USE OF ARABIDOPSIS FOR GENETIC DISSECTION OF PLANT DEFENSE RESPONSES

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ABSTRACT
Arabidopsis thaliana (Arabidopsis) is proving to be an ideal model system for studies of host defense responses to pathogen attack. The Arabidopsis genetic system is significantly more tractable than those of other plant species, and Arabidopsis exhibits all of the major kinds of defense responses described in other plants. A large number of virulent and avirulent bacterial, fungal, and viral pathogens of Arabidopsis have been collected. In the last few years, a large number of mutations have been identified in Arabidopsis that cause a wide variety of specific defense-related phenotypes. Analysis of these mutant phenotypes is beginning to give glimpses into the complex signal transduction pathways leading to the induction of the defense responses involved in protecting plants from pathogen infection.

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Plant defense responses to microbial pathogens have been studied extensively for many years, as a consequence of the agronomic goal of reducing crop damage caused by pathogen attack. Despite the obvious importance of understanding host defense responses, no systematic attempts to genetically dissect these processes were made until the advent of well-characterized Arabidopsis-pathogen model systems. In large part this was due to the relative intractability of genetic analysis in many hosts, caused partly by long generation times and large, polyploid, or repetitive genomes. The following discussion focuses on identification of Arabidopsis genes that function in defense against pathogens, and on what has been learned from the phenotypes caused by mutations in these genes. As shown in Figure 1, plant defense responses can be divided into three general pathways: the gene-for-gene resistance pathway, which functions during responses to avirulent pathogens; the systemic acquired resistance (SAR) pathway, which leads to strong resistance against a variety of pathogens; and pathway(s) that function to limit the spread of virulent pathogens. The ultimate goal of Arabidopsis-pathogen research is to identify all the components of each of these pathways, to understand how each component functions in disease resistance, and to determine how the different pathways are interconnected.

INTRODUCTION

Essentially all of the major aspects of plant defense responses that have been described in other plant hosts have also been observed in Arabidopsis. Arabidopsis responds to pathogen attack by activating similar batteries of chemical and enzymatic defenses and by displaying both gene-for-gene and systemic acquired
Figure 1  Signaling pathways that are activated in response to pathogen attack.

resistance. These aspects of plant defense responses are described briefly below.

The Plant Defensive Arsenal

The potential strategies for plants to inhibit pathogens are limited to structural barriers that prevent pathogens from obtaining sustenance from the host, and enzymatic and chemical defenses that interfere with pathogen metabolism. Plant responses to pathogen attack include synthesis of reactive oxygen species and antimicrobial secondary metabolites, lignification of host cell walls, and activation of a large number of genes including those encoding glucanases, chitinases, thionins, defensins, glutathione-$S$-transferases, lipoxygenase, phenylalanine ammonia lyase, and enzymes of secondary metabolism (46). In addition, avirulent pathogens often trigger the hypersensitive response (HR), a rapid programmed cell death of the plant cells in contact with the pathogen that is thought to limit pathogen access to water and nutrients (2). Evidence supporting the utility of particular defenses in limiting pathogen growth includes observations of antimicrobial activity in vitro and findings that constitutive expression of defense genes in transgenic plants can lead to reduced pathogen growth [for example (3, 10, 45, 67, 70, 74, 80, 91, 93, 101, 102, 115, 116, 119)]. However, the signal transduction pathways controlling activation of defense responses are
not well understood, and much remains to be learned concerning the relative contributions of particular responses to limiting pathogen growth.

**Gene-for-Gene Resistance**

When a plant pathogen interacts with a potential host, it may successfully colonize the host and cause disease, in which case the pathogen is said to be virulent, the host is susceptible, and the interaction is compatible. Alternatively, the plant may respond to the pathogen by rapidly activating a battery of defense responses, interfering with pathogen multiplication, and preventing disease. In this case, the pathogen is said to be avirulent, the host is resistant, and the interaction is incompatible. The outcomes of plant-pathogen interactions often fit a “gene-for-gene” model first proposed by Flor in the 1940s (34). In this model, resistance results when the pathogen carries a particular avirulence gene that corresponds to a particular resistance gene (R gene) in the host. In general, each R gene confers resistance only to pathogens carrying the corresponding avirulence gene. Gene-for-gene resistance responses have been observed in interactions of plants with a wide variety of pathogens, including fungi, bacteria, and viruses. A simple molecular explanation for gene-for-gene resistance is that avirulence genes encode, or direct synthesis of, ligands that bind to receptors encoded by the plant R genes. Ligand binding triggers activation of a signal transduction cascade culminating in expression of defense responses that inhibit the pathogen and confer resistance.

**Systemic Acquired Resistance (SAR)**

Another form of strong disease resistance in plants is systemic acquired resistance, or SAR. Infection by a necrotizing pathogen (i.e. a pathogen that causes host cell death) causes a signal to be transmitted throughout the plant. In response, defense genes are activated in uninfected tissue, and the plant shows resistance to subsequent infection by a wide range of normally compatible pathogens. It is clear that salicylic acid plays a key role in establishment of SAR; resistant tissue contains elevated levels of salicylic acid (68, 72, 82), treatment of plants with salicylic acid induces defense gene expression and resistance (106, 110, 113), and salicylic acid is required in the responding tissue for defense gene expression and resistance (27, 36, 108). The question of whether salicylic acid is the systemic signal has not yet been settled.

