Copia-Like Retrotransposons in the Rice Genome: Few and Assorted

Carlos M. Vicient¹ and Alan H. Schulman^{1,2,*}

¹*Plant Genomics Laboratory, Institute of Biotechnology, University of Helsinki, Viikki Biocenter, P.O. Box 56, FIN-00014 Helsinki, Finland*

²*Crops and Biotechnology, Agrifood Research Finland, Myllytie 10, FIN-31600 Jokioinen, Finland*

(Received: 16 October 2001; accepted: 19 December 2001)

ABSTRACT: We performed a comprehensive analysis of Ty1-*copia*-like retrotransposons in about 10% of the *Oryza sativa* genome and identified 100 elements. These were divided into nine classes by their reverse transcriptase similarity and into 39 families according to the sequences of their long terminal repeats (LTRs). The deduced total number of *copia*-like elements in rice is ~1000, comprising 1.7% of the genome. Solo LTRs and truncated elements comprise an additional 2.3% of the genome. This is low compared with other plants, especially grasses, but comparable to *Arabidopsis*. However, the total number of families in the genome, at least 50, is similar to that in other plants. At least some of these classes are present in the genomes of other grasses, and related elements can be found in dicotyledonous plants. The transcriptional activity of each family was estimated by searching for homologous expressed sequence tags in databases. No correlation was found between the number of homologous expressed sequence tags and family size. In comparison with the *copia* retrotransposons, 222 *gypsy*-like and 66 LINE-like retroelements, as well as five pararetrovirus-like elements, were found, corresponding to about 2220, 660, and 50, respectively, in the genome. A low rate of retrotransposon transcription and a high rate of loss of integrated copies by ectopic, intrachromosomal recombination are proposed as the reasons for the paucity of retrotransposons in the rice genome.

Keywords: Rice, Genome Evolution, *Copia*-like Retrotransposon, *Gypsy*-like Retrotransposon, LINE Element, *Oryza sativa*.

1. INTRODUCTION

Retrotransposons are mobile genetic elements that transpose through reverse transcription of an intermediate RNA.¹ Retrotransposons can be divided into two main classes based on the presence or absence of long terminal direct repeats (LTRs). The LTR retrotransposons are bounded by an LTR at each end and contain an internal region coding for GAG (capsid protein), proteinase, integrase, reverse transcriptase (RT), and RNase H. Retrotransposons are structurally and functionally similar to retroviruses; both groups replicate by reverse transcription of an intermediate RNA. However, LTR retrotransposons are not

infective, because of their lack of an *env* domain encoding an envelope glycoprotein. The LTR retrotransposons, in turn, can be divided into two main groups, the *copia*- and the *gypsy*-like elements. These differ in the order of their coding domains. Although both contain a relatively conserved *rt* domain, sequence comparisons indicate that each group is monophyletic and transcends organismal groupings.² The *gypsy*-like elements are more retroviral in their sequence as well as their coding domain organization. Non-LTR retrotransposons include LINE and SINE elements.³

The LTR retrotransposons are universal and abundant components of eukaryotic genomes in general, and of plant genomes in particular.⁴ Retrotransposons are present in high numbers of copies in plants, especially in those with

*Author to whom correspondence should be addressed.

large genomes, where they show considerable sequence heterogeneity.^{5–8} The processes of transcription and reverse transcription are error prone and lead to this heterogeneity. Furthermore, many retrotransposons within the genome are either integrated in a transcriptionally or translationally incompetent state or become fossils because of accumulation of mutations following integration. Nevertheless, the activity of retrotransposons in somatic tissues has been demonstrated,⁹ and a basal level of expression appears to exist for many elements.¹⁰ Of those retrotransposons quiescent in somatic tissues, many can be activated by stress,^{11,12} protoplast formation,¹³ or tissue culture.^{14,15} Indeed, environmental gradients involving stress have been correlated with retrotransposon-based genome differentiation in wild barley.¹⁶ Translation and formation of virus-like particles have also been directly demonstrated for plant retrotransposons.¹⁷

Unlike DNA transposons, when retrotransposons transpose, the original copy does not excise as part of the process. Retrotransposons can thereby greatly increase their copy numbers and, consequently, their genome size.¹⁸ Whereas retrotransposons account for 50–80% of the large maize genome (*Zea mays* L, $1C = 2.6 \times 10^9$ bp),¹⁹ the small *Arabidopsis thaliana* L. genome ($1C = 1.3 \times 10^8$) contains about 5.5% retrotransposons in the finished sequence (T. Bureau, personal communication). The control or inefficiency of retrotransposition, however, is not the only possible explanation for the paucity of retrotransposons in small genomes. Mechanisms such as recombination between LTRs can eliminate retrotransposon sequences from the genome, generating solo LTRs and other types of deletion products.^{20,21}

Cultivated rice (*Oryza sativa* L.) has one of the smallest genomes ($1C = 4.3 \times 10^8$ bp)²² in the genus *Oryza*, which is composed of more than 20 species that vary 3.5-fold in nuclear DNA content, from 3.43×10^8 to 12.01×10^8 bp among the diploids.¹⁹ Three times that of *A. thaliana*, the rice genome is also among the smallest of the grasses as a whole, which range from 2.0×10^8 bp in *Oropetium thomaeum* to 1.3×10^{10} bp in *Lygeum spartum*.¹⁹ In most grasses, genes comprise less than 20% of the genome,²³ the rest being composed mainly of repetitive DNA. Gene number is thought to be relatively constant within groups of related species, and, consistent with this, most of the 3.6-fold difference in genome size between maize and sorghum was shown to be due to the accumulation of retrotransposons.²⁴

Such comparisons lead to the expectation that the rice genome should contain less repetitive DNA generally and fewer LTR retrotransposons in particular. Early solution hybridization experiments indicated that only half of the rice genome is composed of repetitive sequences.²⁵ Based on the screening of genomic libraries with a conserved retrotransposon probe (ⁱ_{met}tRNA primer), Hirochika and co-workers²⁶ estimated that the rice genome contains about

1000 retrotransposons in total, divided into 32 families. By hybridizing a rice BAC library with *rt* probes representative of different *rt* families, Wang and co-workers,²⁷ however, estimated that the genome contains only about 100 *copia*-like elements. A detailed survey of 910 kb of rice genome (0.2%) revealed two *copia*-like and 10 *gypsy*-like retrotransposons,²⁸ giving a total estimation of 1000 *copia* and 5000 *gypsy* elements in the genome, representing 2.6% and 7.3% of the total genome, respectively. They also detected five non-LTR retrotransposons, giving a total estimate of 2500 elements in the genome.

These estimates span a 100-fold range, leaving the issue of retrotransposon copy number in rice unresolved. Furthermore, although various retrotransposon families have been reported for rice,^{26,29–34} the total number of families present in the genome remains unclear. The current availability of an appreciable part of the sequence of the rice genome provides an excellent and timely opportunity to analyze the evolutionary dynamics of rice retrotransposons. In this study, we examine the sequence abundance and diversity of the rice *copia*-like, LTR-containing retrotransposons.

2. EXPERIMENTAL METHODS

2.1. Isolation of Rice Retrotransposon Sequences

We used a collection of amino acid sequences corresponding to reverse transcriptases of *copia*-like elements.^{5,6,8,35–37} These sequences were used in TBLASTN searches,³⁸ where translated nucleotide databases are queried by a polypeptide string, through the on-line facilities of the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>). The plant division of the Genbank database (Release 125.0, 15.08.2001), specifically all *O. sativa* PAC, YAC, and BAC accessions, which are the annotated long contiguous chromosomal regions, was queried. New reverse transcriptase (RT) sequences were extracted from the database with this approach. These sequences were again used as queries in additional database searches, carrying the process forward iteratively until no new RTs were obtained. The limits of the RT domain were defined according to the method of Xiong and Eickbush,² but only the central, most conserved “core” of this domain was used in multiple alignments, as has been used in previous analyses based on polymerase chain reaction (PCR) amplification.^{5,6} We did not exclude *rt* sequences with frameshifts and stop codons from our analyses. LTR sequence searches were conducted on-line with the BLAST2 program (<http://www.ncbi.nlm.nih.gov/gorf/bl2.html>).

