Fine-Tuning Plant Defence Signalling: Salicylate versus Jasmonate

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Abstract: **Plant defences against pathogens and herbivorous insects form a comprehensive network of interacting signal transduction pathways. The signalling molecules salicylic acid (SA) and jasmonic acid (JA) play important roles in this network. SA is involved in signalling processes providing systemic acquired resistance (SAR), protecting the plant from further infection after an initial pathogen attack. SAR is long-lasting and provides broad spectrum resistance to biotrophic pathogens that feed on a living host cell. The regulatory protein NPR1 is a central positive regulator of SAR. SA-activated NPR1 localizes to the nucleus where it interacts with TGA transcription factors to induce the expression of a large set of pathogenesis-related proteins that contribute to the enhanced state of resistance. In a distinct signalling process, JA protects the plant from insect infestation and necrotrophic pathogens that kill the host cell before feeding. JA activates the regulatory protein COI1 that is part of the E3 ubiquitin ligase-containing complex SCF^{COI1}, which is thought to derepress JA-responsive genes involved in plant defence. Both synergistic and antagonistic interactions have been observed between SA- and JA-dependent defences. NPR1 has emerged as a critical modulator of cross-talk between the SA and JA signal and is thought to aid in fine tuning defence responses specific to the encountered attacker. Here we review SA- and JA-dependent signal transduction and summarize our current understanding of the molecular mechanisms of cross-talk between these defences.**

Key words: **Salicylic acid, jasmonic acid, systemic acquired resistance, pathogenesis-related genes, NPR1, cross-talk.**

Introduction

In their natural environment, plants continuously have to cope with various stress factors, including attack by herbivorous insects and invasion by microbial pathogens. To survive, plants have to respond to each attacker in a rapid and effective way. Disease resistance is dependent on both preformed barriers and inducible defence mechanisms. Preformed barriers, such as thick cell walls and secondary metabolites toxic to the invader, form a passive first line of defence. Upon recognition of the attacker, inducible defences are activated at the site of infection as well as in uninfected distant tissues. Depending on the type of attacker, the plant activates different signalling pathways to synthesize an optimal mixture of defensive compounds. For instance, biotrophic pathogens (pathogens that feed on the living host cell) require a very different type of defence compared to necrotrophic pathogens (pathogens that kill the host cell before feeding). Thus, activation of inducible defences may be very specific to the type of invader encountered. Moreover, cross-talk between different defences has been observed, which is thought to further optimize the specificity of the overall defence response. The signalling molecules salicylic acid (SA) and jasmonic acid (JA) play key roles in this signal interplay. Interestingly, both synergistic and antagonistic interactions between these molecules have been reported that result in enhanced resistance and pathway tradeoff, respectively. Here, we review new developments that have recently emerged in studies on cross-talk between the SA and JA signal in optimizing defence responses. Because a comprehensive overview of the current knowledge of both the SA and JA signalling pathways is beyond the scope of this short review, we aimed to provide a concise discussion on the key factors that appear to be involved in the interplay between SA and JA.

Systemic Acquired Resistance (SAR)

Upon recognition of a pathogen, plants often activate the hypersensitive response, resulting in rapid cell death of infected tissue to kill the pathogen and prevent it from spreading further. In addition to the locally effective hypersensitive response, pathogen recognition also triggers various inducible systemic defences. In plant parts distant from the site of primary infection, systemic responses establish an enhanced defensive capacity against subsequent infection. This biologically induced resistance in systemic tissue is known as systemic acquired resistance (SAR) and has been shown to be effective in many plant species. The attained state of resistance is long-lasting and effective against a broad spectrum of pathogens, including pathogenic bacteria, fungi, oomycetes and viruses (Ryals et al., 1996; Sticher et al., 1997; Durrant and Dong, 2004). SAR is associated with activation of a large number of pathogenesis-related (*PR*) genes in local and systemic tissues (Ward et al., 1991; Maleck et al., 2000). It is generally thought that SAR is the result of concerted action of products encoded

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by many *PR* genes. In plant defence research, *PR* genes serve as powerful molecular markers for the onset of SAR (Ryals et al., 1996).

Salicylic acid mediates SAR signal transduction

It has long been thought that the plant hormone SA functions as a signalling molecule in SAR. Malamy et al. (1990) reported elevated endogenous SA levels in both local and systemic tissues after viral infection of tobacco plants, which correlated with the induction of *PR* genes. Similarly, it was found that levels of SA in the phloem sap of cucumber plants rose after viral or fungal infection (Métraux et al., 1990). Further evidence for the involvement of SA in SAR came from the finding that exogenous application of SA or the SA analogues 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole S-methyl ester (BTH) could induce the same set of *PR* genes (Métraux et al., 1991; Ward et al., 1991; Friedrich et al., 1996; Görlach et al., 1996; Lawton et al., 1996). Transgenic plants carrying the bacterial *nahG* transgene, encoding a bacterial SA hydroxylase that converts SA to inactive catechol, cannot accumulate SA, fail to express *PR* genes, and show enhanced susceptibility to SA resisted pathogens (Gaffney et al., 1993; Delaney et al., 1994; Lawton et al., 1995). Thus, SA is a key signalling molecule in plant defence and is required for activation of SAR.

