Plant retrotransposons: **Turned on by stress**

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All known active plant retrotransposons are largely quiescent during development but activated by stresses, including wounding, pathogen attack and cell culture. This may reflect a survival strategy based on plant biology, or retrotransposons could be the stress-induced generators of genomic diversity proposed by McClintock.

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Current Biology 1996, Vol 6 No 8:959–961

© Current Biology Ltd ISSN 0960-9822

Virtually all genomes contain transposable elements. In some organisms, especially plants, transposable elements or sequences derived from them can exceed 50 % of genomic DNA. Some people believe that these elements are simply parasitic entities that flourish because they can out-replicate their host, the genome of which provides numerous safe havens both within and between genes [1]. According to this view, any useful role assumed by the sequences of a transposable element is rare and fortuitous. In contrast, the idea that transposable elements might flourish because they benefit their host goes back to the discovery of such elements in maize by McClintock (discussed in [2]). In this model, the deleterious effects of transposition could be minimized by maintaining active elements in a quiescent state during normal growth and development. In life-threatening situations, elements could be activated, thereby increasing the mutation rate and restructuring the genome. Populations able to diversify their genomes rapidly in this manner are thought to be more likely to survive and produce progeny.

It was the activation of transposable elements in maize strains undergoing repeated rounds of chromosome breakage that led McClintock to this view. Using a variety of biotic and abiotic stresses, including ultraviolet light and γ radiation, McClintock and investigators who followed in her footsteps were able to identify rare spotted maize kernels, which provide a genetic assay for mutations caused by the activation of transposable elements. The elements activated in these studies were 'Class 2' or DNA elements, a group characterized by short inverted repeats at their termini and, most importantly, transposition *via* a DNA intermediate. That is, the elements usually excise from one site and reinsert elsewhere. Excision during somatic development is what results in unstable phenotypes such as spotted kernels.

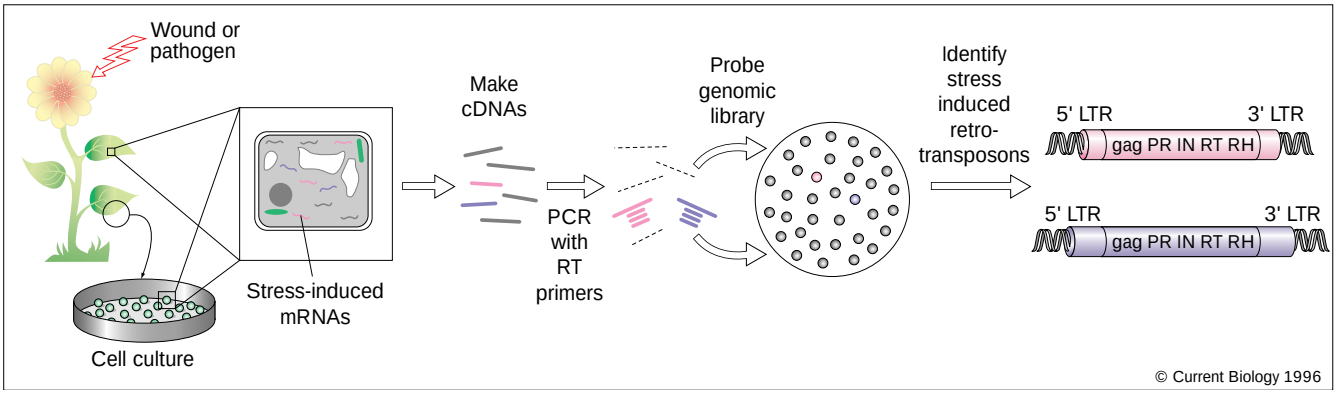
Following up on these early observations, Peschke and colleagues [3,4] found that maize plants regenerated from cell culture contained newly activated DNA elements, specifically *Activator* (*Ac*) and *Suppressor–mutator* (*Spm*) elements, as assayed again by the appearance of rare spotted kernels. Activation of transposable elements during cell culture had been suggested as a mechanism responsible for 'somaclonal variation', the term used to describe the high frequency of mutant plants regenerated from the cultured cells of many plant species. However, it could not be demonstrated that cell-culture-induced mutations were a direct result of the insertion of Class 2 elements.

