Signal transduction in plant immunity

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Significant recent advances in the understanding of plant defense mechanisms include the isolation and characterization of resistance genes against bacterial, fungal and viral pathogens, the identification of genes involved in cell death, and the demonstration of the involvement of reactive oxygen species and salicylic acid in the signal-transduction pathways for expression of induced resistance.

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Abbreviations

HR	hypersensitive response
INA	methyl-2,6-dichloroisonicotinic acid
LRR	leucine-rich repeat
PR	pathogenesis-related
SA	salicylic acid
SABP	SA-binding protein
SAR	systemic acquired resistance
TMV	tobacco mosaic virus

Introduction

Higher plants have developed an immune system that is different from the well-studied antibody system of vertebrates. The plant's 'immune' state, systemic acquired resistance (SAR), can be induced by initial localized infection with pathogens that cause lesions involving host cell death [1]. Once established, resistance is not limited to the specific pathogen used for immunization but extends to a broad range of pathogens, including bacteria, fungi and viruses. Furthermore, SAR persists for several weeks following the initial immunization. This 'immunization' of plants against disease has been applied in agriculture since the beginning of the century [2,3].

Studies using tobacco plants revealed that specific families of genes, often now called SAR genes, were induced systemically after immunization with tobacco mosaic virus (TMV). SAR genes encode pathogenesis-related (PR) proteins, PR-1 (anti-fungal), PR-2 (acidic and basic β-1,3-glucanases), PR-3 (chitinase), PR-4 (anti-fungal), PR-5 (thaumatin-like protein) and PR-8 (acidic and basic class III chitinases) [4,5]. Transgenic plants overexpressing an SAR gene in many, but not all, cases exhibit enhanced protection against at least some pathogens, indicating that these genes may potentially play direct roles in induced resistance [6]. For example, transgenic tobacco constitutively expressing PR-1 is resistant to *Peronospora tabacina* and *Phytophthora parasitica* [7]. Co-expression of a basic chitinase and an acidic glucanase in tobacco enhances protection against *Cercospora nicotianae*, suggesting that induced resistance involves concerted action of multiple protective mechanisms [8•]. In this review, we focus on recent studies of signal transduction mechanisms leading to the induction of disease resistance.

Primary recognition of pathogens

The formation of dry, clearly delimited lesions, involving the rapid collapse of challenged host cells in the hypersensitive response (HR), is thought to contribute to the induction of SAR [2], as well as the limitation of pathogen growth in the expression of localized resistance [9]. The genetic basis of localized disease resistance is described by the 'gene for gene' hypothesis, developed by Flor [10] to account for the inheritance of resistance to flax rust. Incompatibility is specified by the epistatic interactions of sets of paired genes, each pair comprising a resistance (*R*) gene in the host and its corresponding avirulence (*avr*) gene in the pathogen. *R* gene products are thought to be receptors that recognize specific pathogen signal molecules (elicitors or avirulence factors), whereas *avr* genes either directly encode elicitors or encode proteins involved in elicitor production [11].

Several *R* genes have recently been isolated by transposon tagging or positional cloning [12,13••]. The first *R* gene to be cloned was the tomato *Pto* gene, which confers resistance to *Pseudomonas syringae* pv. *tomato* carrying the *avrPto* avirulence gene [14]. *Pto* encodes a serine/threonine protein kinase, suggesting a direct role in signal transduction. *Fen*, a tightly-linked member of the *Pto* family, confers sensitivity to the insecticide fenthion, resulting in cell death in exposed tissues [15•]. Although *Fen* shares 80% identity with *Pto*, and likewise exhibits serine/threonine protein kinase activity, it does not confer resistance to *P. s. tomato* [16,17•,18].

