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ORIGINAL PAPER

Transpositional activation of *mPing* in an asymmetric nuclear somatic cell hybrid of rice and *Zizania latifolia* was accompanied by massive element loss

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Abstract We have reported previously that the most active miniature inverted terminal repeat transposable element (MITE) of rice, *mPing*, was transpositionally mobilized in several rice recombinant inbred lines (RILs) derived from an introgressive hybridization between rice and wild rice (*Zizania latifolia* Griseb.). To further study the phenomenon of hybridization-induced *mPing* activity, we undertook the present study to investigate the element's behavior in a highly asymmetric somatic nuclear hybrid (SH6) of rice and *Z. latifolia*, which is similar in genomic composition to that of the RILs, though probably contains more introgressed alien chromatins from the donor species than the RILs. We found that *mPing*, together with its transposase-donor, *Pong*, underwent rampant transpositional activation in the somatic hybrid (SH6). Because possible effects of protoplast isolation and cell culture can be ruled out, we attribute the transpositional activation of *mPing* and *Pong* in SH6 to the process of asymmetric somatic hybridization, namely, one-step introgression of multiple chromatin segments of the donor species *Z. latifolia* into the recipient rice genome. A salient feature of *mPing* transposition in the somatic hybrid is that the element's activation was accompanied by massive loss of its original copies, i.e., abortive transpositions, which was not observed in previously reported cases of *mPing* activity. These data not only corroborated our earlier finding that wide hybridization and introgression may trigger transpositional activation of otherwise quiescent transposable elements, but also suggest that transpositional mobilization of a MITE like *mPing* can be accompanied by dramatic reduction of its original copy numbers under certain conditions, thus provide novel insights into the dynamics of MITEs in the course of genome evolution.

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Introduction

The miniature inverted-repeat transposable elements (MITEs) were first discovered in plants, then in animals and human, thus pointing to their ubiquity in higher eukaryotes (Feschotte et al. 2002). Structurally, MITEs are reminiscent of non-autonomous DNA transposons (class II element), as they do not encode any transposase. In plants, MITEs can be divided into two major groups: *Tourist*-like and *Stowaway*-like, based on their similarity of terminal inverted repeats (TIRs) and target site duplications (TSDs)

(Feschotte et al. 2002). Sequence analysis of assembled rice chromosomes has revealed that MITEs preferentially reside at low-copy, gene-rich regions (Feng et al. 2002; Huang et al. 2008). This, together with their extraordinary abundance in terms of copy numbers, suggests they likely have played important roles in divergent evolution of plant genes (Zhang et al. 2000; Feng et al. 2002; Jiang et al. 2004; Huang et al. 2008).

The miniature-Ping (*mPing*), a 430 bp endogenous element in the rice genome, contains 15-bp TIRs and produces TAA or TTA target site duplications (TSDs) upon insertion, and hence, is typical of a *tourist*-like MITE (Jiang et al. 2003; Kikuchi et al. 2003; Nakazaki et al. 2003). *mPing* is the most active MITE so far characterized in any organism (Feschotte and Pritham 2007). Because *mPing* has no coding capacity, the transposase required for its transposition is provided in trans by related autonomous element(s). Based on sequence similarly and co-transpositional behavior, two transposase-encoding autonomous elements, *Ping* and *Pong*, were believed to be the transposase donors for *mPing* (Jiang et al. 2003; Kikuchi et al. 2003; Nakazaki et al. 2003), as being experimentally verified recently by transgenic studies in a non-host plant, *Arabidopsis* (Yang et al. 2007). Different from *Ping* which exists in only some of the rice cultivars (Jiang et al. 2003), *Pong* appears to present in all rice cultivars so far studied. However, either *Ping* or *Pong* may play the decisive role for *mPing* activity and survival, depending on genotypes and/or eliciting conditions.

It was found that the copy number of *mPing* varies dramatically between the two cultivated subspecies of rice, *japonica* and *indica*, which were domesticated from a common wild ancestral species, i.e., the common wild rice, *Oryza ruffipogon* (Second 1982; Zhu and Ge 2005). This suggests that *mPing* has been differentially active in transposition during domestication and/or breeding/cultivation of the two subspecies. Indeed, it was recently documented that in some landraces of *japonica* rice, *mPing* has been so active that its copy number can reach to the range of thousands (Naito et al. 2006). Nonetheless, in most cultivars, *mPing* is cryptic under normal conditions, but can be activated to transpose by several stress conditions like tissue culture (Jiang et al. 2003; Kikuchi et al. 2003), irradiation (Nakazaki et al. 2003), and high-pressurization (Lin et al. 2006).

We previously reported that introgressive hybridization between rice and *Zizania latifolia* has induced marked transpositional mobilization of *mPing* and its transposase-donor, *Pong*, with the three recombinant inbred lines (RILs) originally derived from the same single hybrid individual showing dramatically different *mPing* and *Pong* gel-blotting patterns from each other as well as from their recipient parental line (Shan et al. 2005). Because the RILs entailed multiple generations to construct, it remains unknown whether the transpositional patterns were established immediately following hybridization or being accumulated gradually over generations to reach their final equilibrium. To further study the issue of hybridization- and/or introgression-induced *mPing* activity, independently produced plant materials at the earliest generations possible are preferable.

