REVIEW

Signal perception and transduction in plant defense responses

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Plant diseases have major effects on agricultural production and the food supply. Currently, worldwide crop losses due to disease are estimated to exceed $100 billion (Brears and Ryals 1994). Although application of fungicides and pestcides has helped control plant diseases, chemical control is economically costly as well as environmentally undesirable. Therefore, the development of new strategies based on a plant's own defense mechanisms for disease control is critical for sustaining agricultural production and improving our environment and health.

Plant disease resistance and susceptibility are governed by the combined genotypes of host and pathogen and depend on a complex exchange of signals and responses occurring under given environmental conditions. During the long process of host–pathogen coevolution, plants have developed various elaborate mechanisms to ward off pathogen attack. Whereas some of these defense mechanisms are preformed and provide physical and chemical barriers to hinder pathogen infection, others are induced only after pathogen attack. Similar to animal immune responses, induced plant defense responses involve a network of signal transduction and the rapid activation of gene expression following pathogen infection. Recent studies have revealed intriguing parallels between animal and plant defense responses as demonstrated by the structural and functional conservation of some of their signal transduction processes. For example, several plant disease resistance (*R*) genes, which confer recognition and resistance to specific plant pathogens, were shown to encode an amino-terminal domain homologous to the cytoplasmic signaling domain of the mammalian interleukin-1 receptor and *Drosophila* Toll protein (Whitham et al. 1994; Lawrence et al. 1995; Anderson et al. 1997). The interleukin-1 receptor and Toll mediate cytokine-induced activation of NF-κB or Spätzle-induced activation of Dif/Dorsal, respectively, thereby leading to antimicrobial defense responses. In *Drosophila,* the genes *spätzle, Toll, tube, pelle,* and *cactus* are all required for the activation of a defense response that involves, in part, the induction of an antifungal peptide called drosomycin (Lemaitre et al. 1996). Interestingly, plant proteins that share homology with Pelle (e.g., tomato Pto), Cactus (e.g., *Arabidopsis* NPR1/NIM1), and drosomycin (e.g., the radish defensin Rs-AFP1) are also important for disease resistance (Martin et al. 1993; Fehlbaum et al. 1994; Terras et al. 1995; Cao et al. 1997; Ryals et al. 1997). Furthermore, signaling components such as G proteins, NADPH oxidase, H_2O_2, salicylic acid (SA, and aspirin), mitogen-activated protein kinases (MAPK), and Myb transcription factors have been shown to be associated with or participate in both animal and plant defense responses, suggesting the presence of conserved signaling pathways for host defenses in diverse higher eukaryotes.

A key difference between resistant and susceptible plants is the timely recognition of the invading pathogen and the rapid and effective activation of host defense mechanisms. A resistant plant is capable of rapidly deploying a wide variety of defense responses that prevent pathogen colonization. In contrast, a susceptible plant exhibits much weaker and slower responses that fail to restrict pathogen growth and/or spread. As a result, a susceptible plant is often severely damaged or even killed by pathogen infection. The activation of defense responses in plants is initiated by host recognition of pathogen-encoded molecules called elicitors (e.g., microbial proteins, small peptides, and oligosaccharides, etc). The interaction of pathogen elicitors with host receptors (many of which may be encoded by *R* genes) likely activates a signal transduction cascade that may involve protein phosphorylation, ion fluxes, reactive oxygen species (ROS), and other signaling events (see Fig. 3, below). Subsequent transcriptional and/or post-translational activation of transcription factors eventually leads to the induction of plant defense genes (Zhu et al. 1996). In addition to eliciting primary defense responses, pathogen signals may be amplified through the generation of secondary plant signal molecules such as SA (Durner et al. 1997). Both primary pathogen elicitors and secondary endogenous signals may activate a diverse array of plant protectant and defense genes, whose products include glutathione

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S-transferases (GST), peroxidases, cell wall proteins, proteinase inhibitors, hydrolytic enzymes (e.g., chitinases and β-1,3-glucanases), pathogenesis-related (PR) proteins, and phytoalexin biosynthetic enzymes, such as phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS, Hammond-Kosack and Jones 1996). The PR proteins are host-encoded, abundant proteins induced by pathogens and many of them have antimicrobial activity in vitro or when overexpressed in transgenic plants. Phytoalexins are low-molecular-weight, antimicrobial compounds (e.g., phenylpropanoids, terpenoids, etc), whose synthesis is induced following pathogen infection.

At the macroscopic level, induced defense responses are frequently manifested in part as a hypersensitive response (HR), which is characterized by necrotic lesions resulting from localized host cell death at the site of infection (Goodman and Novacky 1994; Fig. 1). Plant cell death occurring during the HR resembles animal programmed cell death (pcd) and may play a role in preventing the growth and spread of the pathogen into healthy tissues (Dangl et al. 1996; Greenberg 1996). In addition to the localized HR, many plants respond to pathogen infection by activating defense responses in uninfected parts of the plant. As a result, the entire plant becomes more resistant to subsequent infections. This systemic acquired resistance (SAR) is long-lasting and often confers broad-based resistance to a variety of different pathogens (Ryals et al. 1996; Delaney 1997).

Over the last few years, significant progress has been made in understanding the signaling processes involved in plant–pathogen interactions. This review will focus on recent developments in the signal recognition, perception, and transduction mechanisms underlying induced plant defense responses.

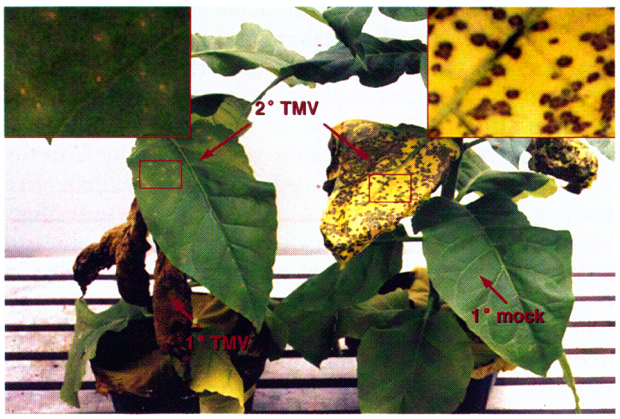

Figure 1. Hypersensitive response and systemic acquired resistance. TMV infection of tobacco plants that carry the *N* resistance gene leads to the hypersensitive response (HR) and subsequent development of systemic acquired resistance (SAR) throughout the plant. In contrast to the mock-infected plant on the right, the plant on the left develops SAR after the primary infection, as shown by the decreased lesion size and number (see *insets*) produced after the secondary infection of upper leaves.

Host recognition of pathogen elicitors

The gene-for-gene specificity

When a plant is infected by a pathogen, the plant must recognize the pathogen and then initiate defense responses to limit the potential damage that can be caused by the pathogen. Host recognition of the invading pathogen often is determined by the so-called "gene-for-gene" interaction between a dominant avirulence (*avr*) gene in the pathogen and a corresponding dominant *R* gene in the host (Flor 1971). For example, the *Cf9* resistance gene in tomato mediates specific resistance only towards the races of the fungal pathogen *Cladosporium fulvum* that carries the *avr9* gene. Therefore, activation of defense responses requires the expression of a matching pair of plant *R* genes and pathogen *avr* genes.

The primary biological function of *avr* genes is believed not to trigger plant defense responses, which could negatively affect pathogen's own survival. Rather, recent studies have shown that some *avr* genes may be important for pathogen fitness and/or pathogenicity (Kearny and Staskawicz 1990; Dangl 1994; Yang et al. 1996). Many *avr* genes have likely originated from virulence genes that contribute to the ability of the pathogen to grow on the host, but which have been subsequently recognized by evolving *R* genes in resistant host plants. Dozens of *avr* genes have been isolated and characterized from bacterial and fungal pathogens. However, biochemical function of their products remains unknown (Leach and White 1996). One exception is the *avrD* gene of *Pseudomonas syringae* pathovar (pv.) *tomato*, which appears to encode an enzyme involved in the production of glycolipid elicitors (Midland et al. 1993).

