Retrotransposon activation followed by rapid repression in introgressed rice plants

Bao Liu and Jonathan F. Wendel

Abstract: Plant retrotransposons are largely inactive during normal development, but may be activated by stresses. Both *copia*-like and *gypsy*-like retrotransposons of rice were activated by introgression of DNA from the wild species *Zizania latifolia* Griseb. The copy number increase was associated with cytosine methylation changes of the elements. Activity of the elements was ephemeral, as evidenced by nearly identical genomic Southern hybridization patterns among randomly chosen individuals both within and between generations for a given line, and the absence of transcripts based on Northern analysis. DNA hypermethylation, internal sequence deletion, and possibly other mechanisms are likely responsible for the rapid element repression. Implications of the retroelement dynamics on plant genome evolution are discussed.

Key words: epigenetics, DNA methylation, genome evolution, retrotransposons, rice, introgression.

Résumé : Chez les plantes, les rétrotransposons sont largement inactifs lors du développement normal mais peuvent être activés suite à des stress. Les rétrotransposons de type *copia* et de type *gypsy* chez le riz ont été activés par suite de l'introgression d'ADN en provenance de l'espèce sauvage *Zizania latifolia* Griseb. Un accroissement du nombre de copies était associé à des changements au niveau de la méthylation des éléments. L'activité des éléments s'est avérée éphémère tel que révélé par la quasi-identité des motifs d'hybridation Southern parmi des clones choisis au hasard au sein d'une même génération ou parmi diverses générations d'une même lignée de même que par l'absence de transcrits en analyse Northern. L'hyperméthylation, la délétion de séquences internes, et potentiellement d'autres mécanismes, sont vraisemblablement responsables de la rapide répression de ces éléments. Les auteurs discutent des implications de la dynamique des rétroéléments sur l'évolution des génomes chez les plantes.

Mots clés : épigénétique, méthylation de l'ADN, évolution du génome, rétrotransposons, riz, introgression.

[Traduit par la Rédaction]

Introduction

Retrotransposons that transpose through reverse transcriptase of RNA intermediates are ancient and ubiquitous constituents of eukaryotic genomes, and have played a significant role in the structure, organization, and evolution of plant genomes (Bennetzen 2000; Kumar and Bennetzen 1999; Wessler et al. 1995). Three major groups of retrotransposons have been identified in plants, namely, *copia*-like, *gypsy*-like and non-long terminal repeat (LTR) retrotransposons. The abundance and distribution of retrotransposons vary greatly among plant species, and appear to correlate with genome size differences.

Almost all plant retrotransposons so far characterized are inactive during normal growth and development but may be activated by stresses, such as wounding, pathogen attacks, and cell culture (Grandbastien 1998; Hirochika 1993; Wessler 1996). Given the prevalence of these phenomena in natural plant populations, as well as the potentially disastrous consequences of uncontrolled retrotransposition, it is not surprising that plant genomes have evolved mechanisms to repress retrotransposon activity. One such likely mechanism is DNA methylation, whereby retroelements are rendered transcriptionally inactive. This has recently been established in *Arabidopsis*, where DNA methylation status and retrotransposon activation have been causally connected (Hirochika et al. 2000).

It was recently demonstrated in interspecific marsupial hybrids that demethylation is associated with retroelement activation and amplification (O'Neill et al. 1998). Thus, in this case it may be that hybridization mimics genomic stress, in that it is perceived by the cellular defense response machinery as an invasion of foreign DNA. Natural wide hybridization is a prominent phenomenon in plants. Although McClintock predicted long ago that wide hybridization may activate cryptic mobile elements (McClintock 1984), no example of this phenomenon is yet reported for plants.

We recently described the production of a series of rice lines introgressed with genetic material from the wild species *Zizania latifolia* Griseb. (Liu et al. 1999*b*). As measured by RFLP (restriction fragment length polymorphism) analysis, the proportion of alien DNA in these introgression lines is minute (<1%), as expected from their pedigree. A salient feature of these lines is that they exhibited heritable morphological traits not found in either the rice or wild parent. Sur-

Corresponding Editor: R.J. Kemble.

Received April 27, 2000. Accepted June 13, 2000. Published on the NRC Research Press web site on September 22, 2000.

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prisingly, extensive genomic DNA methylation changes occurred in these lines (Liu et al. 1999*a*). Given the possible causal association between DNA methylation and retrotransposon activity, the present study was undertaken to investigate whether hybridization has led to retrotransposon activation in these introgression lines. We report that (*i*) DNA introgression from *Z. latifolia* could indeed activate both *copia*- and *gypsy*-like retrotransposons in the rice genome; (*ii*) proliferation of retrotransposons is probably correlated with genomic cytosine demethylation; and (*iii*) de novo methylation, sequence deletion, and possibly other mechanisms are responsible for the rapid repression of the activated elements.

