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Review

Plant LTR-retrotransposons and MITEs: control of transposition and impact on the evolution of plant genes and genomes

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Abstract

Transposons are genetic elements that can move, and sometimes spread, within genomes, and that constitute an important fraction of eukaryote genomes. Two types of transposons, long terminal repeat (LTR)-retrotransposons and miniature inverted-repeat transposable elements (MITEs), are highly represented in plant genomes, and can account for as much as 50–80% of the total DNA content. In the last few years it has been shown that, in spite of their mutagenic capacity, both LTR-retrotransposons and MITEs can be found associated to genes, suggesting that their activity has influenced the evolution of plant genes. In this review we will summarise recent data on the control of the activity and the impact of both LTR-retrotransposons and MITEs on the evolution of plant genes and genomes.

Keywords: Transposable element; Silencing; Stress; Transcription

1. Structural characteristics of retrotransposons and miniature inverted-repeat transposable elements (MITEs)

Transposable elements (TEs) are usually classified in two different groups according to their mode of transposition: class I elements transpose through an RNA intermediate, while class II elements transpose directly via a DNA intermediate. The replicative mode of transposition of retrotransposons can rapidly increase their copy number, which can be extremely high in eukaryote genomes. On the contrary, class II TEs are usually present in a low copy number, probably as a consequence of their 'cut and paste' mechanism of transposition. MITEs constitute a particular type of TEs with characteristics of both class I and class II elements. While their structural characteristics are similar to defective class II elements, their high copy number and the existence of subfamilies showing high sequence and size conservation suggest that they can be amplified from a very limited number of progenitors (Feschotte et al., 2002a).

Retrotransposons are the most widespread class of eukaryotic TE. They can be divided into two principal groups, the long terminal repeat (LTR) and the non-LTR retrotransposons. LTR retrotransposons are further subdivided into the Ty1-*copia* and the Ty3-*gypsy* groups, while non-LTR retrotransposons are subdivided into long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). LTR retrotransposons have long terminal repeats (LTRs) of variable length (from 100 bp to several Kb) that flank the internal coding region. Both Ty1-*copia* and Ty3-*gypsy* groups encode a number of proteins in two major genes, *gag* and *pol* that are synthesised as a polyprotein, which is cleaved into functional peptides by an element-encoded protease. *Gag* encodes structural proteins important for the packaging of retrotransposon RNA while the *pol* gene encodes the enzymatic activities needed for the retrotransposon life cycle. The order in which these enzymatic activities are encoded within the *pol* gene differs between Ty1-*copia* and Ty3-*gyspsy* elements. While integrase precedes the reverse transcriptase, and RNAseH, and is located just downstream of the protease coding capacity in Ty1-*copia* elements, it is located at the end of the *pol* gene in Ty3-*gypsy* elements (Fig. 1). Transcription of

Abbreviations: LTR, long terminal repeat; MITE, miniature inverted-repeat transposable element; TIR, terminal inverted repeat; VLP, virus like particle; PTGS, post-transcriptional gene silencing; TGS, transcriptional gene silencing; ORF, open reading frame; MAR, matrix attachment region.

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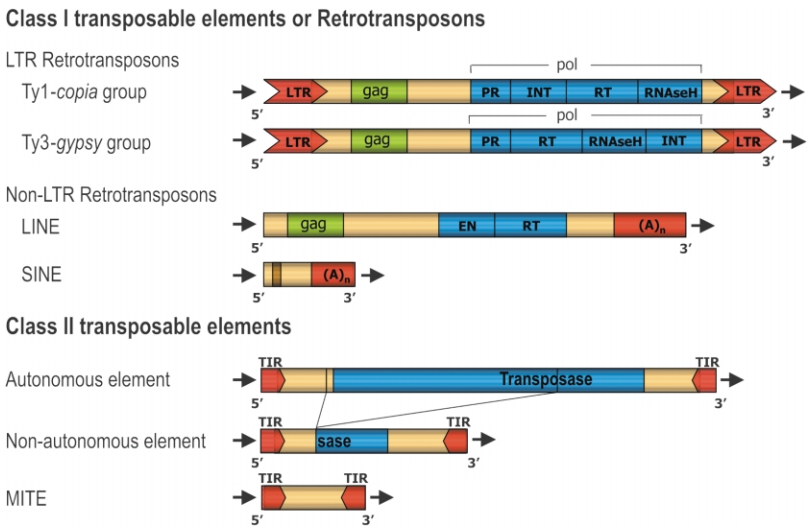

Fig. 1. Structure of the different types of plant transposable elements.

LTR retrotransposons starts at the 5′ LTR and ends at the 3′ LTR, and LTRs usually contain regulatory sequences for both promoting and terminating transcription of the element.

Non-LTR retrotransposons lack LTRs and are transcribed from an internal promoter. LINEs, like LTR-retrotransposons, have *gag* and *pol* genes encoding structural and enzymatic activities, and it has been proposed that LINEs could be the precursors of LTR-retrotransposons (Xiong and Eickbush, 1990). On the contrary the small retrotransposons called SINEs are very different from the rest, and they do not have any coding capacity. SINEs derive from polymerase III transcripts (like tRNAs or 7SL RNAs), and use LINE-specified functions to transpose (Kajikawa and Okada, 2002). Transcription is the first step of retrotransposition, as the synthesised RNA is used as template for reverse transcription to generate a new copy of the element prior to integration. In the case of retroelements with coding capacity (e.g. LTR retrotransposons and LINEs), this RNA is also used as mRNA for the synthesis of the encoded proteins.

Most class II elements transpose by a 'cut and paste' mechanism mediated by a transposase that recognise their short terminal inverted repeated sequences (TIRs). The presence of certain conserved motives within transposases, as well as sequence and length similarities in the TIRs and in the target site duplications generated upon insertion, allow to classify eukaryotic class II transposons in 7 different superfamilies (Robertson, 2002; Feschotte et al., 2002b). Internal deletions within the coding sequences of transposons can generate defective elements that are no longer able to transpose autonomously, but can be transactivated by active transposases expressed by related elements. MITE structure resembles to that of defective class II transposons in the absence of the coding capacity and the presence of TIRs. However, the high copy number and the sequence and size conservation of each MITE subfamily suggest that MITEs can be highly amplified from a limited number of progenitors, which is a characteristic of class I elements. For these reason MITEs remained long time unclassified. Recently, however, a direct link between a MITE family and a potential autonomous element was found suggesting that MITEs are a particular type of defective class II transposons. The example of transposase-encoding element related to a MITE family was found in Arabidopsis, where an element closely related to the *Emigrant* family of MITEs was found to encode a *pogo*-like transposase (Feschotte and Mouches, 2000). Since then, transposase-encoding elements related to most MITE families have been found in plants and other organisms (Feschotte and Wessler, 2002; Feschotte et al., 2002b; Le et al., 2000; Turcotte et al., 2001; Turcotte and Bureau, 2002; Yu et al., 2000; Zhang et al., 2001), and it has been proposed that MITEs are a particular type of defective class II elements mobilised by transposases encoded by their related autonomous elements (Feschotte et al., 2002a,b). Nevertheless, the mechanism by which these elements are amplified remains unknown (Fig. 2).

