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Interplay of signaling pathways in plant disease resistance

Plants are under constant threat of infection by pathogens armed with a diverse array of effector molecules to colonize their host. Plants have, in turn, evolved sophisticated detection and response systems that decipher pathogen signals and induce appropriate defenses. Genetic analysis of plant mutants impaired in mounting a resistance response to invading pathogens has uncovered a number of distinct, but interconnecting, signaling networks that are under both positive and negative control. These pathways operate, at least partly, through the action of small signaling molecules such as salicylate, jasmonate and ethylene. The interplay of signals probably allows the plant to fine-tune defense responses in both local and systemic tissue.

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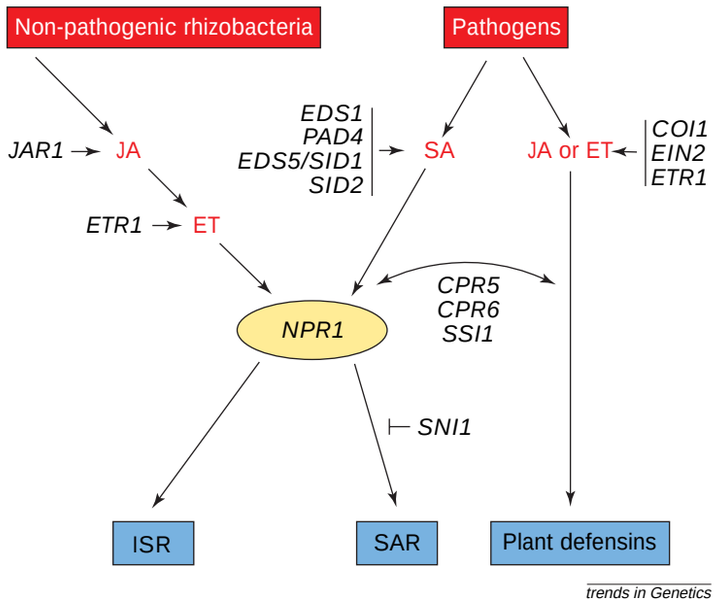
Plants encounter a diverse range of enemies, including microbial pathogens, nematodes and insects, and have evolved countermeasures to resist most potential invaders. Upon pathogen detection, plants activate a number of early responses that lead to the production of a broad spectrum of defensive molecules[1,2]. One of the most effective defenses in plants is mediated by Resistance (*R*) genes that are able to detect specific pathogen races through recognition of pathogen-encoded Avirulence (Avr) proteins[3,4]. *R*-gene-mediated resistance (also termed

FIGURE 1. Overview of local and systemic signaling in *Arabidopsis* disease resistance



A number of resistance pathways (SAR, ISR and plant defensin induction), with different requirements for the signaling molecules salicylic acid (SA), jasmonic acid (JA) and ethylene, and that lead to the induction of different sets of defense-related genes, have been defined in *Arabidopsis*. Recruitment of *NPR1* to these pathways appears to depend on the nature of the pathogen input signal and evidence points to *NPR1* functioning as signal modulator, determining a particular output. *NPR1* function in SAR requires the removal of a negative control by *SNI1*. Analysis of several resistance-upregulating mutations (*cpr5*, *cpr6* and *ssi1*) also points to cross-talk between SA- and JA/ethylene-responsive processes. Non-pathogenic rhizobacteria induce a JA/ethylene-dependent, SA-independent systemic response (ISR) that requires functional NPR1 but induces an as yet undefined set of downstream events. Genes that are known to be required for pathogen-induced SA accumulation and full resistance are shown, as well as signaling components from the JA and ethylene pathways that control pathogen-induced defensin synthesis and establishment of ISR.

gene-for-gene resistance) is commonly, although not invariably, associated with rapid necrosis of plant cells at the site of invasion (the hypersensitive response, or HR) and this results in efficient containment of the pathogen. In evolutionary terms, a plant pathogen would benefit by eliminating these Avr proteins to avoid detection by the plant, unless they contribute substantially to pathogen fitness and virulence, as demonstrated in a number of plant–pathogen interactions[5].

In addition to a localized resistance response, plants have also evolved mechanisms of systemic immunity in which local defenses establish a state of heightened resistance throughout the plant against subsequent attack. This phenomenon, known as systemic acquired resistance (SAR), is effective against a broad spectrum of pathogens and requires the phenolic signaling molecule, salicylic acid (SA)[6]. Correlated with the onset of SAR, plants express a set of pathogenesis-related (PR) proteins, some of which have been shown to have antimicrobial activity *in vitro* or to confer increased resistance when overexpressed in plants[7,8]. The *Arabidopsis NPR1* gene (described in more detail below) is required both for the establishment of SAR and for the SA-induced expression of the *PR-1* gene[9].

