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Plant Disease Resistance Genes: Function Meets Structure

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INTRODUCTION

The coevolution of interacting plants and microbes has given rise to a diverse array of exchanged signals and responses. Microbes that elicit a host response can be met variously with hospitable acceptance (as is the case with symbionts such as nitrogen-fixing *Rhizobium* bacteria), with tardy recognition and moderately effective defenses (as for most interactions that result in disease), or with a strong and rapid defense response that blocks further infection (Dixon and Lamb, 1990; Keen, 1990; Long and Staskawicz, 1993). This latter form of disease resistance forms the subject of this review and is known variously as race-specific resistance, gene-for-gene resistance, or hypersensitive resistance. Activation of gene-for-gene resistance typically depends on specific recognition of the invading pathogen by the plant (Keen, 1990). Numerous individual plant genes have been identified that control gene-for-gene resistance, and these genes are known as resistance (*R*) genes.

Study of gene-for-gene resistance might be justified solely by the intrigue of plant–pathogen coevolution or as a model for signal transduction research in which an organism perceives and responds to its environment. However, the topic takes on greater interest due to its pivotal impact on crop health and food production. Plant diseases cause billions of dollars in lost harvest annually, and in some instances, these losses have severe consequences for humans (Agrios, 1988; Schumann, 1991). One of the most convenient, inexpensive, and environmentally sound ways to control plant disease is to utilize disease-resistant varieties, and plant breeders make extensive use of classically defined *R* genes (Agrios, 1988).

Recent work has revealed the structure of a number of plant *R* genes, and a striking degree of similarity among these genes has been observed. After briefly introducing the subject of *R* genes and avirulence (*Avr*) genes, this review provides an overview of the conserved structural components that are predicted in the proteins encoded by *R* genes. The cloning of *R* genes has stimulated additional research that is also discussed, including structure–function analysis of *R* gene–encoded proteins, isolation of additional *R* genes, identification of functionally related components of the defense signal transduction cascade, and engineering of improved disease resistance in plants.

RESISTANCE GENES, AVIRULENCE GENES, AND PLANT DEFENSE

The plant kingdom contains thousands of *R* genes with specificities for particular viral, bacterial, fungal, or nematode pathogens. Although there are differences in the defense responses induced during different plant–pathogen interactions, some common themes are apparent among *R* gene–mediated defenses. Most significantly, the function of a given *R* gene is dependent on the genotype of the pathogen (Keen, 1990; De Wit, 1992; see also Alfano and Collmer, 1996; Crute and Pink, 1996; Dangl et al., 1996; Hammond-Kosack and Jones, 1996; and Knogge, 1996, in this issue). Plant pathogens produce a diversity of potential signals, and in a fashion analogous to the production of antigens by mammalian pathogens, some of these signals are detectable by some plants. A pathogen gene is called an *Avr* gene if its expression causes the pathogen to produce a signal that triggers a strong defense response in a plant with the appropriate *R* gene (Keen, 1990; De Wit, 1992). However, expressing an *Avr* gene does not stop the pathogen from being virulent on hosts that lack the corresponding *R* gene. The discovery of matched specificity between single host *R* genes and single pathogen *Avr* genes was made by Harold Flor in the 1940s (Flor, 1947) and is the source of the term gene-for-gene resistance. A single plant can have many *R* genes, and a pathogen can have many *Avr* genes.

A strong resistance response is induced when an *Avr* gene and an *R* gene of matched specificity are expressed. A distinguishing hallmark of most gene-for-gene interactions is the activation of a hypersensitive response (HR) in which plant cells in the immediate vicinity of the pathogen undergo programmed cell death as part of the overall defense response (Figure 1; Goodman and Novacky, 1994; Jones and Dangl, 1996). Other features of the resistance response include induced synthesis of antimicrobial metabolites (often referred to as phytoalexins), synthesis of enzymes that can be harmful to the pathogen (such as chitinases and glucanases), and reinforcement of plant cell walls in the infected area (Dixon and Lamb, 1990; Dixon et al., 1994). In addition, responses are generated that have been postulated or proven to be involved in defense signal transduction. These include Ca^{2+} and other ion fluxes, specific changes in protein phosphorylation, the generation of activated oxygen species such as superoxide,