2. Retrotransposon and MITE copy number and plant genome size

Both LTR and non-LTR retrotransposons are widespread in plant genomes where they can reach very high copy numbers. For example, the Ty1-*copia* elements, BARE-1 from barley and Opie-1 and Huck2 from maize are present in 20,000–200,000 copies (Meyers et al., 2001; SanMiguel et al., 1996; Vicient et al., 1999), the Ty3-gypsy Cinful-1 is present in 20,000 copies in the genome of maize, the LINE Del2 is present at 250,000 copies in Lilium (Leeton and Smyth, 1993), and the SINE TS is present at 50,000 copies in tobacco (Yoshioka et al., 1993). Retrotransposons have been found to be present at a high copy number in

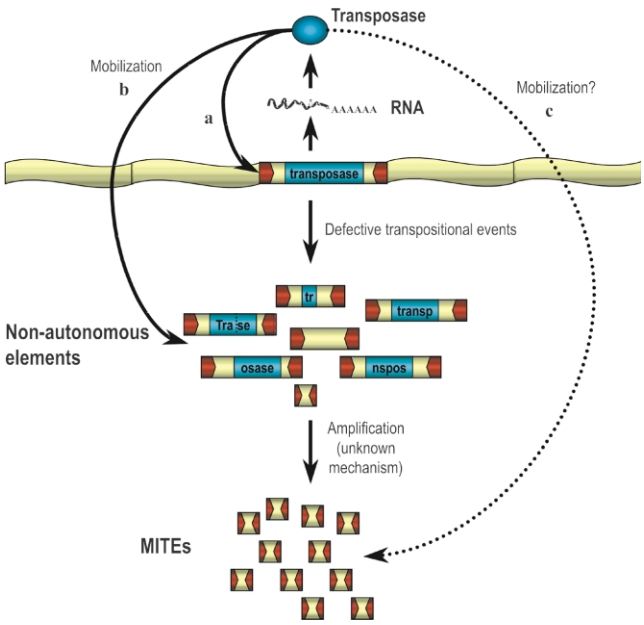

Fig. 2. Model for the origin of MITEs. DNA transposons code for a transposase that directs their mobilization (a). Incomplete transpositional events can generate defective copies, no longer able to autonomously transpose. These elements can be mobilised in trans by transposases coded by their related autonomous elements (b). Some short non-autonomous copies could be amplified by an already unknown mechanism, and generate a family of MITEs. Mobilization of such elements is supposed to be catalysed by the transposase encoded by the autonomous transposon (c) or by related elements.

heterochromatic regions including centromeres (Arabidopsis Genome Initiative, 2000; Feng et al., 2002), but they are also found interspersed with genes. Indeed, gene-flanking regions have been frequently found to contain sequences related to LTR retrotransposons (White et al., 1994). Retrotransposons can insert within pre-existing retrotransposons giving rise to nested structures, as it has been shown analysing the *adh1* region in maize (SanMiguel et al., 1996). The comparison of this region between maize and sorghum showed that the later was devoid of retrotransposons, suggesting that they were inserted after the divergence of these two species. This hypothesis was further confirmed by the analysis of the variability between the two LTRs of these elements that allowed to date the insertions at 2 to 6 million years, thus coinciding with the time of species divergence between sorghum and maize (SanMiguel et al., 1998). This study and the high copy number of retrotransposons in maize compared to sorghum suggest that the difference in genome size between both species is largely due to an important retrotransposon accumulation in maize after the divergence of both species. This is also the case of two *Oryza* species, *O. sativa* and *O. australiensis*, in which the variation in number of RIRE1 copies alone can explain one-third of their differences in genome size (Vicient and Schulman, 2002). In general, differences in retrotransposon content is probably one of the reasons of the high variability of genome size in plants. While in small genomes like *Arabidopsis thaliana* retrotransposons represent only the 4–8% of the genome, in large genomes like maize they can account for more than 50–80% of their DNA content (Kumar and Bennetzen, 1999). The copy number of retrotransposons increases with their activity due to their replicative mechanism of transposition and has probably played a major role in plant genome expansion (Bennetzen and Kellogg, 1997), but the presence of repetitive sequences within the genome can also favour recombination events between them reducing genome size (Devos et al., 2002). In the case of LTR-retrotransposons, it has been shown that recombination between LTRs, to generate solo LTRs, can be an important mechanism to reduce the number of copies of particular retrotransposons (Vicient et al., 1999). Therefore, the copy number of a retrotransposon in a given genome will be the result of its retrotransposition activity but also of the ability of the genome to eliminate the newly inserted copies, and it will be different for each retrotransposon family and each host genome.

MITEs are also abundant in plant genomes, but differently to what it was found for retrotransposons, the analysis of the rice genome has shown that they are preferentially located within euchromatic regions (Feng et al., 2002; Zhang et al., 2000), where they can also form nested structures (Jiang and Wessler, 2001). Most MITE families are present in hundreds of copies of different subfamilies in plant genomes (reviewed in Feschotte et al., 2002b), but in some cases, like the maize *mPIF* element, which is present in more than 6000 copies (Zhang et al., 2001), their copy number can be particularly high. The recent analysis of the sequence of rice chromosome 4 has shown that MITEs constitute almost 50% of the total number of repetitive DNA elements (Feng et al., 2002). On the other hand, although MITEs are very short elements compared to retrotransposons, they can also account for an important fraction of plant genomes. This is the case of the *Stowaway* superfamily of elements that account for 2% of the rice genome (Mao et al., 2000). This high invasivity differentiates MITEs from other class II-related mobile elements that are maintained at a very low copy number within genomes. Whether amplification of MITEs is part of their particular mechanism of transposition or it is an independent phenomenon remains to be elucidated. In any case, the already mentioned relationship of MITEs and class II transposons suggest that they are mobilised by enzymes closely related to class II transposases (Feschotte et al., 2002a). Transposases catalyse both the insertion and the excision of their associated transposons, and thus MITEs should be expected to excise with a certain frequency and, as it is the case of class II transposons, leave excision footprints upon excision. Indeed, the only active MITE described to date, the rice *mPing* element, excises imprecisely and leaves different types of excision footprints (Nakazaki et al., 2003; Kikuchi et al., 2003). Nevertheless, although possible excision footprints have been found for

other MITEs (Yang et al., 2001; Petersen and Seberg, 2000; Casacuberta, unpublished), most MITE insertions seem relatively stable, as particular MITEs are frequently found at the same genomic position in different related species (Wessler, 1998). Abortive transpositions in which excision of an element is not followed by its reinsertion are relatively frequent for some class II transposons like the Ac/Ds elements (Gorbunova and Levy, 2000 and references therein). This mechanism could also account for a loss of MITE elements, and reverse the increase of copy number that seems to accompany their particular mode of transposition.

