

Cross talk between signaling pathways in pathogen defense

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Plant defense in response to microbial attack is regulated through a complex network of signaling pathways that involve three signaling molecules: salicylic acid (SA), jasmonic acid (JA) and ethylene. The SA and JA signaling pathways are mutually antagonistic. This regulatory cross talk may have evolved to allow plants to fine-tune the induction of their defenses in response to different plant pathogens.

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Abbreviations

<i>cet</i>	constitutive expressor of thionin
<i>cev1</i>	constitutive expression of VSP1
CHIB	CHITINASEB
<i>coi1</i>	coronatine insensitive1
<i>eds1</i>	enhanced disease susceptibility1
<i>ein2</i>	ethylene insensitive2
ET	ethylene
<i>fad</i>	fatty acid desaturase
HEL	HEVEIN-LIKE PROTEIN
JA	jasmonic acid
<i>jar1</i>	jasmonic acid resistant1
<i>joe</i>	jasmonate overexpressing
<i>mpk4</i>	mitogen-activated protein kinase4
<i>nahG</i>	salicylate hydroxylase
<i>pad4</i>	phytoalexin deficient4
PDF1.2	PLANT DEFENSIN1.2
PR	pathogenesis-related
SA	salicylic acid
SAR	systemic acquired resistance
<i>sid2</i>	SA induction deficient2
<i>ssi2</i>	suppressor of SA insensitivity2
THI2.1	THIONIN2.1

Introduction

In response to microbial attack, plants activate a complex series of responses that lead to the local and systemic induction of a broad spectrum of antimicrobial defenses [1]. Much progress has been made in understanding the mechanisms by which plants detect and defend themselves against microbial attack. Recent advances have been made in several areas. These include the cloning and characterization of plant disease resistance genes that govern the recognition of specific pathogen strains [2,3], the identification of components involved in the signal transduction pathways coupling pathogen recognition to the activation of defense responses [4,5], and the demonstration that three endogenous plant signaling molecules, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), are involved in plant defense [6,7*].

These signaling molecules are involved in what appear to be two major pathogen defense signaling pathways: an SA-dependent pathway and an SA-independent pathway that involves JA and ET. These pathways do not function independently, but rather influence each other through a complex network of regulatory interactions. A greater understanding of the SA, JA and ET signaling pathways and of how they modulate each other should provide insight into the mechanisms underlying the activation and regulation of defense responses. This may also provide insight into strategies that are used by plant pathogens to alter (e.g. evade or suppress) host defense responses, and thus to promote pathogen virulence and disease production.

In this review, we focus on the signaling pathways that are involved in plant defense against pathogens, and summarize recent studies that provide evidence for regulatory cross talk between these pathways. As many of these studies take advantage of plant signaling mutants, the emphasis of this review is on work that has been carried out in *Arabidopsis thaliana*. However, we note that studies in other systems may reveal that the SA, JA and ET signaling pathways are utilized and/or modulated in different ways in different plant–pathogen interactions.