For *gypsy*-like retroelements, the same method was followed as for the *copia*-like ones, with RIRE2 (accession AB030283.1, nt 3650–4180) used as the initial query sequence to seed the iterative search procedure. For LINE-like retrotransposons, the searches were seeded with the

RT-encoding domains of Li2 (Y13371) and Li4 (Y13370) elements, isolated from rice.³⁹ Integrated pararetroviruses were discovered as a by-product of the search for *gypsy*-like sequences and were distinguished from them by sequence comparisons.

2.2. Sequence Comparisons and Phylogenetic Trees

CLUSTALW of the EMBL European Bioinformatics Institute web page (<http://www.ebi.ac.uk/clustalw/>)⁴⁰ was used to generate multiple sequence alignments of the predicted RT polypeptides, which were edited manually to correct errors. The TREECON 1.3b program⁴¹ was used to generate phylogenetic trees of the predicted polypeptides based on the neighbor-joining method.⁴² Branch reliability in the phylogenetic predictions was tested by bootstrap analysis, based on 1000 repetitions. Homology searches of expressed sequence tags (ESTs) were made as described before.¹⁰

3. RESULTS

3.1. Identification and Frequency of *Copia*-Like Retrotransposons in the Rice Genome

The diversity of the *copia*-like retrotransposons in the genome of *O. sativa* was examined by a systematic search of all rice sequences available in Genbank, representing about 10% of the rice genome. The RT domains were used for query sequences. Because the core domain of RT is highly conserved and sequences of diverse origin have been aligned for phylogenetic analyses, the primary structure is a reliable tool for identifying retrotransposons. The predicted RTs identified in Genbank accessions were iteratively applied in further queries until no new *copia*-like coding regions were found. A total of 182 nonredundant, complete *rt* regions were identified. Of these, 81 corresponded to PCR products, one was a cDNA, and 100 were genomic sequences.

Assuming that the 10% of the genome surveyed is representative of the whole, the total number of *copia*-like retrotransposons in rice would be about 1000, comprising about 1.7% of the total genome. This is likely to be an underestimation of the total number of *copia*-like retroelements, however, because our count includes only elements with complete *rt* domains. In some species such as barley, solo LTRs can contribute as much DNA to the genome as the intact elements themselves.²⁰ In an attempt to estimate how many of these “RT-defective” elements are present in the genome, we searched databases with the BLAST program and the sequences of each rice retrotransposon as queries. By this method, the 1210 *copia*-like solo LTRs and 380 truncated retroelements represent about 0.6% of the genome, bringing the *copia*-like elements to 2.3% of

the nuclear DNA. This is very much more than some previous estimates^{26,27} but more similar to other, sequence-based estimates.²⁸ In our view and that of others,²⁸ the earlier underestimations were due to the use of nucleic acid hybridizations that probably did not detect some of the families present in the genome. Discrepancies in computer-based estimations could be explained by differences in the source and quantity of DNA sequences used.

3.2. Families and Phylogeny of *Copia*-Like Retrotransposons in the Rice Genome

We analyzed the phylogenetic relationships between the 100 rice genomic *rt* domains by constructing a neighbor-joining tree, displayed in Figure 1, on the predicted polypeptides, using the deduced amino acid sequences. Sequences sharing more than 20% amino acid similarity were grouped into classes. The sequences grouped into nine major classes, each containing from one to 31 elements. Elements in the same class in general also share similar (–)-strand (PBS) and (+)-strand (polypurine tract, PPT) primer binding sites. The elements in most of these classes could be subdivided into different families. We used LTR sequence similarity as the criterion to define the families for several reasons. The LTR is the most rapidly evolving region of retrotransposons^{43,44} and therefore gives good phylogenetic resolution. The LTR critically not only determines transcription patterns, which can differ between retrotransposon families,¹¹ but also provides the recognition sites for integration. By our criterion, two elements belong to the same family if their LTR sequences share more than 90% of DNA sequence homology in at least 100 bp.

We examined the flanking sequences of each of the *rt* domains to identify potential LTRs. The LTRs were delimited by the presence of sequences similar to the conserved PBS, expected to be next to the 5' LTR; by the PPT, expected to be adjacent to the 3' LTR¹; and by the direct repeat outside of the LTRs in the host DNA, which are generated by integration. In some cases either it was not possible to identify some of these features or one of the LTRs was truncated, missing, or outside the insert of the clone. Despite these limitations, LTRs were identified for most of the coding domains by comparisons with other members of the same family or related sequences.

We also studied the RIRE1 element in a bit more detail. RIRE1 is a *copia*-like retrotransposon present in high copy number in wild rice *Oryza australiensis* [31]. For the RIRE1 element (family 6C), we found three elements containing RT and LTRs, 25 solo LTRs, and one element with LTRs but a deletion in the RT domain. That gave us a total of 33 LTRs and four internal regions, and an estimation of 330 RIRE1 LTRs in the genome, not very different from the previous estimation of 180 RIRE1 LTRs.²⁹

In all of the cases in which two elements had the same type of LTR, they were located in the same branch of the

Figure 1. Neighbor-joining tree of the reverse transcriptase amino acid sequences of *copia*-like retrotransposons in the rice genome. Genbank accession numbers and the position in the sequence are indicated. To the right of the various lineages, the classes of elements are indicated by numbers, and the families are indicated by letters. Bootstraps were performed with 1000 repetitions; bootstrap values (as a percentage) are shown next to branches dividing classes and families of sequences.

RT-based tree. We therefore believe the RT-based classification to be robust enough to be used to divide the *copia*-like retrotransposons into classes, and we used LTRs only to define within-class families. The only exception were families 7B and 7C, which have highly similar RTs but different LTR sequences. A total of 39 families were identified, as shown in Figure 1. In general, as described in Table 1, families in the same class have similar total sizes, similar LTR lengths, and similar PBS and PPT sequences. For example,

all four families in Class 1 have LTRs longer than 1200 bp and total sizes around 9 kb, whereas the nine families in Class 5 have LTRs between 230 and 474 bp and internal domain lengths between 4.1 and 5.1 kb. The most abundant families are 7C and 7B, with 22 and 11 elements, respectively. For several families, we found only one element.

Some *copia*-like retrotransposons have previously been characterized in rice, and several PCR fragments corresponding to *copia*-like *rt* domains have been sequenced

Table 1. Occurrence of *copia*-Like retrotransposons in *Oryza sativa* genomic sequences.

