SA, JA, ethylene, and disease resistance in plants

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Exciting advances have been made during the past year: isolating mutants affecting plant disease resistance, cloning genes involved in the regulation of various defense responses, and characterizing novel defense signaling pathways. Recent studies have demonstrated that jasmonic acid and ethylene are important for the induction of nonspecific disease resistance through signaling pathways that are distinct from the classic systemic acquired resistance response pathway regulated by salicylic acid.

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Abbreviations

Avr	avirulence
BTH	benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester
eds	enhanced disease susceptibility
HR	hypersensitive response
INA	2,6-dichloroisonicotinic acid
ISR	induced systemic resistance
JA	jasmonic acid
MAP	mitogen-activated protein
ndr	non-race-specific disease resistance
pad	phytoalexin deficient
PR	pathogenesis-related
R	resistance
SA	salicylic acid
SAR	systemic acquired resistance

Introduction

The battle between a plant and an invading pathogen is often dependent on speed. The winner is determined by how quickly the pathogen can proliferate and exert damage, compared to how fast the plant can respond with the necessary levels of defense. If a pathogen is immediately recognized by a plant, for example when a plant has a specific resistance (*R*) gene that interacts with the corresponding avirulence (*avr*) gene from the pathogen, a rapid defense mechanism known as the hypersensitive response (HR) occurs to prevent infection [1–6]. A lack of rapid pathogen recognition often leads to a successful infection. In addition to this immediate, one-on-one contest, plants also employ general resistance mechanisms induced after an HR or during a successful infection to combat secondary infections from a broad spectrum of pathogens or to prevent an existing infection from spreading further. One such general defense mechanism is known as systemic acquired resistance (SAR) [7–9]. SAR induction requires the signal molecule salicylic acid (SA), which accumulates in plants prior to the onset of SAR. Removal of SA in transgenic plants expressing salicylate hydroxylase (encoded by the bacterial *nahG* gene) prevents the establishment of SAR [10]. In some plants such as tobacco, cucumber, and *Arabidopsis*, SA is not only necessary but also sufficient for the induction of SAR. Treatment of these plants with SA or its functional analogs such as 2,6-dichloroisonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) induces SAR [11,12]. SAR is believed to be a result of the concerted activation of pathogenesis-related (*PR*) genes [13–16]; overexpression of a single *PR* gene only provides limited protection to the plant [17–21]. Even though their roles in disease resistance have yet to be clearly demonstrated, expression levels of *PR* genes serve as convenient markers for monitoring SAR.

Characterization of SAR in a variety of plant species has suggested the existence of a complex signaling network that involves many factors affecting various aspects of general disease resistance. It has become evident that plants utilize multiple pathways to transduce pathogenic signals to activate HR, SAR, and other resistance responses, and that SA-mediated SAR is not the only pathway that can lead to broad-spectrum disease resistance. Evidence is emerging that strongly suggests the importance of jasmonic acid (JA) and ethylene as alternative signals in the induction of resistance against microbial pathogens, in addition to their well-characterized roles in the wounding response in plants. In this review, I focus on the genetic and molecular experiments carried out in the past year that define the signaling components in both SA-dependent and SA-independent acquired resistance.

Different signaling pathways leading to the induction of resistance

Genetic screens performed in *Arabidopsis* have generated several classes of mutants which define potential signaling components downstream of the *avr-R* recognition event. One group of mutants, represented by *eds1* (for enhanced disease susceptibility) [22] and *ndr1* (for non-race-specific disease resistance) [23], exhibit altered responses to multiple *avr* signals. The recessive *eds1* mutation lacks the capability for signal transduction from a subset of *R* genes resulting in susceptibility to strains of *Peronospora parasitica* and *Pseudomonas syringae* carrying the corresponding *avr* genes. The *ndr1* mutation appears to influence the function of a different subset of *R* genes causing a loss of resistance to *Pseudomonas syringae* pv. *tomato* DC3000 carrying the bacterial avirulence genes *avrRpt2*, *avrB*, or *avrPph3*. The requirement for EDS1 or NDR1 by different sets of *R* genes seems to be mutually exclusive (JE Parker, personal communication). It has been proposed, therefore, that separate signaling components are used in different *avr-R* interactions. The

