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HAL Id: hal-02704744
https://hal.inrae.fr/hal-02704744v1

Submitted on 28 Sep 2023

Microbial elicitors of plant defence responses activate transcription of a retrotransposon

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Summary

The Tnt1 retrotransposon was isolated from tobacco after transposition. Tnt1 expression is very low at the plant level and under most stress conditions but is strongly induced during protoplast isolation. It is shown here that transcription of the Tnt1 retrotransposon can be activated by several microbial factors having the common ability to elicit a hypersensitive response in tobacco. These elicitors include Onozuka crude extracts from the fungus *Trichoderma viride*, elicitins purified from *Phytophthora* fungal species and culture supernatants of the bacterium *Erwinia chrysanthemi*. Activation of Tnt1 expression correlates with the biological activity of purified elicitins. On detached leaves, dose response to two different elicitins, the highly necrotic cryptogein from *P. cryptogea* and the less toxic capsicein from *P. capsici*, is parallel to the necrotic dose response. A dose of 1 μg cryptogein induces Tnt1 transcription to a high level similar to that induced by Onozuka 1 mg ml^{-1} whilst 10–100-fold more capsicein is required to induce this level. In whole plants treated with cryptogein, activation of Tnt1 transcription is restricted to necrotic organs. In tobacco cell suspension cultures treated with *E. chrysanthemi* culture supernatants, the accumulation of Tnt1 RNA correlates with the accumulation of the phytoalexin capsidiol a few hours later. Two hours after contact with the elicitor, cells accumulate a high level of Tnt1 RNA, this level being almost 10-fold higher than that observed in protoplasts in response to Onozuka 1 mg ml^{-1}. Our results suggest that Tnt1 activation might reflect a local and early plant response to microbial stress.

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Introduction

The transcription and mobility of transposable elements is commonly activated by a number of environmental stresses. Under stress situations both autonomous and cellular mechanisms of control that normally suppress transposition activity can be overcome. This is observed for members of the two major categories of transposable elements that have so far been found in eukaryotes, namely a class of elements transposing via a DNA intermediate and a second class of elements, also called retroviral elements or retrotransposons, transposing via an RNA intermediate through reverse transcription.

Genomic stress by DNA damaging agents, such as UV light, X or γ rays, has been shown to activate transposable elements (Bradshaw and McEntee, 1989; Fedoroff, 1989; Rolfe *et al.*, 1986; Walbot, 1988). Similarly, thermal treatments have been reported to stimulate transposition (Coen *et al.*, 1989; Strand and McDonald, 1985). The production of cell or tissue cultures also commonly results in the activation of transposition (Hirochika, 1993; Junakovic *et al.*, 1988; Peschke *et al.*, 1987; Planckaert and Walbot, 1989). In higher plants, this activation is thought to be involved in the somaclonal variations arising from the traumatic process of cell isolation, calli formation and cell regeneration (Lee and Phillips, 1988), although no clear demonstration of the involvement of one particular step has yet been provided.

An important challenge that plants must face repeatedly is the attack of pathogens. To respond to pathogen infections plants activate several mechanisms that have been shown to contribute to disease resistance. These responses include the hypersensitive reaction (HR) and the accumulation of different compounds such as the plant antibiotics phytoalexins. Phytoalexins accumulate rapidly in response to pathogen infection (Darvill and Albersheim, 1984) and are thought to be involved in disease resistance (Hain *et al.*, 1993). The HR is a rapid localized necrosis of plant cells at the site of infection and this is supposed to limit the spread of invading pathogens (Sequeira, 1983). The HR occurs during incompatible plant–pathogen interactions, i.e. interactions between pathogens and non-host or resistant plants. During plant pathogen interactions, molecular signals called elicitors either produced by the pathogen or the plant induce the HR. Elicitors of plant defence responses have been shown to be either oligosaccharides released from the plant or the pathogen cell walls (Ryan and Farmer, 1991) or proteins produced by the pathogen (De Wit *et al.*, 1985; Ricci *et al.*, 1993).

Until now no microbial factor has been reported to be involved in the activity of a transposable element. However, one class of plant pathogens, the viruses, have been shown possibly to activate transposition (Dellaporta *et al.*, 1984; Johns *et al.*, 1985). The Tnt1 retrotransposon of *Nicotiana tabacum* was isolated after transposition into a nitrate reductase gene (Grandbastien *et al.*, 1989). Previously, we showed that the Tnt1 transcript level is very low or undetectable in plant tissues and under a number of stress conditions, but that it is highly induced during tobacco protoplast isolation (Pouteau *et al.*, 1991). An increase in Tnt1 copy number in established cell lines was also reported (Hirochika, 1993), suggesting that Tnt1 transposition might be activated by tissue culture. The Tnt1 long terminal repeat (LTR) was shown to contain the transcriptional start site of the element (Pouteau *et al.*, 1991) and regulatory sequences sufficient to confer protoplast-dependent induction of transcription (Casacuberta and Grandbastien, 1993). Also, one activator element within this regulatory region was found to interact with protoplast-specific nuclear factors (Casacuberta and Grandbastien, 1993). Induction of Tnt1 transcription in protoplasts results from contact with the Onozuka solution prepared from the fungus *Trichoderma viride* which contains cell wall-degrading enzymes. After heat treatment, this maceration solution, which contains oligosaccharides released from the cell wall, did not retain the ability to activate Tnt1 expression, suggesting that other factors already present in the Onozuka solution or produced during cell wall degradation might be responsible for the observed induction (Pouteau *et al.*, 1991).