**Organization of Plant Defense Pathways**

A summary of plant defense response pathways is shown in Figure 1. Infection by an avirulent pathogen triggers the gene-for-gene resistance pathway, causing rapid activation of plant defense responses, and triggering programmed cell death (the HR). The programmed cell death is followed by synthesis of salicylic
acid in both local and systemic tissues, which causes systemic expression of defense genes and SAR. Infection by a virulent pathogen does not trigger the HR, but does lead to activation of other defense responses, although this generally occurs more slowly than in interactions with avirulent pathogens. Salicylic acid also plays a role in activation of defense responses by virulent pathogens. All of these pathways are amenable to genetic analysis, and Arabidopsis genetics has been used to investigate all of them.

GENE-FOR-GENE RESISTANCE IN ARABIDOPSIS

Identification of Resistance Genes

An early goal of Arabidopsis-pathogen research was to identify Arabidopsis R genes by genetic methods and then clone them using map-based strategies. R genes had been known genetically in other species for many years, and their dramatic effects on pathogen interactions had prompted efforts to clone them to facilitate studies of their mechanisms of action and potential application in crop breeding. The advantages of the Arabidopsis model system made identification of Arabidopsis R genes, followed by map-based cloning, a reasonable alternative approach.

The first hurdle encountered in the R gene cloning projects was that, because Arabidopsis is not a crop plant, few Arabidopsis pathogens had been described. However, observations of naturally occurring infections, and testing of pathogens known to infect related species, soon yielded an extensive collection of bacterial, fungal, oomycete, and viral pathogens [for review, see (18)]. Using these pathogens, two different strategies were used to identify R genes.

One strategy was identification of a pathogen isolate that is virulent on one Arabidopsis accession, followed by screening of other accessions for resistance. The resistant accession was then crossed to the susceptible one, and the F2 progeny were tested to determine whether resistance segregated as a single gene. In this way, many single loci that determine resistance to various pathogens were identified. These include at least 26 genes conferring resistance to various isolates of the biotrophic oomycete pathogen Peronospora parasitica (18, 48, 49, 77, 83, 103), at least 2 conferring resistance to the closely related Albugo candida (50), 5 conferring resistance to the ascomycete Erysiphe cichoracearum (1), 1 conferring resistance to Plasmodiophora brassicae (35), 1 conferring resistance to cauliflower mosaic virus (13), 1 conferring resistance to a geminivirus (65), 1 conferring resistance to turnip crinkle virus (28, 95), 1 conferring tolerance to tobacco ringspot nepovirus (64), and 1 conferring tolerance to Xanthomonas campestris pv. campestris (11, 105). In cases of tolerance, the pathogen grows to the same extent in tolerant and susceptible accessions, but the tolerant accession shows no symptoms.
In the case of some bacterial pathogens, a different strategy was used to identify \( R \) genes. Cloned avirulence genes were used to convert virulent strains into isogenic avirulent strains, which were used to identify \( R \) genes. This approach has the advantage that it yields isogenic pairs of virulent and avirulent strains differing by only a single avirulence gene. Use of such strains in studies of gene-for-gene resistance ensures that observed differences in the host response result from a single avirulence gene-\( R \) gene interaction, and are not affected by differences in the pathogen strains other than the presence or absence of the avirulence gene itself. Screening collections of *Pseudomonas syringae* strains that infect related plant species for those that displayed virulence on Arabidopsis identified several virulent and avirulent strains (23, 24, 32, 112). The two virulent strains that have been most widely used, *P. syringae* pv. *maculicola* 4326 (PsmES4326) (32, 112), and *P. syringae* pv. *tomato* DC3000 (PstDC3000) (19, 112), are virulent on all Arabidopsis accessions on which they have been tested (23, 32, 112). The avirulence genes *avrRpt2*, *avrRpm1*, and *avrRps4* were obtained by transferring cosmid libraries of avirulent strains into virulent strains, and screening for avirulence (24, 32, 47, 112). The avirulence genes *avrB* (99) and *avrPphB* [formerly *avrPph3* (81)] (55) were identified in studies of other plants, and later found to be recognized by some Arabidopsis accessions (53, 96). Certain *Xanthomonas campestris* strains are virulent on Arabidopsis, and an avirulence gene (*avrXca*) has been cloned (75).

Given the availability of isogenic pairs of virulent and avirulent strains, two approaches have been used to identify \( R \) genes corresponding to the avirulent strains. One of these was comparison of different accessions, as described above. This was used to identify the \( R \) genes *RPM1* (24), *RPS3* (53), *RPS4* (47), and *RPS5* (96), corresponding to the avirulence genes *avrRpm1*, *avrB*, *avrRps4*, and *avrPphB*, respectively.