The SAR signal transduction pathway: a central role for NPR1

Several mutant screens have been performed to identify *Arabidopsis* mutants defective in the SA-dependent SAR signal transduction pathway. Strikingly, four independent screens identified mutants that contain a mutation in the regulatory gene *NPR1/NIM1/SAI1 (non-expresser of PR-genes/non-inducible immunity/salicylic acid insensitive)* (Cao et al., 1994; Delaney et al., 1995; Glazebrook et al., 1996; Shah et al., 1997). Mutant *npr1* plants accumulate high levels of SA after infection, but fail to activate *PR* genes and are highly susceptible to a wide range of pathogens. Moreover, wild-type plants show potentiated expression of SAR-related genes after treatment with inducers of SAR, a process known as priming, whereas in mutant *npr1* plants potentiation is lost (Conrath et al., 2001; Kohler et al., 2002). Thus, *NPR1* encodes an important positive regulator that transduces the SA signal in SAR and plays a key role in priming. Cloning of *NPR1* revealed two conserved protein-protein interaction domains within the NPR1 protein: an ankyrin repeat domain and a Broad-Complex, Tramtrack, Bric-a-brac/Poxvirus, Zinc finger (BTB/POZ) domain (Bork, 1993; Aravind and Koonin, 1999). In addition, a nuclear localization signal, multiple conserved cysteine amino acid residues and several putative phosphorylation sites were also identified (Cao et al., 1997; Ryals et al., 1997; Kinkema et al., 2000; Mou et al., 2003).

In wild-type *Arabidopsis* plants *NPR1* is constitutively expressed, although its mRNA levels increase two- to three-fold upon pathogen infection or treatment with SA (Cao et al., 1997). The *NPR1* promoter region contains several W-boxes (consensus sequence: [T]TGAC[C/T]), which function as binding sites for plant-specific WRKY transcription factors. Mutation of these W-boxes completely disrupted binding of WRKY proteins and abolished expression of *NPR1*, resulting in loss of both SA-induced *PR* gene expression and disease resistance (Yu et al., 2001). Thus, WRKY proteins appear to function upstream of NPR1, but downstream of SA in the SAR signal transduction pathway. Transgenic *Arabidopsis* lines overexpressing NPR1 do not exhibit an obvious phenotype, but show enhanced induction of *PR* genes upon pathogen infection. Accordingly, these plants show an enhanced level of disease resistance that is dependent on NPR1 dosage (Cao et al., 1998). Overexpression of *Arabidopsis NPR1* in rice conferred enhanced resistance to the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (Chern et al., 2001). Thus, by overexpressing a key regulator of SAR, plant immunity can be boosted in both monocots and dicots, suggesting that they share at least partly similar defence signalling pathways. Indeed, NPR1 homologues are currently being identified in several different plant species (Durrant and Dong, 2004).

The importance of SA and NPR1 in SAR has long been recognized. But how is the SA signal transduced to NPR1? Overexpression of *NPR1* does not lead to constitutive *PR* gene expression in the absence of SAR induction, indicating that NPR1 requires an activation step to be functional. Several studies have suggested that changes in endogenous SA levels after pathogen infection can affect the redox state of the cell (Chen et al., 1993; Vanacker et al., 2000). Together with the fact that NPR1 contains conserved cysteine residues that are often subject to redox regulation, Mou et al. (2003) investigated the possibility of redox-mediated activation of NPR1 to provide the missing link between accumulation of SA and activation of NPR1. It was elegantly demonstrated that monomeric NPR1 protein links together via intermolecular disulfide bonds at specific cysteine residues to form a high molecular weight oligomeric complex. Pathogen-induced SA accumulation or treatment with SAR inducers results in an early transient increase in cellular reduction potential followed by a rapid decrease in reduction potential. Consequently, the cysteine residues of NPR1 are reduced by hydrolysis of the intermolecular disulfide bonds, thereby releasing monomeric NPR1 from the oligomer (Fig. **1**). Release of NPR1 monomer precedes the activation of *PR* gene expression, suggesting that monomerization is required for activation of *PR* genes. Indeed, solely monomeric NPR1 protein carrying an intact nuclear localization sequence is capable of being translocated into the nucleus to activate *PR* genes, whereas the high molecular weight oligomer is thought to be retained in the cytoplasm (Kinkema et al., 2000; Mou et al., 2003).

