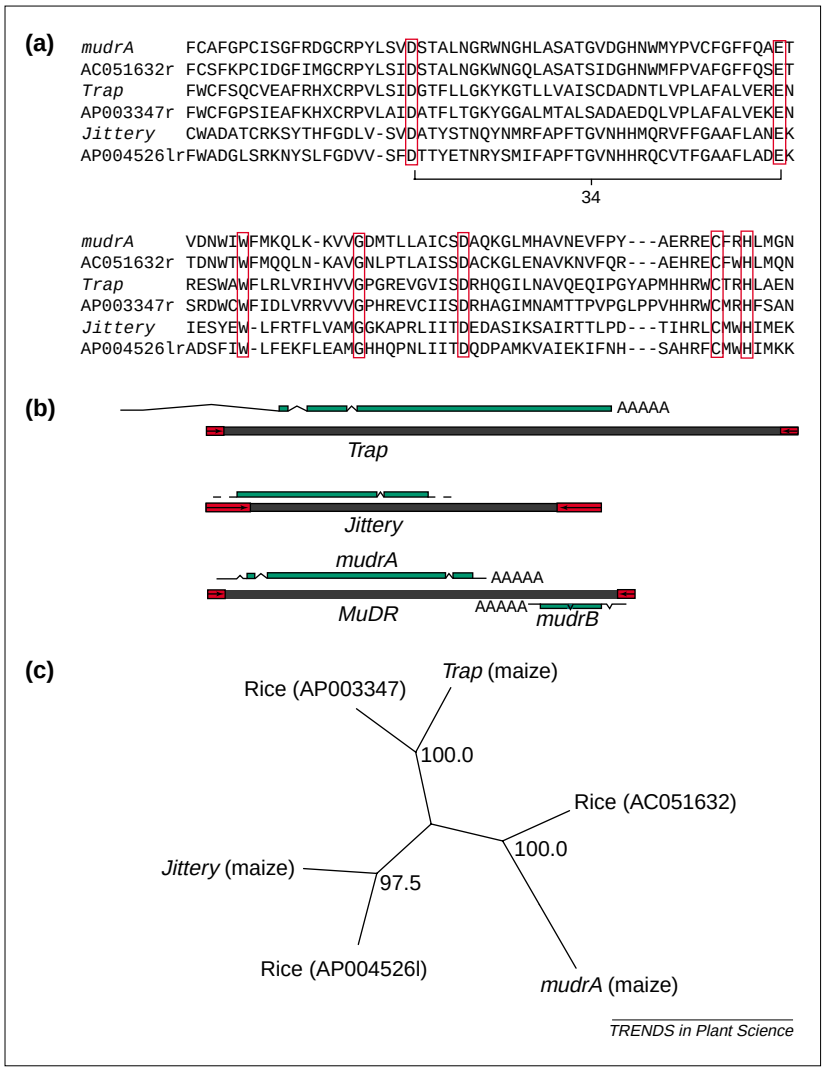

Fig. 2. There are distant relatives of *MuDR* in maize that are more closely related to elements in rice than they are to each other. (a) Sequence alignment of the core regions of the transposase of several *MuDR*-like elements from maize as well as their closest relatives in rice (identified using tBLASTN and designated by their Accession numbers). *Trap* [52] (transposon-associated protein) is encoded as part of a polycistronic message that also encodes a maize homeobox-containing protein. *Jittery* is an active distant relative of *MuDR* in maize (H. Dooner, pers. commun.). The well-conserved D–34–E integrase signature is noted. Other amino acids indicated in red boxes are also well conserved across a wide range of *Mu*-like elements. (b) Structural features of *Trap*, *Jittery* and *MuDR*. Exons are depicted as green boxes, introns are depicted as thin black lines. Terminal inverted repeats are indicated by red boxes. The *Trap* transcript actually initiates outside the element [52]. Neither *Trap* nor *Jittery* carries sequences similar to *mudrB*, nor does any other sequenced *MuDR* homolog. (c) Parsimony tree of these proteins. Numbers on the unrooted tree represent bootstrap values from 100 replicates.

20% of all insertions occur in postmeiotic cells [20]. The alternative replication/duplicative model is also consistent with the data, but it invokes the existence of an additional mode of transposition for which there is no evidence.

Epigenetic regulation

The most consistent molecular correlate for epigenetic silencing of *Mutator* is cytosine methylation of sites within *Mu*-element TIRs [25,38]. In complex lines, both *MuDR* and non-autonomous elements become spontaneously methylated in ~10% of progeny. This methylation is associated with transcriptional silencing of *MuDR* elements and an absence of new insertions [9]. Minimal lines show no evidence of such epigenetic silencing of *MuDR* elements. However, when a minimal line carrying a single *MuDR* element was crossed with an unrelated line, silencing of that element was observed [39]. A silencing factor, *MuKiller*, segregates as a single dominant mendelian locus in these lines. Silenced *MuDR* elements remain silenced in subsequent outcrossing, suggesting that the inactivation is heritable and is not dependent on the continued presence of the silencing locus [40].

