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Functional analysis of the tobacco *Tnt1* retrotransposon

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Abstract

Retroelements represent by far the largest and most widespread class of mobile genetic elements. Representatives of several classes of retrotransposons have been characterized in a broad range of plant species, but only a few of them have been shown to be active. Among these, the tobacco *Tnt1* retrotransposon has been isolated after insertional mutagenesis and is one of the very few to be transcriptionally active. *Tnt1* expression is strongly regulated in a tissue-specific and developmental manner. Moreover, *Tnt1* expression is induced by a range of biotic or abiotic elicitors, which all have in common the ability to induce the plant defense response. Regulatory sequences involved in this elicitor-mediated induction have been located in the LTR U3 region. The link between *Tnt1* activation and the plant defense response might represent an example of the involvement of transposable elements in genome restructurations needed in response to environmental fluctuations such as pathogen attacks.

Introduction

Mobile genetic elements were originally discovered in higher plants by maize cytogeneticists such as Barbara McClintock, who first defined the concept of transposition. Since then, transposable elements of several types have been discovered in a wide range of organisms, from bacteria to higher eukaryotes. They have been divided into two major classes according to their mode of propagation. Class I elements (also defined as 'retroelements') transpose via formation of a daughter copy by reverse transcription of an RNA intermediate, while class II (or 'classical') elements move from DNA to DNA. Retroelements represent by far the largest and most widespread class of mobile genetic elements.

The fundamental characteristic of all transposable elements is their ability to insert at different positions in the genome and to alter the function of the genes with which they become associated. However, the origin and the biological role of these ubiquitous mobile sequences remain largely unknown. Through creation of genetic variability, they might play an important role as a source of genome plasticity needed for evolution. Transposition is not a random process, and is controlled by several regulatory systems originating from the element itself, from the host organism, and from external signals. Duplicative transposition by reverse transcription, in particular, is a highly mutagenic process, and efficient control mechanisms are necessary to allow the coexistence of host genomes and elements competent for transposition. This control is thought to occur all along the transposition process, from transcription of the parental copy up to insertion of the daughter copy.

The first – and the best studied – step regulating retrotransposition is transcription, which controls both the production of the genomic RNA used as a template for reverse transcription and the production of messenger RNA species involved in the synthesis of structural and catalytic proteins necessary for retrotransposition. The importance of transcriptional control is moreover greatly increased by the fact that in many documented cases, the element's transcriptional induction correlates directly with its ability to mutate the gene in which it has inserted, as shown for several *Drosophila* elements, in particular *gypsy* (Corces & Geyer, 1991), but also for *Ty* elements (Boeke & Corces, 1989) and

for mammalian endogenous retroviruses (Wilson, Policastro & Fredholm, 1988).

The transcription of retrotransposons in *Drosophila* is regulated according to specific temporal, tissular and hormonal patterns, but is also dependent on chemical or environmental stresses such as heat shock or tissue culture (for a review see Echalier, 1989). Moreover, the transcription of the *Drosophila* retrotransposons has also been shown to depend on host genes (Boeke & Corces, 1989; Corces & Geyer, 1991). Similarly, the RNA level of the yeast *Ty* elements is regulated by the cell mating type, by pheromone induction, by stresses such as UV light irradiation, but also by several host regulator genes (Boeke, 1989). In vertebrate systems, the transcription level of integrated proviruses is dependent on the host-cell type (Varmus & Brown, 1989) and is also modulated by external agents such as other viral infections, or external chemical and physical stimuli (Chinnadurai, 1991).

However, regulation at the post-transcriptional level is also an important component of the control of retrotransposition. In particular, *copia* transcripts have been shown to accumulate in *Drosophila* tissues and at developmental stages where Virus-Like Particles (VLPs), important intermediates for retrotransposition, are not present (for a review see McDonald *et al.*, 1988). Similarly, although there is a good correlation between the steady state level of transcription of a given *Ty* element and its transposition rate, naturally or artificially induced increases of transposition are often not correlated with increases in transcription levels (Paquin & Williamson, 1988; Curcio & Garfinkel, 1991). Mechanisms of post-transcriptional control of retrotransposition rates are, however, still not clear. They could act at the level of protein processing or stability, or VLP formation. In particular, the production of the relative amounts of the two sets of proteins encoded by retrotransposons, the capsid gag and the catalytic pol proteins, is regulated in most systems. The molecular excess of gag needed to form competent VLPs is in most cases provided by a frameshift between the *gag* and *pol* domains, and the efficiency of the readthrough necessary for the production of the catalytic proteins is thought to be a critical point in the regulation of retrotransposition (Voytas & Boeke, 1993). Other key regulatory steps of retrotransposition might include the control of the priming of DNA synthesis by host factors, and the preferential insertion in non-transcribed regions of the genome, as shown for yeast elements (reviewed in Voytas & Boeke, 1993). Finally, the potential preferential *cis* action of retroviral catalytic proteins, which would reduce the movement of defective elements, has been proposed as another control mechanism by which the mutagenic effect of retrotransposons could be minimized (Curcio & Garfinkel, 1991).