In this work our aim was to determine whether Tnt1 expression could be activated by several microbial factors able to elicit a plant defence response in tobacco, such as elicitins produced by *Phytophthora* fungal species (Ricci *et al.*, 1989) and culture supernatants of the bacterium *Erwinia chrysanthemi*. Interaction between several incompatible pathogen fungal species of the genus *Phytophthora* and the non-host plant tobacco leads to an HR characterized by plant tissue necrosis and restricted growth of the fungus (Bonnet, 1985). A group of 10 kDa proteins named elicitins have been purified from culture media of these *Phytophthora* species (Billard *et al.*, 1988). These proteins are toxic to tobacco cells (Blein *et al.*, 1991) and cause necrosis on tobacco leaves, activating phytoalexin accumulation (Milat *et al.*, 1991) parallel to induction of disease resistance (Ricci *et al.*, 1991). *E. chrysanthemi*, a phytopathogenic bacterium causing soft rotting on a wide range of hosts, elicits a HR in tobacco. This HR has been shown to be correlated with the accumulation of a specific sesquiterpene, capsidiol, a major tobacco phytoalexin (Boccara *et al.*, 1993). In this paper, activation of Tnt1 transcription by elicitins and culture

supernatants of *E. chrysanthemi* is described and the implications of this activation during incompatible interactions of pathogen micro-organisms with tobacco are discussed.

Results

Compared effect of Onozuka and cryptogein

Tnt1 expression was analysed by measuring the GUS activity in leaves of transgenic tobacco plants harbouring a LTR-GUS chimeric gene (Pauls *et al.*, submitted for publication; Pouteau *et al.*, 1991). The LTR-GUS gene consists of the GUS reporter gene (Jefferson *et al.*, 1987) placed under the control of Tnt1 LTR. In a previous report (Pouteau *et al.*, 1991), we showed that the expression of the LTR-GUS gene in transgenic plants is correlated with the accumulation of Tnt1 genomic RNA. These results, and the characterization of the LTR described above, suggest that the LTR contains most regulatory regions involved in Tnt1 expression. Five independent transformants, selected on the basis of their ability to express GUS activity during protoplast isolation, were analysed (see legend to Figure 1). Detached leaves of LTR-GUS transgenic plants were treated with 1 mg ml^{-1} Onozuka solution for 16 h, as described in Experimental procedures. This treatment appeared to induce the GUS activity (Figure 1) to a level similar to that obtained after treatments already described, i.e. maceration of dilacerated leaf tissue with Onozuka for 16 h and protoplast isolation (Pouteau *et al.*, 1991), or vacuum infiltration of leaf tissue with Onozuka and 16 h subsequent incubation (Pouteau, unpublished data). Also, this treatment typically provoked necrosis on tobacco leaves (data not shown).

Cryptogein, an elicitin produced by *P. cryptogea*, is highly necrotic on tobacco (Billard *et al.*, 1988). A dose of cryptogein known to induce a high level of necrosis on expanding detached leaves (Nespoulous *et al.*, 1992) was used to compare the effect of cryptogein to that of Onozuka on the activation of LTR-GUS expression. Purified cryptogein (1 μg) was applied to the petiole of detached leaves which were then supplied with nutrient solution for 16 h, as described in Experimental procedures. Subsequently, for both Onozuka and cryptogein treatments, GUS activities were measured in proximal and distal parts of the leaves. High levels of GUS activity were induced by 1 μg of cryptogein, these levels being essentially similar to that induced by Onozuka solution at 1 mg ml^{-1} (Figure 1). Induction factors in the five independent lines were essentially similar, as shown by standard deviation bars, suggesting that position effects are not responsible for the induction observed. No significant activation was detected before 8 h. GUS expression occurred uni-
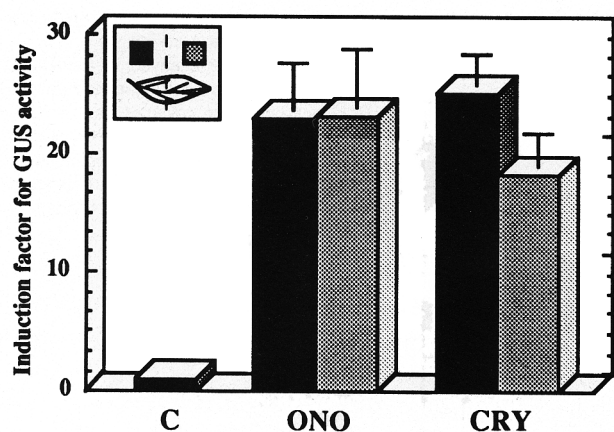
Figure 1. Tnt1 response to Onozuka R10 and cryptogein elicitation. Detached leaves of LTR-GUS transgenic plants were supplied with nutrient solution (C) or with 1 mg ml⁻¹ Onozuka R10 (ONO) for 16 h, or treated with 1 μg cryptogein (CRY) applied to cut petioles and then supplied with nutrient solution for 16 h. GUS activities were then measured in proximal and distal parts of the leaves, except for controls (C) where whole leaves were assayed. Induction factors were calculated as ratios between the levels in treatments and controls. Sixteen to 31 samples from five independent transgenic lines were used for each measurement and the standard deviations are shown.

formly throughout the leaf, as shown by measurements done on different parts of the leaf (Figure 1) or by GUS histochemical staining (data not shown).

