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Interactions Between Signaling Compounds Involved in Plant Defense

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Abstract

To elude or minimize the effects of disease and herbivory, plants rely on both constitutive and inducible defenses. In response to attack by pathogens or pests, plants activate signaling cascades leading to the accumulation of endogenous hormones that trigger the induction of defenses. Salicylic acid (SA), jasmonic acid (JA), and ethylene (E) are plant-specific hormones involved in communicating the attack by many pathogens and pests in a broad range of plant species. SA, JA and E signaling cascades do not activate defenses independently, but rather establish complex interactions that determine the response mounted in each condition. Deployment of defenses is energetically costly, so a trade-off between the activation of resistance against a particular pest or pathogen and down regulation of other defenses is common. Conversely, activation of broad range resistance in response to an initial attack may serve to deter opportunistic agents. Thus, the interaction among SA, JA and E defense signaling pathways can be antagonistic, cooperative or synergistic, depending on the plant species, the combination of organisms attacking the plants, and the developmental and physiological state of the plant. A characterization of the interactions among defense signaling pathways and the determination of the molecular components mediating cross-talk between the different pathways will be essential for the rational design of transgenic plants with increased resistance to disease and/or herbivores without critically compromising other agronomic traits.

Key words: Hormone; Jasmonic acid; Salicylic acid; Ethylene; Defense; Resistance; Disease; Pathogen; Herbivore; Wounding; Cross-talk; Signaling

Introduction

Numerous defense strategies have evolved in plants to protect them from pathogens and herbivores, and in most cases those defenses successfully prevent the establishment of the infection or the trophic interaction. Some defenses are preformed whereas others are inducible upon the attack, probably because they are costly or deleterious for the plant to maintain continuously (Baldwin 1998; Royo and others 1999). The induction of resistance responses indicates that mechanisms to detect pathogens and pests and activate defense reactions against those organisms are present in plants. Pathogen or pest-derived elicitors, receptors and signaling molecules that participate in defense activation and induction

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of genes encoding defense-related products have been described. The signaling steps that link the perception of the cue to the regulation of gene expression required for defense have been studied extensively (Glazebrook 2001; Dong 2001; León and others 2001; Asai and others 2002; Holt and others 2003). These studies have shown that defense-signaling pathways are not linear. Rather, recognition and response to pathogen and pest attack involves, in most cases, parallel circuitry that activates multicomponent downstream defenses. Moreover, it has become obvious that the signaling pathways activating defense responses against different stimuli are not independent but are instead interconnected and establish cooperative, synergistic and antagonistic interactions. The nature of those interactions will eventually determine the output response (Thomma and others 2001; Kunkel and Brooks 2002). It is common in nature for plants to be challenged simultaneously by multiple pathogens and pests activating distinct signaling cascades. The cross-talk between signaling pathways adds plasticity to the defense response, allowing the plant to adjust it depending on the combination of stimuli present. We will review examples of interactions between signaling hormones involved in plant defense, and will discuss some of the possible molecular components that may integrate the signals emanating from those different pathways.

Hormone Interactions In Defense Signaling Against Pathogens

When challenged with a pathogen, plants mount complex responses that involve the activation of different signaling cascades, finally leading to the activation of local and systemic antimicrobial defenses. Three plant-specific hormones, salicylic acid (SA), jasmonic acid (JA) and ethylene (E) are major endogenous signals involved in communicating the presence of an infection and triggering the defense responses in plants. Abscisic acid (ABA) and auxins may also play a role in defense against pathogens. For example, ABA negatively regulates SA-dependent resistance (Audenaert and others 2002) and the *dth9* mutant of *Arabidopsis* that displays reduced sensitivity to auxins shows enhanced susceptibility to *Pseudomonas syringae* and *Peronospora parasitica* (Mayda and others 2000). However, the involvement of ABA and auxins in pathogen defense is much less documented and will not be further discussed here.

The range of pathogen infections signaled through SA and JA or E are partially exclusive. SA has been linked to the response to infection by viruses like tobacco mosaic virus (TMV) and turnip crinkle virus, and by biotrophic bacteria and fungi such as *Pseudomonas*, *Peronospora*, *Erisyphe*, and so on (Delaney and others 1994; Cao and others 1994; Shulaev and others 1995; Thomma and others 1998; Reuber and others 1998; Dewdney and others 2000; Kachroo and others 2000). These plant-pathogen interactions are associated in many cases with the development of a hypersensitive response (HR) that results in death of the infected cells, which may deprive these biotrophic pathogens of nutrients needed to thrive. In contrast, HR-induced cell death would not be efficient and may even be detrimental against necrotrophic pathogens (Govrin and Levine 2000). Other defense mechanisms against necrotrophic pathogens have thus evolved in plants, activated in many cases by JA and E signaling pathways. It has been shown that JA and E signaling are required for resistance to pathogens (mainly necrotrophic or saprophytic) such as *Alternaria*, *Botrytis*, *Septoria*, *Phytium*, *Erwinia*, *Plectosphaerella*, and so on (Knoester and others 1998; Thomma and others 1998, 1999; Berrocal-Lobo and others 2002; Diaz and others 2002). However, ascribing the defense against biotrophic pathogens to SA signaling and against necrotrophic pathogens to JA and E signaling would be an oversimplification. JA and E signaling pathways are involved in defense against the biotrophic pathogens *Erisyphe cichoracearum*, *Erisyphe orontii*, *Oidium lycopersicum*, and *Pseudomonas syringae* (Ellis and Turner 2001; Ellis and others 2002). Conversely, SA is involved in resistance against the necrotrophic fungi *Botrytis cinerea* and *Plectosphaerella cucumerina* (Berrocal-Lobo and others 2002; Audenaert and others 2002; Diaz and others 2002). Moreover, not only have these hormones been shown to participate in activating parallel defenses against the same pathogen, but also many events of cross-talk among the SA, E and JA signaling pathways have been reported and, as described below, shown to be significant in determining the resistance to pathogens.