Materials and methods

Plant material

A series of rice (*Oryza sativa* L.) introgression lines was produced using introgressed genetic material from wild rice (*Z. latifolia* Griseb.) into the inbred rice line 'Matsumae', as described in Liu et al. (1999*b*). For the present study, five introgression lines were used, designated lines 1 through 5, representing the 9th and 11th selfed generations (S_9 and S_{11}). These introgression lines do not contain large chromosomal segments from the wild species, as they were not derived from a sexual hybrid; instead, all lines represent selfed progenies of a single, nonconventional F_1 plant produced via mixed pollination and incompletely understood mechanisms of DNA transfer from the wild to the cultivated species. This F_1 plant had a chromosomal constitution identical to rice (24 normal-looking chromosomes in somatic cells), but showed distinct morphological traits unique to *Z. latifolia*, implying DNA introgression (Liu et al. 1999*b*, and unpublished data). This was confirmed by RFLP analysis showing that some of the lines contain *Z. latifolia*-specific DNA repeats, although overall, these lines were identical with their rice parent in more than 99% of the probe–enzyme combinations analyzed (Liu et al. 1999*b*, and unpublished data).

Extraction of nucleic acids and preparation of Southern and Northern blots

Extraction of genomic DNA from young, expanded leaves of individual plants was as described in Liu et al. (1999*a*). Care was taken to use leaves from the same growth stage, thus minimizing potential DNA methylation differences associated with development. Purified DNA was digested with a pair of isoschizomers (*Hpa*II–*Msp*I), which recognize the same 5′-CCGG-3′ sequence, but vary in their sensitivity to cytosine methylation. To ensure complete digestion, a 3-fold excess of enzymes was used (3–4 U/μg DNA) and the digestions were performed twice. Digested DNAs were fractionated by electrophoresis through 0.8% agarose gels, stained with ethidium bromide, and transferred onto Hybond N^+ membranes by alkaline transfer (Amersham). Total RNA was extracted from young, expanding leaves of individual plants by the RNeasy Plant Minikit (Qiagen, Valencia, Calif.) following the manufacturer's protocol. For Northern analysis, RNAs were fractionated by electrophoresis through 1% agarose–formaldehyde gels in MOPS (morpholinepropanesulfonic acid) buffer, and transferred onto Hybond nylon membranes using standard methods.

Preparation of retrotransposon probes and Southern and Northern hybridization

The reverse transcriptase region of the *copia*-like rice retrotransposon *Tos17* (Hirochika et al. 1996) and the integrase coding region from the *gypsy*-like rice retrotransposon *RCS1* (Miller et al. 1998) were amplified by PCR using rice (*O. sativa* cv. Matsumae) genomic DNA as the template and specific primers designed based on the sequence of the retrotransposons. For *Tos17*, the primers are: forward, 5′-GGCTACCCGTTCTTGGACTATC and reverse, 5′-CTGAGTGGTCCCAAATCAGAC, corresponding to nucleotides 2100–2120 and 2482–2462, respectively, of *Tos17*. For *RCS1*, the primers are forward, 5′-GATGGGTTTGTTTTCAGAGC and reverse, 5′-CAAACCAAATCTCCAGGTTCA, corresponding to nucleotides 9–28 and 934–914, respectively, of *RCS1*. Identities of the PCR products were confirmed by sequencing, which showed that the amplicons recovered were identical to the sequences originally described. A mixture of rice *copia*-like reverse transcriptase fragments was obtained by PCR amplification at low stringency from the rice parent using a pair of degenerate primers (Hirochika et al. 1992). PCR products were purified using the Wizard PCR purification kit (Promega) and labeled with [α–^{32}P]dCTP by the random primer method. Following hybridization, filters were washed at a stringency of 0.2× SSC – 0.1% SDS at 65°C for 2× 30 min. To monitor hybridization signals in Southern blot analysis, care was taken to ensure equal loading of digested genomic DNAs, as revealed both by ethidium bromide staining and hybridization to low-copy RFLP probes (Liu et al. 1999*a*).

Cloning and sequencing of *Tos17* and *RCS1* homologs from the introgression lines

Using genomic DNA from each of the five introgression lines and their rice and *Z. latifolia* parents, *Tos17* and *RCS1* homologs were amplified. PCR products were purified and cloned into the pGEM-T vector (Promega). Randomly selected clones were sequenced using the M13 forward and reverse primers using automated sequencing.

Results

copia-like retrotransposons become activated in the introgression lines

Tos17 is normally an inactive *copia*-like retrotransposon in rice, but may become highly activated under tissue culture conditions (Hirochika et al. 1996), suggesting its potential activation by other stresses. When the reverse transcriptase fragment of *Tos17* was used as a probe and hybridized to a Southern blot containing *Hpa*II- or *Msp*I-digested genomic DNA of the introgression lines and their rice and *Z. latifolia* parents, a significant increase in copy number of the element was observed in all five introgression lines (Fig. 1A). In rice, only one major and two minor bands are present (Fig. 1A, lane 1), whereas numerous major and minor bands are observed in the introgression lines (Fig. 1A, lanes 3–15). Inspection of the Southern blot showed that the intensity of most bands in the introgression lines was several times stronger than that observed in either parent. This phenomenon is unlikely to reflect Mendelian inheritance from parental individuals with exceptionally high copy numbers for these elements, as such individuals have not been observed, and because there is no evidence for extreme copy number variation among individuals in either the rice or wild rice parent. The copy number increase in the introgression lines was so extreme in some cases [e.g., lines 2 (lanes 5–7) and 5 (lanes 13–15)] that a hybridization smear was formed. Based on the strength of the hybridization signal, the number of bands observed, and an assumption that there are two copies of *Tos17* in the rice parent (ranges from 1 to 4 in various rice cultivars, Hirochika et al. 1996), we conservatively estimate that there are 10 to 20 copies of this retroelement in most introgression lines. This increase was also apparent in

