LTR-retrotransposons and MITEs: important players in the evolution of plant genomes

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Retrotransposons are an abundant and ancient component of plant genomes, yet recent evidence indicates that element activity in many modern plants is restricted to times of stress. Stress activation of plant retrotransposons may be a significant factor in somaclonal variation, in addition to providing an important means to isolate new active elements. Long terminal repeat retrotransposons and a second class of elements we have called miniature inverted-repeat transposable elements (MITEs) have recently been found to be associated with the genes of diverse plants where some contribute regulatory sequences. Because of their sequence diversity and small size, MITEs may be a valuable evolutionary tool for altering patterns of gene expression.

Current Opinion in Genetics & Development 1995, 5:814–821

Introduction

Plant transposable element research in the past decade has focused on the DNA elements responsible for unstable mutations. Elucidation of the molecular basis for the often complex and diverse phenotypes produced continues to reveal aspects of normal plant development and gene regulation. In addition, the high level of element activity coupled with their ability to transpose in heterologous species have made DNA elements valuable tools in the tagging and isolation of plant genes. These subjects have been recently surveyed [1–4], so will not be covered in this review.

An unfortunate consequence of the success of heterologous tagging protocols is that few spontaneous mutations have been sought and analyzed in most plant species. This has an important impact on the scope of plant transposable element research because natural mutations provide a rich source of the active mobile element complement in a genome. In *Drosophila melanogaster*, for example, retrotransposons such as *copia*, *gypsy* and *412* are responsible for as much as 80% of spontaneous mutations [5]. In the past few years, this shortcoming in plant studies has begun to be redressed. Through a combination of mutant analysis, characterization of restriction fragment linked polymorphisms (RFLPs) within populations, and PCR and computer-based surveys of plant genes and genomes, virtually all classes of transposable elements have been found in plants. These studies indicate that two classes of elements, long-terminal repeat (LTR) retrotransposons and miniature inverted-repeat transposable elements (MITEs) are important components of plant genes and genomes. It is for this reason that we focus on these element classes in this review.

Retrotransposons

Retrotransposons, like other members of the retroelement family of transposable elements, move via a RNA intermediate. The retroelement family is composed of retroviruses, LTR-retrotransposons, long interspersed nuclear elements (LINEs), short interspersed nuclear elements (SINEs) and processed pseudogenes [6]. LTR-retrotransposons are flanked by long terminal repeats and encode all the proteins required for their transposition: capsid protein (encoded by *gag*), protease, integrase, reverse transcriptase and RNase H. They can be further classified as either *copia*-like or *gypsy*-like, depending on the order of their coding domains [7]. LINEs (also called non LTR retrotransposons) encode most of the same proteins as the LTR retrotransposons, but have no LTRs, having instead a characteristic run of adenosine residues at their extreme 3′ end [7]. SINEs and processed pseudogenes are also terminated by an A-rich tail but, unlike LINEs, they have sequence similarity to host genes. Unlike other retroelements, they do not encode reverse transcriptase.

Abbreviations

Ac—activator; **LINE**—long interspersed nuclear element; **LTR**—long terminal repeat; **MITE**—miniature inverted-repeat transposable element; **RT**—reverse transcriptase; **SINE**—short interspersed nuclear element; **Spm**—Suppressor mutator; **UTR**—untranslated region.

 ISSN 0959-437X

Mutant alleles

To date, all of the retroelements found in mutant alleles in plants have LTR-retrotransposons, including both *copia*-like and *gypsy*-like elements, although the former clearly predominate. Other than the tobacco element *Tnt1*, first isolated from a nitrate reductase mutation in cell culture [8], these elements have all been isolated from maize. An *adh1* mutation yielded the *Bs1* element [9], and five *waxy* mutations yielded four distinct elements: *Stonor* from *wxStonor* [10]; *Hopscotch* from *wxK* [11•]; the B5 family from two independent mutations, *wxB5* and *wxG* [10]; and the *gypsy*-like element *Magellan* from *wxM* [12]. Furthermore, members of two of these element families have turned up in two recently described mutations: *bm-3* contains a *B5* element [13], and *pl-987* results from insertion of a *Magellan* element (PS Cooper, KC Cone, personal communication).