selective use of a signaling component may be based on the structure of an individual *R* gene, with each *R* gene within a class sharing a particular component. Connections between these early signaling components and the downstream SA or JA and ethylene signaling pathways have yet to be established. The recent success in cloning the *EDS1* gene which encodes a lipase (JE Parker, personal communication) and the *NDR1* gene which encodes a protein with two putative transmembrane domains [24••] will certainly lead to new information on the molecular functions of these genes.

A new class of mutants, designated *dnd* (for defense, no death), do not show HR after inoculation with *P. syringae* expressing *avrRpt2* or *avrRpm1* but maintain resistance to this pathogen (AF Bent, personal communication). The identification of *dnd* mutants may imply that cell death is not essential for *avr–R* specific resistance. Another plausible explanation is that the resistance observed in *dnd* mutants is a result of the downstream activation of the SAR response, signified by the constitutively elevated levels of SA and *PR* gene expression in these mutant plants.

Analysis of mutants that form HR-like lesions in the absence of pathogen infection and display enhanced nonspecific pathogen resistance has yielded additional insights into regulation of resistance responses. The *Arabidopsis LSD1* gene encodes a novel zinc finger protein which may respond to superoxide accumulated during the HR to restrict the spreading of cell death [25••]. The maize *Lls1* gene has been found to encode a protein with two conserved substrate binding motifs of aromatic ring-hydroxylation dioxygenases and probably functions to degrade a phenolic mediator of cell death [26••]. The *Mlo* gene of barley is predicted to be a membrane bound protein with at least six membrane-spanning helices [27••]. Further characterization of these genes, in addition to the genes defined by the HR-compromised mutants, may provide clues as to how signals are produced during an HR leading to the induction of systemic resistance. Epistasis analyses between the HR-compromised mutants and the lesion mimic mutants may reveal the hierarchy of the signaling events.

A recent report has shown convincingly that reactive oxygen intermediates generated during an HR may serve as signals mediating the establishment of systemic resistance [28••]. Alvarez *et al.* demonstrated that inoculation of *Arabidopsis* with an avirulent pathogen triggers not only an HR at the site of infection but also induces secondary oxidative bursts in discrete clusters of cells in distant, uninfected tissues, which ultimately lead to the formation of microscopic HR lesions. Both the primary oxidative burst and the reiterative secondary oxidative bursts in the systemic tissues have been shown to be required for the induction of SAR.

Virulent pathogens have also been used to identify components of nonspecific resistance responses. Characterization of these mutants has shown that there is a perception/signaling pathway for virulent pathogens leading to SA accumulation and resistance that is distinct from that triggered by avirulent pathogens. The *eds* mutants were isolated for their enhanced disease susceptibility after infection by the virulent pathogen *P. syringae* pv. *maculicola* ES4326 [29,30••]. Inoculation of these mutant plants with an avirulent pathogen still results in an HR and an SAR response even though there is an upward shift in growth of the challenging pathogen in both control plants, pre-treated with 10 mM magnesium chloride, and induced plants, pre-inoculated with an avirulent pathogen. In *eds5-1*, *PR-1* gene induction by the virulent pathogen *Psm* ES4326 is reduced to 10% of the wild-type levels but is fully restored by SA treatment. The *pad* mutants (for phytoalexin-deficient) are deficient in accumulating camalexin, an indolic phytoalexin, after infection by the virulent pathogen *Psm* ES4326 [31] and *pad4*, specifically, appears to affect a regulatory component in camalexin biosynthesis. The *pad4* mutant displays enhanced susceptibility to *Psm* ES4326 and a number of isolates of *P. parasitica* [32•]. In addition, it exhibits pleiotropic defects in *Psm* ES4326-induced SA accumulation and *PR* gene expression that can be partially reversed by the addition of SA [33••]. The pleiotropic effects of *pad4* are specific to the virulent pathogen *Psm* ES4326 (and also *Pst* DC3000) because the synthesis of camalexin, the accumulation of SA, and the expression of *PR-1* gene induced by *Psm* ES4326 carrying *avrRpt2* are all similar to wild-type in *pad4*.