Asymmetric somatic cell hybridization mediated by protoplast fusion is an alternative approach for the introgression of multiple chromatin segments from a donor species' genome into a recipient one by a single, none-sexual step (Dudits et al. 1987; Kisaka et al. 1994; Xia et al. 2003). By using this method, we obtained a highly asymmetric nuclear somatic cell hybrid of rice and *Z. latifolia* by the "gamma"-fusion protocol (Gleba et al. 1988), namely fusing intact cells of rice and gamma ray-irradiated (with lethal-dose to induce chromatin fragmentation) mesophyll cells of *Z. latifolia* (Liu et al. 1999). One hybrid plant (SH6) was produced, which was partially fertile and molecularly characterized as containing multiple introgressed chromatin segments of the donor species, *Z. latifolia* (Liu et al. 1999). Taking into account of our previous finding on rice × *Z. latifolia* hybridization-induced *mPing* transpositional activity (Shan et al. 2005), this plant thus represents an ideal independent system to confirm and further study the effects of hybridization and introgression on *mPing* activity.

In this paper, we report that both *mPing* and *Pong* were transpositionally activated in the somatic hybrid (SH6) of "rice (cv. Zhonghua8) + *Z. latifolia*" produced previously (Liu et al. 1999), but they remained largely static in a protoplast-derived plant line of the same cultivar (Zhonghua8). We found that a salient feature of *mPing* transpositional behavior in SH6 is the massive loss of the element copies, which is a novel behavior of the element.

Materials and methods

Plant material

A partially fertile and highly asymmetric nuclear somatic cell hybrid plant (SH6) derived from protoplast fusion ("gamma"-fusion)-mediated asymmetric somatic cell hybridization between rice (cv. Zhonghua8) and a local accession of *Zizania latifolia* Griseb. was produced previously (Liu et al. 1999) and used in this study. The somatic cell hybrid (SH6) exhibits heritable, unique traits including apparent hybrid necrosis. The nature of SH6 as a bona fide asymmetric nuclear somatic hybrid was verified by both genomic DNA Southern blotting and gel-blot hybridization with *Z. latifolia* species-specific DNA repeats (Liu et al. 1999).

As an additional control, a protoplast-derived plant line (actually an "escaper" from the same protoplast-fusion experiment) with normal phenotype indistinguishable from the parental donor cultivar (Zhonghua8) was also used.

DNA gel-blot analysis

Genomic DNA was isolated from expanded leaves of individual plants by a modified CTAB method (Kidwell and Osborn 1992) and purified by phenol extractions. Genomic DNA (~3 μg per lane) of the various plants was digested by *Xba*I (New England Biolabs Inc.), separated on a 1% agarose gel, and transferred onto Hybond N^+ nylon membranes (Amersham Pharmacia Biotech) by the alkaline transfer recommended by the supplier. The probe-fragments were PCR amplified by using the following element- or region-specific primers: (a) *mPing* (positions: 6–430; the full length): forward: 5′-GTCACAATGGGGGTTTCACT, reverse: 5′-GGCCAGTCACAATGGCT AGT; (b) *Pong*-specific (positions: 158–1732; a fragment in the region before the first ORF, which bears little homology with *Ping*): forward: 5′- GGGGTGAAACAGCATTGAGA, reverse: 5′-TGTGGTTGCAAAGAAGACCA; and (d) *Ping*-specific (positions: 327–1513; a fragment in the region before the first ORF, bears little homology with *Pong*): forward: 5′-CTACGGAGTACACCGCAACC, reverse: 5′-AATGGATTGCCTACTGCTGACT. Identities of all probe-fragments were verified by sequencing. The fragments were then 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) after washing at a stringency of 0.2 × SSC, 0.1% SDS for 2 × 50 min. The filters were exposed to X-ray film for 1–3 h depending on signal intensity.

PCR-based locus assay on *mPing* and *Pong* excision

To detect possible excisions of *mPing* (AB087615.1), a set of 53 pairs of locus-specific primers each bracketing an intact *mPing* in the standard laboratory cultivar (Nipponbare) for rice, ssp. *japonica* (primer information is available upon request) was designed based on its whole genome sequence (http://rgp.dna.affrc.go.jp), by the Primer 3 software (http://biocore.unl.edu/cgi-bin/primer3/primer3_www.cgi). Eight loci each containing an *mPing* in rice cultivar Zhonghua8 (the parent for the somatic hybrid) were identified by PCR amplification with the whole set of primers (Supplementary Table 1). Two loci in Zhonghua8 flanking the 5′ end of *Pong* were isolated by TAIL-PCR (Liu et al. 1995) using the *Pong* sub-terminal-specific primers as reported (Jiang et al. 2003). The contiguous 3′ flanking sequences of these loci were determined based on the Nipponbare genome sequence by a BlastN search. Two pairs of locus-specific primers for these *Pong*-bracketing loci were designed and listed in Supplementary Table 1. PCR amplifications were performed at annealing temperatures ranging from 58 to 62°C depending on the primer pairs. The amplicons were visualized by ethidium bromide staining after electrophoresis through 2% agarose gels. All identified empty donor sites for *mPing* and *Pong* excisions were isolated and sequenced, together with their corresponding element-containing loci.