Dose effects of cryptogein and capsicein

On the basis of their biological effects, elicitins can be classified into two classes. The two classes are typically represented by capsicein (class α) produced by *P. capsici* and cryptogein (class β), with class β elicitins being 50-fold more active in inducing necrosis than class α elicitins (Nespoulous *et al.*, 1992). The necrosis dose response for cryptogein and capsicein was described on the basis of obvious necrotic areas exhibited by leaves. In this paper, we refer to necrosis on this same basis, as we found essentially the same results (data not shown).

Different quantities of cryptogein and capsicein were applied to the petiole of detached leaves of transgenic plants and GUS activities were measured (Figure 2). After 16 h, the highest level of induction of LTR-GUS expression was observed in leaves showing the highest necrosis symptoms, i.e. leaves treated with 1 μg cryptogein or 100 μg capsicein. The LTR-GUS expression dose response was parallel to the necrosis dose response for both elicitins. For cryptogein, a dose decrease of 1 order of magnitude resulted in more than 60% reduction of the GUS activity. In contrast, the Tnt1 response to a dose decrease of capsicein was more progressive and a reduction of 1–3 orders of magnitude was necessary to cause 50% reduction of the GUS activity. The dose threshold for induction

of Tnt1 expression was 0.1 μg per leaf for cryptogein, this being higher for capsicein, occurring between 0.1 and 10 μg per leaf.

Distribution of Tnt1 expression and HR symptoms

Elicitins applied to decapitated stems cause necrosis on tobacco plants not only at the application site but also on distant leaves (Bonnet, 1985). The distant location of the necrosis induced by cryptogein and capsicein has been shown to be due to the migration of these proteins rather than to the existence of a systemic endogenous signal (Devergne *et al.*, 1992; Zanetti *et al.*, 1992). Elicitin-induced necrotic symptoms in the plant are dependent on leaf position along the stem, being more severe in upper leaves and decreasing progressively to lower leaves as elicitins migrate and become diluted in the plants (Zanetti *et al.*, 1992). Necrosis frequently develops asymmetrically so that only one longitudinal leaf half is affected (Zanetti *et al.*, 1992), probably due to obstruction of some vessels after decapitation (Tyree and Sperry, 1989) and to non-uniform migration of elicitins (Zanetti *et al.*, 1992). We took advantage of this phenomenon to analyse the distribution of GUS activity with respect to this asymmetrical HR-developing pattern. Cryptogein (5 μg) was applied to cut stems of LTR-GUS transgenic tobacco plants after decapitation. For one plant showing asymmetrical development of necrosis, five leaves numbered from top to bottom of the plant were tested. Twenty-four hours later, leaves 1 to 3 showed high necrotic symptoms on their left longitudinal half whereas no symptoms could be detected on their right half. After 48 h, leaf 4 had no symptoms and leaf 5 showed only faint necrotic symptoms on its right longitudinal half. Figure 3 shows that according to the basipetal necrosis gradient previously described, the level of LTR-GUS expression was highest in the upper leaves and decreased progressively to the lower leaves. After 24 h, GUS activity was highest in leaf 1, decreasing progressively to leaf 3. Even after 48 h, almost no activity could be detected in the lowest leaves, 4 and 5. GUS activities were measured independently in both leaf halves. Correlating with the asymmetric necrotic pattern, GUS activity was detected only in the necrotic halves. Similar results were obtained with plants treated with capsicein (Pouteau, unpublished data).

Tnt1 response to culture supernatants of E. chrysanthemi

Concentrated culture supernatant (500 μg of total protein ml⁻¹) of *E. chrysanthemi* strain 4121 was applied to the cut stem of detached expanding leaves of LTR-GUS transgenic tobacco plants. High amounts of elicitor solution
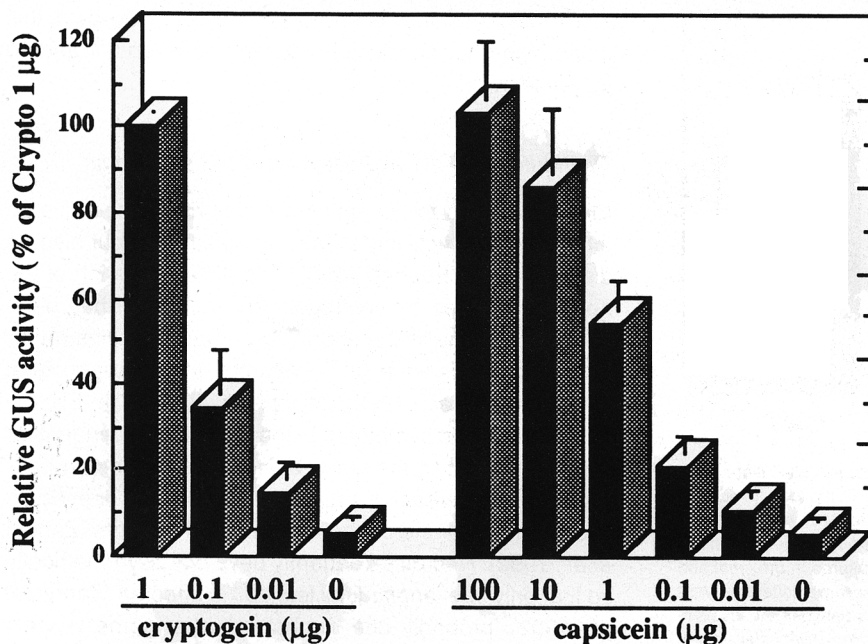
Figure 2. Dose effects of cryptogein and capsicein on Tnt1 expression.