Recent results have shifted attention to the activation of another class of element, the long terminal repeat (LTR) retrotransposons, and their possible involvement in somaclonal variation. Retrotransposons are members of the retroelement or Class 1 family, which also includes retroviruses, long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). For all Class 1 elements, it is the element-encoded transcript (mRNA), and not the element itself, that forms the transposition intermediate. LTR retrotransposons are flanked by long terminal repeats and encode all the proteins required for their transposition. They can be further classified as either *copia*-like or *gypsy*-like, depending on the order of their coding domains. The discussion below focuses exclusively on *copia*-like retrotransposons.

The first active plant retrotransposon was isolated from tobacco by selecting for insertions into the previously cloned nitrate reductase (*NR*) gene. Screening millions of plants for such a rare event is physically impossible. Instead, Grandbastien *et al.* [5] used microbiological techniques to screen millions of protoplasts (plant cells lacking cell walls) for the chlorite-resistant phenotype characteristic of *NR*-deficient cells. Three independent cell lines contained mutant *NR* genes disrupted by a *copia*-like retrotransposon that the authors called *Tnt1* (for 'transposon *Nicotiana tabacum*'). They reasoned that *Tnt1* had to be transcribed during some aspect of cell culture, as mRNA is the transposition intermediate for elements of this class. Sure enough, subsequent studies found that *Tnt1* transcripts were restricted to roots during normal plant development, but were induced during protoplast isolation [6]. Interestingly, the fungal extract used to digest the plant cell walls during protoplast preparation had activated the plant's defense response (called the hypersensitive response) leading to the activation of the promoter in the 5′ LTR of *Tnt1* (see Fig. 1).

For years, *Tnt1* remained the only *bona fide* active plant retrotransposon, despite numerous studies demonstrating that retrotransposons were ancient and abundant residents of all the plant genomes analyzed so far [7,8]. Although

Figure 1



Protocol for isolating stress-induced retrotransposons. RNA isolated from stressed plants or cultured cells is used to synthesize single-stranded DNA, which in turn is the substrate for amplification using the polymerase chain reaction with degenerate primers that recognize conserved sequences in all *copia*-like reverse transcriptase (RT) domains. In the example shown, two different products from two different retrotransposons have been amplified.

Double-stranded PCR products serve as probes to isolate genomic copies of each retrotransposon. Retrotransposons can vary in size, especially in the length of LTRs. They encode all the proteins required for their transposition, including the capsid protein (encoded by gag), protease (PN), integrase (IN), reverse transcriptase and RNase H (RH).

seemingly intact retrotransposons were isolated from several mutant alleles of plant genes, none could be shown to be active on the basis of two criteria, namely the identification of element-encoded transcripts and an increase in element copy number from parent to progeny. This situation changed dramatically when Hirochika [9] designed a protocol to isolate *copia*-like retrotransposons that might be induced during plant cell culture. This procedure (Fig. 1) involved using the polymerase chain reaction (PCR) with primers derived from the conserved sequences of transposon-encoded reverse transcriptases, in order to amplify reverse transcriptase sequences among cDNAs copied from the mRNAs of tobacco cells in culture. Characterization of amplified sequences eventually led to the identification of two retrotransposons, *Tto1* and *Tto2* ('transposon tobacco'), transcripts of which could be detected in cultured but not from the cells of intact normal plants. More importantly, the copy number of these elements increased both in cell culture and in plants regenerated from cultured cells.

The first active retrotransposons from rice have recently been isolated, again using the PCR-based protocol with RNA from cultured cells [10]. Like the *Tto1* and *Tto2* elements of tobacco, transcripts from the rice *Tos10*, *Tos17* and *Tos 19* (transposon *Oryza sativa*) *copia*-like retrotransposons were detected in cell culture but not during normal plant development. Furthermore, the copy number of each element increased in plants regenerated from cultured cells. The *Tos17* element proved most dramatic in this regard, with its copy number increasing from between one and four in most rice cultivars to over thirty in some regenerants. Consistent with this finding was the demonstration that *Tos17* transcription continued during culturing and resulted in the accumulation of new retrotransposition events during prolonged culture.

