Suppression of an Atypically Spliced Rice CACTA Transposon Transcript in Transgenic Plants

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ABSTRACT

OsES1, a rice homolog of the maize En/Spm transposon, is transcribed to produce TnpA-like and TnpD-like transcripts. However, an alternatively spliced form of the TnpA-like transcript, which was found to be suppressed in transgenic plants, was revealed to be due to atypical splicing of a Hipa-like CACTA transposon.

The rice genome sequence (Oryza sativa cv. Nipponbare) was searched for sequences similar to the autonomous maize En/Spm transposon and revealed the 10,693-bp-long low-copy transposon OsES1 (GenBank accession no. AC123523) with imperfect terminal inverted repeats (TIR) ending with the typical CACTA seventh intron (Figure 1; Table 2). In addition, a 1169-base pair defective CACTA-like transposon with a typical trinucleotide target-site duplication is inserted in the last exon. This element shares homology to Hipa, a rice En/Spm-like transposon with characteristic conserved CACTG termini (Panaud et al. 2002; Wang et al. 2003). Indeed, sequence and DNA gel-blot analyses of OsES1 elements in several other rice cultivars revealed a clear association between the occurrence of these modifications and lack of mobility (data not shown).

Sequence and DNA gel-blot hybridization likely correspond to the TnpD (6-kb) and TnpA (2-kb) homologous transcripts, as for the maize En/Spm (Pereira et al. 1986; Figure 2A, lane 1). These transcripts probably arose within the element as revealed by multiple 5’-RACE products (data not shown). However, an additional alternative TnpA transcript of ~2.4 kb was also visible and was confirmed by 3’-RACE experiments, which revealed a 1-kb main fragment and a less abundant 1.4-kb form (Figure 2B, lane 1). In addition, RT-PCR products obtained using the reverse primer in the last exon (L) in combination with the forward primers

Notes

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Figure 1.—Diagram of OsES1. Positions 1 and 10,693 of OsES1 correspond to positions 24,158 and 34,850 in the BAC clone AC123523. Solid boxes and triangles represent exons and terminal regions, respectively. The Helitron (hel) and Hipa-like (hip) transposon insertions are indicated as open triangles. Thick arrows indicate the predicted open reading frames. ORF1 would encode a putative peptide of 1146 aa with 61% similarity to TnpD of maize (S29-329). ORF2 would encode a 528 aa putative peptide partially homologous to TnpA (S28365; 44% similarity over 324 aa). However, a frameshift at position 3317 and a nonsense mutation at position 5093 (indicated by asterisks) are present in TnpD and TnpA, respectively, which interrupts the reading frames. The positions of the primers used for transcription analyses are also shown, along with the probe used for Northern analysis.

F or H were less abundant than the products obtained with primer I (Figure 2C, lanes 2s and data not shown).

Because DNA gel-blot analysis revealed the presence of only one additional OsES1 homologous element in the Nipponbare genome (GenBank accession no. AC-073391; supplementary Figure 1 at http://www.genetics.org/supplemental/), which appeared to be truncated and partially divergent at the nucleotide level, the two TnpA-like transcripts must result from alternative transcription of the OsES1 element. In particular, sequencing of the RACE products (5' and 3') revealed that the most abundant shorter form originates by transcription through the last intron and premature polyadenylation at position 7043, before the Helitron-like insertion. On the other hand, the less abundant longer form contained all the expected exons and was polyadenylated, as predicted, at position 10,216. However, neither the Helitron-like nor the Hipa-like transposon insertions were present in this longer transcript that is a complete form of the TnpA-like product.

The presence of the Helitron-like insertion seems to prevent splicing of the last intron, inducing premature polyadenylation before the insertion site and resulting in the formation of the more abundant short transcript. Such examples of transposon insertions in transcribed regions influencing pre-mRNA processing have been observed in the shrunken2 and waxy genes of maize (VARGONA et al. 1992; LAL et al. 2003).

