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Update on Plant-Pathogen Interactions

Genetic Dissection of Acquired Resistance to Disease

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Plants come in frequent contact with potentially pathogenic fungi, bacteria, and viruses, yet disease results from relatively few of these exposures. In many cases an encounter leaves no obvious trace of its occurrence and the microbe fails to establish itself due to a lack in activation of pathogenicity functions or to highly effective plant defense mechanisms. Other encounters leave evidence of an intense plant-microbe interaction that results in the arrest of pathogen development after attempted colonization. In these cases plant tissues often display activated defense functions that produce antimicrobial compounds, enzymes, and structural reinforcements that may limit pathogen growth (Dixon and Lamb, 1990). These reactions may also be associated with the HR, a localized region of plant cell death around infection sites. An HR may involve just a single cell or it can produce death of extensive regions of tissue. The combination of defense activities can limit infection and prevent it from spreading to other tissues. The effectiveness of these defense strategies is determined both by the rate of host activation of defenses and by the rate of expansion of the pathogen colony. Therefore, in the evolution of plants and their pathogens, traits that permit rapid and effective defense responses should be favored in the evolution of the host, whereas desirable pathogen traits are those that enable rapid growth or facilitate evasion, suppression, or tolerance of host reactions.

Host defense reactions are activated by sensing of the pathogen, and are sometimes mediated by plant R genes that recognize pathogens that possess corresponding *avr* genes (Keen, 1990). Although R genes have obvious adaptive value to plants, the maintenance of *avr* genes by pathogens may seem paradoxical, since their presence can trigger host defense responses. However, in several cases *avr* gene products have been shown to contribute to pathogen virulence on hosts that lack corresponding R genes (see Dangl, 1994, and refs. therein), indicating that, whereas a particular *avr* gene may expose a pathogen to the defenses of one host, it may more importantly enhance its pathogenicity on other susceptible hosts.

In cases in which host defenses are inadequate to constrain pathogen development, multiplication and growth of the invading microbe will tap host resources and can lead to tissue damage and ultimately to pathogen reproduction, any of which can contribute to both immediate and delayed symptoms of disease. Whether a diseased plant succumbs to or recovers from infection depends on both its ability to assemble effective defenses against an established disease-causing organism and on the pathogenic strategy of the etiologic agent. Under conditions favorable for disease, some pathogens cause massive destruction of host tissues, against which there is little defense. By contrast, many intimate biotrophic pathogens produce much less damage and are highly sensitive to host defenses if triggered (Collmer and Bauer, 1994). Furthermore, some pathogens use a "fast in-fast out" strategy, completing their life cycle in a short time, which may enable evasion of host defenses.

SAR

After a plant's recovery from disease, it can display a remarkable reduction in its susceptibility to future infection. This response, known as SAR, results from infection with necrotizing pathogens and leads to whole-plant systemic resistance to the inducing agent, as well as to a broad spectrum of other viral, fungal, and bacterial pathogens (Ross, 1961; Kuc, 1982; Delaney et al., 1994a; Ryals et al., 1994, 1995).

Extensive work to understand the induction and basis of SAR led to the discovery of a diverse array of proteins associated with this response and to the finding that application of SA could produce broad-spectrum disease resistance in plants, like that found in pathogen-induced SAR. Subsequently, SA was shown to accumulate after pathogen inoculation and, when applied to plants, to cause accumulation of SAR-associated gene products after application. Compelling evidence for an essential role of SA in SAR came from experiments with plants transformed with the bacterial *nahG* gene, which encodes salicylate hydroxylase. This enzyme catalyzes the conversion of SA to the inactive compound catechol (Fig. 1). Plants expressing salicylate hydroxylase failed to accumulate SA following pathogen attack, and they were unable to activate SAR genes or to develop resistance against pathogens (Gaffney et al., 1993). The identification of SA and SAR genes and evidence for their roles in SAR was reviewed in an *Update* by Ryals et al. (1994).

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Abbreviations: *avr* gene, avirulence gene; BTH, benzo-(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester; HR, hypersensitive response; INA, 2,6-dichloroisonicotinic acid; NahG plants, transgenic plants containing the *nahG* gene; R gene, resistance gene; SA, salicylic acid; SAR, systemic acquired resistance.

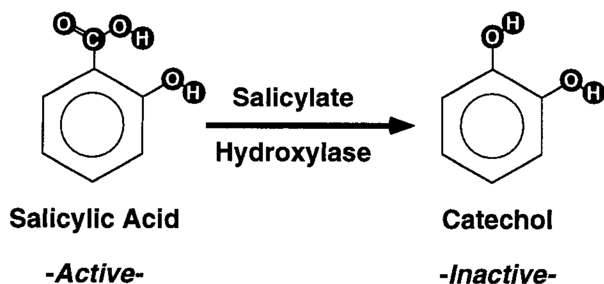

Figure 1. Conversion of SA to catechol by salicylate hydroxylase, encoded by the *nahG* gene from *Pseudomonas putida*. Reaction is accompanied by oxidation of coenzyme NADH, not shown.