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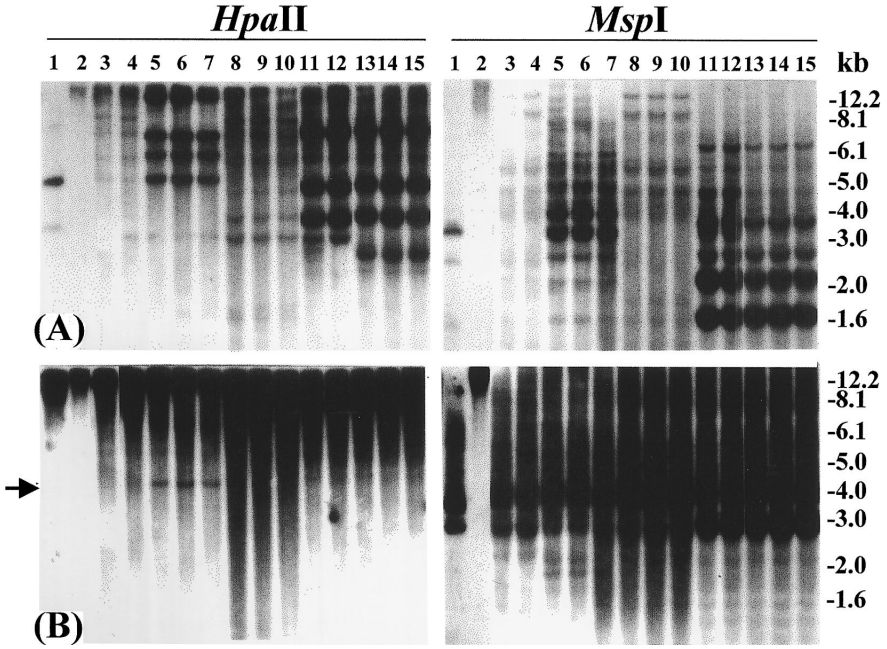
Fig. 1. Activation and methylation changes of retrotransposons in introgressed rice lines. (A) Hybridization of the reverse transcriptase region of a rice *copia*-like element (*Tos17*) to digests of 2–3 individuals each of five introgression lines and their parents. Lanes 1 and 2 are the rice parent ('Matsumae') and *Zizania latifolia*, respectively; lanes 3 and 4 are introgression line 1; lanes 5–7 are line 2; lanes 8–10 are line 3; lanes 11–12 are line 4; lanes 13–15 are line 5. DNAs were digested with a pair of isoschizomers (*Hpa*II–*Msp*I) which vary in their methylation sensitivity. Copy number increase is apparent in the introgression lines relative to their rice parent. Both hypermethylation and demethylation changes relative to rice are evident, as inferred from the sizes of the hybridizing fragments. (B) Hybridization of the integrase region of a *gypsy*-like element (*RCS1*) to digests of the same set of plants as in panel (A). Both copy number increase and demethylation changes also occurred for this element in the introgression lines, as evidenced by stronger hybridization signal and the appearance of smaller novel fragments (e.g., arrow).



Southern blots prepared from *Hin*dIII-digested genomic DNAs (data not shown). Because little hybridization is observed in lanes corresponding to *Z. latifolia* (lane 2 in each figure panel), it is unlikely that the increase in hybridization signal observed in the introgression lines is due simply to detection of introgressed *Z. latifolia* retroelements. Instead, we interpret the results to reflect activation of endogenous rice elements stimulated by introgression. This interpretation is additionally supported by the sequence data presented below.

We also used PCR-amplified reverse transcriptase fragments from *copia*-like retrotransposons as a probe on Southern blots prepared from the same plant materials. Apparent copy number increase was observed in at least two of the five introgression lines, with hybridization patterns that were different than those observed using the *Tos17* probe (data not shown). This indicates that members other than the *Tos17* class of *copia*-like retrotransposons also became activated by interspecific hybridization.

Based on comparison of the banding patterns of the introgression lines with those of the rice parent in both *Hpa*II and *Msp*I digests, it was apparent that extensive cytosine methylation changes occurred at both the inner and the outer cytosines of CCGG sites within or flanking the *Tos17* element. The complex hybridization patterns observed are most readily explained by both de novo hyper- and hypomethylation modifications, probably at multiple CCGG sites (Fig. 1A). Alternatively, the patterns could have been caused by transposition of activated copies of *Tos17* into genomic regions of varying background cytosine methylation levels. However, given the novel appearance of prominent heavily methylated bands (note fragments larger than those corresponding to the original parental fragments in both *Hpa*II and *Msp*I digests) and the transpositional preference of *Tos*17 into undermethylated, low-copy genic regions (Hirochika et al. 1996), our preferred explanation is that retroactivation was accompanied by de novo methylation changes.