Dose effects were assayed in transgenic plants as described in the legend to Figure 1. GUS activities were calculated as percentages of activities induced by 1 µg cryptogein as standards. Repeats for two independent transgenic plants were done and standard deviations corresponding to 6–11 independent repeats are shown.
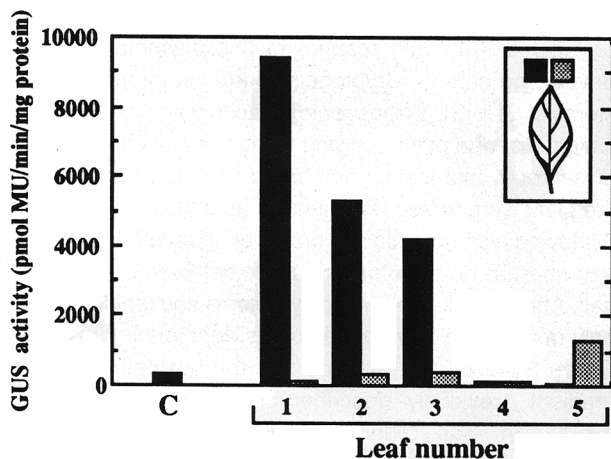

Figure 3. Correlation between Tnt1 expression and HR symptoms. Transgenic plants were decapitated and either 5 µg cryptogein, or water for the control (C), was immediately applied to cut stems. Necrosis developed asymmetrically on leaves of the elicitor-treated plant. Five leaves numbered from top to bottom of the plant were tested. After 24 h, leaves 1 to 3 presented high necrotic symptoms on their left longitudinal half whereas no symptoms could be detected on their right half. After 48 h, leaf 4 had no symptoms and leaf 5 showed only faint necrotic symptoms on its right longitudinal half. GUS activities were measured independently in both leaf halves: the solid bars represent the activities measured in the left half and the stippled bars in the right half of the leaves. In the control experiment (C), whole leaves were assayed and the mean values of measurements are shown. MU = methylumbelliferone.

were needed (more than 100 µg of total protein) to induce the GUS activity. Since the high protein concentration rapidly saturated the absorption capacity of the cut stems, it was difficult to apply large volumes of elicitor and to quantify the response. Whatever the elicitor volume

applied, the GUS activity was always induced to lower levels than the reference response to 1 µg cryptogein (Pouteau and Boccara, unpublished data).

A cell suspension culture of *N. tabacum* cv. Wisconsin 38 was used to avoid this absorption limit. *E. chrysanthemi* concentrated culture supernatant was added to the tobacco cell suspension cultures during exponential growth. Cells were harvested after different periods of treatment and accumulation of both capsidiol and Tnt1 genomic RNA (5.2 kb) was analysed (Figure 4). A significant level of capsidiol was detected in the cell suspension culture 8 h after contact with bacterial supernatant and this level reached a maximum after 16 h, decreasing almost to background level by 24 h (Figure 4a). No Tnt1 transcription could be detected in the cell line, as shown at time point 0 on Figure 4(b). Accumulation of Tnt1 transcript was induced to a high level 2 h after contact with bacterial supernatant, the RNA level being highest after 5 h. This high level was maintained for up to 24 h, but fell to very low levels after 48 h, probably due to the high level of cell death (85.4%). A control experiment with protoplasts isolated after 16 h of maceration (Figure 4b, P), in which Tnt1 expression is known to be induced (Pouteau *et al.*, 1991), was done in parallel. The Tnt1 RNA level in protoplasts was less than 5% of that found in elicited cells (Figure 4), illustrating the high elicitor activity of the bacterial supernatant on Tnt1 transcription in cell suspensions. This important difference might reflect the lower accessibility of elicitors to cells in leaf tissues during maceration compared to cell suspensions. Furthermore, probably due to the use of sublethal doses of elicitors or to their lower toxi-

city, cells are not killed by elicitors in the course of proto-plast isolation. The high level of cell death during elici-tation with bacterial supernatant (see legend to Figure 4) suggests that leakage of cell compounds might be very high and could account for the lower apparent accumula-tion of capsidiol detected in elicited cells compared with protoplasts (about 30% of the maximum level in elicited cells after 16 h of treatment with *E. chrysanthemi* super-natant).

Discussion

Long known in the animal kingdom and in micro-organ-isms, retroviral elements have only been identified recently in the plant kingdom and so far very little informa-tion has accumulated concerning their expression and transposition activity. So far, the Tnt1 tobacco element is the only plant retrotransposon that has been shown to be transcriptionally active (Pouteau *et al.*, 1991). Tnt1 tran-scription is strongly regulated, the level of genomic RNA being low or even undetectable at the plant level. This sit-uation is very different from that observed in the well characterized eukaryote models of yeast (Boeke, 1989) and *Drosophila* (Bingham and Zachar, 1989), suggesting that transcription could be an essential level for the regula-tion of Tnt1 retrotransposition.