Retrotransposon activity is inducible

The retrotransposons responsible for new mutations share a striking and unexpected property: element transcripts have not been detected during normal plant development (with one exception being rare *Tnt1* transcripts in adult roots). In contrast, retrotransposon transcripts can be quite abundant in yeast [14] and *Drosophila* [15], and transcripts of the plant DNA elements *Activator* (*Ac*), *Suppressor mutator* (*Spm*) and *Mutator* are readily detected in most tissues [16–18].

How can retrotransposons that are not active during normal plant development be responsible for a significant fraction of new spontaneous mutations? One answer to this question is that transcription of several plant elements is inducible. Transcription of the *Tnt1* element is induced when tobacco cells are treated with fungal extracts containing cell wall hydrolases (to remove the cell wall and produce protoplasts prior to cell culture initiation) [19]. A component of the fungal extract, called an elicitor, was found to be specifically responsible for induction. *Tnt1* can also be induced by a broad spectrum of microbial and fungal elicitors that are all able to activate the plant defense response — the hypersensitive response [20•].

To test the extent of retrotransposon activation, Hirochika [21] devised a method using PCR with cDNA (reverse transcriptase [RT]-PCR) to identify conserved sequences in the RNA isolated from cultured tobacco cells. Characterization of amplified sequences led to the identification of two retrotransposons, *Tto1* and *Tto2*, whose transcripts could be detected in cultured but not normal plant cells. The first active retrotransposons from rice have been isolated recently using the RT-PCR protocol with RNA from cultured cells ([22]; H Hirochika, K Sugimoto, Y Otsuki, M Kanda, personal communication) — transcripts from three elements, *Tos10*, *17* and *19*, were detected in cultured, but not normal, cells. These results demonstrate that tissue culture conditions can activate the transcription of retrotransposons in evolutionarily diverse monocotyledenous and dicotyledenous plants. Application of the RT-PCR protocol to a wide variety of plant species offers the exciting opportunity of isolating a bumper crop of active retrotransposons.

Somaclonal variation and retrotransposon induction

Somaclonal variation is the term given to the high frequency of mutations encountered among plants regenerated from cell culture [23]. Previous experiments suggested that the activation of dormant DNA elements may have been at least partially responsible for these mutations. *Ac* and *Spm* activity was detected in a few plants derived from cultured embryos lacking all such activity [24,25], but unfortunately, the activated elements could not be recovered and the mechanism of activation was not determined. It was suspected that element activation involved the demethylation of highly methylated (so-called cryptic) elements that may be present in all maize strains [26]. The demonstration that plants regenerated from cultured tobacco or rice cells possess new retrotransposon insertions establishes a connection between transcription induction and retrotransposition (H Hirochika, K Sugimoto, Y Otsuki, M Kanda, personal communication). Of these new insertions it was found that seven of eight insertions of *Tos17* were in single-copy sequences, and four of these were in the coding regions of transcribed genes. Given the frequency of these insertions, it is likely that retrotransposon-induced mutations are a major factor in somaclonal variation.

Variability in sequences that control transcription induction

It is not surprising that the tobacco and rice elements are all induced by cell culture, as expression in cell culture was the basis for their isolation. What is surprising is the nature of other factors that also induce these elements. *Tnt1*, for example (as mentioned above) is induced by microbial and fungal elicitors of the hypersensitive response, but not by viruses that induce this response [20•]. In contrast, *Tto1* and *Tto5* are activated when tobacco is infected with tobacco mosaic virus [22]. Finally, the rice *Tos17* element, unlike other elements tested, remains induced during prolonged cell culture, and there is no evidence that *Tos17* is activated by the hypertensive response (H Hirochiki, personal communication).

Retrotransposon transcripts initiate in the 5′ LTR and terminate in the 3′ LTR. Induction of *Tnt1* and *Tto1* transcription has been shown to be mediated by sequences in their respective 5′ LTRs. A 38 bp repeat in the 574 bp 5′ LTR of *Tto1* is necessary for its induction following viral infection [22]. Contained within this 38 bp repeat is a 13 bp repeat which is involved in the activation of *Tto1* during cell culture [22]. Similarly, the 610 bp 5′ LTR of *Tnt1* contains a 31 bp repeat (called the

BII box) that is necessary for its induction by microbial elicitors [27,28].