Interaction Between JA and E

The resistance to many pathogens like *Erisyphe cichoracearum*, *Erisyphe orontii*, *Oidium lycopersicum*, *Botrytis cinerea* and *Plectosphaerella cucumerina* (Thomma and others 1999; Ellis and Turner 2001; Thomma and others 2001; Berrocal-Lobo and others 2002) requires both JA and E, as demonstrated by

the enhanced susceptibility of loss-of-function mutations in components of these signaling pathways such as *ETHYLENE-INSENSITIVE2* (*EIN2*) and *CORONATINE-INSENSITIVE1* (*COI1*). In addition, exogenous application of the hormones (Thomma and others 1999; Diaz and others 2002) and work with transgenic plants that constitutively activate the ethylene pathway (Berrocal-Lobo and others 2002) demonstrated that both hormones (E and JA) are also sufficient to enhance resistance against several pathogens such as *Botrytis cinerea* and *Plectosphaerella cucumerina*. Moreover, the mutant *cev1* that has constitutively active JA and E signaling shows enhanced resistance to infection by biotrophic fungi (Ellis and Turner 2001). The fact that E and JA signaling pathways mediate resistance against an almost overlapping range of pathogens indicates that they may be activating common defense responses. JA and E may act sequentially, cooperatively or synergistically, depending on the infectious agent. Nonpathogenic rhizosphere-colonizing *Pseudomonas* trigger a systemic tolerance to a broad range of pathogens, the Induced Systemic Resistance (ISR), which requires both E and JA. JA-induced ISR is blocked in E-insensitive mutants but E-induced ISR is not blocked in JA-insensitive mutants, suggesting that JA and E act sequentially to induce this defense response (Pieterse and others 1998).

However, in many cases, JA and E act in a synergistic fashion to activate defenses such as in the induction of some defense-related proteins like the antimicrobial defensin PDF1.2, the basic chitinase PR-3, osmotin and others (Xu and others 1994; Penninckx and others 1998; Lorenzo and others 2003). In addition, the induction of many defense-related proteins is blocked in the JA-insensitive mutant *coi1* and in the E-insensitive mutant *ein2*, demonstrating a basic requirement for both JA and E signaling in their induction (Penninckx and others 1998; Lorenzo and others 2003).

Recently, a mechanistic explanation for the integration of the JA and E signaling pathways at the molecular level has been provided (Figure 1) (Lorenzo and others 2003). It has been shown that the expression of the transcription factor ETHYLENE RESPONSE FACTOR1 (*ERF1*) requires both JA and E signaling and that E and JA treatment have a synergistic effect on *ERF1* expression. The overexpression of *ERF1* results in constitutive expression of defense genes induced simultaneously by JA and E, and not genes regulated differentially by these hormones. Moreover, the overexpression of *ERF1* bypasses the requirement of COI1 and EIN2 for expression of defense genes like *PDF1.2* and *PR-3*,

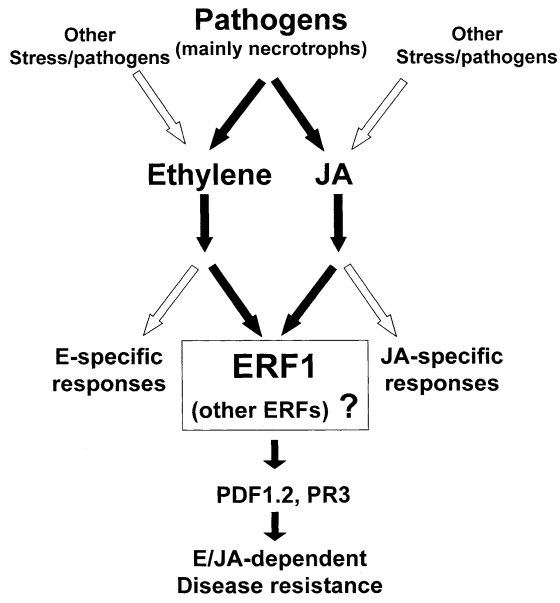

Figure 1. Model for the jasmonate/ethylene-dependent defense response pathway in *Arabidopsis*. Infection by some pathogens induces the synthesis of jasmonic acid (JA) and ethylene (E) and the simultaneous activation of their signaling pathways (black arrows). The signals from both pathways converge on the transcriptional activation of *ERF1*. In turn, ERF1 activates the expression of JA/E-dependent, defense-related genes that prevent disease progression. Other types of stresses or pathogens induce the activation of only one of the signaling pathways (white arrows) and, therefore, of E- or JA-specific responses.

indicating that ERF1 acts downstream of JA and E perception and that it may integrate JA and E signaling pathways (Solano and others 1998; Lorenzo and others 2003). *ERF1* overexpression results in increased resistance against *Botrytis cinerea* and *Plectosphaerella cucumerina* in *Arabidopsis* (Berrocal-Lobo and others 2002), suggesting that it is a significant component of the JA- and E-induced resistance against these pathogens. These data indicate that the regulation of the expression of the ERF1 transcript may be the step integrating the signals emanating from the JA and E pathways to activate the resistance to pathogens such as *Botrytis cinerea* and *Plectosphaerella cucumerina*.

To unequivocally demonstrate this, several questions remain to be answered: (1) Does *ERF1* overexpression complement the increased susceptibility of JA- and E-insensitive mutants to infection by *Botrytis cinerea* and *Plectosphaerella cucumerina*? (2) are *erf1* mutants blocked in JA- and E-dependent gene expression and resistance? *ERF1* belongs to a large family of transcription factors (ERFs, previously known as EREBPs), which may have redun-

dant functions, thus making the functional analysis of *ERF1* more difficult. (3) What is the basis of the regulation of *ERF1* expression by JA and E? The fact that *ERF1* transcript levels are induced synergistically by E and JA suggests that the integration may occur at the level of transcription by regulatory elements in the promoter of *ERF1*, in a similar manner as floral inductive signals are integrated in the promoter of *LEAFY* (Blazquez and Weigel 2000) or elicitor, and UV-light signals converge on the promoter of acyl-CoA oxidase (Logemann and Hahlbrock 2002).

Interaction of SA with JA and E

SA and JA/E are involved in signaling defenses against different sets of pathogens (mainly against biotrophs in the case of SA and mainly against necrotrophs in the case of JA/E), although some overlap exists, as previously indicated. Interestingly, most of the reported cases of cross-talk between the SA and the JA/E signaling pathways are negative interactions, indicating a trade-off between these pathways, such that induction of defenses activated by one concurrently down regulates defenses activated by the other.

Negative Interactions Between SA and JA

The cross-talk between SA and JA occurs both upstream of the production of the hormones and in their downstream signaling pathways. SA treatment blocks JA biosynthesis in tomato and *Arabidopsis* (Peña-Cortes and others 1993; Doares and others 1995; Harms and others 1998; Laudert and Weiler 1998), possibly by preventing the release of the JA-precursor 12-oxo-phytodienoic acid from the chloroplasts. In addition, SA blocks JA signaling, as demonstrated by the antagonistic effect of SA and JA treatment on gene induction (Niki and others 1998) and by the enhanced expression of JA-responsive genes in mutants that have reduced SA accumulation or perception. *Arabidopsis* plants expressing the SA degrading enzyme salicylate hydroxylase (*nahG* gene) show increased accumulation of the JA-responsive antifungal defensin PDF1.2 when challenged with *Alternaria* (Penninckx and others 1996). Moreover, mutations that impair SA signaling, such as *pad4* and *eds4*, show higher sensitivity to inducers of JA-dependent gene expression (Gupta and others 2000). In the *cpr6* mutant, which has higher levels of SA and expresses constitutively SA- and JA-dependent defenses, reducing the levels of SA by crossing in an eds5 mutation or reducing SA-signaling by crossing in an *npr1* mutation results in higher expression of the JA-responsive gene *PDF1.2* (Clarke and others 2000). These results suggest an inhibitory effect of SA both on JA synthesis and JA signaling.