A mutagenesis approach has also been used to identify \( R \) genes corresponding to particular avirulence genes. This strategy was based on the idea that since the avirulent pathogens carried only a single avirulence gene, host resistance was determined by only a single \( R \) gene, so a mutation in this gene would abolish resistance. In the absence of the isogenic pathogen strains, the mutagenesis approach would have been more risky, because naturally occurring avirulent pathogens might be recognized by more than one \( R \) gene, making it unlikely that mutations in a single \( R \) gene would lead to a phenotype. [Similar difficulties can be encountered in trying to identify \( R \) genes in inter-accession crosses. Some *P. parasitica* isolates may be recognized by more than one \( R \) gene in a single accession (49)]. Mutant screening led to the identification of the \( R \) gene *RPS2* corresponding to *avrRpt2* (59, 117) and yielded mutant alleles of *RPS3* (7) and *RPS5* (RF Warren & RW Innes, personal communication). The *rps2*, *rps3*, and *rps5* mutations were shown to affect the \( R \) genes themselves, rather than...
some other component of signal transduction pathways or defense responses, because their effects were specific to the corresponding avirulence genes. For example, \textit{rps2} mutants retained resistance to strains carrying \textit{avrB} (59, 117), and \textit{rps3} mutants retained resistance to \textit{avrRpt2} strains (7). Interestingly, \textit{rps3} mutants were susceptible to \textit{avrRpm1} strains, and complementation testing demonstrated that \textit{RPM1} and \textit{RPS3} were the same gene (7), now referred to as \textit{RPM1} (41). Possible explanations for the apparent dual specificity of \textit{RPM1} include: \textit{RPM1} recognizes a 6-amino acid motif that is shared by \textit{avrB} and \textit{avrRpm1} (22, 99), \textit{avrB} and \textit{avrRpm1} cause synthesis of the same metabolite that is recognized by \textit{RPM1}, or \textit{RPM1} has the capacity to recognize two different signals.

Testing of the ligand-receptor model for \textit{R} gene function requires cloning of the \textit{R} genes. Consequently, several laboratories cloned Arabidopsis \textit{R} genes using map-based strategies. \textit{RPS2} (6, 73), \textit{RPM1} (41), \textit{RPS5} (RF Warren & RW Innes, personal communication), and \textit{RPP5}, a gene specifying resistance to \textit{Peronospora parasitica} isolate \textit{Noco2} (JE Parker, personal communication), are all members of a class of \textit{R} genes that have the following structure: a nucleotide binding site (NBS) in the N-terminal portion and several imperfect leucine-rich repeats (LRR) in the C-terminal portion. Consequently, they are referred to as the NBS-LRR class. Several of the \textit{R} genes cloned from crop species also belong to this class (60, 89, 114).

The sequences of the NBS-LRR class of \textit{R} genes do not in themselves provide obvious support for the ligand-receptor model. They do not resemble known receptors, and there is strong evidence from mutational analysis and in vitro translation-translocation experiments that \textit{RPS2} is cytoplasmic (66). On the other hand, nucleotide binding sites are found in small GTP binding proteins such as Ras, which are involved in signal transduction (104), and leucine-rich repeats are known to mediate protein-protein interactions (58). The cytoplasmic localization of \textit{RPS2} may not be inconsistent with a role as a receptor, because it is now apparent that recognition of bacterial avirulence gene products can occur within the plant cell cytoplasm. Bacterial avirulence genes are sufficient to induce \textit{R} gene-dependent hypersensitive responses if they are expressed in plant cells (40, 66). This is consistent with the finding that bacterial \textit{hrp} genes that are required for both avirulence and virulence appear to encode a bacterial type III protein secretion system (52, 90). In \textit{Yersinia enterocolitica} and other human bacterial pathogens, a type III secretion system transfers proteins from bacterial pathogens into mammalian cells (87).

There is also evidence supporting the idea that other classes of \textit{R} genes encode receptors. In yeast two-hybrid studies, the tomato \textit{R} gene product, Pto, a member of the serine threonine kinase class of \textit{R} genes (69), was found to interact with the avirulence protein AvrPto (92, 100). Other \textit{R} genes appear
to encode membrane-bound receptors (12, 31, 57, 97). The topic of $R$ gene structure and possible function has been extensively reviewed (5, 20, 98).

In the most simplified version of the ligand-receptor model, if any one of a large complement of different $R$ gene products interacts with its ligand, a common signal transduction cascade is initiated that leads to the expression of the hypersensitive response and other defense responses. In this model, all $R$ gene-mediated resistance responses are phenotypically similar. However, careful examination of the morphology of HR lesions in Arabidopsis plants undergoing resistance responses mediated by different *Peronospora parasitica* $R$ genes revealed that there were significant differences among them, suggesting that not all HR responses are the same (18, 48, 49, 83). Furthermore, comparison of the expression patterns of genes induced in response to *P. syringae* strains carrying *avrRpt2* or *avrRpm1* revealed that some genes expressed in response to *avrRpt2*-expressing strains are not induced in response to *avrRpm1*-expressing strains, and vice versa (84, 85).

