REVIEW ARTICLE

Wound signalling in plants

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Abstract

Plants undergoing the onslaught of wound-causing agents activate mechanisms directed to healing and further defence. Responses to mechanical damage are either local or systemic or both and hence involve the generation, translocation, perception, and transduction of wound signals to activate the expression of wound-inducible genes. Although the central role for jasmonic acid in plant responses to wounding is well established, other compounds, including the oligopeptide systemin, oligosaccharides, and other phytohormones such as abscisic acid and ethylene, as well as physical factors such as hydraulic pressure or electrical pulses, have also been proposed to play a role in wound signalling. Different jasmonic acid-dependent and -independent wound signal transduction pathways have been identified recently and partially characterized. Components of these signalling pathways are mostly similar to those implicated in other signalling cascades in eukaryotes, and include reversible protein phosphorylation steps, calcium calmodulin-regulated events, and production of active oxygen species. Indeed, some of these components involved in transducing wound signals also function in signalling other plant defence responses, suggesting that cross-talk events may regulate temporal and spatial activation of different defences.

Key words: Jasmonic acid, systemin, oligosaccharides, protein phosphorylation, local and systemic responses.

Introduction

Plants are sessile organisms, anchored to the ground through the root system for acquisition of nutrients and water, and thus are devoid of any possible avoidance mechanism to prevent injuries caused by chewing insects or larger herbivores. For defence, plants are endowed with pre-existing physical barriers that limit damage, such as the cuticle, and hardened, woody covers that may successfully withstand the aggression of small herbivores, or else have trichomes, thorns and other specialized organs that may further restrict pest access to the more nutritious parts of the plant. Once an injury occurs though, there is no possibility of mobilizing specialized cells devoted to wound healing such as in mammals, as plant cells are encapsulated inside rigid walls. Plants have thus evolved towards the capacity of making each cell competent for the activation of defence responses which largely depend on the transcriptional activation of specific genes. These wound-activated responses are directed to healing of the damaged tissues and to the activation of defence mechanisms that prevent further damage. Most of the induced responses occur in a time window between a few minutes to several hours after wounding, and include the generation/release, perception and transduction of specific signals for the subsequent activation of wound-related defence genes. Proteins encoded by those wound-inducible genes may play one of the following functions: (i) repairing of damaged plant tissue; (ii) producing substances that inhibit growth of the predator insect, i.e. those lowering the digestibility of the plant tissue or producing a toxin; (iii) participating in the activation of wound defence signalling pathways; or (iv) adjusting plant metabolism to the imposed nutritional demands.

A localized injury activates defence mechanisms throughout the plant both in the tissues directly damaged (local response) and in the non-wounded areas (systemic response). Whilst healing or reparation of damaged tissue is a function related to locally activated responses, herbivore deterring defences are deployed both locally

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and systemically. Wound-activated local and systemic responses include metabolic changes and induction of gene expression, but only damaged tissues also undergo a severe disorder of cellular structures, associated with de-compartmentalization and release of stored material, and a drastic loss of water. This review will focus on what is currently known about the signals that are produced and mobilized in the wounded plant, on the different transduction pathways activated by these wound signals and, finally, on the results that the activation of defence gene expression has on the injured plant. The potential and perspectives of the studies on wound signalling for the improvement of plant resistance against pests and pathogens will also be summarized.

Wound signals

Multiple signals and differential induction of gene expression point to the existence of a complex wound signalling network in plants that, in addition may have species-specific variations (Fig. 1). Many structurally different molecules play regulatory roles in wound signalling, including the oligopeptide systemin (Pearce et al., 1991), oligosaccharides released from the damaged cell wall (Bishop et al., 1981), and molecules with hormonal activity such as jasmonates (Farmer and Ryan, 1990), ethylene (O’Donnell et al., 1996), and abscisic acid (Peña-Cortés et al., 1989). However, it has not been possible to identify and define unequivocally the nature of the primary signals that trigger wound-activated defence responses. Frequently, the induction of wound responses requires the simultaneous action of different signals and regulators and, quite often, the qualitative and quantitative participation of any putative signal in the activation of wound responses depends on the plant species as well.