Conversely, negative effects of JA on SA synthesis and/or signaling have also been documented. Constitutive JA-signaling in the *Arabidopsis* mutant *cev1* suppresses SA-dependent defense gene expression (Ellis and others 2002) and, moreover, activation of the JA/E pathway by overexpression of *ERF1* results in a reduced (SA-dependent) resistance to the biotrophic bacteria *Pseudomonas syringae* (Berrocal-Lobo and others 2002). In contrast, mutations in the mitogen-activated protein kinase (MAPK) MPK4 and the steroyl-ACP fatty acid desaturase SSI2 result in JA insensitivity and, concomitantly, in higher constitutive levels of SA, constitutive expression of the SA-responsive PR genes, and constitutive systemic acquired resistance (SAR) to *Pseudomonas syringae* and *Peronospora parasitica* (Petersen and others 2000; Kachroo and others 2001). Mutations in the F-box protein COI1 also provoke JA insensitivity and enhanced accumulation of SA in *Pseudomonas syringae* infected plants, that correlate with a higher resistance to this bacterium (Feys and others 1994; Kloek and others 2001). The elevated levels of SA are responsible for the enhanced resistance of *mpk4* and *coi1* mutants but not of *ssi2* mutants, indicating that JA may negatively interact with both synthesis and signaling of SA required for pathogen defense. On the other hand, the impairment of JA signaling in *mpk4*, *ssi2* and *coi1* mutants is independent of enhanced SA levels, indicating that MPK4, COI1 and SSI2 are involved in both JA signaling and in JA-mediated negative regulation of the SA pathway. It has been speculated that SSI2 may be involved in the generation of a fatty acid-derived signal required along with JA for activating JA-responsive genes while repressing SA signaling, possibly by modulating the MAPK activity of MPK4 (Kachroo and others 2001).

Interestingly, other MAPKs may be involved in a similar cross-talk between SA and JA in tobacco. Silencing of WIPK, a tobacco MAPK, inhibits JA production in response to wounding and blocks the induction of wound-responsive genes (Seo and others 1995). Conversely, WIPK overexpression leads to enhanced JA levels and constitutive expression of the wound-inducible gene *Pin2*. Moreover, SA production and induction of SA-responsive genes by wounding is observed in WIPK-silenced plants but not in wild-type plants. Thus a similar inhibition of SA synthesis by MAPKs involved in JA signaling occurs in tobacco and *Arabidopsis*, indicating that this cross-talk mechanism is present in

distantly related plant species. However, the level at which they act in the signaling cascades may be different in tobacco and *Arabidopsis*. Although both MPK4 and WIPK repress SA synthesis, WIPK activity may be required for *de novo* JA synthesis in response to wounding (Seo and others 1999), whereas MPK4 may be involved in signaling downstream of JA synthesis (Petersen and others 2000). However, it cannot be discounted that MPK4 and WIPK have the same function, because it has not been reported whether JA synthesis is blocked in *mpk4* mutants, and whether WIPK-silenced plants are JA insensitive.

Many cases of negative cross-talk between the JA and SA pathways have been reported and shown to have an effect on disease resistance. However, most experiments were performed in laboratory conditions and in most cases involved exogenous application of the hormones and assay of the resistance to a single pathogen that activates either pathway exclusively. The role of these interactions in resistance of plants in natural or cultivated environments has not been thoroughly tested. Cross-talk between JA and SA should be important in field conditions, where the plant may be challenged with numerous pathogens simultaneously and it has to activate a response that maximizes fitness in each condition. The fact that the interaction between JA and SA is genetically determined by genes such as *SSI2*, *MPK4* and *COI1* is consistent with it being an endogenous regulatory mechanism of the plant and not a result of artificial regulation of the pathways by exogenous application of the hormones.

Some of the most convincing evidence for the biological significance of the negative interaction of SA and JA in pathogen defense derives from experiments suggesting that pathogens may use this cross-talk mechanism to down-regulate host defenses. The *COI1* gene that represses SA-signaling is required for sensitivity to JA but also to its structural analog coronatine, a compound produced by *Pseudomonas syringae* (Feys and others 1994). Coronatine is a virulence factor of *Pseudomonas syringae* (Mittal and Davis 1995), possibly required to activate JA signaling, thus interfering with the activation of SA-dependent defenses. Analogously, Harpin, the proteinaceous elicitor from *Pseudomonas syringae*, activates MPK4 (Desikan and others 2001), which as discussed above may activate JA signaling and repress SA-mediated resistance mechanisms.

Negative Interactions Between SA and E

Examples of negative interaction between E and SA signaling pathways in disease resistance have also been reported. E-insensitive mutants of soybean and tomato showed reduced disease severity in the SA-dependent response to bacterial pathogens such as *Pseudomonas syringae* and *Xanthomonas campestris*, and the fungal pathogen *Fusarium oxysporum* (Hoffman and others 1999; Lund and others 1998), which may just indicate the involvement of E in the development of disease symptoms, or might represent a negative effect of E in SA synthesis or signaling. Crossing in an *ein2* mutation that causes insensitivity to E in the *Arabidopsis* mutant *cpr5* that has higher levels of SA and expresses constitutively SA-dependent defenses, results in even higher levels of SA accumulation (Clarke and others 2000), suggesting that E is inhibiting SA synthesis in that mutant. Conversely, tomato plants expressing the *nahG* gene show an increase in E accumulation upon challenge with *Xanthomonas campestris* (O'Donnell and others 2001). Simultaneous treatment of tomato plants with SA and E blocks the E-induced resistance to *Botrytis cinerea* (Diaz and others 2002).