In this work, we show that transcription of Tnt1 can be greatly stimulated by different microbial elicitors of plant defence responses. The elicitors shown to induce Tnt1 transcription are of different types and from different micro-organisms. Tnt1 expression can be activated by factors of fungal origin (crude extracts from *T. viride* and elicitins purified from *Phytophthora* species), as well as by culture supernatants of the bacterium *E. chrysanthemi*. Elicitins are holoproteins devoid of known enzymatic activ-ities and their biochemical role remains to be elucidated. In contrast, HR elicitors from *T. viride* and *E. chrysanthemi* have been shown to be hydrolytic enzymes which may release active oligosaccharide elicitors. In the case of *T. viride*, an endoxylanase elicits HR in tobacco (Bailey *et al.*, 1990), and in the case of *E. chrysanthemi* strain PMV4121, a pectate lyase has been shown to elicit the accumulation of capsidiol in tobacco cell cultures (Boc-cara *et al.*, 1993). More evidence is required to prove that these purified HR elicitors are responsible for the activa-tion of Tnt1 transcription observed in response to the crude *T. viride* extracts and *E. chrysanthemi* culture supernatants used in this study. Whether the same signal transduction pathways and/or same transcription factors are involved in both HR and Tnt1 activation by these dif-ferent plant defence elicitors also remains to be eluci-dated.

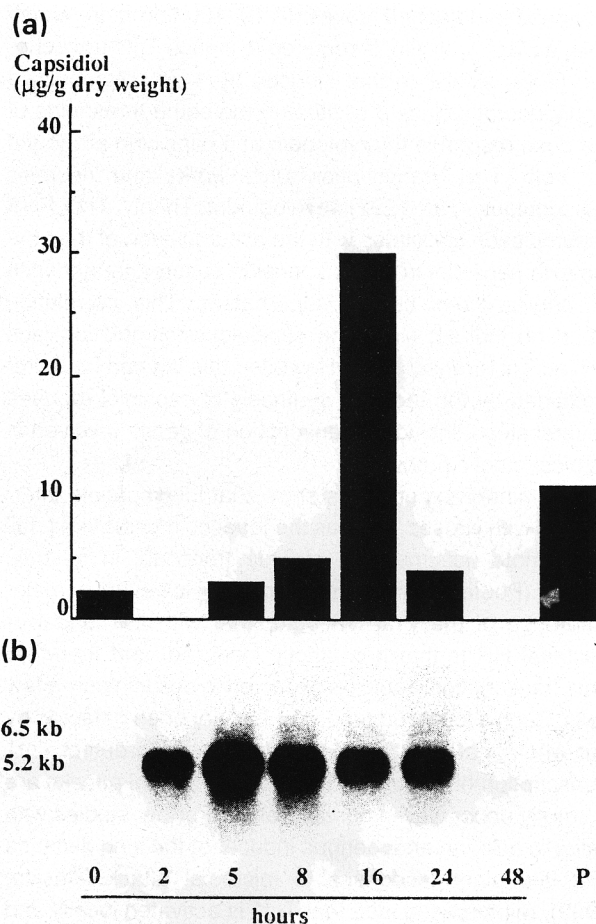Our results suggest that Tnt1 activation specifically

Figure 4. Activation of Tnt1 transcription in tobacco cell suspension cul-tures treated with culture supernatant of *E. chrysanthemi*.

E. chrysanthemi concentrated culture supernatant was added to cell sus-pension cultures of tobacco (time point 0). Cells were harvested after dif-ferent periods of treatment and accumulation of both capsidiol (a) and Tnt1 genomic RNA (5.2 kb; b was analysed). Capsidiol accumulation after 2 h and 48 h of treatment was not determined. A control with freshly isolated protoplast (P) was done in parallel. The 6.5 kb RNA visible in the P lane has been previously supposed to be transcribed from a gene in which Tnt1 had been inserted (Pouteau *et al.*, 1991). Percentage of cell death measured in parallel was 13.8 ± 3.9 after 2 h, 28.4 ± 1.7 after 8 h, 53.3 ± 0.5 after 24 h, and 85.4 ± 1.7 after 48 h.

reflects a local and early plant response to microbial stress, though more evidence is needed to show HR-dependent activation of Tnt1. Firstly, activation of Tnt1 expression by elicitins is restricted to necrotic organs: (i) the same basipetal gradient is observed for both Tnt1 activation and necrosis symptoms in whole plants; (ii) partition of Tnt1 transcription is strictly correlated with the asymmetrical pattern of necrosis development in the plant, showing that it is dependent on elicitin migration. Secondly, the overall pattern of Tnt1 response to elicitins is correlated with the biological necrotic activity of these elicitors. The dose response of Tnt1 to elicitins displaying different levels of toxicity is parallel to the necrotic dose

response in detached leaves: (i) 10–100-fold more capsicein, a class α elicitin, is required to induce Tnt1 transcription levels similar to that induced by 1 μg cryptogein, a highly necrotic class β elicitin; (ii) the same thresholds of the dose response to cryptogein and capsicein are found for both Tnt1 transcription (this work) and necrosis (Nespoulous *et al.*, 1992) development. Thirdly, Tnt1 RNA accumulation correlates with the accumulation of the phytoalexin capsidiol in cell suspension cultures treated with *E. chrysanthemi* culture supernatant. This correlation could be indirect since the capsidiol accumulation lags behind Tnt1 transcription. However, this lag can be better explained by the fact that synthesis of capsidiol requires several steps, including transcription of genes involved in its biosynthetic pathway.