The finding that naturally occurring SA can induce broad-spectrum disease resistance led the Ciba-Geigy Corp. to identify other chemical inducers of SAR. Those efforts resulted in the discovery of the SAR-activating compounds INA and BTH (Métraux et al., 1991; Görlach et al., 1996), which induce the same set of genes and produce the same spectrum of resistance to disease as found in biologically induced SAR, supporting the proposed mode of action of these chemicals through induction of the SAR pathway (Kessmann et al., 1994). It is interesting that INA and BTH can induce resistance in plants that are unable to accumulate SA because of expression of salicylate hydroxylase, showing that the site of activity for these chemicals is either at or downstream from the site of SA action in the signal transduction pathway leading to SAR (Delaney et al., 1994b; Vernooij et al., 1995; Lawton et al., 1996).

MULTIPLE ROLES OF SA

Additional studies of *Arabidopsis thaliana* and tobacco plants expressing the salicylate hydroxylase (*nahG*) gene revealed two dramatic alterations in their resistance to disease, outside of the defect in SAR induction described above. First, a variety of fungal, bacterial, and viral pathogens grew to much higher levels in SA-depleted plants compared with wild-type plants (Delaney et al., 1994b). This hypersusceptibility to pathogens was accompanied by more severe symptoms of disease. Second, salicylate hydroxylase plants exhibited a breakdown in genotype-specific or gene-for-gene resistance. This was demonstrated by inoculating Arabidopsis plants containing the *nahG* gene with genetically incompatible strains of bacterial and fungal pathogens. The growth of these normally avirulent pathogen strains was correlated with reduced induction of SAR genes in the SA-depleted plants (Delaney et al., 1994b). Together, experiments with NahG Arabidopsis and tobacco plants indicated a function of SA in several modes of disease resistance. These roles include activation of SAR, expression of gene-for-gene resistance, and limitation of disease caused by virulent pathogens. Because each of these forms of resistance are diminished in NahG plants, their full expression appears to depend on inducible mechanisms mediated by SA accumulation. Evidence for the involvement of a common signal transduction pathway for these responses came from studies of the Arabidopsis *nim1* mutant, which exhibits most of the defects in resistance found in NahG plants. Plants with mutations in the *NIM1* gene failed to respond to SA application, yet still accumulated SA in response to pathogen induction (Delaney et al., 1995, and described below). Thus, the *NIM1* gene product identifies a multifunctional, inducible defense pathway that responds to pathogen perception and is modulated by SA accumulation.

The functions of SA described above depend in part on signaling through the *NIM1* gene product. However, a separate role in defense has been proposed for SA, based on biochemical studies of an SA-binding protein, which turns out to be a catalase enzyme (Chen et al., 1993). Upon SA binding, the activity of this catalase is diminished, which may thus promote the accumulation of H_2O_2. Despite early speculation (Chen et al., 1993), recent evidence indicates that an SA-sensitive catalase is unlikely to play a role in SAR (Bi et al., 1995; Neuenschwander et al., 1995). First, the concentration of SA in nondiseased tissues expressing SAR is at least 1 order of magnitude lower than the binding constant of SA with the SA-binding catalase. Second, in NahG plants unable to accumulate SA, application of physiologically relevant amounts of H_2O_2 failed to induce SAR genes. However, because much higher levels of SA accumulate in pathogen-infected tissues, inhibition of catalase at these sites may yield significant quantities of H_2O_2, possibly promoting HR-associated cell death (Levine et al., 1994; but see Glazener et al., 1996). Thus, although it is not likely to be involved in SAR, an SA-sensitive catalase may participate in pathogen-induced host cell death. Other roles have been suggested for H_2O_2 in plant defense, including the cross-linking of cell-wall proteins, which has been proposed to strengthen cell walls against pathogen ingress (Bradley et al., 1992).

Upon perception of pathogens by plants, a clear role of SA is indicated for induction of SAR, full expression of genotype-specific resistance, and modulation of disease severity. At least part of these responses depends on SA signaling through NIM1. Key questions persist regarding how this activation occurs and about the functions of the NIM1-inducible defense pathway, including: How is pathogen recognition achieved and how does it activate the pathway? How important is the HR in producing resistance, and does it produce signals that activate the NIM1 pathway? In addition to signaling through NIM1, what other roles does SA have? Finally, how does SA signaling through NIM1 act to produce SAR, modulate disease severity, and contribute to gene-for-gene resistance?

By combining molecular genetics, cell biology, and pathology, these questions are being examined using *A. thaliana* as a model genetic system for host-parasite interactions. A diverse array of pathogens have been found that cause disease in Arabidopsis (reviewed by Dangl, 1993; Mauch-Mani and Slusarenko, 1993), and many Arabidopsis R genes have been found that condition resistance against specific bacterial, fungal, and viral isolates (Staskawicz et al., 1987; Holub et al., 1994; Kunkel, 1996). Because SAR and disease can be manipulated easily in Arabidopsis

(Uknes et al., 1993; Cameron et al., 1994), many genetic investigations of SAR and other host responses to pathogens have been performed using this plant.