3. Retrotransposons and MITEs as mutagens

The movement of transposons, and in particular that of LTR-retrotransposons and MITEs, can generate a great variety of mutations in plant genomes. It was the characterisation of an insertional mutant in the maize *Adh* gene that allowed the first description of a retrotransposon in plants, the *Bs1* element (Johns et al., 1985). In a similar way, Tnt1, the first active retrotransposon described in plants, was also isolated after its insertion within the tobacco *Nitrate reductase* gene (Grandbastien et al., 1989). Since then, many examples of mutant phenotypes generated by retrotransposon insertions within coding sequences have been characterised (see for example Vignols et al., 1995; Takano et al., 2001). But the insertion of retrotransposons in non-coding sequences can also generate mutations. Their insertion within introns can result in tissue specific alternative splicing leading to the production of fully active or truncated proteins in different tissues (Marillonnet and Wessler, 1997; Leprince et al., 2001; Varagona et al., 1992), and the insertion of LTR-retrotransposons in non-coding regions close to genes can also modify their transcription or transcriptional termination due to the presence in their LTR of transcriptional promoters, regulators, and terminators. This ability of retrotransposons to generate mutations has been recently used as a tool to generate mutant collections in rice (Hirochika, 2001). On the other hand, although MITEs are miniature elements, their insertion can also generate mutations. Indeed, *Tourist*, the first MITE family described, was initially identified as an insertion within the maize *waxy* gene leading to a mutant phenotype (Bureau and Wessler, 1992), and the *mPing* element, the first active MITE described has been shown be to responsible for a *slender glume* mutant allele in rice (Nakazaki et al., 2003).

This capacity to generate mutations of both retrotransposons and MITEs seem somehow contradictory with the high copy number those elements can reach within fully viable plant genomes. The first possible explanation to this dilemma is the high prevalence of polyploidy in plants that can buffer the mutagenic activity of TEs. Indeed, the proportion of angiosperms that have experienced one or more episodes of chromosome doubling in their evolutionary history might be more than 70% (Wendel, 2000). In addition, most MITE and retrotransposon copies present in plant genomes are probably defective elements that are no longer able to transpose and generate mutations. This is particularly clear for LTR-retrotransposons. Although the number of LTR-retrotransposons described in plants is very high (Kumar and Bennetzen, 1999) and continues to increase with the completion of genome projects, evidences for recent activity has only been obtained for a handful of them. Insertion polymorphisms between closely related species (Pearce et al., 2000; Kumar and Bennetzen, 1999), or among different varieties and populations (Vicient et al., 1999; Kalendar et al., 2000), have been obtained for a number of elements. In some cases, virus like particles (VLPs), proteins or a low level of transcription, has been detected, suggesting a low level of transpositional activity for some elements (Jaaskelainen et al., 1999; Vicient et al., 2001a, 2001b). Nevertheless, a high level of expression associated to a copy number increase within a genome has only been shown for a very few number of elements. The tobacco retrotransposon Tnt1 can be activated generating new insertions (Melayah et al., 2001), the tobacco Tto1 retrotransposon actively transposes in cell culture (Hirochika, 1993), and the rice Tos17 element can also increase its copy number in tissue culture conditions (Hirochika, 1997). Although retrotransposition seems to have been important in the evolutionary history of many plant genomes, very few plant retrotransposons have maintained their transpositional capacity during evolution.

A similar situation is found for MITEs. Although MITEs are abundant in plant genomes and the number of different MITE families described has greatly increased in the last few years (see Feschotte et al., 2002b for a review), and some of these families show insertion polymorphisms among individuals or populations of the same species (see for example Casa et al., 2000; Casacuberta et al., 1998), only one active MITE family, the rice *mPing* element, has been characterised to date (Jiang et al., 2003; Kikuchi et al., 2003; Nakazaki et al., 2003).

4. Control of TEs by silencing mechanisms

The existence of a high number of sequences within plant genomes that can be considered as remnants of mobile elements reveals the existence of efficient transposon inactivating mechanisms. Indeed, eukaryote genomes seem to have developed mechanisms to reduce the activity of mobile elements and control their mutagenic activity. Among them, silencing mechanisms are probably the most general and effective. Silencing was first described in transgenic plants, but related phenomena have now been described in a broad range of normal organisms. Post-transcriptional gene silencing (PTGS) is a sequence-specific RNA degradation that probably constitutes a general antiviral defence mechanism in plants, while the promoter

inactivation mechanism named transcriptional gene silencing (TGS), could be a mechanism primarily directed to abolish transcription of mobile elements (Vance and Vaucheret, 2002; Vaucheret and Fagard, 2001). Different factors influence the induction of TGS, but the presence of multiple copies of the target sequence seems to be a major factor leading to gene silencing. In the least few years examples of TE inactivation by high copy number-induced silencing have been reported. For example, the activity of the *Drosophila* I element (a LINE retrotransposon), is repressed by the introduction of multiple copies of a transgene expressing a small internal region of this element (Jensen et al., 1999), and the tobacco Tto1 retrotransposon becomes silent in Arabidopsis after several rounds of retrotransposition leading to a copy number increase (Hirochika et al., 2000). On the other hand, the presence of short interfering RNA (siRNA), a mediator of silencing, corresponding to retrotransposon sequences (Hamilton et al., 2002; Llave et al., 2002), also confirms that these elements are indeed targeted by genome silencing mechanisms.

Silenced promoters are hypermethylated and have an increased resistance to DNAse I, suggesting that they form secondary DNA structures that attract methylation and heterochromatin components (Vaucheret and Fagard, 2001). Consisting with this, it has been shown that mutations affecting different chromatin remodelling factors reactivate silent mobile elements (Miura et al., 2001; Singer et al., 2001; Wright and Voytas, 2002), as do mutants of paramutation, a phenomenon closely related to gene silencing mechanisms (Lisch et al., 2002). Moreover, it has been shown that the inactivation of the tobacco Tto1 retrotransposon in Arabidopsis, which is associated to its hypermethylation, can be reversed in a methylation deficient context (Hirochika et al., 2000).

The strong hypermethylation of silenced elements can accelerate their mutation rate rendering them definitively inactive. On the other hand, the chances for an evolutionary loss of a TE increase with the time it is maintained inactive. In yeast it has been shown that recombination between LTRs allows a high turn over of Ty retrotransposons, and the maintenance of small populations of active elements (Jordan and McDonald, 1999). In plants, phylogenetic analysis of the retrotransposon Reverse Transcriptase (RT) gene showed evidences of purifying selection in species with low copy numbers of Ty1-copia elements, suggesting also a high turn-over of low copy number populations of retrotransposons (Navarro-Quezada and Schoen, 2002). Nevertheless, plants also contain very high copy number retrotransposon families that are in most cases inactive, and that have not been eliminated, probably because plant genomes can support huge variations in their genome content without important consequences.

The high copy number that MITEs and retrotransposons can attain in plant genomes, as well as the existence of a few active MITE and retrotransposon families, suggests that some elements can escape to the genomic control mechanisms. TGS is directed against repeated sequences, and its effect is the inactivation of the promoters contained within these sequences. Non-autonomous defective TEs are a particular type of transposons in which the mobilised sequence is different from the one that codes for the enzyme needed for transposition. In the case of MITEs, the transposase-encoding element is probably present in one or very few copies, while the transposing and proliferating unit, the MITE itself, reaches very high copy number. Under this situation, silencing mechanisms will be directed towards the MITE, which cannot be inactivated by TGS because it is not transcribed, while the very low copy number transposase-encoding element will not be a target of silencing. As the transposase-encoding element is supposed to share the terminal sequences with its deletion derivate, the MITE, a MITE-directed TGS could also affect the expression of the transposase. Nevertheless, it has been suggested that MITEs could be transactivated by transposases encoded by related elements other that the source element, with very limited sequence similarity with the MITE itself (Feschotte et al., 2002b).