gypsy-like retrotransposons become activated in the introgression lines

RCS1 is a *gypsy*-like retrotransposon located exclusively at centromeric regions of all grasses that have been studied (Miller et al. 1998). When a fragment from the integrase coding region of *RCS1* was used to probe a Southern blot carrying *Hpa*II- or *Msp*I-digested genomic DNA of the introgression lines, an increase in copy number was observed in all five introgression lines (Fig. 1B). This was particularly obvious for line 3, in which the copy number was elevated by at least 2–3-fold. Given the original high copy number of this element in rice, i.e., ~130 copies per haploid genome (Miller et al. 1998), the Southern blot analyses indicate that hybridization and DNA introgression created several hundred new *gypsy*-like retroelements in some lines, probably as a result of replicative transposition.

Apparently, cytosine methylation changes also occurred in at least some copies of *RCS1* in all five introgression lines. This mainly involved hypomethylation of both the inner and

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the outer cytosines at the CCGG sites, as indicated by the novel appearance of smaller-sized smears in both enzyme digests (below ~7.0 kb in *Hpa*II digests and below 3.0 kb in *Msp*I digests) in all five introgression lines. Several prominent bands appeared in all five introgression lines but were absent from both parents (e.g., arrow in Fig. 1B); these presumably represent hypomethylated copies of *RCS1*. This suggests that activation and amplification of *RCS1* in the introgression lines might have been associated with hypomethylation of the elements.

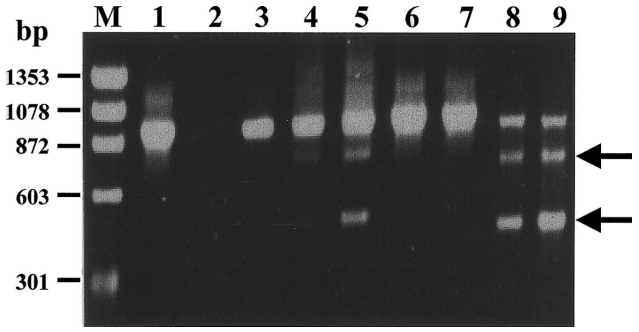
Zizania latifolia homologs of *RCS1* also exist in high copy number and appear to be heavily methylated at both the inner and the outer cytosines of the CCGG sites, as evidenced by the single high-molecular-weight fragment observed in Southern blot analysis (Fig. 1B, lane 2). As was the case for the *copia*-like elements, it is conceivable that *Z. latifolia* contributed copies of *RCS1* homologs to the introgression lines, as opposed to the interpretation that the copy number increase is due to retroelement activation. Although we cannot exclude this possibility, the methylation changes observed and the fractional genomic composition of the introgression lines (>99% *O. sativa*) suggest that this is not likely to be the primary cause for copy number amplification.

Sequence analysis shows complete sequence conservation but occasional internal deletions accompanying retroelement activation

To study whether the changed hybridization patterns in the introgression lines were the result of sequence variation in the probe regions of *Tos17* and *RCS1*, corresponding fragments were amplified from genomic DNA of all five introgression lines, and the rice and *Z. latifolia* parents. For *Tos17*, all introgression lines generated fragments of the same size as the rice parent (382 bp). PCR amplification from *Z. latifolia* yielded less product, but it was of the same size (data not shown). For *RCS1*, all five introgression lines produced fragments of the size expected from the rice data (897 bp). In addition, two of the introgression lines (2 and 3) yielded two smaller fragments (ca. 850 and 446 bp, respectively), neither of which was detected in the rice parent, even after two rounds of amplification (Fig. 2). Using the specific primers for *RCS1*, no amplification product was produced from *Z. latifolia*, suggesting divergence in one or both primer regions.

We cloned the PCR fragments into the pGEM-T vector. For *Tos17*, one clone from each of the rice and *Z. latifolia* parents, and three randomly sampled clones from the normal-sized fragment of each of the five introgression lines were sequenced. All clones of the introgression lines (15 clones) showed 100% identity with that of the rice parent, as well as to the corresponding region of *Tos17* (GenBank Acc. No. D88394) reported by Hirochika et al. (1996), indicating that no sequence change occurred in the probe region of *Tos17* in the introgression lines during transposition. The *Z. latifolia Tos17* homologs showed a low level of homology to *Tos17* of rice, with the terminal parts (positions 2100–2188 and 2453–2482) having more than 80% sequence identity to *Tos17*, while the internal part exhibited no significant homology (Fig. 3). The fact that none of the 15 sequences from the introgression lines showed similarity to this

Fig. 2. PCR amplification, using sequence-specific primers, of the integrase region of a *gypsy*-like element (*RCS1*) from rice (lane 1), *Zizania latifolia* (lane 2), and the introgression lines (lanes 3–7). Lanes 8 and 9 are second-round amplifications on PCR products of line 2 (lane 4) and line 3 (lane 5). Evidently, the normal-sized fragment (926 bp) was amplified from the rice parent and all introgression lines, but not from the wild species *Z. latifolia*. In addition, two smaller-sized fragments of ~850 and 435 bp were amplified from introgression lines 2 and 3, respectively; this is more obvious after the second-round amplification.