MUTATIONS AFFECTING PLANT RESPONSE TO PATHOGEN

To find genes with products that control or contribute to SAR and other host responses to pathogens, mutant screens have been designed to identify plants with defects in pathogen sensing, regulation of HR formation, or SA-mediated responses that lead to SAR gene induction and resistance. Results from these mutant screens will be discussed in turn, although some mutant phenotypes are pleiotropic and affect more than one response (see Table I for a summary of mutants). Because NahG and *nim1* plants show defects in expression of gene-for-gene resistance, this resistance mode also appears to rely in part on SA-inducible mechanisms. Therefore, mutations that affect transduction of R-gene-mediated signals will be considered here, although the R genes themselves will not, since they have recently been reviewed elsewhere (Staskawicz et al., 1995; Kunkel, 1996).

Mutants in Transduction of R-Gene-Mediated Signals

Mutations disrupting genetically determined resistance can either prevent the sensing of pathogens or interfere with the activation of defenses that follow pathogen recognition. In the former class are mutants with inactivated R genes, which are unable to express resistance to specific pathogen strains that possess cognate avirulence genes. By contrast, mutants defective in steps downstream of pathogen recognition may simultaneously lose resistance to several pathogens, provided that the resistance mechanisms that act against these organisms utilize common signal transduction pathways. The latter phenotypic class is represented by the *ndr1* (nonrace-specific disease resistance) mutant, which was found to be susceptible to *Pseudomonas syringae* pv *tomato* (*Pst*) strains containing any of four cloned avirulence genes, as well as to several isolates of the downy mildew fungus *Peronospora parasitica* (Century et al., 1995). The loss of resistance to these widely divergent pathogens suggests that the *ndr1* mutation identifies a common signal transduction pathway that mediates resistance against both fungal and bacterial pathogens. However, *ndr1* plants retain resistance to some *Peronospora* races, suggesting that some parts of the sensory apparatus are competent to act in these mutants.

Mutants That Fail to Regulate HR Cell Death

Several kinds of screens have yielded mutants that spontaneously form HR-like necrotic lesions. Many mutants in this class also express biochemical markers associated with SAR, such as elevated SA levels, high-level expression of SAR genes, and resistance to bacterial and fungal pathogens. The simultaneous occurrence of HR-like lesions and

Table I. *Genes affecting expression of acquired resistance or response to pathogen-derived signals in Arabidopsis*
Host R genes are not included.

Gene Symbol	Description	References	Phenotype of Mutants or Transgenic Plants
nahG (transgene)	In planta depletion of endogenous SA, from transgenic expression of salicylate hydroxylase	Gaffney et al., 1993; Delaney et al., 1994a	1. Suppressed induction of SAR 2. Hypersusceptibility to pathogens 3. Reduced expression of gene-for-gene resistance
NDR1	Nonrace-specific disease resistance	Century et al., 1995	Failure to express some forms of genetically determined resistance
LSD1, LSD2, LSD3, LSD4, LSD5, LSD6, LSD7, ACD2	Lesions simulating disease response, Accelerated cell death	Dietrich et al., 1994 Weymann et al., 1995 Greenberg et al., 1994	Activation of defense pathways in the absence of a pathogen trigger, causing expression of: 1. hypersensitive cell death lesions 2. SAR SA removal shown to suppress lesion formation in *lsd6* and *lsd7* but not in *lsd2* or *lsd4* (Weymann et al., 1995; Hunt et al., 1996)
CIM2, CIM3, CPR1	Constitutive immunity, Constitutive expresser of PR genes	Lawton et al., 1993; H. Steiner and J. Ryals, unpublished data; Bowling et al., 1994	Constitutive expression of SAR genes and expression of resistance; some shown to accumulate SA
NIM1, NPR1	Noninducible immunity, Nonexpresser of PR genes	Delaney et al., 1995, Cao et al., 1994	*npr1* and *nim1* unable to activate SAR genes or disease resistance following pathogen or chemical induction *nim1* plants also shown to be able to accumulate SA and to support growth of incompatible strains of *P. parasitica*
EDS loci (eight)	Enhanced disease susceptibility	Glazebrook et al., 1996; Parker et al., 1996	Hypersusceptibility to virulent *P. syringae* pv *maculicola* strains

other markers of SAR in such mutants supports the view that these mutants respond as though they falsely perceive pathogen in its absence. Included in this category are the Arabidopsis *lsd* genes (lesions simulating disease response): *lsd1*, *lsd2*, *lsd3*, *lsd4*, and *lsd5* (Dietrich et al., 1994), *lsd6*, and *lsd7* (Weymann et al., 1995) and (accelerated cell death-2) *acd2* (Greenberg et al., 1994) mutants. These lines were identified by their visible lesion phenotype (*lsd1*, *lsd3*, *lsd4*, and *lsd5*), their constitutive high-level expression of SAR genes (*lsd2*, *lsd6*, and *lsd7*), or their propensity to form HR lesions following inoculation with virulent *Pst* strains (*acd2*).