Recently, a negative interaction between SA and E on gene regulation has also been documented and a putative cross-talk mechanism unraveled. SA treatment inhibits E-induced expression of the defensive genes glucanase B and osmotin in tomato (Gu and others 2000). E-induced expression of these genes is likely mediated by the putative transcription factor Pti4 that binds to the E-responsive GCC box present in their promoters. Surprisingly, both E and SA induce *Pti4* expression. Conceivably, the repression of E-induced glucanase B and osmotin expression by SA occurs by blocking the activity of Pti4. Consistent with this hypothesis, overexpression of Pto, that phosphorylates and activates Pti4, overcomes the inhibition of expression of glucanase B and osmotin by SA (Gu and others 2000). Pti4 may thus serve to integrate signals from the E and SA pathways. Interestingly, Pti4 is related to the protein ERF1 that, as discussed above, integrates signals from the JA and E pathways. However, *ERF1* transcription is positively regulated by both JA and E, whereas *Pti4* transcription is positively regulated by E and SA, and its activity may be represssed by SA. *Pti4* overexpression in *Arabidopsis* increases resistance to *Erysiphe orontii* and tolerance to *Pseudomonas syringae*. The enhanced resistance correlates with increased expression of SA-regulated *PR1* and *PR2* genes as well as JA and E-regulated *PDF1.2* (Gu and others 2002). Therefore the induced expression of *Pti4* by both SA and E may serve to activate downstream defense genes from both pathways. Moreover, SA may modulate activity of Pti4 and

thus divert Pti4 toward activation of SA-responsive genes (Gu and others 2002).

Positive Interactions of SA with JA and E

Cases of sequential, cooperative and synergistic interactions between SA and JA or E in defense responses to pathogens have also been reported. In tomato, infection with *Xanthomonas campestris* triggers the accumulation of E and SA, and E-insensitive or SA-deficient tomato mutants exhibit a large reduction in disease symptoms but not in bacterial growth (O'Donnell and others 2001). E accumulation peaks earlier than SA accumulation. Moreover, SA accumulation is blocked in the E-insensitive *Nr* mutant and in the E-underproducing ACC deaminase (ACD)-depleted line, indicating that E signaling is required for SA accumulation in response to *Xanthomonas campestris* infection. Exogenous application of SA to *Nr* or ACD-deficient plants restores necrosis in infected tissues, indicating that E and SA act sequentially to regulate the response to this pathogen (O'Donnell and others 2001). Simultaneous activation of SA-dependent SAR and JA/E-dependent ISR induces cooperative protection against a subsequent infection by *Pseudomonas syringae* through parallel activation of complementary defense responses (van Wees and others 2000). SA-dependent SAR and JA/E-dependent ISR also confer protection against *Xanthomonas campestris* in *Arabidopsis* (Ton and others 2002).

Loss-of-function mutations in SA, JA and E signaling pathways have revealed their interactions in pathogen defense. SA, JA and E cooperate in the plant response to *Plectosphaerella cucumerina* since mutations in the E or JA pathways or depletion of SA enhance susceptibility of *Arabidopsis* plants to this fungus (Berrocal-Lobo and others 2002). In *Arabidopsis*, SA and E signaling are required for resistance to the yellow strain of cucumber mosaic virus (Takahashi and others 2002) and JA and E are required in the *cpr5* and *cpr6* mutants for SA-dependent and NPR1-independent enhanced resistance to *Peronospora parasitica* and *Pseudomonas syringae* pv *maculicola* (Clarke and others 2000).

Several mutants that have constitutively active SA and JA/E pathways have been reported, demonstrating that simultaneous activation of these pathways is possible. The *cet1*, *cet2*, *cet3* and *cet4* mutants of *Arabidopsis* show constitutive SA- and JA-dependent defense responses, probably by affecting a step in a signaling cascade prior to a divergence that separates the specific responses to each of the hormones (Nibbe and others 2002). The *Arabidopsis hrl1* mutant shows constitutive expression of SA and JA/E-responsive defense genes, increased accumulation of SA and E, and enhanced resistance against *Pseudomonas syringae* and *Peronospora parasitica* (Devadas and others 2002). SA signaling positively regulates the expression of JA/E-responsive genes in the *hrl1* mutant and, conversely, E positively regulates the expression of SA-responsive genes. An important finding in that work is that SA has opposite effects on the expression of JA/E-responsive genes, depending on the concentration of hormones applied. Therefore the concentration of endogenous E, JA and SA will determine the outcome of their interactions. This may explain why SA and JA/E signaling pathways have been reported to interact in opposite fashion within the same tissue of a single species such as *Arabidopsis*.

Consistent with these positive interactions among SA, JA and E, a microarray analysis of *Arabidopsis* response to *Alternaria*, JA, E and SA treatments showed that these signals mostly cooperate rather than antagonize (Schenk and others 2000). A synergistic effect of SA, E and JA on expression of defense-related proteins has been reported (Xu and others 1994) and E has been shown to potentiate the sensitivity to SA in *Arabidopsis* (Lawton and others 1994).

Hormone Interactions In Defense Signaling Against Herbivores

Plants utilize both constitutive and inducible defenses to protect themselves from herbivory. Constitutive defenses include physical barriers such as cell walls, cuticles, callose, trichomes and thorns, as well as stored secondary metabolites that inhibit herbivore growth and development. However, many of the defenses are activated only after the initial attack. These induced defenses include both accumulation of phytoalexins and proteins with defensive properties (Bowles 1990), such as proteinase inhibitors (Pins) that inactivate digestive proteinases of the foraging organisms and confer resistance against them (Hilder and others 1987; Ryan 1990). Importantly, the accumulation of defense proteins such as Pins occurs both at the tissues damaged by the herbivore as well as in non-damaged systemic tissues, conferring overall protection to the plant (Green and Ryan 1972). Mechanical wounding of the plants reproduces many of the responses induced by feeding herbivores, including the local and systemic accumulation of Pins and other defensive compounds (Green and Ryan 1972; Bergey and others 1996).

The study of the wound response in solanaceous plants has unraveled many of the molecular events that participate in signaling of mechanical damage, which later have been found to be largely common to those that signal herbivore feeding. Those studies have revealed the involvement of oligouronides (OGAs), the peptide systemin (SYS), and the hormones JA, E and ABA in wound signaling in solanaceae and other plants such as *Arabidopsis* (Bishop and others 1981; Farmer and Ryan 1990, 1992; Pearce and others 1991; McGurl and others 1992; Hildmann and others 1992; O'Donnell and others 1996; Rojo and others 1998, 1999; Stintzi and others 2001; Park and others 2002). Moreover, experiments indicating the role of SYS and JA in defense against herbivores have also been reported. For instance, antisense repression of the prosystemin gene in tomato reduces resistance to *Manduca sexta* larvae (Orozco-Cardenas and others 1993), whereas overexpression of prosystemin enhances resistance to *Tetranychus urticae* and *Frankliniella occidentalis* (Li and others 2002). In a similar way, exogenous application of JA induces resistance to a broad range of herbivores (Baldwin 1998; Omer and others 2000; Thaler and others 2002). The tomato mutant *defenseless-1* (*def-1*) does not accumulate JA in response to wounding and it is more susceptible than wild type plants to *Manduca sexta* and *Tetranychus urticae* (Howe and others 1996; Li and others 2002). *Arabidopsis* mutants defective in JA synthesis and perception are more susceptible to attack by *Bradysia impatiens* and *Spodoptera littoralis* (McConn and others 1997; Stotz and others 2002).

