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Short communication

Mrs, a new subfamily of *Tourist* transposable elements

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Abstract

We have characterised a new family of repetitive sequences that we have named *Mrs* (maize repetitive sequences). *Mrs* elements are associated with different maize genes and seem to be specific for the genome of *Zea* species. *Mrs* elements are short, AT-rich and contain terminal inverted repeats (TIRs). The sequence of their TIRs, as well as the fact that they are flanked by short repetitions that tend to be TAA, allows us to propose *Mrs* as a new subfamily of *Tourist* transposable elements.

Transposable elements are usually divided into two classes: class I elements that transpose via an RNA intermediate, and class II elements that transpose via a DNA intermediate. Transposition is often a deleterious process for the genome. The insertion of a transposable element within a gene generates in most cases the inactivation or the deregulation of the gene, and usually leads to a detectable mutant phenotype. Unstable mutant phenotypes are due to the excision of the transposable element from its site, which sometimes restores the wild-type activity of the gene. As class I elements excise very rarely they are thought to be more mutagenic than class II elements. However, only class I elements have been frequently found associated with normal eukaryote genes [13].

In the past few years a new class of transposable elements has been described in plants. These elements, which are known as MITEs (miniature inverted-repeat transposable elements) have frequently been found in normal plant genes [13]. MITEs share characteristics with both class I and class II transposable elements and it is not known at present whether they transpose by a DNA intermediate or by a retrotransposition-related mechanism. The *Tourist* element, originally called MISD [14], was the first transposable element described belonging to the MITE family. This element is present in one-third of the genomic maize sequences submitted to the databases, as well as in a large fraction of the rice, sorghum and barley gene sequences submitted [3]. Similarly, the other MITE element described, named *Stowaway*, has been found in association with normal genes of both monocotyledonous and dicotyledonous plants [4, 12]. Here we describe a new family of maize repetitive sequences that we have named *Mrs* (maize repetitive sequences). *Mrs* elements display some of the characteristics of *Tourist* MITE elements and can be found in association with functional genes.

A short DNA sequence frequently present in maize genes

During the characterisation of a genomic clone corresponding to CHT (an embryo-expressed maize gene homologous to a rice cDNA supposed to be related to chilling tolerance, accession number X96760), a short intronic sequence was found to have a high level of sequence similarity with different maize promoter sequences (Table 1). A sequence of 359 bp of the first intron of the CHT1 gene is 87% similar to a sequence located 480 bp upstream of the transcription start site of the maize chalcone flavone isomerase gene [6]. It also exhibits 86% similarity with a sequence located at position −360 nt within the ferredoxin-NADP reductase-

binding protein gene [10] and shows 68% similarity with a sequence located at −460 nt within the *cab48* gene promoter [7]. A database search revealed a homologous sequence (69% similarity) present in a defective Spm element named *Irma*, which contains non-Spm sequences between Spm ends [9]. This dSpm element seems to be mobile as it has been found as an insertion in two different maize genes [9, 11]. The similarities and locations of the different sequences within the genes are summarized in Table 1. We have named this sequence *Mrs* (maize repetitive sequence).

