Mobilized retrotransposon \textit{Tos17} of rice by alien DNA introgression transposes into genes and causes structural and methylation alterations of a flanking genomic region

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\textit{Tos17} is a \textit{copia}-like endogenous retrotransposon of rice, which can be activated by various stresses such as tissue culture and alien DNA introgression. To confirm element mobilization by introgression and to study possible structural and epigenetic effects of \textit{Tos17} insertion on its target sequences, we isolated all flanking regions of \textit{Tos17} in an introgressed rice line (Tong35) that contains minute amount of genomic DNA from wild rice (\textit{Zizania latifolia}). It was found that there has been apparent but limited mobilization of \textit{Tos17} in this introgression line, as being reflected by increased but stable copy number of the element in progeny of the line. Three of the five activated copies of the element have transposed into genes. Based on sequence analysis and Southern blot hybridization with several double-enzyme digests, no structural change in \textit{Tos17} could be inferred in the introgression line. Cytosine methylation status at all seven \textit{CCGG} sites within \textit{Tos17} was also identical between the introgression line and its rice parent (Matsumae)-all sites being heavily methylated. In contrast, changes in structure and cytosine methylation patterns were detected in one of the three low-copy genomic regions that flank newly transposed \textit{Tos17}, and all changes are stably inherited through selfed generations.

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Retrotransposons, also called class I mobile elements, transpose via reverse transcription of RNA intermediates, and are major genomic components in eukaryotes. The most prominent retrotransposons in plants are the LTR (long terminal repeat) retroelements that can be further divided into \textit{copia}- and \textit{gypsy}-like classes (reviewed by Kumar and Bennetzen 1999; Bennetzen 2000). Although functionally intact, LTR-retrotransposons usually contain sequences encoding all proteins required for their retrotransposition, but they are largely quiescent during normal growth and development (Wessler 1996; Grandbastien 1998; Kumar and Bennetzen 1999). Nevertheless, accumulating evidence indicates that some of the plant retrotransposons are inducible; that is, they are responsive to, and often transcriptionally and/or transpositionally activated by various biotical and abiotical stresses such as wounding, pathogen attack, cell culture induction, environmental cues (e.g. drought) and interspecies introgression (Hirochika et al. 1996; Hirochika et al. 1997). It was found that \textit{Tos17} is active only during callus culture and immediately becomes inactive upon plant regeneration (Hirochika et al. 1996; Hirochika 1997; Grandbastien 1998; Kalendar et al. 2000; Liu and Wendel 2000). Because some of these stresses are prevalent in natural plant populations, and because uncontrolled retrotranspositions could potentially disrupt the host’s genome, it is not surprising that plant genomes have evolved genetic and/or epigenetic mechanisms to tightly control their activity. On the other hand, even limited activation and mobilization of retrotransposons may have bearing on the host genome, particularly if the elements transpose into or near functional genes. Apart from gene inactivation, the insertion of LTR elements may also cause changes in structure and/or epigenetic states of the flanking sequences and lead to alterations in expression (Whitelaw and Martin 2001; Kashkush et al. 2003).

\textit{Tos17} is an endogenous \textit{copia}-like retrotransposon in rice (Hirochika et al. 1996). The number of copies of \textit{Tos17} is very low, ranging from 1 to 4 in various cultivars growing under normal conditions, but can be significantly elevated by tissue culture (Hirochika et al. 1996; Hirochika 1997). It was found that \textit{Tos17} is active only during callus culture and immediately becomes inactive upon plant regeneration (Hirochika et al. 1996; Hirochika 1997, 2001), thus suggesting developmentally regulated efficient element silencing. Nevertheless, the mechanism for silencing of \textit{Tos17} is not clear, neither is its possible influence on flanking sequences after retrotransposition.

We have found that \textit{Tos17} could also be mobilized by sexual introgression into the rice genome of
genomic DNA from wild rice (*Zizania latifolia*); copy number of the element in some introgression lines was markedly increased though insertion sites have not been isolated (*LIU* and *WENDEL* 2000). Also, similar to the case in tissue culture (*HIROCHIKA* et al. 1996), *Tos17* was also likely inactivated after retro-transposition in the introgression lines (*LIU* and *WENDEL* 2000).