A different form of systemic resistance was discovered more recently in plants responding to non-pathogenic strains of the root-colonizing bacterium *Pseudomonas fluorescens*. This has been referred to as induced systemic resistance (ISR) and is also effective against multiple pathogen types[10]. Interestingly, ISR is independent of SA and is not associated with activation of *PR-1* expression. Instead, ISR requires the operation of signaling pathways responding to the plant growth hormones jasmonic acid (JA) and ethylene. Treatment of transgenic *Arabidopsis* plants expressing the bacterial SA-degrading enzyme salicylate hydroxylase (referred to as NahG plants) with either JA- or ethylene- induced ISR, clearly establishing the SA-independent nature of this phenomenon[10]. Although not involving SA, elaboration of ISR was found to require the *NPR1* gene, suggesting a broader role for *NPR1* in systemic plant defenses. A schematic diagram in Figure 1 provides an overview of the components involved in local and systemic signaling in various plant–pathogen interactions and incorporates *NPR1* as a central player in these processes.

This review focuses on recent genetic analyses of plant mutants that compromise or enhance plant disease resistance, and their usefulness in unraveling mechanisms of plant–pathogen recognition and resistance pathway utilization. We assess the contribution of SA in disease resistance, as well as the participation of JA- and ethylene-dependent pathways. What emerges from these studies is a complex signaling network involving cross-talk between different pathways. Also, the mode of plant defense signaling appears to be influenced both by the structural type of R protein mediating specific plant–pathogen recognition and the pathogen's biotrophic or necrotrophic lifestyle. Recent insights into certain biochemical aspects of plant defense, in particular the roles of reactive oxygen intermediates (ROI) and nitric oxide, can be found in some excellent reviews[11,12,13].

R gene-dependent signaling and potentiation mechanisms

Plant R proteins confer specific recognition of a pathogen *avr* gene product but must also serve as a signal transducer to elicit downstream defenses. A large number of plant *R* genes specifying resistance to bacterial, fungal, viral or nematode pathogens and aphids, have now been cloned[3,4]. Surprisingly, despite the widely different modes of pathogen colonization, analysis of the structural features of R proteins reveals the existence of only a limited number of sequence motifs. These include putative protein-interaction/recognition domains such as leucine-rich repeats (LRR) and leucine zippers (LZ), signaling functions such as a kinase domain or nucleotide-binding site (NB), and the TIR domain, defined by homology to the intracellular effector domains of the *Drosophila* Toll and human interleukin-1 receptors. Recruitment of this rather limited repertoire of motifs suggests that the processes underlying R protein-specified recognition of unrelated pathogens are mechanistically highly conserved and probably engage a restricted number of downstream defense pathways. Precisely how perception of an Avr protein primes defense signaling is, however, still unclear.

Mutational dissection of *R* gene-mediated resistance and systemic immunity in plants such as tomato, barley and *Arabidopsis*, has provided an initial genetic framework for defense pathway utilization, establishing the hierarchies and interplay of particular signaling molecules. In several instances, ancillary signaling genes are found to