**Mutations Affecting Gene-for-Gene Resistance**

A priori, it is reasonable to expect that screens for mutants that fail to display resistance to a pathogen carrying an avirulence gene should yield mutations in the corresponding $R$ gene, in genes encoding components of the signal transduction pathway leading to expression of resistance, and in the genes required for the responses that cause resistance. However, most of the mutations identified in such screens are in the $R$ genes (7, 59, 117). This could be due to functional redundancy of downstream components, lethality of mutations in downstream components, or to target size effects. Despite this difficulty, genes that appear to act downstream from $R$ genes in gene-for-gene resistance have been identified.

A mutation in *NDR1* allows *PstDC3000* strains carrying any of the avirulence genes, *avrRpt2*, *avrB*, *avrRpm1*, or *avrPphB*, to grow to the same extent as *PstDC3000* in infected leaves (17). In addition, several *P. parasitica* isolates that are incompatible on Columbia (the accession in which *ndr1* was isolated) are compatible on the *ndr1* mutant (17). Interestingly, although *ndr1* plants failed to develop an HR in response to *PstDC3000/avrRpt2*, they did show HR-like lesions in response to infection by bacteria carrying *avrB*, *avrRpm1*, or *avrPphB*, despite the fact that growth was not limited (17). These phenotypes show that *NDR1* functions in resistance responses mediated by several different $R$ genes and suggest that the HR does not necessarily lead to resistance. *NDR1* has been cloned, and appears to encode a transmembrane protein (KS Century, AD Shapiro, PP Repetti, D Dahlbeck & BBJ Staskawicz, personal communication).

The *eds1* mutant was identified by screening mutagenized plants of accession Wassilewskija (Ws) for plants susceptible to *P. parasitica* isolate Noco2 (76). Ws is resistant to Noco2 because it carries the $R$ gene *RPP14* (83).
Complementation testing proved that *eds1* is not an allele of *RPP14* (76). Many *P. parasitica* isolates that are incompatible on Ws are compatible on *eds1*, including an isolate from Brassica that is incompatible on all tested Arabidopsis accessions (76). In addition, although *eds1* abolishes *RPS4*-mediated resistance, it has no effect on resistance mediated by *RPS2, RPM1*, or *RPS5* (JE Parker, personal communication). A simple interpretation of these phenotypes is that *R* gene-mediated resistance responses can be divided into two classes: those that require *EDS1* function, and those that do not.

A screen for mutants with defects in *RPS5*-mediated resistance identified two genes other than *RPS5* that are required for resistance to *PstDC3000/avrPphB* (RF Warren & RW Innes, personal communication). One of these affects resistance mediated by *RPS5*, but not resistance mediated by *RPS2, RPM1*, or *RPS4*, while a mutation in the other completely abolishes *RPS5*-mediated resistance, and partially blocks resistance mediated by other *R* genes, including *RPP4*, a *P. parasitica R* gene (RF Warren & RW Innes, personal communication). This latter result is consistent with the idea that there is partial redundancy in some components of *R* gene signaling pathways (RF Warren & RW Innes, personal communication).

**SYSTEMIC ACQUIRED RESISTANCE (SAR)**

Occurrence of gene-for-gene programmed cell death in one part of a plant often leads to development of SAR throughout the plant. Infection by virulent pathogens that cause host cell necrosis can also lead to SAR (61, and references therein). Presumably, dying cells emit a signal that activates systemic expression of defense responses. Mutational analysis is an ideal tool for identifying the regulatory factors responsible for control of SAR signaling.

**Characterization of SAR in Arabidopsis**

Establishment of SAR in Arabidopsis is correlated with the expression of the defense-related genes *PR1* (pathogenesis related protein 1), *PR5* (pathogenesis related protein 5), and *BGL2* (1,3-β-glucanase) (106). A large number of biotic and abiotic stimuli induce systemic resistance to a variety of pathogens (14, 15, 61, 62, 71, 106, 107). As in other plants, treatment of Arabidopsis with salicylic acid (SA) or its structural analogs 2,6-dichloroisonicotinic acid (INA) (106) and benzothiadiazole (BTH) (62) causes increased expression of *PRI* and resistance to pathogens.

Conclusive proof that SA is required for defense gene expression and disease resistance during SAR came from analysis of transgenic plants expressing the bacterial salicylate hydroxylase gene, *nahG*. Salicylate hydroxylase converts SA to catechol, which is inactive in SAR signaling. Transgenic *nahG* plants
fail to accumulate SA, express PR1, or become resistant to subsequent pathogen attack in response to infection by SAR-inducing pathogens (27, 36). Interestingly, nahG plants are also more susceptible to local infection by both virulent and avirulent pathogens, suggesting that SA plays a central role in regulation of defense responses during these interactions, as well as during SAR (27).