To confirm mobilization of *Tos17* by introgression, and to study the possible role of cytosine methylation on the element’s activity, particularly the potential influence of element insertion on structure and epigenetic state of the target sequences, we selected an introgression line (Tong35) wherein *Tos17* was activated but its transposition was very limited, hence indicating rapid and efficient repression of its activity. The isolation of all possible genomic regions flanking *Tos17* (original and newly transposed) in this line, has enabled the confirmation of element mobilization induced by alien DNA introgression, and the analysis on genomic structure and methylation status of both *Tos17* itself and its targeted flanking sequences. We report that sequence structure and cytosine methylation state of all copies of *Tos17* itself in the introgression line remain the same hypermethylation as that of the rice parent. In contrast, heritable alterations in both structure and methylation level of one of the three low-copy sequences flanking newly transposed *Tos17* have occurred in the introgression line. Possible causes for the changes are discussed.

**MATERIAL AND METHODS**

**Plant materials**

Production of a series of rice lines with introgressed genomic DNA from wild rice *Zizania latifolia* was described previously (*LIU* et al. 1999b). For the present study, one introgression line (Tong35) which exhibits novel morphological characteristics in multiple traits from its rice parent (Matsumae), and which was characterized by genome-wide DNA fingerprinting as possessing ≤0.1% genomic DNA from *Z. latifolia* (unpubl. data), was used. Another reason to use Tong35 is based on preliminary studies indicating that there has been limited mobilization (thus implicating efficient silencing) of the retrotransposon *Tos17* in this line following *Zizania* DNA introgression (see RESULTS).

**Isolation of *Tos17*-flanking sequences**

Insertion sites of *Tos17* in Tong35 and Matsumae were isolated by the thermal asymmetric interlaced PCR (TAIL-PCR) described by *LIU* et al. (1995). Three nested *Tos17* 3'-LTR-specific primers, namely T17TAIL2, T17TAIL3 and T17TAIL4, described by *YAMAZAKI* et al. (2001) were used, together with each of the three arbitrary primers, AD1, AD2 and AD3, as reported by *LIU* et al. (1995), for PCR amplification. PCR products from the 3rd-round amplification were purified, and either directly sequenced or ligated to the PTGM-T vector (Promega) and sequenced with vector primers. Similarity searches of the isolated sequences were conducted with the BlastX program at the NCBI website.

**Preparation of genomic Southern blots**

Genomic DNAs were isolated from fully expanded leaves at the same developmental stage of Tong35 and its rice parental line Matsumae by a modified CTAB method (*KIDWELL* and *OSBORN* 1992), and purified by chloroform extractions. Because both parental and the introgression lines are genetically homogeneous (*LIU* et al. 1999b), leaves from several individuals were pooled. For *Tos17* copy number estimation, genomic DNAs of Tong35 and Matsumae were digested respectively by *EcoRI* and *HindIII* that have no site within *Tos17* (Fig. 1). For detecting possible structural changes within *Tos17*, two types of analysis were conducted. First, two regions (Fig. 1) of the element, i.e. region I (including 5’-LTR and part of the PBS region, total length 296 bp) and region II (part of the RT/RNase H gene, total length 666 bp) were isolated by PCR from genomic DNAs of Tong35 and Matsumae respectively with specific primers. The primers for region I are *LTRP1*: 5’-CTGATATAGTGGGCCATGTC-3’ (nucleotide positions 28–48) and *LTRP2*: 5’-GGCGGTCA-ACGACAATC-3’ (nucleotide positions 323–366); primers for region II are *RTPI*: 5’-GCTACCCGGTTTGGGTAT-3’ (nucleotide positions 2817–2837) and *RTPI*: 5’-CGAAATCGGAGCAC-3’ (nucleotide positions 3483–3463). For each region, five randomly chosen clones were sequenced and compared. Second, genomic DNAs of the two lines were digested with *Bst*I (located in each of the LTRs and thus to delineate the internal fragment) plus each of five other enzymes including *Pst*I, *Spe*I, *Xba*I, *Bam*HI and *Xho*I, which have unique restriction sites within the element. For assaying cytosine methylation status at the CCGG sites within *Tos17*, genomic DNAs were cut with *Bst*I plus either *Hpa*II or *Msp*I, and hybridized with an internal fragment of *Tos17* as a probe. For assaying cytosine methylation status of flanking sequences, genomic DNAs were digested with *Hpa*II or *Msp*I alone and *Hpa*II or *Msp*I with other enzymes (usually the same as the ones used for probe preparation to exclude confounding effect of *Tos17*, thus ensuring the
analyzed regions are bona fide flanking sequences, see RESULTS), and hybridized with each of the flanking sequences as probes. Digested DNAs were run through 1% agarose and transferred onto Hybond N\textsuperscript{+}/C27 nylon membrane (Amersham Pharmacia Biotech) by the alkaline transfer.