Z. latifolia sequence suggests that few, if any copies of the *Z. latifolia* homolog of *Tos17* were present in the introgression lines.

For *RCS1*, one clone each from the rice and *Z. latifolia* parents, and two randomly chosen clones from each of the normal-sized fragments from all five introgression lines were sequenced. Results indicated that all the normal-sized clones of the introgression lines (10 clones) showed 100% identity with the rice parental clone and to the corresponding region of the original *RCS1* (AF078903) characterized by Miller et al. (1998). This indicates again that no sequence change occurred in the probe region of *RCS1* in the introgression lines. Sequencing of the 446-bp fragments of lines 2 and 3 showed that fragments of the two lines were identical to each other, and that the small fragments resulted from an internal deletion of 491 bp (Fig. 3). The deleted region was either from positions 190–681 or from 193–684 of *RCS1*, the ambiguity arising from the presence of identical TCG trinucleotides at both flanking regions (deletion sites). Attempts to clone the ~850-bp fragments of both lines were unsuccessful. When using this excised fragment as a template for further PCR amplification, the 446-bp fragment was always produced, indicating that the ~850-bp fragment may be a dimer of 446-bp fragments.

Retroelement activation is ephemeral

Despite the dramatic increase in copy number of both *Tos17* and *RCS1* retrotransposons, including some apparently demethylated copies in the introgression lines, banding patterns for two or three randomly chosen individual plants for each line were always identical or nearly so (Fig. 1). This suggested that the changed patterns were stably inherited, a suggestion confirmed by subsequent experiments using later-generation selfed progeny of the five introgression lines. In all cases, individuals from the S_{11} generation yielded nearly identical hybridization profiles to those from corresponding S_9 plants, indicating no further transposition of the elements (data not shown). The stable inheritance of

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the changed hybridization patterns in all introgression lines implies that the retrotransposons were silenced by the S_9 generation (earlier generations were unavailable for analysis). As additional evidence, we performed Northern analysis on both parents and all five introgression lines from the S_9 generation, using *Tos17* and *RCS1* probes. No transcript of the expected size was detected in any individual, suggesting low to no retroelement transcriptional activity.

Discussion

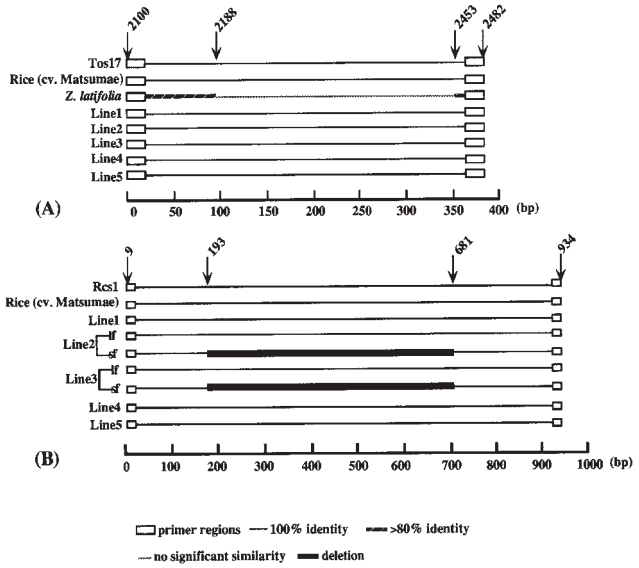
Rice retrotransposon activation is induced by foreign DNA introgression

Hybridization is a prominent feature of natural plant populations, and one that often leads to speciation, either through polyploidization (Leitch and Bennett 1997; Soltis and Soltis 1999; Wendel 2000) or at the diploid level (Arnold 1997; Rieseberg 1995). It is tantalizing to speculate that in at least some cases speciation and (or) adaptation have been facilitated by retroelement activation and its effects on gene expression. While at present no data causally connect adaptation and (or) speciation with retroelement mobilization, this possibility is suggested by the well-known phenomenon of hybrid dysgenesis in *Drosophila* (Kidwell and Lisch 1997) and by recent data from marsupials, where retroelements were activated by interspecific hybridization (O'Neill et al. 1998). In the latter case, the authors suggested that this activation promoted karyotypic evolution, thereby enhancing reproductive isolation.

Here we have shown that retroelements may also be activated by hybridization in plants. Each of the two primary classes of plant retroelements, i.e., *copia*-like and *gypsy*-like, were similarly effected by *Zizania* introgression, demonstrating that the effects of hybridization on element amplification conceivably extend to a variety of plant transposable elements. Whether or not this mobilization has led to adaptation is an open question, as the plant lines used were artificial constructs maintained under cultivation. Nonetheless, we note that each of the introgression lines were morphologically distinct from the parents, suggesting that element activation has led to morphological novelties. Further experimentation is needed to evaluate the effects of individual transposition events, both with respect to potential effects on morphological or physiological attributes, and in terms of reproductive fitness under a diverse array of selective conditions. In addition, it would appear fruitful to extend these types of analyses to natural plant populations, focusing on well-documented examples of hybridization and introgression (Arnold 1997; Rieseberg 1995; Rieseberg and Wendel 1993).