Element	Size	LTR	Accession	Position	5' flank	3' flank	PBS	PPT
Class 1								
1A1	6850	1199/1221	AC027661.1	30253-39522	TCTCA TGCAAT	TGCAAT AGTTT	AGTGGTATCAGCCT	TCATCAAGGGGAGAT
1A2	6853	1190/1219	AC068654.2	143638-152889	TAGCC TTAAT	TTAAT CTTCTA	AGTGGTATCAGTCT	TCATCAAGGGGAGAT
1A3	8894	231/1197	AC074283.3	68152-78473	CGATATCCAG	CCTCTCATGT	AGTGGTATTAGCCA	TCATCAAGGGGAGAT
1A4	6778	1174/1167	AC078839.4	102270-110504	GCGC CGGAA	CGGAA TAGTG	AGTGGTATCAGCCT	TCATTGAGGGGAGAT
1A5	6820	1213/1212	AP002069.1	68612-77856	ATATGT AGAT	AGAT ATCCA	AGTGGTATCAGCTT	TCATTAAGGGGAGAT
1A6	6801	1214/1156	AP002971.2	14644-22261	CTCT ATTG	ATTG CATAT	AGTGGTATCAGCCT	TCATCAAGGGGAGAT
1A7	6781	1215/1220	AC021891.6	65191-74406	AAGTC ATTAA	ATTAA ACTTT	AGTGGTATCAGCCT	TCATCAAGGGGAGAT
1A8	6596	812/1145	AP003073.1	126193-132708	CGGAGGTTCC	ATTGGGCGTT	AGTGGTATCAGCCT	TCATCAAGGGGAGAT
1B1	5672	1618/1630	AP002538.2	44996-53915	ACCTT AGTT	AGTT CTCCT	ATTGGTATCAGCCC	CCATCAAGGGGAGAT
1C1	8074	1424/1429	AC084319.5	70748-85600	CACAA AAAA	AAAA CAGTA	ATTGGTATCGGCGA	CCAAAAGAGGGAGAT
1C2	8208	1432/134	AC091238.2	71552-71326	TTAGTCTATC	CCCCCCCCC	n.a.	n.a.
1D1	6209	1794/1677	AP000969.1	23381-33059	CCTTG AGCAC	AGCAC TTATA	ATTGGTATGAGCCT	CTAAAAAGGGGAGAT
1E1	8831	1580/1585	AC069324.3	132725-144720	TCTCTAGCCT	TTTCATCGGG	AGTGGTATCAGCTT	CCAAAAGGGGAGAT
1E2	8645	1198/1396	AP003205.2	49512-49734	GGGGA GTGAT	GTGAT CTCAG	n.a.	AAAAAAGGGGAGAT
1E3	8004	1669/n.a.	AP003199.3	29060-36796	AAAACGCTGG	TTCATCTTTT	AGTGGTATCAGCTT	n.a.
Class 2								
2A1	5069	226/226	AC087181.8	83222-88742	CGTGCGATCA	GGATGGAACC	TGGTATCAGAGCTTG	ATCAGAATCCATCCC
2B1	4398	206/172	AC068654.2	23236-28011	GGGAG GGCAC	GGCAC CACCG	TGGTATCAGGCTAT	ATGATTGAGGGGGGC
2C1	4424	226/226	AP003289.3	122532-127407	GAGTG AGCAA	AGCAA GACAA	TGGTATCAGAGCTTT	TATGATTGAGGAGGG
2D1	4356	209/209	AP002843.2	139482-144255	GTGTT AGCCT	AGCCT CCTTC	TGGTATCAGAGCATA	TGTGATTGAGGAGGA
20	4426	177/177	AC087544.2	66943-71722	ATGAT ATCCG	ATCCG CATGG	TGGTATCAGAGCCTC	TGTGATTGAGGAGGAG
2F1	4347	180/183	AP002093.1	28354-33063	TAGTT TGCCA	TGCCA AAACC	TGGTATCAGAGCTCC	TAAGATTGAGGGGAG
2F2	4387	191/191	AC074283.3	19794-24562	AGGT GGTTC	GGTTC ATACA	TGGTATCAGAGCTTA	TGCAATTGAGGGGAG
2G1	4379	218/218	AC0091123.	7841-8181	AACGG CGTCC	CGTCC GATTT	TGGTATCAGAGCAAA	TGAGATTGAGGGGAG
2H1	3579	73/-	AP003046.2	100588-104239	TATGTATGTA	TGTAGCGCCC	TGGTATCAGAGCTTC	AGAGTAGGAATGAATG
2I1	4410	221/221	AC016781.6	26002-30853	GTGAG CTCCG	CTCCG CGTTG	TGGTATCAGAGACCC	TAAGATTGAGGGGAG
2I2	5437	220/220	AC016781.6	72407-78283	CTGCA CACGT	CACGT GTCCC	TGGTATCAGAGCTGT	TAAGATTGAGGGGAG
2J1	4330	184/205	AC069300.7	73013-77731	GTCAA GTCCC	GTCCC TCCTG	TGGTATCAGAGCTCC	TCAGATTGAGGGGAG
Class 3								
3A1	3928	138/138	AC087545.2	80982-85185	TTCTT CTGCT	CTGCT TTCTT	TGGTATCAGAGCAATG	TGAGTTTGAAGGGGG
Class 4								
4A1	3116	135/135	AP002872.2	90440-90179	CAGCT AGACA	AGACA TATTT	n.a.	TTAGACTGAGGGGAG
4B1	4672	199/235	AF161269.1	108089-113194	TAAAC CAATT	CAATT GACTT	TGGTATCAGAGCTAG	TGCGACTGCGGCGGG
4B2	6042	201/203	AC027661.2	138699-145144	GATCA ACTAT	ACTAT ATATT	TGGTATCAGAGTTCT	TACGACTGCGGCGGG
4C1	2105	221/221	AC079037.3	33655-36201	GAAG CGCCC	CGCCC AGTGC	TGGTATCAGTACCCT	TGCGACTGCGGGGGG
4C2	4276	239/221	AC073166.7	104290-109025	TGTT CTTCT	CTTCT ATTGT	TGGTATCAGTACCCT	TGCGACTGCGGGGGG
4C3	4277	221/221	AC025098.4	12386-17104	TCTT ATATG	ATATG TTTAT	TGGTATCAGTACCCT	TGCGACTGCGGGGGG
Class 5								
5A1	4200	361/361	AC037197.2	66391-71188	CCGT GACCT	GACCT CGCG	ACTGGTATCAGCGC	AAATTAAGGGGAGTT
5B1	4334	472/476	AL512543.1	49668-54949	CAAAG GGCAC	GGCAC ACCTA	AGTGGTATCAGACAC	TAATTAAGGGGAGAT
5C1	5138	477/477	AC021891.6	56044-62135	TAGTG ATCAG	ATCAG CCCGT	AGTGGTATCAGCTT	GAATTAAGGGGAGTT
5D1	4325	439/439	AP001366.1	57570-62772	CGTG CAGGT	CAGGT GGTGT	ATTGGTATCAGACAG	AGATTAGGGGGAAT
5E1	4185	415/415	AC080019.7	55421-59541	CCTG AGGAC	AGGAC GTGAT	TTTGGTATCAGCTT	AGATTAGGGGGAAT