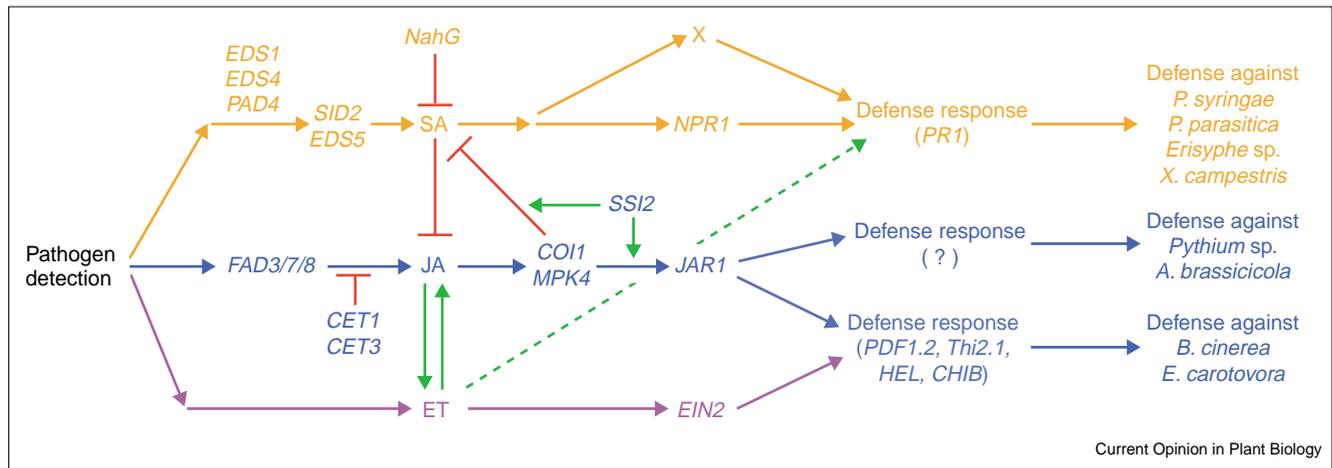
Pathogen defense response pathways

To date, three signaling molecules, SA, JA and ET, are known to play key roles in various aspects of plant defense. These include defense against abiotic stresses, such as wounding and exposure to ozone, as well as defense against insect and microbial attack [8,9]. Our current understanding of the roles of the SA, JA and ET signaling pathways in pathogen defense has been summarized in several recent reviews [4,6,7*,10–12]. Thus, we provide here only a brief overview of the defense pathways before exploring the regulatory interactions among these pathways.

Salicylic-acid-mediated defenses

SA has long been known to play a central role in plant defense against pathogens. SA levels increase in plant tissue following pathogen infection, and exogenous application of SA results in enhanced resistance to a broad range of pathogens [13]. Genetic studies have shown that SA is required for the rapid activation of defense responses that are mediated by several resistance genes, for the induction of local defenses that contain the growth of virulent pathogens, and for the establishment of systemic acquired resistance (SAR). SAR is a state of heightened defense that is activated throughout the plant following primary infection by pathogens that elicit tissue damage at the site of infection [13]. Several pathogenesis-related (*PR*) genes whose expression is dependent on SA are commonly used as reporters of SA-dependent defenses.

Figure 1



A working model of the SA, JA and ET pathogen defense pathways in *Arabidopsis thaliana*. The *SID2* and *EDS5* genes appear to be directly involved in SA biosynthesis [16,63], whereas the *EDS1*, *EDS4* and *PAD4* genes regulate SA synthesis [4]. The placement of *EDS5* downstream of *EDS1*, *EDS4* and *PAD4* is based on studies indicating that the expression of *EDS5* is dependent on these genes [64]. The relative placement of *SID2* and *EDS5* with respect to each other has not been determined. The placement of *COI1* and *MPK4* early in the JA signaling pathway is based on reports that mutations in these genes block JA signaling [46•,50]. However, the phenotypes of these mutants are not identical; although both mutants exhibit defects in pollen development, *mpk4* mutants constitutively express SA-dependent genes [46•], whereas *coi1* mutants do not [48•]. The placement of *COI1* and *MPK4* with respect to each other in the JA signaling pathway awaits further molecular and genetic experiments. *JAR1* is tentatively placed downstream of *COI1* and *MPK4* because

of the observations that JA signaling is only partially blocked in the *jar1* mutant and that *jar1* plants do not exhibit enhanced resistance to *P. syringae* [48•]. *SSI2* is proposed to function downstream of *COI1* and *MPK4*, as the *ssi2* mutation does not completely block JA signaling (*ssi2* plants produce pollen and express *THI2* in response to JA) [47•,49]. However, as the S-ACP DES fatty-acid desaturase encoded by *SSI2* may be a member of a gene family [47•], this placement is tentative. Positive regulatory interactions between these signaling pathways are indicated by green arrows, antagonistic interactions by red lines. The hypothesis that *SSI2* modulates cross talk between the JA and SA pathways is indicated by the short green arrows [47•]. The dashed green arrow indicates potential positive interactions between the ET and SA pathways. Putative positive interactions between the SA and JA pathways, and potential negative interactions between the ET and SA pathways, are not shown.

A. thaliana mutants that are impaired in the production of SA (e.g. *enhanced disease susceptibility1* [*eds1*], *eds4*, *eds5*, *phytoalexin deficient4* [*pad4*] and *SA induction deficient2* [*sid2*]), as well as transgenic plant lines that can't accumulate elevated levels of SA because of their expression of the SA-degrading enzyme salicylate hydroxylase (*NahG*), exhibit enhanced disease susceptibility to a variety of pathogens. These include the fungal pathogens *Peronospora parasitica* and *Erysiphe* sp., and the bacterial pathogen *Pseudomonas syringae* (Figure 1; [5,14–16]). Likewise, plants that carry mutations in *NON-EXPRESSOR OF PR1* (*NPR1*), a gene that encodes a signaling component that functions downstream of SA, exhibit enhanced susceptibility to these pathogens [5,14,15]. In general, pathogens that are controlled by SA-dependent defense responses colonize the apoplast and multiply within host tissue for several days before causing plant cell death and tissue damage.