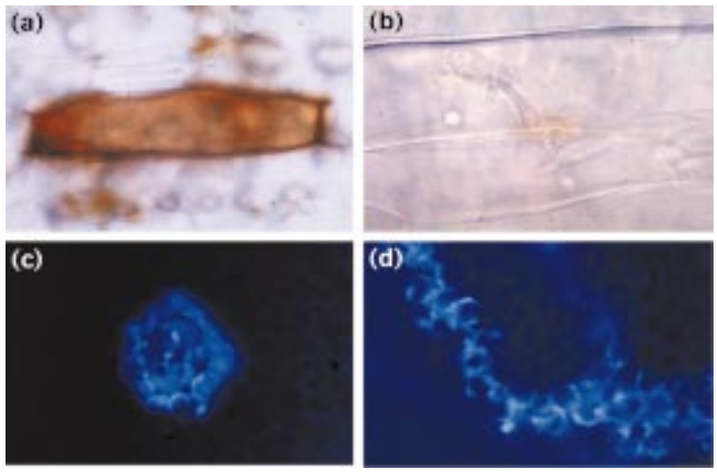
be necessary for the function of multiple *R* genes, reinforcing the notion that common processes operate downstream of plant–pathogen recognition. For example, mutational analysis of the interaction between barley and the powdery mildew fungus *Erysiphe graminis* f sp *hordei* uncovered two genes, *Rar1* and *Rar2*, that are essential for the function of the *R* gene *Mla-12* (Refs 14,15). The *rar1* mutation compromises the function of many other, but not all, *R* genes recognizing different powdery mildew isolates, placing wild-type *Rar1* at a point of pathway convergence for a subset of barley *R* genes[16,17]. *Rar1* was cloned and shown to encode the founding member of a novel class of eukaryotic zinc-binding protein[18]. Significantly, *Mla-12*-specified resistance to *E. graminis* in epidermal cells is accompanied by a biphasic accumulation of the ROI species, H_2O_2, the first burst occurring at the site of attempted fungal penetration and a more extensive secondary burst occurring at the whole cell level and coinciding with a single-cell HR[18] (Fig. 2a). Two phases of H_2O_2 generation have also been observed in plant cell suspension cultures after challenge with avirulent *Pseudomonas syringae* strains[19,20]. The first transient and weaker burst occurs in both compatible and incompatible *P. syringae* interactions, whereas the second burst was specific to *R* gene-mediated recognition. In *rar1* mutants, the second H_2O_2 burst and single-cell HR are greatly attenuated[18] (Fig. 2b), suggesting a role for barley *Rar1* in priming whole-cell H_2O_2 accumulation, leading to plant cell death and containment of the pathogen.

So far, the most extensive genetic studies in plant pathology have utilized the model plant *Arabidopsis*, a natural host plant to all the major classes of phytopathogens (viruses, bacteria, fungi and nematodes). Phenotypic analysis of the *Arabidopsis* disease resistance signaling mutants, *eds1* and *ndr1*, showed that they were indispensable for the function of distinct classes of *R* gene[21] (Fig. 3). Moreover, a correlation was found between the particular predicted R protein structure and its signaling mode. Thus, mutations in *EDS1*, encoding a lipase-like protein[22], abolished resistance mediated by *R* genes of the TIR-NB-LRR class, whereas *ndr1* mutants suppressed resistance conferred by LZ-NB-LRR type *R* genes. Interestingly, *RPP8*, an LZ-NB-LRR type *R* gene conferring isolate-specific resistance to the oomycete pathogen *Peronospora parasitica*[23], was not strongly suppressed by either *eds1* or *ndr1*[21]. Further analysis by McDowell *et al.* showed that this was not due to redundant engagement of both signaling components but rather to utilization of another, as yet undefined, signaling pathway[24] (see Fig. 3 for an overview).

In other cases, screens for mutants impaired in *R* gene-mediated resistance have identified genes that are specifically required for the function of individual *R* genes, highlighting the existence of highly discriminatory R protein-associated components. In tomato, for example, *rcr3* mutants specifically compromise the function of *Cf-2* but not *Cf-5*, *R* genes that recognize different pathogen races of *Cladosporium fulvum*, although the respective R proteins are 93% similar at the amino acid level[25]. In *Arabidopsis*, mutations in *PBS1* suppress resistance to the bacterial pathogen *P. syringae* mediated by the *R* gene *RPS5*, but do not affect other *R* genes tested[26].

The screen for suppressors of *RPS5*-mediated resistance revealed two further mutations, *pbs2* and *pbs3*[26]. Whereas *PBS1* is required specifically for *RPS5* function, mutations in *PBS2* affect the function of multiple *R* genes recognizing different *P. syringae* races with a phenotypic spectrum similar to that of the *Arabidopsis ndr1* mutant (Fig. 3). However, *PBS2* and *NDR1* have at least one non-overlapping activity, as *pbs2* plants permit heavy sporulation of the *P. parasitica* isolate *Wand1*, whereas *ndr1* is unaffected in its resistance to this isolate[26]. Thus, *Arabidopsis* is able to deploy a set of partially overlapping defense pathways depending on the particular set of pathogen-derived signals. In contrast, the *pbs3* mutation compromises resistance mediated by all *R* genes tested so far. It also allows increased colonization by virulent *P. syringae* strains, suggesting that *PBS3* functions in the plant's basal resistance machinery.