Mutants with Aberrant Regulation of SAR Genes

In the first screens to identify mutants defective in regulation of SAR, RNA was examined from mutagenized (M_2) seedlings using northern blots hybridized to SAR gene probes. These searches led to the discovery of several mutants with constitutive expression of SAR genes and resistance to pathogens. These were called constitutively immune or *cim* mutants (screen described by Lawton et al., 1993), and they could be placed into two categories, depending on whether they appeared normal or displayed leaves with necrotic lesions. The *cim* mutants with necrotic lesions were later renamed *lsd* (*lsd2*, *lsd6*, and *lsd7*) mutants and are described above. Like *lsd* mutants, lesion-free *cim* lines were resistant to pathogens (H.-Y. Steiner and J. Ryals, unpublished results), probably because of their constitutive expression of SAR genes.

An alternative method for identifying SAR gene-expression mutants utilized transgenic plants containing the β-1,3-glucanase (PR-2) promoter fused to a *uidA* reporter gene that encodes GUS. Mutants with elevated expression of PR-2 were found by staining M_2 plants with a fluorogenic substrate to show GUS activity. This approach led to the identification of the *cpr1* (constitutive expressor of PR genes) mutant, which, like *lsd* and *cim* mutants, also exhibited resistance to fungal and bacterial pathogens (Bowling et al., 1994). Another mutant screen using the same GUS reporter system was conducted on M_2 plants after treatment with INA to find mutants that failed to show chemical induction of the PR-2 promoter. This led to the identification of the *npr1* (nonexpresser of PR genes) mutant, which fails to show SA or INA induction of either SAR genes or resistance (Cao et al., 1994).

Mutants Affecting Expression of Acquired Resistance

To target genes that couple SA signaling to the induction of pathogen resistance, screens were developed to identify mutants susceptible to *Peronospora* infection despite pretreatment with SAR-inducing chemicals. At least four independent lines with mutations in the *NIM1* (noninducible immunity) gene were identified by their SA-, INA-, and BTH-insensitive phenotype (Delaney et al., 1995). Because these chemicals fail to activate SAR genes or pathogen resistance in *nim1* plants, each of them must act through NIM1 induction of the SAR pathway. Like *nim1*, *npr1* mutants also show a loss of response to SA and INA, suggesting that both mutations may identify a common gene. However, not enough details of the *npr1* phenotype are available to permit extending this comparison.

It is interesting that *nim1* plants support growth of normally incompatible races of *Peronospora*, indicating a role for the NIM1 pathway in expression of genetically determined resistance. This result is consistent with our earlier findings with salicylate hydroxylase-expressing plants and indicates that the SA-dependent component of gene-for-gene resistance is mediated by signaling through the *NIM1* gene product (Delaney et al., 1994b, 1995).

Because pathogen-inoculated *nim1* plants retain the ability to accumulate SA and yet no longer show induction of SA-responsive genes, the wild-type *NIM1* gene product is believed to couple these events. The *NIM1* gene product may act as an SA receptor, provided that it also interacts with INA and BTH, since each of these SAR-inducing chemicals fails to function in *nim1* mutants (Delaney et al., 1995; Lawton et al., 1996). Alternatively, the *NIM1* gene product may act downstream of reception of the SA, INA, and BTH signals.

Effect of SA on Phenotypes of Mutants

The screens described above have permitted identification of at least a dozen genes that affect the expression of SAR (Table I). Some of the genes will soon be cloned, which will open new doors to understanding molecular mechanisms controlling SAR and other host responses to pathogens. However, based on mutant phenotypes and their sensitivity to application or removal of SA, inferences can be made about the role of some of the genes' products relative to SA action. For example, *nim1* and *npr1* mutants fail to exhibit SA induction of SAR genes and resistance (Cao et al., 1994; Delaney et al., 1995), suggesting that these mutations cause pathway disruption downstream of SA. This view is supported by the finding that *nim1* plants retain the ability to accumulate SA in response to pathogens and yet cannot respond to this signal (Delaney et al., 1995). By contrast, whereas *lsd*, *cpr1*, and *cim* mutants show constitutive activation of the SAR pathway and exhibit elevated SA levels, depletion of SA by *nahG* expression suppressed SAR gene expression and resistance phenotypes in all lines examined (Bowling et al., 1994; Weymann et al., 1995; Hunt et al., 1996). These observations indicate that the *lsd*, *cpr1*, and *cim* mutations tested are disrupted upstream of SA in the signaling pathway.

However, experiments with *lsd2*, *lsd4*, *lsd6*, and *lsd7* plants revealed interesting differences in the response of these mutants to depletion of SA. Although *nahG* expression suppressed SAR gene activation and resistance phenotypes in all lines, striking differences were observed in the expression of the lesion phenotype. Upon introduction of the salicylate hydroxylase gene, *lsd2* and *lsd4* lines maintained their lesion-forming phenotype (Hunt et al., 1996), whereas *lsd6* and *lsd7* lines showed NahG suppression of lesion formation. It is interesting that the suppression of lesions observed in *lsd6*/NahG plants could be reversed by

application of INA or SA (Weymann et al., 1995). This differential response to SA depletion indicates differences in the basis for the mutant phenotypes in *lsd* mutants. Because wild-type plants do not form HR lesions following application of SA, HR development appears to be determined by steps separate from or upstream from the site of SA action in the signal transduction pathway leading to SAR. It is therefore curious that lesion formation in several *lsd* lines can be influenced by application of SA or INA (Dietrich et al., 1994; Weymann et al., 1995), which suggests that some kind of mechanism may exist to link processes downstream from SA action to feedback upon the upstream response of lesion formation (Weymann et al., 1995, T.P. Delaney, unpublished observations). Such a feedback loop may modify plant perception or response to pathogen after SAR has been induced.