Characteristics of all these mutants suggest the existence of separate components involved in transducing diverse signals generated by different pathogens. PAD4 and EDS5 may be regulators controlled by signals produced during a virulent infection while NDR1, EDS1, DND and LSD1 may represent components involved in the *avr*-induced HR, with NDR1 and EDS1 responding to distinct *avr-R* interactions. Without further knowledge of the signal molecules, however, classification of these signal transducing components can only be done arbitarily. A component identified in an avirulent pathogen-induced response, such as *eds1*, may also affect the response to a virulent pathogen. Nevertheless, all these components seem to be involved in early signaling events. Whether they play a direct role in the induction of SAR or other general resistance responses has yet to be determined.

SA-dependent signaling pathway

In tobacco, *Arabidopsis*, and cucumber, an increase in SA concentration leads to the onset of SAR [34–36], but in other plants such as potato and rice, high levels of SA are detected even under noninducing conditions [37,38•,39]. It has been questioned whether SA is a signal required for SAR in potato and rice, and if it is, how is

the resistance response is activated in these plants with constitutively high levels of SA. Using transgenic potato plants expressing bacterial salicylate hydroxylase (which metabolises SA), it has recently been demonstrated that SA is required for arachidonic acid-induced resistance to *Phytophthora infestans* in potato. The resistance, however, is induced as a result of an increase in sensitivity to SA rather than an increase in SA synthesis [40••]. The mechanism of this induction is unknown; it could occur by increasing the accessibility of SA to a receptor, or by inducing synthesis or activity of an SA receptor.

Biochemical approaches used to identify components involved in the transduction of the SA signal have produced promising candidates. An SA binding protein has been identified [41•] which has a 150-fold higher affinity for SA than catalase: catalase being the first SA binding protein isolated using ^{14}C-labeled SA. Protein phosphorylation and dephosphorylation have been indicated in various defense responses including SA-regulated *PR* gene expression [42]. Consistent with these results, a mitogen-activated protein (MAP) kinase has been shown to be activated in an SA-induced tobacco suspension cell extract [43••]. The sequence of this p48SIP kinase (SA-induced protein kinase) is distinct from other plant MAP kinases previously implicated in stress responses.

In addition to the biochemical approaches, genetic screens performed in *Arabidopsis* have uncovered one locus, *NPR1* (for nonexpresser of PR genes; also called *NIM1* or *SAI1*), that clearly functions downstream of SA [29,44–46]. *NPR1* has been cloned independently by two research groups [47••,48••]. Sequence analysis of the gene and its twelve mutant alleles has shown that NPR1 contains a functionally important ankyrin-repeat domain which may be involved in protein–protein interaction. The carboxyl end of NPR1, where a nuclear localization signal (NLS) resides, has also been shown to be essential for protein function. The importance of this NLS has been demonstrated by the strict nuclear localization of a biologically active NPR1-GFP fusion protein after SAR induction (M Kinkema, X Dong, unpublished data). SAR induction not only modestly increases NPR1 levels, but also is required for activation of the NPR1 protein. Overexpression of NPR1 in *Arabidopsis* confers significant resistance to strains of *P. syringae* and *P. parasitica* that are normally virulent on wild-type *Arabidopsis* [49••]. By measuring the induction kinetics of the *PR* genes during the infection, it has been demonstrated that *PR* genes are not constitutively expressed in the NPR1-overexpressing lines and that pathogen infections cause a stronger, but not faster, expression of these genes compared to wild-type. These characteristics make NPR1 a favorable target for genetic engineering of disease resistance in crops, because constitutive activation of SAR would be likely to have detrimental effects on plant growth and also might result in high selection pressure for more virulent pathogens. There are still many questions that remain to be answered with regard to the molecular function of NPR1. For example, it is unknown if NPR1 regulates *PR* gene expression through activation of a positive transcription factor, or inactivation of a negative transcription factor. To completely understand NPR1 function, the NPR1-interacting component(s) will have to be identified and characterized.