Jasmonic-acid-dependent defenses

JA, a fatty-acid-derived signaling molecule, is involved in several aspects of plant biology including pollen and seed development, and defense against wounding, ozone, insect pests and microbial pathogens [8,10,17]. *A. thaliana* mutants that are impaired in JA production (e.g. *fatty acid*

desaturase [*fad3*]/*fad7*]/*fad8* triple mutants) or perception (e.g. *coronatone insensitive1* [*coi1*] and *jasmonic acid resistant1* [*jar1*]) exhibit enhanced susceptibility to a variety of pathogens, including the fungal pathogens *Alternaria brassicicola*, *Botrytis cinerea*, and *Pythium* sp., and the bacterial pathogen *Erwinia carotovora* (Figure 1; [18,19•,20–22]). These pathogens employ a common virulence strategy that involves rapidly killing plant cells to obtain nutrients, and thus are often referred to as 'necrotrophs' [23]. Several JA-dependent genes that encode pathogenesis-related proteins, including *PLANT DEFENSIN1.2* (*PDF1.2*), *THIONIN2.1* (*THI2.1*), *HEVEIN-LIKE PROTEIN* (*HEL*) and *CHITINASEB* (*CHIB*), are commonly used to monitor JA-dependent defense responses [10].

Several mutants that exhibit enhanced or constitutive JA responses (i.e. *constitutive expression of VSP1* [*cev1*], *cev1*, and several *constitutive expressor of thionin* [*cet*] and *jasmonate overexpressing* [*joe*] mutants) have been isolated recently [24•–27•]. The number of loci defined by these mutants has not yet been determined. *cet1* and *cet3* plants accumulate elevated levels of JA, suggesting that these mutants define negative regulators of JA biosynthesis (Figure 1; [26•]). The basis for the constitutive JA-response phenotypes of the remaining mutants has not been reported, and

thus could involve either increased JA synthesis or increased flux through the JA signaling pathway. The *coi1* mutant exhibits increased resistance to *Erysiphe* sp. [24*], but the response to pathogen infection of the other constitutive JA mutants has not been investigated. However, it seems likely that constitutive JA-signaling mutants would exhibit enhanced resistance to necrotrophic pathogens that are normally controlled by the JA pathway. Consistent with this hypothesis, *A. thaliana* plants that overexpress a JA-biosynthetic gene constitutively express *PDF1.2* and exhibit enhanced resistance to *B. cinerea* [28*].

Ethylene-dependent responses

The role of ET in plant defense is somewhat controversial as it contributes to resistance in some interactions [22,29] but promotes disease production in others [30–32]. For example, the *ethylene insensitive2 (ein2)* mutant of *A. thaliana* exhibits increased susceptibility to *B. cinerea* [29] and *E. carotovora* [22], but decreased symptoms when infected with virulent isolates of *P. syringae* or *Xanthomonas campestris* pv. *campestris* [30]. Similar divergent effects of ethylene insensitivity on disease development have also been observed in soybeans [32].

In *A. thaliana*, the pattern of altered pathogen responses for the *ein2* ET-signaling mutant generally parallels the patterns observed for the *coi1* and *jar1* JA-signaling mutants. For example, the expression of several JA-dependent defense genes (i.e. *PDF1.2*, *THI2.1*, *HEL* and *CHIB*) also requires *EIN2* [22,33]. The JA and ET signaling pathways are also both required for the induction of induced systemic resistance (ISR), a form of systemic resistance that is triggered by the root-colonizing bacterium *P. fluorescens* [11]. These observations have led to the development of simple models in which ET and JA are placed together in a single signaling pathway. However, these models are likely to be too simple, as the JA and ET signaling pathways have also been shown to modulate each other.

Cross talk among pathogen defense signaling pathways

There is a growing body of literature that reports that the JA, SA and ET defense signaling pathways do not function independently. Rather, they are involved in a complex signaling network in which the different pathways influence each other through positive and negative regulatory interactions. Below and in Figure 1, we summarize the results of studies, carried out primarily in *A. thaliana*, that provide evidence for cross talk among the SA, JA and ET signaling pathways. Incorporating the results from these studies into a single model is difficult as several different plant signaling mutants, pathogen systems and defense reporter genes have been used. Thus, it is often hard to compare the results from different studies directly.