The means by which silencing occurs is unknown. It might involve a derivative of *MuDR* or *hMuDR* sequences, or possibly a plant gene involved in the control of repetitive sequences. There is also evidence for the involvement of non-*Mutator* genes in at least the maintenance of the silenced state. The *mop1* mutation prevents the epigenetic silencing associated with paramutation (the process by which one allele of a gene can heritably alter the expression characteristics of another allele of that gene) [41]. This mutation also prevents methylation of *Mu1*- and *MuDR*-element TIRs in lines lacking *MuDR* elements or those carrying only silenced *MuDR* elements. Furthermore, over several generations, this mutation can cause reactivation of a previously silenced *MuDR* element [42]. Thus, both *MuDR*/*Mu* methylation and inactivation are regulated by some of the same genes that are involved in the regulation of paramutable host genes. Interestingly, the same phenomenon has been observed in *Arabidopsis*: MULEs have been reactivated in a line that was homozygous for a mutation in *ddm1*, a SWI2/SNF2 chromatin remodeling gene that reduces levels of cytosine methylation in the *Arabidopsis* genome by 70% [31]. Interestingly, the *ddm1* mutation, like *mop1*, causes loss of both symmetrical and nonsymmetrical cytosines [43].

Evolution

Although *Mutator* activity has only been described in maize and *Arabidopsis*, recent genome-sequencing projects have made it clear that *Mu* elements are widespread among plants. Complete MULEs (containing *mudrA* homologs, long TIRs and direct repeats at the point of insertion) have been identified in *Arabidopsis*, rice, barley, sorghum and lotus [14,15,31] (D. Lisch, unpublished). In addition, *mudrA*-homologous sequences have been detected in all major subfamilies of grasses [44]. Many of these sequences are found in expressed-sequence-tag databases, suggesting that expressed *mudrA* homologs are a common feature of plant genomes. Furthermore, multiple almost-identical copies of several elements have been identified in species with completely sequenced genomes [14,15]. Together, these data suggest that MULEs are or have recently been active in a wide range of angiosperms.

MULEs can be widely diverged from each other. For instance, *Jittery* (an element in maize) is an