Although MITEs cannot be inactivated by TGS, silencing-associated processes, such as methylation, could influence its ability to transpose. Methylation has been shown to modify the capacity of the TIRs of different class II transposons to bind the transposase, and thus influence their transposon competence (Benito and Walbot, 1997; Ros and Kunze, 2001). Holomethylated Ac/Ds elements cannot bind Ac transposase and are unable to transpose, while hemimethylated elements bind the transposase with high affinity and transpose actively (Ros and Kunze, 2001). It has been suggested that the preference of Ac transposase for hemimethylated DNA could explain the link between Ac/Ds transposition and DNA replication (Wang et al., 1996). It is tempting to hypothesise that a similar replication-dependent transposition could help MITEs to attain the extremely high copy number they present in eukaryote genomes.

In any case, the amplification process leading to a new MITE family, which has been suggested to represent the last step of the life cycle of a transposon preceding its immediate death (Feschotte et al., 2002b) could also be interpreted as part of a strategy allowing their spread and maintenance within a genome.

Very short defective elements related to LTR-retrotransposons, known as TRIMs have also been described (Witte et al., 2001), although a direct link to an autonomous element has not been found yet. Nevertheless, in this case, and differently to what happens with MITEs, these short elements are supposed to transpose through an RNA intermediate, and can thus be inactivated. In addition, not only the short defective elements but also retrotransposons themselves can reach very high copy numbers. Active retrotransposons have thus to escape silencing by other mechanisms. Individual elements located in particular locations within chromosomes could perhaps be less sensible to inactivation by silencing allowing them to amplify. Related to this, it has been shown that the repeated induction of a

promoter located nearby induces the progressive demethylation and de-silencing of a particular copy of the mouse IAP retrotransposon (Barbot et al., 2002). Nevertheless, the effect of particular chromosomal locations on silencing cannot explain the simultaneous transcription of multiple copies of a retrotransposon family. This is the case, for example, of the tobacco retrotransposon Tnt1 that is expressed as a population of related but different sequences originated by the concomitant transcription of many different elements (Casacuberta et al., 1995). Another possibility is that their high sequence variability could help LTR-retrotransposons to escape silencing. Interestingly, it has been shown that the sequence variability of the tobacco Tnt1 retrotransposon is not homogeneously distributed along the sequence, the promoter, which is the target of the TGS mechanisms, being the most variable region (Vernhettes et al., 1998) (Fig. 3). Nevertheless, it seems difficult that the sequence variability displayed by retrotransposons could be sufficient to escape the extremely efficient TGS mechanisms, which are able to detect and inactivate repeated sequences as short as 90 nt (Vaucheret et al., 1998). A particularity of the very few plant retrotransposons that have maintained their ability to transpose is that they are active only under stress situations (Grandbastien, 1998). Different reasons can be invoked to explain this association of transposon mobility with stress (see Section 5), but it would well be that the genomic silencing mechanisms are somehow relaxed under these situations, allowing TEs to temporally escape to the genomic control. Interestingly, it has recently been shown that a cold stress can lead to a severe demethylation and activation of a retrotransposon related sequence in maize (Steward et al., 2002).

5. Stress activation of plant LTR-retrotransposons and MITEs

Transcription is the first step of the retrotransposition process and seems to be a major controlling step for plant retrotransposons. Transcription, and subsequent transposition, is only detectable under certain conditions that in all cases can be considered as stress conditions. In the case of Tnt1, the three different subfamilies described, Tnt1A, Tnt1B and Tnt1C (Vernhettes et al., 1998), are all transcribed under stress situations associated to plant defence reactions (Casacuberta et al., 1997; Beguiristain et al., 2001) (Fig. 3). Tnt1A is transcribed in roots, and strongly induced in leaves treated with the fungal elicitor cryptogein or methyl jasmonate (Pouteau et al., 1994; Vernhettes et al., 1997; Beguiristain et al., 2001) while Tnt1C transcription can be induced in leaves treated with salicylic acid or 2,4-D, and Tnt1B is transcribed in cell cultures (Beguiristain et al., 2001), In a similar way, Tto1 expression is induced by wounding and cell culture associated stresses (Hirochika, 1997; Takeda et al., 1999), and Tos17 activity is also strongly induced in cell culture (Hirochika, 1997). Environmental stresses can also activate retrotransposition. It has been shown that sharp microclimate changes can modify the copy number of the BARE-1 retrotransposon in wild barley (Kalendar et al., 2000).

Transcriptional regulation of both Tnt1 and Tto1 has been studied in some detail and shown to be strictly controlled. The promoter of Tnt1A contains two different boxes, located within the U3 region of the LTR, that have been shown to be important for the element's transcription and that show sequence similarities with plant defence promoters (Vernhettes et al., 1997). One of these boxes specifically interacts in vivo with proteins that are induced in defence-related stresses (Vernhettes et al., 1997). Tnt1B and Tnt1C are also expressed in tobacco under different stress situations and differ from Tnt1A in their U3 sequence that probably contains the sequences needed to control their expression (Beguiristain et al., 2001). In the case of Tto1, a 13-bp motif has been identified as a cis-regulatory sequence associated to the induction of Tto1 expression in defence-related stresses (Takeda et al., 1999). Interestingly, this motif specifically binds different MYB transcription factors, one of which, that has been named LBM1, is identical to

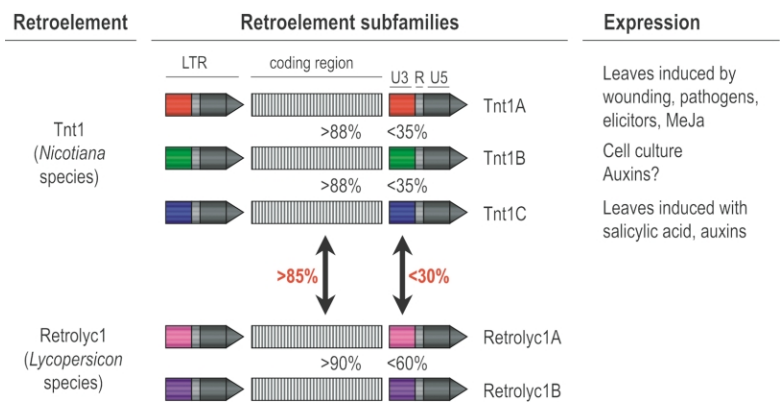

Fig. 3. Evolution of stress regulated promoters in Tnt1-related retrotransposons. Percentages of identity between U3 regions (coloured boxes) and coding regions (based on RT domain) between different subfamilies are indicated in black and between Tnt1 and Retrolyc1 elements in red. Stress conditions under which the three different Tnt1 subfamilies are expressed are also indicated.

a previously described MYB-1 factor induced by virus infection (Sugimoto et al., 2000). The overexpression of another of these MYB transcription factors, NtMYB2, activates transcription of both Tto1 and the PAL defence-related gene in tobacco (Sugimoto et al., 2000). Moreover, extended homologies are found between promoters of Tto1 and an asparagus defence gene, AoPR1 (Takeda et al., 1999). All these results suggest that both Tnt1 and Tto1 are activated in defence-associated stresses because their promoters are similar to that of plant defence genes and bind the same defence induced transcription factors.