Isolation of de novo *mPing* insertion sites and additional excision sites in the somatic cell hybrid by transposon-display (TD)

Transposon display or TD (Casa et al. 2000; Van den Broeck et al. 1998) was performed by the protocol similar to that modified by Jiang et al. (2003) except using silver staining for band visualization (Wang et al. 2005). Briefly, 300 ng of rice total genomic DNA was digested by *Mse*I. Adaptors (5′-GACGATGAGTCCTGAG and 5′-TACTCAGGACTCAT) were ligated to the digested DNAs. Pre-amplifications were first done with an *mPing* internal primer *mPing*-f (5′-GCTGACGAGTTTCACCAGGATG) and *Mse*I+0 (5′-GATGAGTCCTGAGTAA). These reaction products served as templates in the selective amplifications with *Mse*I+3 or 2 selective base pairs and another *mPing* internal primer, *mPing*-r (5′-TGTGCATGACACACCAGTG), using single *Mse*I+C primer amplification as control (Fig. 4). Novel bands appeared in Zh-regenerant and SH6 amplified with an *mPing*-specific primer (*mPing*-r) and *Mse*I primers but absent in the control (Zhonghua8) were considered as putative *mPing* de novo insertions and isolated for sequencing. Likewise, bands present in the control but absent in Zh-regenerant or SH6 were considered as putative *mPing* excisions and also isolated for sequencing. The insertions and excisions were then confirmed by PCR amplification using *mPing*-flanking primers designed as described above.

Results

Indication for transpositional activation of *mPing* and *Pong* in the highly asymmetric somatic cell hybrid of rice-*Zizania latifolia* (SH6) based on gel-blot analysis

The somatic cell hybrid (SH6) of rice (ssp. *japonica*, cv. Zhonghua 8) and *Zizania latifolia*, which was produced by the "gamma"-fusion method (Gleba et al. 1988) is highly asymmetric in the sense that it contains the complete (or nearly so) genome of rice (the recipient parent) but only

minute amount of integrated chromatin segments from the donor species, *Z. latifolia* (Liu et al. 1999). Thus, SH6 is very similar in genomic constitution to that of the set of rice-*Z. Latifolia* recombinant inbred lines or RILs (Wang et al. 2005), though probably contains a larger amount of alien chromatins from the donor species than the RILs (Liu et al. 1999). However, the processes for producing the two kinds of plant lines are fundamentally different and also involved two different rice cultivars as recipient parents. Given that *mPing* and *Pong* were found as transpositionally mobilized in the set of rice-*Z. latifolia* RILs (*Ping* does not exist in these lines), and introgressive hybridization was postulated as the major elicitor for the elements' mobilization (Shan et al. 2005), this somatic cell hybrid apparently serves as an ideal independent experimental system to confirm the finding as well as to further investigate the effect of hybridization of rice by *Z. latifolia* on the activity of *mPing*, *Pong*, and *Ping* (if exist in the recipient cultivar).

Because none of the three elements, *mPing*, *Ping*, and *Pong*, contains a *Xba*I restriction site (Kikuchi et al. 2003), DNA gel-blot hybridization with this enzyme-digest should enable a conservative estimation on the elements' copy number and changing patterns, and hence, their possible transpositional activity. Three randomly selected individuals from the rice parent (cv. Zhonghua8), a protoplast-derived plant line (designated as Zh-regenerant, an escaper from the same gamma-fusion experiment) of Zhonghua8, the somatic cell hybrid (SH6), and one individual of *Zizania latifolia* were subjected to DNA gel-blot analysis as described in "Materials and methods". Marked changes in the banding patterns of both *mPing* and *Pong* in the somatic cell hybrid (SH6) compared with its rice parent were evident (Fig. 1a, b). No hybridization signal was detected when using the *Ping*-specific probe (amplified from cv. Nipponbare) on the same blot (data not shown), indicating absence of *Ping* in Zhonghua8, and hence, in Zh-regenerant and SH6.