JA- and ethylene-dependent signaling pathway

An exciting new development in the past year is the discovery of SA-independent pathway(s) that also lead to broad-spectrum, systemic resistance. Both JA and ethylene have been shown to be important for the induction of these alternative responses. Fortunately, there already exists a wealth of information about the role of JA in plants response to wounding and insect attack [50]. The wound-induced octadecanoid pathway results in the synthesis of the signal molecule JA and subsequent activation of proteinase inhibitor genes. In addition to JA, ethylene has recently been shown to be a required, though not sufficient, signal that acts with JA to induce the wound response in tomato [51]. Ethylene is produced rapidly and transiently in leaves after injury or upon induction by JA or cell wall oligosaccharide fragments. Inhibition of ethylene production or sensitivity through mutation, antisense technology, or the use of chemical inhibitors all negatively affect induction of the JA pathway.

The JA regulated wound- and insect-induced pathway has long been thought to be involved in conferring resistance to microbial pathogens because it is also induced by oligosaccharide fragments released by the action of lytic enzymes produced by an invading pathogen; however, the role of ethylene and JA in plant disease resistance seemed at times to be ambiguous. For example, in the *Arabidopsis* ethylene-insensitive mutant *ein2*, disease symptoms caused by *Pst* DC3000, *Psm* 4326, and *Xanthomonas campestris* pv. *campestris* were shown to be reduced while growth of these pathogens remained unaffected [52,53]. In the same ethylene-insensitive mutant, the involvement of ethylene in SA-mediated SAR was ruled out because SA- and INA-induced *PR-1* gene expression and resistance to *P. parasitica* were shown to be unaffected by the mutation [54]. In fact, the basal levels of *PR-1* mRNA seemed to be higher in the *ein2* mutant than in the wild-type. Similar results have recently been obtained using the tomato *Never ripe* mutant impaired in ethylene perception [55•]. In another example, the *Arabidopsis coi1* mutants identified for their insensitivity to coronatine and methyl JA (a volatile form of JA) were shown to be resistant to a bacterial pathogen producing the phytotoxin coronatine [56]; however, this result is inconsistent with the hypothesis that methyl JA induces disease resistance. These results can be explained if the ethylene/JA pathway negatively affects the SA pathway. When the ethylene/JA pathway is blocked in the *ein2* and *coil* mutants, the SA pathway may be slightly upregulated, leading to elevated

levels of *PR* gene expression and increased tolerance to bacterial pathogens.

A more clear-cut connection between systemic disease resistance, JA and ethylene signaling molecules has recently been established in *Arabidopsis* by the characterization of the systemically inducible antimicrobial peptides, thionin Thi2.1 and defensin PDF1.2 [57,58]. The expression of *Thi2.1* is induced by methyl JA, silver nitrate, and *Fusarium oxysporum* f. sp. *matthiolae*, but not by SA [57]. The antimicrobial activity of thionin has been demonstrated in *Arabidopsis* by the enhanced resistance to *F. oxysporum* sp *matthiolae* observed in transgenic plants overexpressing the *Thi2.1* gene [59•]. The *PDF1.2* gene is induced by JA, ethylene, rose bengal, and the non-host pathogen *Alternaria brassicicola* [58]. The induction of *PDF1.2* by *A. brassicicola* is inhibited in the JA-insensitive mutant *coi1* and in the ethylene-insensitive mutant *ein2*, indicating that JA and ethylene are required for the induction. *PDF1.2* expression, however, is unaffected in *nahG* transgenic plants which are unable to accumulate SA, in the *npr1* mutant which is insensitive to SA induction, or in the *cpr1* mutant which has elevated levels of SA and constitutive SAR. It is evident that PDF1.2 and Thi2.1 represent end products of a systemically expressed resistance pathway(s) that is distinct from the SA-dependent SAR and is potentially regulated by both JA and ethylene.