The continued retrotransposition of *Tos17* in cell culture was of particular interest because it provided a mechanism for the higher frequency of somaclonal variation observed in plants regenerated from long-term cultures compared to short-term cell cultures [11]. As such, *Tos17* became an excellent candidate for the agent responsible for a significant fraction of the variation seen in regenerated rice plants. This notion was strengthened when it was found that *Tos17* displayed a striking preference for single-copy sequences as insertion sites, despite the fact that a large fraction of the rice genome is repetitive [10]. Of eight target sites sequenced, four had significant amino-acid similarity to known genes (one insertion was in the phytochrome A gene) and three of the remaining four were single-copy sequences. These data provide the first evidence that retrotransposons may be responsible for a large fraction of the mutations induced during cell culture. Furthermore, they provide encouragement that the PCR-based protocol can be used to identify cell culture-induced elements from virtually any plant.

With these elements in hand, investigators should be able to use a variety of transposon-tagging strategies to isolate the genes responsible for interesting phenotypes among plants regenerated from the cultured cells of a wide spectrum of plant species. There is a certain irony to the prospect of somaclonal variation becoming useful again to plant molecular biologists. It was initially hailed as a valuable source of mutants, but it quickly became a thorn in the side of scientists using transgenic plants regenerated from cell culture to isolate what they mistakenly thought were mutations due to the insertion of foreign transposons. Knowledge of the elements causing these 'unwanted' mutations may now pave the way for the comeback of somaclonal variation as a valuable resource to plant biologists.

Cell culture is not the only stress that activates plant retrotransposons. As mentioned above, activation of Tnt1 was a consequence of using a fungal extract to prepare leaf protoplasts. Bacterial and viral infections that lead to induction of the plant-defense hypersensitive response also induce *Tnt1* transcription [12]. This can be demonstrated visually by fusing the promoter of *Tnt1*, which resides in the 5′ LTR, to the reporter gene *GUS*; *GUS* encodes β-glucuronidase, the enzymatic product of which can be visualized histochemically. In this way histochemical staining could be seen at the wound site but not at distant sites, indicating that *Tnt1* is not systemically induced. Modification of the PCR-based assay to accommodate mRNA from pathogen-infected or chemically treated plants should facilitate the isolation of additional retrotransposons that are activated under specific stress regimens.

A central question remains: why are elements largely inactive during normal development but induced by stress? From a purely mechanistic point of view, the answer seems to be that induction is mediated by *cis*-acting elements in the 5′ LTRs. When fused to the *GUS* reporter gene, the *Tnt1* 5′ LTR accurately reports the transcription pattern of the *Tnt1* element [12]. That is, *GUS* staining is seen only in roots and at the site of wounds or pathogen attack. The 'BII repeat' region within the 5′ LTR is required for element induction and contains several *cis*-acting elements that have been identified previously in plant defense-response genes. The same LTR–*GUS* fusion gene is also induced by stress in tomato and *Arabidopsis*. However, unlike the situation in tobacco, where expression is confined to roots, the 5′ LTR promotes reporter gene expression in the flowers of these heterologous hosts. Moreau-Mhiri *et al.* [13] suggest that *Tnt1* induction in tobacco flowers may have been selected against because it would have increased the germinal mutation frequency. 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. Thus, transcription of *Tnt1* and other plant retrotransposons in these cells could be catastrophic, because each transcript represents a potential new insertion (usually into a gene), some in lineages that will go on to produce gametes. Thus, retrotransposition during normal development may reduce the fertility of the host to such an extent that elements allowing such transposition are quickly eliminated from the population.

If elements are not active during normal development, they must be active at some other time if they are to survive. From the elements' point of view (so to speak), stress activation may provide a means for both vertical and horizontal transmission. Vertical transmission could occur if stress-activated elements retrotranspose in lineages that give rise to gametes. Horizontal transmission might occur if the pathogen serves both to activate element transcription and to provide a vector for the movement of transcripts to another host [14]. The recent demonstration that the tobacco elements *Tnt1* and *Tto1* can move in the genomes of *Arabidopsis* and rice [15,16], respectively, makes such a scenario more plausible. That is, if element-encoded transcripts can get into another organism, they would probably not be restricted by a requirement for host-specific factors.

From the point of view of plant evolution, the relative silence of transposable elements during normal development and their activation by stress is consistent with the genome-restructuring role envisioned for transposable elements by McClintock. Such a role is also bolstered by the demonstration that *Tos17* inserts preferentially into single-copy sequences. Furthermore, the recent identification of retrotransposon-derived sequences in the flanking regulatory regions of many normal plant genes [17] suggests that *copia*-like retrotransposons may have fulfilled this role in the past.

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