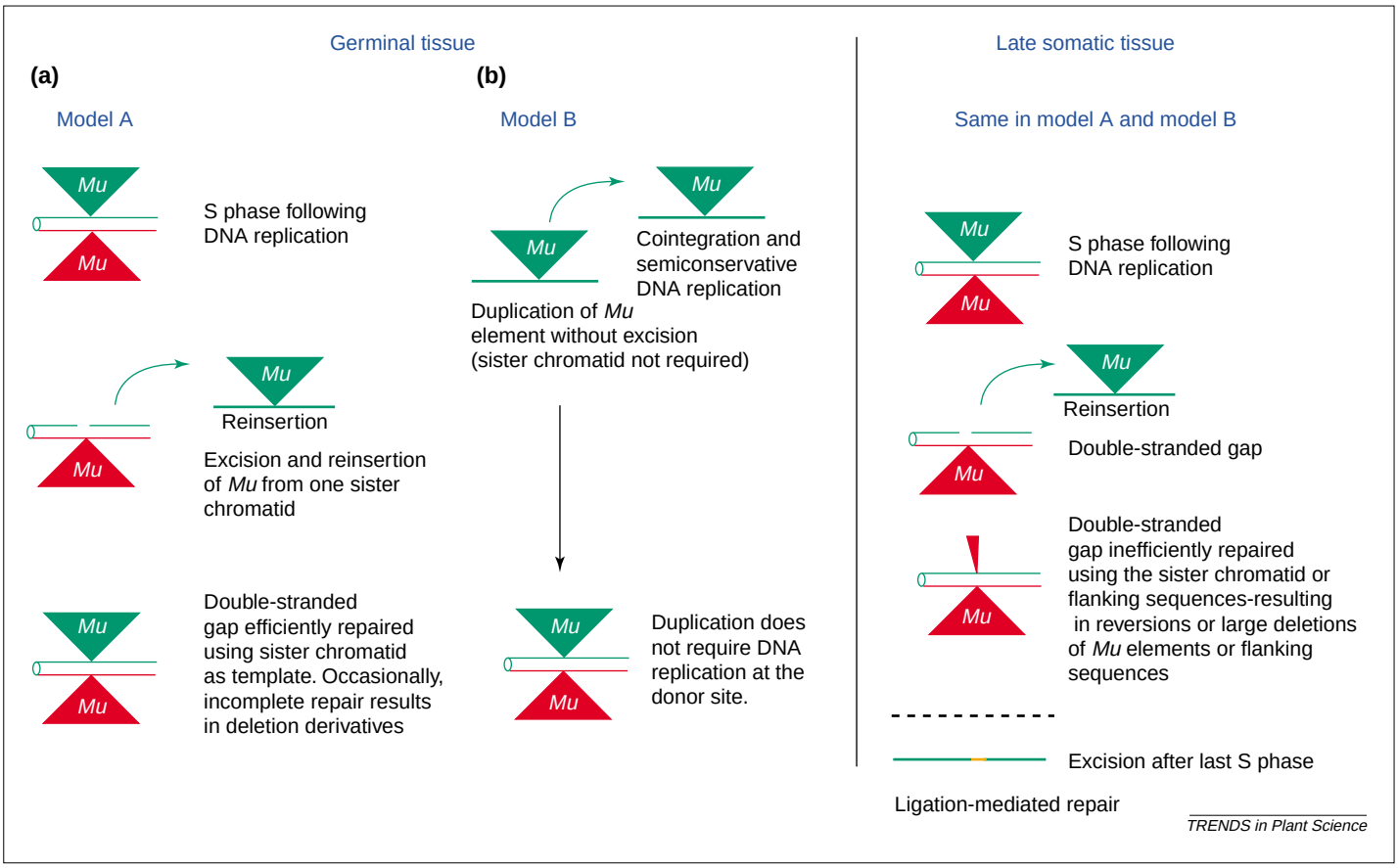

Fig. 3. Models for developmental regulation of *Mu*-element transposition. (a) The gap-repair model [34] suggests that the double-stranded gap left when *Mu* elements excise is resolved in different ways at different times. In germinal cell lineages, gaps are efficiently repaired using the sister chromatid (red), duplicating the element. Interruption of gap repair results in deletions within the element at direct repeats, as have been observed [36]. Late in the development of somatic tissue there is a shift from efficient to inefficient template-mediated repair or to simple ligation after the last S phase, resulting in the observed spectrum of footprints [3]. (b) The replicative/duplicative model [3] suggests that an inefficient form of gap- or ligation-mediated repair is restricted to late somatic tissue. In the germinal cells, gap repair is replaced by a duplication mechanism that uses semiconservative DNA replication, as is observed for some bacterial transposons. This duplication does not involve the production of a double-stranded gap and thus cannot result in reversions in tissue in which it occurs.

active MULE that is more similar to sequences in *Arabidopsis* and rice than it is to *MuDR* (H. Dooner, pers. commun.; Fig. 2). Like all other characterized MULEs (with the exception of *MuDR*), *Jittery* lacks a *mudrB* gene. Interestingly, it also appears to excise without duplicating, similar to the *MuDR* elements that lack *mudrB*. Remarkably, two of the MULEs most closely related to *Jittery* in *Arabidopsis* appear to be host genes rather than transposons; mutations in them cause defects in the far-red-light response pathway [45,46].

Distribution of any given subfamily of MULEs is patchy [44], which could be a result of horizontal transfer of MULEs or of differential loss of many paralogous elements. In either case, it is clear that the evolutionary history of this family of transposable elements differs markedly from that of its hosts.

Future directions

Although the basic requirements for activity (a permissive genetic background and full-length *mudrA* and *mudrB* genes) are well established, the mechanisms that regulate *MuDR* activity are not well understood. With respect to developmental regulation, it will be necessary to understand what kinds of post-transcriptional modification *MuDR* proteins undergo at various stages of development and how those modifications affect *Mu*-element activity and its consequences. Analysis of variants of *MuDR* or mutations in host genes that modify the observed tight regulation of *Mu* would be also be useful. These variants should make it possible to distinguish between models for developmental regulation. Much of the required work involves detailed biochemical and molecular analysis of phenomena that have currently only been described using classical genetic approaches.

With respect to epigenetic regulation, it will be important to understand exactly what changes occur during the process of silencing, because these changes might involve specific pathways, such as RNA interference or chromatin remodeling. For instance, are aberrant RNA molecules present in plants that are undergoing silencing? Are changes in chromatin such as histone deacetylation or resistance to DNase-I digestion associated with silencing? Do *hMuDR* elements or their products play a role in the process of silencing? However, beyond the phenomenology of silencing, isolation of the genes encoding *MuKiller*, *mop1* and other modifiers of silencing will provide the most important clues to how epigenetic silencing can be initiated and

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maintained in maize. In other species, such as *Arabidopsis* [47], *Caenorhabditis elegans* [48] and *Chlamydomonas reinhardtii* [49], it has been the isolation of mutations in genes that affect silencing that has led to the most rapid progress in understanding the mechanism of silencing, and the same will undoubtedly be true in maize.

Finally given the availability of transformable *mudrA* and *mudrB* genes, it should be possible to establish the system in a heterologous species or, alternatively, to activate an endogenous *Mutator* system. An active *Mutator* system could provide an invaluable tool for mutagenesis and gene isolation in any species in which it can be developed.

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