It seems, therefore, that the general rule is for tight control of the activity of retrotransposons, which is necessary to preserve the viability of the host genome. The way in which this control is established seems, however, to be different in each case, and is probably the result of a long coevolution of each particular family of elements and their host organism. Transposable elements represent, therefore, fascinating models for the study of regulatory processes.

Plant retrotransposons: an overview

Although retroelements have only recently been discovered in higher plants, representatives of several classes of retrotransposons have now been characterized in a broad range of plant species, monocots as well as dicots (for a review see Grandbastien, 1992; Smyth, 1993; see Table 1 for a compilation of plant retrotransposons reported to date). Moreover, recent studies have shown that copia-type retrotransposons are ubiquitous components of plant genomes (Flavell *et al.*, 1992; Voytas *et al.*, 1992; Hirochika & Hirochika, 1993).

Since most plant elements reported to date have been isolated as inactive insertions, little is known about how plant retrotransposons function, but because of their structural similarities to animal and yeast retrotransposons, the mechanisms of transcription and transposition of plant elements are most probably very similar to those characterized for the former. However, to date, direct evidence for transpositional activity, through insertional mutagenesis, has only been obtained for the maize *Bs1* element (Johns, Mottinger & Freeling, 1985), the tobacco *Tnt1* element (Grandbastien, Spielmann & Caboche, 1989), the *Tnp2* element of *Nicotiana plumbaginifolia* (Vaucheret *et al.*, 1992), and the tobacco *Tto1* element, whose copy number was shown to increase during tissue culture (Hirochika, 1993), and which was also characterized in our laboratory after insertional mutagenesis (Grappin, unpublished). Indirect evidence of mobility has also been reported for the wheat *WIS-2* element, for which insertional polymorphism has been obtained between

Table 1. An overview of characterized plant retrotransposons. Retrotransposons have been listed in chronological order of their first report. References corresponding to each element or family can be found in Grandbastien (1992), except for: *Tos* (Hirochika, Fukuchi & Kikuchi, 1992), *Stonor and G(B5)* (Varagona, Purugganan & Wessler, 1992), *BARE-1* (Manninen & Shulman, 1993; Suoniemi, Arna & Schulman, 1994), *Tnp2* (Vaucheret *et al.*, 1992), and *Tto1* (Hirochika, 1993; Grappin, unpublished). *A. thaliana* = *Arabidopsis thaliana*; *N. plumba* = *Nicotiana plumbaginifolia*; nr = non reported.

Name	Species	Type	Copy number	Transposition	Expression
LTR retrotransposons					
Cin1	maize	solo LTR	1000	*nr*	*nr*
Bs1	maize	?	2–3	YES (Adh gene)	*nr* (link with viral infection?)
Wis-2	wheat	copia	200	YES	*nr* (anther culture?)
Ta	*A. thaliana*	copia	10 fam. of 2–3 copies	*nr*	*nr*
Tnt1	tobacco	copia	>100	YES (NR gene)	plant defense response + developmental regulation
del1	lily	Ty3	>13,000	*nr*	*nr*
Tst1	potato	copia	1	*nr*	*nr*
Tms1	alfalfa	?	?	*nr*	*nr*
PDR1	pea	?	50	*nr*	*nr*
IFG7	pine	Ty3	10,000	*nr*	*nr*
Tos	rice	copia	1000	*nr*	*nr*
Stonor	maize	?	?	*nr*	*nr*
G(B5)	maize	?	?	*nr*	*nr*
BARE-1	barley	copia	5000	*nr*	somatic tissues + tissue cult.
Tnp2	*N. plumba*	copia	?	YES (NR gene)	*nr*
Tto1	tobacco	copia	30	YES (NR gene)	tissue culture
Retrotransposons or non-LTR-retrotransposons					
Cin4	maize	LINE	50–100	*nr*	*nr*
del2	lily	LINE	250,000	*nr*	*nr*

plants regenerated from anther cultures (Moore *et al.*, 1991). Furthermore, direct transcriptional activity has only been shown for the tobacco *Tnt1* (Pouteau *et al.*, 1991) and *Tto1* (Hirochika, 1993) elements, and very recently for the barley *BARE-1* element (Suoniemi, Arna & Schulman, 1994) (see Table 1).

Since transcription is probably a key control of the retrotransposition process, the analysis of the expression of plant retrotransposons will provide fundamental information concerning the biological role of these elements and their importance in the evolution of plant genomes. We have therefore engaged in a detailed study of the conditions of expression of *Tnt1* as well as of the molecular basis of its expression and regulation.