Ethylene has been investigated as a possible signaling molecule in SAR. Ethylene treatment of tobacco caused accumulation of chitinase and glucanase, but did not lead to heightened disease resistance (63). Two Arabidopsis mutants that do not respond to ethylene, etr1 and ein2, were used to test the idea that ethylene is involved in SAR signal transduction. Both etr1 (8) and ein2 (44) mutants developed SAR as judged by PR gene expression and pathogen resistance following treatment with SA or INA, suggesting that ethylene is not required for SAR signal transduction after the point at which SA and INA act (63). The subsequent finding that an etr1 mutant developed SAR in response to infection by PstDC3000/avrRpt2, as judged by systemic expression of PR1 and resistance to a virulent isolate of P. parasitica, demonstrated that ethylene is not the major signal molecule in SAR signal transduction (61). However, the level of PR1 expression in etr1 mutants during SAR was lower than the level in wild-type plants, suggesting that ethylene may play some role in SAR regulation. Other evidence that ethylene plays a role in activation of defense responses is described below.

**Mutations Affecting Control of SAR**

Mutants that fail to develop SAR and mutants that constitutively express SAR have been isolated. A screen for mutants that failed to exhibit increased expression of a BGL2-β-glucuronidase reporter gene in response to SA treatment yielded the npr1-1 (nonexpresser of PR genes) mutant (15). Three additional npr1 alleles were isolated in direct screens for mutants that display enhanced susceptibility to the virulent bacterial pathogen PsmES4326 (16, 38). Six alleles of nim1 (noninducible immunity) were identified in an independent screen for plants that failed to develop resistance to P. parasitica in response to INA treatment (26, 88). The nim1 and npr1 mutants were recently shown to be allelic, as was an independently isolated allele, called sai1 (25, 94). For the remainder of the review, this gene is referred to as NPR1/NIM1.

Plants with npr1/nim1 mutations do not develop SAR, defined as resistance to usually virulent pathogens, in response to infection by pathogens that induce SAR, or treatment with SA, INA, or BTH (15, 26, 94). These mutants also fail to express the SAR-associated genes, PR1, PR5, and BGL2, in response to SA, INA, or BTH treatment (15, 26, 62, 94). The phenotypes of npr1/nim1 mutants are not limited to SAR. They are more susceptible than wild-type plants to infection by virulent (15, 38) or avirulent (94) P. syringae strains, and they
allow sporulation of *P. parasitica* isolates that are incompatible on *NPR1/NIM1* parental accessions (26). In *npr1/nim1* mutants, SA accumulation in response to pathogen infection is slightly greater than it is in wild-type plants (26, 94), but expression of *PR1* in infected leaves is greatly reduced (26, 38, 88, 94). These phenotypes show that *NPR1/NIM1* acts in the SAR signal transduction pathway at a point downstream from SA. They also reinforce the conclusion from the phenotypes of *nahG* plants, that SA signaling is important for defense responses to local infections by both virulent and avirulent pathogens, as well as in SAR.

*NPR1/NIM1* was recently cloned as a result of two independent map-based cloning projects (16, 88). From DNA sequence analysis, it appears to encode a cytoplasmic protein containing ankyrin repeat motifs (16, 88). Ankyrin repeats are commonly involved in protein-protein interactions. The *npr1-1* mutation alters a conserved histidine residue that is thought to play an important role in the structure of ankyrin repeats, suggesting that this motif, and therefore interaction with another protein, is important for *NPR1/NIM1* function (16). Ryals et al (88) also detected limited homology to the mammalian transcription factor inhibitor *IκB*. It is not clear whether the regions of *IκB* homology are important for the function of *NPR1/NIM1*. *IκB* functions in binding the transcription factor NF-κB, thereby preventing NF-κB from entering the nucleus and activating transcription [for review, see (4)]. It does not seem likely, however, that *NPR1/NIM1* is simply a functional homolog of *IκB* because *npr1/nim1* mutations are recessive, suggesting that they cause loss of protein function (88). Loss-of-function mutations in *IκB* should cause constitutive expression of regulated genes, not failure to induce expression, as is observed in *npr1/nim1* mutants (88). However, it is possible that *NPR1/NIM1* acts in a manner analogous to *IκB*, by inhibiting the action of a transcriptional repressor, rather than a transcriptional activator (88).

Several mutants that exhibit constitutive expression of SAR have been isolated. These include *cpr1* (9), *cpr6* (J Clarke, S Bowling & X Dong, personal communication), and *cim3* (H-Y Steiner, S Uknes, K Weymann, D Chandler, S Potter, E Ward & J Ryals, personal communication). The *cpr* and *cim* mutants were identified by screening for constitutive expression of a *BGL2-GUS* reporter gene (9), or for constitutive expression of *PR1* (H-Y Steiner, S Uknes, K Weymann, D Chandler, S Potter, E Ward & J Ryals, personal communication), respectively. All of these mutants display decreased susceptibility to pathogen attack, elevated SA levels, and constitutive expression of *PR1, PR5*, and *BGL2* (9; J Clarke & X Dong, personal communication; H-Y Steiner, S Uknes, K Weymann, D Chandler, S Potter, E Ward & J Ryals, personal communication). Unlike the lesion-mimic mutants described in the next section, *cpr* and *cim* mutants do not develop spontaneous lesions in the absence of
pathogen attack (8a; H-Y Steiner, S Uknes, K Weymann, D Chandler, S Potter, E Ward & J Ryals, personal communication). Addition of the nahG transgene to cpr1 or cim3 mutants suppresses their phenotypes, indicating that cpr1 and cim3 act upstream of SA in the SAR pathway, and that the elevated SA levels in the mutants cause the gene expression and pathogen resistance phenotypes (9; H-Y Steiner, S Uknes, K Weymann, D Chandler, S Potter, E Ward & J Ryals, personal communication). The cpr1 mutation is recessive, suggesting that CPR1 acts to repress salicylic acid accumulation (9). The cpr6 and cim3 mutations are dominant, so it is likely that these mutations cause constitutive activity of factors that are positive regulators of SA accumulation (J Clarke & X Dong, personal communication; H-Y Steiner, S Uknes, K Weymann, D Chandler, S Potter, E Ward & J Ryals, personal communication).