OVERVIEW

Based on these results, a general model of an SA-responsive regulatory pathway that controls several forms of disease resistance can be developed (Fig. 2). A variety of inputs can activate the pathway, which upon induction leads to hypersensitive cell death and accumulation of SA. Elevated SA is directly or indirectly sensed by the *NIM1* gene product to produce several forms of resistance that leads to SAR, limits pathogen growth, and contributes to gene-for-gene resistance. Activation of this pathway may reinforce itself through some kind of feedback mechanism described above.

A significant challenge is to understand the means by which plants sense pathogens in the absence of R genes. Recognition can be mediated by host R genes if corresponding *avr* genes are present in the pathogen. However, defense pathways can also be activated by virulent pathogens that are not recognized via host R genes, suggesting that other pathogen surveillance mechanisms exist (Fig. 2A). Following recognition of virulent pathogens, such pathways may mediate resistance mechanisms that attenuate the severity of disease. Evidence for such resistance mechanisms can be found in NahG plants (Delaney et al., 1994b) or *eds* (enhanced disease susceptibility) mutants (Glazebrook et al., 1996). In each case, whether due to SA depletion or mutation in *EDS* loci, pathogens exhibit supervirulence, implicating the existence of resistance mechanisms that constrain the development of disease on wild-type plants.

The sensory mechanisms that plants use to detect virulent pathogens are unknown. However, clues may come from analysis of mutants that activate the SAR pathway in the absence of pathogen inducers. In particular, mutants that spontaneously form HR-like lesions and show activated defense functions may be informative because they exhibit markers suggesting whole-pathway activation and thus may identify genes involved with the primary sensory apparatus. This phenotypic class is represented in Arabidopsis by the seven *lsd* mutants and *acd2*. Lesion phenotypes associated with disease resistance have also been found in other plant species, such as tomato containing wild *autogenous necrosis* alleles, *mlo* barley, and maize plants harboring certain derivatives of the rust resistance *Rp1* locus (Johal et al., 1995, and refs. therein). A large number of genes exist that if mutated can produce a lesion mutant phenotype; in maize, for example, more than 200 lesion-mimic loci have been estimated to exist (Dietrich et al., 1994; Johal et al., 1995, and refs. therein), some of which may also display markers of SAR. Therefore, many genes may be capable of effecting input into pathways that lead to cell death and activate SAR pathways. Some of these may be R gene alleles that signal constitutively (Pryor, 1987), whereas others may be genes whose products can impinge on other surveillance mechanisms.

Non-R-gene-mediated pathogen sensing may be achieved by host monitoring of metabolite balances and energy levels, with excessive deviations indicating the presence of parasites. This model seems plausible, because

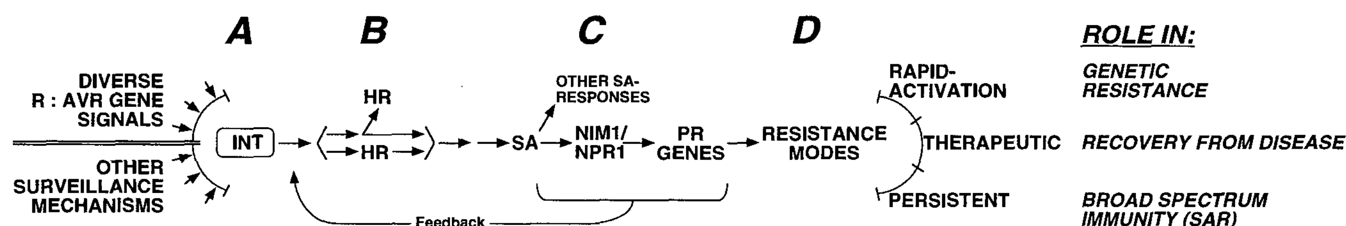

Figure 2. Model signal transduction pathway that regulates acquired resistance. The pathway is activated by pathogen sensing mediated by host R genes, pathogen *avr* gene interactions, or by other surveillance mechanisms such as those mentioned in the text (A). These disparate signals are collected by some kind of integrator (INT) to activate at least one common pathway defined by its downstream components. Subsequent defense responses may include HR cell death, which may or may not be required for signaling (B). SA accumulation acts as a signal that requires the *NIM1* gene product to cause induction of pathogenesis-related (PR) gene induction and several forms of resistance. SA may also play other roles in plant defense, outside of *NIM1*-mediated signaling (C). Among the defense functions activated by *NIM1* signaling are those that act rapidly and play a role in gene-for-gene resistance, those that act during a primary disease cycle and may act therapeutically to attenuate disease severity, and those that lead to long-term SAR to produce broad-spectrum persistent immunity against subsequent infection (D). Studies described in "Effect of SA on Phenotypes of Mutants" provide evidence for a feedback loop that can modify the lesion phenotype of some *lsd* mutants and presumably wild-type plants. The origin of the putative feedback circuit is downstream of the site of SA action, and it modifies steps upstream from those that determine lesion formation.