Continued

Table 1. Continued

5F1	4336	436/436	AP003435.1	40436-45643	CCTCC AGGTC	AGGTC CGTAT	AGTGGTATCAGGCCT	GCTTAGGGGGGATT
5G1	4339	250/268	AL442110.1	38597-43453	CACAA CACCC	CACCC ATAGG	ATTGGTATCAGGCTC	GGATTAGGGGAGAAT
5G2	4414	268/268	AL442110.1	95120-100070	GGCTC ATATT	ATATT TCTCA	ATTGGTATCAGGCTC	GGATTAGGGGAGAAT
5H1	4937	229/286	AC079736.1	85122-90573	TAGCT TATAA	TATAA AAGTA	ATTGGTATCAGCAA	AGACTTAGAAGAGAA
5I1	4965	350/350	AB026295.2	160208-165872	AGGTC GTATC	GTATC ACTTT	TCTGGTATCAGCAA	AGACTTAGGGGAGAA
5I2	3252	n.a./131	AC074354.8	130053-133141	n.a.	CTCGAGTCGA	n.a.	n.a.
5I3	4978	362/362	AP002836.1	135483-141185	AGGTC CACAT	CACAT GACTC	TCTGGTATCAGGCCA	AGACTTAGGGGAGAA
5I4	4974	363/363	AC084763.4	95584-101284	ACGTT GCAAT	GCAAT TACAC	TCTGGTATCAGGCCA	AGACTTAGGGGAGAA
5I5	4947	396/395	AP000836.1	56024-61762	TTATC AAATG	AAATG TACTA	TCTGGTATTAGGCCA	AGACTTAGGGGAGAA
Class 6								
6A1	6087	402/402	AP003338.2	90661-97551	AAGAA TCCAC	TCCAC CAGAT	ATGGTATCAGAGCACC	CGATCAAGGGGAGAA
6A2	6105	401/402	AP002845.1	42644-49551	CTGTA TGCTT	TGCTT AACAT	ATGGTATCAGAGCACC	CGATCAAGGGGAGAA
6A3	6117	405/403	AC091238.2	117675-124599	GGGCG CGCAC	CGCAC CAGAT	ATGGTATCAGAGCACC	CGATCAAGGGGAGAA
6B1	5417	483/482	AF161269.1	87038-87390	AAGTT ACTCA	ACTCA TGGTT	ATGGTATCAGAGCAAC	AGATCAAGGGGAGAA
6B2	5471	492/492	AP003607.3	7372-13826	CTCAT CCGAC	CCGAC CTCCT	TTGGTATCAGAGCAAC	AGATCAAGGGGAGAA
6B3	5453	485/485	AP003631.2	16304-22726	AAGAA GGGCA	GGGCA ATGAT	ATGGTATCAGAGCAAC	AGATCAAGGGGAGAA
6C1	6102	1399/1341	AP003257.3	2488-11329	GGGGG GTAAT	GTAAT AACCA	n.a.	AGTGCAATGGGAGAC
6C2	5212	1017/1079	AC084831.6	66126-73433	AGGATC GTCG	GTCG TAGGC	GGTGCTAGCAATCTG	AGTGCAAGTGGAGAC
6C3	6209	216/1368	AP003203.2	3488-3911	ACGAGCTCAC	TGCTATCGTA	GTGGTATCAGAGCCAC	AGTGCAAGTGGAGAC
Class 7								
7A1	4458	858/858	AP003537.2	115701-121875	GGAGG GATAT	GATAT AACCA	TTCCAGAAACCTTAG	TGAAATTGGTGGGAT
7B1	4468	986/983	AP003213.3	139586-146023	TGGTC GCGAT	GCGAT TATTA	ATCCAAAAACCTGATA	GAAACTTGGTGGGAT
7B2	4463	961/990	AP002486.1	83267-88211	ATGTC TAAATC	TAAATC TTTAC	ATCCAAAAACCTGATA	GAAATTTGGTGGGAT
7B3	4470	1503/1001	AP002843.2	43107-52941	TGGGA GTTCG	GTTCG TCGTC	ATCCAAAAACCTGATA	GAAATTTGGTGGGAT
7B4	4468	978/963	AC025296.1	158281-164690	TTAGCT ATA	ATA TACTGGT	ATCCAAAAACCTGATA	GAAATTTGGTGGGAT
7B5	4468	976/998	AP003256.1	153934-160376	CCGCCGCCAT	GTTGTCTATA	ATCCAAAAACCTGATA	GAAATTTGGTGGGAT
7B6	4626	395/1066	AP003257.3	76175-83456	TGGAG TAAAC	TAAAC ATGAT	n.a.	GAAATTTGGTAGGGAT
7B7	2330	118/119	AC051624.6	25238-29317	TTGCTGCTTC	AGCTATTAGA	n.a.	n.a.
7C1	4469	965/965	AC023240.8	100409-106808	GGGG AGTCC	AGTCC GGCAA	ATCCAAAAACCTGATA	AAAATTTGGTGGGAT
7C2	4461	965/965	AP000969.1	113971-120362	ATGTC CATAT	CATAT GGGCTC	ATCCAAAAACCTGATA	AAAATTTGGTGGGAT
7C3	4471	1148/1003	AC083945.3	37356-43978	CCGGA CATAC	CATAC AGCAT	GTCCAAAAACCTGATA	GAAATTTGGTAGGGAT
7D1	4504	968/979	AP002883.1	52500-58741	CGCTC TAATT	TAATT TAATT	ATCCAAAAACCTTATA	AGATTTGGTGGGAT
7D2	4501	975/985	AP003144.1	85756-92221	ATGAA CTGGC	CTGGC AGTGA	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D3	4501	980/995	AP003021.2	89259-95734	AGGCA GTGGG	GTGGG CATAC	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D4	4501	970/968	AP003202.2	44254-50692	TGGCT CTGAC	CTGAC ATCGC	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D5	4500	968/978	AC073405.2	72924-79369	TGAA TAAT	TAAT TAATAAC	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D6	4501	913/958	AP002872.2	58702-65073	AGGTA GAACA	GAACA AACCA	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D7	4501	900/926	AP003118.2	23292-29618	GAGAG GAACT	GAACT AATAT	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D8	4501	869/973	AP000836.1	10914-17390	AGGCT GCAAT	GCAAT TAGTT	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D9	4501	963/978	AP003143.2	124008-130449	ACTAA TATGC	TATGC TTTAT	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D10	4501	938/977	AP003504.1	61735-68150	CAGGT GTGAG	GTGAG CATCT	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D11	4501	941/980	AC087546.1	95510-101931	GCCTG CGATT	CGATT TTCTG	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D12	4499	780/964	AC087546.1	28857-33217	GGTGGGGGAT	GTCCTCCCCC	ATCCAAAAACCTTATT	AGATTTGGAGGGGAT
7D13	4498	861/969	AC087546.1	160052-162204	TAGCA CAATC	CAATC TACCA	ATCCAAAAACCTTATT	AGATTTGGTGGGAT
7D14	4501	971/983	AP002914.2	136611-143065	CTGAA GAAG	GAAG AGGAA	ATCCAAAAACCTTATC	AGATTTGGTGGGAT
7D15	4501	953/968	AC087192.1	88449-94870	CACAA GAAT	GAAT TGCCC	ATCCAAAAACCTTATT	AGATTTGGTGGGAT

Continued

Table 1. Continued

7D16	3866	958/962	AP003104.2	23131-28916	CAATACTTTA	CTTTAACGGA	ATCCAAAAACCTTATT	AGATTGGTGGGGAT	
7D17	4477	915/916	AC087797.5	13217-19524	CTAATGACTA	GACTATTAT	ATCCAAAAACCTTATT	AGATTGGTGGGGAT	
7D18	2909	710/950	AF172282.1	129515-134083	TACATATATA	ATATTGCT	n.a.	AGATTGGTGGGGAT	
7D19	1923	n.a./953	AL512543.1	81114-1119	n.a.	ACTCC	CCAGG	n.a.	AAATTGGTGGGGAT
7D20	4501	957/978	AC087852.9	50420-56855	ATGCTTTAAC	TTAAC	TGTGA	ATCCAAAAACCTTATT	AGATTGGTGGGGAT
Class 8									
8A1	4082	319/319	AC069324.3	8813-10028	GTGCTGGG	GGGTGGAG	AGTGGTATCAGCTT	GTCTCCAGGTGAGTT	
8A2	4108	249/46	AC079853.2	126402-130804	CGGTCTCC	CCCGTAG	AGTGGTATCAGCTT	GTCTCAAGGTGAGTT	
8A3	4109	316/329	AP003263.2	179142-183895	GGCGCTTACC	CTACC	TCCAC	AGTGGTATTAGCTT	GTCTCAAGGTGAGTT
8A4	4108	259/297	AP003683.2	49289-53952	GGGCTTAC	TCGAGGAGGC	AGTGGTATCAGCTT	GTCTCAAGGTGAGTT	
8B1	4546	262/262	AP002747.2	39882-44951	GGGTGCTTC	CTTCCT	AGTGGTATCAGCAT	GTCTCAAGGTGAGAT	
8B2	4567	247/259	AP003311.2	168526-173598	ACAGTGGG	GGGATAGA	AGTGGTATCATGCAT	GTCTCAAGGTGAGAT	
8B3	4488	263/263	AC090056.2	33496-38509	CTGGTCCACG	CCACGAACGC	AGTGGTATCAGCAT	GTCTCAAGGTGAGAT	
Class 9									
9A1	7036	296/295	AP002871.2	94011-101637	GTACTATGCA	TATACTACTT	CGTTATTAGCAGAGG	TTCGCCCAAGGGGAG	
9A2	6970	295/295	AC084404.8	105862-113421	GATTCTGCT	CTGCTGTCCG	CGTTATCAGCAGAGG	TTCACCCCAAGGGGAG	
9B1	7191	275/257	AF172282.1	9612-17334	TCTTCTTTA	GTAAAGTCT	CGTTATCAGCACTTC	TTCGATCAAGGGGAG	
9B2	5087	n.a./252	AP002866.1	161415-166753	n.a.	TTTTT	TACAG	n.a.	TTCGATCAAGGGGAG
9B3	3477	n.a./261	AP002969.1	240-3977	n.a.	GATCA	GACCA	n.a.	TTCGATCAAGGGGAG