The presence of protein-protein interaction domains in NPR1 suggests that it exerts its function through physical interaction with other proteins. Yeast-two-hybrid analysis, using NPR1 as bait, yielded members of the TGA/OBF subclass of basic domain/Leu zipper (bZIP) transcription factors (Zhang et al., 1999; Després et al., 2000; Zhou et al., 2000). TGA transcription factors bind to *as-1* motifs that play a crucial role in the activation of *PR* genes (Lebel et al., 1998; Niggeweg et al., 2000). Thus, TGA factors may provide a direct link between nucleus-targeted NPR1 and *PR* gene induction. Further evidence for this link came from biochemical and *in vivo* imaging studies that showed physical interaction between NPR1 and TGA2 *in planta* (Subramaniam et al., 2001; Fan and Dong, 2002). Although TGA1 and TGA4 failed to interact with NPR1 in yeast-two-hybrid assays, it was recently reported that treatment with SA induces interaction with NPR1 *in planta* (Després et al., 2003). Interaction correlated with reduction of TGA cysteine residues that form an intramolecular disulfide bond, indicating the importance of the oxidation state of these

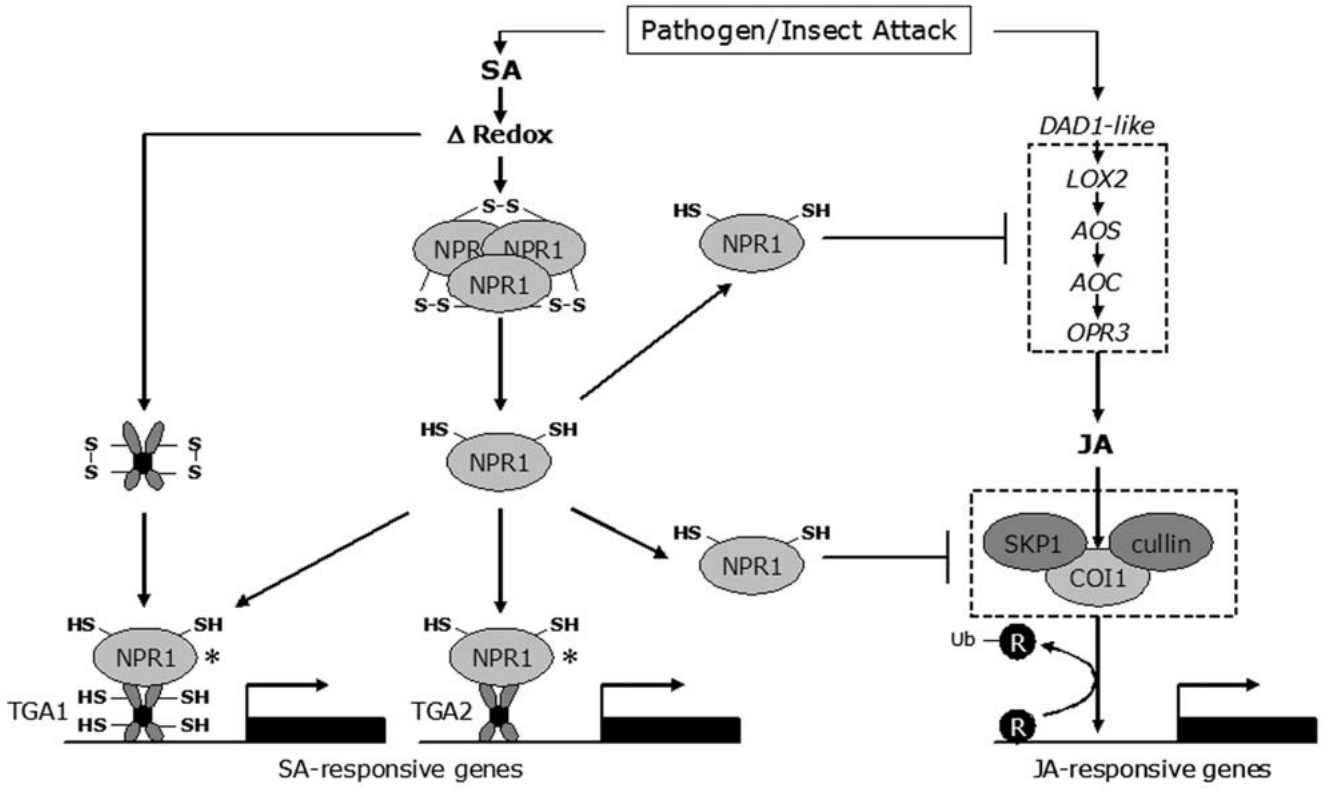

Fig. 1 Proposed schematic model illustrating the central role of NPR1 in the regulation of SA-induced suppression of JA-dependent defence signalling. SA induces changes in the cellular redox potential, thereby promoting the reduction of intermolecular disulfide bonds at specific cysteine residues of the NPR1 oligomer and the subsequent release of NPR1 monomer. NPR1 monomer translocates to the nucleus where it interacts with TGA transcription factors. Some TGA transcription factors (e.g., TGA1) require the SA-induced reduction of intramolecular disulfide bonds for interaction with nuclear NPR1 monomer, whereas other TGA transcription factors do not (e.g., TGA2). By contrast, cytosolic NPR1 monomer is involved in inhibition of JA signalling. The model proposes that NPR1-mediated inhibition of JA signalling occurs through suppression of the transcription of JA biosynthesis genes, as well as through preventing the SCF^{COI1} complex from targeting repressors (R) of JA-responsive genes for ubiquitin (Ub)-proteasome-mediated degradation. Asterisk indicates nuclear NPR1 monomer.