FIGURE 2. The *Arabidopsis pad4* and barley *rar1* mutations compromise *R* gene function



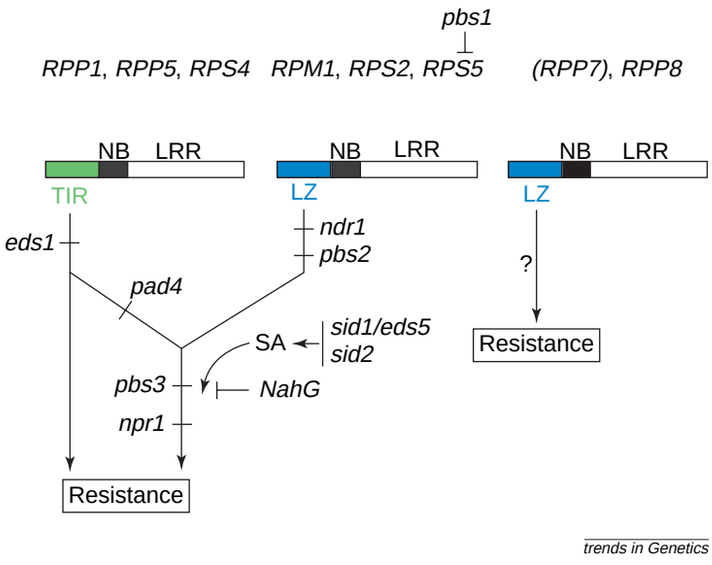
Epidermal cells of wild-type resistant barley plants respond to *Erysiphe graminis* f.sp *hordei* infection with two distinct phases of ROI generation. H_2O_2 accumulation (monitored by staining with diaminobenzidine) is first observed at the site of cell wall appositions and is followed by a whole cell response (a). In *rar1*, the initial limited H_2O_2 burst is retained (b) but whole cell H_2O_2 accumulation is greatly diminished[18] (pictures kindly provided by Ken Shirasu and Paul Schulze-Lefert). Wildtype resistant *Arabidopsis* plants respond to *Peronospora parasitica* infection with massive deposition of callose (shown by staining with aniline blue) in cells undergoing an HR (c). In *pad4* mutant plants the pathogen elicits an attenuated HR and is able to grow beyond the site of penetration, causing a spreading plant cell necrotic response (d) (B.J. Feys and J.E. Parker, unpublished).

Salicylic acid and plant defense

The small defense signaling compound SA has previously been shown to play a central role in plant disease resistance, both in the establishment of SAR and the elaboration of local defense responses in the attacked tissue. Analyses of an expanding panel of *Arabidopsis* disease resistance mutants with respect to SA levels, as well as the isolation of mutants with defects in SA accumulation, are helping to further elucidate the role of SA in plant resistance.

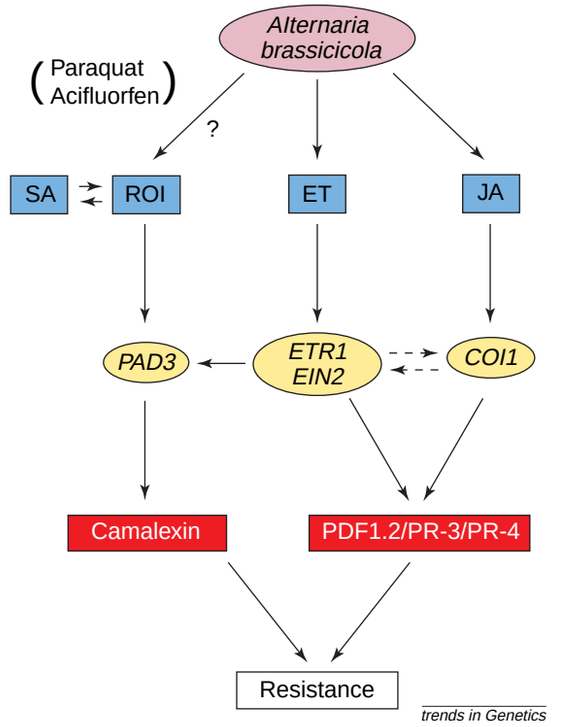
Mutations in the *Arabidopsis* gene, *PAD4*, give rise to enhanced disease susceptibility to a compatible *P. syringae* strain and lead to deficiencies in pathogen-induced accumulation of both SA and camalexin, an indole phytoalexin with antimicrobial activity[27,28]. Infection of *pad4* plants with certain incompatible *P. parasitica* isolates also reveals an inability of the mutant to consolidate an *R* gene-mediated hypersensitive response, resulting in a