a plant's balance of certain metabolites and energy sources must be shifted as a parasite consumes them for its own profit. Because cells possess mechanisms for monitoring and regulating homeostasis, they may use the same systems for detecting parasites. This kind of sensory mechanism could provide a pathogen-nonspecific means for detecting infection, without the need for pathogen-specific R genes. Although this model is speculative, supporting evidence may be found in experiments with transgenic plants that express genes that may interfere with normal metabolism, including cholera toxin (Beffa et al., 1995), a bacterial proton pump (Mittler et al., 1995), a variant ubiquitin gene (Becker et al., 1993), or invertase (Herbers et al., 1996). In each case expression of the transgene caused an *lsd*-mutant-like phenotype, with development of necrotic lesions accompanied by induction of defense genes and resistance against pathogens.

Because many pathways contribute to homeostasis, the apparent abundance of *lsd*-like mutants may reflect the large number of genes that compose these pathways. Therefore, any mutation that perturbs such balances may cause the plant to falsely perceive a pathogen in its absence, thus triggering defensive reactions that produce HR lesions and SAR. If this model is correct, then characterization of Arabidopsis *LSD*, *ACD2*, or other genes with related mutant phenotypes may identify metabolic systems that are monitored in pathogen surveillance.

Following pathogen perception, host cells may induce localized cell death leading to the HR. However, SAR genes and resistance can be induced by compatible pathogens that do not evoke an HR, indicating that cell death is not essential for downstream signaling (Kuc, 1982). Therefore, it is not clear whether HR formation is an integral part of the SAR pathway or whether it is directed by a divergent pathway from that leading to SA accumulation and NIM1 signaling (Fig. 2B). The importance of the HR as a primary defense mechanism may be called into question because of the observations that NahG, *nim1*, *npr1*, and *ndr1* plants retain the ability to form an HR despite the loss of resistance to pathogens.

In both distal, uninfected leaves undergoing SAR and in infected leaves, SA accumulation is required for activation of defense genes and possibly other processes (Fig. 2C). Other roles for SA may include inhibition of an SA-sensitive catalase (Chen et al., 1993), which may be important at sites of high pathogen load and SA accumulation, but appears unlikely to play a role in signaling through the SAR pathway (Neuenschwander et al., 1995). Experiments with NahG plants described above indicate that SA accumulation is both necessary and sufficient for SAR gene induction and SAR. Furthermore, the hypersusceptibility of NahG plants to many pathogens indicates that SA signaling plays a therapeutic role in constraining development of virulent pathogens in primary disease cycles. Finally, NahG plants are significantly impaired in expressing gene-for-gene resistance, indicating that SA signals contribute to this response. Results with Arabidopsis *nim1* mutants are consistent with observations of NahG plants, with *nim1* plants also being defective in the activation of SAR genes and showing compromised expression of gene-for-gene resistance. Together, the panoply of defense activities mediated by SA accumulation appear to utilize NIM1 signaling for expression (Fig. 2D).

FUTURE PROSPECTS

Investigation into the genetic regulation of acquired resistance has been facilitated by a wealth of information describing the induction and response of SAR. In addition, the availability of molecular markers such as the SAR genes has permitted this pathway to be monitored and has enabled reporter gene strategies to be used for the isolation of mutants. The SAR pathway can be easily manipulated by applying or removing SA or through the use of existing mutant lines. Finally, a number of powerful pathosystems have been developed recently that permit increasingly elegant studies to explore the subtleties of host-parasite interactions. Together, these features make the SAR pathway a powerful system for understanding plant signal transduction mechanisms. This pathway may have unique features not found in animal systems, such as those that regulate plant cell death, enable pathogen surveillance, and utilize SA as a signaling molecule.

In addition to providing valuable information about plant signal transduction mechanisms, the study of SAR is likely to provide several new strategies for achieving disease control in agriculture. Although most of the genetic analysis has been conducted in Arabidopsis, SAR is likely to function in many plant species and has already been observed in a wide variety of dicot and monocot plants. By exploiting endogenous plant defense mechanisms for disease control, we can reduce our reliance on fungicides and produce crops more efficiently.

Several strategies are likely to yield advances toward this goal. First, by understanding the nature of the signaling molecules that induce resistance in planta, we can develop new agrochemicals that mimic the action of SA and induce resistance following application. This strategy has been exploited by Ciba-Geigy Corp. using the BTH compound to control downy mildew in wheat (Görlach et al., 1996) as well as other crop diseases. Second, natural variation among plants can be identified to find genotypes with more effective inducible defense systems or constitutively activated resistance, such as the Arabidopsis *lsd*, *cim*, *acd2*, and *cpr* mutants. Therefore, by promoting these traits in plant breeding programs, we can produce new varieties with enhanced disease resistance. Finally, the genes that regulate inducible defense pathways, such as *NIM1*, *NPR1*, or those that regulate SA plant synthesis, can be isolated and manipulated to produce varieties that express SAR or other defenses at an optimal place and time, controlled by the regulation schemes engineered into such plants.