cysteines for the interaction with NPR1 (Fig. **1**). Furthermore, it was shown that *in vivo* TGA2 and TGA3 are recruited to *as-1* motifs of the *PR-1* promoter in response to SA in a NPR1-dependent fashion (Johnson et al., 2003). Besides biochemical studies, genetic analysis of a triple *tga* mutant provided conclusive evidence for the involvement of TGA transcription factors in SA/NPR1-mediated SAR. The *tga6 tga2 tga5* triple mutant exhibited loss of INA-induced gene expression and INA-induced protection against pathogen infection (Zhang et al., 2003). Hence, NPR1 interacts differentially with members of the TGA class of transcription factors and regulates their binding to *PR* gene promoters. The NPR1-dependent order of recruitment of TGA transcription factors to *PR* gene promoters and their individual functions are, however, still largely unclear. It is important to note that cellular redox alterations not only play a crucial role in the regulation of NPR1, but also in the activation of certain TGA transcription factors, likely providing additional specificity in the regulation of defence gene expression (Fig. **1**).

Jasmonic Acid-Dependent Defence Signalling

Besides defences dependent on SA and NPR1, defence signalling pathways that are independent of these molecules have been described. Attack by necrotrophic pathogens, as well as herbivorous insects, elicits the production of a large chemically diverse set of oxygenated fatty acids (oxylipins) that can be potent regulators of defence signalling. Especially oxylipins, known as the jasmonates, orchestrate a large set of defence responses. Interestingly, the signalling molecule JA and other jasmonates generate a specific signal signature depending on the type of stress. For instance, compared to mechanical wounding, wounding caused by herbivorous insects elicits overlapping, yet distinct signalling events (Reymond et al., 2000). These and other findings indicate that the JA-regulated wound response is modified by recognition of specific elicitors from the herbivore (Kessler and Baldwin, 2002). In addition to playing a key role in plant defence, JA is involved in a wide range of developmental processes in the plant, such as pollen maturation, flower and fruit development, vegetative sink and storage regulation, photosynthesis, senescence, and root growth (Creelman and Mulpuri, 2002; Turner et al., 2002). Thus, JA and precursors of JA that are synthesized during JA biosynthesis have an important function as signalling mol-

ecules in various processes, including plant defence (Farmer et al., 2003).

Jasmonate biosynthesis (the octadecanoid pathway)

Jasmonate synthesis occurs through the octadecanoid pathway and begins with the release of linolenic acid from the chloroplast membrane, a process thought to be catalyzed by a phospholipase (Creelman and Mulpuri, 2002). Mutant screens in *Arabidopsis* resulted in the identification of male-sterile *dad1 (defective anther dehiscence 1)* mutant plants in which fertility could be rescued by the application of JA or linolenic acid (Ishiguro et al., 2001). *DAD1* encodes a JA-inducible chloroplast-localized phospholipase A1 and *in vitro* DAD1 protein was indeed capable of hydrolyzing phospholipids. However, *DAD1* is predominantly expressed in stamen filaments and thus may not contribute to JA synthesis in plant defence. Sequence alignment revealed 11 *DAD1*-like genes encoded by the *Arabidopsis* genome. Some of these may be involved in defence, but their function awaits further characterization. Moreover, the *Arabidopsis* genome encodes 10 patatin-related phospholipase A genes, which may be involved in JA biosynthesis (Holk et al., 2002). After linolenic acid has been released from the membrane, it is subsequently oxygenated by lipoxygenases (LOX) to a hydroperoxy derivative (Feussner and Wasternack, 2002). The resultant 13-hydroperoxy-octadecatrienoic acid is dehydrated by allene oxide synthase (AOS) to an unstable allene oxide intermediate, which undergoes cyclization to form a cyclopentenone ring-containing 12-oxo-phytodienoic acid (OPDA), mediated by allene oxide cyclase (AOC) (Laudert et al., 1996; Laudert and Weiler, 1998; Ziegler et al., 2000; Turner et al., 2002). OPDA is reduced in a process catalyzed by OPDA reductase 3 (OPR3), yielding a cyclopentanone intermediate (Schaller et al., 2000). Two mutant alleles of *OPR3* were identified in *Arabidopsis*. Mutant *dde1 (delayed dehiscence 1)* and *opr3 (oxo-phytodienoic acid reductase 3)* plants accumulate high levels of OPDA upon wounding, but fail to produce JA (Sanders et al., 2000; Stintzi and Browse, 2000). Characteristically, *dde1/opr3* mutant plants are male-sterile, but can be rescued by application of JA, whereas OPDA is ineffective in this respect. The octadecanoid pathway concludes with three rounds of β-oxidation to yield the best-known jasmonate family member, the 12-carbon signalling hormone jasmonic acid (Creelman and Mulpuri, 2002; Turner et al., 2002). Enzymes involved in JA biosynthesis are mostly localized to the chloroplast, except for OPR3, which is localized to peroxisomes, where β-oxidation is thought to occur (Turner et al., 2002; Stenzel et al., 2003). It is at present unclear how intermediates of JA biosynthesis move between the chloroplast and the peroxisomes.