For *mPing*, the most dramatic change in the blotting pattern of SH6 is loss of hybridization fragments, though gain of at least four novel fragments are also discernible (Fig. 1a). In SH6, the hybridization patterns of both *mPing* and *Pong* among the three random individuals are identical (Fig. 1a, b), suggesting that the changes occurred earlier and then being stably inherited after becoming homologous, given that the SH6 plants were at the 3rd selfed generation. Notably, difference in a single band was detected in the *mPing* hybridization patterns among the three random individuals of the rice parent cv. Zhonghua 8 (Fig. 1a). This raised an concern that *mPing* might be intrinsically unstable in this rice cultivar as in a previously reported case; which would then provide an alternative explanation to the *mPing* pattern in the somatic cell hybrid (Fig. 1a). To clarify this issue, we further investigated the extent of this "natural

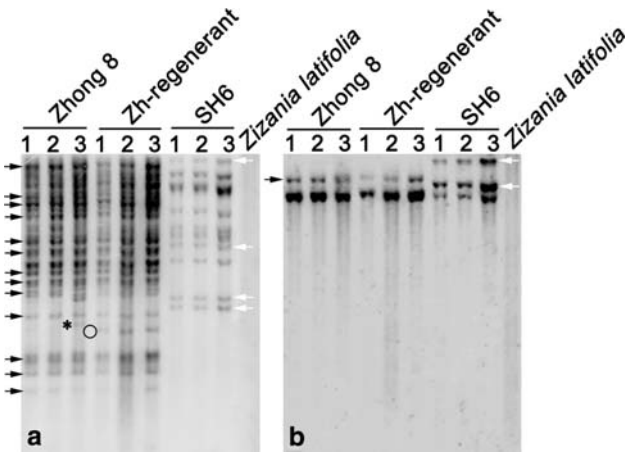

Fig. 1 Transpositional mobilization of *mPing* and *Pong* in a somatic cell hybrid of "rice + *Z. latifolia*"(SH6) as revealed by DNA gel-blot analysis. **a** Hybridization of *mPing* to a blot containing *Xba*I-digested DNA of three random individuals of the somatic hybrid (SH6), its rice parent (Zhonghua8), the protoplast-derived regenerant Zh-regenerant (escaper) of Zhonghua 8, and one individual of *Z. latifolia*. *mPing* does not contain a *Xba*I restriction site, and hence, the changing hybridization patterns most likely reflect presence or absence of intact element members at the particular loci, rather than internal structural changes. *Black arrows* denote loss of parental bands in the somatic hybrid, whereas the *white arrows* point to novel bands appeared in the somatic hybrid (not all are labeled). An *asterisk* and a *circle*, respectively, refer to variant bands attributable to parental heterozygosity and possible effect of the protoplast preparation and/or culture process. **b** Hybridization of the *Pong*-specific fragment to a blot with *Xba*I-digested DNA of the various plants as in **a**. Marked alteration in the banding pattern of *Pong* in the somatic hybrid versus that of the rice parent and the protoplast-regenerant (identical with that of parent) is evident. Loss and gain of bands were also denoted by *black* and *white arrows*, respectively

polymorphism" of *mPing* patterns within this rice cultivar by analyzing 24 more random individuals by the same gel-blotting, and we found only monomorphic patterns for both *mPing* and *Pong* (data not shown). This suggests that in this rice cultivar, *mPing* activity, if any, is very weak under normal conditions, as in most rice cultivars, and hence, cannot be a major contributing factor to the dramatically altered pattern in the somatic cell hybrid (Fig. 1a). Among the three individuals of the protoplast-derived escaper, Zh-regenerant, minor difference in the banding patterns for *mPing* was also clear. In addition, they all were different from their parental line by at least one band (marked), suggesting probable weak activation of the element by the protoplast isolation and/or cell culture process. No alteration was detected for *Pong* among the parental individuals or between the escaper and the parent, but both loss of at least one parental band and gain of two bands were evident in the somatic cell hybrid (Fig. 1b). These changing patterns of loss and gain of hybridization fragments for both *mPing* and *Pong* in the somatic cell hybrid is consistent with mobilization of the type II DNA transposons including MITEs via the

"cut-and-paste" model (Casacuberta and Santiago 2003; Feschotte and Pritham 2007). Because no homolog of *mPing* exists in the donor species *Z. latifolia* (Shan et al. 2005; Fig. 1), and also only minor changes for *mPing* and no change for *Pong* were detected in the protoplast-derived escaper (Zh-regenerant), the evidence is compelling to allow us to tentatively conclude here that, similar to the situation of the set of recombinant inbred lines (RILs) of rice—*Z. latifolia* (Shan et al. 2005), the asymmetric somatic cell hybridization, i.e., the introgression of multiple chromatin segments of *Z. latifolia* into the genome of rice (Liu et al. 1999) is likely responsible for triggering the transpositional activation of *mPing* and *Pong* in the somatic cell hybrid genome.

Validation of transpositional activity of *mPing* and *Pong* by detection of excisions and insertions in the somatic cell hybrid