FIGURE 3. Dissection of *R* gene-mediated signaling pathways in *Arabidopsis*



Genetic analysis of NB-LRR type *R* genes in *Arabidopsis* has shown that R proteins with an amino-terminal TIR domain predominantly signal through *EDS1*, whereas LZ-containing R proteins require *NDR1* and *PBS2* to initiate defense responses. Interestingly, RPP8, an LZ-NB-LRR type R protein, functions independently of these two pathways. *RPP7*, an *R* gene that has not yet been cloned, confers isolate-specific resistance to *P. parasitica* through a novel pathway that may or may not be shared with *RPP8* (Ref. 24). Mutations in *PAD4* cause a partial loss of *RPP5*-mediated resistance to *P. parasitica* in contrast to the *eds1* mutant, which shows a full loss of *RPP5* function. Both *EDS1* and *PAD4* operate upstream of pathogen-induced SA accumulation[28] (M.A. Newman and J.E. Parker, unpublished data), which in turn is defined by the genes *SID1*/*EDS5* and *SID2*. *NPR1* is a key modulator of SA responses that functions downstream of SA perception[34]. Mutations in *PBS3* affect *R* genes of both the TIR and LZ class and have therefore been placed at a point of convergence between both pathways. The effect of the *pbs3* mutation on SA levels, as well as its position relative to *NPR1*, is unknown. The requirement for *PBS1* was shown to be specific for *RPS5*.

FIGURE 4. Signaling in the resistance response of *Arabidopsis* to *Alternaria brassicicola*



The necrotrophic fungus *A. brassicicola* induces a set of defensive compounds upon infection of *Arabidopsis*. Analysis of available *Arabidopsis* signaling mutants has allowed a fine dissection of the components involved in *A. brassicicola* resistance. Mutations in the camalexin biosynthetic enzyme, *PAD3*, lead to partial loss of resistance to this plant pathogen. The signals that are generated upon pathogen infection and that lead to camalexin accumulation are, however, unknown. Camalexin biosynthesis is not induced by external application of JA, ethylene or SA, although SA is necessary for pathogen-induced accumulation of camalexin[45,56]. Certain herbicides, such as paraquat and acifluorfen, that are known to cause oxidative stress, can induce camalexin biosynthesis[45,56], suggesting that ROI may be involved in camalexin production by *A. brassicicola*, possibly in conjunction with SA. *A. brassicicola* infection also leads to production of the plant hormones JA and ethylene. Mutational analysis has shown that induction of the plant defensin *PDF1.2* requires the concomitant activation of the JA and ethylene signaling pathways. Interestingly, mutations in *COI1*, but not in *ETR1* or *EIN2*, cause a partial loss of resistance, suggesting that induction of *PDF1.2* is not required for resistance. A degree of cross-talk exists between the pathway leading to camalexin accumulation and the ethylene pathway, as mutations in *EIN2* cause a reduction in *A. brassicicola*-induced camalexin accumulation[45].

characteristic trailing necrotic reaction as the pathogen repeatedly overcomes host defenses (Fig. 2d; B.J. Feys and J.E. Parker, unpublished). This suggests that *PAD4* is involved in the reinforcement or potentiation of the local plant resistance response that is required for complete containment of the pathogen. The *PAD4* gene was cloned and found to encode another member of the L-lipase class of plant protein that includes EDS1[29], suggesting that this type of activity may have been recruited in plants specifically for defense signaling. *EDS1* is also required for SA accumulation upon *P. syringae* infection (M.A. Newman and J.E. Parker, unpublished data). Moreover, although *PAD4* and *EDS1* function upstream of SA accumulation (Fig. 3), their mRNA levels are upregulated by applications of SA[22,29], reinforcing earlier biochemical studies that demonstrate a capacity of SA to potentiate plant defense signaling, probably in combination with other molecules[20,30].

A different screen performed by Nawrath and Métraux aimed to identify *Arabidopsis* mutants that are impaired in SA accumulation upon pathogen challenge and uncovered two new loci, *SID1* and *SID2* (for salicylic acid-induction deficient)[31]. Like *pad4*, the *sid* mutants also show increased susceptibility to both virulent and avirulent *P. syringae* strains and *P. parasitica* isolates. However, unlike *pad4* they are not deficient in camalexin accumulation when challenged with a compatible *P. syringae* strain, revealing a further bifurcation of downstream signaling processes. Also, whereas *PAD4* encodes a regulatory component of SA accumulation, analyses suggest that *SID1* and *SID2* are more likely to encode proteins directly controlling SA biosynthesis. Complementation tests have shown *sid1* to be allelic to *eds5*, an *Arabidopsis* mutant exhibiting enhanced disease susceptibility towards both *P. syringae* pv *maculicola* and the powdery mildew fungus, *Erysiphe orontii*[32], pointing to a role for *SID1*/*EDS5* in general defense against a broad spectrum of pathogens.