Genetic studies have shown that JA and ethylene are essential signals in another resistance response known as induced systemic resistance (ISR) that is established after treatment with various strains of root-colonizing biocontrol bacteria *Pseudomonas fluorescens* (CMJ Pieterse, personal communication). ISR is effective against the virulent *Pst* DC3000 and *F. oxysporum* f. sp. *raphani* and functions independently of SA and *PR* gene expression [60,61•]. Intriguingly, during ISR, *PDF1.2* is not induced, suggesting that JA and ethylene may regulate ISR through a pathway different from that which includes PDF1.2 and Thi2.1. ISR, therefore, appears to be a novel systemic resistance response in plants that can be triggered in roots by rhizobacteria and is regulated by JA and ethylene.

Interactions between different resistant pathways

The antagonistic relationship between the JA and SA pathways has been well documented in the studies of wound responses in plants [50,62]. The inhibitory effect of SA on the proteinase inhibitor genes is partially overcome by pretreatment of plants with both JA and ethylene, but not by either signal alone [51]. This further supports the notion that both ethylene and JA are required signals for wound responses and implies that SA inhibits not only the synthesis of JA and ethylene but also a signaling step downstream of JA and ethylene.

Interactions between the different pathways are also evident in plant resistance responses to microbial organisms. For example, in tobacco, treatments with SA and the pathogenic *Erwinia carotovora* or *Erwinia*-derived elicitors seem to induce two distinct pathways leading to the expression of separate sets of genes [63•]. *Erwinia*-derived elicitors antagonize the SA-mediated induction of *PR* genes while SA inhibits the induction of *Erwinia*-activated genes. Such antagonism suggests the presence of common regulatory components for both pathways, and possibly signifies the evolution of a mechanism designated to prioritize different resistance responses.

Common regulatory components have been found in separate signaling pathways. In the root-colonizing bacterium-induced ISR, which is SA-independent but JA- and ethylene-dependent, the function of NPR1 has been shown to be essential (CMJ Pieterse, personal communication). This implies that NPR1 not only plays a key regulatory role in SAR but also participates in the JA- and ethylene-regulated, SA-independent ISR. This is consistent with the detection of high levels of NPR1 in roots (H Cao, M Kinkema, X Dong, unpublished data) where ISR is induced and where *PR* gene expression and SAR are absent [64].