The less abundant TnpA-like spliced product can be produced by splicing of the intron containing the Helitron and transcription through the last exon. The Hipa-like transposon might be expected to excise by activity

<table>
<thead>
<tr>
<th>Exon</th>
<th>Begin</th>
<th>End</th>
<th>Primer</th>
<th>Position</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>420</td>
<td>895</td>
<td>A</td>
<td>514</td>
<td>F</td>
</tr>
<tr>
<td>2</td>
<td>1,215</td>
<td>3,458</td>
<td>B, C</td>
<td>2112, 2927</td>
<td>R, F</td>
</tr>
<tr>
<td>3</td>
<td>3,537</td>
<td>3,845</td>
<td>D</td>
<td>3560</td>
<td>F</td>
</tr>
<tr>
<td>4</td>
<td>3,918</td>
<td>4,038</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4,116</td>
<td>4,973</td>
<td>E</td>
<td>4431</td>
<td>R</td>
</tr>
<tr>
<td>6</td>
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<td>5,253</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5,353</td>
<td>5,530</td>
<td>F, G2, G</td>
<td>5371, 5388, 5525</td>
<td>R, R</td>
</tr>
<tr>
<td>8</td>
<td>5,782</td>
<td>6,153</td>
<td>H</td>
<td>5836</td>
<td>F</td>
</tr>
<tr>
<td>9</td>
<td>6,229</td>
<td>6,342</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6,417</td>
<td>6,755</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>6,844</td>
<td>6,957</td>
<td>I</td>
<td>6881</td>
<td>R</td>
</tr>
<tr>
<td>12</td>
<td>8,548</td>
<td>9,974</td>
<td>L1, L</td>
<td>8554, 9855</td>
<td>F, R</td>
</tr>
<tr>
<td>3'-UTR</td>
<td>9,975</td>
<td>10,216</td>
<td>L2</td>
<td>10202</td>
<td>R</td>
</tr>
</tbody>
</table>

Prediction of exon/intron boundaries was performed with GENSCAN (BURGE and KARLIN 1997; http://genes.mit.edu/GENSCAN.html).

*F, forward; R, reverse.

Predicted sites not confirmed experimentally.

Splicing of this exon also can occur using alternative donor sites at positions 4210 and 4680.

In most transcripts, this donor site is skipped and transcription proceeds until the alternative polyadenylation site at position 7043.
TABLE 2
Transposon insertions in OsES1

<table>
<thead>
<tr>
<th>Transposon Type</th>
<th>Insertion Position</th>
<th>Insertion Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helitron-like</td>
<td>(position 7106–8385)</td>
<td>tcatatttatca...CGATACATGCCGTATAGGCGGCTACCTTTCTAG tgatactaatag</td>
</tr>
<tr>
<td>Hipa-like</td>
<td>(position 8586–9757)</td>
<td>aga CACTGTTAGGAAAAACATCTTTTGGTGGT...CCGGGACTAAAAAGCATTTCTCCACCAGTG aga</td>
</tr>
</tbody>
</table>

Transposon sequences are represented in uppercase letters, with the terminal conserved sequences (TIRs in the case of Hipa) double underlined. The palindrome in the 3’ terminal region of the Helitron is single underlined. Boldface lowercase letters in the flanking genomic sequences represent the target insertion site (duplicated in the case of Hipa).

of a trans-activating transposase from an autonomous partner. Although several hundred Hipa-homologous sequences have been shown to be present in the Nipponbare genome (Panaud et al. 2002; Wang et al. 2003), no transpositional activity has yet been demonstrated. The Hipa sequence described by Panaud et al. (2002) has a size compatible with it being an autonomous element (10,539 bp) and shares sequence identity in the TIRs and the subterminal regions with the Hipa-like insertion in OsES1, indicating that they belong to the same family (Figure 3A). RT-PCR analysis revealed that the two predicted ORFs coding for putative peptides homologous to TnpD-like transposases (ORF1) or to putative transposases and hydroxyproline-rich glycoproteins (ORF2) within this Hipa element are transcribed (Figure 3, B and C). However, attempts to demonstrate the occurrence of excision of the Hipa-like transposon from OsES1 by PCR amplification of an empty donor site proved unsuccessful, even when appropriate conditions to reveal rare events were used (data not shown). Moreover, the absence of a molecular footprint at the site of the Hipa-like insertion in the long TnpA-like transcript would require the occurrence of a perfect excision event, known in plants to be exceptional compared to footprint-leaving excisions (Saedler and Ners 1985; Coen et al. 1989). Thus it is unlikely that excision of the Hipa-like element from the OsES1 element occurs.