Cross talk between the JA and ET signaling pathways

Several studies provide evidence for positive interactions between the JA and ET signaling pathways. Both JA and ET

signaling are required for the expression of the defense-related gene *PDF1.2* in response to infection by *A. brassicicola* [34], and for the expression of *PDF1.2*, *HEL*, and *CHIB* in response to treatment with *E. carotovora* culture filtrates [22]. Further, when exogenously applied together to plant tissue, JA and ET appear to function synergistically to induce *PDF1.2*, *HEL* and *CHIB* in *A. thaliana* [22,33], and *osmotin* and *PR1b* in tobacco [35]. Evidence that JA and ET coordinately regulate many other defense-related genes was obtained in an *A. thaliana* microarray experiment that monitored gene expression in response to various defense-related stimuli. In this study, nearly half of the genes that were induced by ET were also induced by JA treatment [36]. Not surprisingly, the study revealed that JA and ET also independently regulate separate sets of genes. Little evidence exists suggesting antagonistic interactions between the JA and ET defense pathways.

Cross talk between the SA and ET signaling pathways

Limited data suggest both positive and negative regulatory interactions between the ET and SA signaling pathways. In tomato, the development of disease symptoms following infection by *X. campestris* pv. *vesicatoria* requires both SA and ET, and the accumulation of SA in infected plants is dependent on ET synthesis [37]. Results from the microarray experiment mentioned previously suggest that, in *A. thaliana*, SA and ET may function together to coordinately induce several defense-related genes [36]. Although the induction of SA-dependent expression of *PR* genes does not require an intact ET signaling pathway in *A. thaliana*, exposure to ET has been reported to potentiate the SA-mediated induction of *PR-1* in this species [38]. However, genetic data from the same study suggest that the ET signaling pathway also negatively affects SA-dependent responses: the basal level of *PR-1* mRNA appears to be significantly elevated in *ein2* plants [38]. These data, which appear contradictory at first, may reflect the complexity of regulatory cross talk between the SA and ET signaling pathways.

Cross talk between the SA and JA signaling pathways

The interactions between SA and JA signaling appear to be complex, and there is evidence for both positive and negative interactions between these pathways. However, the primary mode of interaction between these pathways appears to be mutual antagonism. The inhibitory effect of SA on JA signaling in tomato is well substantiated [39–41]. Several recent genetic studies also provide evidence for an antagonistic effect of SA on JA signaling in *A. thaliana*. The *eds4* and *pad4* mutants, which are impaired in SA accumulation, exhibit enhanced responses to inducers of JA-dependent gene expression [42*]. In the *cpr6* mutant, which accumulates elevated levels of SA and constitutively expresses both SA- and JA-dependent defenses, reducing the level of SA by crossing in an *eds5* mutation results in a further increase in *PDF1.2* expression [43*].

There is growing evidence that JA also antagonizes SA signaling. Studies in tobacco reveal that JA inhibits the

expression of SA-dependent genes [44]. Treatment of tobacco plants with elicitors produced by *E. carotovora*, which we now know activates JA signaling in *A. thaliana* [22], resulted in inhibited expression of SA-dependent genes [45]. The characterization of three JA-signaling mutants, *mitogen-activated protein kinase4* (*mpk4*), *suppressor of SA insensitivity2* (*ssi2*) and *coi1*, has provided genetic evidence that JA signaling also negatively regulates the expression of SA-mediated defenses in *A. thaliana* [46••–48••]. In addition to exhibiting impaired JA signaling, *mpk4* and *ssi2* plants constitutively express SA-mediated defenses and exhibit enhanced resistance to *P. syringae* and *P. parasitica* [46••,47••,49]. Importantly, the impairment of JA signaling in these mutants is not due to an inhibitory effect of elevated levels of SA; JA-dependent gene expression was also impaired in *mpk4 nahG* and *ssi2 nahG* plants that do not accumulate high levels of SA [46••,47••]. Thus, constitutive SA signaling in the *mpk4* and *ssi2* mutants is likely due to loss of an antagonistic effect of JA signaling on the SA pathway. *MPK4* is predicted to encode a mitogen-activated (MAP) kinase that is required for JA-dependent gene expression. The *SSI2* gene encodes a steroyl-ACP fatty-acid desaturase, which is hypothesized to catalyze the synthesis of a fatty-acid-derived signal that is involved in mediating both JA signaling and negative cross talk between the JA and SA pathways (Figure 1; [47••]).