More than 100 copies of *Tnt1* elements are found in tobacco [8]. By sequencing a random sample of parts of these elements, it has been determined that the number of BII boxes is highly variable [29••]. Specifically, the repeat motif is particularly susceptible to deletions that are predicted to either alter the degree of inducibility or render the element uninducible. Thus, sequences involved in transcription induction may be highly mutable and this may facilitate either their diversification or their elimination. In this way, actively transcribed elements may give rise to new elements with altered patterns of regulation or, more commonly, to inactive copies. Casacuberta *et al.* [29••] point out that the BII box deletions are reminiscent of similar lesions that occur during retroviral integration. In those instances, the error-prone process of reverse transcription has been shown to be responsible. Two *Magellan* elements isolated from recently isolated mutations have also sustained large deletions ([12]; PS Cooper and KC Cone, personal communication), demonstrating that the production of defective elements may be a frequent consequence of plant retrotransposon insertions.

It is not yet known whether the transcription of any of the maize elements isolated from mutant alleles is inducible. However, the finding that transcription of the tobacco elements *Tto1* and *Tto5* is activated by viral infection [22] may help to explain how the defective maize element *Bs1* is able to retrotranspose. *Bs1* cannot promote its own retrotransposition because part of the element's reverse transcriptase gene has apparently been replaced with a fragment of a cellular gene by a process reminiscent of the transduction of oncogenes by retroviruses [30,31,32•]. Presumably, retrotransposition of *Bs1* into the *adh1* gene, occurring in a plant infected with an unrelated virus [33], was facilitated by reverse transcriptase activity provided in *trans* [34]. At first, it was thought that this activity was encoded by an autonomous member of the *Bs1* family, but no such element has been detected to date. Perhaps *Bs1* has survived by utilizing the reverse transcriptase activity of retrotransposons in the maize genome that are induced by viral infection. It should be noted, however, that this scenario also depends on the simultaneous presence of *Bs1* transcripts during viral infection, as element-encoded transcripts are intermediates in retrotransposition.

Plant retrotransposons: ubiquitous and diverse

Given the extremely low activity and restricted expression of the retrotransposons described above, one might get the impression that retrotransposition is tightly regulated and that retrotransposons cannot establish a significant presence in plant genomes. In fact, recent studies demonstrate that retrotransposon-derived sequences are an abundant component of many plant genomes. These studies rely on either the detection of degenerate retrotransposons or the isolation of highly repeated sequences without regard to their origin.

The results of a PCR-based survey to detect *copia*-like retrotransposons have revealed that this element class is ubiquitous in plant genomes [35–37]. Furthermore, sequences isolated from individual plants are usually extremely diverse, far more so than similar sequences amplified from *Drosophila* and yeast [38,39]. Copy number alone cannot explain the greater diversity, because the extremely small *Ta* family of *copia*-like retrotransposons of *Arabidopsis thaliana* are also highly diverse [38]. It is more likely that this sequence heterogeneity reflects an ancient association between plants and *copia*-like retrotransposons.

Computer-based sequence similarity searches using copia-like elements as query sequences have uncovered retrotransposon insertions in the 5′ and 3′ regions of >30 normal plant genes ([11•]; S White, S Wessler, unpublished data). Fixation of retrotransposon sequences near normal genes, coupled with the degenerate nature of these sequences, is taken as additional evidence for an ancient association between *copia*-like retrotransposons and plant genomes. Failure to detect similar associations between this element class and the genes of yeast and *Drosophila*, where retrotransposons are abundant, could indicate a more recent invasion of retrotransposons into these genomes. Alternatively, selection for small genome size or differences in genome architecture might preclude similar associations in yeast and *Drosophila*. It may be relevant that plant genes are now known to be separated by huge regions of intergenic repetitive sequences. Of a 280 kb region containing the maize *adh1* gene, 197 kb comprises at least 37 classes of middle and highly repetitive DNA. A significant fraction of these sequences are thought to be of retrotransposon origin [40]. Perhaps the retrotransposon sequences found in 5′ and 3′ flanking regions of genes represent the borders of these huge intergenic domains.