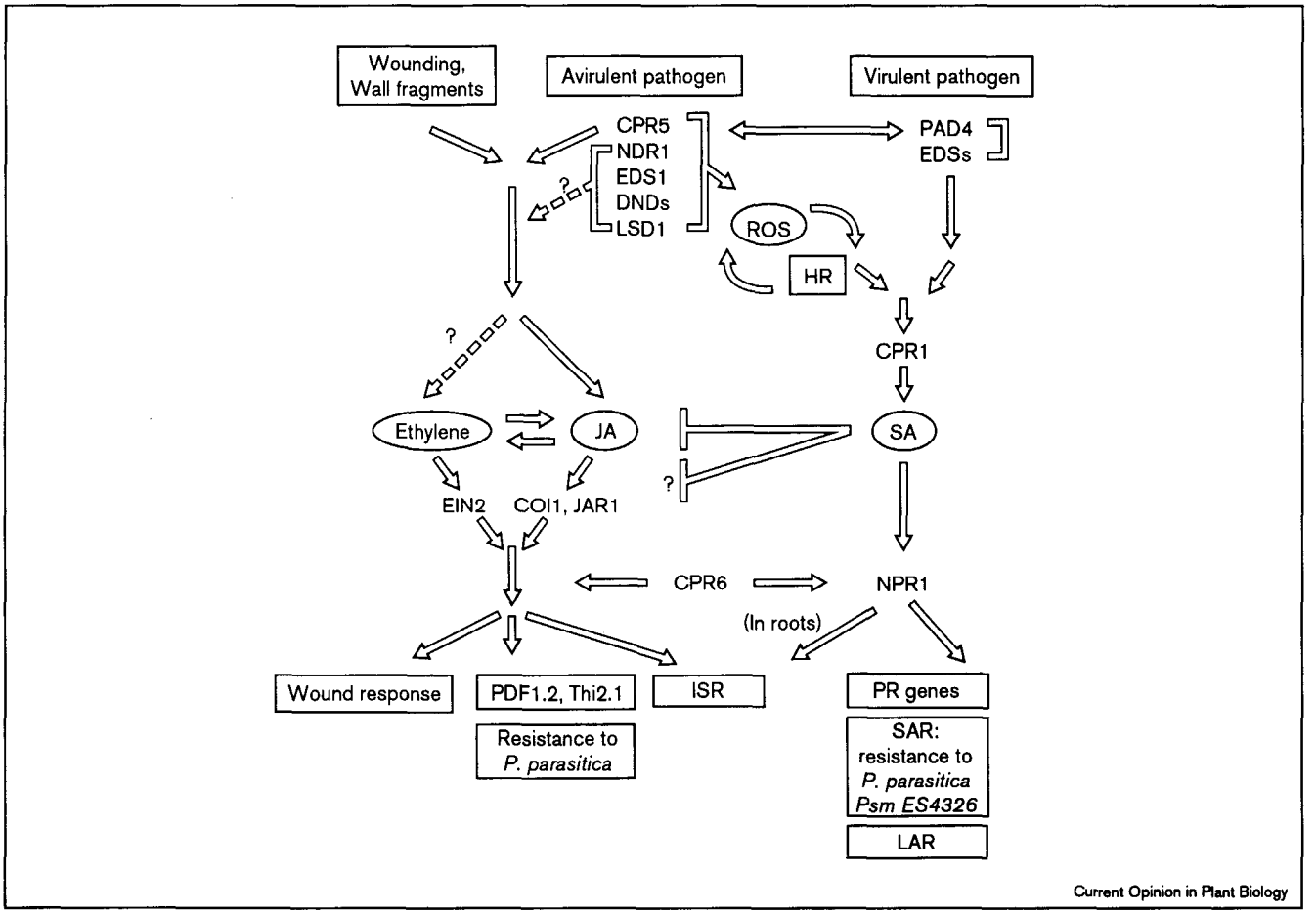
Other components potentially shared by the SA-induced SAR and the JA- and ethylene-induced disease resistance pathways have been identified through epistasis analyses between *Arabidopsis* mutants with enhanced resistance and mutants with compromised defense responses. The *cpr5* mutant expresses both the *PR* genes and the *PDF1.2*, and *Thi2.1* genes, and is resistant to *Psm* ES4326 and *P. parasitica* Noco2. In the *cpr5:npr1* double mutant, the expression of *PR* genes and resistance to *Psm* ES4326 are abolished while the expression of *PDF1.2* and resistance to *P. parasitica* remain unaffected [65••]. These results suggest that a mutation in a single gene can upregulate an NPR1-dependent pathway and an NPR1-independent pathway, both of which can provide resistance to *P. parasitica*. This conclusion is supported by the epistasis analysis performed in the *cpr1:npr1* double mutant. In *cpr1*, where only the NPR1-dependent pathway is activated, the enhanced resistance to *P. parasitica* is completely abolished by *npr1* in the *cpr1:npr1* double mutant (SA Bowling, X Dong, unpublished data).