Preliminary experiments show that incompatible interactions with viruses, such as the tobacco mosaic virus, fail to promote accumulation of Tnt1 transcript in necrotic leaves (Pouteau, unpublished data). However, a major difference of the viral HR compared with the fungal or bacterial HR is that it is strictly localized, and therefore Tnt1 transcription would be expected to occur in only a few cells. Such a localized response has not been detected by our whole leaf RNA assays and attempts to detect Tnt1 transcription histologically in response to viral attacks are currently under way in our laboratory. Further studies with salicylic acid, an endogenous inducer of the long distance and late plant responses to microbial attack (Raskin, 1992), reinforce the idea that Tnt1 is activated locally and early, since salicylic acid fails to activate Tnt1 transcription (Pouteau, unpublished data).

Since transposition of retrotransposons is dependent on transcription, activation of Tnt1 transcription by microorganisms eliciting HR is expected to result in the activation of transposition. Indeed, activation of Tnt1 transcription during protoplast isolation (Pouteau *et al.*, 1991) might be the origin of the transposition events shown after protoplast-derived cell culture (Grandbastien *et al.*, 1989). The high copy number of Tnt1 elements in tobacco (more than 100 copies) prevents direct study of the correlation between transcription and transposition, and we are currently setting up an indirect transposition assay that may help to answer this intriguing question.

What could be the biological significance of Tnt1 activation by plant–pathogen interactions? Tnt1 might be indirectly activated by cell perturbation occurring during the HR. Alternatively, we cannot exclude the possibility that the localized activation of high levels of Tnt1 transposition could contribute to cell death (HR) and therefore be part of the localized plant defence response. In addition, as suggested by McClintock (1984), Tnt1 activation could be an example of the involvement of transposable elements in the organism's response to genomic stress, forcing the

genome to restructure itself in order to survive. Evidence supporting such an evolutionary role for transposable elements is growing, overshadowing the concept of selfish DNA (McDonald, 1990). The finding that transposable elements can be classified on a taxonomic basis with sequences involved in gene horizontal transfer and antimutator genes might also be relevant to this idea (Médigue *et al.*, 1991). Bursts of transposition, activated in response to microbial stress, could lead to bursts of diversity, requiring the activity of antimutator genes for fixation of the acquired variations. Whether the genetic transmission of elicitor-induced transposition could contribute to genome evolution is currently being addressed in our laboratory.

Experimental procedures

Plant material

The transgenic plants of *Nicotiana tabacum* cv. Samsun used contain the LTR-GUS fusion gene which consists of the complete LTR plus a sequence coding for the first 25 amino acids of the open reading frame of Tnt1 fused in frame with the GUS open reading frame (Pauls *et al.*, submitted for publication; Pouteau *et al.*, 1991). The plants were grown in a shaded greenhouse under reduced daylight intensity.

Elicitor assays on detached leaves

Expanding detached leaves (10–15 cm long) were collected from vegetative plants having 8–16 leaves and petioles were immediately dipped into nutrient solution (Coïc and Lesaint, 1971). Just before application of elicitors, petioles were cut with a blade to a length of approximately 2 cm. For Onozuka assays, leaves were supplied with 1 mg ml^{-1} Onozuka R10 (Yakult Biochemicals Co., Ltd) in water, or with nutrient solution as a control, by directly dipping the petiole into 150 ml of solution in a flask for 16 h. For elicitin assays, different doses (0.01, 0.1, 1, 10, 100 μg) of purified cryptogein or capsicein in 10 μl of water were applied to cut petioles. After complete absorption of the elicitin solution, the petiole was dipped into 150 ml of nutrient solution in a flask for 16 h. For all elicitor assays, leaves were kept at room temperature in the dark (Nespoulous *et al.*, 1992). After 16 h, leaves were frozen in liquid nitrogen.

Elicitin assays on whole plants

Transgenic plants having 8–10 leaves were decapitated and either 5 μg cryptogein in 10 μl water or 10 μl water for the control were applied to cut stems (Ricci *et al.*, 1989). Plants were kept for 48 h in the shaded greenhouse. A plant showing typical asymmetrical development of necrosis on leaves was chosen. Leaves 1 to 3, numbered from top to bottom, were collected 24 h after treatment, and leaves 4 and 5 were collected after 48 h.

GUS assays

Leaf tissues were ground in liquid nitrogen with a mortar and pestle. Extracts were made in GUS buffer and the GUS activity was measured according to Jefferson *et al.* (1987). The protein concentration in the extracts was determined following Bradford (1976).

Cell suspension culture

Cell suspension cultures of *N. tabacum* cv. Wisconsin 38 clone 19-3 (Gregorini and Laloue, 1980) were grown in a slightly modified Murashige and Skoog medium as described in Gregorini and Laloue (1980) on a rotary shaker (150 rpm) in a growth chamber at 25°C under constant white light (1500 Lux). Cell suspensions were subcultured every 2 weeks.

Elicitor assays on cell suspension cultures

Supernatant was obtained from strain PMV4121 of *Erwinia chrysanthemi*, deleted of its major pectate lyase encoding genes (Favey *et al.*, 1992), grown in M9 minimal medium (Miller, 1972) containing 0.4% polygalacturonate. This culture suspension was 100-fold concentrated by acetone precipitation (60% v/v), resuspended in 10 mM acetate buffer pH 5.6 and extensively dialysed with the same buffer. Protein (20 µg) from PMV4121 concentrated supernatant (500 µg of total protein ml⁻¹) was added to 10 ml of exponentially grown (8-day-old) cell suspension culture. Cells were then collected at different times, 0, 2, 5, 8, 16, 24, and 48 h after treatment, pelleted and frozen in liquid nitrogen for RNA extraction, or mixed with an equal volume of 95% ethanol for capsidiol quantification. As a control, tobacco protoplasts were prepared as described by Chupeau *et al.* (1974) and isolated after 16 h of maceration of leaf tissue.