Plant R *genes*

It has long been postulated that plant *R* genes encode receptors for the recognition of specific elicitors or ligands encoded directly or indirectly by pathogen *avr* genes (Gabriel and Rolfe 1990). During the past five years, at least a dozen *R* genes have been isolated by map-based cloning or transposon tagging from various plant species (Bent 1996; Jones 1996; Baker et al. 1997). Although these *R* genes confer specific resistance to different viral, bacterial, fungal, or nematode pathogens, interestingly, most of them encode one or more common structural motifs. Except for *Pto* from tomato, all cloned *R* genes that confer gene-for-gene specificities contain leucine-rich-repeats (LRRs). LRRs have been implicated in protein–protein interactions in animals (Kobe and Deisenhofer 1995) and may also be responsible for recognitional specificity in gene-for-gene interactions (Bent 1996; Jones 1996). Other common motifs include serine/threonine kinase domains (e.g., *Pto* of tomato and *Xa21* of rice; Martin et al. 1993; Song et al. 1995), nucleotide binding sites (e.g., *RPS2* and *RPM1* of *Arabidopsis*, *N* of tobacco, *I2C* of tomato, *L6* and *M* of flax; Bent et al. 1994; Mindrinos et al. 1994; Whitham et al. 1994; Grant et al. 1995; Lawrence et al. 1995; Anderson et al. 1997; Ori et al. 1997), leucine zippers (e.g., *RPS2* and *RPM1* of *Ara-*

bidopsis) and Toll/interleukin-1 receptor-like domains (e.g., *N* of tobacco, *L6* and *M* of flax). Such structural conservation within *R* genes from a wide range of plant taxa suggests the presence of a common molecular mechanism underlying many gene-for-gene interactions. Based on sequence homologies within conserved motifs, several putative resistance genes have been isolated from soybean, potato, and wheat (Kanazin et al. 1996; Leister et al. 1996; Yu et al. 1996; Feuillet et al. 1997).

The predominance and diverse specificities of LRR-containing *R* genes may be explained partly by the presence of their internal repetitive sequences, which are potentially active sites for genomic recombination. Furthermore, plant *R* genes are flanked frequently by multiple homologous alleles (Lawrence et al. 1995; Dixon et al. 1996; Jia et al. 1997). Therefore, the LRR-containing *R* genes may undergo active intragenic and intergenic recombination that would contribute signficantly to the evolution of new specificities. Recent cloning and analyses of the flax *M* gene, the tomato *I2C* gene, and their mutant alleles suggest that genomic recombination within the LRR-encoding region may be important for the generation of structurally altered *R* genes with, possibly, new resistance specificities (Anderson et al. 1997; Ori et al. 1997).

Interestingly, repetitive sequences are also found in some of the *avr* genes from plant pathogens. Members of a *Xanthomonas* avirulence/pathogenicity (*avr/pth*) gene family, which consists of >26 genes and is the largest *avr* gene family known to date (Gabriel et al. 1996), encode nearly identical, leucine-rich, 34-amino-acid tandem repeats that determine avirulence and pathogenic specificities (Herbers et al. 1992; Yang et al. 1994). Both intragenic and intergenic recombination have been demonstrated to be involved in the rapid generation of new specificities in this *avr* gene family (De Feyter et al. 1993; Yang and Gabriel 1995b). Therefore, genomic recombination among homologous sequences appears to play an important role during the reciprocal evolution of gene-for-gene interactions.

Interaction between R *and* avr *gene products*

Based on sequence analyses, it has been predicted that some of the plant *R* gene products are localized extracellularly (Fig. 2). For example, the tomato *Cf2* and *Cf9* genes confer resistance to specific races of the fungal pathogen *C. fulvum* and encode glycoproteins with putative extracellular LRRs, a transmembrane region, and a small cytoplasmic domain (Jones et al. 1994; Dixon et al. 1996). As Avr4 and Avr9 elicitors, which are small cysteine-rich proteins (<15 kD), are secreted from *C. fulvum* into the intercellular fluid of infected plant tissues (De Wit 1995), they could interact readily with the extracellular LRR domains of Cf proteins. However, a direct interaction between Avr and Cf proteins has yet to be demonstrated. The rice *Xa21* gene also encodes a protein with an extracellular LRR motif as well as an intracellular serine/threonine protein kinase domain (Song et al. 1995). Thus, it is possible that the AvrXa21 elicitor directly interacts with the Xa21 receptor-like kinase. Unfortunately, the identity of this Avr elicitor is unknown and the *avrXa21* gene has yet to be isolated from the bacterial pathogen *Xanthomonas oryzae*.

Unlike Xa21 and Cf proteins, the products of other cloned plant *R* genes do not contain transmembrane domains and appear to be localized intracellularly. An intracellular location poses no problem for *R* gene products to interact with the elicitors of viral pathogens, which multiply inside plant cells. But how intracellular receptors interact with specific elicitors from extracellular pathogens was initially perplexing. Low-molecular-weight elicitors like glycolipids may be taken into host plant cells and interact with intracellular receptors; however, no Avr protein has been found to be secreted by bacterial plant pathogens.

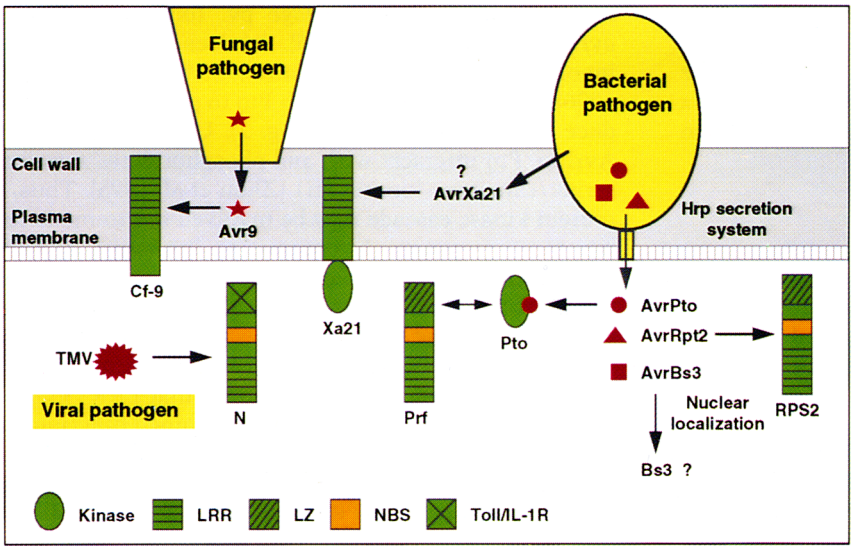

Figure 2. Molecular recognition in gene-for-gene interactions. Race-specific elicitors encoded by fungal, bacterial, and viral avirulence genes are specifically recognized by extracellular or intracellular receptors encoded by corresponding plant resistance genes. As in the case of AvrPto–Pto interaction, a direct interaction between *Avr* and *R* products likely determines the gene-for-gene specificity and initiates plant defense responses in many plant–pathogen interactions. Question marks indicate unknown elicitors or receptors. LRR, leucine-rich repeats; LZ, leucine zipper; NBS, nucleotide-binding site; Toll/IL-1R, Toll- and interleukin-1 receptor-like domain.

The mechanism by which putative protein elicitors encoded by *avr* genes interact with intracellular *R* gene products was elucidated by the discovery of the type III protein secretion pathway in bacterial pathogens. In animal pathogenic bacteria, such as *Yersinia, Shigella,* and *Salmonella,* the type III secretion pathway is used to transfer virulence proteins directly into animal host cells (Stephens and Shapiro 1996). The *Hrp* (hypersensitive response and pathogenicity) gene cluster found in phytopathogenic bacteria also encodes components of the type III secretion pathway. Expression of *Hrp* genes is required for bacterial *avr* gene products to elicit an HR on resistant plants carrying the corresponding *R* genes and for pathogenicity of the pathogen on susceptible plants (Alfano and Collmer 1996). Some of the *Hrp* gene products appear to be involved in the formation of a pilus-like bridge between bacteria and plant cells (Roine et al. 1997), thereby providing an avenue for delivering Avr protein elicitors (or virulence factors) into host cells, where they can interact with intracellular receptors.