One might suggest that instead of induction by hybridization, element amplification in the introgression lines reflects other phenomena. For example, pathogen attack, tissue culture, and wounding are known to promote retroactivation in some cases (Grandbastien 1998; Wessler 1996). In our study, we note that (*i*) the rice parent ('Matsumae') is a genetically pure line; (*ii*) the introgression lines were maintained by selfing; (*iii*) no physical (e.g., irradiation), chemical, or tissue culture treatments were used in the production of the initial hybrid plant from which all introgression lines descended (Liu et al. 1999*b*); (*iv*) all 11 generations of the introgression lines and the rice parent were grown under the same greenhouse conditions, without apparent environmental stresses such as heat shock or drought; and (*v*) although pathogen attack was not monitored, there were no obvious infestations in any generation. Taken together, we conclude that activation of retrotransposons in the introgression lines was likely due to the introduction of *Z. latifolia* DNA.

Fig. 3. Sequence comparison among clones of the reverse transcriptase region of *Tos17* (A) and the integrase region of *RCS1* (B) from rice (cv. Matsumae), *Zizania latifolia* and the introgression lines. lf, long fragment; sf, short fragment.



Although results from marsupials and rice show that hybridization may induce mobilization of dormant retroelements, it appears that this response is not an automatic consequence of all natural hybridizations. For example, hybridization in nonmarsupial animal groups was not associated with retroelement activation (Roemer et al. 1999). Similarly, in some hybridization-derived polyploid plants, such as triticale and hexaploid oat, retroelements were found to be largely confined to the subgenome from which they were isolated, suggesting their relative inactivity (Katsiotis et al. 1996; Pearce et al. 1997). On the other hand, retroelements specific to one of the diploid progenitors of tetraploid cotton have spread to the other subgenome since polyploid formation (Hanson et al. 1999). An unanswered question concerns the genomic and environmental conditions under which hybridization does and does not elicit retroelement activation.

Activated retrotransposons experience cytosine methylation changes

In all introgression lines, the reverse transcriptase regions of the new copies of *Tos17* have increased cytosine methylation relative to wild-type copies in the parental rice line, although the smaller hybridization fragments observed (Fig. 1A) also indicate that some copies underwent demethylation. Because the integrase region of *RCS1* was heavily methylated in rice, only demethylation was potentially observable in the introgression lines using our South-

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ern hybridization techniques (Fig. 1B). In both cases, it appears that methylation modification occurred predominantly in the transposed or new copies, whereas the original parental copies of the elements were retained in at least some introgression lines (Fig. 1). Because the two restriction endonucleases employed differ in their methylation sensitivity, we were able to infer that methylation modification occurred at both cytosines. Thus, in rice lines introgressed with foreign DNA, retroelements are not only activated, but they subsequently become methylated. These results parallel recent observations from the tobacco retrotransposon *Tto1* when it is introduced into *Arabidopsis* (Hirochika et al. 2000).

Repression of activated retrotransposons

As noted above, several lines of evidence indicate that both classes of retroelements in the rice introgression lines are no longer active. One likely mechanism for retroelement repression is methylation. In this respect, the causal connection between cytosine methylation and retroelement activity has been established in *Arabidopsis*, wherein hypermethylation in the coding region of *gag* efficiently silenced activity of tissue culture-activated *Tto1* elements (Hirochika et al. 2000). Given the similarity between these results and the patterns in rice, we speculate that hypermethylation of *Tos17* in the introgression lines likely contributed to silencing of this element. While it is possible that some of the hypermethylated fragments observed in Southern blots resulted from transposition into heavily methylated genomic regions, a more plausible interpretation is that newly integrated retroelements became subsequently repressed via intrinsic methylation mechanisms.

Several apparently demethylated copies of the element appear to exist in the introgression lines, as deduced from the smaller fragments in the Southern blots. Because data show that elements are no longer active in the introgression lines, we suspect that these hypomethylated copies are repressed by either hypermethylation elsewhere in the element (e.g., the 5′ LTR), or by some other silencing mechanism (Matzke and Matzke 1998). For example, in *Drosophila*, where DNA methylation does not exist, silencing of transgenes and probably also endogenous transposable elements is dependent on the chromodomain polycomb group of proteins (Wolffe and Matzke 1999).

This suggestion that newly integrated retroelements may become silenced by means other than DNA methylation is supported by the observation of de novo sequence deletion in the integrase region of *RCS1* (see results). The short PCR fragments were reproducibly amplified from introgression lines 2 and 3, neither of which could be amplified from either the rice or *Z. latifolia* parent (Fig. 2). Moreover, this deleted fragment was not found in a broader survey of DNA samples from approximately 30 rice cultivars (data not shown). Therefore, we conclude that the deletion occurred de novo in the introgression lines. Perhaps it is more than coincidental that sequence deletion only was observed in the two lines that show the most significant copy number increase of the *RCS1* element (Fig. 1B). Our data reinforce the idea that multiple and mutual exclusive silencing systems may be responsible for repression of activated retroelements.