It has been proposed that retrotransposons could have captured plant defence promoters from normal genes or inversely, that they could have provided their inducible promoters to some plant defence genes (Grandbastien et al., 1997; Takeda et al., 1999). The distribution of particular successful promoters throughout the genome is a suggestive hypothesis to explain the co-ordinate regulation of groups of genes (e.g. plant defence genes). Nevertheless, evolutionary analysis of Tnt1 promoters seem to indicate that they have not evolved outside the rest of the retrotransposon sequence (Vernhettes et al., 1998), which suggests that the similarities among retrotransposon plant defence gene promoters could be the result of a convergent evolution. Retrotransposons are structurally and functionally very similar to retroviruses and it has been proposed that, as retroviruses, they could display a high sequence plasticity allowing them to rapidly evolve parts of their sequence, and acquire stress associated promoters (Casacuberta et al., 1997). In agreement with that possibility, it has been recently shown that the high variability of Tnt1 U3 region has allowed to this family of elements to evolve three different stress inducible promoters in tobacco (Beguiristain et al., 2001), and that the Tnt1-related element Retrolyc1, has evolved different promoters in tomato (Araujo et al., 2001) (Fig. 3). The driving force for the selection of stress promoters could be that stress is a rare event and thus stress induced TEs will transpose few enough to not compromise host genome viability. On the other hand, the variability that the movement of TEs generates could also help to rapidly evolve the genome when facing a situation to which it is not well adapted, as it was initially proposed by McClintock (McClintock, 1984).

MITE transposition also seems to be induced by stress. The first active MITE described, the rice *mPing* element, increases its copy number in cell cultures (Jiang et al., 2003), and excises and reinserts at new locations in anther-derived calli (Kikuchi et al., 2003). Although the transposase responsible for *mPing* mobilisation has not been described yet, these data suggest that it is probably induced by stress.

6. Impact of retrotransposons and MITEs on the evolution of genes and genomes

Besides the mutagenic effect of TE insertion, transposition could generate variability useful for evolution. Transposition of LTR-retrotransposons and MITEs seems to have been a major player in plant gene evolution, as both types of elements have been frequently found associated to genes in maize (Wessler et al., 1995).

Although most MITEs insert within a TA or a TAA sequence, and, in general they seem to target very high AT-rich regions for integration, they have often been found close to transcribed sequences (Wessler et al., 1995; Yang et al., 2001; Feschotte et al., 2002a). Nevertheless, the Arabidopsis *Emigrant* element was found to be inserted relatively far from genes, and a survey of the published genomic sequence failed to detect transposon insertions in *Arabidopsis thaliana* coding regions (Le et al., 2000). This apparent contradiction has recently been solved for the *Emigrant* element. A phylogenetic analysis has shown that while young *Emigrant* sequences are located far from genes, the ancient *Emigrant* insertions are often associated to genes, suggesting that *Emigrant* elements preferentially insert far from open reading frames (ORFs), but the elements closely linked to genes are more frequently maintained during

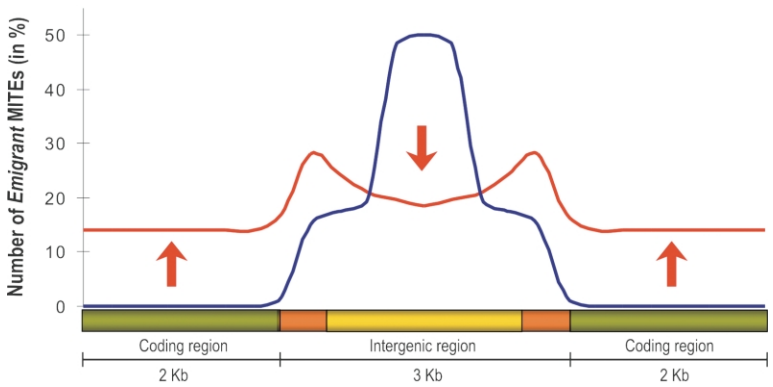

Fig. 4. Maintenance of *Emigrant* elements close to genes during Arabidopsis evolution. Blue line represents the percentage of young *Emigrant* elements found at a particular location with respect to Arabidopsis coding regions. Red line represents the percentage of old *Emigrant* elements found at a particular location with respect to Arabidopsis coding regions. Green boxes represent coding regions; orange boxes represent 0.5 Kb regions close to coding regions supposed to contain regulatory sequences; yellow box represents intergenic regions. Arrows show the shift of *Emigrant* distribution with time.

evolution (Santiago et al., 2002) (see Fig. 4). This is reminiscent of what has been shown for the Alu family of SINEs in the human genome. Alus tend to insert in AT rich regions, and recently transposed Alu subfamilies are found in poor-gene regions, while ancient Alu subfamilies are found preferentially in GC-rich regions closely associated to genes (International Human Genome Sequencing Consortium, 2001). This shift of MITE distribution with time could suggest that the elements close to genes have been positively selected during Arabidopsis evolution. Alternatively, it could be the result of a more frequent elimination of MITEs located far from genes. Indeed, in the case of human Alus it has been proposed that recombination plays a major role on removing them, re-creating insert-free alleles, and that this process is less frequent in gene-rich regions due to the adverse effects that deletions and unequal recombinations could have in these regions (Medstrand et al., 2002). Whatever the mechanism could be this concentration with time in gene-rich regions could be a particularity of short elements like Alus and MITEs, as it has been shown that LTR retrotransposon insertions present a totally opposite dynamics in the human genome (Medstrand et al., 2002).

The insertion of MITEs within genes can modify the promoter and terminator sequences, as well as the translational start and coding sequences (Wessler et al., 1995; Yang et al., 2001; Santiago et al., 2002; El Amrani et al., 2002). LTR-retrotransposon sequences are also frequently found associated to genes (Wessler et al., 1995), suggesting that the modification of the regulation of the expression of target genes due to the presence within the LTRs of promoter and terminator sequences has been an important mechanism in the evolution of plant genes. On the other hand, the insertion of retrotransposons in intergenic regions can also modify the expression of adjacent genes. It has been recently shown that interespecific hybridisations can reactivate the transcription of the wheat WIS2 retrotransposon, which can drive the readout synthesis of new transcripts from adjacent sequences including sense and antisense strands of genes located nearby, resulting on the silencing or the activation of these genes (Kashkush et al., 2003).

A particular case of rapidly evolving gene loci is that of plant resistance genes. Genes conferring race-specific resistance are often clustered in the genome forming large tandem repeats of highly polymorphic genes. It has been shown that the rice *Xa21* gene family contains a high number of TEs (including LTR-retrotransposons and MITEs) inserted within the different genes, and it has been proposed that besides the unequal exchange between the different copies, the high variability needed to evolve new resistance specificities is generated by the TEs insertions (Richter and Ronald, 2000).