Analysis of these mutants highlights a recurring theme in plant–pathogen recognition. It is apparent that

mutations such as *eds1*, *pad4*, *npr1*, *sid1* and *sid2*, suppressing or partially suppressing *R* gene-mediated responses, also cause enhanced disease susceptibility (eds) to a range of virulent pathogens. It is therefore likely that common features exist between signaling processes involved in *R-avr* gene-mediated resistance (normally associated with the HR) and restriction of virulent pathogens during disease. These mutations add to an expanding array of *eds* mutant loci identified in *Arabidopsis*, demonstrating that growth of virulent pathogens is actively limited by host plant defenses that are not necessarily associated with the HR[32,33]. It is quite plausible that R-Avr recognition events have evolved to somehow potentiate the activities of a pre-existing 'basal resistance' machinery.

Phenotypic similarities of *pad4*, *sid1/eds5* and *sid2*, allied with defects in SA accumulation, suggest the involvement of numerous genes in SA-dependent plant defenses. One closely scrutinized *Arabidopsis* defense signaling gene that operates downstream of SA accumulation, is *NPR1*, originally identified in mutational screens for defects in responses to SA or SA analogues and representing a central component of SAR[6]. Further examination of *npr1* mutations in the context of different plant defense responses and mutant backgrounds reveals *NPR1* to be a more versatile modulator of downstream resistance pathways than initially envisaged. What emerges in several plant–pathogen interactions is a separation of *NPR1* from SA-dependent processes. For example, analysis of *PR1* gene activation by *Erysiphe orontii* was abolished in a NahG background but only partially compromised in the *npr1* mutant[32], indicating the existence of an SA-dependent, but *NPR1*-independent pathway. Also, as mentioned above, *NPR1* was found to be an essential component of induced systemic resistance (ISR) elicited by root-colonizing rhizobacteria, a process that functions independently of SA but requires JA and ethylene signaling[10]. Genetic dissection of this pathway provides evidence that *NPR1* has a dual role in systemic resistance mechanisms mediated by either SA or JA and ethylene (Fig. 1). It also strengthens the idea that pathogen-derived molecules influence the recruitment of common defense signaling components such as *NPR1* into particular resistance pathways depending on the type of input signals the plant perceives.

Clues to the molecular basis of *NPR1* function come from analysis of its predicted protein sequence, showing the presence of putative ankyrin repeats[34], a ubiquitous motif known to mediate a diverse range of protein–protein interactions. Consequently, a modulating role of *NPR1* in multiple pathways may be governed by pathogen-specific signals that cause selective association of *NPR1* with other proteins to drive activation of the required set(s) of defense genes. *NPR1* was recently shown to interact, through its ankyrin repeats, with members of the TGA family of transcription factors[35,36,37]. A subset of these specifically bind SA-responsive promoter elements in the *Arabidopsis PR-1* promoter, suggesting a direct link between *NPR1* and transcriptional activation of *PR-1*. This model is supported by cellular localization studies showing that NPR1 localizes to the nucleus[37]. In a screen for genetic suppressors of *npr1*, Li *et al.* identified the recessive *sni1* mutation on the basis of restored *PR-1* expression and establishment of SAR and pathogen resistance after application of the SA analogue INA, indicating that *SNI1* functions as a negative regulator in the establishment of SAR[38] (Fig. 1). The *sni1* mutants are supersensitive to induction of *PR-1* by SA. Nevertheless, untreated *sni1* or *sni1 npr1* plants fail to exhibit enhanced resistance to virulent strains of *P. syringae* or *P. parasitica*, indicating the requirement for an activation step that is logically independent of both *NPR1* and *SNI1*. SNI1 was also shown to localize to the nucleus when transiently expressed in onion epidermal cells[38]. Thus, SNI1 may act as a transcriptional repressor of SAR and NPR1 possibly removes SNI1 repression upon activation by SA, thereby allowing transcription factors of the TGA family to drive expression of *PR-1*.