Many retrotransposons have been identified as extremely abundant sequences in some plants. These studies indicate that retrotransposons can attain phenomenal copy numbers and their amplification may be primarily responsible for some very large plant genomes. *BARE1*, for example, a *copia*-like retrotransposon in barley, is present in ~50 000 copies [41]. Similarly, the *Bis*-1 family of *copia*-like retrotransposons may account for 5% of the wheat genome [42].

The incredible potential of amplification via retrotransposition in plants is dramatically illustrated by members of the genus *Lilium*. The 14 species have enormous genomes of 30–45 Mkb [43], the size of which may be largely the result of unrestrained retrotransposition. The 13 000 copies of the 9.35 kb *gypsy*-like retrotransposon *del1* comprises 120 000 kb or 0.4% of the genome of *L. henryi* [44]. *L. longiflorum* has >40 000 copies of *del1* which account for at least 1% of its 34 Mkb genome [45]. Comparison of the copy number of *del1* with

the phylogeny of *Lilium* suggests that *del1* amplification has occurred in sudden sporadic bursts [46]. The LINE *del2*, which predominates in other lily species, may be the most abundant transposable element in nature. Incredibly, 4% of the *L. speciosum* genome comprises 250 000 copies of this 4.45 kb element [45].

The plant retrotransposon paradox

From the examples cited above, it is clear that plant retrotransposon activity has been very high in both the distant and recent past. Yet, retrotransposon activity in modern plants appears to be extremely low: most active elements isolated to date are not transcribed during normal development. Perhaps retrotransposon activity has to be restrained during somatic development because, at that time, plants are uniquely susceptible to the deleterious effects of retrotransposition. After all, unlike animals which set aside a germ-line very early in development, plant germ cells derive from somatic (meristematic) cells that continue to divide throughout development. Transcription of retrotransposons in these cells could be catastrophic, because each transcript represents a potential new insertion. As cells divide, element copy number can theoretically increase exponentially in lineages that will go on to produce gametes.

At least two models can be employed to explain why retrotransposons are not transcribed during development. The first model proposes that retrotransposons are epigenetically silenced, perhaps by a mechanism analogous to the reversible inactivation of DNA elements or transgenes. *Ac*, *Spm* and *Mutator* are frequently maintained in the maize genome in a reversibly inactive form. Inactivation correlates with the methylation of element sequences (reviewed in [4]). To date, the methylation state of most plant retrotransposons has not been examined in either normal or cultured cells. The existence of cellular mechanisms that reversibly inactivate plant transgenes has received a great deal of attention recently. Gene silencing is the general name given to what is probably a collection of interrelated phenomena that prevent gene expression either at the transcriptional or post-transcriptional level (reviewed in [47,48]). It has been suggested that gene silencing is actually a manifestation of a global system that has evolved to repress the activity of endogenous transposable elements [49]. It remains to be determined whether active retrotransposons are silenced by similar processes during plant development.

The second model predicts that the LTRs of plant retrotransposons contain transcriptional silencers or that they lack *cis*-elements recognized by transcriptional activators during normal development. This possibility is easier to reconcile with the finding that the LTRs of *Tnt1* and *Tto1* contain *cis*-elements necessary for the induction of transcription in cultured or stressed cells [22,28]. Furthermore, constructs containing the LTR of *Tnt1* do not promote transcription if they are not induced. Given the diversity of the active elements currently available (no two LTRs have significant sequence similarity), it is difficult to imagine how they have all evolved LTRs that go unrecognized during plant development. On the other hand, retrotransposon expression during normal development may reduce the fertility of the host to such an extent that those elements are quickly eliminated from the population.

The significance of retrotransposon induction

The activation of some plant retrotransposons during viral infection may represent a mechanism which promotes their horizontal transfer to a new host [38]. In accordance with this view, plant viruses, which are known to package cellular mRNAs, could serve as vectors for the horizontal transfer of retrotransposon transcripts.