Mrs *has some of the features of a transposable element*

No similarity was found between *Mrs* sequences and any other repetitive sequences described to date. Nevertheless, *Mrs* sequences have some of the characteristics of a transposable element. Hybridization of maize DNA with *Mrs*1 shows that *Mrs* elements are dispersed in the maize genome (data not shown). The ends of *Mrs* elements are inverted repeated sequences of 14 bp (see Fig. 1A). Terminal inverted repeats (TIRs) are characteristics of DNA transposons and also of the new class of short mobile elements called MITEs. *Mrs* elements are, like MITEs, AT-rich and contain repeated sequences that could allow them to form stable secondary structures (see Table 1). In addition, *Mrs* TIRs have similarities with those of *Tourist* elements (see Fig. 1B) and, like *Tourist*, *Mrs* elements are flanked by 3 bp repetitions which are often TAA (see Fig. 1A). These similarities are particularly significant since the sequence of the TIRs and the size, and sometimes the sequence, of the target site duplication generated upon integration are believed to be specific for each family of transposable elements that share integration machinery. *Tourist* elements have been classified into 4 different subfamilies according to their sequences. *Tourist* A elements are short (135 bp on average) and contain several copies of a GGATT motif [2]. *Tourist* B elements are usually larger than A elements (240 bp on average) and are characterised by the presence of polyA-polyT sequences near the TIRs, and by a short sequence called box I. In addition, the elements belonging to this subfamily contain a characteristic 100 bp sequence that has been named domain I [3]. Class C elements are similar to class B elements but they contain an additional domain I′, which is 70% homologous to domain I and is found in opposite orientation [3]. *Tourist* D elements do not contain any of the characteristic domains described above and they are variable in length [3]. In addition to internal sequence similarities, the different *Tourist* subfamilies are defined by a consensus TIR sequence that is characteristic for each subfamily, even though the consensus TIR sequences are 60% homologous between all different *Tourist* subfamilies. *Mrs* internal sequences are not similar to those of any *Tourist* subfamilies, nor do they have any of the motifs that are characteristic of some of these elements. Thus, *Mrs* elements do not belong to any of the subfamilies of *Tourist* elements described to date. The homology that the consensus *Mrs* TIR sequences display with *Tourist* TIR sequences (65%), the preference for a TAA target site, in addition to the sequence characteristics described before, led us to propose *Mrs* elements as a new subfamily of *Tourist* elements.

Polymorphic presence of Mrs *elements*

The maize genome contains two genes encoding the embryo-expressed CHT gene (R. Roca, this laboratory, personal communication). To assess whether *Mrs*1 was present in the homologous gene, we amplified by PCR the intron that contains *Mrs*1 with oligonucleotides corresponding to flanking exonic sequences. The primers amplified a band of 1750 bp, which coincides with the expected size for the CHT1 region, and a band of 1200 bp. These two bands were able to hybridize with an exonic CHT probe, while only one of them hybridized with a probe corresponding to *Mrs*1 (data not shown). This strongly suggests that while both homologous genes were amplified with the primers, the *Mrs* insertion was present in only one of them. The cloning and sequencing of both PCR products showed that the introns of the two CHT genes are 80% identical except for an insertion of 362 bp in the CHT1 gene. This additional sequence can be easily explained by the insertion of the *Mrs*1 element which generated a duplication of a short TTA sequence (Fig. 2). The polymorphic presence of *Mrs*1 and the target site duplication found confirms the mobile nature of the *Mrs* elements. We have cloned a partial cDNA from a maize embryo cDNA library corresponding to a CHT gene. The sequence of this cDNA exactly coincides with the one deduced from the CHT1 gene (data not shown). The sequences of CHT1 and CHT2 genes are only 80% identical within the region analysed, suggesting that the cDNA obtained corresponds to the CHT1 gene, and that the presence of the *Mrs* insertion within an intron does not inactivate this gene.

MITE elements are associated with many different plant genes. *Stowaway* elements have been described

Table 1. Characteristics of the different *Mrs* elements found in maize (1). Only partial sequences are available for these elements.

Element	Location	Position	Size	%Homology with *Mrs*1	%AT	ΔG° kcal/mol	Ref.
*Mrs*1	CHT1	Intron	359 bp	–	68	−72.8	this paper
*Mrs*2	CHI	5′-flanking (−482)	– (1)	87	70	−78.5	[6]
*Mrs*3	Fnrbp	5′-flanking (−362)	– (1)	86	71	−54.2	[10]
*Mrs*4	cab48	5′-flanking (−458)	358 bp	68	77	−59.2	[7]
*Mrs*5	Irma	Non-Spm region	359 bp	69	72	−72.6	[9]