Wound Signaling in Solanaceous Species

Work on the wound-induced synthesis of Pins and other defense-related proteins in members of the solanaceae led to the proposal of a model for the transmission of the wound signal to the vicinity of the damaged tissues and also over long distances to activate defense throughout the plant (Farmer and Ryan 1992). That linear model, and subsequent refinements thereof (Doares and others 1995; Bergey and others 1996), suggests that SYS produced in the wounded tissues by processing of prosystemin and/or OGAs derived from cell wall pectin polysaccharides, activate a lipase that releases linolenic acid from the membranes, which serves as a precursor for JA synthesis. An E burst also occurs, probably due to the wound-induced expression of its biosynthetic genes (Liu and others 1993; Bouquin and others 1997). The accumulation of JA and E then signals the induction of *Pin* genes (O'Donnell and others 1996). The sequence of events proposed is consistent with the induction of JA and E synthesis observed in wounded tomato plants or in plants treated with SYS or OGAs (Doares and others 1995; O'Donnell and others 1996). A phospholipase A activity is induced by wounding and OGA or SYS treatment in tomato (Narvaez-Vasquez and others 1999). Moreover, JA and E synthesis and sensitivity are required downstream of SYS and OGAs for induction of *Pin2*. JA and E signaling pathways thus interact at two levels to induce *Pin* genes: (1) JA and E reciprocally activate their synthesis (O'Donnell and others 1996; Sivasankar and others 2000); and (2) simultaneous E and JA signaling are required for wound-induced *Pin* expression, suggesting that integration of the two signals downstream of their generation is essential for wound-induced gene expression.

The role of ABA in wound signaling in solanaceous species is more controversial. Although ABA perception is required for the wound induction of *Pins* (Carrera and Prat, 1998), it may not be a primary signal for perception of mechanical damage (Birkenmeier and Ryan 1998). SA has also been shown to interact with the wound-signaling network in tomato. SA inhibits wound-induced Pin accumulation by blocking JA and E synthesis (Peña-Cortes and others 1993; Doares and others 1995; O'Donnell and others 1996). SA-inhibition of wound-induced *Pin* gene expression is overcome by treating with E and JA (O'Donnell and others 1996) but not with JA alone (Doares and others 1995). However, SA is not produced in wounded wild type plants, indicating that it does not play a direct role in wound signaling. SA may be important to regulate the defensive response of wounded plants if pathogen infection develops at the wound sites (see below). Evidence has been provided that the SA pathway is shut off in wounded tissues by a genetically determined, JA-dependent mechanism supporting the biological significance of SA interaction with wound signaling. *Arabidopsis* mutants with reduced sensitivity to JA accumulate SA after wounding and have enhanced resistance to pathogens (Sano and others 1994; Seo and others 1995; Petersen and others 2000; Kachroo and others 2001; Kloek and others 2001).

Wound Signaling in *Arabidopsis*

The study of wound signaling in *Arabidopsis* has led to a model that is more complex than that laid out for solanaceous species (León and others 2001) (Figure 2). It has been determined that mechanical damage activates at least two separate signaling pathways in *Arabidopsis* and that cross-talk between

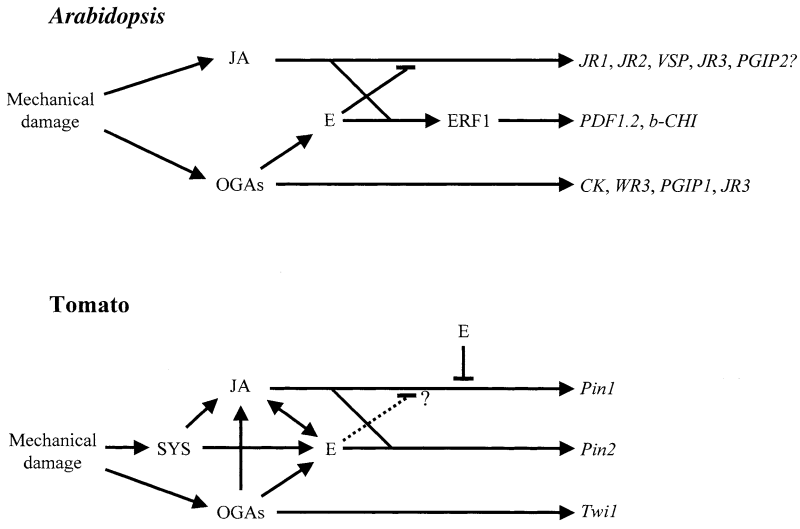

Figure 2. Comparison of wound signaling pathways in *Arabidopsis* and tomato. Similar molecules transduce the wound signal in both species, although some distinctions in signal generation are apparent. In tomato, processing of prosystemin to SYS or release of OGAs from the cell wall may lead to increased accumulation of JA and E, which reciprocally activate their own synthesis, and signal the activation of genes like *Pin2*. In *Arabidopsis* no functional homologue of prosystemin has been found so far. The accumulation of E is independent on JA and may be triggered by OGAs. Arrows indicate positive regulation and blunted lines negative regulation. The dashed lines indicate potential effect. References on the genes depicted in the models are included in the text.

these pathways determines local and systemic responses to wounding (Rojo and others 1999). Genetic and biochemical evidence fully support the existence of at least two separate pathways (Titarenko and others 1997; Rojo and others 1998; León and others 1998; Rojo and others 1999; Reymond and others 2000; Ferrari and others 2003). One of the pathways induces genes such as *WR3*, *CK* and *PGIP1*, and is activated in the vicinity of the wound sites probably by OGAs released from wounded cell walls in a JA and E-independent manner (Rojo and others 1999; Ferrari and others 2003). The other pathway induces genes such as *VSP*, *JR1* and *JR2*, and is mainly active in systemic, non-damaged tissues of the wounded plant through a JA-dependent pathway (Titarenko and others 1997; Rojo and others 1999). Although JA accumulation is much higher in wounded tissues than in systemic, non-damaged tissues of *Arabidopsis* (Laudert and Weiler 1998; Rojo and others 1999; Stintzi and others 2001), JA-responsive genes are induced to higher levels in systemic tissues, indicating that a wound-derived short-range signal is inhibiting JAresponsive genes in wounded tissues.

Evidence has been presented implicating OGAs and E in down regulation of the JA signaling pathway in wounded tissues. OGAs are short-range signals that may be produced by wound-induced polygalacturonases (Bergey and others 1999).