The results of recent experiments suggest that if elements can get into another organism they would probably not be restricted by a requirement for host-specific factors. Retrotransposition of the tobacco *Tnt1* element in *A. thaliana* [50••] was the first example of the transposition of a retrotransposon after introduction into a heterologous host species (in plants or animals). Interestingly, the proliferation of *Tnt1* in *A. thaliana* indicates that the small genome of this plant is not a consequence of an inability to support retrotransposition. Perhaps even more surprising was the capacity of the tobacco *Tto1* element to retrotranspose in the distantly related monocot species rice [22]. Like these retrotransposons, the maize DNA elements *Ac* and *Spm* can also transpose in a wide variety of distantly related species (reviewed in [2]). It appears that host factors involved in both transposition and retrotransposition are conserved throughout higher plants, a factor that may favor their horizontal movement.

To date, only two elements, *Tto1* and *Tto5* have been shown to be activated by viral infection [22]. Other conditions of induction, such as cell culture or microbial elicitors, do not fit as easily into a horizontal transfer scenario. Alternatively, elicitors, cell culture, viral infection and the plant defense response can all be described as stress conditions. As such, the relative silence of plant retrotransposons during normal development and their activation by stress is consistent with the role envisioned for transposable elements by McClintock [51]. That is, elements may be entities that exist in a quiescent state in the genome, but that can be activated to promote genome restructuring when an organism's survival is threatened. With the identification of retrotransposon-derived sequences near many plant genes [11•], the role, if any, of this element class in genome evolution can now be tested directly.

MITEs

A new class of element in plant genes

As discussed above, retrotransposons are found in the 5′ and 3′ flanking regions of many plant genes, often <1 kb from transcription start or stop sites [11•]. Only a single instance is known of a plant retrotransposon in an intron [52]. In contrast, two classes of retroelements, LINEs and SINEs, have been found to predominate in mammalian genes. An enormous number of elements can be found in a single gene; for example, 17 LINE-1 elements are found within the 65 kb mouse β-globin complex [53], and the 57 kb human hypoxanthine phosphoribosyltransferase gene contains 49 copies of the SINE *Alu* [54]. The identification of SINEs in both dicot and monocot genes indicates that this class of element is able to retrotranspose in the genomes of diverse plants [55–57] and even attain very high copy numbers (e.g. 50 000 copies of the *TS* family in tobacco [55]). On the basis of current data, however, SINEs do not appear to be prevalent in all plant genomes; for example, only two SINEs have been found in the hundreds of grass genes sequenced to date [57].

Rather than containing large numbers of SINEs or LINEs like mammalian genes, many plant genes harbor a different class of mobile element. A 128 bp insertion in the maize *wxB2* allele led to the identification of what may be a new class of element that is frequently associated with the genes of diverse flowering plant species. The *B2* insertion is a member of a larger family, called *Tourist*, found in maize genes and in the genes of the other cereal grasses, barley, sorghum and rice [58,59]. Many *Tourist* elements are only distantly related to each other (with 50–70% sequence identity), indicating that they transposed long ago and have subsequently diverged. Two recent insertions (from the mutant alleles *wxB2* and *hm1::dHbr-1062* [60]) serve to define *Tourist* subfamilies (*B2* and *Hbr*) that are still active in maize. These subfamilies, each with >10 000 copies, are themselves distantly related.

An insertion in a sorghum *Tourist* element led to the discovery of a second family of elements, called *Stowaway*, in the genes of both monocots and dicots [61•]. *Tnr1*, a repetitive sequence found in ~3500 copies in rice is also a member of the *Stowaway* family [62]. *Tourist* and *Stowaway* share structural, but not sequence, similarity. For instance, both are short (*Tourist* 113–343 bp and *Stowaway* 80–323 bp), have no coding potential, have conserved terminal inverted repeats, have potential to form DNA secondary structure, and have target site preference (*Tourist*, TAA; *Stowaway* for TA).

The predominance of short inverted repeat element families such as *Tourist* and *Stowaway* in plant genomes has been suggested by a systematic computer analysis of repeated sequences in the non-coding regions of rice genes (T Bureau, P Ronald, S Wessler, unpublished data). This study identified nine repeat families, including four new families of short inverted-repeat elements. Together, these elements are the most prevalent type of transposon found in the rice genes surveyed (and possibly in the genes of most flowering plants). We refer to all of these diverse families (including *Tourist* and *Stowaway*) as MITEs. The sequences and structures of MITEs are clearly distinct from those of SINEs or LINEs. Thus, although mobile elements are associated with both mammalian and plant genes, the identity of these elements is strikingly different.