Although the changing hybridization patterns of both *mPing* and *Pong* in the somatic cell hybrid can be most parsimoniously explained by their transpositional mobilizations, an alternative cause cannot be confidently ruled out solely based on the DNA gel-blotting data. This potential alternative cause is genomic rearrangement at chromosomal regions involving *mPing* or *Pong*, followed by homogenization in the selfed progenies. For example, alien chromatin integration is conceivably involving recombinational loss of the rice parental sequences, thus, if the *mPing* or *Pong* element happened to reside within the deleted sequences, then, loss of element copy numbers would have been similarly reflected in the gel blots as in Fig. 1. Thus, we roughly assessed the extent of loss of chromatin segments from the rice parent (Zhonghua8) in the somatic hybrid (SH6) relative to the rice parent (Zhonghua8) and the escaper (Zh-regenerant), by the amplified fragment length polymorphism (AFLP) at >1000 random genomic loci. We found that, first, for both types of variable bands (loss and gain) resolvable by AFLP, SH6 indeed showed much more incidences than those of the escaper (Zh-regenerant), suggesting the occurrence of extensive genomic changes as a result of somatic hybridization (Supplementary Fig. 1); second, of the two types of variable bands, loss occurred at much higher frequencies than gain (22.45 vs. 11.84%), suggesting that there were likely numerous deletions of the rice genetic material (Supplementary Fig. 1). Nonetheless, it should be noted that a loss of parental band in SH6 revealed by AFLP could also be the result of a single or a few base alteration(s) at the restriction and/or selective nucleotide bases.

Bear in mind the occurrence of extensive genomic changes including possible loss of chromatin from the rice parent in the somatic cell hybrid (SH6), described above, it is important to distinguish the two possibilities as a cause for the loss of *mPing* copies in this plant (Fig. 1). Therefore, it is necessary to test if the loss and gain events were *confined to mPing* and *Pong*, i.e., via element excisions and insertions, or involving larger genomic regions including the elements (random genomic rearrangements including sequence deletions). We thus designed a set of locus-specific primer pairs (53 in total) each bracketing an *mPing* based on the whole genome sequence of the standard rice (ssp. *japonica*) laboratory cultivar Nipponbare (http://rgp.dna.affrc.go.jp). Eight pairs of primers were identified as containing *mPing* in the rice cultivar Zhonghua8. PCR amplification using these eight pairs of locus-specific, *mPing*-containing primers on genomic DNA of the protoplast escaper (Zh-regenerant) indicated that none of the primer pairs amplified a smaller-sized product indicative of *mPing* excision in these plants, thus corroborating the DNA gel-blotting results, and both showed large stability of *mPing* during the protoplast preparation and cell culture processes in Zhonghua8. In a sharp contrast, all eight *mPing*-encompassing primer pairs generated smaller-sized amplification products in the somatic cell hybrid (SH6), and the size difference (based on molecular size markers) between the original larger bands (*mPing*-containing, from Zhonghua8) and the smaller bands were consistent with the precise loss of *mPing* (e.g., Fig. 2a), which was confirmed by sequencing (Supplementary Table 1). Amplification of two pairs of *Pong*-containing primers on the protoplast-regenerant and the somatic cell hybrid showed that one pair (TAIL-PongL3) amplified a normal, larger-sized fragment from the protoplast-regenerant, but smaller-sized fragment from SH6, suggesting its excision in the somatic cell hybrid (Fig. 2b); the other pair (TAIL-Pong1) amplified a smaller-sized fragment in both Zh-regenerant and SH6, suggesting the excision of *Pong* from this locus was most likely due to protoplast isolation and/or the cell culture process (Fig. 2c). For both primer pairs, sequencing confirmed that the smaller-sized amplification products were resulted from precise excision of *Pong* instead of random genomic rearrangements (Supplementary Table 1). In addition, the sequence analysis of all *mPing*- and *Pong*-empty donor sites together with their corresponding element-containing loci isolated from parental line Zhonghua8 indicated that, apart from one *mPing*-locus (mPL4) that left a single nucleotide base (A) footprint upon excision, all the rest excised loci, of either *mPing* or *Pong*, have left no footprints (Supplementary Table 1). This result of lack of an excision footprint after *mPing* excision is consistent with that found in the rice-*Zizania* RILs (Shan et al. 2005), as well as with the recent results in *mPing* mobilization when being introduced into the *Arabidopsis* genome, which showed that the great majority (83%) of *mPing* excisions were precise (Yang et al. 2007). The *precise nature* in the loss of *mPing* from the somatic cell hybrid (SH6) ruled out alternative