**Mobile signals**

Systemin is an 18 amino acid peptide generated from a larger protein precursor called prosystemin (McGurl and Ryan, 1992). Although several lines of evidence point to it as a primary long-distance transmittable signal in plants (Pearce et al., 1991; recently reviewed in Ryan and Pearce, 1998, and in Ryan, 2000), the existence and function of systemin or a related peptide has so far been documented only in solanaceous plants (Constabel et al., 1998). The constitutive activation of wound responses, including the transcriptional activation of two families of well characterized proteinase inhibitor (pin) genes in transgenic tomato plants overexpressing the prosystemin gene (McGurl et al., 1994) indicates an essential role for systemin in local and systemic wound-activated
responses. The suppression of wound-induced pin expression by antisense inhibition of the prosystemin gene (McGurl et al., 1992) and the concomitant effects on wound-induced gene expression of mutations affecting systemin signalling (Howe and Ryan, 1999) lend further support to this hypothesis. Moreover, grafting experiments with wild-type and prosystemin over-expressing transgenic plants, and the mobility of exogenously applied systemin out of wound sites are consistent with a role for systemin as a long-distance signal for wound-activated gene expression in tomato. Other cues, however, have also been proposed as primary long-distance transmittable signals, such as electrical pulses (Wildon et al., 1992; Herde et al., 1995) and hydraulic waves (Malone and Alarcón, 1995). Both action and variation potentials induce pin gene expression in tomato (Stankovic and Davies, 1996). As it has been reported that systemin can evoke electrical responses at the plasma membrane (Moyen and Johannes, 1996), it is likely that both chemical and physical signals may participate in the early steps of wound signalling and also that regulatory interactions between them may occur.

**Local signals**

At early times after wounding, plants transiently produce reactive oxygen species (ROS), including the superoxide anion in the damaged tissue (Doke et al., 1991) and hydrogen peroxide, both locally and systemically (Orozco-Cárdenas and Ryan, 1999). Production is maximal at several minutes after wounding for superoxide and at 4–6 h for hydrogen peroxide, and then declines (Doke et al., 1991; Orozco-Cárdenas and Ryan, 1999). H$_2$O$_2$ is generated locally and systemically in non-wounded plants treated with systemin (Orozco-Cárdenas and Ryan, 1999) suggesting that it may be involved in systemin-related responses. In addition, systemin may potentiate plant cells for a further oxidative burst in oligogalacturonide-elicited cultured tomato cells (Stennis et al., 1998).

Upon injury there is a release of oligogalacturonides derived from plant cell walls (Benhamou et al., 1990). Small oligosaccharides are well characterized elicitors of defence responses (Côté and Hahn, 1994). Oligogalacturonides and fungal-derived chitosan have both been shown to activate wound-inducible pin gene expression in solanaceous plants (Doares et al., 1995b). Although oligosaccharides may originate from mechanical disruption of plant cell walls, a wound-inducible polygalacturonase gene recently described in tomato (Bergey et al., 1999) may also be responsible for the production of endogenous oligogalacturonides upon wounding. Considering that the polygalacturonase gene is also systemin-inducible, oligogalacturonides may thus represent an intermediate step in signalling the activation of local responses following systemin production at the wound site. Moreover, oligogalacturonides elicit an oxidative burst in tomato cells (Stennis et al., 1998) suggesting that, at least in tomato, the sequence wound-systemin-oligogalacturonides-ROS may belong to the same signalling pathway. Oligosaccharides have a very limited mobility, hence their activity as a wound signal is likely to be restricted to responses close to the damaged areas of wounded leaves (Baydoun and Fry, 1985). However, since systemin can induce polygalacturonase activity in systemic tissues, the possibility cannot be ruled out that some oligosaccharide-dependent responses may also be functional in areas of the plant remote from the damaged site.