Another tomato *R* gene, *Cf-9*, which confers resistance to the fungus *Cladosporium fulvum* carrying the *avr9* avirulence gene, was cloned by transposon tagging [19••]. *Cf-9* encodes a putative membrane-anchored extracytoplasmic protein with a leucine-rich repeat (LRR) domain. These LRRs are found in many proteins involved in protein–protein interactions and *Cf-9* may encode a receptor for the elicitor peptide, which is a fragment of the *avr9* gene product. Interestingly, the *Cf-9* LRR domain is strikingly similar to an inhibitor of fungal polygalacturonases involved in pathogenesis. Several other *R* genes also encode proteins with variant LRR motifs. The *Arabidopsis Rps2* and *Rpm1* genes, which confer resistance to *P. syringae*, were isolated by map-based

cloning [20•,21•,22••], and the tobacco *N* gene and the flax L^6 gene, which confer resistance to TMV and the fungus *Melampsora lini*, respectively, were cloned by transposon tagging [23•,24•]. *Rpm1* is of interest because it confers resistance to *P. syringae* expressing either *avrRpm1* or *avrB*, the sequences of which are unrelated [22••]. Hence, *Rpm1* may determine dual specificities.

Despite conferring resistance to bacterial, viral, and fungal pathogens, respectively, these four *R* gene products show significant homology, with the *N* and *Rps2* products being strikingly similar. In addition to LRR motifs in their carboxy-terminal halves, these proteins, unlike the *Cf-9* product, contain a putative P-loop nucleotide-binding site in their amino-terminal half. L^6 also contains a putative signal peptide, suggesting a function in signal reception at the cell surface, whereas *N* and *Rps2* are most likely cytoplasmic, as they lack leader peptide sequences. Both L^6 and *N* gene transcripts undergo alternative splicing resulting in full and truncated gene products [24•,25], and it will be of interest to determine the functions of the alternative products.

A further example of an *R* gene product with P-loop and LRR domains is that encoded by the tomato *Prf* gene, which is tightly linked to *Pto* and *Fen*. Mutations in *Prf* result in both susceptibility to *P. s. tomato* and insensitivity to fenthion, indicating that *Prf* functions in both the *Pto* and *Fen* protein kinase pathways [26••]. The functional interaction between *Prf* and *Pto* suggests a binary model for perception of the microbial signal and initiation of a phosphorylation cascade to activate defense responses, and the recently cloned rice *Xa21* gene, which confers resistance to bacterial blight, encodes a protein containing both components, with the putative extracellular LRR ligand-binding and intracellular protein kinase catalytic domains separated by a hydrophobic putative transmembrane domain [27••]. Intriguingly, the *Arabidopsis Rpk5* gene encodes a functional receptor protein kinase in which the putative extracellular domain is highly related to the acidic PR-5 protein [28•]. Although the biological function of *Rpk5* has not yet been determined, the incorporation of a defense-related protein as the putative ligand-binding domain of a receptor, reminiscent of the relationship between *Cf-9* and the polygalacturonase inhibitor protein, suggests a function in microbial perception. It will be of great interest to see whether other *R* genes encode products that fall into the emerging classes within the 'ligand-binding input/protein kinase output' model, and whether co-option of defense protein domains into signal-transduction systems is a strategy commonly used in plants to create coupled recognition–response systems for microbial ligands.

Cell death in disease resistance

Several groups have isolated *Arabidopsis* mutants that give accelerated cell death (*acd*) or spontaneous occurrence of lesions simulating disease resistance response (*lsd*). For example, in the absence of pathogens, the *acd2* mutant spontaneously develops apparently typical HR lesions, in which the transcripts of defense genes such as glutathione *S*-transferase (*GST1*), *PR-1*, *PR-5*, β-1,3-glucanase (*BGL2*), *PAL1* and lipoxygenase accumulate at least 10-fold [29]. HR-like lesions were also elicited by infection with normally virulent *P. syringae* pv. *maculicola* ES4326, which is unable to evoke an HR in wild-type plants. Infection of *acd2* induces *GST1*, *PAL1* and *PR-1* transcripts and the natural product camelexin, which is an *Arabidopsis* phytoalexin, the levels of which correlate with resistance levels similar to those in wild-type plants induced for SAR [30]. Thus, observed lesions in the *acd2* plants induced spontaneously or by virulent pathogens show physiological attributes similar to those induced by avirulent pathogens.