Structural features of the tobacco *Tnt1* retrotransposon

Tnt1 was the first transposable element characterized in *Solanaceae* species, and was isolated from the tobacco (*Nicotiana tabacum*) genome in the course of a gene-trapping experiment designed to isolate active transposable elements through their insertional mutagenic activity. The nitrate reductase (NR) structural gene was used as a target gene, because a very simple direct procedure for isolating NR-deficient mutants from tobacco cell cultures was available, by *in vitro* selection of spontaneous chlorate-resistant cellular clones, followed by plant regeneration (Grafe, Marion-Poll & Caboche, 1986). *Tnt1* was found inserted into exons of the NR gene in three independent NR-deficient mutants (Grandbastien, Spielmann & Caboche, 1989). It is interesting to note that multiple transposition events

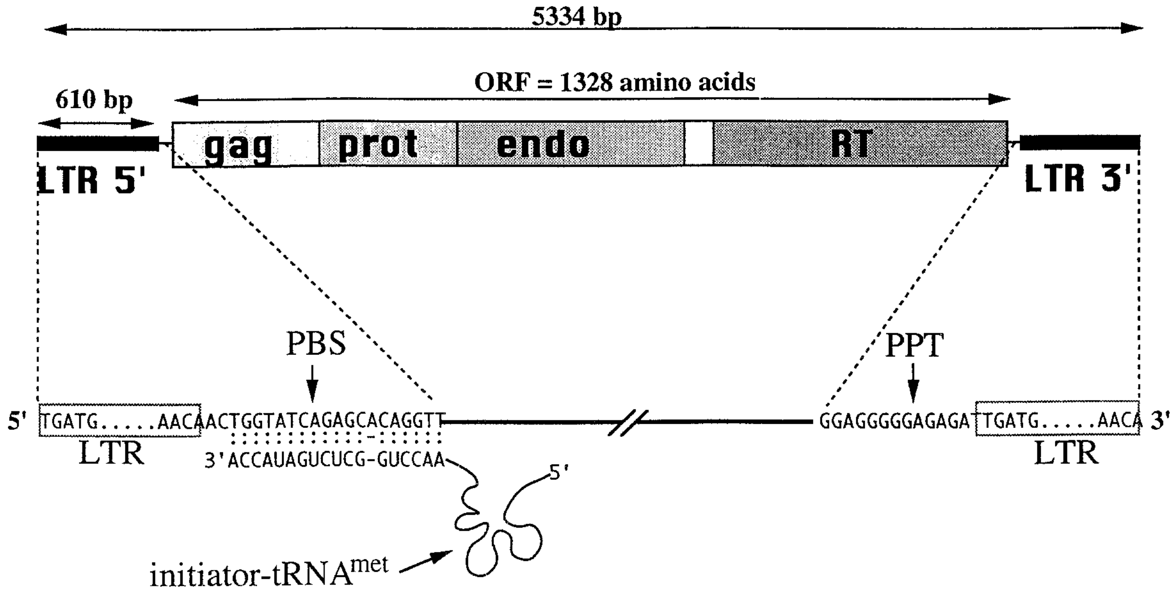

Fig. 1. Structural features of the tobacco *Tnt1* retrotransposon. The structure of the *Tnt1-94* mobile copy (Grandbastien, Spielmann & Caboche, 1989) is represented. Shaded boxes indicate regions of extensive amino acid homologies with the *Drosophila copia* element. Symbols are detailed in the text. Sequences of *Tnt1* Primer Binding Site (PBS) and Polypurine Tract (PPT) are shown below, as well as the complementarity between *Tnt1* PBS and the 3' end of bean and wheat initiator tRNA^{met}.

into the coding sequence of the NR gene, corresponding to insertions of different families of transposable elements, including the *Tto1* retrotransposon, were also obtained during this experiment (Grappin, unpublished). Selection for NR-deficient mutants was also used for the isolation of several mobile transposable elements in the closely related species *Nicotiana plumbaginifolia* (Vaucheret *et al.*, 1992; Meyer *et al.*, 1994), and for the isolation of several families of transposable elements from the fungal plant pathogen *Fusarium oxysporum* (Daboussi, Langin & Brygoo, 1992).