It is important to note that expression of the octadecanoid enzymes DAD1, LOX2, AOS, AOC, and OPR3 is inducible by jasmonic acid, wounding, and disease (Bell and Mullet, 1993; Kubigsteltig et al., 1999; Mussig et al., 2000; Ishiguro et al., 2001; Spoel et al., 2003; Stenzel et al., 2003). This provides the plant with a clever regulatory potential of a feed-forward loop in JA biosynthesis to amplify signalling. The enzymes involved in JA biosynthesis are highly abundant in the leaves of *Arabidopsis*. Nevertheless, only after biotic or abiotic stress is JA accumulation observed. To explain this apparent discrepancy, it has been proposed that changes in the sequestration of octadecanoid enzymes and substrate availability upon stress play a key role in the regulation of JA biosynthesis. Alternatively, stress-induced post-translational modifications of octadecanoid enzymes may be important (Stenzel et al., 2003). Thus, stress-induced accumulation of JA by pre-existing octadecanoid enzyme activity precedes the induction of genes encoding JA biosynthetic genes. Besides JA, intermediates of the JA biosynthesis pathway have been proposed to regulate gene expression. Mutant *opr3* plants, capable of accumulating OPDA but not JA, are still resistant to insect infestation and infection by necrotrophic pathogens (Reymond et al., 2000; Stintzi et al., 2001), all of which are attackers that are resisted through JA-dependent defences. Moreover, treatment of mutant *opr3* plants with OPDA results in the activation and repression of a specific set of genes. Notably, transcription of some of these genes could be regulated by JA as well, whereas others were regulated solely by OPDA (Stintzi et al., 2001). This indicates that OPDA has similar signalling capacities as JA, but also distinct novel regulatory capacities. OPDA is a cyclopentenone jasmonate, which can regulate gene expression through electrophilic activity, a function that JA lacks (Farmer et al., 2003). A previously discovered large pool of galactolipid esterified OPDA present in plastids may be an important source of OPDA in plant defence signalling (Stelmach et al., 2001). The ability of other JA biosynthesis intermediates to regulate stress-induced gene expression is at present unclear, but may prove to be important for proper functioning of responses mediated by the JA signalling pathway.

Signalling downstream of JA biosynthesis

After biosynthesis of JA, the signal is most likely perceived and transduced by specific receptors for JA. However, to date, such receptors have not been found. Nevertheless, mutants impaired in signalling downstream of JA biosynthesis have been identified in several genetic screens. These screens cleverly made use of JA as a potent inhibitor of root growth during plant development to select mutants that are unresponsive to JA or coronatine, a biologically active structural analogue of JA (Staswick et al., 1992; Feys et al., 1994). These screens have identified mutant alleles of the genes *COI1 (coronatine insensitive 1)* and *JAR1 (jasmonate resistant 1)*. Mutant *coi1* plants are insensitive to coronatine- and JA-induced inhibition of root growth, fail to express JA-responsive genes, and are highly susceptible to insect infestation and pathogen infection, indicating that *COI1* is a central positive regulator of JA signalling (Feys et al., 1994; Thomma et al., 1998; Li et al., 2004b). *COI1* encodes a 66-kD protein containing an N-terminal F-box motif and 16 leucine-rich repeats (Xie et al., 1998). F-box proteins function as receptors that specifically recruit regulatory proteins as substrates for the ubiquitin-proteasome-mediated protein degradation pathway. Because COI1 is a positive regulator of JA signalling, it is thought to act as a selective derepressor of JA-responsive genes by targeting repressor proteins for degradation. Recruitment of such repressors is mediated by SCF complexes, consisting of SKP1, cullin, and an F-box protein that provides substrate selectivity. Importantly, COI1 was recently found to interact with SKP1 and cullin1 proteins *in planta*, suggesting that COI1 is indeed part of a SCF^{COI1} ubiquitin-ligase complex. The functionality of this complex was proven by reducing the expression of one of the complex members by RNAi, resulting in decreased JA-responsive gene expression (Xu et al., 2002). Interestingly, COI1 was also found to interact with a histone deacetylase, possibly a repressor of transcrip-

tion, strengthening the view that COI1 derepresses JA-responsive genes by targeting repressor proteins for ubiquitination and subsequent degradation (Devoto et al., 2002; Xu et al., 2002). Recently, it was demonstrated that SCF^{COI1} directly interacts with the COP9 signalosome (CSN) *in vivo* (Feng et al., 2003). CSN plays important roles in many developmental processes and serves a regulatory role in ubiquitin-proteasome-mediated protein degradation. In analogy to mutant *coi1* plants, mutants impaired in CSN function exhibit reduced inhibition of root growth in response to JA and lack expression of JA-responsive, *COI1*-regulated genes. Hence, SCF^{COI1} and CSN complexes appear to function together to regulate JA-dependent responses, probably by targeting a repressor of JA signalling for proteasome-mediated degradation.