Recent studies have shown that several bacterial Avr proteins are indeed recognized inside host cells. Introduction of the *P. syringae avrB* or *avrRpt2* gene into *Arabidopsis* by transient or stable transformation elicits an HR specifically in the plants carrying the corresponding *RPM1* and *RPS2* resistance genes (Gopalan et al. 1996; Leister et al. 1996). Scofield et al. (1996) and Tang et al. (1996) reported a similar effect when *avrPto* of *P. syringae* pv. *tomato* is expressed in tomato plants carrying the *Pto* resistance gene. Using the yeast two-hybrid system, they further demonstrated a direct physical interaction between AvrPto and Pto. Mutations in AvrPto or Pto that disrupted their interaction in yeast also abolished the resistance response in plants. Thus, the direct interaction of AvrPto with Pto determines the gene-for-gene specificity and initiates plant defense responses. In addition to these *Pseudomonas* Avr proteins, AvrBs3 from *Xanthomonas campestris* pv. *vesicatoria* is also recognized inside plant cells following delivery via the *Hrp*-encoded type III secretion system. The *avrBs3* gene belongs to the family of *Xanthomonas avr/pth* genes that contain functional nuclear localization signals (Yang and Gabriel 1995a). Recently, AvrBs3 was shown to require its nuclear localization signals to elicit the resistance response, suggesting the involvement of a nuclear factor(s) in AvrBs3 perception (van den Ackerveken et al. 1996).

Plant receptors for nonspecific elicitors

In addition to specific resistance determined by the gene-for-gene interaction, plant defense responses can be activated without a matching pair of *avr* and *R* genes. Many fungal and bacterial oligosaccharides, proteins, and glycoproteins can function as nonspecific elicitors to induce defense responses in the plants carrying no specific *R* genes (Benhamou 1996; Hahn 1996). Host recognition of general fungal elicitors is likely mediated by high affinity receptors present in plasma membranes. For instance, a 91-kD parsley plasma membrane protein has been identified as a receptor for the fungal protein elicitor from *Phytophthora megasperma* (Nürnberger et al. 1995). Recently, a 70-kD soybean β-glucan elicitor-binding protein has been purified from the membrane fraction of soybean root cells, and its cDNA isolated (Umemoto et al. 1997). Expression of the cDNA in tobacco suspension cultures and in *Escherichia coli* conferred β-glucan elicitor-binding activity. Furthermore, an antibody against the recombinant protein was shown to inhibit binding of β-glucan elicitors to soybean membranes and to block the induction of phytoalexins, suggesting that this protein functions as a receptor to mediate the action of the fungal elicitor.

A central role for protein phosphorylation in intracellular signal transduction

Protein kinases and phosphatases play a pivotal role in the signal transduction processes leading to cell growth, differentiation, and responses to environmental stimuli. Mounting evidence indicates that phosphorylation and dephosphorylation are also essential to early as well as later events along the signaling pathways leading to plant defense responses. Fungal elicitors trigger rapid and transient protein phosphorylation in parsley and tomato cell suspension cultures (Dietrich et al. 1990; Felix et al. 1991). Protein kinase inhibitors such as K-252a and staurosporine block these elicitor-induced changes in protein phosphorylation and prevent the induction of plant defense responses. In contrast, protein phosphatase inhibitors, such as calyculin A, cantharadin, and okadaic acid, mimic elicitor action to activate defense responses (Felix et al. 1994; Levine et al. 1994; Mackintosh et al. 1994).

The discovery that the tomato *Pto* and rice *Xa21* resistance genes encode serine/threonine protein kinases further suggests a central role for protein phosphorylation in signal perception and transduction in disease resistance (Martin et al. 1993; Song et al. 1995). The intracellular Pto kinase requires an LRR-containing cytoplasmic protein, encoded by the closely linked *Prf* gene, for resistance against *P. syrinage* pv. *tomato* expressing *avrPto* (Salmeron et al. 1996). Prf does not appear to be involved in the specific recognition of AvrPto (Tang et al. 1996), but it may interact with Pto to perceive and transduce the avirulence signal (Fig. 2). Upon activation by AvrPto, Pto interacts with and phosphorylates a second serine/threonine kinase, Pti1 (Zhou et al. 1995). Thus, a protein kinase cascade may be involved in *Pto*-mediated resistance responses. In contrast to *Pto,* most *R* genes encode LRR motifs, but not a kinase domain. These *R* genes may interact with a Pto-like kinase for signal transduction. The rice *Xa21* resistance gene encodes a receptor-like kinase that contains an intracellular kinase domain as well as a putative extracellular LRR (Song et al. 1995); thus it may possess the combined functions of both Pto and Prf. In addition to Xa21, a few other receptor-like protein kinases have been implicated in plant disease resistance. The PR5K receptor-like kinase of *Arabidopsis* contains an extracellular domain homologous

to the antimicrobial PR-5 protein, suggesting its possible involvement in the perception of pathogen signals (Wang et al. 1996). Recently, a receptor-like kinase (SFR2) belonging to the *S* gene family, which mediates self-incompatibility in *Brassica oleracea*, was shown to be rapidly induced by wounding and bacterial infection (Pastuglia et al. 1997). Therefore, it may also play a role in signaling plant defense responses.

Protein phosphorylation/dephosphorylation is not only involved in early signal perception but also in downstream events leading to the activation of plant defense genes (Fig. 3). For example, elicitor-induced phosphorylation of the nuclear factor PBF-1 is required to increase its binding activity and thereby activate the potato *PR-10a* gene (Després et al. 1995). A functional homolog of mammalian protein kinase C has recently been shown to be involved in this phosphorylation process (Subramamiam et al. 1997). Similarly, phosphorylation of G/HBF-1, a soybean bZIP transcription factor, by a bacterial pathogen-induced serine/threonine kinase enhances its binding activity to the chalcone synthase *chs15* promoter (Dröge-Laser et al. 1997). Some of these transcription factors may be regulated by the MAPK cascade, which has been implicated in plant stress signal transduction (Jonak et al. 1996; Mizoguchi et al. 1996). Treatment of tobacco cells with fungal elicitors was shown to transiently activate a 47-kD putative MAPK via tyrosine phosphorylation (Suzuki and Shinshi 1995). Activation of this 47-kD kinase was inhibited by staurosporine and the Ca^{2+} channel blocker Gd^{2+} (gadolinium, a lanthanide), suggesting that upstream kinases and Ca^{2+} might be involved in the activation of this kinase. In addition, a wound-induced MAPK (WIPK) gene was isolated from tobacco (Seo et al. 1995). Transgenic plants with depressed WIPK activity exhibited increased SA levels and induction of the acidic *PR-1* and *PR-2* genes upon wounding. Because wounding wild-type tobacco does not induce SA production or *PR* gene expression, these results suggest that protein phosphorylation mediates cross-talk between wound- and pathogen-induced signaling pathways.

Protein kinases and phosphatases are also involved in the development of HR, SAR, and the SA-mediated induction of *PR-1* gene expression in tobacco plants. Okadaic acid was shown to inhibit the TMV-induced HR (Dunigan and Madlener 1995) and to block SA-induced *PR-1* gene expression (Conrath et al. 1997). Phosphorylation of both a soluble and a plasma membrane-associated protein was induced during the development of SAR (Ye et al. 1995). Recent studies have demonstrated that SA induces a rapid and transient activation of a 48-kD protein kinase in tobacco (Zhang and Klessig 1997). Based on sequences obtained from the purified kinase and its encoding cDNA, this SA-induced protein (SIP) kinase was shown to be a distinct member of a MAPK family. It will be interesting to see whether the SIP kinase phosphorylates any transcription factors involved in the induction of known defense genes.

Ion fluxes, oxidative burst, and other early signaling events

In addition to protein phosphorylation, early signaling events in plant defense responses may involve ion channels, ROS, GTP-binding proteins, phospholipases and/or other signaling components (Fig. 3). Various fungal and bacterial elicitors have been reported to trigger fluxes of H^+, K^+, Cl^-, and Ca^{2+} across the plasma membrane (Atkinson et al. 1990, 1996; Mathieu et al. 1991; Bach et al. 1993; Kuchitsu et al. 1993; Popham et al. 1995). In parsley suspension cells, a transient influx of Ca^{2+} and H^+ and an efflux of K^+ and Cl^- are initiated within two to five minutes after the addition of a fungal oligopeptide elicitor (Hahlbrock et al. 1995). Ca^{2+} channel blockers were shown to inhibit ion fluxes as well as defense responses induced by fungal and bacterial elicitors (Nürn-