TE insertion outside genes can also contribute to genome evolution. It has been shown that MITEs often coincide with sequences showing matrix attachment region (MAR) activity, and it has been proposed that some MITEs could act as MARs isolating their neighbouring genes (Tikhonov et al., 2000). On the other hand, plant centromere and pericentromeric regions often contain retrotransposons that could be important for the functionality of these regions (Pelissier et al., 1996; Fukui et al., 2001; Cheng et al., 2002; Jiang et al., 2002). A centromere specific LTR-retrotransposon has been described in rice (Cheng et al., 2002) and other grasses (Miller et al., 1998) suggesting that these elements can play a role in plant chromosome organisation. Indeed, it has been recently shown that the maize centromere-specific retroelement can interact with the kinetochore protein CENH3 (Zhong et al., 2002). Interestingly, it has been shown that the interspecific cross between two different mammalian species to generate an hybrid, activates the transposition of a centromere specific retrotransposon, and it has been suggested that this could facilitate rapid karyotypic evolution (O'Neill et al., 1998).

7. Concluding remarks: McClintock revisited

Insertion of TEs can modify the expression or the coding capacity of genes and thus transposition can be an extremely deleterious event. For this reason, since the work of Barbara McClintock in the forties, the role of transposable elements (TEs) has been the object of an intense debate. McClintock's idea of mobile elements being active genome remodelling machines in response to stress (McClintock, 1984), was countered by those considering TEs as merely selfish or parasite elements (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). During the last 20 years examples of TEs fulfilling important roles in genomes have been described (Pardue et al., 1996) but it has appeared clearly that it is not necessary to suppose a benefit for the host to explain the presence within genomes of most TEs. Still, many evidences have shown that a high number of transposons are indeed activated by stress and that their mobility has reshaped eukaryote genomes in many ways.

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References

Arabidopsis Genome Initiative, 2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408, 796–815.

Araujo, P.G., Casacuberta, J.M., Costa, A.P., Hashimoto, R.Y.,

Grandbastien, M.A., Van Sluys, M.A., 2001. Retrolyc1 subfamilies defined by different U3 LTR regulatory regions in the *Lycopersicon* genus. Mol. Genet. Genomics 266, 35–41.

Barbot, W., Dupressoir, A., Lazar, V., Heidmann, T., 2002. Epigenetic regulation of an IAP retrotransposon in the aging mouse: progressive demethylation and de-silencing of the element by its repetitive induction. Nucleic Acids Res. 30, 2365–2373.

Beguiristain, T., Grandbastien, M.A., Puigdomenech, P., Casacuberta, J.M., 2001. Three Tnt1 subfamilies show different stress-associated patterns of expression in tobacco. Consequences for retrotransposon control and evolution in plants. Plant Physiol. 127, 212–221.

Benito, M.I., Walbot, V., 1997. Characterization of the maize Mutator transposable element MURA transposase as a DNA-binding protein. Mol. Cell Biol. 17, 5165–5175.

Bennetzen, J.L., Kellogg, E.A., 1997. Do plants have a one-way ticket to genomic obesity? Plant Cell 9, 1509–1514.

Bureau, T.E., Wessler, S.R., 1992. Tourist: a large family of small inverted repeat elements frequently associated with maize genes. Plant Cell 4, 1283–1294.

Casa, A.M., Brouwer, C., Nagel, A., Wang, L., Zhang, Q., Kresovich, S., Wessler, S.R., 2000. Inaugural article: the MITE family heartbreaker (Hbr): molecular markers in maize. Proc. Natl. Acad. Sci. USA 97, 10083–10089.

Casacuberta, E., Casacuberta, J.M., Puigdomenech, P., Monfort, A., 1998. Presence of miniature inverted-repeat transposable elements (MITEs) in the genome of Arabidopsis thaliana: characterisation of the Emigrant family of elements. Plant J. 16, 79–85.

Casacuberta, J.M., Vernhettes, S., Audeon, C., Grandbastien, M.A., 1997. Quasispecies in retrotransposons: a role for sequence variability in Tnt1 evolution. Genetica 100, 109–117.

Casacuberta, J.M., Vernhettes, S., Grandbastien, M.A., 1995. Sequence variability within the tobacco retrotransposon Tnt1 population. EMBO J. 14, 2670–2678.

Cheng, Z., Dong, F., Langdon, T., Ouyang, S., Buell, C.R., Gu, M., Blattner, F.R., Jiang, J., 2002. Functional rice centromeres are marked by a satellite repeat and a centromere-specific retrotransposon. Plant Cell 14, 1691–1704.

Devos, K.M., Brown, J.K., Bennetzen, J.L., 2002. Genome size reduction through illegitimate recombination counteracts genome expansion in Arabidopsis. Genome Res. 12, 1075–1079.

Doolittle, W.F., Sapienza, C., 1980. Selfish genes, the phenotype paradigm and genome evolution. Nature 284, 601–603.

El Amrani, A., Marie, L., Ainouche, A., Nicolas, J., Couee, I., 2002. Genome-wide distribution and potential regulatory functions of AtATE, a novel family of miniature inverted-repeat transposable elements in Arabidopsis thaliana. Mol. Genet. Genomics 267, 459–471.

Feng, Q., Zhang, Y., Hao, P., et al., 2002. Sequence and analysis of rice chromosome 4. Nature 420, 316–320.

Feschotte, C., Mouches, C., 2000. Evidence that a family of miniature inverted-repeat transposable elements (MITEs) from the Arabidopsis thaliana genome has arisen from a pogo-like DNA transposon. Mol. Biol. Evol. 17, 730–737.

Feschotte, C., Wessler, S.R., 2002. Mariner-like transposases are widespread and diverse in flowering plants. Proc. Natl. Acad. Sci. USA 99, 280–285.

Feschotte, C., Jiang, N., Wessler, S.R., 2002a. Plant transposable elements: where genetics meets genomics. Nat. Rev. Genet. 3, 329–341.

Feschotte, C., Zhang, X., Wessler, S.R., 2002b. In: Craig, N., Craigie, R., Gellert, M., Lambowitz, A. (Eds.), Miniature Inverted-repeat Transposable Elements (MITEs) and their relationship with established DNA transposons, Mobile DNA II, American Society of Microbiology Press, Washington, DC, pp. 1147–1158.

Fukui, K.N., Suzuki, G., Lagudah, E.S., Rahman, S., Appels, R., Yamamoto, M., Mukai, Y., 2001. Physical arrangement of retrotransposon-related repeats in centromeric regions of wheat. Plant Cell. Physiol. 42, 189–196.

Gorbunova, V., Levy, A.A., 2000. Analysis of extrachromosomal Ac/Ds transposable elements. Genetics 155, 349–359.

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

Grandbastien, M.A., Lucas, H., Morel, J.B., Mhiri, C., Vernhettes, S., Casacuberta, J.M., 1997. The expression of the tobacco Tnt1 retrotransposon is linked to plant defense responses. Genetica 100, 241–252.

Grandbastien, M.A., Spielmann, A., Caboche, M., 1989. Tnt1, a mobile retroviral-like transposable element of tobacco isolated by plant cell genetics. Nature 337, 376–380.

Hamilton, A., Voinnet, O., Chappell, L., Baulcombe, D., 2002. Two classes of short interfering RNA in RNA silencing. EMBO J. 21, 4671–4679.