In addition to COI1, JAR1 was identified in screens for JA-insensitive mutants. Mutant *jar1* plants are unresponsive to JA (Staswick et al., 1992), exhibit strongly reduced expression of JA-responsive genes, and show impaired resistance to pathogens (Staswick et al., 1998; Tiryaki and Staswick, 2002). Structural fold-prediction analysis of JAR1 revealed that this protein belongs to the acyl adenylate-forming firefly luciferase superfamily of proteins that function as enzymes catalyzing the activation of carboxyl groups of a variety of substrates. JAR1 specifically adenylates JA, suggesting that this biochemical modification is important for the signalling function of JA (Staswick et al., 2002). Gas chromatography-mass spectrometry analysis of JA-amido conjugates in wild-type versus mutant *jar1* plants suggested that *jar1* plants are impaired in synthesis of JA-isoleucine conjugates. Accordingly, *in vitro* synthesized JAR1 was capable of forming amido conjugates between JA and isoleucine. JA-isoleucine, but not other JA-amido conjugate, was effective in inhibiting root growth, demonstrating the biological activity of this signalling molecule (Staswick and Tiryaki, 2004). Although the exact involvement of JA-isoleucine in plant defence has yet to be determined, it is now becoming clear that this is a key signalling molecule of the JA response pathway produced by the JA-amino synthetase JAR1. Instead of screening for mutants that show loss of JA signalling, several screens have now been performed to identify mutants that exhibit constitutive JA signalling phenotypes and may thus represent negative regulators. These efforts have resulted in the identification of *cet (constitutive expression of thionin)* (Hilpert et al., 2001), *cev (constitutive expression of VSP1)* (Ellis and Turner, 2001; Ellis et al., 2002), and *cex (constant expression of JA-inducible genes)* (Xu et al., 2001) mutants. These mutants require further characterization to elucidate their precise function in JA signalling.

Signal Interplay Between Different Induced Defences

Signal synergies and trade-offs in plant defence

Defence responses dependent on SA are often effective against biotrophic pathogens, whereas defences dependent on JA are mostly effective against necrotrophic pathogens and insects. To achieve an effective state of resistance after recognition of the invader, plants are thought to fine-tune different defence signalling pathways by means of synergistic and antagonistic interactions (Pieterse et al., 2001 a). Recent findings indicate that cross-communication between signals may provide a cost-efficient regulatory potential for mounting defences specifically targeted to the invader encountered. A synergistic effect was reported between SA-dependent SAR and rhizobacteria-mediated induced systemic resistance (ISR). ISR is activated by root-colonizing, non-pathogenic fluorescent *Pseudomonas* spp. and provides broad spectrum resistance to pathogen attack (Pieterse et al., 2001 b, 2002). ISR signal transduction is dependent on an intact JA/ethylene response and, interestingly, requires the function of the regulatory protein NPR1 (Pieterse et al., 1998). Thus, SAR and ISR signalling pathways are distinct in their requirement for SA and JA/ethylene, yet unite in their downstream requirement of NPR1. However, SAR and ISR signalling pathways are thought to converge downstream of NPR1, because both types of induced resistance result in the activation of different sets of defence genes (Van Wees et al., 1999; Verhagen et al., 2004). Simultaneous activation of SAR and ISR results in enhanced resistance to pathogenic *P. syringae* pv. *tomato* DC3000 compared to either defence response alone (Van Wees et al., 2000). This indicates that signal synergism between SAR and ISR boosts defence without apparent competition for NPR1 availability. A cooperative interaction between SA, JA and ethylene was also recently observed in a susceptible tomato disease response. Bacterial pathogen infection of mutant and transgenic plants impaired in JA signalling indicated that early JA signalling must precede the accumulation and action of ethylene and SA in the disease response (O'Donnell et al., 2003). These findings indicate a cooperative and sequential interaction among JA, ethylene and SA in disease development in tomato.