Preparation of probes and Southern hybridization

A portion (666 bp) of the RT/RNaseH region of Tos17 was isolated by PCR, as described above, and used as a probe for Tos17. Probes for each of the flanking sequences of the Tos17 3’ LTR were prepared by firstly isolating the fragments by TAIL-PCR described above, and then, digesting the PCR products with proper enzymes that were adjacent to, but downstream of, the 3’-LTR of Tos17 (Fig. 4), and finally, eluting the fragments of the expected sizes. For isolation of a probe residing at the region of sequence T14-2 that flank 5’-LTR of Tos17, a pair of primers specific to this region was designed based on the whole genome sequence of the standard laboratory japonica rice cultivar, Nipponbare (http://rgp.dan.affrc.go.jp); these are: T14FLK5'-1 (5’-CAGGGATTTGCCATC-TATGC) and T14FLK5'-1 (5’-TGCAATTGTGTTGG-TGTACC). All probes were gel-purified and labeled with fluorescein-11-dUTP by the Gene Images random prime labeling module (Amersham Pharmacia Biotech). Hybridization signal was detected by the Gene Images CDP-Star detection module (Amersham Pharmacia Biotech). Pre-hybridization, hybridization and post-hybridization washing conditions are as described by LIU and WENDEL (2000).

RESULTS

Tos17 is a copia-like retrotransposon that was first isolated by HIROCHIKA and colleagues (1996) from cDNA of rice callus. Using a cDNA fragment of the reverse transcriptase (RT) region of Tos17 (accession no. D85876) as a query, we searched the Genbank database by BlastN, and found a 139 kb rice BAC genomic DNA fragment (nbxb0019M20, accession no. AC087545) located on Chromosome 10; a region of this fragment shows perfect match with the RT region of Tos17. Further analysis identified the two identical LTRs, the 5 bp targeting site duplication (CTCCT; HIROCHIKA et al. 1996) and all intact components for a typical copia-like retrotransposon, thus indicates that this BAC clone encompasses the complete sequence of Tos17. Based on this sequence information, we identified seven HpaII/MspI restriction sites (CCGG) within Tos17, and one BstXI site within each of its LTRs (Fig. 1).
Tos17 was mobilized in introgression line Tong35

We previously surveyed about 20 rice lines introgressed by wild rice, *Zizania latifolia* Griseb., by Southern blot hybridization with *Eco*RI/Tos17 (RT fragment), and found that about half of the lines (including Tong35) possess increased copy numbers of the element (unpubl. data). To confirm that there had been mobilization of Tos17 in introgression line Tong35, we digested genomic DNA of this line and its rice parent Matsumae with two enzymes (*Eco*RI and *Hind*III) that have no restriction site within the element (Fig. 1). Therefore, copy number of Tos17 could be conservatively estimated by Southern blot hybridization in the two digests using an internal fragment of Tos17 as a probe (Fig. 1). It was found that in *Hind*III digest, four new bands in Tong35 are detectable compared with Matsumae (Fig. 2a), indicating that there are at least four new retrotransposition events. The *Eco*RI digest revealed two new bands (Fig. 2a). This discrepancy between the enzymes is probably because some of the retrotranspositions occurred in genomic regions remote from *Eco*RI sites, and thus the fragments are too large to be revealed by the conventional Southern blotting method. Because in our earlier studies, no change in copy number of *Tos17* was found in >30 randomly selected individuals of the rice parent (Matsumae), and because the homologue of *Tos17* in wild rice *Z. latifolia* showed less than 80% identity to *Tos17* in the probe region (thus undetectable at the stringency of Southern hybridization used) (Liu and Wendel 2000), it could be concluded that the increase in copy number of *Tos17* in introgression line Tong35 is the result of activation of the original cryptic *Tos17* in Matsumae by DNA introgression from wild rice (*Z. latifolia*). Nevertheless, activity of the element has been apparently transit, as no further change in its copy number was observed in three individuals of selfed progeny of the introgression line (Fig. 2b).