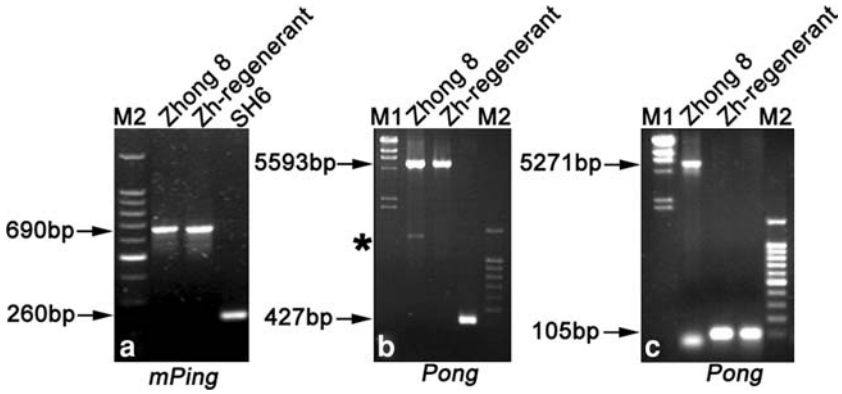

Fig. 2 Examples of *mPing* and *Pong* excisions in the somatic cell hybrid (SH6) as revealed by locus-specific PCR amplifications and ethidium bromide-staining of products on agarose gels. **a** PCR amplification with *mPing*-bracketing locus-specific primer pair TmpL2 on template DNAs of Zhonghua8, Zh-regenerant, and SH6. Size of the smaller-sized band coincided with deletion of a full-length *mPing* copy from the larger-sized band, as validated by sequencing. **b** and **c** are PCR amplifications with two *Pong*-bracketing locus-specific primer pairs (TAIL-Pong1 and TAIL-Pong3) on the same template DNAs as in **a.** The smaller-sized bands were also resulted from precise loss of *Pong*, as validated by sequencing. The asterisk in **b** indicates a non-specific band. That all PCR products being amplified from orthologous loci between the parent and the somatic cell hybrid is confirmed by sequencing both the larger- and smaller-sized bands (Supplementary Table 1). M1 and M2 are, respectively, the Lambda-*Hin*dIII digest and the 100 bp DNA ladder size maker (products of the TaKaTa Biotech, Japan)

mechanisms (e.g., recombinational loss of rice chromatin segments) as a cause for massive reduction of the element's copy number.

Despite the massive loss of *mPing* copies, de novo insertion of *mPing* likely also occurred in the somatic cell hybrid as judged by the appearance of novel bands in the DNA gel-blotting patterns for both elements (Fig. 1a). To confirm this, we performed *mPing*-specific transposon display (TD) analysis (Casa et al. 2000; Van den Broeck et al. 1998). Several novel bands present only in the somatic cell hybrid were visualized in the TD profiles (Fig. 4), and which were isolated and sequenced. Sequence analysis of these bands isolated from SH6 enabled identification of 10 fragments containing at their 5′ end the stretch of nucleotides of *mPing* (Supplementary Table 2). The contiguous upstream sequences putatively flanking complete members of *mPing* at these loci were deduced from the whole genome sequence of Nipponbare (http://rgp.dna.affrc.go.jp), and hence, again enabled the design of locus-specific primers bracketing a copy of *mPing* (as described above). PCR amplifications using these putative *mPing*-containing primers in both the Zhonghua8 parental line and the protoplast-regenerant produced only smaller-sized bands expected for absence of *mPing* at each of the identified loci, whereas larger-sized bands expected to contain a member of *mPing* were amplified from SH6 (Fig. 3a and data not shown). By sequencing the full length (*mPing*-containing) of the isolated larger-sized bands, we found that they all indeed contained the complete TIRs (GGCCAGTCACAATGG) and the TSDs (TAA or TTA) characteristic of newly transposed *mPing* (Jiang et al. 2003; Kikuchi et al. 2003; Nakazaki et al. 2003) (Supplementary Table 2). From the TD profiles,

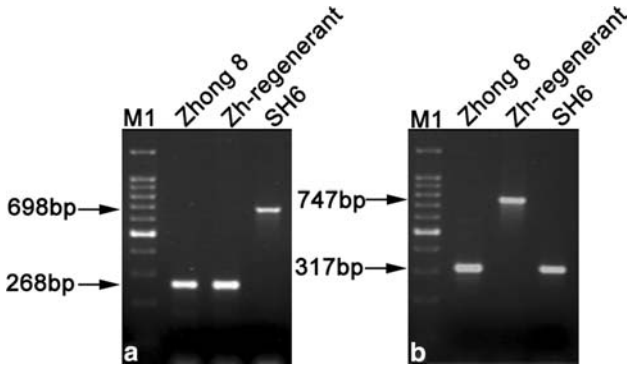

Fig. 3 Examples of *mPing* de novo insertions in the somatic cell hybrid (SH6) as revealed by locus-specific PCR amplifications and ethidium bromide staining of products on agarose gels. **a** A typical de novo *mPing* insertion in SH6 (locus TmpL9). **b** A de novo *mPing* insertion only in the Zhong-regenerant by the locus-specific primer TmPL14. The novel larger-sized bands in both **a** and **b** coincide with insertion of a full-length *mPing* copy at each of the loci (Supplementary Table 2). M is the 100 bp DNA ladder size marker (TaKaRa Biotech, Japan)

we also cloned a *mPing*-containing locus in the protoplast regenerant, which was absent from Zhonghua8 (Fig. 3b), suggesting the insertion of this particular *mPing* copy was caused by the protoplast isolation and/or the cell culture process. From the TD profiles, we also cloned five additional *mPing*-containing loci from the parental cultivar Zhonghua8. These five primer pairs generated only large-sized bands identical with those from the parental line in the Zh-regenerant, but they all produced smaller-sized products in the somatic cell hybrid (SH6), and sequence analysis revealed that they were again precise *mPing* excisions (Supplementary Table 1).