Capsidiol quantification

Capsidiol was extracted twice from tobacco culture medium with ethyl acetate and quantified as indicated by Nemestothy and Guest (1990).

RNA isolation and gel blot analysis

Total RNA was prepared from cells according to Verwoerd *et al.* (1989). Tnt1 RNA was analysed using 10 µg of total RNA on a formaldehyde denaturing gel. Gel blot hybridization with a Tnt1 specific probe of 852 bp corresponding to the gag domain of the Tnt1 coding sequence was performed as described by Pouteau *et al.* (1991).

Acknowledgements

References

Bailey, B.A., Dean, J.F.D. and Anderson, J.D. (1990) An ethylene biosynthesis-inducing endoxylanase elicits electrolyte leakage and necrosis in *Nicotiana tabacum* cv. Xanthi leaves. *Plant Physiol.* **94**, 1849–1854.

Billard, V., Bruneteau, M., Bonnet, P., Ricci, P., Pernollet, J.C., Huet, J.C., Vergne, A., Richard, G. and Michel, G. (1988) Chromatographic purification and characterization of elicitors of necrosis on tobacco produced by incompatible *Phytophthora* species. *J. chromatogr.* **44**, 87–94.

Bingham, P.M. and Zachar, Z. (1989) Retrotransposons and the FB transposon from *Drosophila melanogaster*. In *Mobile DNA* (Berg, D.E. and Howe, M.M., eds). Washington: American Society for Microbiology, pp. 485–502.

Blein, J.P., Milat, M.L. and Ricci, P. (1991) Responses of cultured tobacco cells to cryptogein, a proteinaceous elicitor from *Phytophthora cryptogea*. *Plant Physiol.* **95**, 486–491.

Boccara, M., Vedel, R. and Dufresne, M. (1993) Plant defense elicitor protein produced by *Erwinia chrysanthemi*. In *Mechanisms of Plant Defense Responses* (Fritig, B. and Legrand, M., eds). Dordrecht: Kluwer Academic Publishers, p. 166.

Boeke, J.D. (1989) Transposable elements in *Saccharomyces cerevisiae*. In *Mobile DNA* (Berg, D.E. and Howe, M.M., eds). Washington: American Society for Microbiology, pp. 335–374.

Bonnet, P. (1985) Réactions différentielles du tabac à 9 espèces de *Phytophthora. Agronomie*, **5**, 801–808.

Bradford, M.M. (1976) A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein dye binding. *Anal. Biochem.* **72**, 248–254.

Bradshaw, V.A. and McEntee, K. (1989) DNA damages activate transcription and transposition of yeast Ty retrotransposons. *Mol. Gen. Genet.* **218**, 465–474.

Casacuberta, J.M. and Grandbastien, M.A. (1993) Characterisation of LTR sequences involved in the protoplast specific expression of the tobacco Tnt1 retrotransposon. *Nucl. Acid Res.* **21**, 2087–2093.

Chupeau, Y., Bourgin, J.P., Missonier, C., Dorion, N. and Morel, G. (1974) Préparation et culture de protoplastes de divers *Nicotiana. C.R. Acad. Sci. Paris*, **278**, 1565–1568.

Coen, E.S., Robbins, T.P., Almeida, J., Hudson, A. and Carpenter, R. (1989) Consequences and mechanisms of transposition in *Antirrhinum majus*. In *Mobile DNA* (Berg, D.E. and Howe, M.M., eds). Washington: American Society for Microbiology, pp. 413–436.

Coïc, Y. and Lesaint, C. (1971) Comment assurer une bonne nutrition en eau et ions minéraux en horticulture. *Hortic. Fr.* **8**, 11–14.

Darvill, A.G. and Albersheim, P. (1984) Phytoalexins and their elicitors: a defense against microbial infections in plants. *Ann. Rev. Plant Physiol.* **35**, 243–275.

Dellaporta, S.L., Chomet, P.S., Mottinger, J.P., Wood, J.A., Yu, S.M. and Hicks, J.B. (1984) Endogenous transposable element associated with virus infection in maize. *Cold Spring Harbor Symp. Quant. Biol.* **49**, 321–328.

Devergne, J.C., Bonnet, P., Panabières, F., Blein, J.P. and Ricci, P. (1992) Migration of the fungal protein cryptogein within tobacco plants. *Plant Physiol.* **99**, 843–847.

De Wit, P.J.G.M., Hofman, A.E., Velthuis, G.C.M. and Kuc, J.A. (1985) Isolation and characterization of an elicitor of necrosis isolated from intercellular fluids of compatible interactions of *Cladosporium fulvum* (syn. *Fulvia fulva*) and tomato. *Plant Physiol.* **77**, 642–647.

Favey, S., Bourson, C., Bertheau, Y., Kotoujansky, A. and

Boccara, M. (1992) Purification of the acidic pectate lyase and nucleotide sequence of the corresponding gene (*pelA*) of *Erwinia chrysanthemi* strain 3937. *J. Gen. Microbiol.* **138**, 499–508.

Fedoroff, N.V. (1989) Maize transposable elements. In *Mobile DNA* (Berg, D.E. and Howe, M.M., eds). Washington: American Society for Microbiology, pp. 375–411.