**Mobilized Tos17 in introgression line Tong35 transposed into genes**

By sequencing more than 30 clones from the 3rd-round TAIL-PCR amplification products of introgression line Tong35, six different sequences that all possess a complete LTR (138 bp) of *Tos17* at their 5’-termini were identified. Because of the positions of the TAIL-PCR primers (all upstream of the 138 bp 3’ LTR; Yamazaki et al. 2001), presence of the *Tos17* LTR confirmed authenticity of theses sequences as bona fide *Tos17*-flanking genomic regions. We believe that most probably all regions that flank *Tos17* in Tong35 have been isolated, because according to the Southern analysis, the element number in this line is less that five (Fig. 2 and above section). In addition, one of the sequences (T10-1) is believed to be the original *Tos17* flanking region, as it is identical to the only LTR-containing sequence isolated from the parental line Matsumae after sequencing nine clones from the 3rd-round TAIL-PCR products. This is also in accord with the Southern hybridization results showing that there is likely only one copy of *Tos17* in Matsumae (Fig. 2; Liu and Wendel 2000).

Based on BlastX analysis, three of the five newly transposed *Tos17*-flanking sequences bear significant similarity to known-function genes that are located on Chromosome 5 and 10 (Table 1). Interestingly, all three genes are those that encode for metabolic enzymes, i.e. DNA-directed RNA polymerase, beta-glucosidase, and myrosinase (Table 1). The rest two regions that are located respectively on Chromosome 11 and 3, showed no significant homology to known genes. Analysis on the 5-bp targeting sites revealed no consensus motif at the nucleotide level (Table 1). Thus, the isolation of additional five distinct *Tos17*-flanking sequences from introgression line Tong35, as compared with only one sequence from its rice parent Matsumae, confirmed that there had been mobilization of *Tos17* following alien DNA introgression from *Z. latifolia*.

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Fig. 2. Copy number estimation of *Tos17* in the rice parent, cv. Matsumae (lane 1) and introgression line Tong35 (lane 2) by Southern blot analysis using genomic DNAs digested by *Hind*III and *Eco*RI that do not have restriction site within the element (a). Genomic DNAs from three randomly chosen individuals from progeny of Matsumae and Tong35 were also digested with *Hind*III to test for stability/heretability of the copy number of *Tos17* (b). The blots were probed with a fragment of the reverse transcriptase (RT) region of *Tos17*. New bands each should represent a retrotransposition event in the introgression line are marked by arrowheads.
Absence of genetic and epigenetic changes within Tos17 in introgression line Tong35

Because activity of Tos17 was rapidly repressed in the introgression line Tong35, as was reflected by stability of the element copy number among different individuals taken from two successive selfed generations (Fig. 2b and data not shown), and by the lack of element transcript based on northern blot analysis, it is interesting to test possible genetic and/or epigenetic changes within the element. To detect possible structural changes in all copies of Tos17 in Tong35, two regions of the element (Fig. 1), i.e. the 5’-LTR region (region I) and the RT region (region II) were amplified by sequence-specific primers and cloned. Five arbitrarily selected clones for each region of Tong35 were sequenced and aligned to those amplified from Matsumae and also to the BAC clone (nbxb0019M20, accession no. AC087545) that encompasses Tos17. No evidence for base changes was obtained in any clones for either region. In addition, using the RT fragment (Fig. 1) to probe Southern blots carrying genomic DNAs digested with BstXI alone and in combination with HpaII or MspI, which is expected if none of the seven CCGG sites within any Tos17 copy is digestible by HpaII or MspI (Fig. 1 and 3a). It is noted however that compared with BstXI digest, there are several faint bands in both lines cut with BstXI+HpaII or BstXI+MspI (Fig. 3a), suggesting that no structural change within any copies of Tos17 in introgression line Tong35.