**Hormonal imbalance**

The roles of systemin and oligogalacturonides in activating wound responses have been tightly linked in solanaceous plants to the local and systemic accumulation of high levels of jasmonic acid (JA) and abscisic acid (ABA), plant hormones that mediate wound-activated gene expression through an apparently unified signal transduction pathway (Peña-Cortés et al., 1995; Bergey et al., 1996). These increases are probably due to wound-induced, de novo synthesis of the hormones which, in the case of ABA, may relate to the desiccation of the wounded leaf (Reymond et al., 2000). It is not known which step in ABA biosynthesis is activated by mechanical damage. As in water-stressed plants, elevated ABA levels in wounded plants may be related to an increase in the enzymes catalysing the rate-limiting steps of the pathway, zeaxanthin epoxidase and 9-cis-epoxy-carotenoid dioxygenase. On the other hand, JA is synthesized through the octadecanoid pathway from its α-linolenic acid precursor. Since application of linolenic acid to tomato plants induces the expression of the same set of genes as JA itself, it is assumed that fatty acid release from complex membrane lipids is a checkpoint in JA biosynthesis. The step catalysed by allene oxide synthase (AOS) appears to be rate-limiting in JA production as well, since both AOS mRNA and protein are present at very low levels in healthy plants and accumulate to high levels upon wounding, following a time-course that precedes the increase in JA concentration.

In response to mechanical injury, plants also produce ethylene by activating transcription of the corresponding biosynthetic genes (Liu et al., 1993; Bouquin et al., 1997). Ethylene is proposed to potentiate systemin-activated wound signalling through the octadecanoid pathway in tomato (O’Donnell et al., 1996).

In contrast, auxins have a negative effect on wound-induced gene expression. The endogenous levels of indole-acetic acid decline upon wounding in tobacco (Thornburg and Li, 1991), and recovery of the initial
levels of active auxins has been proposed as a mechanism to limit the duration of the response to wounding (Rojo et al., 1998).

In the model plant Arabidopsis thaliana, however, oligogalacturonides activate gene expression through a JA-independent pathway (Titarenko et al., 1997). Two other lines of evidence point to the existence of separate wound signalling pathways in different plant species: (i) the differential patterns of gene expression in local and systemic tissues of wounded tomato plants (Dalkin and Bowles, 1989; Lightner et al., 1993) that are not consistent with a single JA-dependent signalling pathway; and (ii) the identification of wound-inducible genes that are not induced by JA in Arabidopsis (Titarenko et al., 1997; Nishiuichi et al., 1997) and tomato plants (O’Donnell et al., 1998). The characterization of JA-dependent and -independent wound signalling pathways in Arabidopsis (Fig. 1) that are regulated in opposite directions by common elements including reversible protein phosphorylation (Rojo et al., 1998) and Ca²⁺/calmodulin events (León et al., 1998) fully confirmed this proposition (see below). In addition, the action of JA and ABA activating the expression of wound-responsive genes, initially proposed to function through a common, systemin-activated, signalling pathway in solanaceous plants (Hildmann et al., 1992; Peña-Cortés et al., 1989, 1996), has been uncoupled in species such as barley, potato or Arabidopsis (Lee et al., 1996; Dammann et al., 1997; J León and J Sánchez-Serrano, unpublished results). Although ABA perception is necessary for the wound-induced expression of proteinase inhibitors in tomato (Carrera and Prat, 1998), ABA does not appear to be a primary signal in wound signalling (Birkenmeier and Ryan, 1998).

In contrast to its role in solanaceous plants, ethylene acts in Arabidopsis as a cross-talk regulator between JA-dependent and -independent wound signalling pathways determining local versus systemic wound-induced gene expression (Rojo et al., 1999). There is an oligogalacturonide-mediated repression of the JA-dependent pathway that is exerted locally, through the production and perception of ethylene in damaged tissues, but not systematically, where the JA-dependent signalling pathway remains fully operative. Thus, oligosaccharide-responsive genes are expressed close to the wound site whilst the largest accumulation of transcripts derived from JA-responsive genes is observed in the systemic tissues. In contrast, other genes, like the defensin PDF1.2, requires both JA and ethylene perception for attaining full expression upon pathogen challenge (Penninckx et al., 1998). Thus, a complex network, responsive to the input of different primary wound signals and resulting in the activation of distinct functional responses, is likely to underlie inducible defence gene activation in plants.