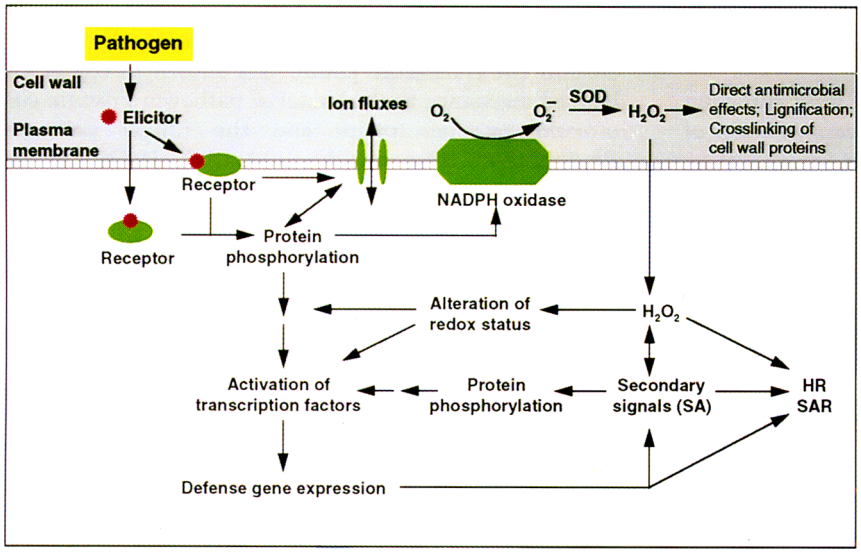

Figure 3. A simplified model for signal transduction in plant defense responses. Host recognition of pathogen elicitors initiates early signaling events such as protein phosphorylation/dephosphorylation, ion fluxes and oxidative burst. Subsequent transcriptional and/or posttranslational activation of transcription factors leads to induction of plant defense genes such as *GST* and *PAL*, and biosynthesis of endogenous secondary signals such as SA. In addition, the activated NADPH oxidase complex generates reactive oxygen species (ROS) such as $O_2\cdot^-$ and H_2O_2 that alter the redox status of plant cells and affect defense signaling. SA, ROS, as well as defense genes, all contribute to the development of HR and SAR during plant–pathogen interactions. SOD, superoxide dismutase.

berger et al. 1994; Atkinson et al. 1996). Conversely, Ca^{2+} ionophores or increases in extracellular Ca^{2+} level activated defense gene expression in tobacco (Suzuki et al. 1995), suggesting that Ca^{2+} plays an important role in signaling defense responses. In soybean cells, anion channel blockers have been shown to effectively inhibit the fungal elicitor-induced Ca^{2+}/H^+ influxes and the expression of defense genes (Ebel et al. 1995).

Elicitor-induced ion fluxes are likely mediated by protein phosphorylation and dephosphorylation events. Protein kinase inhibitors, such as K252a, can block ion fluxes and medium alkalinization induced by fungal and bacterial elicitors (Felix et al. 1991; Popham et al. 1995). By contrast, treating tomato cells carrying the *Cf5* resistance gene with the fungal Avr5 elicitor induced a phosphatase-catalyzed dephosphorylation of the plasma membrane H^+–ATPase (Vera-Estrella et al. 1994a). This led to a significant increase in H^+–ATPase activity and a subsequent acidification of the extracellular medium. Decreases in H^+–ATPase activity by rephosphorylation were shown to be mediated by a Ca^{2+}/calmodulin-dependent kinase that in turn is activated by a Ca^{2+}-dependent protein kinase C-like kinase (Xing et al. 1996). Thus, reversible phosphorylation appears to provide a mechanism to prevent prolonged stimulation of the H^+–ATPase, which could otherwise result in cell death. Interestingly, transgenic tobacco plants expressing a bacterial proton pump exhibited programmed cell death and induction of defense genes, consistent with the involvement of H^+ flux in signaling defense responses (Mittler et al. 1995). Furthermore, several fungal toxins have been known to target the plasma membrane H^+–ATPase. Fusicoccin, the major phytotoxin from the peach and almond pathogen *Fusicoccum amygdali*, stimulates H^+–ATPase activity through modulation of its receptor, a member of the 14-3-3 superfamily of eukaryotic proteins (De Boer 1997). The fungal toxin NIP1 from the barley pathogen *Rhynchosporium secalis*, which functions pleiotropically as a specific Avr elicitor on host plants carrying the *Rrs1* resistance gene (Rohe et al. 1995), also activates the plasma membrane H^+–ATPase (Wevelsiep et al. 1993). Therefore, perturbation of H^+ flux appears to be a common mode of action by virulent toxins and avirulent elicitors.

Another striking event occurring early after pathogen infection is the rapid production and accumulation of ROS, such as $O_2^{\cdot -}$ and H_2O_2, known as the oxidative burst (Doke et al. 1996; Low and Merida 1996; Tenhaken et al. 1995). Elicitation of the oxidative burst by pathogen elicitors appears to be mediated by multiple signaling cascades and may be associated with the activation of G proteins, Ca^{2+} influx, H^+/K^+ exchange, induction of phospholipases, and protein phosphorylation (Low and Merida 1996). The generation of ROS is likely dependent on the activation of a plasma membrane NADPH oxidase similar to that present in mammalian phagocytes. Specific inhibitors of the mammalian NADPH oxidase (e.g., diphenylene iodonium) prevent plant ROS induction by pathogen elicitors. Antibodies to various components of the mammalian NADPH oxidase cross-react with plant proteins of similar molecular mass (Dwyer et al. 1995; Tenhaken et al. 1995; Desikan et al. 1996). Moreover, a rice gene highly homologous to *gp91phox*, the main catalytic subunit of mammalian NADPH oxidase, has been isolated (Groom et al. 1996). Recently, specific Avr elicitors of *C. fulvum* were shown to increase NADPH oxidase activity by promoting the translocation of cytosolic components of the oxidase to the plasma membrane of tomato cells (Xing et al. 1997). This assembly process involves a Ca^{2+}-dependent protein kinase that phosphorylates cytosolic components of the oxidase and facilitates their translocation to the plasma membrane. In addition, H_2O_2 may also be produced by oxalate oxidases (Zhang et al. 1995b) and cell wall peroxidases (Bolwell et al. 1995) during plant defense responses.

Two distinct phases of ROS production have been observed during plant–pathogen interactions. The first burst occurs within minutes in both susceptible and resistant interactions, whereas the second, sustained burst occurs within a few hours of infection and only in a resistant interaction (Levine et al. 1994; Baker and Orlandi 1995). For example, Chandra et al. (1996) have shown recently that the second burst only occurs when tomato cells carrying the *Pto* resistance gene are challenged with *P. syringae* pv. *tomato* carrying *avrPto*. Either no burst or only a first burst is observed in the remaining three combinations of susceptible interactions, indicating that the second burst is correlated with race-specific resistance. However, the Pto kinase is not required for the oxidative burst initiated either by the nonhost pathogen *P. syringae* pv. *tabaci* (for which tomato is not a host for the growth of any races or pathovars of this pathogen) or by nonspecific elicitors such as oligogalacturonides. These results suggest the presence of multiple signaling cascades that activate the plasma membrane NADPH oxidase.

Superoxide anion and H_2O_2 generated during the oxidative burst play multiple roles in plant defense responses. During an HR, a highly localized accumulation of H_2O_2 was found in the lettuce cell walls adjacent to invading bacteria (Bestwick et al. 1997). In addition, constitutive expression of an H_2O_2-generating glucose oxidase in the transgenic potato was shown to confer enhanced resistance to the bacterial pathogen *Erwinia carotovora* pv. *carotovora* and the fungal pathogen *Phytophthora infestans* (Wu et al. 1995). H_2O_2 was also demonstrated to have direct antimicrobial activity (Peng and Kuc 1992) and to contribute to cell wall reinforcement by stimulating lignification and crosslinking of cell wall hydroxyproline-rich glycoproteins (Bradley et al. 1992; Brisson et al. 1994). Furthermore, superoxide anion and H_2O_2 may act as a secondary messenger to induce plant defense-related genes (Levine et al. 1994; Green and Fluhr 1995) and hypersensitive host cell death (Doke 1983a,b; Doke and Ohashi 1988; Levine et al. 1994; Dangl et al. 1996). Most recently, elicitor-stimulated superoxide anion from the oxidative burst was shown to be essential in triggering defense gene activation and phytoalexin synthesis in parsley (Jabs et al.

1997). In mammals, transcription factors such as NF-κB and AP-1 are known to be redox regulated (Sen and Packer 1996). It is possible that ROS may activate defense gene expression by altering the redox status of plant cells, thereby modulating the activity of redox-sensitive transcription factors. In addition, H_2O_2 was shown to increase SA biosynthesis in tobacco by stimulating benzoic acid-2 hydroxylase activity (Léon et al. 1995). The action of ROS may also be mediated through changes in cytosolic Ca^{2+} levels and the generation of lipid peroxides (Price et al. 1994; León et al. 1995). Thus, although their mechanism(s) of action is poorly understood, ROS undoubtly play an important role in signaling plant defense responses.