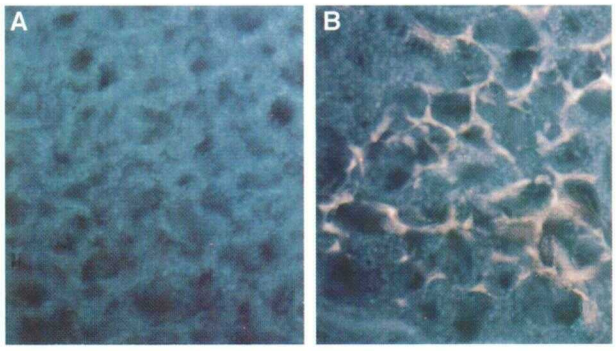

Figure 1. The Hypersensitive Response.

Early and extensive activation of defenses in response to an avirulent pathogen is shown. Arabidopsis plants expressing the *R* gene *RPS2* were inoculated with isogenic *Pseudomonas syringae* pv *tomato* strains differing only by the presence of *avrRpt2*, an *RPS2*-specific *Avr* gene. Bacteria were introduced into leaf mesophyll, and after 20 hr, tissue samples were removed, fixed, and visualized by fluorescence microscopy (Klement et al., 1990). Approximately 40 leaf mesophyll cells can be seen in each panel.

(A) Leaf tissue infected by a virulent pathogen. No defense response is apparent in this sample; instead, these leaves developed disease symptoms 3 to 4 days after inoculation.

(B) Leaf tissue infected by a pathogen expressing *avrRpt2*. Visible aspects of the HR include cell collapse and the release of autofluorescent phenolic compounds.

and the production or release of salicylic acid (Dixon et al., 1994). Other reviews in this issue address these components of plant defense in more detail (Dangl et al., 1996; Hammond-Kosack and Jones, 1996; Ryals et al., 1996).

For the present discussion, it is important to note that many of the above biochemical responses can also be induced in the absence of gene-for-gene resistance, during interactions in which disease develops. Responses such as chitinase expression or phytoalexin biosynthesis make incremental contributions that may slow pathogen growth but often do not block disease (e.g., see Broglie et al., 1991; Maher et al., 1994; Zhu et al., 1994). Conversely, *R* gene products activate a wide array of defense responses in a manner that confers highly effective disease resistance. It is also important to distinguish race-specific elicitors produced as a result of *Avr* gene expression from non-race-specific elicitors. Non-race-specific elicitors, such as fungal or plant cell wall fragments, induce plant responses such as phytoalexin synthesis that help to minimize disease, but these elicitors are in a conceptually (and possibly mechanistically) distinct category from *Avr* gene–derived elicitors that trigger *R* gene–mediated plant defense responses (Dixon et al., 1994).

MOLECULAR ISOLATION OF RESISTANCE GENES

What, then, does an *R* gene encode? An elicitor–receptor model was proposed >20 years ago to account for gene-for-gene resistance (reviewed in Gabriel and Rolfe, 1990). In this model, *Avr* genes encode elicitors that serve as ligands for the receptors encoded by *R* genes. This model may turn out to be valid in some cases (discussed below), but it did little to facilitate the isolation of *R* genes or their gene products. Success in the isolation of *R* genes was not achieved until the development of technologies for cloning plant genes of unknown structure or molecular function. Maize transposable elements offered the first strong hope for success, but early efforts were thwarted by a high spontaneous mutation rate for the targeted *R* genes, such as *Rp1* of maize (Bennetzen et al., 1988). These efforts did, however, spawn a still-unfolding body of work on the mechanisms of *R* gene mutability and its biological significance in the generation of new resistance specificities (e.g., Sudapak et al., 1993; see also Crute and Pink, 1996, in this issue, and the discussion below).