^aSize for left/right LTR.
^bShading indicates direct repeat formed on integration.
^ch.a., data not available.

from rice.^{30,45,46} A new RT sequence alignment was prepared, including a representative of each of the 39 families as well as the other rice *rt* sequences that are available in Genbank. We also included, in this alignment, some RT sequences from known elements of species other than *O. sativa*. The alignment was used to produce a phylogenetic tree, shown in Figure 2. Many of the families discovered by our analysis of the *O. sativa* sequence are novel, having no representatives in Genbank. Of these, families 9A and 9B are fully distinct from all other RT clades. Only one deeply branching clade in the tree shown in Figure 2, the one including Tst1 (potato), PDR1 (pea), and Tos10 (rice), has both a rice member and no family member from our analysis. In total, five to 10 additional families are present among the cloned PCR fragments in the database but not in the genomic sequences we have scanned. This suggests that the total number of families in rice genome is probably more than 50.

Conversely, all of the previously characterized *Oryza* retrotransposons could be assigned to one of the families we found: RIRE1 belongs to family 6C, Tos17 to 3A, and Retrofit probably to a new family in class 2. These results were verified by comparisons at the DNA level. Some of the PCR fragments can also be assigned to some of the families, or at least to classes. For example, Tos6 can be placed in 6A, Tos15 in 7A, and Tos14 in 7B or 7C. Some of the RT classes are not represented by the cloned PCR

fragments in the database (classes 1, 5, and 9). This could reflect a sampling problem in the PCR methods used to produce the *rt* sequences, but the classes not represented in the database are not necessarily the least abundant. That suggests that the PCR protocols used for *copia*-like *rts* cannot amplify certain classes of *rt*, producing an underestimation of the sequence variability of *copia*-like elements.

Regarding retrotransposons from other plants, similarities with rice sequences were not high but were nevertheless sufficient to associate the elements with one or more rice classes. Opie2 (maize), Endovir1 (*Arabidopsis thaliana*), and SIRE-1 (soybean) are related to class 1. SIRE-1 and Endovir1 are retrovirus-like retrotransposons, meaning that they contain a region upstream of the *pol* gene that is similar to an *env* gene. The presence of an envelope domain expands the internal domain, and, interestingly, it is class 1 elements in rice that are the ones with longer internal domains. Hopscotch (maize) is associated with class 2, Stonor (maize) with class 6, and BARE-1 (barley) with 6C. Finally, the retrotransposons Tto1 (tobacco), Tnt1 (tobacco), and Ta1 (*A. thaliana*) are related to class 8.

3.3. Expression of Copia-Like Retrotransposons in Rice

To search globally for expression of the retroelement families identified by their *rt* sequences, all of the elements

Figure 2. Neighbor-joining tree of the rice and other plant *copia*-like reverse transcriptase amino acid sequences. Bootstraps were performed with 1000 repetitions; branches with bootstrap values under 30% are shown as unresolved. Representatives of the rice families from Figure 1 are shown in bold. The PCR clones from the database are Ros,⁴⁵ Rrt,⁴⁶ Tos,³⁰ RICTOSRT1 (accession D12825), RICCOPIA (M94492), OSCOPIA (M94492), TOSRT4 (D12826.1), Hua1A-2 (AF349737), Hs3-2 (AF349740), Hs3-3 (AF349741), Nongken58s-1 (AF349742), Nongken58s-2 (AF349743), Nongken58s-3 (AF349744), Yahual-1 (AF349745), Yahual-3 (AF349747), Zhenhsan97A-1 (AF349748), Zhenhsan97A-3 (AF349750), and Zhenhsan97A-2 (AF349749). The characterized retrotransposons are Tst1 (X52287), PDR1 (AJ243356), Opie2 (U68408), Endovir1 (AY016208), SIRE-1 (AF053008), Evelknievel (AF039376), Hopscotch (U12626), Retrofit (U72726), Tos17 (D88394), Tpv2 (AJ005762), Stonor (AF082133), RIRE1 (D85598), *BARE*-1 (Z17327), *Osrl* (AB046118), *Tto1* (D12827), *Tal* (X13291), and *Tnt1* (X13777).

Table 2. Rice *copia*-Like retrotransposon families and their matching grass ESTs.

Class	Family	ESTs										
		Oryza ^a						Sorghum	Zea	Triticum	Hordeum	Secale
		Total	V	F	G	S	T					
1	A	5	1	—	1	—	3	—	—	—	2	—
	B	2	—	—	1	—	1	—	—	—	—	—
	C	—	—	—	—	—	—	—	1	—	—	—
	D	1	1	—	—	—	—	1	1	—	—	—
	E	—	—	—	—	—	—	—	5	—	—	—
2	A	5	3	1	—	—	1	—	—	—	—	—
	B	—	—	—	—	—	—	—	—	—	—	—
	C	—	—	—	—	—	—	—	—	—	—	—
	D	—	—	—	—	—	—	—	—	—	—	—
	E	2	2	—	—	—	—	—	—	2	1	—
	F	—	—	—	—	—	—	—	—	—	1	—
	G	—	—	—	—	—	—	—	—	—	—	—
	H	—	—	—	—	—	—	—	—	—	—	—
	I	3	1	1	—	1	—	—	1	—	—	—
	J	—	—	—	—	—	—	—	—	—	1	—
3	A	2	—	—	—	—	2	—	—	1	—	—
4	A	—	—	—	—	—	—	2	—	—	—	—
	B	3	1	—	1	—	1	—	—	—	3	—
	C	—	—	—	—	—	—	—	1	—	—	—
5	A	—	—	—	—	—	—	—	—	—	—	—
	B	2	—	2	—	—	—	—	—	—	—	—
	C	1	—	1	—	—	—	—	—	—	—	—
	D	1	—	—	—	1	—	—	—	—	—	—
	E	—	—	—	—	—	—	1	—	—	—	—
	F	1	—	1	—	—	—	—	—	—	—	—
	G	—	—	—	—	—	—	—	—	—	—	—
	H	2	—	2	—	—	—	6	1	—	—	—
	I	5	5	—	—	—	—	3	1	1	3	—
6	I	—	—	—	—	—	—	—	—	—	—	—
	B	9	1	4	1	—	3	1	—	1	—	—
	C	9	—	2	2	—	5	—	—	—	7	—
7	A	—	—	—	—	—	—	—	—	—	—	—
	B	6	—	2	—	3	1	1	1	1	—	—
	C	2	—	1	—	—	1	1	—	—	—	—
	D	—	—	—	—	—	—	—	1	—	4	1
8	A	1	1	—	—	—	—	1	—	—	—	1
	B	2	1	1	—	—	—	—	—	—	—	—
9	A	—	—	—	—	—	—	—	—	—	—	—
	B	1	—	—	1	—	—	—	—	—	1	—

^aV, vegetative tissues; F, floral tissues; G, grain and related tissues; S, stress-induced tissues; T, tissue culture.

identified were used as query sequences against the EST database. The matches were analyzed and are displayed in Table 2. Only ESTs from grasses were considered. A total of 65 rice ESTs were identified with a high probability of match, in addition to 23 from barley, 17 from sorghum, 13 from maize, six from wheat, and two from rye. The number of rice ESTs similar to each of the families was not correlated with their genomic copy numbers. For example, 6B and 6C are the most represented in the EST databases, although we detected only three copies of each, whereas the most abundant in the genomic sequences, family 7D,

does not have any rice-related EST. The 2A family, which has only one element in the genomic sequences, has six related ESTs.