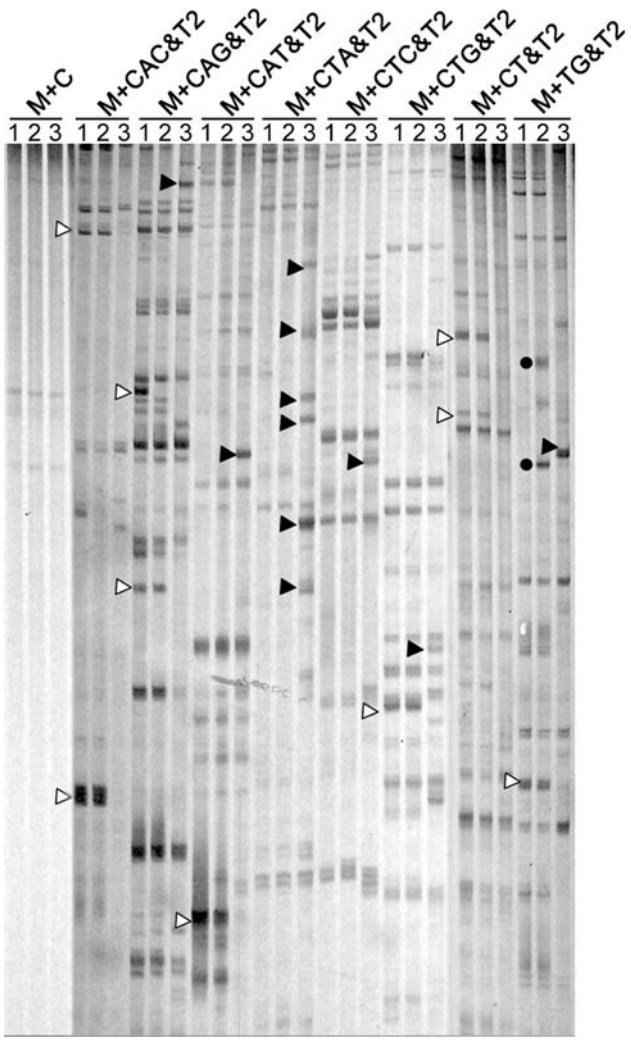

Fig. 4 Examples of a transposon display (TD) profile of *mPing* on genomic DNAs of the rice parental line Zhonghua8 (lane 1), the protoplast regenerant (Zh-regenerant) (lane 2) and the somatic cell hybrid (SH6) (lane 3). The left-most three lanes are amplifications without adding the *mPing*-specific primer, indicating very faint amplification by the *Mse*I-adapter primer alone, and hence, validating the feasibility of the silver-staining-based TD assay. The three or two selective bases in the *Mse*I-adapter-primers are indicated. The *empty* and *solid arrowheads*, respectively, denote loss of parental bands or gain of novel bands in SH6 only or in both SH6 and Zh-regenerant. The two *solid circles* refer to two novel bands appeared only in the Zh-regenerant. Note that not all changed bands are labeled. Sequencing of a subset of bands representing loss or gain verified that they all are, respectively, bona fide *mPing* excisions and insertions

To test the possibility that the smaller-sized bands amplified from the somatic cell hybrid (SH6), in the locus-specific assays, were actually originated from introgressed chromatin segments of *Zizania*, we performed PCR amplifications using *Zizania* DNA as a template under identical conditions (Methods) with all the 13 pairs of *mPing*-containing primers in the rice parental line (Zhonghua8). Results indicated that seven pairs of primers failed to amplify a product within the expected size-ranges from *Zizania* DNA, and the rest six primer pairs, though produced amplification products in the expected size ranges, the amplification products are of different sizes from those amplified from the somatic cell hybrid (SH6) (Supplementary Fig. 2). Further sequencing of these PCR products of *Zizania* and then comparing with the corresponding amplicons from the somatic cell hybrid (SH6) as along with the reference sequences of Nipponbare (http://rgp.dna.affrc.go.jp) indicated that the bands of *Zizania* origin showed either no homology at all or high levels of sequence divergence from those of rice origin including SH6 (Supplementary Table 1). Thus, this analysis has unequivocally verified the smaller-sized bands from SH6 as bona fide rice chromosomal sequences, and hence, validating the *mPing* excisions.

Discussion

We have shown in this study that the rice endogenous MITE *mPing* and its transposase-encoding partner *Pong* were transpositionally mobilized in a highly asymmetric somatic cell hybrid of "rice + *Z. latifolia*" we produced previously (Liu et al. 1999). A common feature of this somatic cell hybrid and the set of recombinant inbred lines (RILs) of rice and *Z. latifolia* (Wang et al. 2005) is that they all have 24 normal-looking rice chromosomes in their somatic cells, i.e., they only contain gnomically integrated chromatin segments, rather than independent chromosomes or chromosomal fragments, derived from the wild donor species *Z. latifolia* (Liu et al. 1999; Wang et al. 2005). Thus, the *mPing*/*Pong* transpositional activity in SH6 as compared with its rice parent (cv. Zhonghua8) should be caused by *Z. latifolia* DNA integration and/or the protoplast preparation (enzymatic hydrolysis) and the followed cell culture process. Both the DNA gel-blotting and transposons-display (TD) data have indicated that the *mPing*/*Pong* banding-patterns in a protoplast-derived escaper (Zh-regenerant) remained nearly the same as those of the parental line Zhonghua8, suggesting that the effect of protoplast preparation and cell culture on the transpositional activity of *mPing* and *Pong* was minor. This is consistent with previous findings that *japonica* rice cultivars (e.g. Nipponbare) often are not responsive to somatic tissue culture with regard to *mPing*, *Ping* and *Pong* transpositional activity (Jiang et al. 2003). Taken together, it can be concluded that the original somatic cell hybridization process and/or integration of chromatin segments of *Z. latifolia* into the rice genome is the most likely conceivable cause for the transpositional mobilization of *mPing* and *Pong* in the somatic cell hybrid.