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

Hirochika, H., 1997. Retrotransposons of rice: their regulation and use for genome analysis. Plant. Mol. Biol. 35, 231–240.

Hirochika, H., 2001. Contribution of the Tos17 retrotransposon to rice functional genomics. Curr. Opin. Plant Biol. 4, 118–122.

Hirochika, H., Okamoto, H., Kakutani, T., 2000. Silencing of retrotransposons in arabidopsis and reactivation by the ddm1 mutation. Plant Cell 12, 357–369.

International Human Genome Sequencing Consortium, 2001. Initial sequencing and analysis of the human genome. Nature 409, 860–921.

Jaaskelainen, M., Mykkanen, A.H., Arna, T., Vicient, C.M., Suoniemi, A., Kalendar, R., Savilahti, H., Schulman, A.H., 1999. Retrotransposon BARE-1: expression of encoded proteins and formation of virus-like particles in barley cells. Plant J. 20, 413–422.

Jensen, S., Gassama, M.P., Heidmann, T., 1999. Taming of transposable elements by homology-dependent gene silencing. Nat. Genet. 21, 209–212.

Jiang, N., Bao, Z., Temnykh, S., Cheng, Z., Jiang, J., Wing, R.A., McCouch, S.R., Wessler, S.R., 2002. Dasheng: a recently amplified nonautonomous long terminal repeat element that is a major component of pericentromeric regions in rice. Genetics 161, 1293–1305.

Jiang, N., Bao, Z., Zhang, X., Hirochika, H., Eddy, S.R., McCouch, S.R., Wessler, S.R., 2003. An active DNA transposon family in rice. Nature 421, 163–167.

Jiang, N., Wessler, S.R., 2001. Insertion preference of maize and rice miniature inverted repeat transposable elements as revealed by the analysis of nested elements. Plant Cell 13, 2553–2564.

Johns, M.A., Mottinger, J., Freeling, M.A., 1985. A low copy number, copia-like transposon in maize. EMBO J. 4, 1093–1102.

Jordan, I.K., McDonald, J.F., 1999. Tempo and mode of Ty element evolution in Saccharomyces cerevisiae. Genetics 151, 1341–1351.

Kajikawa, M., Okada, N., 2002. LINEs mobilize SINEs in the eel through a shared 3′ sequence. Cell 111, 433–444.

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

Kashkush, K., Feldman, M., Levy, A.A., 2003. Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nat. Genet. 33, 102–106.

Kikuchi, K., Terauchi, K., Wada, M., Hirano, H.Y., 2003. The plant MITE mPing is mobilized in anther culture. Nature 421, 167–170.

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

Le, Q.H., Wright, S., Yu, Z., Bureau, T., 2000. Transposon diversity in Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA 97, 7376–7381.

Leeton, P.R., Smyth, D.R., 1993. An abundant LINE-like element amplified in the genome of Lilium speciosum. Mol. Gen. Genet. 237, 97–104.

Leprince, A.S., Grandbastien, M.A., Meyer, C., 2001. Retrotransposons of the Tnt1B family are mobile in *Nicotiana plumbaginifolia* and can induce alternative splicing of the host gene upon insertion. Plant Mol. Biol. 47, 533–541.

Lisch, D., Carey, C.C., Dorweiler, J.E., Chandler, V.L., 2002. A mutation

that prevents paramutation in maize also reverses Mutator transposon methylation and silencing. Proc. Natl. Acad. Sci. USA 99, 6130–6135.

Llave, C., Kasschau, K.D., Rector, M.A., Carrington, J.C., 2002. Endogenous and silencing-associated small RNAs in plants. Plant Cell 14, 1605–1619.

Mao, L., Wood, T.C., Yu, Y., Budiman, M.A., Tomkins, J., Woo, S., Sasinowski, M., Presting, G., Frisch, D., Goff, S., Dean, R.A., Wing, R.A., 2000. Rice transposable elements: a survey of 73,000 sequence-tagged-connectors. Genome Res. 10, 982–990.

Marillonnet, S., Wessler, S.R., 1997. Retrotransposon insertion into the maize waxy gene results in tissue-specific RNA processing. Plant Cell 9, 967–978.

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

Medstrand, P., Van De Lagemaat, L.N., Mager, D.L., 2002. Retroelement distributions in the human genome: variations associated with age and proximity to genes. Genome Res. 12, 1483–1495.

Melayah, D., Bonnivard, E., Chalhoub, B., Audeon, C., Grandbastien, M.A., 2001. The mobility of the tobacco Tnt1 retrotransposon correlates with its transcriptional activation by fungal factors. Plant J. 28, 159–168.

Meyers, B.C., Tingey, S.V., Morgante, M., 2001. Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. Genome Res. 11, 1660–1676.

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

Miura, A., Yonebayashi, S., Watanabe, K., Toyama, T., Shimada, H., Kakutani, T., 2001. Mobilization of transposons by a mutation abolishing full DNA methylation in Arabidopsis. Nature 411, 212–214.

Nakazaki, T., Okumoto, Y., Horibata, A., Yamahira, S., Teraishi, M., Nishida, H., Inoue, H., Tanisaka, T., 2003. Mobilization of a transposon in the rice genome. Nature 421, 170–172.

Navarro-Quezada, A., Schoen, D.J., 2002. Sequence evolution and copy number of Ty1-copia retrotransposons in diverse plant genomes. Proc. Natl. Acad. Sci. USA 99, 268–273.

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

Orgel, L.E., Crick, F.H., 1980. Selfish DNA: the ultimate parasite. Nature 284, 604–607.

Pardue, M.L., Danilevskaya, O.N., Lowenhaupt, K., Slot, F., Traverse, K.L., 1996. Drosophila telomeres: new views on chromosome evolution. Trends Genet. 12, 48–52.

Pearce, S.R., Knox, M., Ellis, T.H., Flavell, A.J., Kumar, A., 2000. Pea Ty1-copia group retrotransposons: transpositional activity and use as markers to study genetic diversity in Pisum. Mol. Gen. Genet. 263, 898–907.

Pelissier, T., Tutois, S., Tourmente, S., Deragon, J.M., Picard, G., 1996. DNA regions flanking the major Arabidopsis thaliana satellite are principally enriched in Athila retroelement sequences. Genetica 97, 141–151.

Petersen, G., Seberg, O., 2000. Phylogenetic evidence for excision of Stowaway miniature inverted-repeat transposable elements in triticeae (Poaceae). Mol. Biol. Evol. 17, 1589–1596.

Pouteau, S., Grandbastien, M.A., Boccara, M., 1994. Microbial elicitors of plant defence responses activate transcription of a retrotransposon. Plant J. 5, 535–542.

Richter, T.E., Ronald, P.C., 2000. The evolution of disease resistance genes. Plant Mol. Biol. 42, 195–204.

Robertson, H.M., 2002. In: Craig, N., Craigie, R., Gellert, M., Lambowitz, A. (Eds.), Molecular evolution of DNA transposons, Mobile DNA II, American Society of Microbiology Press, Washington, DC.

Ros, F., Kunze, R., 2001. Regulation of activator/dissociation transposition by replication and DNA methylation. Genetics 157, 1723–1733.