in both monocotyledonous and dicotyledonous plant genes [4] and *Tourist* elements have been found in association with the genes of many cereals [3]. Presence of the different subfamilies of *Tourist* elements depends on the genome studied. *Tourist* B elements have only been found in sorghum genes, while all the *Tourist* elements found in rice genes belong to the *Tourist* C subfamily of elements [3]. We were thus interested in analysing the presence of *Mrs* elements in different cereal genomes. The slot blot hybridizations in Fig. 3 show that *Mrs* elements are present in the genomes of the different *Zea* species analysed while they seem to be absent from *Sorghum* genome. The *Zea mays* W64 genome contains about 100 copies of the *Mrs* element. The *Mrs* copy number is similar in the genome of the other *Zea* species analysed ranging from 50–100 copies in *Zea mexicana* to 500–1000 copies in the Palomero Toluqueño race of *Zea mays*. We have investigated the presence of *Mrs*1 in the CHT1 gene in all these genomes by amplifying the intronic sequence that contains the insertion in the maize CHT1 gene with a pair of primers that specifically amplifies the intron of CHT1 gene. We amplified a single band in all the genomes tested including *Sorghum*, even though the amplified band was shorter in this genome (Fig. 4). These bands hybridized with a probe corresponding to CHT1 exonic sequences while only the bands corresponding to amplifications performed with DNA from the genus *Zea* were able to hybridize with an *Mrs*1 element probe (Fig. 4). The band amplified from *Sorghum* DNA failed to hybridize with the probe corresponding to the *Mrs*1 element. This confirms that *Mrs*1 is not inserted into the CHT1 intron in this species. This was further reinforced by the sequencing of the *Sorghum* band (data not shown). The distribution of *Mrs* elements seems thus different from those of the other *Tourist* subfamilies of elements. *Mrs* activity could be more recent than those of other subfamilies of *Tourist* elements as *Sorghum*, which is one of the genera more closely related to *Zea*, seems to be devoid of *Mrs* sequences.

Mrs *elements have been found associated to normal maize genes*

Tourist elements have been found associated with normal plant genes. Elements of this family have been described in the 5′- and 3′-flanking regions, and in intronic sequences [2, 3]. Four of the 5 *Mrs* here described lie within normal maize genes. The fifth, *Mrs*5, has been found as an insertion within a non-*Spm* central sequence of a defective *dSpm*. The occurrence of mobile genetic elements inserted in other ones has been widely described and is understood to be a mechanism by which the deleterious effects of insertions are minimized. The *Stowaway* MITE element has initially been described as an insertion within a *Tourist* element [4], which demonstrates that MITE elements can also integrate within other transposons.

Three of the *Mrs* elements described here have been found within the promoter region of functional maize genes. The close proximity to the transcriptional start site (less than 500 nt) indicates that the insertion of these elements could probably modify the transcriptional regulation of the genes. Unfortunately, no detailed reports are available on the promoters of these genes that could confirm this aspect. Nevertheless, a 1100 bp region of the *cab48* gene containing the 358 bp *Mrs*4 element is sufficient to drive a high level of transcription in leaf derived protoplasts [7]. Interestingly the *cab48* promoter displays an important sequence similarity with the *cab-m1* gene promoter which lacks this insertion [1]. It would be very interesting to compare the regulation of cab-m1 and *cab48* genes in order to determine the effect of *Mrs* elements insertion in maize gene promoters.

*Mrs*1 has been found as an insertion in an intron of a normal maize gene. Different *Tourist* elements have