Another mutant, *acd1*, which also develops rapid, spreading lesions in response to virulent *P. s. maculicola*, is unable to control the rate or extent of cell death under a variety of conditions that induce senescence [31]. Likewise, Dietrich *et al.* [32] isolated six *lsd* mutants with spontaneous lesion phenotypes in *Arabidopsis*. Five exhibited characteristics associated with disease-resistance responses, including autofluorescence, callose deposition and induction of *PR-1* gene transcripts, suggesting the involvement of *lsd* genes in the activation of defense mechanisms. The *lsd1* mutant shows a hair-trigger response to bacterial and fungal pathogens, such that once lesion formation is initiated upon infection, it spreads throughout the entire leaf. Furthermore, *lsd1* shows resistance to virulent fungi comparable with that obtained by induction of SAR.

Transgenic tobacco plants expressing a bacterial proton pump gene (*bO*) also form spontaneous lesions [33•]. Various defense mechanisms are activated, including the accumulation of *PR* and *PAL* gene transcripts and the production of autofluorescent material, and the transgenic plants exhibit systemic resistance to viral and bacterial pathogens. Accumulation of DNA 3′-hydroxyl groups during cell death in *bO* plants was noted, but it remains to be determined whether this reflects programmed DNA processing characteristic of apoptotic cell death or general degradation associated with necrosis. Moreover, although the identification of *acd* and *lsd* mutants implies some form of genetic control over plant cell death, the phenotypes of these mutations and the *bO* transgenotes might result from activation of default cell death programs evoked by dysfunction of key cellular processes unrelated to the HR signal pathway, in line with the high frequency with which such mutations are recovered.

Role of salicylic acid in resistance and immunity

Resistance can be induced by chemical activators such as salicylic acid (SA) or methyl-2,6-dichloroisonicotinic acid (INA) [5,34]. In tobacco and cucumber, endogenous SA increases to high levels locally and to some extent systemically upon pathogen immunization [35,36]. Tobacco

plants expressing a bacterial gene, *NahG*, encoding SA hydroxylase, which degrades SA to catechol, are unable to establish SAR, confirming that SA functions as an essential endogenous signal [37]. When a *NahG* genotype scion is grafted on to wild-type rootstocks, as expected neither SAR nor PR gene expression is detected in leaves of the scion following immunization of leaves of the stock [38•]. In the reciprocal grafting experiment, however, plants that have a *NahG* genotype rootstock still develop systemic resistance and *PR* gene induction in the wild-type scion following immunization of leaves below the graft junction, indicating that SA is not required as a mobile signal.

Interestingly, the local lesions in TMV-infected leaves of *NahG* plants are significantly larger than in control plants. *NahG* expressed in *Arabidopsis* also leads to enhanced susceptibility to virulent pathogens and suppression of hypersensitive resistance to normally avirulent pathogens [39••]. These data indicate that SA functions in the local activation of resistance mechanisms, and this function might contribute to the effective expression of SAR following challenge with a normally virulent pathogen.

Role of hydrogen peroxide in disease resistance

An SA-binding protein (SABP) from tobacco has been purified and the corresponding gene cloned [40,41]. Nucleotide sequence analysis reveals that SABP is a catalase. SA, albeit at relatively high concentrations (1 mM), specifically inhibits this catalase *in vitro* and induces a 40% increase in the level of H_2O_2 *in vivo*. Injection of 1 mM H_2O_2 causes the accumulation of PR-1 proteins, a putative marker for SAR, and it has been proposed that SA, by inhibiting SABP, causes an accumulation of H_2O_2 as a signal for SAR. SABP and SA-inhibitible catalase activity are also found in *Arabidopsis*, tomato and cucumber [42], and SABP/catalase antisense transgenic lines are known to constitutively express PR-1 genes, suggesting that decreased catalase activity *in vivo* leads to PR gene induction [43•]. In addition, INA and structural analogs of INA, which are able to induce PR gene expression and SAR, also inhibit catalase activity *in vitro* [44•].