The first cloning of an *R* gene was instead achieved through transposon tagging of *Hm1* from maize, an *R* gene that is functionally distinct from classic *Avr* gene–dependent *R* genes. *Hm1* confers resistance to Race 1 strains of the fungal pathogen *Cochliobolus carbonum* (Johal and Briggs, 1992). In a conclusive set of studies, *Hm1* was found to encode an NADPH-dependent reductase that inactivates the potent plant toxin produced by these fungal strains (Johal and Briggs, 1992; Meeley et al., 1992). Unfortunately, studies of *Hm1* did not suggest a structure or function for classically defined *R* genes because the toxin-degrading strategy of *Hm1* does not involve pathogen *Avr* genes, induction of hypersensitive plant cell death, or other hallmarks of gene-for-gene interactions. Toxin production is a very common component of pathogen virulence, however, and work on *Hm1* outlined an important mechanistic paradigm for natural or engineered plant disease resistance (see Walton, 1996, in this issue).

The maturation of positional cloning (chromosome walking) and heterologous transposon tagging technologies provided feasible approaches to the isolation of *R* genes, and a number of successes have now been reported (this subject is also reviewed in Lamb, 1994; Briggs, 1995; Dangl, 1995; Michelmore, 1995; and Staskawicz et al., 1995). Table 1 lists the cloned *R* genes for which published reports are available. *Pto* of tomato, which confers resistance against *Pseudomonas syringae* pv *tomato* bacteria expressing the *Avr* gene *avrPto*, was the first *Avr* gene–specific *R* gene to be isolated (Martin et al., 1993). A positional cloning strategy was used to isolate *Pto*, and this gene was found to encode a protein with similarity to serine–threonine protein kinases.

Surprisingly, the next five *R* genes that were isolated bore a striking similarity to each other but had no resemblance to *Pto*. A new class of *R* genes that encode proteins containing leucine-rich repeat (LRR) domains was defined by the essentially simultaneous cloning of *RPS2* of Arabidopsis (Bent et al., 1994; Mindrinos et al., 1994), *N* of tobacco (Whitham et al., 1994), *L6* of flax (Lawrence et al., 1995), *Cf-9* of tomato (Jones et al., 1994), and *Prf* of tomato (Salmeron et al., 1996). Subsequently, *RPM1* of Arabidopsis (Grant et al., 1995), *Cf-2* of tomato (Dixon et al., 1996), and *Xa21* of rice (Song et al., 1995) were also isolated and found to encode proteins with

LRR domains (Table 1). Sequence analysis has revealed a number of structural themes in addition to the LRR, and new twists are added to these themes with the characterization of each additional cloned *R* gene. The structural domains found in *R* gene products merit detailed attention and are discussed in the following paragraphs. The first and most striking theme to emerge, however, is that *R* genes from diverse plant species with specificity for a wide variety of viral, bacterial, and fungal pathogens often encode structurally similar proteins. This similarity suggests a high degree of mechanistic conservation among the pathways that plants use to trigger defense responses.

STRUCTURAL DOMAINS OF RESISTANCE GENE PRODUCTS

Serine-Threonine Kinases

The cloning and characterization of *Pto* demonstrated the central role of kinase-mediated signal transduction in gene-for-gene plant disease resistance. Modulation of phosphorylation state is one of the most common mechanisms that living organisms use to control protein activity, and protein kinases have been reviewed extensively (e.g., see Pawson, 1994). Sequence data are available for a tremendous number of biochemically confirmed protein kinases (Hanks and Quinn, 1991; Fantl et al., 1993). Eleven subdomains and 15 invariant amino acid residues characteristic of protein kinases have been identified, and regions conserved among kinases that phosphorylate serine–threonine residues have also been determined (Hanks et al., 1988). The derived amino acid sequence of the *Pto* gene contains these conserved domains, and Pto has been shown to exhibit protein kinase catalytic activity in vitro (Loh and Martin, 1995; Rommens et al., 1995a). The N terminus of Pto contains a potential myristoylation site that could provide a membrane anchor for this primarily hydrophilic protein (Martin et al., 1993). A number of findings related to serine–threonine kinases, including the discovery of Fen, Pti1, and Xa21, are introduced later in this review.