Overlapping requirements for jasmonate, ethylene and camalexin in resistance

Several independent analyses have now established a requirement for the plant wound response regulator, JA, in defenses against certain pathogens[39]. The involvement of another plant hormone, ethylene, in plant–pathogen interactions has also been vigorously debated. Recently, however, it has become evident that participation of ethylene may lie more in the control of disease symptom expression than in determining absolute plant resistance or susceptibility phenotypes. For example, reduced ethylene-sensitive soybean mutants produced less severe chlorotic symptoms when challenged with virulent strains of *P. syringae*, whereas virulent strains of the fungi *Septoria glycines* and *Rhizoctonia solani* caused more severe symptoms[40]. Similarly, mutations in the ethylene response regulator *EIN2* cause enhanced susceptibility of *Arabidopsis* to the necrotrophic fungus *Botrytis cinerea*[41]. Therefore, the ethylene pathway may impinge either positively or negatively on symptom development, depending on the particular type of plant–pathogen interaction.

One of the most informative studies showing the involvement of both JA and ethylene in plant disease resistance has examined infection of *Arabidopsis* plants with the necrotrophic fungus *Alternaria brassicicola*. This particular pathogen causes necrotic lesions and elicits an increase in endogenous levels of JA and ethylene, as well as accumulation of the plant defensin, PDF1.2, which has antifungal activity towards *A. brassicicola in vitro*[42,43]. Analysis of mutants affecting the JA, ethylene or SA pathways has permitted a genetic dissection of the requirements for *A. brassicicola* resistance in *Arabidopsis* and evaluation of the relevance of elevated *PDF1.2* expression (Fig. 4). *PDF1.2* mRNA and protein levels are induced by exogenous applications of JA or ethylene, but not by SA[42]. However, *A. brassicicola*-induced *PDF1.2* expression requires the concomitant activation of both the JA and ethylene signaling pathways, suggesting tight cooperation between these two signaling processes[42]. Mutations in *NPR1* or expression of NahG were shown to have no effect on either *PDF1.2* expression or resistance to *A. brassicicola*, whereas mutations in the JA response regulator *COI1* abolished resistance and *PDF1.2* mRNA accumulation, establishing the resistance mechanism as SA-independent[44]. Interestingly, mutations in the ethylene signaling component *EIN2*, whilst abolishing pathogen-induced *PDF1.2* expression, had no effect on resistance[41], questioning the critical importance of *PDF1.2* as an antifungal agent in this plant–pathogen combination.

In *Arabidopsis*, synthesis of the phytoalexin camalexin is not induced by applications of JA or ethylene, whereas

SA is necessary, although not sufficient, for its accumulation[45]. Importantly, *A. brassicicola* infection induces camalexin synthesis[45]. The *Arabidopsis PAD3* gene, previously identified as a necessary component of camalexin accumulation, encodes a putative cytochrome P450 monooxygenase with similarity to maize enzymes required for synthesis of the indole-derived secondary metabolite 2,4-dihydroxy-1,4-benzoxazin-3-one[46], suggesting that *PAD3* most probably encodes a camalexin biosynthetic enzyme. This finding has allowed scrutiny of *pad3* mutants for the requirement of camalexin in plant defense against various pathogens. Interestingly, *pad3* mutant plants were found to be susceptible to *A. brassicicola* but were unaffected in their response to virulent *P. syringae* pv *maculicola*, which also induces camalexin synthesis[45,47]. Clearly, camalexin is a crucial antifungal compound in the *Arabidopsis–A. brassicicola* interaction, even though its role in other plant–pathogen responses is unclear. Again, analysis of the complexities of *Arabidopsis* defense signaling in response to *A. brassicicola* infection reveals the plant's capacity to integrate various pathways depending on the type of pathogen it is encountering (Fig. 4).

Interplay of plant defense signaling pathways

The previous sections have illustrated the multiplicity of signaling processes that impinge on plant defense and that signal relay may depend on the particular R protein type conferring resistance, as well as the lifestyle of the pathogen. The question of how different signaling circuits cross-talk with each other now arises. Recent analyses illustrate the plant's ability to fine-tune responses to particular pathogens in order to activate appropriate subsets of downstream defenses.