These results suggest that possible genomic contamination in the EST databases has not been sufficient to make the distribution of elements in them mirror that in the genome. Rather, the EST frequencies are indicative of transcriptional activity of the families of *copia*-like retrotransposons in rice. These results beg the question of why highly prevalent elements, whose abundance ultimately results from expression, should be rare among

the ESTs. This discordance may be explained in part by the fact that the tissues used for mRNA isolation were not the ones in which the abundant families were most highly expressed. Conversely, it is well known that rare retrotransposons such as Tos17 can be highly expressed under particular conditions.³⁰ Only two ESTs similar to Tos17 (family 3A) were found; both are derived from tissue culture cDNA libraries. Furthermore, the finding of ESTs from other grasses matching rice retroelement RT classes or families indicates that some of these are both present and expressed in all grasses and is consistent with earlier, broader analyses.¹⁰

3.4. Identification and Frequency of Other Classes of Retrotransposons in the Rice Genome

Gypsy-like elements are the other major class of LTR retrotransposons, and some perspective on their occurrence in the rice genome is useful for comparison with the *Copia*-like elements. Using a method similar to that for *Copia*-like elements, we were able to estimate the number of *gypsy*-like and LINE-like retroelements based on their *rt* domains. We found a total of 222 *gypsy*-like elements, in the fraction of the genome surveyed, indicating about 2220 *gypsy*-like elements in the genome as a whole. This is twice the number of *Copia*-like ones, compared with previous estimates of a three- to fivefold greater abundance.^{28,47} Two thousand copies, at an average size of 7.7 kb, comprise about 3.6% of the genome, although this may be an underestimation because we did not consider solo LTRs and truncated elements. The *Copia*-like retrotransposons and their deletion derivatives, together with the *gypsy*-like elements, amount to more than 4800 copies or about 5.9% of the genome, at an average spacing of one element every 84 kb.

Our search also produced 66 LINE-like (non-LTR) retrotransposons, indicating about 660 LINEs in the genome, and five integrated pararetroviruses. LINE elements have previously been reported as features of the *Oryza* genome.^{39,48} Pararetroviruses, lacking their own integrase, have been found in the genomes of both banana⁴⁹ and tobacco.⁵⁰ Our identification of a few sequences in the rice genome shows that the presence of pararetrovirus sequences in the genome is not unique to tobacco and banana.

4. CONCLUSIONS

That genome sequencing projects are qualitatively changing our understanding of the mechanisms of evolution of retrotransposons has been demonstrated for *Saccharomyces cerevisiae*,^{51,52} *Caenorhabditis elegans*,^{53,54} and *A. thaliana*.^{55,56} The increasing genomic sequence information for rice now provides an excellent opportunity to understand the evolution and diversity of transposable elements in grasses. Grasses are interesting subjects because

of the wide range in the size of their genomes and the prevalence of their retrotransposons. In this work, we concentrated on the analysis of *Copia*-class elements, which includes Ty1 of yeast.

Our analysis is based on about 10% of the rice genome. A limitation of the data, true also for the “finished” *A. thaliana* genome, is that centromeres, which tend to be rich in repetitive DNA (in some cases including transposable elements),^{33,57–60} are relatively underrepresented in the database. While the final word on the rice genome awaits complete annotation of the entire genome, including centromeres, the available data may be sufficient to give a good general view of the genome, excluding the centromeres and telomeres.

Our analysis of the data yields three main conclusions. First, although the number of retrotransposons in rice is considerable, it is still very low compared with that in other members of the grass family and in other plants generally. The contribution of retrotransposons to the genome share in rice is similar to that in *A. thaliana*. Although copy number is relatively low, variability is not, with at least 39 different families grouped into at least nine classes. A high degree of heterogeneity in *Copia*-like retrotransposons appears, from other available data,^{5,6,8,30,35,36,45,61–63} to be typical for plants, including *A. thaliana*. Finally, our and others' data⁵⁶ indicate that related classes of *Copia*-like retrotransposons can be found in several plant species, within the genomes of both monocots and dicots. This indicates that *Copia*-like elements already comprised distinct families of elements before the divergence of the monocots and dicots, placing their origin at more than 200 million years ago, and that some of these ancient families have persisted until the present day.

Despite the persistence of diverse families of *Copia*-like elements in the rice genome, their copy number is exceptionally low compared with that in other cereals. For example, RIRE1 belongs to the same family (6C) as *BARE*-1 and *Wis*2 from barley and wheat.^{31,64} This family is highly abundant in both species^{20,65} but is represented by only about 30 copies in rice (about 90 if we consider all class 6 elements together). In the wild rice *Oryza australiensis*, with a genome size double that of rice, the number of RIRE1 copies is in the thousands.²⁹ The variation in the number of RIRE1 copies alone can explain about one-third of the difference in size between the genomes of *O. sativa* and *O. australiensis*. A comparison of a contiguous region of the barley and rice genomes⁶⁶ indicates that the larger genome of barley can be attributed at least in part to the integration of retrotransposons into currently retrotransposon-poor regions of the rice genome. The key issue emerging from these comparisons is whether the genome of cultivated rice never expanded through gain of retrotransposon copies, or whether, instead, it expanded but then lost the copies integrated earlier.

The steady-state copy number of a retrotransposon depends on the balance between the rates of gain through retrotransposition and loss through mutation and through various forms of deletion. Retrotransposition requires transcription, reverse transcription, and, ultimately, integration. In general, retrotransposons in rice seem to be poorly transcribed compared with elements in other grasses and, by the lack of copy number variation between cultivars,⁶⁷ fairly quiescent integrationally. Only 0.98‰ (0.98 per thousand) of all of the rice ESTs in the databases matched retrotransposons, compared with the 3.12‰ for barley, 2.49‰ for maize, and 1.26‰ for wheat.¹⁰ All rice retrotransposons examined by Hirochika and co-workers³⁰ were inactive under normal growth conditions, and only three were activated by tissue culture. One of these, Tos17 (family 3C) is rare, despite its potential activity.³⁰ Family 6C (RIRE1) is the second most common among the ESTs after 6B, suggesting that it, at least, should be transcriptionally active compared with other families, but the family's genomic prevalence is not commensurately great.

Not only may rice retrotransposons not be particularly actively transcribed; they also may be lost through ectopic or unequal recombination. Intrachromosomal recombination between retrotransposon LTRs can remove the internal domain of an element, producing a solo LTR. Recombination between LTRs not of the same copy can remove, as well, intervening DNA and generate a solo LTR.^{4,21} This process appears to be common for retrotransposons in barley^{20,68} and to play a role in modulating copy number in populations of wild barley, *H. spontaneum*.¹⁶ The maize genome, however, appears to contain relatively few solo LTRs.¹⁸

We are able to derive some conclusions from our data regarding recombinational loss of retrotransposons in rice. For the RIRE1 element (family 6C), we found three elements containing RT and LTRs, 25 solo LTRs, and one element with LTRs but a deletion in the RT domain. This amounts to a total of 33 LTRs and four internal regions, or 330 RIRE1 LTRs in the genome as a whole, comparable to the previous estimate of 180 RIRE1 LTRs.²⁹ Hence, the rice genome has an LTR:internal domain ratio for RIRE1 of 8.25:1 instead of the 2:1 expected if there were no solo LTRs. Considering all *copia*-like retroelements in the genome, our data yield an LTR:internal domain ratio of 3.6:1 and suggest that more *copia*-like retrotransposons than are currently in the genome may have been lost by recombination. Furthermore, although for the *gypsy*-like RIRE2 we find relatively few solo LTRs and a LTR:internal domain ratio of 2.3, the centromere of chromosome 5 in rice contains many repeats similar to solo LTRs of *gypsy*-like RIRE3, RIRE7, and RIRE8.⁵⁹ These calculations and data together indicate that intrachromosomal, ectopic recombination between LTRs is driving much of the loss of retroelements from the rice genome.