MITEs contribute regulatory sequences to plant genes

Much of the speculation concerning the role of plant transposable elements in gene and genome evolution stems from the many examples of mutations caused by mobile element insertions [63]. Clearly DNA elements such as *Ac*, *Spm* and *Mutator* have the potential to alter gene expression patterns by, for example, behaving as introns [64] or creating new promoters with altered tissue specificity [65]. The contribution of these elements to normal gene expression may be minimal, because *Ac*, *Spm* and *Mutator* sequences are rarely found near normal wild-type genes (TE Bureau, unpublished data). Most MITEs, on the other hand, have been identified in the non-coding regions of normal genes, where several appear to provide regulatory sequences involved in transcription initiation and polyadenylation [58,59,61•]. Despite these examples, MITE contribution to normal gene expression is equally uncertain because it is not as yet known whether the insertion of any MITE has altered the pre-existing pattern of gene expression. A *Tourist* element that furnishes the core promoter (TATA box and transcription start site) of the maize auxin binding protein 1 (*abp1*) gene may afford such an opportunity. Its absence in some of the wild progenitors of maize will permit a comparison of closely related *abp1* genes that differ, in part, by the presence of the *Tourist* element (TE Bureau, S Wessler, unpublished data).

Another opportunity to firmly establish a role for MITEs in altering gene expression patterns may be found at the other end of the gene. Many members of the active *Tourist* subfamily *Hbr* have been discovered in the 3′ untranslated region (UTR) of transcribed maize genes (J Gray, G Johal, S Briggs, personal communication). Perhaps by coincidence, the original *Hbr* element was also a 3′ UTR insertion that inactivated the *hm1* gene [60]. The association of *Hbr* family members with the 3′ UTR of normal and mutant genes may signal a role for this subfamily in the regulation of mRNA stability or even translation initiation.

How might MITEs transpose?

Although MITE families such as *Tourist* and *Stowaway* have no sequence similarity, their shared structural features suggest that they transpose by a common mechanism. Whether that mechanism involves a DNA or RNA intermediate is not yet known. Characteristics of both families could be used to argue for either of

these modes. Like most DNA elements, for example, MITEs have short conserved terminal inverted repeats. If they are DNA elements, then they must be mobilized by transposase activity encoded by another element or genetic locus. Furthermore, no MITE has been shown to excise, which may indicate low transposase activity or that the available MITEs are no longer active.

Infrequent excision and conserved secondary structure are features that these elements share with retroelements, specifically SINEs. Similarly, very high copy number has been attained only by retroelements, as each transcript is a potential transposition intermediate and each insertion is essentially permanent. However, if MITEs are retroelements, it is unclear how they are transcribed because they appear to lack promoters, nor do they have terminal poly (A) tracts like LINEs or SINEs. Once transcribed, reverse transcriptase activity would also have to be provided in *trans*. With no obvious mode of transcription, reverse transcriptase or insertion, it seems unlikely that MITEs are retroelements. If they transpose via a DNA intermediate, they would represent the only DNA elements that are frequently associated with normal genes. More importantly, their mechanism of transposition has allowed them to attain higher copy numbers than all previously described DNA elements.

Conclusions

Whereas SINEs and LINEs are frequently encountered in mammalian genes, LTR-retrotransposons and MITEs appear to predominate in the genes of most flowering plants. It will be interesting to see whether this difference between mammalian and plant genes reflects an evolutionary accident or whether their distinct lifestyles have led to the proliferation of distinct element classes. Similarly, the inactivity of retrotransposons during normal plant development may reflect a unique adaptation to the plant lifestyle where retrotranspositions occurring during normal development can be inherited. For each active element, it remains to be determined whether repression of transcription is an active process, mediated by a mechanism akin to gene silencing, or a passive process, involving selection for LTRs that cannot initiate transcription in unstressed plant cells. From a practical point of view, stress induction and the ability to be mobilized in heterologous plant backgrounds are features of plant retrotransposons that should hasten their isolation and analysis in the future.

Acknowledgements

We thank Steve Briggs and Hirohiko Hirochika for providing results prior to publication and Lane Arthur for critical reading of the manuscript. Our work has been supported by a research grant from the National Institutes of Health to SR Wessler.

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