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

Gregorini, G. and M. Laloue, M. (1980) Biological effects of cytokinin antagonists 7-(pentylamino) and 7-(benzylamino)-3-methylpyrazolo(4,3-d)pyrimidines on suspension-cultured tobacco cells. *Plant Physiol.* **65**, 363–367.

Hain, R., Reif, H.J., Krause, E., Langebartels, R., Kindl, H., Vornam, B., Wiese, W., Schmelzer, E., Schreier, P.H., Stöcker, R.H. and Stenzel, K. (1993) Disease resistance results from foreign phytoalexin expression in a novel plant. *Nature*, **361**, 153–156.

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

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

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

Junakovic, N., Di Franco, C., Best-Belpomme, M. and Echalier, G. (1988) On the transposition of copia-like nomadic elements in cultured *Drosophila* cells. *Chromosoma*, **97**, 212–218.

Lee, M. and Phillips, R.L. (1988) The chromosomal basis of somaclonal variation. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 413–437.

McClintock, B. (1984) The significance of responses of the genome to challenge. *Science*, **226**, 792–80.

McDonald, J.F. (1990) Macroevolution and retroviral elements. *BioScience*, **40**, 183–191.

Médigue, C., Rouxel, T., Vigier, P., Hénaut, A. and Danchin, A. (1991) Evidence for horizontal gene transfer in *Escherichia coli* speciation. *J. Mol. Biol.* **222**, 851–856.

Milat, M.L., Ducruet, J.M., Ricci, P., Marty, F. and Blein, J.P. (1991) Physiological and structural changes in tobacco leaves treated with cryptogein, a proteinaceous elicitor from *Phytophthora cryptogea. Physiol. Biochem.* **81**, 1364–1368.

Miller, J.H. (1972) *Experiments in Molecular Genetics.* New York: Cold Spring Harbor Laboratory Press.

Nemestothy, G.S. and Guest, D.I. (1990) Phytoalexin accumulation, phenylalanine ammonia lyase activity and ethylene biosynthesis in fosetyl-Al treated resistant and susceptible tobacco cultivars infected with *Phytophthora nicotianae* var.

nicotianae. Physiol. Mol. Plant. Pathol. **37**, 207–219.

Nespoulous, C., Huet, J.C. and Pernollet, J.C. (1992) Structure-function relationships of α and β elicitins, signal proteins involved in the plant-*Phytophthora* interaction. *Planta*, **186**, 551–557.

Peschke, V.M., Phillips, R.L. and Gengenbach, B.G. (1987) Discovery of transposable element activity among progeny of tissue culture-derived maize plants. *Science*, **238**, 804–807.

Planckaert, F. and Walbot, V. (1989) Molecular and genetic characterization of Mu transposable elements in *Zea mays*: behaviour in callus culture and regenerated plants. *Genetics*, **123**, 567–578.

Pouteau, S., Huttner, E., Grandbastien, M.A. and Caboche, M. (1991) Specific expression of the tobacco Tnt1 retrotransposon in protoplasts. *EMBO J.* **10**, 1911–1918.

Raskin, I. (1992) Role of salicylic acid in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **43**, 439–463.

Ricci, P., Bonnet, P., Huet, J.C., Sallantin, M., Beauvais-Cante, F., Bruneteau, M., Billard, V., Michel, G. and Pernollet, J.C. (1989) Structure and activity of proteins from pathogenic fungi *Phytophthora* eliciting necrosis and acquired resistance in tobacco. *Eur. J. Biochem.* **183**, 555–563.

Ricci, P., Panabières, F., Bonnet, P., Maia, M., Ponchet, M., Devergne, J.C., Marais, A., Cardin, L., Milat, M.L. and Blein, J.P. (1993) Proteinaceous elicitors of plant defense responses. In *Mechanisms of Plant Defense Responses* (Fritig, B. and Legrand, M., eds). Dordrecht: Kluwer Academic Publishers, pp. 121–135.

Rolfe, M., Spanos, A. and Banks, G. (1986) Induction of yeast Ty element transcription by ultraviolet light. *Nature*, **319**, 339–340.

Ryan, C.A. and Farmer, E.E. (1991) Oligosaccharide signals in plants: a current assessment. *Ann. Rev. Plant Physiol. Mol. Biol.* **42**, 651–674.

Sequeira, L. (1983) Mechanisms of induced resistance in plants. *Ann. Rev. Microbiol.* **37**, 51–79.

Strand, D.J. and McDonald, J.F. (1985) Copia is transcriptionally responsive to environmental stress. *Nucl. Acids Res.* **13**, 4401–4410.

Tyree, M.T. and Sperry, J.S. (1989) Vulnerability of xylem to cavitation and embolism. *Ann. Rev. Plant Physiol. Mol. Biol.* **40**, 19–38.

Verwoerd, T.C., Dekker, B.M.M. and Hoekema, A. (1989) A small-scale procedure for the rapid isolation of plant RNAs. *Nucl. Acids Res.* **17**, 2362.

Walbot, V. (1988) Reactivation of the mutator transposable element system following gamma irradiation of seed. *Nature*, **336**, 242–244.

Zanetti, A., Beauvais, F., Huet, J.C. and Pernollet, J.C. (1992) Movement of elicitins, necrosis-inducing proteins secreted by *Phytophthora* sp., in tobacco. *Planta*, **187**, 163–170.