Implications for genome evolution

Retrotransposon activation followed by rapid repression may have several implications for gene and genome evolution, and hence adaptation. The most obvious consequence of a burst of retrotransposition following natural hybridization is insertional mutagenesis. Although some retrotransposons have evolved a propensity to insert into nongenic, heterochromatin regions (Kumar and Bennetzen 1999; Zou and Voytas 1997), some retrotransposons, such as *Tos17*, preferentially transpose into genes (Hirochika et al. 1996). It is possible that some small fraction of insertions modify gene structure or expression in such a way that fitness is enhanced under certain environmental conditions (Kalendar et al. 2000; Wendel and Wessler 2000). The recent discovery that many wild-type plant genes have retrotransposon-derived sequences in their regulatory regions (Bennetzen 2000; Wessler 1998) underscores the role that retrotransposon insertions may have played in generating novel structure or expression patterns during evolution. In the present work, many novel morphological traits appeared in the introgression lines (Liu et al. 1999*b*); it would be of interest to explore whether any of these are causally connected to *Tos17* or *RCS1* insertional activity.

Notwithstanding the potential adaptive significance of transpositional activity, it seems probable that organisms will have evolved efficient retrotransposition repression mechanisms because of the deleterious consequences of unchecked retrotransposition. Little is known about most aspects of the responsible silencing mechanisms, and of their dynamics following hybridization-mediated retroelement release. It may be that bursts of retroelement activity are anomalous genetic accidents, caused by stress and (or) the genomic shock of hybridization. The rapidity with which the cellular machinery is mobilized to check a retrotransposon invasion will likely vary widely among organisms and situations; important parameters probably include the nature and quantity of foreign DNA introduced, the degree of retroelement activation, the nature of the released retroelements, and the relative efficiency of the intrinsic silencing mechanisms. In the present study, apparent genomic stability was established by the S_9 generation, and potentially occurred by the time of one of the first few generations following hybridization. We are presently designing follow-up experiments to explore the consistency and timing of the silencing response.

A final implication of the present work concerns the contribution of retrotransposable elements to genome size. Recent studies have demonstrated that retrotransposons comprise a major component of plants with large genomes. For example, in maize (genome size ~3200 Mbp), 50–80% of the nuclear DNA consists of retrotransposons and their relics (SanMiguel et al. 1996), and in barley (genome size ~4870 Mbp), a single retroelement, *BARE*-1, makes up ~3% of the nuclear genome (Vicient et al. 1999). On the other hand, rice, with its relatively small genome (genome size ~420 Mbp), has a greatly reduced proportional representation of retrotransposons in its genome. The copy number of the *copia*-group of retrotransposons in rice is only ~100 per haploid genome, a feature suggested to reflect a predominant evolutionary history of relative inactivity (Wang et al. 1999).

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However, as is evident from the present and other studies, *copia*-like retroelements may become activated under certain conditions. This raises the possibility that the distinctively low copy number and restricted genomic distribution of the rice retrotransposons reflects highly efficient silencing systems. This possibility, in fact, has been suggested to explain the difference in retrotransposon silencing between *Arabidopsis* (small genome) and tobacco (large genome): the *Tto1* retrotransposon is hypermethylated and silenced in *Arabidopsis* if its copy number reaches 10–15, but it remains active in tobacco cell lines with a copy number of up to 300 (Hirochika et al. 2000). Further understanding of silencing mechanisms in both small and large genomes may help us understand the impressive genome size differences that exist among even closely related species.

Acknowledgements

We thank G.L. Tang and X.F. Li for technical advice. This work was supported by the United States–Israel Binational Science Foundation and the National Natural Science Foundation of China.

References

Arnold, M.L. 1997. Natural Hybridization and Evolution. Oxford University Press, New York.

Bennetzen, J.L. 2000. Transposable element contributions to plant gene and genome evolution. Plant Mol. Biol. **42**: 251–269.

Grandbastien, M.-A. 1998. Activation of plant retrotransposons under stress conditions. Trends Plant Sci. **3**: 181–187.

Hanson, R.E., Islam-Faridi, M.N., Crane, C.F., Zwick, M.S., Czeschin, D.G., Wendel, J.F., Mcknight, T.D., Price, H.J., and Stelly, D.M. 1999. Ty1-*copia*-retrotransposon behavior in a polyploid cotton. Chromosome Res. **8**: 73–76.

Hirochika, H. 1993. Activation of tobacco retrotransposons during tissue culture. EMBO J. **12**: 2521–2528.

Hirochika, H., Fukuchi, A., and Kikuchi, F. 1992. Retrotransposon families in rice. Mol. Gen. Genet. **233**: 209–216.

Hirochika, H., Sugimoto, K., Otsuki, Y., and Kanda, M. 1996. Retrotransposons of rice involved in mutations induced by tissue culture. Proc. Natl. Acad. Sci. U.S.A. **93**: 7783–7788.

Hirochika, H., Okamoto, J., and Kakutani, T. 2000. Silencing of retrotransposons in *Arabidopsis* and reactivation by the *ddm1* mutation. Plant Cell, **12**: 357–368.