Leucine-Rich Repeats

LRRs are multiple, serial repeats of a motif ~24 amino acids in length (Kobe and Deisenhofer, 1994; see also references in Table 1). LRRs contain leucines or other hydrophobic residues at regular intervals and can also contain regularly spaced prolines and asparagines. The crystal structure for one LRR-containing protein, porcine RNase inhibitor, has been determined. In this protein, the LRRs generate a tertiary structure resembling a fist or a curved spring, with each curled finger representing a single LRR (Kobe and Deisenhofer, 1994). In porcine RNase inhibitor, these repeats are abnormally long (28 to 29 amino acids each), however, and it has been speculated that LRR domains with shorter repeat lengths will adopt a structure more closely resembling a β-helical array (e.g., Yoder et al., 1993). In either case, it seems likely that functional specificity in LRRs resides less in the conserved hydrophobic residues (which are oriented internally to provide the characteristic structure) than in the intervening, exposed amino acids. For many published LRR-encoding sequences, the individual repeats often include residues that do not match the LRR consensus, and in some cases this degeneracy is sufficient to suggest deviation from a highly regular structure. In addition, the postulated LRR region of some *R* gene products is bisected by a short stretch of amino acids that appears unlikely to form an LRR structure.

Table 1. Cloned Plant Disease Resistance Genes

R Gene[a]	Plant	Pathogen	*Avr* Gene	Structure[b]	Reference
Hm1	Maize	*Cochliobolus carbonum*	None	Toxin reductase	Johal and Briggs (1992)
Pto	Tomato	*Pseudomonas syringae* pv *tomato*	*avrPto*	Protein kinase	Martin et al. (1993)
Xa21	Rice	*Xanthomonas campestris* pv *oryzae*	Unknown	LRR, protein kinase	Song et al. (1995)
RPS2	Arabidopsis	*P. s. tomato*	*avrRpt2*	LRR, NBS, LZ	Bent et al. (1994); Mindrinos et al. (1994)
RPM1	Arabidopsis	*P. syringae pv maculicola*	*avrRpm1*, *avrB*	LRR, NBS, LZ	Grant et al. (1995)
Prf	Tomato	*P. s. tomato*	*avrPto*	LRR, NBS, LZ	Salmeron et al. (1996)
N	Tobacco	Tobacco mosaic virus	Unknown	LRR, NBS	Whitham et al. (1994)
L6	Flax	*Melampsora lini*	Unknown	LRR, NBS	Lawrence et al. (1995)
Cf-9	Tomato	*Cladosporium fulvum*	*Avr9*	LRR	Jones et al. (1994)
Cf-2	Tomato	*C. fulvum*	*Avr2*	LRR	Dixon et al. (1996)

[a] This list includes race-specific *R* genes for which a sequence had been published at the time of manuscript revision.
[b] Structure refers to protein structure motifs recognizable in the derived amino acid sequences of the listed genes: leucine-rich repeat (LRR; Kobe and Deisenhofer, 1994), nucleotide binding site (NBS; Saraste et al., 1990), and leucine zipper (LZ; Alber, 1992).

In terms of function, the LRR domains of proteins from yeast, Drosophila, humans, and other species have been shown to mediate protein–protein interactions (reviewed in Kobe and Deisenhofer, 1994). Examples include interaction between enzymes and enzyme inhibitors (such as with RNase and RNase inhibitor), interaction between intracellular components of a signal transduction cascade (as between ras and adenylate cyclase in yeast), and the binding of peptide hormones by transmembrane receptors (exemplified by the receptors for gonadotropin and follicle-stimulating hormone).

