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.

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. syringae [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 Rp2 and Rp1 genes, which confer resistance to P. syringae, were isolated by map-based

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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 (thiamatin-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.

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cloning [20*,21*,22**], and the tobacco N gene and the flax
L6 gene, which confer resistance to TMV and the fungus
Mellampsora lini, respectively, were cloned by transposon
tagging [23*,24*]. Rpml is of interest because it confers
resistance to P. syringae expressing either avrRpm1 or avrB,
the sequences of which are unrelated [22**]. Hence, Rpml
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. L6 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
L6 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 gluthionine
S-transferase (GST1), PR-1, PR-5, β-1,3-glucanase (BGL2),
PAL1 and lipoxigenase 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 H₂O₂ in *tis*. Injection of 1 mM H₂O₂ 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 H₂O₂ as a signal for SAR. SABP and SA-inhibitable 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 H₂O₂ is detected in tobacco expressing SAR [45*–46]. Second, although H₂O₂ 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 H₂O₂ is suppressed in *NahG* plants, suggesting that SA acts downstream of H₂O₂ induction. Moreover, injection of 1 M H₂O₂ 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. 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 H₂O₂ as a signal downstream of SA in the pathway leading to SAR. On the other hand, H₂O₂ treatment induces SA accumulation in tobacco and *Arabidopsis* leaves [45*–48,49], and H₂O₂ stimulates the activity of benzoic acid 2-hydroxylase, which catalyzes the formation of SA [49].

Although H₂O₂ 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 H₂O₂ 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 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 H₂O₂ 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 H₂O₂ 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 H₂O₂ 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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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


Rice basic chitinase and alpha acidic glucanase are co-expressed at high levels in transgenic tobacco. The combination of the two transgenes substantially enhances protection against a fungal pathogen, *C. nicotianae*.


An excellent review of disease-resistance genes, which summarizes data from many of the following references.


Although *Fen* encodes a protein kinase closely related to that encoded by the linked Pto resistance gene, and can cause cell death when stimulated by the insecticide fenthion, *Fen* does not duplicate the Pto function in disease resistance. This has important implications for signal specificity, and raises the question of whether *Fen* is an orphan resistance gene for which the corresponding avr gene has not yet been identified.


A novel transient gene expression system using potato virus X is developed to study the functions of the Pto and *Fen* gene products.


The tomato *Cf-9* gene is tagged by a maize transposable element. A transgenic tomato expressing *avr9*, but not *Cf-9*, is crossed with a line containing *avr9* and a *Da* element. Only mutants carrying *De* inactivated *Cf*-9 survive as the interaction of *avr9* and *Cf-9* gene products results in widespread HR. This is of interest in relation to the engineering genetically acquired resistance. First fungal *R* gene cloned, LRR but no P-loop in the product.


Along with [20*] describes an R gene encoding a product with LRR and P-loop regions.


This paper describes an R gene, which encodes a protein with LRR and P-loop domains, and which interacts with two distinct avr genes, suggesting dual specificity.


Describes the first R gene to a viral pathogen to be cloned, and which contains both LRR and P-loop domains.

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