Intracellular wound signalling

As depicted in the previous section, wound signalling in plants is complex involving a whole array of molecules with regulatory activity on inducible defence responses. Although several wound signalling pathways have already been proposed to function in plants, little information is available on the molecular components responsible for the perception and transduction of these signals.

Receptors

As in any other transduction pathway, the first step in wound signalling should be the perception of the signal by a specific receptor. To date, a systemin-binding protein (Scheer and Ryan, 1999) and a membrane-associated uronide-binding phosphoprotein (Reymond et al., 1996) have been identified and partially characterized, but their function in wound signalling remains uncertain. The identified systemin-binding protein appears to have protease activity, and may thus be involved in degrading systemin once its function is fulfilled. A putative systemin receptor has been identified in tomato cells (Scheer and Ryan, 1999). The N-terminal part of systemin is required for binding to this receptor while the C-terminal part is involved in activating cell responses (Meindl et al., 1998; Dombrowski et al., 1999). However, no putative receptor has been identified for JA, one of the major inducers of gene activation in response to wounding.

Second messengers

The limited knowledge on perception events makes difficult the elucidation of early components of the wound signalling pathways. Nevertheless, it is well known that wound signal molecules promote rapid membrane-associated events such as depolarization of the membrane with a concomitant proton influx (Thain et al., 1995; Moyen and Johan, 1996). Elevation of intracellular levels of calcium (Moyen et al., 1998) and changes in the pattern of protein phosphorylation (Schaller and Oecking, 1999) are also part of the responses to wounding in tomato plant cells. In Arabidopsis, transgenic plants expressing aequorin (Knight et al., 1993) have been used to detect increases in intracellular calcium levels upon wounding. Although in opposite ways, both JA-dependent and oligogalacturonide-dependent JA-independent wound signal transduction pathways are regulated by mobilization of calcium from intracellular stores, and by calmodulin-related activity (León et al., 1998). In tomato, it has been reported that the expression of a wound- and systemin-inducible calmodulin gene may be associated with activation of wound-responsive genes (Bergey and Ryan, 1999). All these data support the implication of calcium and calcium-binding proteins in the regulation of wound responses.
that of the WR genes induced in *Arabidopsis* through the JA-independent, oligosaccharide-dependent signalling pathway. Okadaic acid induces WR gene expression in non-challenged plants and, moreover, a staurosporine-sensitive protein kinase activity is required for full activation of WR genes upon wounding.

**Jasmonates, a second wave of wound signals**

After the early events that occur within the first minutes after wounding, comprising the production and perception of primary signals, activation of ion channels at the plasma membrane, and reversible protein phosphorylation, mechanisms are activated to generate a second wave of wound-related signals likely directed to either propagate defence responses or to activate different defence functions in systemic tissues. Among these secondary signals, are oxylipins, and especially JA, widespread key regulators of wound-activated gene expression (see Fig. 1 in the accompanying review by Schaller; Schaller, 2001).