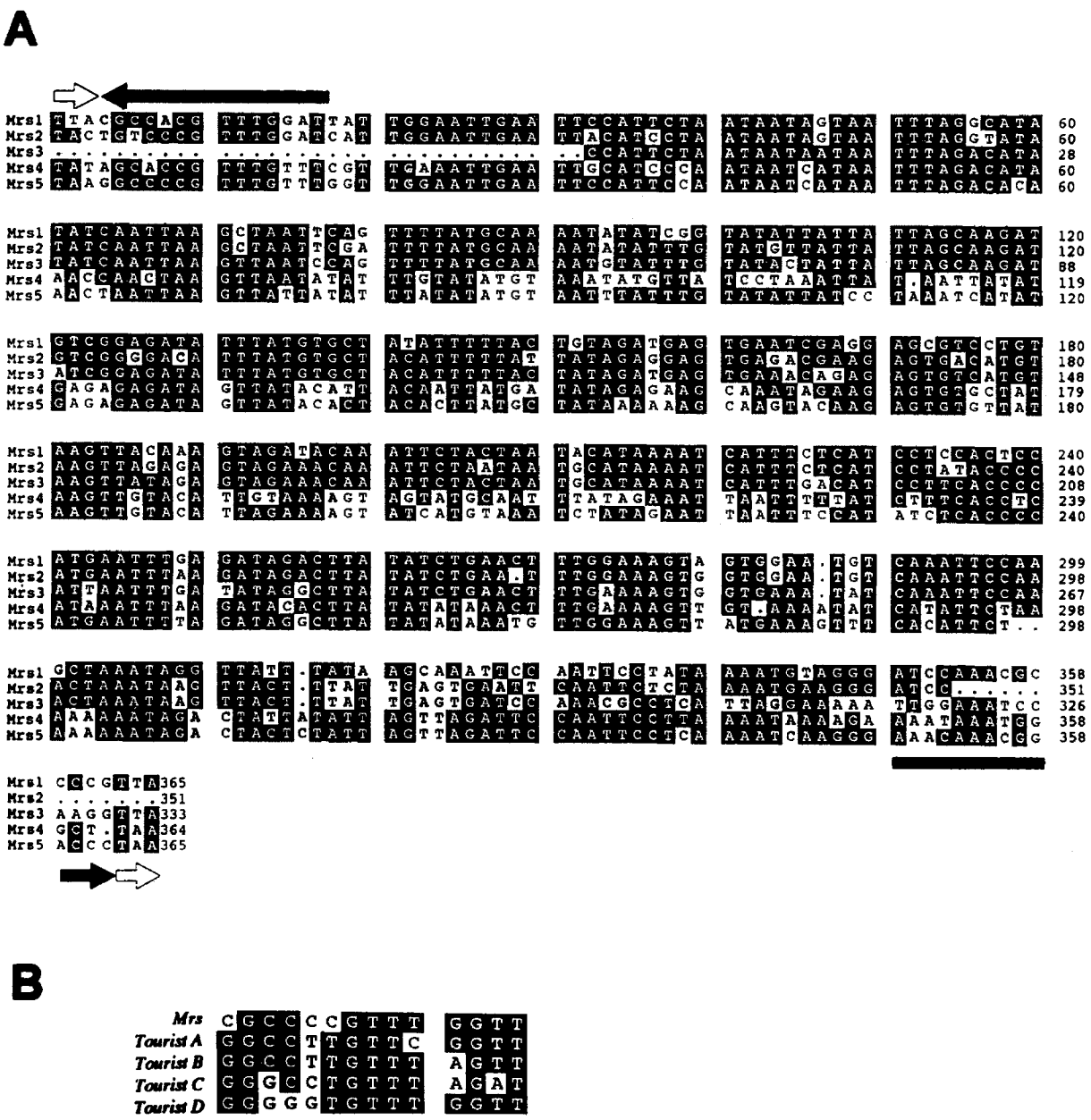

Figure 1. **A.** Multiple sequence alignment of *Mrs* sequences. Target sites duplications are shown by open arrows and terminal inverted repeats (TIRs) by solid arrows. Conserved nucleotides are indicated by white letters on a black background. **B.** Comparison of the *Mrs* elements consensus TIR sequence with the consensus TIR sequences of the different families of *Tourist* elements. Conserved nucleotides are indicated by white letters on a black background.

been found in introns [2, 3]. The insertion in introns has also been reported for other transposable elements and, in some cases, they can behave as introns capable of perfect splicing out from the primary transcript [5]. Little is known about the mechanism of MITE transposition, and it is not clear whether these elements transpose by a DNA intermediate or whether they are retrotranscribed from an RNA before its integration in the genome. As discussed elsewhere, these elements have characteristics of both types of elements [13]. The high copy number of MITE elements, as well as the fact that no MITE element has been shown to excise,

Figure 2. Alignment of CHT1 and CHT2 sequences. Only sequences flanking *Mrs*1 insertion are shown. Terminal inverted repeats are given in bold and the target site duplication is underlined. CHT1 and CHT2 fragments were amplified by PCR from W64 maize genomic DNA using two nucleotides corresponding to sequences located within the first (5′-AGCAAGGCATATGGGAC-3′) and the fifth (5′-AGAGGGTGCAACACTGC-3′) exons of the CHT gene. PCR products were cloned in a pBluescript (Stratagene) T-vector, constructed as described [8] and sequenced by standard procedures.