Besides signal synergy between SA- and JA-dependent defence responses, cases of antagonism between these two signalling molecules have also been reported. For instance, mutation of *COI1* results in resistance to certain bacterial pathogens, due to elevated SA levels and the associated activation of *PR* genes (Kloek et al., 2001). This suggests that the JA signal can be a potent inhibitor of SA-dependent signalling. Similarly, it was recently shown that infection with a virulent bacterial pathogen induces systemic susceptibility to a subsequent attack. This effect was attributed to the inhibition of SA signalling by the pathogen-produced JA analogue coronatine (Cui et al., 2005). By contrast, there is evidence that the SA signal can strongly inhibit JA-dependent defence signalling. The JA-resisted tobacco hornworm *Manduca sexta* inflicted more damage on SAR-induced tobacco plants compared to control plants (Preston et al., 1999). Moreover, tobacco plants silenced for the expression of the phenylpropanoid biosynthesis gene *PAL (phenyl ammonia-lyase)* exhibit reduced SAR against TMV, but exhibited enhanced resistance to insect infestation. Conversely, plants overexpressing *PAL* were more resistant to TMV, whereas resistance to insect attack was lost (Felton et al., 1999). Activation of the SAR signalling pathway by the SA analogue BTH was shown to have similar effects on insect resistance. BTH treatment of tomato plants reduced resistance to both larvae of the corn earworm *Helicoverpa zea* and the beet armyworm *Spodoptera exigua* (Stout et al., 1999; Thaler et al., 1999). It should be noted, however, that these examples may not exclusively represent cross-talk between SA and JA signalling pathways, because defence against insects may also involve other signalling molecules than JA (Felton and Korth, 2000; Reymond et al., 2000; Kessler and Baldwin, 2002). Accordingly, SA or SA analogues do not affect resistance to all herbivorous insects (Thaler et al., 2002). The biological significance of cross-talk between SA- and JA-dependent defence responses still awaits further elucidation.

Molecular mechanisms of cross-talk between SA- and JA-dependent defences

Antagonism between SA and JA signalling pathways has previously been observed in pharmacological experiments in which SA and JA or mimicking compounds were exogenously applied to plant tissue. In tobacco, methyl-JA (MeJA)-induced expression of basic *PR* genes was inhibited by SA treatment, whereas MeJA treatment inhibited SA-induced expression of acidic *PR* genes expression (Niki et al., 1998). Moreover, in tomato SA and BTH were shown to repress the JA/wound-induced activation of genes encoding proteinase inhibitors (Doherty et al., 1988; Doares et al., 1995; Fidantsef et al., 1999). Similarly, in *Arabidopsis,* MeJA-induced *VSP (vegetative storage protein)* gene expression is inhibited by SA treatment (Van Wees et al. 1999).

How is cross-talk between SA- and JA-signalling pathways regulated? Although cross-talk between the SA and JA signal may be regulated differently depending on the plant species, recent work on *Arabidopsis* indicates a crucial role for the regulatory protein NPR1. Upon simultaneous treatment with SA and MeJA of *Arabidopsis* wild-type plants, SA strongly suppressed JA-responsive gene expression. In contrast, SA had no inhibitory effect on MeJA-induced gene expression in mutant *npr1* plants, indicating that NPR1 is required for the SA-mediated suppression of JA-responsive gene expression. Using mutant *npr1* plants expressing a fusion protein of NPR1 and the hormone binding domain of the rat glucocorticoid receptor, which allows control of the nucleocytoplasmic localization of this fusion protein, we showed that nuclear localization of NPR1 is not required for SA-mediated suppression of MeJA-induced expression of the plant defensin *PDF1.2* (Spoel et al., 2003). Thus, in marked contrast to its nuclear function in SA-dependent activation of *PR*-genes, NPR1 regulates suppression of JA signal transduction through a function in the cytosol. To date, it is unclear whether the presumably cytoplasmic-localized oligomeric conformation or the monomeric conformation of NPR1 is involved in this process. Mutations in NPR1 that result in constitutive monomerization (Mou et al., 2003) do not affect JA-induced *VSP1* gene expression in the absence of SA, whereas constitutive *PR* gene expression is observed (Spoel, S. H. and Dong, X., unpublished results). This implies that SA is required to activate NPR1 for suppression of JA signalling. Therefore, it seems likely that NPR1 monomer, released by SA-mediated changes in cellular redox potential, is involved in cross-talk.

Some research has focused on the level at which the JA signalling pathway is inhibited by SA/NPR1-dependent signalling. In *Arabidopsis,* SA treatment results in NPR1-mediated suppression of MeJA-induced *LOX2* gene expression, implying that the capacity to synthesize JA is limited. Indeed, wild-type plants accumulated high levels of JA in response to pathogen infection, whereas plants co-suppressed for *LOX2* failed to accumulate significant amounts of JA, indicating that expression of *LOX2* may be a limiting factor in JA biosynthesis (Spoel et al., 2003). In tomato leaves, wound-induced accumulation of JA was inhibited by treatment with aspirin, an acetyl derivative of SA. However, application of JA or the JA precursor 12-oxo-PDA rescued tomato leaves from the inhibitory effect of aspirin, whereas application of precursors upstream of 12-oxo-PDA had no effect (Peña-Cortés et al., 1993). These results suggest that formation of 12-oxo-PDA, catalyzed by the second enzyme of the octadecanoid pathway, AOS, is inhibited by SA. Later studies in flax demonstrated that SA and aspirin were indeed able to reduce the wound-induced accumulation of *AOS* mRNA transcripts (Harms et al., 1998). We previously observed that JA-induced expression of *OPR3* is also inhibited by SA in an NPR1-dependent way (Spoel, S. H., Pieterse, C. M. J., and Dong, X., unpublished results). Taken together, SA is capable of inhibiting the expression of at least three enzymes involved in JA biosynthesis and at least two of these are suppressed through NPR1 (Fig. **1**). These data demonstrate that inhibition of JA signalling occurs at least in part at the level of transcription, although the effect of SA/NPR1 on enzyme activities is still largely unknown. Besides inhibition of JA biosynthesis, NPR1 appears to inhibit JA signalling downstream of JA formation, because SA/NPR1 inhibit the expression of several JA-responsive genes induced by exogenous application of MeJA (Spoel et al., 2003).