During the past several years, studies have uncovered many important details concerning the regulation of SAR. Among the challenges that will occupy workers over the next several years are to understand R-gene-dependent and -independent pathogen-sensing mechanisms, to elucidate the regulation and roles of hypersensitive cell death,

and to further define the systems with which SA interacts. In addition, further dissection of the signal transduction pathways that control SAR and other inducible defense systems will be a priority, as will the understanding of the mechanisms by which defense functions interfere with pathogen fitness and survival.

NOTE ADDED IN PROOF

We recently determined that *NIM1* and *NPR1* are the same gene. This was established in a cross between recessive *nim1*-1 and *npr1*-2 alleles (latter obtained from J. Glazebrook; Glazebrook et al., 1996). F1 and F2 progeny of that cross failed to complement, as determined by the lack of INA induction of PR-1 gene expression, indicating that both mutations lie in the same gene (T. Milos, G. Rairdan, and T. Delaney, unpublished data). Figure 2 was modified to reflect this finding.

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LITERATURE CITED

Becker F, Buschfeld E, Schell J, Bachmair A (1993) Altered response to viral infection by tobacco plants perturbed in ubiquitin system. Plant J **3:** 875–881

Beffa R, Szell M, Meuwly P, Pay A, Vogeli Lange R, Métraux JP, Neuhaus G, Meins F Jr, Nagy F (1995) Cholera toxin elevates pathogen resistance and induces pathogenesis-related gene expression in tobacco. EMBO J **14:** 5753–5761

Bi Y-M, Kenton P, Mur L, Darby R, Draper J (1995) Hydrogen peroxide does not function downstream of salicylic acid in the induction of PR protein expression. Plant J **8:** 235–245

Bowling SA, Guo A, Cao H, Gordon AS, Klessig DF, Dong X (1994) A mutation in Arabidopsis that leads to constitutive expression of systemic acquired resistance. Plant Cell **6:** 1845–1857

Bradley DJ, Kjellbom P, Lamb CJ (1992) Elicitor and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell **70:** 21–30

Cameron RK, Dixon R, Lamb C (1994) Biologically induced systemic acquired resistance in *Arabidopsis thaliana*. Plant J **5:** 715–725

Cao H, Bowling SA, Gordon AS, Dong X (1994) Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell **6:** 1583–1592

Century KS, Holub EB, Staskawicz BJ (1995) *NDR1*, a locus of *Arabidopsis thaliana* that is required for disease resistance to both a bacterial and a fungal pathogen. Proc Natl Acad Sci USA **92:** 6597–6601

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science **262:** 1883–1886

Collmer A, Bauer DW (1994) *Erwinia chrysanthemi* and *Pseudomonas syringae*: plant pathogens trafficking in extracellular virulence proteins. *In* J Dangl, ed, Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms, Vol 192. Springer-Verlag, Berlin, pp 43–78

Dangl JL (1993) Applications of *Arabidopsis thaliana* to outstanding issues in plant-pathogen interactions. Int Rev Cytol **144:** 53–83

Dangl JL (1994) The enigmatic avirulence genes of phytopathogenic bacteria. *In* JL Dangl, ed, Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms, Vol 192. Springer-Verlag, Berlin, pp 99–118

Delaney TP, Friedrich L, Kessmann H, Uknes S, Vernooij B, Ward E, Weymann K, Ryals J (1994a) The molecular biology of systemic acquired resistance. *In* MJ Daniels, JA Downie, AE Osbourn, eds, Advances in Molecular Genetics of Plant-Microbe Interactions, Vol 3. Kluwer Academic, Dordrecht, The Netherlands, pp 339–347

Delaney TP, Friedrich L, Ryals JA (1995) Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance. Proc Natl Acad Sci USA **92:** 6602–6606

Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, Ryals J (1994b) A central role of salicylic acid in plant disease resistance. Science **266:** 1247–1250

Dietrich RA, Delaney TP, Uknes SJ, Ward ER, Ryals JA, Dangl JL (1994) Arabidopsis mutants simulating disease response. Cell **77:** 565–577

Dixon RA, Lamb CJ (1990) Molecular communication in interactions between plants and microbial pathogens. Annu Rev Plant Physiol Plant Mol Biol **41:** 339–367

Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H, Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. Science **261:** 754–756

Glazebrook J, Rogers EE, Ausubel FM (1996) Isolation of Arabidopsis mutants with enhanced disease susceptibility by direct screening. Genetics **143:** 973–982

Glazener JA, Orlandi EW, Baker CJ (1996) The active oxygen response of cell suspensions to incompatible bacteria is not sufficient to cause hypersensitive cell death. Plant Physiol **110:** 759–763

Görlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel K-H, Oostendorp M, Staub T, Ward E, Kessmann H, Ryals J (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell **8:** 629–643