To test possible cytosine methylation alterations at the seven CCGG sites within Tos17 (Fig. 1) of the introgression line, genomic DNAs of Tong35 and Matsumae were digested with BstXI alone and BstXI plus HpaII or MspI, and hybridized with the RT fragment as a probe. Clearly, in both Matsumae and Tong35, all seven CCGG sites within Tos17 are heavily methylated at both the inner and outer cytosines because a prominent ~4.0 kb band was detected in BstXI+HpaII and BstXI+MspI, which is expected if none of the seven CCGG sites within any Tos17 copy is digestible by HpaII or MspI (Fig. 1 and 3a). It is noted however that compared with BstXI digest, there are several faint bands in both lines cut with BstXI+HpaII or BstXI+MspI (Fig. 3a), suggesting...

Table 1. Tos17-flanking sequences in introgression line Tong35.

| Clone | Size (bp) | Chromosome location | Target sequence (5 bp) | Putative protein function predicted by BLSTX | Accession number | E-value |
|-------|-----------|---------------------|------------------------|---------------------------------------------|------------------|---------|
| T10-1* | 701       | chr.10              | CTCCT                  | Unknown                                     |                  |         |
| T14-2  | 808       | chr.5               | GAAGC                  | DNA-directed RNA polymerase II largest subunit from Arabidopsis thaliana | CAA37130         | 1e-12   |
| T24-1  | 539       | chr.10              | GTAGT                  | Beta-glucosidase from Vitis vinifera         | AAK72100         | 2e-1    |
| T25-1  | 636       | chr.10              | GTAGT                  | Putative myrosinase precursor from Arabidopsis thaliana | AAG52628         | 4e-10   |
| T25-2  | 724       | chr.11              | GAGGC                  | Unknown                                     |                  |         |
| T26-2  | 696       | chr.3               | AGACC                  | Unknown                                     |                  |         |

*Determined by BlastN against the IRGP database (http://rgp.dan.affrc.go.jp).
*Original flanking sequence of Tos17, which was also isolated from parental line Matsumae.

Fig. 3. Detection of cytosine methylation alterations within and flanking Tos17 in the rice parent, cv. Matsumae (lane 1) and introgression line Tong 35 (lane 2). (a) Genomic DNAs of Matsumae and Tong35 were digested with BstXI along or in combination with HpaII/MspI and hybridized against the RT probe of Tos17. No difference exists between the rice parent and the introgression line in any of the enzyme digests. (b) Genomic DNAs of Matsumae and Tong35 were digested with HpaII or MspI and hybridized against the RT probe of Tos17. Loss of parent band (marked by a solid circle) and appearance of novel bands (marked by arrows) in the introgression line in both digests indicate differential methylation levels at both CG and CNG sites of the Tos17-flanking genomic regions.
slight demethylation at some CCGG sites, probably in only some of the leaf cells, as there is only one copy of Tos17 in Matsumae. Consequently, no change was observed between Tong35 and Matsumae in either enzyme combination (Fig. 3a), indicating absence of methylation changes at the CCGG sites, or more likely, the heavily methylated state has been completely restored. Nevertheless, when using HpaII or MspI alone, marked difference between Tong35 and Matsumae in both enzyme digests were detected (Fig. 3b), indicating either that the activated copies of Tos17 had transposed into genomic regions with different methylation status at the CCGG sites, or de novo structural and/or cytosine methylation changes had occurred in the element-flanking regions in the introgression line.