SanMiguel, P., Gaut, B.S., Tikhonov, A., Nakajima, Y., Bennetzen, J.L., 1998. The paleontology of intergene retrotransposons of maize. Nat. Genet. 20, 43–45.

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

Santiago, N., Herráiz, C., Goñi, J.R., Messeguer, X., Casacuberta, J.M., 2002. Genome-wide analysis of the *Emigrant* family of MITEs of *Arabidopsis thaliana*. Mol. Biol. Evol. 19, 2285–2293.

Singer, T., Yordan, C., Martienssen, R.A., 2001. Robertson's Mutator transposons in A. thaliana are regulated by the chromatin-remodeling gene Decrease in DNA Methylation (DDM1). Genes Dev. 15, 591–602.

Steward, N., Ito, M., Yamaguchi, Y., Koizumi, N., Sano, H., 2002. Periodic DNA methylation in maize nucleosomes and demethylation by environmental stress. J. Biol. Chem. 277, 37741–37746.

Sugimoto, K., Takeda, S., Hirochika, H., 2000. MYB-related transcription factor NtMYB2 induced by wounding and elicitors is a regulator of the tobacco retrotransposon Tto1 and defense-related genes. Plant Cell 12, 2511–2528.

Takano, M., Kanegae, H., Shinomura, T., Miyao, A., Hirochika, H., Furuya, M., 2001. Isolation and characterization of rice phytochrome A mutants. Plant Cell 13, 521–534.

Takeda, S., Sugimoto, K., Otsuki, H., Hirochika, H., 1999. A 13-bp cis-regulatory element in the LTR promoter of the tobacco retrotransposon Tto1 is involved in responsiveness to tissue culture, wounding, methyl jasmonate and fungal elicitors. Plant J. 18, 383–393.

Tikhonov, A.P., Bennetzen, J.L., Avramova, Z.V., 2000. Structural domains and matrix attachment regions along colinear chromosomal segments of maize and sorghum. Plant Cell 12, 249–264.

Turcotte, K., Bureau, T., 2002. Phylogenetic analysis reveals stowaway-like elements may represent a fourth family of the IS630-Tc1-mariner superfamily. Genome 45, 82–90.

Turcotte, K., Srinivasan, S., Bureau, T., 2001. Survey of transposable elements from rice genomic sequences. Plant J 25, 169–179.

Vance, V., Vaucheret, H., 2002. RNA silencing in plants - defense and counterdefense. Science 292, 2277–2280.

Varagona, M.J., Purugganan, M., Wessler, S.R., 1992. Alternative splicing induced by insertion of retrotransposons into the maize waxy gene. Plant Cell 4, 811–820.

Vaucheret, H., Fagard, M., 2001. Transcriptional gene silencing in plants: targets, inducers and regulators. Trends Genet. 17, 29–35.

Vaucheret, H., Beclin, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J.B., Mourrain, P., Palauqui, J.C., Vernhettes, S., 1998. Transgene-induced gene silencing in plants. Plant J. 16, 651–659.

Vernhettes, S., Grandbastien, M.A., Casacuberta, J.M., 1998. The evolutionary analysis of the Tnt1 retrotransposon in Nicotiana species reveals the high variability of its regulatory sequences. Mol. Biol. Evol. 15, 827–836.

Vernhettes, S., Grandbastien, M.A., Casacuberta, J.M., 1997. In vivo characterization of transcriptional regulatory sequences involved in the defence-associated expression of the tobacco retrotransposon Tnt1. Plant Mol. Biol. 35, 673–679.

Vicient, C.M., Jaaskelainen, M.J., Kalendar, R., Schulman, A.H., 2001a. Active retrotransposons are a common feature of grass genomes. Plant Physiol. 125, 1283–1292.

Vicient, C.M., Kalendar, R., Schulman, A.H., 2001b. Envelope-class retrovirus-like elements are widespread, transcribed and spliced, and insertionally polymorphic in plants. Genome Res. 11, 2041–2049.

Vicient, C.M., Schulman, A.H., 2002. Copia-like retrotransposons in the rice genome: few and assorted. Genome lett. 1, 35–47.

Vicient, C.M., Suoniemi, A., Anamthawat-Jonsson, K., Tanskanen, J., Beharav, A., Nevo, E., Schulman, A.H., 1999. Retrotransposon BARE-1 and Its Role in Genome Evolution in the Genus Hordeum. Plant Cell 11, 1769–1784.

Vignols, F., Rigau, J., Torres, M.A., Capellades, M., Puigdomenech, P., 1995. The brown midrib3 (bm3) mutation in maize occurs in the gene encoding caffeic acid O-methyltransferase. Plant Cell 7, 407–416.

Wang, L., Heinlein, M., Kunze, R., 1996. Methylation pattern of Activator transposase binding sites in maize endosperm. Plant Cell 8, 747–758.

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

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

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

White, S.E., Habera, L.F., Wessler, S.R., 1994. Retrotransposons in the flanking regions of normal plant genes: a role for copia-like elements in the evolution of gene structure and expression. Proc. Natl. Acad. Sci. USA 91, 11792–11796.

Witte, C.P., Le, Q.H., Bureau, T., Kumar, A., 2001. Terminal-repeat retrotransposons in miniature (TRIM) are involved in restructuring plant genomes. Proc. Natl. Acad. Sci. USA 98, 13778–13783.

Wright, D.A., Voytas, D.F., 2002. Athila4 of Arabidopsis and Calypso of soybean define a lineage of endogenous plant retroviruses. Genome Res. 12, 122–131.

Xiong, Y., Eickbush, T.H., 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J. 9, 3353–3362.

Yang, G., Dong, J., Chandrasekharan, M.B., Hall, T.C., 2001. Kiddo, a new transposable element family closely associated with rice genes. Mol. Genet. Genomics 266, 417–424.

Yoshioka, Y., Matsumoto, S., Kojima, S., Ohshima, K., Okada, N., Machida, Y., 1993. Molecular characterization of a short interspersed repetitive element from tobacco that exhibits sequence homology to specific tRNAs. Proc. Natl. Acad. Sci. USA 90, 6562–6566.

Yu, Z., Wright, S.I., Bureau, T.E., 2000. Mutator-like elements in Arabidopsis thaliana. Structure, diversity and evolution. Genetics 156, 2019–2031.

Zhang, Q., Arbuckle, J., Wessler, S.R., 2000. Recent, extensive, and preferential insertion of members of the miniature inverted-repeat transposable element family Heartbreaker into genic regions of maize. Proc. Natl. Acad. Sci. USA 97, 1160–1165.

Zhang, X., Feschotte, C., Zhang, Q., Jiang, N., Eggleston, W.B., Wessler, S.R., 2001. P instability factor: an active maize transposon system associated with the amplification of Tourist-like MITEs and a new superfamily of transposases. Proc. Natl. Acad. Sci. USA 98, 12572–12577.

Zhong, C.X., Marshall, J.B., Topp, C., Mroczek, R., Kato, A., Nagaki, K., Birchler, J.A., Jiang, J., Dawe, R.K., 2002. Centromeric retroelements and satellites interact with maize kinetochore protein CENH3. Plant Cell 14, 2825–2836.