JA is a derivative of α-linolenic acid (18:3, Δ9,12,15), the most abundant fatty acid in the membrane lipids of leaves. Wound responses that are mediated through the action of jasmonates require the production of unesterified fatty acids as substrates for the octadecanoid pathway. During the first hours after wounding, plants accumulate phosphatidic acid and unesterified fatty acids that are released from lipids presumably by the action of wound-inducible phospholipases of types D and A2 (Conconi et al., 1996; Lee et al., 1997; Ryu and Wang, 1998; Narváez-Vásquez et al., 1999). These classes of phospholipases are activated by calcium (Ryu and Wang, 1996) and modulated by reversible phosphorylation (Stratmann and Ryan, 1997). However, although the participation of these kinds of lipases in JA-mediated gene expression has been widely invoked, such a phospholipase has not been fully identified, and neither the corresponding gene cloned nor its function in wound-activated responses proved. In parallel to animal structural analogs of JA, the prostaglandins, it is assumed that a phospholipase A2 activity releases linolenic acid from complex lipids to provide the unesterified fatty acid substrate for lipooxygenases, which introduce molecular oxygen with stereospecificity for the 13-C position, thus starting the octadecanoid pathway (Vick, 1993). Hydro-peroxide utilization is a branch point in this biosynthetic pathway. Fatty acid hydroperoxides can either be reduced to the corresponding alcohols by the action of peroxigenases or converted to divinyl ethers by the action of a divinyl ether synthase. Both divinyl ethers and peroxigenase-derived hydroxy and epoxyalcohols may participate in plant defence responses (Blé, 1998; Weber et al., 1999). Alternatively, hydroperoxide lyase-mediated cleavage of fatty acid hydroperoxides
leads to the production of 12C-oxoacids and 6C-aldehydes. Derivatives of the 12C-oxoacids such as traumatin and unsaturated 6C-aldehydes such as hexalins play a function in plant defence against wounding and pathogens, respectively (Vick, 1993; Croft et al., 1993). On the other hand, the 13-hydroperoxy-linolenic acid is a substrate for allene oxide synthase (AOS) and cyclase, yielding 12-oxo-phytodienoic acid (12-oxo-PDA), a precursor in JA formation. 12-oxo-PDA may in itself be an active compound, at least in some physiological responses such as tendril coiling in *Bryonia dioica*. The evidence gathered so far suggests that 12-oxo-PDA synthesis occurs in chloroplasts. However, the next enzyme in the JA biosynthetic pathway (Creelman and Mullet, 1997), PDA-reductase, has a cytosolic location. For JA production, 12-oxo-PDA has thus to be transported to the cytosol for further processing that includes three rounds of β oxidation, and this is likely to occur in peroxisomes (for a more detailed overview on JA synthesis see the review by Schaller in this issue; Schaller, 2001).

Events that lead to the synthesis of oxylipins from fatty acids through the octadecanoid pathway are relatively well characterized and most of the genes encoding enzymes of the biosynthetic pathway have already been cloned (reviewed by Creelman and Mullet, 1997, and León and Sánchez-Serrano, 1999). Wounding activates the expression of most of the genes encoding enzymes of the JA biosynthetic pathway. The expression of ω3 fatty acid desaturase, 13-lipoxygenase, AOS, and 12-oxo-PDA reductase, is induced in concert both upon wounding and after exogenous application of JA, suggesting that a positive feedback mechanism leads to the high levels of JA present in damaged plants. As the enzymes involved in the JA pathway may combine to catalyse reactions with linoleic (18:2) and hexadecatrienoic (16:3) acids, the activity of this route is likely to yield a family of related products, collectively termed jasmonates. Among them, active oxylipins with wound-related functions have been identified (Gundlach and Zenk, 1998). A new family of jasmonate-related regulators including dinor-oxo-phytodienoic acid, produced from 16:3 plastid-derived hexadecatrienoic acid, accumulate in wounded leaves of *Arabidopsis* and potato (Weber et al., 1997), and have been suggested to induce enzyme activities of the JA biosynthetic pathway, thus potentiating JA-dependent wound responses. Oxylipins may be further modified by glycosylation, methylation and amino acylation giving rise to compounds with slightly different activities. Some of these modifications may also serve to eliminate their biological activity.