An unexpected finding from this study is the transposition-associated massive loss of *mPing* copies from the original loci in the somatic cell hybrid (SH6) compared with its

rice parent (cv. Zhonghua8). Because this feature is incongruent with a transposon's inherent property, a concern is raised that the loss of *mPing* might be due to alternative mechanisms. Two alternative causes are conceivable: (1) recombinational loss of the rice chromatin segments containing the element copies subsequent to somatic hybridization; (2) the smaller-sized PCR fragments were originated from Zizania chromatin integrated into the rice genome in the somatic hybrid. However, we consider that both possibilities can be confidently ruled out based on the following two lines of evidence. (1) The observation by locus-specific PCR analysis that 12 out of the 13 *mPing* deletion events in the somatic cell hybrid were exclusively confined to the element per se while leaving the flanking regions intact, indicating that recominational loss of rice parental chromatin cannot be as a cause for the *mPing* elimination. (2) The failure to amplify a product from *Zizania* DNA by some (7/13) of the *mPing*-relevant, locus-specific primers, and the lack of homology between the amplicons from *Zizania* and SH6 by the rest primers unequivocally testified that the smaller-sized PCR products amplified from SH6 (relative to the larger-sized PCR products from its parental rice line, Zhonghua8) were not from integrated chromatin of *Zizania* in the somatic hybrid (SH6), but of rice origin. Therefore, the loss of *mPing* copies in SH6 is most likely due to their active excisions upon or subsequent to the somatic cell hybridization event, followed by abortive insertion of some of the excised copies into new chromosomal loci. Parental heterozygosity and changes attributable to the protoplast preparation/cell culture process were minimal, as being reflected by a single band change in one of the three randomly chosen parental individuals (but no change in a set of additional 24 random individual plants tested) and alterations in the three individuals of the protoplast-regenerated plant Zh-regenerant (escaper) was also minor.

Abortive transposition, which refers to excision of a mobile element that is not followed by reinsertion, may occur for some class II transposons, e.g., the *AC/DS* elements in maize (Gorbunova and Levy 2000), the introduced *Drosophila* mariner element *Mos1* in *Caenorhabditis elegans* (Bessereau et al. 2001), and a recently identified *Mutator* transposon called *Jittery* (Xu et al. 2004). Nonetheless, this transpositional behavior was not observed for *mPing* mobilization in any of the previously reported case (Jiang et al. 2003; Kikuchi et al. 2003; Nakazaki et al. 2003; Shan et al. 2005). Thus, the question arises as to what host factor(s) might have been compromised in the somatic cell hybrid (SH6) to cause the greatly decreased incidence of *mPing* reinsertion after excision? In this respect, a previous study has elegantly documented that genetic mutation in one *Arabidopsis* locus called *IAE1* greatly increased the excision frequency (by 550-fold) of the introduced *AC* element, but with very low frequencies of reinsertion by the excised element copies (Jarvis et al. 1997). This study has thus explicitly implicated that the excision and reinsertion frequencies for a given transposon can be genetically controlled by distinct host factor(s) or host genotypes. As shown by gel-blotting in the previous study (Liu et al. 1999) and the AFLP results in this study, extensive genomic instabilities occurred in the somatic hybrid (SH6), thus, it can be imaged that if one or more of the host factor(s) responsible for controlling the *mPing* excision and reinsertion properties were mutated, then we might expect to see the altered behavior of *mPing* in this particular case. Alternatively, the rice parental genotype (Zhonghua8) may simply become a "defective" host with regard to *mPing* reinsertion capacity when being used for the somatic cell hybridization (e.g., due to a natural mutation), which can be tested in further experiments by inducing *mPing* activity through other means. Irrespective of mechanisms, the sheer extent to which abortive transpostions may occur for *mPing* under certain circumstances might suggest an explanation for the exceptionally low-copy number of this MITE as compared with other characterized plant MITEs (Feschotte et al. 2002), as well as for the conspicuous copy number difference in *mPing* among the various groups of rice cultivars (Jiang et al. 2003; Huang et al. 2008). In addition, results of this study have provided additional evidence in support of McClintock's insight that plant transposable elements though often remain quiescent under normal conditions can be instigated to transpose under various stress conditions (McClintock 1984).

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