LESION-MIMIC MUTANTS

SAR is triggered by programmed cell death resulting from gene-for-gene resistance reactions, and by cell death caused by necrotizing virulent pathogens. Many lesion-mimic mutants, which develop lesions as a result of spontaneous cell death, have been isolated from Arabidopsis (29, 42, 43, 111; M Spaly & JT Greenberg, personal communication; S Bowling, J Clarke & X Dong, personal communication) and other species (56, 109). In a subset of the Arabidopsis mutants, including acd2 (43), lsd1 through lsd7 (29, 111), cpr5 (8a), and 10 complementation groups of newly isolated acd mutants (M Spaly & JT Greenberg, personal communication), lesion formation is associated with elevated SA levels, expression of PR1, and resistance to virulent pathogens. Many other lesion-mimic mutants, including acd1 (42), do not exhibit these SAR-related phenotypes (M Spaly & JT Greenberg, personal communication). This section describes lesion-mimic mutants that display defense response phenotypes.

Phenotypes of Lesion-Mimic Mutants

Studies of acd2 (43), lsd1 through lsd7 (29, 111), and cpr5 (8a) mutants showed that they share the common phenotypes of elevated SA levels, constitutive expression of defense genes such as PR1, and enhanced resistance to virulent pathogens. In other respects, they show significant differences. The acd2, lsd1, lsd3, lsd5, and cpr5 mutants are recessive, whereas lsd2, lsd4, lsd6, and lsd7 are dominant. In acd2 mutants, lesions appear first on the older leaves, so it is possible to study plants before and after lesion formation, and lesion tissue as well as healthy tissue from lesioned plants. Studies of acd2 mutants showed that formation of lesions is required for systemic accumulation of SA, expression of PR1, and resistance to PsmES4326 (43).
The *lsd1* mutant differs from the other lesion-mimics in that once lesions are initiated, they spread and completely destroy the affected leaf, whereas the extent of lesions in the other mutants is limited (29). Superoxide is a necessary and sufficient condition of lesion spread in *lsd1* mutants (54). Therefore, *LSD1* appears to define a function that responds to superoxide, or a signal derived from it, to inhibit programmed cell death and expression of defense responses (54). This observation is consistent with a role for LSD1 in limiting the extent of programmed cell death during the hypersensitive response, because synthesis of reactive oxygen species is an early event in gene-for-gene resistance responses (46). *LSD1* was recently cloned (30). It is expressed at low levels, and encodes a zinc finger protein (30). Consequently, it has been proposed that LSD1 functions in regulation of transcription, either by repressing a death pathway, or activating a death-inhibiting pathway (30).

**Analysis of Lesion-Mimic Mutations**

Different laboratories have applied different criteria to sort lesion-mimic mutants into broad categories and to place them in signal transduction pathways. The *nahG* transgene was introduced into the *lsd* mutants to determine the relationship between the high SA levels in these mutants and lesion formation, defense gene expression, and disease resistance. In *lsd2* and *lsd4* mutants, *nahG* does not suppress lesion formation, but does suppress defense gene induction and disease resistance (51). Therefore, the dominant mutations *lsd2* and *lsd4* define factors that act upstream of SA to promote lesion formation, and defense gene induction and disease resistance depends on SA, as expected from previous studies using *nahG* plants (51). In contrast, *nahG* suppresses lesion formation, as well as gene expression and pathogen resistance phenotypes, in *lsd1*, *lsd6*, and *lsd7* mutants (111; U Neuenschwander, RA Dietrich, JA Ryals & JL Dangl, personal communication). Lesion formation in *lsd1* and *lsd6* is restored by treatment with INA (111; U Neuenschwander, RA Dietrich, JA Ryals & JL Dangl, personal communication). This result could be explained by a feedback loop in SAR signaling such that lesions cause SA accumulation, but other signaling factors downstream from SA are also required for lesion formation (111).

The *npr1-1* mutation was used to determine whether lesion formation in *cpr5* is dependent on NPR1/NIM1 function. Lesion formation was not affected in *cpr5 npr1-1* double mutants, whereas *PR1* gene expression and resistance to *PsmES4326* was lost (8a), suggesting that lesion formation in *cpr5* is not NPR1/NIM1-dependent, but that *PR1* expression and *PsmES4326* resistance are. Curiously, the *cpr5 npr1-1* double mutant retained the resistance to *P. parasitica* strain Noco2 observed in the *cpr5* single mutant, suggesting that
cpr5-induced resistance to Noco2 is mediated by an NPR1/NIM1-independent mechanism (8a).