GTP-binding proteins have also been suggested to participate in the induction of ion fluxes and the oxidative burst by some fungal elicitors (Legendre et al. 1992; Vera-Estrella et al. 1994a,b; Xing et al. 1997). Further suggestion that GTP-binding proteins are associated with defense signaling has come from studies of tobacco transformed with the cholera toxin A1 subunit (Beffa et al. 1995). The A1 subunit irreversibly blocks the GTPase activity of G proteins, resulting in the sustained activation of the downstream signaling pathway. Transgenic plants expressing cholera toxin contained high levels of SA, expressed constitutively *PR* genes, and exhibited enhanced resistance to a bacterial pathogen. Similarly, when a Ras-related, small GTP-binding protein was overexpressed in tobacco, wounding induced abnormal accumulation of SA, expression of acidic PR proteins, and enhanced resistance to TMV (Sano et al. 1994).

Endogenous secondary signals in plant disease resistance

Salicylic acid

Following the early signaling events activated by pathogen attack, the elicitor signals are often amplified through the generation of secondary signal molecules such as SA, ethylene, and jasmonates. A large body of evidence indicates that SA plays a critical role in the activation of defense responses. Increases in the levels of SA and its conjugates have been associated with the activation of resistance responses in a wide variety of plant species. These increases slightly precede or parallel the expression of *PR* genes in both the infected tissue as well as the uninfected tissues exhibiting SAR (Malamy et al. 1990; Métraux et al. 1990; Rasmussen et al. 1991; Uknes et al. 1993; Summermatter et al. 1995; Dempsey et al. 1997). In addition, exogenous application of SA to tobacco and *Arabidopsis* induces the same set of PR genes as those activated during SAR development (Ward et al. 1991; Uknes et al. 1992, 1993).

Several studies have also demonstrated that when SA accumulation is prevented, resistance is compromised. Transgenic tobacco and *Arabidopsis* plants unable to accumulate SA because of the expression of the *Pseudomonas putida nahG* gene, which encodes salicylate hydroxylase, exhibit poor induction of *PR* genes after pathogen infection and fail to develop SAR (Gaffney et al. 1993; Delaney et al. 1994). In addition, these plants are more susceptible to primary infection by normally avirulent pathogens. This NahG phenotype appears to be caused specifically by the reduction in SA levels, since resistance could be restored by treatment with 2,6-dichloroisonicotinic acid (INA) or benzothiadiazole (BTH), two functional analogs of SA that are not substrates for salicylate hydroxylase (Delaney et al. 1994; Vernooij et al. 1995; Freidrich et al. 1996; Lawton et al. 1996). It also was demonstrated recently that inhibition of PAL, the first enzyme in the SA biosynthetic pathway, caused otherwise resistant *Arabidopsis* plants to become susceptible to avirulent strains of the fungal pathogen *Peronospora parasitica*. Resistance in these PAL-inhibited plants could be restored by the application of SA or INA (Mauch-Mani and Slusarenko 1996).

SA appears to be crucial for the activation of some of the defense responses, however it is currently unclear whether it is the long-distance signal that activates SAR in the uninoculated leaves. Grafting experiments have suggested that SA is not the mobile signal. Although NahG tobacco rootstocks fail to accumulate SA, they are able to generate a long-distance signal that activates SAR in the wild-type scion (grafted) leaves (Vernooij et al. 1994). In contrast, the demonstration that nearly 70% of the SA detected in the upper, uninoculated leaves of TMV-infected tobacco is transported from the inoculated leaf suggests that SA could be the mobile signal (Shulaev et al. 1995). Interestingly, recent experiments suggest that SAR may be induced by methyl salicylate, a volatile compound that is synthesized from SA in the inoculated leaf and converted back to SA in the uninoculated leaves (Shulaev et al. 1997). Further studies are clearly required to clarify SA's relationship to the long distance SAR signal.

Efforts to identify the SA receptor have led to the identification of several proteins that interact with SA (Durner et al. 1997). Most of these are either iron sulphur- or heme-containing proteins. The first SA-binding protein identified in tobacco was a catalase (Chen et al. 1993). The discovery that SA inhibited catalase's H_2O_2-degrading activity and H_2O_2-induced *PR* gene expression led to the proposal that one mechanism of SA's action was to elevate the level of H_2O_2 or H_2O_2-derived ROS, which then serve as intermediates in the SA signaling pathway (Chen et al. 1993). Supporting this model was the observation that SA also inhibited the activity of ascorbate peroxidase, the other major H_2O_2-scavenging enzyme (Durner and Klessig 1995). Moreover, other inducers of resistance, namely INA and BTH, also inhibited catalase and ascorbate peroxidase activity (Conrath et al. 1995; D. Wendehenne, J. Durner, Z. Chen, and D.F. Klessig, in prep.).

In contrast, recent reports have provided evidence that defense responses, such as *PR-1* induction during the HR and SAR, are not activated by elevated H_2O_2 levels resulting from SA-mediated inhibition of catalase and ascorbate peroxidase. No decrease in catalase activity

was detected in pathogen-inoculated leaves of tobacco and *Arabidopsis* plants (Bi et al. 1995; Summermatter et al. 1995). In addition, the level of SA in uninfected tissues appears to be too low to effectively inhibit catalase and ascorbate peroxidase, and no increases in H_2O_2 were detected during the development of SAR (Bi et al. 1995). Furthermore, transgenic tobacco plants exhibiting drastically reduced catalase expression, attributable to cosuppression or synthesis of antisense RNA, failed to constitutively accumulate elevated levels of *PR-1* mRNA or protein (Chamnongpol et al. 1996; Takahashi et al. 1997). Moreover, although H_2O_2 induced the expression of the *PR-1* genes in wild-type tobacco leaves, it was ineffective in NahG plants (Bi et al. 1995; Neuenschwander et al. 1995). These results strongly argue that the elevated H_2O_2 levels produced by SA-mediated inhibition of catalase and ascorbate peroxidase do not directly activate defense responses. Rather, H_2O_2-activated expression of *PR-1* appears to be mediated by SA. Indeed, application of H_2O_2, albeit at very high levels, induced SA synthesis in tobacco (León et al. 1995; Neuenschwander et al. 1995) as well as *Arabidopsis* (Summermatter et al. 1995).

Although SA-mediated inhibition of catalase and ascorbate peroxidase activity does not lead to *PR* gene expression via elevated levels of H_2O_2, it may play other roles in the defense response. Inhibition of catalase and ascorbate peroxidase by the very high levels of SA found at infection sites may lead to increases in H_2O_2, which might initiate necrotic lesion formation by activating a cell death program. In addition, SA interaction with catalase and ascorbate peroxidase in the upper, uninoculated tissue may activate defense responses via the generation of free radicals. SA is thought to inhibit catalase and ascorbate peroxidase by serving as a one-electron-donating substrate; in the process, SA free radicals may be produced (Durner and Klessig 1996). Phenolic free radicals are potent initiators of both lipid peroxidation and protein oxidation. Increases in lipid peroxidation have been observed within and surrounding the HR induced by *C. fulvum* and TMV infection of tomato and tobacco, respectively (Hammond-Kosack and Jones 1996; May et al. 1996; M. Anderson, Z. Chen, and D.F. Klessig, unpubl.). Furthermore, lipid peroxides, the products of lipid peroxidation, were shown to induce *PR-1* gene expression. Because lipid peroxidation is a self-perpetuating chain reaction, small amounts of SA free radical could be sufficient to activate defense responses in uninfected tissue without a readily discernible inhibition of catalase or ascorbate peroxidase activities.

Very recently, a 25-kD soluble SA-binding protein termed SABP2 has been identified in tobacco leaves (Du and Klessig 1997). The affinity of SABP2 for SA (K_d=90 nM) is ~150 times higher than that of catalase. Because it exhibits a greater affinity for SA and its biologically active analogs, as opposed to its inactive analogs, which do not induce *PR* genes and enhanced resistance, SABP2 might play a role in SA-mediated disease resistance. Furthermore, SABP2 has a 15-fold greater affinity for the plant defense activator BTH than for SA. This is consistent with BTH's greater efficacy in inducing plant defense responses (Friedrich et al. 1996; Lawton et al. 1996; Du and Klessig 1997). However, the role of SABP2 in disease resistance needs to be evaluated further using molecular genetic approaches.