Exogenous treatment with OGAs blocks wound and JA-induced activation of JA-responsive genes (Rojo and others 1999), and E may mediate this negative effect of OGAs on JA signaling (Rojo and others 1999). OGAs induce E synthesis in tomato and *Arabidopsis* (O'Donnell and others 1996; Rojo and others 1999). Moreover, E-insensitive mutants of *Arabidopsis* show an increased induction of a subset of JA-responsive genes in wounded tissues that is also observed in tissues treated with JA or even simultaneously with JA and OGAs, further supporting the requirement of E for the observed effects of OGAs on wound-induced gene expression (Rojo and others 1999; Ellis and Turner 2001). Exogenous E treatment also compromises JA-induced expression of those genes in *Arabidopsis* (Rojo and others 1999; Matsushima and others 2002; Lorenzo and others 2003). Interestingly, E treatment also blocks the JA-induced formation of ER-derived protease precursor vesicles in wild type *Arabidopsis* but not in an E-insensitive mutant (Matsushima and others 2002), indicating that the antagonistic interaction between JA and E also has effects at the cellular level. Most importantly, the antagonistic effects of JA and E on defense gene expression influence the resistance of the plant to herbivory. It has been shown that resistance of *Arabidopsis* to *Spodoptera littoralis* is mediated by JA (Stotz and others 2002) and is enhanced in E-insensitive mutants and decreased by treatment with etephon, an E-releasing compound, suggesting that E down regulates this JA-dependent defense response. Recently, E has been shown to negatively regulate the JA-induced expression of glucosinolate biosynthetic genes and the accumulation of certain glucosinolates in *Arabidopsis* (Mikkelsen and others 2003), which are compounds that function in defense against herbivores and microbes.

The negative interaction between JA and E is, however, not restricted to *Arabidopsis*. A similar negative effect of E in the wound- and JA-induced accumulation of lectin genes in damaged leaves of *Griffonia simplicifolia* has been reported (Zhu-Salz-

man and others 1998), although it has not been tested whether OGAs have the same effect. In *Nicotiana attenuata*, feeding by *Manduca sexta* larvae induces an E burst that inhibits wound and JA-induced nicotine production. The negative regulation of E on JA-induced nicotine production is likely to occur at the level of transcription of nicotine biosynthetic enzymes (Winz and Baldwin 2001).

The cross talk between OGA-dependent and JA-dependent signaling pathways may account for the different spatial patterns of wound-induced gene expression observed in *Arabidopsis*. Genes induced by the OGA-dependent pathway are active close to wound sites and may thus have a role in wound healing or defense against pathogens that may enter through wounds. Genes induced by the JA-dependent pathway are induced mainly in non-damaged tissues and may have a role in providing protection against further herbivore attack. The *JR3* gene defines a third class of wound-induced genes that is activated by both OGA-dependent and JA-dependent pathways and is equally induced in damaged and systemic tissues of the plant (León and others 2001; Rojo and Sanchéz-Serrano unpublished results). *JR3* encodes a hydrolase of auxin conjugates that may be involved in releasing active auxins during wounding, which could in turn negatively regulate the activation of other wound-responsive genes (Kernan and Thornburg 1989; Rojo and others 1998).

Unlike other OGA-dependent wound-responsive genes, the induction of *JR3* by OGAs or wounding requires JA-perception, as it is blocked in a JA-insensitive *coil* mutant (Rojo and Sanchez-Serrano, unpublished). Other genes like the defensin *PDF1.2*, a basic chitinase, or *ERF1* are synergistically induced by JA and E and may define a fourth class of wound-responsive gene. The expression of *PDF1.2* and *ERF1* is likely to be regulated differently than that of *JR3*, because E-sensitivity is essential for the expression of the former (Penninckx and others 1998; Lorenzo and others 2003). The pattern of expression of these genes in plants treated with OGAs and in wounded plants may provide further insight into the regulation of wound-induced gene expression.

Two additional examples of genes activated by alternative wound signaling pathways have been reported in *Arabidopsis*. *RNS1* encodes an RNAse that accumulates both locally and systemically upon wounding in a JA- and OGA-independent (LeBrasseur and others 2002), and possibly OPDA-dependent manner (Stintzi and others 2001). The induction upon wounding of an *Arabidopsis* arginine decarboxylase involved in polyamine biosynthesis appears to be subject to regulation by both ABA and JA signaling (Perez-Amador and others 2002). In addition, a new player in wound signaling in *Arabidopsis* has to be introduced (Stintzi and others 2001), because part of the effects ascribed previously to JA, including the COI1-dependent induction of some defense-related genes and the resistance to certain insects and pathogens, are mediated by the JA precursor OPDA. The *Arabidopsis opr3* mutant, defective in an OPDA reductase enzyme, accumulates OPDA, but not JA in response to wounding. However, it has similar resistance to *Bradysia impatiens* and *Alternaria brassicicola* as wild-type plants. Moreover, OPDA induces the expression of COI1-dependent and COI1-independent wound-responsive defense genes in the *opr3* mutant independently of JA synthesis. JA, OPDA and possibly other oxylipin molecules may act in concert to regulate defense gene expression and resistance against insects and pathogens (Stintzi and others 2001).

Similarities and Differences in Wound Signaling Pathways

Although the model for wound signal transduction initially proposed in tomato accounts for the observed sequence of signal production in wounded plants and the downstream activation of a few marker genes like *Pin2*, there is mounting evidence that wound signaling also involves additional pathways in solanaceous species. A unique pathway is not sufficient to explain the differences in the pattern of proteins accumulating in local and systemic tissues of wounded tomato plants (Lightner and others 1993; Strassner and others 2002). Upon wounding, a tomato wound-responsive glucosyl transferase gene, *Twi1*, is induced at much higher levels in damaged than in systemic tissues. However, *Twi1* is not induced by JA or SYS treatment and, moreover, its induction upon wounding is independent of SYS and E (O'Donnell and others 1998). *Twi1* could thus be the first example of the presence in tomato of a pathway similar to the OGA-dependent and JA-independent one described in *Arabidopsis*, although this possibility remains to be tested. The tomato wound-responsive *Pin1* gene is induced by wounding, SYS and JA treatment (Bergey and others 1996). In contrast to *Pin2*, which requires both E and JA signaling for its induction by wounding or SYS treatment, the constitutive expression of *Pin1* in prosystemin overexpressing tomato plants is blocked by E treatment and enhanced by the inhibitor of E perception 1-methylcyclopro-

pene (Diaz and others 2002). These results indicate that *Pin1* is induced by a JA-dependent pathway that is antagonistically regulated by E, in a manner that resembles the induction of the woundresponsive genes *JR1*, *JR2* or *VSP* in *Arabidopsis*. It remains to be tested whether wound- or JA-induction of *Pin1* is blocked by E and OGAs, as is the case with its putative *Arabidopsis* counterparts.