Tnt1 is 5.3 kb long and has typical features of retroviral-like elements (see Fig. 1). It is flanked by short duplications of 5 bp of the NR coding sequences and contains two perfect long terminal repeats (LTRs) terminated by the palindromic TG..CA sequence, as well as a single open reading frame (ORF) of 1328 amino acids with homologies to the gag-pol coding domains of retroviral elements. Sequences corresponding to the primer sites used by retroviral-like elements for DNA synthesis by reverse transcription are also found in untranslated areas of *Tnt1*: a Primer Binding Site (PBS) sequence nearly complementary to the 18 bp at the 3′ end of the initiator tRNA^{met} of bean and wheat (Gauss & Sprinzl, 1983) is found adjacent to the 5′ LTR, and a 11 bp Polypurine Tract (PPT) is found adjacent to the 3′ LTR. It is interesting to note that a sequence complementary to the initiator tRNA^{met} is also found in most other plant retrotransposons characterized so far. From N-terminus to C-terminus, the unique *Tnt1* ORF shows extensive amino acid homologies to copia gag (structural core proteins), prot (protease involved in maturation of gag polyproteins), endo (endonuclease involved in integration into the host DNA) and reverse transcriptase (RT) domains (see Fig. 1). Within these regions are found patches of very high homology with short domains shown to be conserved in all retroelements, including a DNA binding site (typical Zn finger) in the gag domain, and a potential protease active site. *Tnt1* is a member of the *Ty1-copia* retrotransposon family, and shows a very similar overall organization, as well as extensive amino acid homologies (up to 42% for the endo domain) with the *Drosophila copia* element.

Tnt1 is found in high copy number (estimated at more than 100) in the tobacco genome, and hybridization studies have shown that most family members have a conserved overall structure. Two different *Tnt1* insertions in the NR gene were sequenced, and shown to be 99% identical at both the nucleotide and amino acid levels (Longuet, unpublished). The LTR of a third insertion was also shown to be closely related to those of the two first elements (Grappin, unpublished). The

differences between the three inserted copies are too high to be explained by the average error rate of reverse transcriptase (Varmus & Brown, 1989), suggesting that the three elements originate from reverse transcription of three different parental copies. *Tnt1* has also been shown to hybridize to the DNA of several *Nicotianae* species, as well as to that of some other *Solanaceae* species, particularly in the genus *Lycopersicon* (Grandbastien *et al.*, 1991).

Regulation of *Tnt1* expression

Tnt1 was isolated after transposition, which indicates that the three different cloned elements, if not necessarily autonomous, are at least transcribed in the tobacco genome. *Tnt1* expression in tobacco was studied by transcript analysis and by analysis of the expression of a reporter gene placed under control of the LTR, known to contain the promoter and some of the regulatory sequences of retrotransposons. This LTR-GUS translational fusion, in which the *Tnt1* LTR, plus the untranslated leader sequence and the first 25 amino acids of the element's ORF, were fused to the β-glucuronidase (GUS) reporter gene, allowed analysis of the LTR promoter activity, both by quantitative biochemical testings, and by tissue-specific histochemical colorations (Jefferson, Kavanagh & Bevan, 1987). *Tnt1* expression was also studied in tomato (*Lycopersicon esculentum*), another *Solanaceae* species, and in *Arabidopsis thaliana*, which belongs to the more distant *Brassicae* family, after introduction of the LTR-GUS construct in these heterologous species.

Basal level of expression of Tnt1

Tnt1 expression was found to be strongly regulated in a tissue-specific and developmental manner, which indicates that transcriptional control might be the major step in the regulation of *Tnt1* transposition. In tobacco, no expression is detected in most tissues of the mature plant, except in roots (Pouteau *et al.*, 1991). A similar regulation of the expression of the *Tnt1* promoter was found in tomato and in *Arabidopsis*. In both species, it is poorly expressed in young foliar tissues, but clearly induced in roots, especially in the root crown (Moreau, unpublished; Morel & Lucas, unpublished). In tomato, its expression is increased in foliar tissues developed after floral induction (Moreau, unpublished). Expression was also clearly detected, both in tomato and in *Arabidopsis*, in certain floral organs, such as anthers and the stigma (Moreau, unpublished; Morel & Lucas, unpublished).

Very interestingly, no smaller spliced transcript was detected in tobacco tissues in which *Tnt1* is transcribed, in contrast to the closely related *Drosophila copia* element, which uses a transcript splicing mechanism to overexpress the structural gag proteins. This observation can, however, be correlated to recent analysis of the *Saccharomyces pombe Tf1* gypsy-type element, which also contains a single ORF and is thought to overexpress the gag proteins through selective degradation of the catalytic pol proteins (Levin, Weaver & Boeke, 1993).

Activation of Tnt1 *expression during the plant defense response*

In addition to its tissue-specific and developmental regulation, *Tnt1* expression is strongly induced in protoplasts freshly isolated from tobacco leaf mesophyll (Pouteau *et al.*, 1991). The *Tnt1* transcript level declines rapidly after protoplast isolation, suggesting that tissue culture in itself is not the inducing factor. *Tnt1* protoplast-specific expression was indeed shown to result mainly from the presence in the protoplast isolation medium of crude extracts of the pathogenic fungus *Trichoderma viride* (Pouteau *et al.*, 1991). Extracts of *T. viride* induce in tobacco a necrotic response known as the Hypersensitive Reaction (HR), which generally occurs during the interaction between a pathogen and a resistant plant, and corresponds to the induction of a number of plant genes involved in the plant defense reaction (Keen, 1990).