Even though SA is required for resistance to many pathogens, SAR can develop independent of SA in some cases. In *Arabidopsis,* root inoculation with the bacteria *P. fluorescens* induces systemic resistance to *Fusarium oxysporum* as well as *P. syringae* pv. *tomato* in the absence of SA accumulation and SA-mediated *PR* gene expression (Pieterse et al. 1996). Additionally, *Cf2* or *Cf9* gene-mediated resistance to *C. fulvum* in tomato does not appear to require SA accumulation, as resistance appears to be unaffected by presence of the *nahG* gene (Hammond-Kosack and Jones 1996). Similarly, the systemic induction of several *PR* genes in tobacco by the soft-rot pathogen *Erwinia carotovora* and the systemic induction of plant defensins (antimicrobial peptides that share homology with animal defensins such as drosomycin of the fruitfly) in *Arabidopsis* after *Alternaria brassicicola* infection occurred equally well in wild-type and NahG plants (Penninckx et al. 1996; Vidal et al. 1997).

Ethylene, jasmonates, and systemin

Besides SA, ethylene, jasmonic acid (JA), and systemin have also been implicated as important secondary signals for plant defense responses. Ethylene levels have been shown to increase during the HR (De Laat and Van Loon 1982; Enyedi et al. 1992; Hammond-Kosack and Jones 1996). Additionally, ethylene treatment induces the expression of *PAL* and the basic *PR* genes, as well as several wounding-induced genes (Boller et al. 1983; Mauch et al. 1984; Vogeli et al. 1988). Ethylene can also enhance the SA-induced expression of *PR-1* in *Arabidopsis* (Lawton et al. 1994). Conversely, inhibitors of ethylene biosynthesis suppressed TMV-induced expression of *PR* genes (Van Loon 1983) and ethylene action inhibitors blocked the SA-mediated expression of PR-3 proteins in tobacco (Raz and Fluhr 1992). By contrast, genetic evidence suggests that the induction of plant defense responses is ethylene independent. For example, the ethylene-insensitive *Arabidopsis ein1, ein2,* and *etr1* mutants exhibit wild-type levels of resistance to pathogen attack (Bent et al. 1992; Lawton et al. 1995).

The activation of defense responses after mechanical wounding and insect attack appears to be mediated by systemin, an 18-amino-acid peptide, as well as by JA and its ester, methyl jasmonate (MeJA), collectively termed jasmonates. Jasmonates are produced from the major plant plasma membrane lipid linolenic acid via the octadecanoid biosynthetic pathway. This pathway is analogous to the eicosanoid pathway in which the major animal plasma membrane lipid arachidonic acid is converted to prostaglandins, leukotrienes, and related compounds that often mediate localized stress responses in animal cells. Wounding, systemin, and jasmonates induce the expression of proteinase inhibitors (PI) I and II, PAL, and JIP60 (Pearce et al. 1991; Farmer and Ryan

1992; Gundlach et al. 1992; Reinbothe et al. 1994a). The PI proteins reduce herbivory and insect attack by inhibiting key degradative enzymes, whereas JIP60 has been proposed to reduce pathogen attack by mediating polysome dissociation (Reinbothe et al. 1994b). Transgenic tomato plants with lowered systemin expression, due to antisense inhibition, were shown to accumulate significantly less PI protein after wounding (McGurl et al. 1992). In addition, the systemin-induced accumulation of PI proteins in tomato could be blocked by inhibition of JA or MeJA biosynthesis (Farmer et al. 1994). Based on these results, the signal-activating wounding defenses appear to be transduced initially through systemin and subsequently through the octadecanoid biosynthetic pathway.

Several lines of evidence suggest the presence of cross talk between the SA, ethylene, and systemin/jasmonate defense signaling pathways. For example, in tobacco overexpressing the rice *rgp1* gene, which encodes a Ras-related, small GTP-binding protein, or the tobacco *WIPK* gene, which encodes a MAPK, wounding causes the aberrant accumulation of SA and *PR* mRNAs. In contrast, these responses are not induced by wounding untransformed plants (Sano et al. 1994; Seo et al. 1995). The most striking evidence for cross talk, however, comes from a phenomenon known as potentiation. Potentiation occurs when the signals associated with one defense pathway positively affect the magnitude and kinetics of defense responses associated with a different pathway. Several elicitor- and wounding-induced defense responses, including H_2O_2 production, *PAL* expression, and cell death, can be potentiated by levels of SA that, by themselves, are too low to induce these effects (Kauss et al. 1992; Fauth et al. 1996; Mur et al. 1996; Shirasu et al. 1997). Similarly, ethylene can potentiate the SA-mediated induction of *PR-1* gene expression in *Arabidopsis* (Lawton et al. 1994). The simultaneous treatment of tobacco seedlings with SA and MeJA has also been shown to superinduce the accumulation of *PR-1* transcript compared to that observed with SA or MeJA alone (Xu et al. 1994). However, the significance of this potentiation is unclear since aspirin (and hence probably SA) blocks JA biosynthesis and activity (Peña-Cortés et al. 1993; Doares et al. 1995a,b). Future analyses using the jasmonate- and ethylene-insensitive mutants of *Arabidopsis* (Guzman and Ecker 1990; Feys et al. 1994; Ecker 1995) should help define the role these compounds have in both local and systemic resistance to microbial pathogens.

Integration of signaling pathways and activation of plant defense genes

The initial perception and early signal transduction events may be distinct for various pathogen elicitors. However, many of these signals are probably integrated into one of a few terminal pathways that lead to the transactivation steps involved in the interaction between activated transcription factors and pathogen-responsive *cis* elements in the promoters of defense genes. A single pathogen elicitor may activate multiple transcription factors that interact with different *cis* elements in the same or different promoters, leading to induction of many defense genes. Several pathogen-responsive *cis* elements have been identified and the corresponding DNA-binding proteins isolated (Zhu et al. 1996). Some of these transcription factors are transcriptionally and/or post-translationally activated by pathogen infection or treatment with secondary signals, such as SA.

A number of plant defense genes (e.g., maize *PRms*, asparagus *AoPR1*, and potato *PR-10a*) contain an elicitor-responsive TTGACC element (Raventós et al. 1995). This element is also present as the W boxes in the parsley *PR-1* gene promoter (Meier et al. 1991; note: parsley *PR-1* is not related to tobacco *PR-1*, rather it belongs to the *PR-10* family of intracellular *PR* genes that are wound-inducible). Three parsley cDNA clones encoding the W-box binding proteins have been isolated by Southwestern screening (Rushton et al. 1996). These proteins contain zinc finger motifs and belong to the WRKY family of plant transcription factors. Their mRNA levels were up- or down-regulated on treatment with a fungal protein elicitor, suggesting that they play a role in signaling parsley *PR-1* gene activation.

Transcription of the parsley *PR-2* gene is stimulated rapidly by fungal or bacterial elicitors and mediated by an 11-bp *cis* element (CTAATTGTTTA) present in its promoter. The cDNA clones encoding a homeodomain protein that specifically binds to this element have been isolated from both parsley and *Arabidopsis* (Korfhage et al. 1994). This homeodomain protein may be involved in transcriptional regulation of the parsley *PR-2* gene.

A 10-bp TCA (TCATCTTCTT) element is present in the promoters of many stress-inducible genes, including tobacco *PR* genes (Goldsbrough et al. 1993). A 40-kD tobacco nuclear protein binds to this TCA element in an SA-dependent manner. However, this TCA element was neither sufficient nor required for SA-mediated induction of the tobacco *PR-2d* promoter in vivo (Shah and Klessig 1996). By contrast, a 25-bp SA-responsive element was identified by in vivo analysis of the *PR-2d* promoter. This element contains the sequence TCGACC, which is similar to the elicitor-responsive TTGACC element found in the parsley *PR-1* promoter. As the induction of TTGACC element-containing genes by pathogens, elicitors, or wounding is potentiated by SA pretreatment, related factors such as members of WRKY family might be involved in SA activation of the tobacco *PR-2d* gene.