Several recent observations challenge the catalase inhibition model for SA induction of SAR. First, no accumulation of H_2O_2 is detected in tobacco expressing SAR [45•,46]. Second, although H_2O_2 induces PR-1 gene expression in a dose-dependent manner, the level of induction is much weaker than in response to SA or INA. Third, PR-1 gene induction by H_2O_2 is suppressed in *NahG* plants, suggesting that SA acts downstream of H_2O_2 induction. Moreover, injection of 1 M H_2O_2 does not induce enhanced protection against TMV. Bi *et al.* [47•] also showed that the irreversible catalase inhibitor 3-amino-1,2,4-triazole is only a weak inducer of PR-1. In both tobacco and *Arabidopsis*, no significant changes in catalase activity are detected following immunization with *P. s. syringae* [47•,48], and inhibition of catalase activity is not observed in leaf disks preincubated with concentrations of SA that induce PR-1.

Overall, these data question the biological significance of catalase inhibition by SA and the role of H_2O_2 as a signal downstream of SA in the pathway leading to SAR. On the other hand, H_2O_2 treatment induces SA accumulation in tobacco and *Arabidopsis* leaves [45•,48,49], and H_2O_2 stimulates the activity of benzoic acid 2-hydroxylase, which catalyzes the formation of SA [49].

Although H_2O_2 might not be a second messenger in SAR, it seems to play an important role in HR. Hydrogen peroxide from the oxidative burst elicited by microbial elicitors or an avirulent pathogen triggers multiple defense responses in soybean, including oxidative cross-linking of a proline-rich cell-wall protein, leading to toughening of the cell wall, and induction of cellular protectant genes such as glutathione *S*-transferase [50,51,52••]. Moreover, the massive and prolonged oxidative burst induced by avirulent pathogens triggers localized hypersensitive cell death [52••].

Interestingly, physiological concentrations of SA dramatically accelerate and enhance H_2O_2 accumulation in soybean cells in response to avirulent *P. s. glycinea* (K Shirasu, H Nakajima, RA Dixon, C Lamb, unpublished data) leading to marked potentiation of events downstream of the oxidative burst, including glutathione *S*-transferase induction and cell death. Blockage of phenylpropanoid biosynthesis in response to avirulent *P. s. glycinea* by addition of the specific PAL inhibitor AOPP, inhibited avirulence gene-dependent induction of H_2O_2 accumulation and cell death. These responses can be rescued by the addition of SA, although somewhat higher levels are needed than in the absence of AOPP, and the lag is not decreased, suggesting that other phenylpropanoid products may also contribute. The potentiating effects of SA are not mimicked by the catalase inhibitor 3-AT. Moreover, SA also potentiates PAL and chalcone synthase induction by avirulent *P. s. glycinea*, indicating that SA functions at an early point in the signal pathway before the divergence of branch pathways for antimicrobial defense gene induction and activation of the oxidative burst. At higher concentrations, SA alone will induce H_2O_2 production, and this effect, as well as the potentiated response to *P. s. glycinea*, is blocked by the protein kinase inhibitor K252A. Moreover, SA at low concentrations has a dramatic synergistic effect on the stimulation of H_2O_2 production and cell death by the protein phosphatase type 2A inhibitor cantharidin. Thus, SA may potentiate a phosphorylation cascade early in the signal pathway for induction of the HR.

Salicylic acid binding protein mutants

Several *Arabidopsis* mutants that fail to establish acquired resistance have been isolated. *npr1* (non-expressor of PR genes) [53] and *nim1* (non-inducible immunity) [54•] are

both insensitive to strong chemical inducers of SAR, such as INA and SA, in relation to the induction of SAR. Following inoculation with the incompatible fungus *Peronospora parasitica*, the *nim1* mutant still accumulates SA but fails to inhibit growth of the pathogen [54•]. Similarly, *npr1* mutants fail to express PR genes locally and instead form less confined lesions upon infection with pathogens [53]. The other type of SAR mutant isolated is a constitutive expressor of SAR, and is associated with elevated levels of SA and resistance to normally virulent pathogens [55•].

Conclusions

The past few years have witnessed the emergence of an understanding of the general circuitry underlying induced resistance, and several key players have been identified. The task ahead of us is to build on these advances to bring the circuitry into focus at the molecular level. We need to define how *R* gene products function in the perception of microbial avirulence signals, the molecular interactions underlying transduction of these signals for local activation of the oxidative burst and defense gene transcription, the molecular mechanisms of cell death in the HR, and how systemic signals are generated and, in turn, perceived.

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