If recombinational loss of retrotransposons is a factor shaping the rice genome, it may explain the high degree of heterogeneity we find among *copia*-like elements. It is thought that retrotransposon heterogeneity in plants is a consequence of their prevalence.⁶⁹ For rice as well as for *A. thaliana*,³⁵ the currently high diversity but low copy number therefore may be a remnant of an earlier, larger genome and population of retrotransposons. The long-term evolutionary dynamics of mobile elements remain a matter of debate,^{4,70} and the factors controlling gain and loss of retrotransposons need to be clarified. Detailed analysis of whole genomes, such as that of *O. sativa*, in comparison with genomic regions of related species,⁶⁶ is expected to shed some light on genome dynamics and the processes underlying it.

Acknowledgments: This work was carried out with support from the Academy of Finland under projects 44404 and 47581.

References and Notes

1. A. Kumar and J. Bennetzen, Plant retrotransposons, *Annu. Rev. Genet.* **33**, 479 (1999).
2. Y. Xiong and T. H. Eickbush, Origin and evolution of retroelements based upon their reverse transcriptase sequences, *EMBO J.* **9**, 3353 (1990).
3. T. Schmidt, LINEs, SINEs and repetitive DNA: non-LTR retrotransposons in plant genomes, *Plant Mol. Biol.* **40**, 903 (1999).
4. J. Bennetzen, Transposable element contributions to plant gene and genome evolution, *Plant Mol. Biol.* **42**, 251 (2000).
5. A. J. Flavell, D. B. Smith, and A. Kumar, Extreme heterogeneity of Ty1-*copia* group retrotransposons in plants, *Mol. Gen. Genet.* **231**, 233 (1992).
6. H. Hirochika and R. Hirochika, Ty1-*copia* group retrotransposons as ubiquitous components of plant genomes, *Jpn. J. Genet.* **68**, 35 (1993).
7. A. Suoniemi, J. Tanskanen, and A. H. Schulman, *Gypsy*-like retrotransposons are widespread in the plant kingdom, *Plant J.* **13**, 699 (1998).
8. D. F. Voytas, M. P. Cummings, A. K. Konieczny, F. M. Ausubel, and S. R. Rodermel, *Copia*-like retrotransposons are ubiquitous among plants, *Proc. Natl. Acad. Sci. USA* **89**, 7124 (1992).
9. A. Suoniemi, A. Narvanto, and A. H. Schulman, The BARE-1 retrotransposon is transcribed in barley from an LTR promoter active in transient assays, *Plant Mol. Biol.* **31**, 295 (1996).
10. C. M. Vicient, M. Jääskeläinen, R. Kalendar, and A. H. Schulman, Active retrotransposons are a common feature of grass genomes, *Plant Physiol.* **125**, 1283 (2001).
11. T. Beguiristain, M. A. Grandbastien, P. Puigdomènech, and J. M. Casacuberta, Three Tnt1 subfamilies show different stress-associated patterns of expression in tobacco. Consequences for retrotransposon control and evolution in plants, *Plant Physiol.* **127**, 212 (2001).
12. S. R. Wessler, Turned on by stress. Plant retrotransposons, *Curr. Biol.* **6**, 959 (1996).
13. M.-A. Grandbastien, Activation of retrotransposons under stress conditions, *Trends Plant Sci.* **3**, 181 (1998).
14. H. Okamoto and H. Hirochika, Efficient insertion mutagenesis of *Arabidopsis* by tissue culture-induced activation of the tobacco retrotransposon Tto1, *Plant J.* **23**, 291 (2000).
15. M. A. Grandbastien, C. Audeon, J. M. Casacuberta, P. Grappin, H. Lucas, C. Moreau, and S. Pouteau, Functional analysis of the tobacco Tnt1 retrotransposon, *Genetica* **93**, 181 (1994).