Another SA-responsive element is the activator sequence-1 (*as-1* or *ocs*), which was identified initially in the *35S* promoter of cauliflower mosaic virus (CaMV), and the *nos* and *ocs* (two opine synthase genes) promoters of *Agrobacterium*, respectively (Ellis et al. 1987; Bouchez et al. 1989; Lam et al. 1989). The *as-1* element was also found in the promoters of stress-induced plant genes such as *GST* (Ellis et al. 1993; Ulmasov et al. 1994; Chen et al. 1996; van der Zaal et al. 1996), and it rapidly responds to various signals, including SA, auxin, jasmonates, and H_2O_2 (Kim et al. 1993; Qin et al. 1994; Ulma-

sov et al. 1994, 1995; Zhang and Singh 1994). Several members of the TGA family of bZIP transcription factors have been isolated from tobacco and *Arabidopsis* and shown to bind the *as-1* element (Katagiri et al. 1989; Zhang et al. 1993; Miao et al. 1994). Transgenic plant analyses have also linked TGA transcription factors with *as-1* element activity (Neuhaus et al. 1994; Rieping et al. 1994). A zinc finger DNA-binding protein from *Arabidopsis* was found to bind to a distinct site next to the *as-1* element in the *35S* promoter; binding of this protein significantly enhanced the *as-1* binding activity of TGA factors (Zhang et al. 1995a). In addition, *as-1* binding activity appears to be regulated by the phosphorylation status of the binding factor(s) and/or an inhibitory protein (Jupin and Chua 1996).

The promoters of many ethylene-inducible *PR* genes (e.g., basic *PR-1, PR-2,* and *PR-3*) contain a conserved ethylene-responsive 11-bp GCC box (TAAGAGCCGCC; Eyal et al. 1993). Tobacco ethylene-responsive element binding proteins (EREBPs) have been isolated and shown to bind specifically to the GCC box sequence (Ohme-Takagi and Shinshi 1995). The expression of EREBP mRNAs is induced by ethylene, but individual EREBPs exhibit different patterns of expression. Recently, several tomato proteins, which interact with the kinase encoded by the *Pto* resistance gene, were shown to share extensive homology with the EREBPs and specifically bind to the GCC box. Thus, there may be a direct link between specific recognition by an *R* gene product, protein phosphorylation, and transcriptional activation of plant defense genes (Zhou et al. 1997). Interestingly, tobacco EREBPs and tomato Pti4/5/6 are homologous to the homeotic protein APETALA2 that controls flower and seed development in *Arabidopsis* (Jufuku et al. 1994).

A ubiquitous *cis* element present in many plant genes is the G box (CACGTG), which is bound by the GBF (G box binding factor) family of bZIP transcription factors (Menkens et al. 1995). The G box is also present in the promoters of several plant defense genes such as *PAL* and *CHS,* and is thought to mediate responses to diverse environmental stimuli including light, elicitors, and redox changes. The H box (CCTACC) is another elicitor-responsive *cis* element frequently found in the promoters of phenylpropanoid biosynthetic genes such as *PAL* and *CHS*. Two H box binding proteins (KAP-1 and KAP-2) have been purified from bean suspension cultures (Yu et al. 1993). Elicitation with glutathione does not affect the overall activity of KAP-1 or KAP-2 in the cells. However, there is a rapid increase in the specific activities of both factors in the nuclear fraction, suggesting that they are translocated to the nucleus after elicitation, where they may play a role in the activation of defense genes. Recently, a soybean bZIP transcription factor called G/HBF-1 was isolated and shown to bind both the G box and an adjacent H box in the proximal region of the bean *chs15* promoter (Dröge-Laser et al. 1997). Although its mRNA and protein levels do not increase during the induction of defense genes, G/HBP-1 is rapidly phosphorylated in elicited soybean cells. Phosphorylation of G/HBP-1 in vitro also enhances binding to the *chs15* promoter, suggesting its involvement in the activation of plant defense genes.

Interestingly, the H and G boxes are very similar to Myb- and Myc-binding sites, respectively, and can be recognized by the plant Myb- and Myc-like transcription factors involved in phenylpropanoid biosynthesis (Grotewold et al. 1994; Sablowski et al. 1994). Recently, a TMV-inducible *myb* gene (*myb1*) was isolated from tobacco (Yang and Klessig 1996). Expression of this gene was activated rapidly (within 15 min) by exogenous SA, and its activation preceeded induction of the acidic *PR-1* genes. Tobacco *PR-1* gene promoters contain both Myb- and Myc-binding sites. The recombinant Myb1 protein was able to specifically bind to an H box-like Myb-binding site present in the promoter of the *PR-1a* gene. These results suggest that the tobacco *myb1* gene encodes a signaling component downstream of SA that may participate in transcriptional activation of *PR* genes and plant disease resistance. As is the case for several of the transcription factors mentioned above, the role of Myb1 in activation of plant defense genes remains to be rigorously established using in vivo assays, such as transgenic plant analysis.

Genetic approaches to dissect signaling pathways for plant defense responses

Mutational analysis has been a powerful tool for identifying the components of various signaling pathways in organisms as diverse as yeast, *Caenorhabditis elegans,* and *Drosophila.* Among plants, the crucifer *Arabidopsis thaliana* serves as an excellent model organism to identify mutants with altered defense responses (Dangl et al. 1996; Ryals et al. 1996). Currently, mutations in *Arabidopsis* that either constitutively activate defense responses (*acd2, lsd, cpr1, cep1, cim3;* Bowling et al. 1994; Detrich et al. 1994; Greenberg et al. 1994; Weymann et al. 1995; Klessig et al. 1996; Ryals et al. 1996) or compromise defense responses (*npr1, nim1, sai1, ndr1, pad4, eds;* Cao et al. 1994; Century et al. 1995; Delaney et al. 1995; Glazebrook et al. 1996, 1997; Parker et al. 1996; Shah et al. 1997) have been identified using various genetic screens.

All of the *Arabidopsis* mutants with constitutively activated defense responses accumulate elevated levels of SA, express elevated levels of *PR* genes, and exhibit enhanced resistance to pathogens (Table 1). In addition, *acd2, cep1,* and the *lsd* group of mutants develop spontaneous lesions that resemble an HR (Dietrich et al. 1994; Greenberg et al. 1994; Weymann et al. 1995; H. Silva and D.F. Klessig, unpubl.). The constitutive expression of *PR* genes and the enhanced resistance phenotypes of the *lsd, cpr1, cep1,* and *cim3* mutants were shown to require constitutively elevated levels of SA, as they were suppressed by the *nahG*-encoded salicylate hydroxylase (Bowling et al. 1994; Weymann et al. 1995; Dangl et al. 1996; Ryals et al. 1996; H. Silva and D.F. Klessig, unpubl.). However, expression of *nahG* suppressed the spontaneous lesion phenotype only in *lsd1, lsd6,* and *lsd7* and not in *lsd2* and *lsd4* (Weymann et al. 1995;

Table 1. A. thaliana *mutants with altered disease resistance*

Mutant[a]	Dominant/ recessive	SA levels[b]	Comments	References
npr1/sai1/nim1/ eds-5/eds-53	recessive	normal	allelic; SA/INA/BTH insensitive; enhanced susceptibility to avirulent pathogens; normal HR; Npr1 contains ankyrin repeats and shares some homology with IκB	Cao et al. (1994, 1997); Delaney et al. (1995); Ryals et al. (1997); Shah et al. (1997)
ndr1	recessive	N.D.	INA responsive; enhanced susceptibility to pathogens; normal HR to most avirulent pathogens	Century et al. (1995)
eds1	recessive	N.D.	INA responsive; enhanced susceptibility to pathogens	Parker et al. (1996)
eds-9(pad4), eds-47(pad2)	recessive	N.D.	nonallelic; SA responsive; enhanced susceptibility to pathogens; deficient in camalexin accumulation	Glazebrook et al. (1996, 1997)
eds-2,-4,-6,-8,-40, -42 and *-48*	recessive	N.D.	nonallelic; SA responsive; enhanced susceptibility to pathogens	Glazebrook et al. (1996)
acd2, cep1, lds1, lsd3, and *lsd5*	recessive	elevated	constitutively high expression of PR genes; develop spontaneous lesions and have enhanced resistance to pathogens; *nahG* suppresses all *lsd1* phenotypes; *acd2, lsd1, lsd3,* and *lsd5* are nonallelic; *cep1* is nonallelic to *acd2*	Dietrich et al. (1994); Greenberg et al. (1994); Klessig et al. (1996); Dangl et al. (1996); H. Silva and D.F. Klessig (unpubl.)
lsd2, lsd4, lsd6, and *lsd7*	dominant	elevated	nonallelic; constitutively high expression of PR genes; develop spontaneous lesions and have enhanced resistance to pathogens; all mutant phenotypes suppressed by *nahG* in *lsd6* and *lsd7*; only SAR phenotype suppressed by *nahG* in *lsd2* and *lsd4*	Dietrich et al. (1994); Weymann et al. (1995)
cpr1	recessive	elevated	constitutively high expression of PR genes; no spontaneous lesions; mutant phenotype suppressed by *nahG*	Bowling et al. (1994)
cim3	dominant	elevated	constitutively high expression of PR genes; no spontaneous lesions; mutant phenotype suppressed by *nahG*	Ryals et al. (1996)

[a]*npr* (non expresser of PR genes); *sai* (salicylic acid insensitive); *nim* (noninducible immunity); *eds* (enhanced disease susceptibility); *pad* (phytoalexin deficient); *ndr* (non-race-specific disease resistance); *acd* (accelerated cell death); *lsd* (lesion-simulating disease); *cpr* (constitutive expressor of PR genes); *cep* (constitutive expression of PR genes); *cim* (constitutive immunity).
[b](N.D.) Not determined.