So how does NPR1 suppress JA signalling? In animal cells, the BTB-containing protein MEL-26 is required for *in vivo* proteasome-mediated degradation of MEI-1, a microtubule severing protein, during meiosis-to-mitosis transition. Interestingly, it was shown that MEL-26 functions as a substrate-specific adaptor that integrates the functional features of both SKP1 and F-box proteins into a single protein to target MEI1 for degradation. Analysis of a large family of BTB-containing proteins in *Caenorhabditis elegans* suggested that BTB proteins may generally function as substrate-specific adaptors for the proteasome (Furukawa et al., 2003; Pintard et al., 2003; Xu et al., 2003; Pintard et al., 2004). In analogy to MEL-26, the NPR1 protein contains a BTB domain and an additional protein-protein interaction domain (Aravind and Koonin, 1999). Thus, it is plausible that NPR1 may also function as a substrate-specific adaptor that specifically targets positive regulators of JA signalling for proteasome-mediated degradation. In addition to its BTB domain, NPR1 contains an ankyrin-repeat domain and shows striking structural similarity to the animal inhibitory protein IκB (Cao et al., 1997; Ryals et al., 1997). Like NPR1, IκB in animals functions in the cytosol where it prevents nuclear localization of the transcription factor NF-κB, thereby regulating synthesis of prostaglandins, the structural analogues of JA, in animal innate immunity (Baldwin, 1996; Newton et al., 1997; Hatada et al., 2000). It is likely that NPR1 is also involved in the inhibition of a positive direct or indirect transcriptional regulator of JA signalling, possibly by inhibiting its nuclear localization. For instance, NPR1 may interfere with the removal of repressors of JA signalling by the SCF^{COI1} complex, as proposed in Fig. **1**.

In addition to the regulatory protein NPR1, other factors have been implicated as major players of cross-talk between SA- and JA-dependent defence signalling. A genetic screen for suppressors of the *npr1-5* mutation led to the identification of *SSI1 (suppressor of SA insensitivity 1)* (Shah et al., 1999). Mutant *ssi1 npr1-5* plants constitutively express both SA-dependent *PR* genes and the JA-dependent *PDF1.2* gene. Introduction of the bacterial *NahG* gene completely suppressed all the phenotypes associated with the *ssi1* mutation, including constitutive expression of JA-dependent *PDF1.2* gene expression. Moreover, treatment of mutant *ssi1 npr1-5 nahG* plants with an SAR inducer rescued *PR* gene expression and surprisingly also *PDF1.2* gene expression (Shah et al., 1999). These data indicate

that the SA- and JA-dependent signalling pathways do not function independently of each other and that the *SSI1* gene may be an important molecular switch that regulates cross-talk between SA and JA. Analysis of overexpression and antisense suppression of WRKY70, a member of the plant-specific WRKY transcription factor family described above, indicated that it is an activator of SA-dependent genes, whereas it functions as a repressor of JA-dependent genes (Li et al., 2004a). Epistasis analysis suggested that WRKY70 functions downstream of NPR1 and could thus represent a link between NPR1 and repression of JA-responsive genes. It will be interesting to address whether these newly emerging regulators of cross-talk function together with NPR1 to regulate signal interplay between SA and JA.

Future Prospects

Plant defence signalling represents a complex network of integrated signals in which SA and JA play important roles. In this review, we aimed to provide a short overview of the current knowledge of interplay between SA and JA signalling pathways. The challenges for the future lie in beginning to understand why and how different signalling pathways interact. The biological significance of cross-talk or trade-offs between defences is not always intuitive. Antagonism and synergism between defence signalling molecules may provide the plant with the regulatory potential to specifically optimize its defence responses to a pathogen, but may also prove to be a disadvantage if multiple pathogens with different life-styles and infection strategies are encountered. In addition to the significance of signal interplay, acquiring comprehensive knowledge of the mechanisms underlying cross-talk is important. As in the case for NPR1, this may attribute new functions to known components of defence signalling pathways, but may also result in the identification of novel components that were previously unidentified. A difficulty in future studies may be that the mechanisms underlying cross-talk between the SA and JA signal appear to be different depending on the plant species. Facing these challenges will provide new insight in the complexity of defence signalling and will prove to be useful in developing novel strategies for crop protection.

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