Kalendar, R., Tanskanen, J., Immonen, S., Nevo, E., and Schulman, A.H. 2000. Genome evolution in wild barley (*Hordeum spontaneum*) by *BARE*-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proc. Natl. Acad. Sci. U.S.A. **97**: 6603–6607.

Katsiotis, A., Schmidt, T., and Heslop-Harrison, J.S. 1996. Chromosomal and genomic orgnization of *Ty1-copia*-like retrotransposon sequences in the genus *Avena*. Genome, **39**: 410–417.

Kidwell, M.G., and Lisch, D. 1997. Transposable elements as sources of variation in animals and plants. Proc. Natl. Acad. Sci. U.S.A. **94**: 7704–7711.

Kumar, A., and Bennetzen, J.L. 1999. Plant retrotransposons. Annu. Rev. Genet. **33**: 479–532.

Leitch, I.J., and Bennett, M.D. 1997. Polyploidy in angiosperms. Trends Plant Sci. **2**: 470–476.

Liu, B., Piao, H., Zhao, F., Zhao, J., Liu, Z., and Huang, B. 1999*a*. DNA methylation changes in rice induced by *Zizania latifolia* Griseb. DNA introgression. Hereditas, **131**: 75–78.

Liu, B., Piao, H.M., Zhao, F.S., Zhao, J.H., and Zhao, R. 1999*b*. Production and molecular characterization of rice lines with introgressed traits from a wild species of *Zizania latifolia* Griseb. J. Genet. Breed. **53**: 279–284.

Matzke, M.A., and Matzke, A.J.M. 1998. Epigenetic silencing of plant transgenes as a consequence of diverse cellular defense responses. Cell. Mol. Life Sci. **54**: 94–103.

McClintock, B. 1984. The significance of responses of the genome to challenge. Science, **226**: 792–801.

Miller, J.T., Dong, F.G., Jackson, S.A., Song, J.Q., and Jiang, J. 1998. Retrotransposon-related DNA sequences in the centromeres of grass chromosomes. Genetics, **150**: 1615–1623.

O'Neill, R.J.W., O'Neill, M.J., and Graves, J.A.M. 1998. Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. Nature, **393**: 68–72.

Pearce, S.R., Harrison, G., Heslop-Harrison, J.S., Flavell, A.J., and Kumar, A. 1997. Characterization and genomic organization of the Ty1-*copia* group retrotransposons in rye (*Secale cereale*). Genome, **40**: 617–625.

Rieseberg, L.H. 1995. The role of hybridization in evolution: Old wine in new skins. Am. J. Bot. **82**: 944–953.

Rieseberg, L.H., and Wendel, J.F. 1993. Introgression and its consequences in plants. *In* Hybrid Zones and the Evolutionary Process. *Edited by* R. Harrison. Oxford University Press, New York. pp. 70–109.

Roemer, I., Grützner, F., Winking, H., Haaf, T., Orth, A., Skidmore, L., Antczak, D., and Fundele, R. 1999. Global methylation in eutherian hybrids. Nature, **401**: 131–132.

SanMiguel, P., Tikhonov, A., Jin, Y.K., Motchoulskaia, N., Zakharov, D., Melake-Berhan, A., Springer, P.S., Edwards, K.J., Lee, M., Avramova, Z., and Bennetzen, J.L. 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science, **274**: 765–768.

Soltis, D.E., and Soltis, P.S. 1999. Polyploidy: Origins of species and genome evolution. Trends Ecol. Evol. **9**: 348–352.

Vicient, C.M., Suoniemi, A., Anamthawat-Jonsson, K., Tanskanen, J., Beharav, A.E.N., and Schulman, A.H. 1999. Retrotransposon *BARE*-1 and its role in genome evolution in the genus *Hordeum*. Plant Cell, **11**: 1769–1784.

Wang, S.P., Liu, N., Peng, K.M., and Zhang, Q.F. 1999. The distribution and copy number of *copia*-like retrotransposons in rice (*Oryza sativa* L.) and their implications in the organization and evolution of the rice genome. Proc. Natl. Acad. Sci. U.S.A. **96**: 6824–6828.

Wendel, J.F. 2000. Genome evolution in polyploids. Plant Mol. Biol. **42**: 225–249.

Wendel, J.F., and Wessler, S.R. 2000. Retrotransposon-mediated genome evolution on a local ecological scale. Proc Natl. Acad. Sci. U.S.A. **97**: 6250–6252.

Wessler, S.R. 1996. Plant retrotransposons: Turned on by stress. Curr. Biol. **6**: 959–961.

Wessler, S.R. 1998. Transposable elements associated with normal plant genes. Physiol. Plant. **103**: 581–586.

Wessler, S.R., Bureau, T.E., and White, S.E. 1995. LTR-retrotransposons and MITEs: Important players in the evolution of plant genomes. Curr. Opin. Genet. Dev. **5**: 814–821.

Wolffe, A.P., and Matzke, M.A. 1999. Epigenetics: Regulation through repression. Science, **286**: 481–486.

Zou, S., and Voytas, D.F. 1997. Silent chromatin determines target preference of the *Saccharomyces* retrotransposon Ty5. Proc. Natl. Acad. Sci. U.S.A. **94**: 7412–7416.

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