It is still not known how the release and further accumulation of jasmonates in the cytoplasm are subsequently perceived, or how and where jasmonates play their functions as activators of defence gene expression. The availability of JA-insensitive mutants of *Arabidopsis* (Feys et al., 1994; Berger et al., 1996) has allowed the elucidation of the role of JA in the transduction of many wound-activated responses. A better understanding of the precise roles of the affected genes will be achieved when they are cloned and their functions characterized. Regarding this, cloning of COJI gene (Xie et al., 1998), by complementation of the coil-1 mutation of *Arabidopsis* that confers male sterility, JA insensitivity and inactivation of the JA-mediated wound signalling (Feys et al., 1994; Titarenko et al., 1997), has revealed it as an F-box protein that may function by recruiting regulators of wound defence and pollen development for modification by ubiquitination (Xie et al., 1998) and subsequent degradation. However, more information is still necessary on how this kind of modification may regulate subsequent JA-dependent gene expression and, especially, on the steps linking JA production with signal transduction pathways that finally control JA-mediated plant development and defence responses to environmental stresses.

**Cross-talk between wound signalling and other plant defence responses**

Often plants undergoing the onslaught of an injury-causing agent are targets for further stress factors. Pathogen infection is greatly facilitated in damaged tissues. Wound sites are easily accessible to penetration by pathogens. Thus, it is likely that wounding first triggers specific defence responses, but also that these responses may, somehow, activate specific developmental programmes that prepare the plant to resist further attack by pathogens. Wounded parts of the plant can be affected in different degrees, but if damage is large enough to severely limit viability of these organs, it may for instance be advantageous to promote its abscission. A whole range of evidence points to the existence of cross-talk between wound- and pathogen-activated signalling pathways. Some of these regulatory interactions actually operate on the production of signals implicated in different pathways. For instance, transgenic tobacco plants over-expressing either a rice Ras-related small GTP-binding protein gene or the MAP kinase WIPK gene (Sano et al., 1994; Seo et al., 1995) accumulate salicylic acid (SA) in response to wounding, a phenomenon that leads to an enhanced resistance to pathogens that does not occur in wounded wild-type plants. Interactions between wound- and pathogen-activated defence responses are, in general, negative, leading to an inverse relationship between resistance to pathogen micro-organisms and insect herbivory (Felton et al., 1999). However, both inhibition of wound-activated JA-mediated responses by SA (Peña-Cortés et al., 1993; Doares et al., 1995a) or pathogens (Preston et al., 1999), and activation of JA-mediated wound responses by the products of
cell-wall-degrading enzymes of the plant pathogen *Erwinia carotovora* (Norman et al., 1999), have been reported. It has also been reported that simultaneous treatment of tobacco seedlings with SA and JA, or JA and ethylene leads to an overinduction of PR1 compared to that observed in seedlings treated with any of them alone (Xu et al., 1994). On the other hand, Arabidopsis mutants impaired in JA sensitivity are less resistant to pathogens (Feys et al., 1994) suggesting that JA perception is also necessary for activating defences against certain pathogens. Whether different defence signalling pathways activated in response to different stress factors may converge or not is still controversial (Van Wees et al., 2000), but it has been reported that responses activated by gene-for-gene plant-pathogen interactions and wounding or mechanical injury are interlinked at the level of MAP kinases in tomato (Romeis et al., 1999). These kinases are closely related to the previous MAP kinases induced by wounding (WIPK) and SA (SIPK) in tobacco (Seo et al., 1995; Zhang and Klessig, 1997, 1998) suggesting that integration of wound- and pathogen-activated responses at the level of MAP kinase-catalysed phosphorylation may be widely functional in plants.

**Perspectives and conclusion**

Identifying and characterizing receptors for wound signals is likely to be a main focus of future research in the field of plant responses to wounding. Elucidating the compartmentalization and cell type specificity of the different wound signalling pathways is likely to follow suit. Promoter elements and the corresponding transcription factors directing wound-activated gene expression will provide useful tools for genetic manipulation of plants towards enhanced resistance to stress. Finally, it will also be of major interest to unravel the regulatory connections between wound-activated signalling pathways and signal transduction pathways triggered by other stress factors. Particularly, cross-talk processes that potentiate more than one signalling pathway could be essential targets to make plants more resistant to different stresses. The combination of genetic approaches (Howe and Ryan, 1999) and powerful molecular techniques such as DNA microarray hybridization (Reymond et al., 2000) are likely to yield exciting results in a not too distant future.

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