Analyses of the dominant, gain-of-resistance *Arabidopsis* mutant *cpr6* define another possible point of interaction between the SA-regulated and the JA- and ethylene-regulated pathways [66••]. Similar to *cpr5*, the *cpr6* mutant displays enhanced levels of *PR* and *PDF1.2* gene expression. In the *cpr6:npr1* double mutant, however, neither *PR* nor *PDF1.2* gene expression is significantly affected by *npr1*. Even though it is difficult to determine the precise position of *cpr6* in the disease resistance signaling network, the data suggest a dual role for *cpr6* in regulating both the *PR* and *PDF1.2* genes. Perhaps the wild-type CPR6 works with NPR1 to induce the expression of *PR* genes, and the dominant *cpr6* mutation renders

the protein NPR1-independent. The interplay between CPR6, NPR1 and the two distinct signaling pathways has been further suggested by the complete suppression of *PDF1.2* expression by INA treatment observed in *cpr6*, in comparison to only a partial suppression of *PDF1.2* expression in the *cpr6:npr1* double mutant. A functional NPR1 protein seems to be required for INA to sequester all the mutant cpr6 protein thereby preventing activation of *PDF1.2*.

The relationship between the SA-regulated SAR and the JA- and ethylene-regulated disease resistance needs to be explored further using mutants of both pathways. For example, epistasis analysis between the *cpr5* or *cpr6* mutant and the ethylene-insensitive mutant, *ein2*, or the jasmonic acid-non-responsive mutant, *jar1* [67], will be carried out and the results will be compared with those of *cpr5:npr1* or *cpr6:npr1*. Preliminary analysis of the *cpr5:ein2* and *cpr6:ein2* double mutants have indicated that a full

Figure 1



A working model of pathways leading to broad-spectrum disease resistance in plants. The *Arabidopsis* genes defined by the mutants described in this review are presented. After plant–pathogen recognition, multiple signaling components (CPR5, NDR1, EDS1, DNDs, LSD1) are employed that lead to the formation of reactive oxygen species (ROS) and HR, and the synthesis of SA. NDR1 and EDS1 are involved in transducing signals from separate subsets of *avr-R* interactions, the relative positions of other genes have yet to be determined. It is also unknown whether these genes (except *CPR5*) are involved in the JA- and ethylene-mediated resistant responses. (This ambiguity is represented by a dashed line.) Virulent pathogens can also induce SA synthesis and local resistance, probably through separate regulatory components such as PAD4 and EDSs. Further downstream, the CPR1-SA-NPR1 linear pathway has been demonstrated by various genetic analyses. The pathway of JA and ethylene is proposed on the basis of the wound response in tomato [51], showing that JA and ethylene induce each other's synthesis and that JA and ethylene are both required for activating the wound response, *PDF1.2* and *Thi2.1* gene expression, resistance to *P. parasitica*, and ISR. It remains to be determined whether these responses (shown in rectangular boxes) share regulatory components downstream of JA and ethylene. The SA pathway and the JA/ethylene pathway may interact antagonistically, with SA inhibiting both the synthesis and the signal transduction of JA and ethylene (illustrated with blocked lines). The possible involvement of NPR1 in the SA-independent but JA- and ethylene-dependent ISR is intriguing. Further characterization of NPR1 and another *Arabidopsis* component CPR6, which is involved in regulating the expression of both *PR* and *PDF1.2* genes, should provide new insight into the relationship of these pathways.

induction of *PDF1.2* in *cpr5* and *cpr6* requires the function of EIN2 and probably ethylene (JD Clarke, X Dong, unpublished data).

Conclusions

Isolation of different classes of mutants affecting nonspecific disease resistance and characterization of various plant defense responses have revealed a complex and interesting network of pathways that function both separately and together to render broad-spectrum protection to plants. At the center of this network are the signal molecules SA, JA, and ethylene. A model, with the *Arabidopsis* genes described in this review highlighted, is represented here to stimulate more discussion (Figure 1). After plant–pathogen recognition, multiple signaling components (CPR5, NDR1, EDS1, DNDs, LSD1) are employed that lead to the formation of reactive oxygen species (ROS) and HR, and the synthesis of SA. While NDR1 and EDS1 are involved in transducing signals from separate subsets

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