The expression of the *Tnt1* promoter was shown to be also induced in tobacco by other factors of microbial origin known to induce the plant defense response, such as elicitins, small proteins produced by *Phytophthora* fungal species, which elicit a necrotic reaction on tobacco, and are involved in the induction of disease resistance (Ricci *et al.*, 1993). Moreover, the induction by cryptogein and by capsicein, elicitins produced respectively by *P. cryptogea* and *P. capsici*, correlates tightly with the biological necrotic activity of both elicitins (Pouteau, Grandbastien & Boccara, 1994). *Tnt1* expression was also shown to be highly induced by the application, on tobacco cell cultures, of culture supernatants of the bacterium *Erwinia chrysanthemi* (Pouteau, Grandbastien & Boccara, 1994).

Tnt1 expression in tobacco is thus induced by different microbial factors, which all have in common the ability to elicit the plant defense response. The

expression of the *Tnt1* promoter was also shown to be induced in tomato and *Arabidopsis* by *T. viride* extracts, in tomato by cryptogein and by extracts of the pathogenic fungus *Cladosporium fulvum*, as well as in *Arabidopsis* by infections by the bacterium *Pseudomonas syringae*, or by several abiotic factors known to induce plant defense response (Moreau, unpublished; Morel & Lucas, unpublished). In all three species studied, a localized induction was observed after wounding, which is also known to induce some of the plant defense genes. It appears thus that the induction of the *Tnt1* promoter by microbial factors is maintained in heterologous species. Interestingly, however, its expression in *Arabidopsis* was also found to be induced by hormonal treatment with auxin-type compounds (Pauls *et al.*, 1994), which is not the case in tobacco, and indicates that there is also some host-specific differential control of the expression of *Tnt1*.

To summarize, our results show that *Tnt1* expression is induced by a range of biotic or abiotic elicitors, which all have in common the ability to induce plant defense genes, and suggest that there is a link between the induction of *Tnt1* and the induction of the plant defense response. The plant defense response is characterized by the derepression of batteries of different defense response genes, leading to the production of different compounds such as Pathogenesis-Related (PR) proteins (Ahl, 1983; Gianinazzi, 1984; Bol, Linthorst & Cornelissen, 1990), or plant antibiotics termed phytoalexins (Darwill & Albersheim, 1984), which all contribute to restricting pathogen development. The signalling mechanisms leading to the induction of these plant defense genes are not yet well understood, and vary depending on the plant-pathogen interaction. Moreover, several defense response genes are also induced during successful pathogen attacks, and can often also be triggered by other external stimuli such as wounding, chemicals, and by plant development factors (Hahlbrock & Scheel, 1989).

In order to elucidate the possible relationship between *Tnt1* activation and the different factors of the plant defense response, it seems therefore crucial to analyze *Tnt1* expression in well defined plant-pathogen interactions, such as those known as gene-for-gene reactions, in which the outcome of the pathogen attack, leading to resistance or to successful infection, is determined by specific genetic interations between the plant and the pathogen (Keen, 1990). It might therefore be possible to test *Tnt1* activation in situations leading to resistance or to sensitivity, and to establish a possible correlation between *Tnt1* activation and the differential

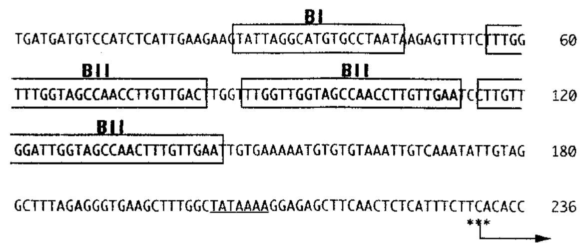

Fig. 2. Nucleotide sequence of the U3 region of the *Tnt1* LTR. The sequence shown corresponds to the U3 region of the *Tnt1–94* mobile copy (Grandbastien, Spielmann & Caboche, 1989). The first TATA box is underlined and the major transcriptional start is shown by asterisks and an arrow (Pouteau *et al.*, 1991). Sequences corresponding to the BI and BII elements are boxed.

induction of plant defense genes which usually characterizes these specific interactions.

Molecular basis of *Tnt1* expression

Transient expression assays and stable transformation studies have shown that the major *cis* acting sequences controlling *Tnt1* expression are located within the U3 region of the 5′ LTR. This U3 region is able to confer, to a minimal heterologous promoter, protoplast-associated expression in transient expression analysis (Casacuberta & Grandbastien, 1993), as well as protoplast- and cryptogein-specific induction in stably transformed tobacco plants (Casacuberta, unpublished). A detailed analysis of the LTR U3 region revealed two particularly interesting sequences, that we named BI and BII (Fig. 2), and that could be at least in part responsible for the elicitor-induced expression of *Tnt1*. Both sequences can act as transcriptional activators of a minimal heterologous promoter, and the BII sequence has been shown by gel retardation assays to interact specifically with tobacco nuclear factors induced during protoplast preparation (Casacuberta & Grandbastien, 1993). Moreover, the 31 bp tandemly repeated BII sequence contains short motifs that are homologous to H-boxes (Loake *et al.*, 1992) and to other *cis* acting elements (Warner, Scott & Draper, 1993) shown to be involved in the elicitor-mediated induction of several plant genes, thus confirming the possible implication of this sequence in the elicitor-associated induction of *Tnt1* expression.