Analysis of a broader set of wound-responsive genes in tomato is revealing in that the increasing number of wound signaling pathways described in *Arabidopsis* may be present in a wider range of species than previously thought (Figure 2). However, some level of divergence in wound signaling exists between solanaceous species and *Arabidopsis*. This is made patently clear by the differences in signal generation between these species. In tomato, JA and E reciprocally activate their synthesis. In contrast, in *Arabidopsis*, E induces JA synthesis (Laudert and Weiler 1998) but JA does not induce E synthesis (Rojo and others 1999). No homologue of prosystemin has been identified in the *Arabidopsis* genome, and no effect of SYS on expression of *Arabidopsis* wound-responsive genes has been reported. However, the peptide hormones involved in wound signaling in tobacco are unrelated in sequence to SYS (Pearce and others 2001), and thus it cannot be excluded that a functional homologue of SYS, albeit highly divergent in sequence, may be present in *Arabidopsis*. In tomato, treatment with OGAs or fungal-derived chitosan oligosaccharides induces the synthesis of JA and E and the expression of *Pin2* (Doares and others 1995; O'Donnell and others 1996). In *Arabidopsis*, chitosan treatment induces accumulation of E but not of JA (Rojo and others 1999). The effect of OGAs in JA and E synthesis in *Arabidopsis* remains to be tested. JA synthesis in *Arabidopsis* occurs mainly in wounded leaves (Laudert and Weiler 1998; Rojo and others 1999; Stintzi and others 2001) where OGAs are thought to be released. Moreover, OGA treatment induces the expression of the JA biosynthetic gene allene oxide synthase (Norman and others 1999), suggesting that OGAs may induce JA accumulation in *Arabidopsis*. Thus, OGAs may interact at two levels with JA by, on the one hand, activating its synthesis while, on the other, blocking its signal transduction.

The opposite effect that OGAs may have on JA synthesis and signaling in *Arabidopsis* could seem paradoxical, but JA has higher mobility than OGAs and thus, JA may be transported from wounded tissues and activate JA-responsive genes in systemic, nondamaged tissues where OGAs would not be present. Recent experiments in tomato support a role for JA synthesized in wounded tissues as the systemic signal that activates expression of wound-responsive genes (Li and others 2002). Although JA is required in both damaged and systemic tissues for induction of *Pin2*, JA synthesis in tomato occurs mainly in wounded tissues, where its biosynthetic genes are induced (Strassner and others 2002). By using reciprocal grafts of JA-insensitive (*jai-1*) and JA-deficient plants (*spr-2*), it has been shown that in wounded tissues, JA synthesis but not JA-perception is required for transmission of the systemic signal to unwounded tissues and induction of *Pin2* expression. Reciprocally, JA perception but not JA synthesis is required in the undamaged tissues for the activation of *Pin2*, indicating that JA or a JA-precursor molecule downstream of the step catalyzed by *spr-2* is being transported to the systemic tissues. These results are consistent with the much higher induction of JA-biosynthesis enzymes and JA-accumulation in wounded leaves than in systemic leaves (Strassner and others 2002). These data also indicate that SYS is not the systemic wound signal in tomato. Because SYS acts upstream of JA synthesis, if SYS were the systemic signal, JA synthesis would still be required in the systemic tissues. Still, it is possible that SYS is involved in the generation or amplification of the systemic signal (McGurl and others 1994; Li and others 2002).

Herbivory-Specific Responses

As expected, the response to herbivore feeding is not entirely identical to the wound response, indicating that herbivore-derived signals are regulating the reaction. Differences in gene expression between wounded and infested plants have been reported (Korth and Dixon 1997; Reymond and others 2000; van der Ven and others 2000). In *Arabidopsis*, SA is involved in the response to aphid feeding but not in wound signaling (Moran and Thompson 2001). Major differences are also observed in the volatile signature emitted by mechanical and herbivore-damaged plants (Pare and Tumlinson 1997; Shen and others 2000; Arimura and others 2000; De Moraes and others 2001; Kessler and Baldwin 2001). These herbivore-induced volatiles may have both direct and indirect roles in plant defense. Volatiles produced by lima bean leaves infested with spider mites (*Tetranychus urticae*) activate defense gene expression in neighboring uninfested leaves, resulting in a reduced suitability as food sources for *Tetranychus urticae* (Arimura and others 2000). Volatiles also have an indirect role in defense by attracting parasitoids of

the foraging pest (Pare and Tumlinson 1999; Kessler and Baldwin 2001) or repelling females and thus reducing oviposition (Kessler and Baldwin 2001; De Moraes and others 2001).

Interestingly, cross-talk between herbivory and wound signaling pathways has been reported. Herbivore-derived molecules can affect the production of endogenous wound signals such as E and JA. The oral secretion of *Manduca* larvae contains fatty acid-amino acid conjugates that can serve as precursors for JA synthesis (Halitschke and others 2001). In *Nicotiana*, feeding by *Manduca sexta* larvae or application of their oral secretions leads to dramatic increases in E and JA accumulation relative to wounded plants (McCloud and Baldwin 1997; Kahl and others 2000; Winz and Baldwin 2001). The generated E burst reduces JA-induced nicotine production, but not JA-induced release of terpenoid volatiles. This differential effect of E may have implications for the indirect defense by nicotine-sensitive parasitoids against this nicotine-tolerant pest. In this way, *Manduca* larvae feeding on tobacco would contain lower levels of nicotine, making them more susceptible to parasitoid attack. Furthermore, *Phaseolus lunatus* plants infested with *Tetranychus urticae* induce the synthesis of E also in neighboring plants by activating the transcription of E biosynthetic genes (Arimura and others 2002).

Hormone Interactions Mediating Cross-Talk Between Pathogen and Herbivore-Induced Defenses

Resistance responses are complex traits that involve large changes in metabolism, and may impose major energetic costs to the plant. Thus, a trade-off may be established, with the deployment of certain defenses resulting in the deactivation of others. In particular, the resistance against pathogens or herbivores is subject to a reciprocal trade-off that involves interaction between different signaling pathways (Felton and Korth 2000).

A well-characterized example is the trade-off established in tobacco between defense to TMV and to insect attack. Tobacco plants carrying the N resistance gene inoculated with TMV accumulate SA and activate SAR to TMV. The inoculated plants show a reduced wound-induced synthesis of JA and nicotine, and increased feeding by larvae of *Manduca sexta* (Preston and others 1999). Transgenic tobacco plants with suppressed expression of phenylalanine ammonia-lyase (*PAL*) have lower SA levels and reduced SAR to TMV. In turn, these plants accumulate higher amounts of JA in response to grazing by *Heliothis virescens* and show enhanced systemic resistance to further feeding by *H. virescens* (Felton and others 1999). Conversely, PAL overexpressers have increased levels of SA and enhanced SAR. Moreover, larvae of *H. virescens* feeding on these plants show reduced weight gain although larval mortality is not significantly affected.