Changes in structure and cytosine methylation in a genomic sequence flanking a newly transposed Tos17 in the introgression line

For detailed study on structural and/or methylation changes in flanking sequences of Tos17, probes for each of the six flanking sequences (including the original) were prepared by digesting the corresponding clones from the 3rd-round TAIL-PCR products of the introgression line, with enzymes downstream of the 3' LTR of Tos17, that have unique restriction sites within the sequenced regions (Fig. 4). In this way, the 3'-LTR of Tos17 is completely eliminated from the probes. For each of the studied sequences, genomic DNAs of Matsumae and Tong35 were digested with HpaII or MspI plus the enzyme used for probe preparation, described above. Two of the sequences (T24-1 and T25-1) produced strong signals and smeared hybridization patterns characteristic of repetitive sequences, thus precluding detection of possible changes in an individual copy into which Tos17 had inserted in the introgression line. Of the three low-copy and genic sequences that produced distinct banding patterns, two sequences (T25-2, T26-2) showed no difference between Tong35 and Matsumae, in either single- or double-enzyme digest, indicating absence of structural or methylation alterations in the introgression line. Similarly, the original flanking sequence of Tos17 (T10-1) also showed identical patterns between Matsumae and Tong35 in BamHI, BamHI+HpaII or BamHI+MspI digests. In contrast, one low-copy flanking sequence (T14-2, the putative RNA pol II large subunit, Table 1) showed apparent changes in both structure and cytosine methylation patterns in the introgression line (Fig. 5). For the analysis of this sequence, two enzymes (BbvI I and EcoRV) that have unique restriction sites within the sequenced region of T14-2 (808 bp) were used both alone and coupled with a pair of methylation-sensitive isoschizomers, HpaII/MspI, to digest the introgression line Tong35 and its parent Matsumae. In the BbvI I digestions, no change is detected between Tong35 and Matsumae when cut with BbvI I alone, but demethylation at the CpG site(s), being reflected by disappearance of two parental bands and appearance of a new band in BbvI I/HpaII digest, occurred in Tong35 (Fig. 5a). More complicated and drastic changes are detected in the EcoRV digestions. Specifically, two bands showed difference between Tong35 and Matsumae when cut with EcoRV alone (Fig. 5b, denoted by arrowheads), indicating structural changes involving the recognition site(s) of EcoRV. In EcoRV+HpaII and EcoRV+MspI double digests, conspicuous changes in the banding patterns have occurred in Tong35 compared with Matsumae, most likely due to hyper- and hypomethylation alterations at the CCGG sites (Fig. 5b, indicated by circles).

The foregoing shows that both structural and methylation changes occurred in a low-copy genic sequence (T14-2) that flanks the 3' LTR of Tos17 in the introgression line. To test if changes also occurred at the region that flanks the 5' LTR of Tos17, a probe was prepared by PCR amplification with region-specific primers (MATERIAL AND METHODS), and hybridized against Southern blots carrying DNA digested with HpaII or MspI plus HphI (whose restriction site being near the beginning.

Fig. 4. Diagrammatic illustration of the six Tos17 3' LTR-flanking regions isolated from introgression line Tong35. Enzymes shown were selected that are immediately downstream of the 3' LTR and that have unique restriction site within the sequenced length (indicated by base pairs). Each of the enzymes were used alone and in combination with HpaII/MspI to digest genomic DNAs of Matsumae and Tong35 and respectively hybridized against each of the probes prepared by digesting PCR products by the given enzymes.
of the 5’ LTR of Tos17). It was found that no change in hybridization patterns between Matsumae and Tong35 were detected in HphI or HphI/HpaII digests, but an extra band appeared in HphI/MspI digest, thus indicating methylation changes only at the CpNpG site of this genomic region in Tong35 (Fig. 5c).

Because Tong35 is a 10-generation-old, genetically homogeneous line, we suspect that the changes are most likely heritable. To confirm this, DNA was extracted from pooled plants of Tong35 respectively at the 11th- and 12th-generations, and digested with EcoRV, EcoRV/HpaII and EcoRV/MspI. When the blot was probed with the same T14-2 probe, identical hybridization patterns as generation 9 (Fig. 5b) were observed, thus verifying stable meiotic transmission of the structural and methylation alterations.

DISCUSSION

LTR retrotransposons are ubiquitous in eukaryotes and particularly prominent in plants with large genomes (Sanmiguel et al. 1998). In rice, the copia-like LTR retrotransposons also account for 17% of the genomic sequence (McCarthy et al. 2002). Apart from their abundance, some LTR retrotransposons are also found to reside in or near wild-type plant genes (White et al. 1994), thus underscoring possible roles that LTR retrotransposons may have played in both structural and expressional evolution of plant genes and genomes.