It is interesting to note that a recent PCR analysis of the U3 regions found in the tobacco *Tnt1* family has shown that the number of BII sequences is highly

variable (from zero to four) in the total *Tnt1* genomic population, but is much more constant (three or four) in the population of actively transcribed *Tnt1* elements (Casacuberta, unpublished). Similarly, the BII sequence is found in three or four tandem repeats in the three *Tnt1* elements found inserted into the NR gene (Longuet, unpublished; Grappin, unpublished). These results confirm the importance of the BII sequence for *Tnt1* expression. Deletion of this sequence, which seems to be a frequent event, could lead to the inactivation of particular copies. This accumulation of defective copies could also contribute to the control of *Tnt1* transpositional activity, as proposed for *Ty1* (Curcio & Garfinkel, 1991).

Tnt1 as a genetic tool

Classical plant transposable elements, such as the maize *Ac* element, are widely used as genetic tools, in particular for the cloning of genes by gene-tagging (Balcells, Swinburne & Coupland, 1991). However, classical elements show preference for transposition into genes located nearby their original insertion site, and successful gene-tagging experiments require thus the availability of multiple insertion sites located on different chromosomes. Retrotransposons, whose transposition cycle includes a cytoplasmic intermediate, are expected to transpose to loci that are not linked to the original insertion site, and may therefore be interesting as alternatives to conventional plant elements. Due to the high copy number of *Tnt1* related elements detected in the tobacco genome, its utilisation as a gene tag seems difficult in its original host. However, *Tnt1* may be useful in heterologous plant species, such as *Arabidopsis*, which do not contain sequences that are closely related to *Tnt1*, and in which *Tnt1* is expressed. Recent results indicate that *Tnt1* is able to transpose in *Arabidopsis* (Lucas *et al.*, 1994). We have also shown that substitution of the U3 LTR sequences by heterologous enhancer sequences can alter the specificity of regulation of *Tnt1* (Pauls *et al.*, 1994). Since the replacement of these U3 sequences is expected to preserve the element's transpositional ability (Boeke *et al.*, 1985), modified *Tnt1* elements expressed in a suitable manner could be constructed. However, the site of integration of replicated copies of *Tnt1* will have to be analyzed in detail, to determine if *Tnt1* could be used as an efficient gene-tagging tool in *Arabidopsis*.

Conclusion and perspectives

The link between *Tnt1* expression and the plant defense response appears now as an increasingly clearer emerging picture. However, as the plant defense response is itself a complex process, the mechanisms involved are still not clear. The further characterization of *Tnt1* inducing conditions will hopefully provide a better understanding of this connection. In addition, the recent characterization of LTR sequences involved in *Tnt1* elicitor-associated induction could allow the molecular identification of the cell factor(s) involved in this regulation, which might represent the possible point linking *Tnt1* induction and the plant defense response.

The biological significance of this specific induction remains puzzling. It might represent a fortuitous ancestral capture of plant regulatory sequences by this family of mobile elements, and could have been maintained because it represents a rare situation, allowing this retrotransposon to move without affecting host viability. In addition, *Tnt1* induction during pathogen attack might favor contact between the element's cytoplasmic forms and different microbial agents, which could increase the possibility of horizontal transmission of the element, and would allow it to colonize new hosts. No answer to these crucial points will, however, be possible until evidence is provided that induction of transcription is correlated to induction of transposition.

Nevertheless, it seems hard to conceive that the maintenance of such a specific regulation would not have evolved to become somehow useful for the host plant. In most systems described, external signals or stresses represent important regulatory factors. Interestingly, the small handful of active plant retrotransposons seems to be expressed in very specific conditions only, with the notable exception of the barley *BARE-1* element, which has very recently been shown to be also expressed in unstressed leaves. The tobacco *Tto* elements are cryptic in the plant, but are expressed and transpose during tissue culture, while the wheat *WIS-2* element has been shown to transpose after anther culture. The maize *Bs1* element has transposed in plants submitted to viral infection, although the link between *Bs1* mobility and infection has not been demonstrated yet. All these activating conditions constitute examples of what McClintock (1984) called 'genomic shocks', which could be specifically involved in genome restructurations needed in response to environmental fluctuations. This possibility would indeed be particularly important for plants,

which cannot move to escape external constraints, one of the most important being the repeated attack of pathogens.