Greenberg J, Guo A, Klessig D, Ausubel F (1994) Programmed cell death in plants: a pathogen-triggered response activated coordinately with multiple defense functions. Cell **77:** 551–563

Herbers K, Meuwly P, Frommer WB, Métraux JP, Sonnewald U (1996) Systemic acquired resistance mediated by the ectopic expression of invertase: possible hexose sensing in the secretory pathway. Plant Cell **8:** 793–803

Holub EB, Beynon LJ, Crute IR (1994) Phenotypic and genotypic characterization of interactions between isolates of *Peronospora parasitica* and accessions of *Arabidopsis thaliana*. Mol Plant-Microbe Interact **7:** 223–239

Hunt MD, Neuenschwander UH, Delaney TP, Weymann KB, Friedrich LB, Lawton KA, Steiner H-Y, Ryals JA (1996) Recent advances in systemic acquired resistance research. Gene (in press)

Johal GS, Hulbert SH, Briggs SP (1995) Disease lesion mimics in maize: a model for cell death in plants. BioEssays **17:** 685–692

Keen NT (1990) Gene-for-gene complementarity in plant-pathogen interactions. Annu Rev Genet **24:** 447–463

Kessmann H, Staub T, Hofmann C, Maetzke T, Herzog J, Ward E, Uknes S, Ryals J (1994) Induction of systemic acquired resistance in plants by chemicals. Annu Rev Phytopathol **32:** 439–459

Kuc J (1982) Induced immunity to plant disease. BioScience **32:** 854–860

Kunkel BN (1996) A useful weed put to work: genetic analysis of disease resistance in *Arabidopsis thaliana*. Trends Genet **12:** 63–69

Lawton K, Uknes S, Friedrich L, Gaffney T, Alexander D, Goodman R, Métraux J-P, Kessmann H, Ahl Goy P, Gut-Rella M, Ward E, Ryals J (1993) The molecular biology of systemic acquired resistance. *In* B Fritig, M Legrand, eds, Mechanisms of Defense Responses in Plants. Kluwer Academic, Dordrecht, The Netherlands, pp 410–420

Lawton KA, Friedrich L, Hunt M, Weymann K, Delaney TP,

Kessmann H, Staub T, Ryals J (1996) Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway. Plant J **10:** 71–82

Levine A, Tenhaken R, Dixon R, Lamb C (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell **79:** 583–593

Mauch-Mani B, Slusarenko A (1993) Arabidopsis as a model host for studying plant-pathogen interactions. Trends Microbiol **1:** 265–267

Métraux JP, Ahl Goy P, Staub T, Speich J, Steinemann A, Ryals J, Ward E (1991) Induced resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens. *In* H Hennecke, DPS Verma, eds, Advances in Molecular Genetics of Plant-Microbe Interactions, Vol 1. Kluwer Academic, Dordrecht, The Netherlands, pp 432–439

Mittler R, Shulaev V, Lam E (1995) Coordinated activation of programmed cell death and defense mechanisms in transgenic tobacco plants expressing a bacterial proton pump. Plant Cell **7:** 29–42

Neuenschwander U, Vernooij B, Friedrich L, Uknes S, Kessmann H, Ryals J (1995) Is hydrogen peroxide a second messenger of salicylic acid in systemic acquired resistance? Plant J **8:** 227–233

Parker JE, Holub EB, Frost LM, Falk A, Gunn ND, Daniels MJ (1996) Characterization of *eds1,* a mutation in Arabiodopsis suppressing resistance to *Peronospora parasitica* specified by several different *RPP* genes. Plant Cell **8:** 2033–2046

Pryor A (1987) The origin and structure of fungal disease resistance genes. Trends Genet **3:** 157–161

Ross AF (1961) Systemic acquired resistance induced by localized virus infections in plants. Virology **14:** 340–358

Ryals J, Lawton KA, Delaney TP, Friedrich L, Kessmann H, Neuenschwander U, Uknes S, Vernooij B, Weymann K (1995) Signal transduction in systemic acquired resistance. Proc Natl Acad Sci USA **92:** 4202–4205

Ryals J, Uknes S, Ward E (1994) Systemic acquired resistance. Plant Physiol **104:** 1109–1112

Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JDG(1995) Molecular genetics of plant disease resistance. Science **268:** 661–667

Staskawicz BJ, Dahlbeck D, Keen NT, Napoli C (1987) Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea.* J Bacteriol **169:** 5789–5794

Uknes S, Winter AM, Delaney T, Vernooij B, Morse A, Friedrich L, Nye G, Potter S, Ward E, Ryals J (1993) Biological induction of systemic acquired resistance in Arabidopsis. Mol Plant-Microbe Interact **6:** 692–698

Vernooij B, Friedrich L, Ahl Goy P, Staub T, Kessmann H, Ryals J (1995) 2,6-Dichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. Mol Plant-Microbe Interact 8: 228–234

Weymann K, Hunt M, Uknes S, Neuenschwander U, Lawton K, Steiner H-Y, Ryals J (1995) Suppression and restoration of lesion formation in Arabidopsis *lsd* mutants. Plant Cell **7:** 2013–2022