As outlined above, the signaling molecules SA and JA control the expression of mostly non-overlapping sets of responses and a number of studies have revealed antagonistic effects of SA application on wound- and/or JA-induced gene expression[48,49]. These observations were extended in a more recent analysis of tobacco plants, which showed an inverse relationship between the level of phenylpropanoid compounds, including SA, and the induction of systemic resistance to insect feeding, mediated by JA[50]. It is likely that mutual antagonism between the SA and JA/ethylene pathways allows the plant to prioritize responses effectively through subtle differences in the kinetics of accumulation or distribution of particular signaling molecules. Opportunities for cross-pathway dialogue have been elegantly represented in the 'tunable dial' model of Reymond and Farmer[39], allowing for both synergistic and antagonistic relationships between signal molecules, depending on their relative concentrations. Indeed, *Arabidopsis* plants are able simultaneously to activate both the SA-dependent SAR pathway and JA/ethylene-dependent ISR responses, resulting in increased resistance against virulent *P. syringae* pv *tomato* strains[51].

Other genetic experiments in *Arabidopsis* have provided further insights to the complexities of pathway interactions in plant disease resistance. For example, a screen for mutants constitutively expressing the SAR marker gene *BGL2*, a β-glucanase, identified the recessive *cpr5* and dominant *cpr6* mutants that produce constitutively high levels of SA and, intriguingly, express both SA- and JA-dependent marker genes[52,53]. The mutant plants also exhibit increased resistance to virulent *P. syringae* and *P. parasitica* strains. All of these phenotypes are SA-dependent but differ in their requirement for *NPR1*. In a screen for suppressors of the *npr1* mutation, Shah *et al.* identified the dominant *ssi1* mutant, which completely bypasses *NPR1* function. Interestingly, *ssi1* mutants constitutively express the JA-dependent marker gene *PDF1.2* in an SA-dependent manner[54], suggesting that wild-type SSI1 protein, together with CPR5 and CPR6, may participate in signal communication between SA- and JA-dependent pathways (Fig. 1). The above data are significant because they show, yet again, that SA-dependent processes can be uncoupled from *NPR1*. They also provide genetic evidence for the existence of both positive and negative switches that may control the interplay between SA- and JA-dependent defenses. Effective execution of a disease resistance pathway that elicits cell death in a discrete patch of plant cells during the HR certainly requires exquisite control by both positive and negative regulators to gauge signal fluxes and establish thresholds. The *cpr5* and *sni1* mutants, together with a large class of mutations causing spontaneous HR-like lesions (so-called lesion mimic mutants[55]), reveal the existence of many potential negative regulators of plant cell death. A number of these may function in feedback regulation at various points of the plant defense response to prevent highly destructive and energy-consuming reactions.

Conclusion

Phenotypic and molecular analyses of plant mutants compromised in disease resistance have provided a first glimpse of the complexities of pathway utilization and signal communication in plant–pathogen recognition. This complexity no doubt equips the plant with the flexibility to respond to a particular pathogen by activating appropriate subsets of defenses and suppressing inappropriate responses. Further studies have extended the roles of previously characterized genes, such as a requirement for *NPR1* in JA/ethylene-mediated ISR, whilst new mutational screens have identified possible genetic switches, such as the *CPRs* and *SSI1*, which may be involved in fine-tuning pathway utilization. Genetic dissection has also shown JA signaling to be integral to the plant's defense signaling repertoire and that multiple genes are involved in balancing the activation of either SA-or JA-mediated resistance.

The precise molecular mechanisms of plant–pathogen recognition remain elusive, although a wealth of sequence variation in *R* genes has allowed the assignment of putative Avr protein binding domains. Identification of additional genes that are required for the function of individual *R* genes, however, suggests that a simple receptor–ligand model in R-Avr protein recognition is probably too simplistic. Interestingly, many of the signaling mutations, such as *npr1*, *pad4*, and *eds1*, that fully or partially suppress *R* gene-mediated resistance to various pathogens, exhibit enhanced disease susceptibility in compatible *Arabidopsis*–pathogen interactions, revealing common threads between mechanisms invoked during *R-avr* gene-mediated resistance and the less well defined processes that restrict growth of compatible pathogens during disease.

Ultimately, further progress in understanding plant disease resistance will depend on the integration of complementary approaches. As new mutations are characterized and tested for genetic interaction with known mutations,

it is becoming clear that a much more comprehensive set of marker genes is required for pathway dissection. Here, microarray technologies will play a major role in genetic dissection of disease resistance signaling. A large number of the newly described genes defined by mutation in *Arabidopsis* have not yet been cloned but the imminent completion of the *Arabidopsis* genome sequence already allows rapid map-based cloning and will yield a flurry of new disease signaling proteins and targets for biochemical analysis. Understanding the regulatory processes in plant disease resistance will, in turn, provide a fresh perspective on how to combat some of the world's most destructive plant pathogens.

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