The acd mutants have been sorted into two classes: those that accumulate the Arabidopsis phytoalexin, camalexin, in the lesions, and those that do not (M Spaly & JT Greenberg, personal communication). Since camalexin synthesis is not associated with SAR (118; J Glazebrook, unpublished data), these results suggest that some lesion mimic mutations may activate other defense-related pathways in addition to SAR.

Suppressor genetics are being used to identify other factors affecting control of cell death pathways. All of the phenotypes of the lsd1 mutant are suppressed by a recessive mutation called phx21 (54). Presumably, PHX21 is an activator of cell death that functions downstream from lsd1 in a cell death regulatory pathway (54). Seven suppressors of lsd5 have been isolated (J-B Morel & JL Dangl, personal communication). At least four of these compromise pathogen resistance as single mutants (J-B Morel & JL Dangl, personal communication), and at least one also suppresses lsd4 and partially suppresses lsd6 (J-B Morel, M Hunt, JA Ryals & JL Dangl, personal communication). These results suggest that many suppressors of lesion-mimic mutants define factors with central roles in defense response signaling pathways (21).

Several types of mutations could cause a lesion-mimic phenotype, besides mutations in regulators of the programmed cell death and SAR pathways. Some transgenic plants expressing foreign genes that are unrelated to plant-microbe interactions display lesion-mimic, constitutive expression of defense genes, and pathogen resistance phenotypes [see Dangl et al (21) for a list of these]. These observations, and the fact that lesion-mimic mutants define a large number of complementation groups, raise the possibility that some lesion-mimic mutations may define genes that are only peripherally related to defense response signaling (29). However, the discovery that lsd1 could encode a transcriptional regulator, and the early results from characterization of lsd suppressors show that studies of lesion-mimic mutants do provide insight into the regulatory pathways controlling programmed cell death and SAR.

OTHER MUTANTS WITH ALTERED DISEASE RESISTANCE

The genes discussed so far are thought to play roles in pathogen recognition or signaling in gene-for-gene resistance responses or in establishment of systemic acquired resistance. Questions that have not yet been addressed include: To what extent do particular defense mechanisms contribute to limiting pathogen growth? How is the slow activation of defense responses that occurs during
infection by virulent pathogens controlled? and How do these responses limit pathogen growth? This section describes efforts to address these questions by targeted screens for mutants with defects in particular defense mechanisms, and by broad screens for mutants with enhanced susceptibility or enhanced resistance to virulent pathogens.

**Phytoalexin-Deficient Mutants**

As mentioned above, phytoalexins are antimicrobial compounds produced by plants in response to pathogen attack (78). Five different complementation groups of phytoalexin-deficient (pad) mutants have now been identified (37–39). None of the pad mutants significantly compromises RPS2- or RPM1-mediated resistance, suggesting that camalexin is not an important component of these gene-for-gene resistance responses (37, 39). However, mutations in pad1, pad2, and pad4, but not pad3 or pad5, cause significantly enhanced susceptibility to the virulent bacterial pathogens PsmES4326 and PstDC3000 (37, 39). Possible explanations for this result include: (a) Camalexin does not contribute significantly to limiting growth of PsmES4326 and PstDC3000, but the pad1, pad2, and pad4 mutations exert pleiotropic effects on other defense responses, and (b) camalexin does limit growth of the bacterial pathogens, but antimicrobial camalexin biosynthetic precursors accumulate in pad3 and pad5 mutants, compensating for the loss of camalexin (37). In support of the former model, PAD4 must encode a regulatory factor, because if challenge with Cochliobolus carbonum is used as the camalexin-inducing stimulus, pad4 does not exhibit a camalexin defect (39). Furthermore, the expression of PR1 in response to PsmES4326 infection of pad4 plants is much lower than in wild-type plants, demonstrating that the pad4 mutation has pleiotropic effects on regulation of defense responses (N Zhou & J Glazebrook, unpublished data). Interestingly, pad4 is susceptible to several P. peronospora isolates that are incompatible on the parent accession, Columbia (39). Taken together, the phenotypes of pad4 suggest that PAD4 plays a signaling role that is important in responses to compatible P. syringae strains and incompatible P. parasitica isolates.

**Enhanced Susceptibility Mutants**

A direct screen for mutants that display the sort of enhanced susceptibility phenotypes observed in some of the pad mutants and in npr1/nim1 mutants should yield mutants in components of signal transduction pathways controlling expression of defense responses, as well as mutations in the “effector genes” that limit pathogen growth. This screen is potentially more powerful than screens for pad, npr/nim, cim, cpr, or lsd/acd mutants, because it does not rest on any
assumptions about which signaling pathways or downstream responses are important for disease resistance, allowing identification of previously unknown resistance mechanisms. In screens for mutants exhibiting enhanced disease susceptibility (eds) in response to infection by PsmES4326, at least 11 complementation groups have been identified, including npr1, pad2, pad4, and at least 8 previously unidentified genes (38, 86; EE Rogers, S Volko & FM Ausubel, unpublished data). Presumably, if an eds screen were carried out using a different pathogen, a similarly large set of mutants would be identified, and these might only partially overlap with the set obtained using PsmES4326, since it seems likely that different pathogens might trigger different signal transduction cascades, and display differing sensitivity to particular plant defense responses. An eds screen using the obligate biotrophic fungal pathogen Erysiphe sp. isolate MGH is under way, and has already yielded several promising putative mutants (TL Reuber & FM Ausubel, unpublished data).