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References

Ahl, P., 1983. Aspects génétiques et moléculaires de la résistance (réaction d'hypersensibilité) chez les *Nicotiana*. Thèse de Docteur es Sciences, Université de Genève.

Balcells, L., J. Swinburne & G. Coupland, 1991. Transposons as tools for the isolation of plant genes. Trends Biotechnol. 9: 31–36.

Boeke, J. D., 1989. Transposable elements in *Saccharomyces cerevisiae*, pp. 335–374 in Mobile DNA, edited by D. Berg and M. M. Howe. American Society for Microbiology, Washington D.C., USA.

Boeke, J. D. & V. G. Corces, 1989. Transcription and reverse transcription of retrotransposons. Annu. Rev. Microbiol. 43: 403–434.

Boeke, J. D., D. J. Garfinkel, C. A. Styles & G. R. Fink, 1985. Ty elements transpose through an RNA intermediate. Cell 40: 491–500.

Bol, J. F., H. J. M. Linthorst & B. J. C. Cornelissen, 1990. Plant pathogenesis-related proteins induced by virus infection. Annu. Rev. Phytopathol. 28: 113–138.

Casacuberta, J. M. & M.-A. Grandbastien, 1993. Characterization of LTR sequences involved in the protoplast specific expression of the tobacco Tnt1 retrotransposon. Nucleic Acids Res. 21: 2087–2093.

Chinnadurai, G., 1991. Modulation of HIV-enhancer activity by heterologous agents: a minireview. Gene 101:165–170.

Corces, V. G. & P. K. Geyer, 1991. Interactions of retrotransposons with the host genome. Trends Genet. 7: 86–90.

Curcio, M. J. & D. J. Garfinkel, 1991. Regulation of retrotransposition in *Saccharomyces cerevisiae*. Mol. Microbiol. 5: 1823–1829.

Daboussi, M. J., T. Langin & Y. Brygoo, 1992. Fot1, a new family of fungal transposable elements. Mol. Gen. Genet. 232: 12–16.

Darwill, A. G. & P. Albersheim, 1984. Phytoalexins and their elicitors – A defense against microbial infection in plants. Ann. Rev. Plant Phys. 35: 243–275.

Echalier G., 1989. *Drosophila* retrotransposons: interactions with genome. Advances in Virus Research 36: 33–105.

Flavell, A. J., E. Dunbar, R. Anderson, S. R. Pearce, R. Hartley & A. Kumar, 1992. Ty1-copia group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucleic Acids Res. 20: 3639–3644.

Gauss, D. & M. Sprinzl, 1983. Compilation of tRNA sequences. Nucleic Acids Res. 11: r1.

Gianinazzi, S., 1984. Genetic and molecular aspects of resistance induced by infections or chemicals, pp. 321–342 in Plant-Microbe Interactions, Molecular and Genetic Perspectives, Vol. 1, edited by T. Kosuge and E. W. Nester. Macmillan, New York, USA.

Grafe, R., A. Marion-Poll & M. Caboche, 1986. Improved in vitro selection of nitrate reductase-deficient mutants on *Nicotiana plumbaginifolia*. Theor. Appl. Genet. 73: 299–304.

Grandbastien, M.-A., 1992. Retroelements in higher plants. Trends Genet. 8: 103–108.

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

Grandbastien, M.-A., A. Spielmann, S. Pouteau, E. Huttner, M. Longuet, K. Kunert, C. Meyer, P. Rouzé & M. Caboche, 1991. Characterization of mobile endogenous copia-like transposable elements in the genome of *Solanaceae*, pp. 333–343 in Plant Molecular Biology 2, edited by R. G. Herrmann and B. Larkins. Plenum Press, N. Y., USA.

Hahlbrock, K. & D. Scheel, 1989. Physiology and molecular biology of phenylpropanoid metabolism. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40: 347–369.

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

Hirochika, H., A. Fukuchi & F. Kikuchi, 1992. Retrotransposon families in rice. Mol. Gen. Genet. 233: 209–216.

Hirochika, H. & R. Hirochika, 1993. Ty1-copia group of retrotransposons as ubiquitous components of plant genomes. Jpn. J. Genet. 68: 35–46.

Jefferson, R. A., T. A. Kavanagh & M. W. Bevan, 1987. GUS fusions: β-glucuronidase as sensitive and versatile gene fusion marker in higher plants. EMBO J. 6: 3901–3907.

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

Keen, N. T., 1990. Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. 24: 447–463.

Levin, H. L., D. C. Weaver & J. D. Boeke, 1993. Novel gene expression mechanism in a fission yeast retroelement: Tf1 proteins are derived from a single primary translation product. EMBO J. 12: 4885–4895.