The hormone receptor example is clearly attractive with respect to the elicitor–receptor model of gene-for-gene resistance, and it brings forth the hypothesis that the LRR domain of some *R* gene products may serve as the binding domain for a ligand produced due to *Avr* gene activity. Alternatively, LRRs may facilitate the interaction of *R* gene products with other proteins that participate in defense signal transduction (see, for instance, the models proposed in Dixon et al., 1996). The products of LRR *R* genes fall into a number of discrete classes (discussed below), and it would not be surprising to find that the LRR domain performs substantially different functions in these different proteins. To date, published experimental support for the functional importance of LRR domains in resistance comes from their conservation in a number of different *R* genes and from the identification of mutant alleles of *RPS2* and *RPM1* that are nonfunctional due to single amino acid changes within the encoded LRR region (Bent et al., 1994; Mindrinos et al., 1994; Grant et al., 1995).

Nucleotide Binding Sites

Many resistance genes that encode LRRs also encode amino acid sequences with strong similarity to known nucleotide binding sites (NBSs, also referred to as P-loops; see Table 1). These NBS domains occur in diverse proteins with ATP or GTP binding activity, such as ATP synthase β subunits, ras proteins, ribosomal elongation factors, and adenylate kinases (Saraste et al., 1990; Traut, 1994). Even more than LRRs, NBS domains in other proteins have been the subject of thorough structure–function analyses. This work has included multiple determinations of crystal structure, detailed physical–chemical characterization of protein–ligand interactions, and kinetic analysis of proteins containing single amino acid substitutions in their NBS domains (Saraste et al., 1990; Traut, 1994). The consensus NBS of some proteins can be broadly defined to encompass not only the P-loop (amino acid consensus GXXXXGK[T/S]) but also the distally located kinase 2 and kinase 3 domains (Traut, 1994).

The presence of the highly conserved NBS domain in some *R* gene products suggests that nucleotide triphosphate binding is essential for the functioning of these proteins. Preliminary support for this hypothesis is emerging from work in a number of laboratories. Site-specific mutations that alter key residues within the proposed NBS have been found to eliminate the HR-inducing function of *RPS2* in transient assays (F. Katagiri, personal communication) and in stably transformed Arabidopsis plants (A.F. Bent, unpublished results). In *N* of tobacco, >20 NBS amino acid substitution mutations have been constructed (B. Baker, personal communication). Although most of these mutations eliminate function, a smaller number cause partial loss-of-function and/or dominant negative effects and will serve as interesting subjects for further analysis.

The mechanistic role of NBS domains in the activation of plant defense remains unknown. Important challenges for the future will be to document nucleotide triphosphate binding and possible hydrolysis and to unravel the function of these processes in the activity of *R* gene products. Nucleotide triphosphate binding may, for example, alter the interaction between *R* gene products and other members of the defense signal transduction cascade. Exciting progress can be expected in light of the extensive knowledge available from work on NBS domains of other proteins.

Leucine Zippers

Within the NBS–LRR subclass of *R* genes, there are still further subgroupings. *RPS2*, *RPM1*, and *Prf*, three genes that encode resistance against *P. syringae* pathovars, all encode probable leucine zipper (LZ) sequences between the N terminus and the NBS and LRR domains (Table 1). In other proteins, these heptad repeat sequences (consensus XXXYXXL, where Y represents a hydrophobic residue) facilitate protein–protein interactions by promoting the formation of coiled-coil structures (Alber, 1992). LZs are well known for their role in homo- and heterodimerization of eukaryotic transcription factors, but similar coiled-coil domains foster interactions between proteins with many other functions. These include, for example, myosins and G protein β and γ subunits (Lupas et al., 1991; Hamm and Gilchrist, 1996). Once again, in other proteins, these motifs have been subjected to detailed structural and functional characterization (such as crystal structure determination), and once again we have little understanding of their role in *R* gene function. Work is in progress to test whether *R* gene products can undergo homodimerization, as are experiments such as yeast two-hybrid library screens (Fields and Song, 1989) to search for other proteins that may interact with *R* gene products through their LZ regions.

Toll/Interleukin-1 Receptor Similarity

N and *L6* form a second subgroup of NBS–LRR *R* genes in that they encode a large N-terminal domain with similarity to the cytoplasmic signaling domain of the Drosophila Toll protein and mammalian interleukin-1 receptors (IL-1R; Whitham et al., 1994; Lawrence et al., 1995). Recently, the *RPP5 R* gene from Arabidopsis has also been cloned and placed in this class of *R* genes (J. Parker and J. Jones, personal communication).