McClintock envisioned more than a decade ago that interspecies hybridization imposes a “genomic shock” to the plant genome and may instigate activation of dormant transposons (McClintock 1984). This prediction is now supported by empirical data, including the classical phenomenon of hybrid disgenesis in Drosophila (Kidwell and Lisch 1997), and recent demonstrations in both animal and plant interspecific hybrids (O’Neill et al. 1998; Kashkush et al. 2002, 2003). Although mechanisms for the phenomena is presently mysterious, it was proposed that interspecies hybridization may compromise the intrinsic epigenetic controlling machinery in parents and thus leading to derepression of mobile elements in hybrids (Comai 2000).

That the rice endogenous cryptic retrotransposon Tos17 can be activated by tissue culture has been well-established (Hirochika et al. 1996;
HIROCHIKA 1997; YAMAZAKI et al. 2001). We showed earlier that Tos17 could also be transpositionally activated by wide hybridization and alien DNA introgression from wild rice, *Zizania latifolia* (LIU and WENDEL 2000). In the present work, we have confirmed this phenomenon by isolating genomic regions that flank newly transposed Tos17 copies in an introgression line, Tong35. Moreover, we have shown that, in accord with the situation in tissue culture (HIROCHIKA et al. 1996), mobilized copies of Tos17 in the introgression line have also preferentially inserted into cellular genes (Table 1), thus implicating the potential impact of mobilized Tos17 on structure and expression of the targeted genes.

DNA methylation has been proposed, and in several cases documented, to play an important role in genome defense including taming activity of mobile elements (YODER et al. 1997; FINNEGAN et al. 1998; COLOT and ROSSIGNOL 1999; HIROCHIKA et al. 2000; LINDROTH et al. 2001; MARTIENSSON and COLOT 2001; MIURA et al. 2001; OKAMOTO and HIROCHIKA 2001). As an intact and potentially active element, the copy number of Tos17 is exceptionally low, ranging from one to four in various rice cultivars (HIROCHIKA 1997). This paradoxical nature of the element strongly suggests a tight control on its activity by epigenetic mechanisms. The observation that all seven CCGG sites within Tos17, including both LTR and protein-coding regions, are heavily methylated (Fig. 1 and 3a) may indicate that activity of the element is repressed by cytosine hypermethylation. If this is the case, the fact that all copies of Tos17 in the introgression line Tong35 are all heavily methylated at the time of study (generation 10) (Fig. 3) may suggest that at the initial stages of wide hybridization and introgression when Tos17 was active, the element was likely demethylated, then it was completely remethylated leading to or followed by inactivation. Unfortunately, earlier generations of the introgression line is not available for studying methylation dynamics of the element. Nevertheless, our recent observation that tissue culture-induced activation of Tos17 is accompanied by demethylation at the CCGG sites within Tos17, whereas inactivation is associated with near complete remethylation seems to corroborate this possibility (LIU et al. 2004).

It has been demonstrated in animals that foreign DNA integration could cause methylation alterations in host sequences adjacent to and even remote from the insertion sites (HELLER et al. 1995; REMUS et al. 1999; MULLER et al. 2001). We have found that methylation status in several cellular genes and transposon sequences underwent heritable changes in some rice lines with introgressed DNA from *Zizania latifolia* (LIU et al. 1999a). Because of their unique structures, i.e. the presence of inverted or direct repeats, the insertion of mobilized transposons and retrotransposons may be particularly potent in causing structural and/or methylation changes of the flanking sequences. In the present work, of the three low-copy regions that enabled assay on possible structural and methylation changes, one region exhibited heritable structural and/or methylation alteration flanking both the 3’ and 5’ of Tos17 (Fig. 5).

We recently found that similar methylation changes in flanking regions also occurred in tissue culture-mobilized Tos17 members (LIU et al. 2004). Because genetic and epigenetic changes on potentially coding sequences may effect structure and expression of cellular genes, the observation of this study implicates that natural and artificial wide hybridizations not only allow the flow of genetic variability but may also potentiate formation of de novo genetic and epimutations as a result of cryptic mobile element activation and its associated DNA methylation changes.

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