16. R. Kalendar, J. Tanskanen, S. Immonen, E. Nevo, and A. H. Schulman, Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence, *Proc. Natl. Acad. Sci. USA* 97, 6603 (2000).
17. M. Jääskeläinen, A.-H. Mykkänen, T. Arna, C. Vicient, A. Suoniemi, R. Kalendar, H. Savilahti, and A. H. Schulman, Retrotransposon BARE-1: expression of encoded proteins and formation of virus-like particles in barley cells, *Plant J.* 20, 413 (1999).
18. P. SanMiguel, B. S. Gaut, A. Tikhonov, Y. Nakajima, and J. L. Bennetzen, The paleontology of intergene retrotransposons in maize, *Nat. Genet.* 20, 43 (1998).
19. M. D. Bennett and I. J. Leitch, Plant DNA C-values Database (<http://www.rbgkew.org.uk/cval/homepag e.html>) (2001).
20. C. M. Vicient, A. Suoniemi, K. Ananthawat-Jónsson, J. Tanskanen, A. Beharav, E. Nevo, and A. H. Schulman, Retrotransposon BARE-1 and its role in genome evolution in the genus, *Hordeum*, *Plant Cell* 11, 1769 (1999).
21. D. Petrov, Slow but steady: reduction of genome size through biased mutation, *Plant Cell* 9, 1900 (1997).
22. K. Arumuganathan and E. D. Earle, Nuclear DNA content of some important plant species, *Plant Mol. Biol. Rep.* 9, 208 (1991).
23. A. Barakat, N. Carels, and G. Bernardi, The distribution of genes in the genomes of Gramineae, *Proc. Natl. Acad. Sci. USA* 94, 6857 (1997).
24. A. P. Tikhonov, P. J. SanMiguel, Y. Nakajima, N. M. Gorenstein, J. L. Bennetzen, and Z. Avramova, Colinearity and its exceptions in orthologous adh regions of maize and sorghum, *Proc. Natl. Acad. Sci. USA* 96, 7409 (1999).
25. V. G. Deshpande and P. K. Ranjekar, Repetitive DNA in three Gramineae species with low DNA content, *Hoppe Seylers Z. Physiol. Chem.* 361, 1223 (1980).
26. H. Hirochika, A. Fukuchi, and F. Kikuchi, Retrotransposon families in rice, *Mol. Gen. Genet.* 233, 209 (1992).
27. S. Wang, N. Liu, K. Peng, and Q. Zhang, 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. USA* 96, 6824 (1999).
28. K. Turcotte, S. Srinivasan, and T. Bureau, Survey of transposable elements from rice genomic sequences, *Plant J.* 25, 169 (2001).
29. R. Nakajima, K. Noma, H. Ohtsubo, and E. Ohtsubo, Identification and characterization of two tandem repeat sequences (TrsB and TrsC) and a retrotransposon (RIRE1) as genome-general sequences in rice, *Genes Genet. Syst.* 71, 373 (1996).
30. H. Hirochika, K. Sugimoto, Y. Otsubo, H. Tsugawa, and M. Kanda, Retrotransposons of rice involved in mutations induced by tissue culture, *Proc. Natl. Acad. Sci. USA* 93, 7783 (1996).
31. K. Noma, R. Nakajima, H. Ohtsubo, and E. Ohtsubo, RIRE1, a retrotransposon from wild rice *Oryza australiensis*, *Genes Genet. Syst.* 72, 131 (1997).
32. H. Ohtsubo, N. Kumekawa, and E. Ohtsubo, RIRE2, a novel gypsy-like retrotransposon from rice, *Genes Genet. Syst.* 74, 83 (1999).
33. N. Kumekawa, H. Ohtsubo, T. Horiuchi, and E. Ohtsubo, Identification and characterization of novel retrotransposons of the gypsy type in rice, *Mol. Gen. Genet.* 260, 593 (1999).
34. N. Kumekawa, N. Ohmido, K. Fukui, E. Ohtsubo, and H. Ohtsubo, A new gypsy-type retrotransposon, RIRE7: preferential insertion into the tandem repeat sequence TrsD in pericentromeric heterochromatin regions of rice chromosomes, *Mol. Genet. Genomics* 265, 480 (2001).
35. A. Konieczny, D. F. Voytas, M. P. Cummings, and F. M. Ausubel, A superfamily of *Arabidopsis thaliana* retrotransposons, *Genetics* 127, 801 (1991).
36. P. L. VanderWiel, D. F. Voytas, and J. F. Wendel, Copia-like retrotransposable element evolution in diploid and polyploid cotton (*Gossypium* L.), *J. Mol. Evol.* 36, 429 (1993).
37. M. Yañez, I. Verdugo, M. Rodriguez, S. Prat, and S. Ruiz-Lara, Highly heterogeneous families of Ty1/copia retrotransposons in the *Lycopersicon chilense* genome, *Gene* 222, 223 (1998).
38. S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, Basic local alignment search tool, *J. Mol. Biol.* 215, 403 (1990).
39. S. E. Kubis, J. S. Heslop-Harrison, C. Desel, and T. Schmidt, The genomic organization of non-LTR retrotransposons (LINEs) from three Beta species and five other angiosperms, *Plant Mol. Biol.* 36, 821 (1998).
40. J. D. Thompson, D. G. Higgins, and T. J. Gibson, Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice, *Nucleic Acids Res.* 22, 4673 (1994).
41. Y. Van de Peer and R. De Wachter, TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment, *Comput. Appl. Biosci.* 10, 569 (1994).
42. N. Saitou and M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, *Mol. Biol. Evol.* 4, 406 (1987).
43. D. H. Lankenau, P. Huijser, E. Jansen, K. Miedema, and W. Hennig, DNA sequence comparison of micropia transposable elements from *Drosophila hydei* and *Drosophila melanogaster*, *Chromosoma* 99, 111 (1990).
44. N. V. Lyubomirskaya, I. R. Arkhipova, Y. V. Ilyin, and A. I. Kim, Molecular analysis of the gypsy (mdg4) retrotransposon in two *Drosophila melanogaster* strains differing by genetic instability, *Mol. Gen. Genet.* 223, 305 (1990).
45. Y. Matsuoka and K. Tsunewaki, Evolutionary dynamics of the Ty1-copia group retrotransposons in grass shown by reverse transcriptase domain analysis, *Mol. Biol. Evol.* 16, 208 (1999).
46. S. Wang, Q. Zhang, P. J. Maughan, and M. A. Saghai Maroof, Copia-like retrotransposons in rice: sequence heterogeneity, species distribution and chromosomal locations, *Plant Mol. Biol.* 33, 1051 (1997).
47. L. Mao, T. C. Wood, Y. Yu, M. A. Budiman, J. Tomkins, S. Woo, M. Sasinowski, G. Presting, D. Frisch, S. Goff, R. A. Dean, and R. A. Wing, Rice transposable elements: a survey of 73,000 sequence-tagged connectors, *Genome Res.* 10, 982 (2000).
48. K. Noma, E. Ohtsubo, and H. Ohtsubo, Non-LTR retrotransposons (LINEs) as ubiquitous components of plant genomes, *Mol. Gen. Genet.* 261, 71 (1999).
49. G. Harper, J. O. Osuji, J. S. Heslop-Harrison, and R. Hull, Integration of banana streak badnavirus into the Musa genome: molecular and cytogenetic evidence, *Virology* 255, 207 (1999).
50. J. Jakowitsch, M. F. Mette, J. van Der Winden, M. A. Matzke, and Matzke, A. J. Matzke, Integrated pararetroviral sequences define a unique class of dispersed repetitive DNA in plants, *Proc. Natl. Acad. Sci. USA* 96, 13241 (1999).
51. J. M. Kim, S. Vanguri, J. D. Boeke, A. Gabriel, and D. F. Voytas, Transposable elements and genome organization: a comprehensive survey of retrotransposons revealed by the complete *Saccharomyces cerevisiae* genome sequence, *Genome Res.* 8, 464 (1998).
52. I. K. Jordan and J. F. McDonald, Comparative genomics and evolutionary dynamics of *Saccharomyces cerevisiae* Ty elements, *Genetica* 107, 3 (1999).
53. T. Oosumi, B. Garlick, and W. R. Belknap, Identification of putative nonautonomous transposable elements associated with several transposon families in *Caenorhabditis elegans*, *J. Mol. Evol.* 43, 11 (1998).
54. N. J. Bowen and J. F. McDonald, Genomic analysis of *Caenorhabditis elegans* reveals ancient families of retroviral-like elements, *Genome Res.* 9, 924 (1999).
55. Q. H. Le, S. Wright, Z. Yu, and T. Bureau, Transposon diversity in *Arabidopsis thaliana*, *Proc. Natl. Acad. Sci. USA* 97, 7376 (2000).
56. I. Marín and C. Lloréns, Ty3/Gypsy retrotransposons: description of new *Arabidopsis thaliana* elements and evolutionary perspectives derived from comparative genomic data, *Mol. Biol. Evol.* 17, 1040 (2000).

57. M. G. Francki, Identification of Bilby, a diverged centromeric Ty1-*copia* retrotransposon family from cereal rye (*Secale cereale* L.), *Genome* 44, 266 (2001).
58. N. Kumekawa, T. Hosouchi, H. Tsuruoka, and H. Kotani, The size and sequence organization of the centromeric region of *Arabidopsis thaliana* chromosome 5, *DNA Res.* 7, 315 (2000).
59. K. Nomomura and N. Kurata, The centromere composition of multiple repetitive sequences on rice chromosome 5, *Chromosoma* 110, 284 (2001).
60. G. G. Presting, L. Malysheva, J. Fuchs, and I. Schubert, A Ty3/gypsy retrotransposon-like sequence localizes to the centromeric regions of cereal chromosomes, *Plant J.* 16, 721 (1998).
61. Y. Matsuoka and K. Tsunewaki, Wheat retrotransposon families identified by reverse transcriptase domain analysis, *Mol. Biol. Evol.* 13, 1384 (1996).
62. Y. Matsuoka and K. Tsunewaki, Presence of wheat retrotransposons in *Gramineae* species and the origin of wheat retrotransposon families, *Genes Genet. Syst.* 72, 335 (1997).
63. V. Kapitonov and J. Jurka, Molecular paleontology of transposable elements from *Arabidopsis thaliana*, *Genetica* 107, 27 (1999).
64. C. M. Vicient, R. Kalendar, K. Ananthawat-Jonsson, and A. H. Schulman, Structure, functionality, and evolution of the BARE-1 retrotransposon of barley, *Genetica* 107, 53 (1999).
65. G. Moore, H. Lucas, N. Batty, and R. Flavell, A family of retrotransposons and associated genomic variation in wheat, *Genomics* 10, 461 (1991).
66. J. Dubcovsky, W. Ramakrishna, P. J. SanMiguel, C. S. Busso, L. Yan, B. A. Shiloff, and J. L. Bennetzen, Comparative sequence analysis of colinear barley and rice bacterial artificial chromosomes, *Plant Physiol.* 125, 1342 (2001).
67. H. Hirochika, Retrotransposons of rice: their regulation and use for genome analysis, *Plant Mol. Biol.* 35, 231 (1997).
68. K. Shirasu, A. H. Schulman, T. Lahaye, and P. Schulze-Lefert, A contiguous 66 kb barley DNA sequence provides evidence for reversible genome expansion, *Genome Res.* 10, 908 (2000).
69. B. Charlesworth, Genetic divergence between transposable elements, *Genet. Res.* 48, 111 (1986).
70. W. J. Miller, J. F. McDonald, and W. Pinsker, Molecular domestication of mobile elements, *Genetica* 100, 261 (1997).