JA and SA signaling mutants in *Arabidopsis* have also revealed compensatory regulations between defense responses to microbes and herbivores. The JA- and OPDA-insensitive *Arabidopsis coi1* mutant shows enhanced SA production and resistance when infected by *Pseudomonas syringae*, and reduced resistance to *Alternaria brassicicola* infection and feeding by larvae of *Spodoptera littoralis* and the dipteran *Bradysia impatiens* (Kloek and others 2001; Stintzi and others 2001; Stotz and others 2002). By contrast, *npr1*, *pad4*, *eds5*, *sid2* and *nahG* mutants that display defects in SA-dependent defenses for resistance to microbes show enhanced resistance to *Spodoptera littoralis* and *Trichoplusia ni* (Stotz and others 2002; Cui and others 2002).

Similar results have been obtained in tomato by exogenous induction of defense responses. Treatment of tomato plants with benzothiadizole, an SA-mimicking compound, increases resistance to *Pseudomonas syringae* and concurrently attenuates the JA-induced expression of anti-herbivore proteins Pin2 and polyphenol oxidase, and compromises resistance to larvae of *Spodoptera exigua* and *Helicoverpa zea* (Fidantsef and others 1999; Thaler and others 1999). Conversely, the application of JA to tomato plants enhances resistance to *Spodoptera exigua* and concomitantly reduces the induction of the pathogenesis-related protein P4 and compromises resistance to *Pseudomonas syringae*. Simultaneous application of both JA and SA results in lower resistance to both pest and pathogen as compared with plants treated with each elicitor alone, indicating that reciprocal negative cross-talk between the two response pathways occurs. However, plants treated with both elicitors showed higher resistance against both agents than untreated plants, indicating that concurrent activation of both resistance pathways is possible, albeit attaining lower levels of protection than when single pathways are activated.

Because many plant pathogens utilize wounded tissues as sites of entry to establish infection, concurrent activation of defenses against pests and pathogens may have a beneficial effect on the fitness of the plant. Moreover, activation of anti-herbivore defenses in pathogen-infected plants could fend off potential opportunistic pests. Indeed, wild-type *Arabidopsis* plants infected with HR-inducing avirulent strains of *Pseudomonas syringae* become

more resistant to attack by larvae of *Trichoplusia ni* (Cui and others 2002). In contrast, disease-causing virulent strains failed to elicit HR and the increase in pest resistance. Incompatible interactions with avirulent strains induce the accumulation of SA, which when applied exogenously antagonizes the resistance to *Trichoplusia ni*. Thus, an HR-derived signal has to override any SA-mediated increase in susceptibility to *Trichoplusia ni*.

Future Directions

Hormones and endogenous signals that activate defenses against pests and pathogens interact at many levels, in many cases in opposite ways, depending on the plant species and the concentrations of the compounds or the organisms attacking the plant. Defense responses against biotic stresses also cross-talk with responses to other abiotic stresses and to developmental and physiological cues. The complex interactions between defense signaling pathways have only recently started to be unraveled. To develop a comprehensive picture of the pathways and responses triggered when a plant is challenged by pathogens and/or pests, several milestones will have to be reached. A fundamental step will be to determine the precise spatial and temporal concentrations of signaling molecules such as SA and JA in response to the different stimuli. This will provide a tentative framework of the signaling pathways engaged under each condition in which to integrate data from upstream and downstream events.

The development of whole genome tools will be invaluable for dissecting the complex circuitry that links the different external stimuli to the accumulation of endogenous signals and further, to the downstream output responses. In particular, whole genome transcription profiling will provide, in some cases, the level of resolution required to determine which pathways are affected by each stimuli and what interactions are established among them (Reymond and others 2000, 2001; Schenk and others 2000; Sasaki and others 2001; Strassner and others 2002; Swidzinski and others 2002; Lorenzo and others 2003). The development of interaction maps for the whole proteome of plants such as *Arabidopsis* will aid in the identification of the components of signaling cascades and consequently of the molecular integrators that mediate cross-talk between the different pathways.

Evidence for a role of transcription factors such as ERF1 and Pti4 in integrating signals from different pathways is already available, and has been discussed in this review. Other components mediating interactions of defense signaling pathways may be identified by a candidate-based approach. In yeast and animals, MAPK cascades are typical modules integrating signals derived from different pathways. MAPK cascades have also emerged as possible integrators of cross-talk between signaling pathways involved in plant defense against pathogens and other stresses such as wounding, osmotic stress or ozone damage (Zhang and Klessig 2001; Jonak and others 2002). A large set of MAPKs, including WIPK, SAMK, MPK3, SIPK, SIMK, MMK2, MMK3, MPK4 and MPK6, have been shown to be induced by various elicitors and biotic and abiotic stresses, suggesting that they may mediate interplay between responses to these different stimuli. The cross-regulation may be at the activity level, as many of these stimuli have been shown to regulate MAPK activity (Zhang and Klessig 2001; Jonak and others 2002). In particular, the activities of SIPK and WIPK from tobacco and related proteins from tomato are induced by Cf9/avr9 resistance signaling, elicitors, mechanical wounding and SA (Romeis and others 1999), suggesting that they may be a target for cross-talk of different defense signaling pathways. Alternatively, the integration of the different signals converging on MAPK cascades may occur by regulating the transcription of MAPKs. In plants the transcription of some MAPKs is regulated by the stimuli they transduce (Seo and others 1995; Mizoguchi and others 1996; Bögre and others 1997; Romeis and others 1999). To test the possible role of MAPK cascades as mediators of cross-talk between defense signaling pathways, the effect of simultaneous activation of the JA, E and SA pathways on the activity or transcription of different MAPKs will have to be analyzed.

The ubiquitin/proteasome-signalosome degradation pathway is a common step in almost every signaling cascade analyzed in plants and thus may also serve to mediate interaction between pathways. A few components of this pathway have already been implicated in regulating host and non-host disease resistance, and responses to JA and SA (Xie and others 1998; Xu and others 2002; Schwechheimer and others 2002; Azevedo and others 2002; Austin and others 2002; Kim and Delaney 2002; Peart and others 2002). For instance, COI1, an F-box protein that is part of an ubiquitin ligase SCF complex, may mediate cross-talk between SA and JA signaling pathways (Feys and others 1994; Kloek and others 2001). It is likely that research efforts in the near future will reveal new implications of targeted protein degradation in stress signaling.

The molecular components regulating cross-talk between alternative defense pathways will be primary targets for breeding plants with a desired complement of resistance traits. To this end, it will be fundamental to analyze the responses of plants in natural and agronomic environments, which in many cases include simultaneous aggression by several biotic and abiotic stresses.

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