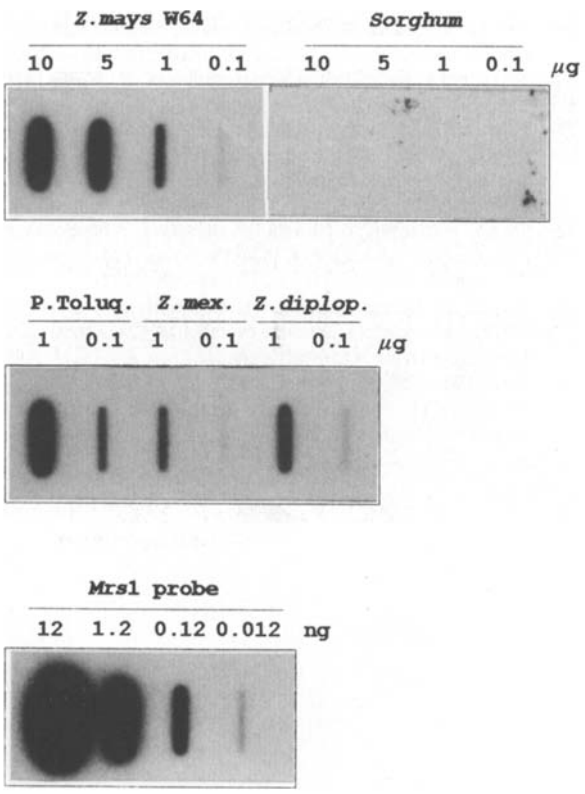

Figure 3. Analysis of the copy number of *Mrs* elements in different species. The species from which DNA was obtained and the amount of DNA used is shown on the top of each panel. Filters were hybridized with a *Mrs*1 probe at medium stringency (20 mM Na_2HPO_4 pH 7.2, 1% SDS, 1 mM EDTA at 50 °C).

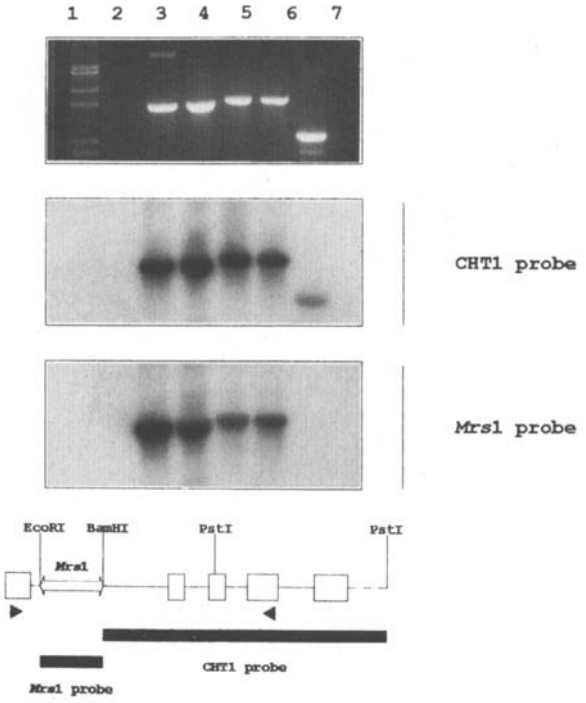

Figure 4. Specific amplification of the *Mrs*1 CHT1 region in different species CHT1 was specifically amplified using the same oligonucleotide corresponding to a sequence located within the first exon described in Fig. 2 and a second one (5′-CCCTCAATTGGACGAG-3′) corresponding to a sequence located within the fourth exon of the CHT1 gene. Upper box: electrophoresis of the PCR products obtained; 1, molecular weight marker; 2, negative control amplification and amplifications obtained with DNA from; 3 , *Z. mays* (W64); 4, *Z. mays* (P. Toluqueño); 5, *M. mexicana*; 6, *Z. diploperenni*; 7, *Sorghum*. Central box: hybridization with a CHT1 probe. Lower box: hybridization with a *Mrs*1 probe. A scheme with the position of the primers and the probes used is shown below. Open boxes indicate exons, solid lines indicate introns and dashed lines indicate a long DNA sequence. Open bars flanked by arrows indicate the *Mrs*1 insertion. Solid arrows indicate the position of primers used to amplify specifically CHT1 and solid bars indicate the positions of the probes used for hybridizations.

seem to indicate a retrotransposition-related mechanism. If MITEs transpose via an RNA intermediate only the elements present in transcribed regions will be able to transpose, since they do not seem to contain internal promoters. The presence of MITE elements within introns, as is the case for the *Mrs*1 element, could thus be especially significant.

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