Dangl et al. 1996; Ryals et al. 1996). Thus, lesion formation may be induced through multiple pathways. Furthermore, lesion formation appears to be regulated by a feedback loop since it was restored in *nahG*-expressing *lsd1* and *lsd6* mutants by the application of INA (Weymann et al. 1995; Dangl et al. 1996).

Although elevated SA levels have a causal role in the enhanced resistance observed in the above mutants, it is unclear whether they are a result of mutations in genes that participate in the resistance signaling pathway. Alternatively, the increase in SA levels may be caused by mutations in SA biosynthetic genes, or they may be a nonspecific response to stress. It was recently demonstrated that the leaves of *lsd1* plants accumulate elevated levels of superoxide prior to the development of spontaneous lesions (Jabs et al. 1996). Furthermore, lesion formation could be induced by injecting *lsd1* leaves with a superoxide-generating system; this treatment did not cause lesions on the leaves of wild-type plants. As lesion formation could be prevented by simultaneously injecting *lsd1* leaves with the superoxide-generating system and superoxide dismutase, these plants may be deficient in some factor or signal that regulates the accumulation of and/or response to superoxide and thereby prevents runaway cell death. The *LSD1* gene has been cloned recently (Dietrich et al. 1997). The predicted protein contains three zinc finger domains, suggesting that it may be a transcription factor that negatively regulates the cell death pathway.

Metabolic stress generated by the inappropriate expression/repression of a wide variety of genes in plants can also lead to phenotypes similar to those of the *Arabidopsis lsd* mutants. Transgenic tobacco expressing either the bacterio-opsin gene, which encodes a bacterial proton pump, or a mutant derivative of ubiquitin, which interferes with ubiquitin-dependent proteolysis, develop

spontaneous HR-like lesions, show enhanced resistance to pathogens, accumulate high levels of SA, and constitutively express *PR* genes (Becker et al. 1993; Mittler et al. 1995). These same characteristics were also observed in transgenic tobacco expressing a subunit of cholera toxin or a yeast vacuolar invertase gene (Beffa et al. 1995; Herbers et al. 1996). Thus, identifying the biochemical and physiological alterations in these transgenic plants and the *Arabidopsis* mutants, as well as cloning the respective *Arabidopsis* genes, should provide new information about the signaling components through which SA-mediated disease resistance can be activated.

A variety of *Arabidopsis* mutants whose resistance to pathogens is compromised have also been identified. The *ndr1* mutant was identified by screening for loss of resistance to an avirulent pathogen, whereas the *eds* group of mutants was identified in screens that permitted enhanced pathogen growth (Century et al. 1995; Glazebrook et al. 1996; Parker et al. 1996). Except for *eds5* and *eds53*, which are allelic with *npr1*, *sai1* and *nim1* (Glazebrook et al. 1996; Cao et al. 1997; Ryals et al. 1997; Shah et al. 1997), the remaining *eds* mutants and *ndr1* exhibit normal SA/INA inducibility of *PR* gene expression and hence are not defective in SA signaling (Glazebrook et al. 1996; Parker et al. 1996; Ryals et al. 1996). The *NDR1* and *EDS* genes may encode components of other signal transduction pathways involved in pathogen recognition. Alternatively, they may encode proteins involved in the synthesis of antimicrobial compounds. For example, the *eds9* (*pad4*) and *eds47* (*pad2-2*) mutants are defective in the accumulation of the phytoalexin camalexin (Glazebrook et al. 1996, 1997).

The allelic *npr1*, *sai1*, and *nim1* mutants, by contrast, were identified initially by their insensitivity to SA or INA (Cao et al. 1994; Delaney et al. 1995; Shah et al. 1997). These mutants fail to express *PR* genes at high levels after SA treatment and exhibit increased susceptibility to bacterial and fungal pathogens (Cao et al. 1994; Delaney et al. 1995; Shah et al. 1997). Additionally, mutants containing the *nim1-1* and *sai1-1* alleles were shown to be nonresponsive to INA and BTH (Lawton et al. 1996; Shah et al. 1997). This finding correlates with the available biochemical evidence suggesting that these compounds are functional analogs of SA that induce defense responses via the SA signaling pathway (Conrath et al. 1995; Durner and Klessig 1995; Vernooij et al. 1995; Görlach et al. 1996; Malamy et al. 1996; Du and Klessig 1997; D. Wendehenne, J. Durner, Z. Chen, and D.F. Klessig, in prep.).

The recessive nature of the various mutant *npr1* alleles suggests that *NPR1* encodes a positive regulator of the SA signal transduction pathway (Delaney et al. 1995; Cao et al. 1997; Shah et al. 1997). Furthermore, NPR1 appears to act downstream of SA in the defense signaling pathway, since the *npr1* mutants do not respond to exogenous SA and the *sai1-1* and *nim1-1* mutants accumulate elevated levels of SA upon pathogen infection (Delaney et al. 1995; Shah et al. 1997). Interestingly, the levels of SA detected in the *sai1-1* mutants after infection with *P. syringae* pv. *syringae* were 10–15 times higher than those seen in wild-type plants, which provides additional evidence that SA accumulation is normally under feedback regulation (Shah et al. 1997).

The *NPR1* gene was cloned recently and shown to encode a 60-kD protein with ankyrin repeats and some homology to the mammalian IκB protein (Cao et al. 1997; Ryals et al. 1997). The significance of the ankyrin repeats in Npr1 function is highlighted by the demonstration that a histidine to tyrosine alteration in the third ankyrin repeat is responsible for the *npr1-1* mutant phenotype (Cao et al. 1997). In mammals, ankyrin repeats have been demonstrated to participate in protein–protein interactions, such as those between IκB and NF-κB or 53BP2 with the tumor suppressor p53 (Gorina and Pavletich 1996; Krappmann et al. 1996). By analogy, Npr1 may interact with another protein(s) to transduce the SA signal.

Conclusions

A combination of genetic, molecular, and biochemical approaches have led to important new insights into the signal perception and transduction processes in plant defense responses. The recent cloning of plant disease resistance genes, in particular, has greatly advanced our understanding of the recognition and perception mechanisms involved in race-specific disease resistance. The striking conservation of certain motifs, such as LRRs, among nearly all cloned *R* genes responsible for recognizing diverse plant pathogens, suggests that a prevalent mechanism of signal recognition may underlie the gene-for-gene specificity. Although initial perception mechanisms may differ, many pathogen elicitors appear to trigger a common network of signaling pathways that coordinate the overall defense responses. Such signaling processes involve protein phosphorylation, ion fluxes, cellular redox modulation, secondary signal amplication, and transcriptional activation of plant defense genes. Intriguingly, many signaling components, such as Toll/interleukin-1 receptor-like domain, G proteins, NADPH oxidase, H_2O_2, MAP kinases, IκB, and Myb transcription factors, appear to be conserved in higher eukaryotes and may mediate host defense responses in both animal and plant systems. Even antimicrobial peptides such as plant defensins and some pathogenesis-related proteins such as *PR-1* are conserved in eukaryotes as components of host defense systems (Fang et al. 1988; Broekaert et al. 1995; Murphy et al. 1995). Recently, the plant hypersensitive cell death triggered by pathogen attack was shown to share many of the cytological and biochemical characteristics associated with animal programmed cell death which is involved in not only development, but also elimination of transformed or infected cells (Dangl et al. 1996; Greenberg 1996). Therefore, elucidation of plant defense signaling mechanisms should provide crucial information to enhance our understanding of host–pathogen interactions in general, as well as to develop new strategies for plant disease control.

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