Like screens for acd or lsd mutants, the usefulness of mutants obtained from the eds screen is compromised by the possibility that mutations affecting cellular metabolism in ways not directly relevant to defense responses might cause an eds phenotype. The finding that the eds screen yielded alleles of genes previously known to play roles in defense responses shows that at least several eds mutants are bona fide defense response mutants. Furthermore, characterization of eds mutants has revealed that eds4 (J Glazebrook, unpublished data) and eds5 (86) mutants exhibit reduced expression of PR1 in response to PsmES4326 infection, and that eds4 also exhibits reduced expression of PR5 (J Glazebrook, unpublished data). Complementation testing has shown that EDS4 and EDS5 are different genes (38). Unlike npr1/nim1 mutations, the eds4 and eds5 mutations do not affect expression of PR1 in response to salicylic acid treatment (38). Therefore, one possibility is that EDS4 and EDS5 regulate defense gene expression in the SA pathway, but at a point upstream from SA. An alternative possibility is that they affect an SA-independent pathway(s) that contributes to regulation of defense genes.

**Enhanced Resistance Mutants**

Screens that are the direct opposite of the eds screen described above were performed by infecting plants with extremely high doses of virulent P syringae strains and screening for plants that appeared more resistant than wild-type plants (CA Frye & RW Innes, personal communication; S Volko & FM Ausubel, personal communication). Four nonallelic mutants, named edr1 through edr4, were isolated on the basis of enhanced disease resistance to PstDC3000 (CA Frye & RW Innes, personal communication). The edr mutants do not display constitutive PR1 expression, so they are not cim/cpr or acd/lsd type mutants (CA Frye & RW Innes, personal communication). Growth of PstDC3000 in the
**edr** mutants is much lower than in wild-type plants, but not as low as growth of PstDC3000 carrying an avirulence gene (CA Frye & RW Innes, personal communication). The **edr1** mutant also displays enhanced resistance to the fungal pathogen *Erysiphe cichoracearum* (CA Frye & RW Innes, personal communication). Additional putative **edr** mutants have been isolated on the basis of enhanced resistance to PsmES4326 (S Volko & FM Ausubel, personal communication).

**USING MUTANTS TO DEFINE SIGNALING PATHWAYS**

The existence of a large collection of mutants with defects in different defense-related signaling pathways makes it possible to use the mutants to determine which pathways are controlling an observed response. For example, camalexin synthesis in response to PsmES4326 infection was not affected by **npr1/nim1** mutations (38, 118), and salicylic acid treatment did not induce high levels of camalexin (118). However, **nahG** transgenic plants failed to synthesize camalexin in response to PsmES4326 infection (118). Taken together, these results suggest that salicylic acid is necessary, but not sufficient for activation of camalexin synthesis, and that salicylic acid effects are mediated by at least two pathways, one that requires NPR1/NIM1 and one that does not (118).

Another study addressed the local and systemic expression of the Arabidopsis defensin gene **PDF1.2** in response to challenge by the incompatible fungus *Alternaria brassicicola* (79). **PDF1.2** was not expressed in response to salicylic acid treatment, but was expressed in response to ethylene or methyl jasmonate (79). Expression of **PDF1.2** was not affected by the **nahG** transgene, or by **npr1/nim1** or **cpr1** mutations (79). However, the **ein2** (ethylene-insensitive) or **coi1** (methyl jasmonate–insensitive) (33) mutations greatly reduced **PDF1.2** expression both locally and systemically (79). Therefore, local and systemic expression of **PDF1.2** is controlled through a methyl jasmonate–dependent pathway, which may also require ethylene (79). Interestingly, an **acd2** mutant displayed systemic expression of **PDF1.2**, suggesting that lesion formation in **acd2** plants activates both the salicylic acid pathway and the methyl jasmonate pathway (79).

**CONCLUDING REMARKS**

Genetic analysis in Arabidopsis is a powerful tool for dissecting plant defense response pathways. A large number of loci that affect various aspects of defense responses have been isolated. In many cases, interesting mutants have been isolated quite recently and have not been thoroughly characterized. As mutants are exchanged among different laboratories, and characterized with respect to...
their effects on gene-for-gene resistance, SAR, and limiting growth of virulent pathogens, it will become easier to place the genes defined by mutations into different pathways. In addition, it is quite likely that some of the mutations isolated in different screens will prove to be allelic. The challenge for the next few years will be to use the defense response mutants to understand the roles of the various genes in plant defense response pathways.

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