Alternatively, removal of the Hipa-like transposon could be a phenomenon occurring at the RNA level rather than at the DNA level, with transcription read through the Hipa-like element followed by processing out the element from the primary transcript. Splicing of transposable elements from pre-mRNA has been previously described for defective members of the Ac/Ds and En/Spm families in maize, suggesting that they may function as novel introns (reviewed by Weil and Wessler 1990). Most relevant in many cases, splicing was suppressed by the presence of the autonomous partner, presumably as a consequence of the binding of the transcriptional partners...
trans-acting transposases to the termini of the defective element. In all cases, canonical donor and acceptor sites from the host DNA or (cryptic sites) from the element itself were employed, which never led to the complete deletion of transposon sequences. In the case reported here, however, the process leads to the perfect restoration of the original donor site. A similar situation was reported by Giroux et al. (1994) in which the exact removal of a $Ds$ transposon and one copy of the eight-nucleotide target-site duplication occurred occasionally (<5% frequency) as a result of RNA splicing from an exon of the maize $sh2-m1$ allele. Also in this case, however, the random incidence of consensus GT/AG splicing sites (Goodall and Filipowicz 1991) within the repeats of the target-site duplication could allow the alternative splicing. In this respect, elimination of the defective $Hipa$-like element from OsESI does not resemble a “true” splicing event, as no common splice recognition sequences that could generate the final product are present in the surrounding region. A similar example of atypical mRNA processing was described for the removal of a 144-bp exonic portion of an opioid receptor in human melanomas (Mayer et al. 2000), although the underlying mechanism remains unclear. Atypical splicing in the absence of canonical splice sites was also revealed for the 13th intron of the $FCA$ gene in Arabidopsis (Macknight et al. 1997), suggesting that this alternative mechanism of intron excision might exist in plants.

ALTERNATIVE TRANSCRIPTION OF OsESI IS SUPPRESSED IN TRANSGENIC PLANTS

Remarkably, the longer TnpA-like transcript was absent in transgenic rice plants, independently of the introduced construct (Figure 2 and data not shown). In analogy with the suppression mechanism invoked for the maize $En/Spm$ transposase (Gierl et al. 1985), $Hipa$ transposases activated from cryptic or silent elements in the transgenic lines could bind to the termini of the...
related Hipo-like element in the last exon of OsES1, inhibiting transcript readthrough and subsequent splicing. This differential activation could be induced by the tissue-culture steps during the transformation procedure, as demonstrated for other transposable elements like Ac/Ds and retrotransposons (e.g., in rice Hirochika et al. 1996; Ks et al. 2002). Indeed, transcription of Hipo transposons was already demonstrated to be strongly activated by biotic stress (He et al. 2000), although no correlation with an increased transpositional activity has yet been revealed. RT-PCR experiments performed in four transgenic lines, to monitor the occurrence of changes in transcription of the putative Hipo autonomous element (Panaud et al. 2002), did not reveal a clear difference compared to the wild type (Figure 3C). Nevertheless, due to the considerable amount of Hipo-like elements in the rice genome and to their evolutionary divergence (Wang et al. 2003), it might be expected that another member of the family with closest homology to the defective element in OsES1 mediates the suppression. Possibly, the TnpD-homologous ORF1 product exerts the effect by binding the CACTG termini of the Hipo-like element, rather than the ORF2 product, which has no evident homology with TnpA-like transposases/DNA-binding proteins.

In conclusion, although the transpositional activity of rice En/Spm-like elements has yet to be proven, their transcriptional behavior is affected by tissue-culture-related stress conditions and displays the “Suppressor” effect, to which this transposon system owes its name (McClintock 1954). This effect on influencing the expression of genes was what Barbara McClintock fore-saw as one of the primary roles of these “controlling elements.” This example is probably the tip of the iceberg, revealed by genomics methods, providing a regulatory role for transposable elements that compose the bulk of complex plant genomes.

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