The *coi1* mutant also exhibits enhanced expression of SA-dependent defenses and enhanced resistance to *P. syringae* [48••,50]. However, unlike *mpk4* and *ssi2* mutants, *coi1* plants do not exhibit constitutive expression of SA-dependent defenses. Rather, the SA-mediated defense pathway is sensitized in *coi1* plants, such that SA-dependent defenses are hyperactivated in response to attack by *P. syringae* [48••]. These findings are consistent with the hypothesis that the JA signaling pathway negatively regulates the expression of SA-dependent defenses. *COI1* encodes an F-box protein that is hypothesized to regulate JA-signaling by inactivating negative regulators of JA-mediated responses [51]. The observation that a JA-insensitive tomato mutant (i.e. *jai1*) [17] exhibits enhanced resistance to *P. syringae* suggests that JA antagonizes SA-dependent pathogen defenses in tomato as well as in *A. thaliana* (G Howe, personal communication).

There is limited evidence for positive interactions between the JA and SA pathways. Results from early experiments with tobacco indicate that SA and JA act synergistically to induce *PR1b* expression [35]. In *A. thaliana*, microarray analysis of plants that had been exposed to a variety of defense-inducing treatments has revealed that more than 50 defense-related genes are co-induced by SA and JA [36], suggesting that the two signals coordinately regulate these genes.

Benefits and potential costs of cross talk between pathogen defense signaling pathways

The evolutionary significance of multiple plant defense pathways that modulate each other has been the subject of

much discussion [5,7•,10,52]. Separate signaling pathways may have evolved to allow plants to fine-tune their defense responses, such that they deploy the appropriate combination of defenses against specific pathogens according to their virulence strategies. Mutually antagonistic interactions between the JA and SA pathways might further ensure that inappropriate defenses are not activated in response to certain pathogens. For example, as SA can promote programmed plant cell death under certain conditions [37,53], it might be advantageous for the plant to prevent the activation of SA signaling in response to attack by necrogenic pathogens, which utilize cell-death inducing toxins as virulence factors.

Although this highly regulated and complex defense response network must provide an evolutionary advantage to plants, it could also lead to increased vulnerability to some pathogens. Several plant pathogens are known to produce plant signaling molecules, such as ET and auxin [54,55], or to generate compounds that function as molecular mimics of these molecules [10,56]. For several pathogens, synthesis of these compounds has been demonstrated to contribute to virulence [56,57]. In most cases, however, the role of pathogen-derived plant signals in virulence is unclear. Production of these molecules may provide a mechanism by which pathogens can modulate host signaling and physiological processes, thus rendering host tissue more suitable for pathogen colonization, growth and symptom production. For example, the *P. syringae* phytotoxin coronatine, which structurally and functionally resembles methyl JA [58], has been proposed to promote virulence by inhibiting host defenses [59,60]. The findings that *coi1* mutants exhibit both coronatine insensitivity [50] and enhanced SA-signaling in response to infection by *P. syringae* [48••] are consistent with the prediction by Reymond and Farmer [10] that *P. syringae* may utilize coronatine to activate the JA signaling pathway, thereby interfering with the induction of SA-dependent signaling. This could inhibit or delay defenses, thus providing the pathogen with a window of opportunity during which it can colonize host tissue.

Conclusions and future directions

Plant defense responses are regulated through a network of signaling pathways that are currently known to involve at least three endogenous plant signaling molecules: SA, JA and ET. Over the past several years, it has become evident that the JA and SA pathways are mutually antagonistic. This has been proposed to be central to the plant's ability to fine-tune the induction of plant defenses in response to different plant pests and pathogens. However, our understanding of the individual signaling pathways that are involved in pathogen defense and the mechanisms through which they regulate each other is limited. Ongoing and future experiments will involve genetic, genomic, molecular, biochemical and pharmacological approaches to identify additional components in these signaling pathways, and to elucidate the molecular mechanisms through which they modulate each other.

Recent studies indicate that defense signaling may be even more complex than is portrayed in this review, and that additional plant signaling pathways are likely to be involved in regulating pathogen defense. For example, in tomato, the induction of the defense genes *Pti4* and *Pti5* (which encode ethylene-responsive element binding protein-like transcription factors) in response to infection by *P. syringae* is independent of SA, JA and ET [61]. In *A. thaliana*, characterization of the *detachment9* (*dth9*) mutant, which exhibits both insensitivity to auxin and enhanced susceptibility to *P. parasitica* and *P. syringae*, suggests that at least some components of auxin signaling may play a role in pathogen responses [62**]. Future studies investigating the signaling pathways that govern the expression of defense in these systems should contribute further to our knowledge of the elaborate mechanisms that regulate defense responses in plants.

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