Loake, G. J., O. Faktor, C. J. Lamb & R. A. Dixon, 1992. Combination of H-box and G-box cis elements is necessary for feed-forward stimulation of a chalcone synthase promoter by the phenylpropanoid-pathway intermediate p-coumaric acid. Proc. Natl. Acad. Sci. 89: 9230–9234.

Lucas, H., J.-B. Morel, M. Caboche & M.-A. Grandbastien, 1994. The tobacco retrotransposon Tnt1 is functional in the heterologous host *Arabidopsis thaliana*. Keystone Symposium on Transposition and site-specific recombination: mechanism and biology, Abstract F 172, January 21–28, 1994, Park City, Utah, USA.

Manninen, I. & A. H. Schulman, 1993. BARE-1, a copia-like retroelement in barley (*Hordeum vulgare* L.). Plant Mol. Biol. 22: 829–846.

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

McDonald, J. F., D. J. Strand, M. R. Brown, S. M. Paskewitz, A. K. Csink & S. H. Voss, 1988. Evidence of host-mediated regulation of retroviral element expression at the post-transcriptional level, pp. 219–234 in Eukaryotic Transposable Elements as Mutagenic Agents, edited by M. E. Lambert, J. F. McDonald and I. B. Weinstein. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

Meyer, C., S. Pouteau, P. Rouzé & M. Caboche, 1994. Isolation and molecular characterization of dTnp1, a mobile and defective transposable element of *Nicotiana plumbaginifolia*. Mol. Gen. Genet. 242: 194–200.

Moore, G., H. Lucas, N. Batty & R. Flavell, 1991. A family of retrotransposons and associated genomic variation in wheat. Genomics 10: 461–468.

Paquin, C. E. & V. M. Williamson, 1988. Effect of temperature on Ty transposition, pp. 235–244 in Eukaryotic Transposable Elements as Mutagenic Agents, edited by M. E. Lambert, J. F. McDonald and I. B. Weinstein. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

Pauls, P.K., K. Kunert, E. Huttner & M.A. Grandbastien, 1994. Expression of the tobacco Tnt1 retrotransposon promoter in heterologous species. Plant Mol. Biol. (In press).

Pouteau, S., M.-A. Grandbastien & M. Boccara, 1994. Microbial elicitors of plant defense responses activate transcription of a retrotransposon. The Plant Journal 5: 535–542.

Pouteau, S., E. Huttner, M.-A. Grandbastien & M. Caboche, 1991. Specific expression of the tobacco Tnt1 retrotransposon in protoplasts. EMBO J. 10: 1911–1918.

Ricci, P., F. Panabières, P. Bonnet, N. Maia, M. Ponchet, J.-C. Devergne, A. Marais, L. Cardin, M. L. Milat & J.-P. Blein, 1993. Proteinaceous elicitors of plant defense responses, pp. 121–135 in Mechanisms of Plant Defense Responses, edited by B. Fritig and M. Legrand. Kluwer Academic Publishers, N.L.

Smyth, D. R., 1993. Plant retrotransposons, pp. 1–15 in Control of Plant Gene Expression, edited by D. P. S. Verma. CRC Press, Boca Raton, USA.

Suoniemi, A., T. Arna & A. H. Schulman, 1994. Analysis of barley retrotransposon BARE-1 expression. Keystone Symposium on Transposition and site-specific recombination: mechanism and biology, Abstract F 224, January 21–28, 1994, Park City, Utah, USA.

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

Varmus, H. & P. Brown, 1989. Retroviruses, pp. 53–108 in Mobile DNA, edited by D. Berg and M. M. Howe. American Society for Microbiology, Washington D.C., USA.

Vaucheret, H., A. Marion-Poll, C. Meyer, J.-D. Faure, E. Marin & M. Caboche, 1992. Interest in and limits to the utilization of reporter genes for the analysis of transcriptional regulation of nitrate reductase. Mol. Gen. Genet. 245: 259–268.

Voytas, D. F. & J. D. Boeke, 1993. Yeast retrotransposons and tRNAs. Trends Genet. 9: 421–427.

Voytas, D. F., M. P. Cummings, A. Konieczny, F. M. Ausubel & S. R. Rodermel, 1992. Copia-like retrotransposons are ubiquitous among plants. Proc. Natl. Acad. Sci. 89: 7124–7128.

Warner, S. A. J., R. Scott & J. Draper, 1993. Isolation of an asparagus intracellular PR gene (AoPR1) wound-responsive promoter by the inverse polymerase chain reaction and its characterization in transgenic tobacco. The Plant Journal 3: 191–201.

Wilson, M. C., P. F. Policastro & M. Fredholm, 1988. Regulation of expression and transposition of murine endogenous retroviral elements, pp. 131–144 in Eukaryotic Transposable Elements as Mutagenic Agents, edited by M. E. Lambert, J. F. McDonald and I. B. Weinstein. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.