IL-1R respond to the cytokine IL-1 and cause the Rel-family transcription factor NF-κB to become activated (Kuno and Matsushima, 1994), whereas Toll is a receptor involved in the establishment of dorsal–ventral polarity that also causes a Rel-family transcription factor (Dorsal) to be released and activated (Morisato and Anderson, 1995). A working hypothesis is that *N* and *L6* activate plant defenses by a mechanism similar to those used by Toll and IL-1R.

In addition to sequence similarities, there are striking (and perhaps even meaningful) parallels between the pathways controlled by N, L6, Toll, and IL-1R. For example, one role of NF-κB is to stimulate production of activated oxygen, and an oxidative burst is widely observed in gene-for-gene defense responses (see Dangl et al., 1996; Hammond-Kosack and Jones, 1996, in this issue). Furthermore, NF-κB activity is modulated by salicylic acid compounds such as aspirin, and salicylic acid is an important downstream signal molecule in plant defense responses (Kopp and Ghosh, 1994; Ryals et al., 1995; Ryals et al., 1996, in this issue). Both NF-κB and Drosophila Dif (another Rel-related transcription factor) are involved in antimicrobial host responses. Lastly, the ability of Toll to release the transcription factor Dorsal is dependent on Pelle, a protein kinase with significant similarity to the *Pto R* gene product of tomato (Morisato and Anderson, 1995). The significance of these similarities is only starting to be addressed, but there is a sense that further elements of conservation across very divergent taxa will be discovered.

Small Regions of Similarity

Returning to the sequence similarity among the NBS–LRR *R* genes, a few additional points merit attention. Although genes such as *RPS2*, *RPM1*, *N*, and *L6* are sufficiently divergent to be almost entirely dissimilar at the nucleotide level, they share many small pockets of similarity within long colinear stretches of the encoded amino acid sequences (Grant et al., 1995; Staskawicz et al., 1995). Along with the well-known amino acid motifs discussed above, many other small but conserved motifs can be found (Grant et al., 1995; Staskawicz et al., 1995). These poorly understood regions of conservation are likely to represent functionally relevant sites, and they take on added significance as useful landmarks in the isolation of *R* gene homologs (discussed below).

Non-NBS, Predicted Extracellular LRR Proteins

Cf-9 and Cf-2 from tomato do not contain an apparent NBS and thereby form a third major group within the LRR-encoding *R* genes (Table 1; Jones et al., 1994; Dixon et al., 1996). The bulk of the *Cf-9* gene encodes 28 LRRs. Further analysis of the derived amino acid sequence suggests that these LRRs are normally extracellular: the N terminus of Cf-9 includes a probable signal peptide sequence for transport across membranes. Moreover, the C terminus of the protein consists of an apparent transmembrane domain and a short 28–amino acid tail that is probably cytoplasmic. The LRRs encoded by *Cf-9* (and *Cf-2*) conform more tightly to a consensus LRR sequence than do those of the NBS–LRR *R* genes, and a conserved glycine residue occurs within the LRRs; this glycine residue is also found in the other proteins that carry a putative extracellular LRR domain. The *Cf-2* gene product is very similar to that of *Cf-9*, which is perhaps not surprising because both genes encode resistance against specific races of the pathogen *Cladosporium fulvum*. The final nine LRRs of Cf-2 in particular are nearly identical to LRRs of Cf-9, clearly indicating the relatedness of these genes.

Surprisingly, the *Cf-2* locus has been shown to contain two separate genes that encode products that differ by only three amino acid residues. Transformation of a tomato cultivar lacking *Cf-2/Avr2*–mediated resistance was used to demonstrate that either one of these *Cf-2* genes is fully functional (Dixon et al., 1996). The implications of these findings with respect to *R* gene evolution and the generation of recognitional specificity are discussed below.

Transmembrane Receptor Kinases

The final class of *R* genes to be considered here solidifies the relationship between *R* genes that encode LRR proteins and those that encode protein kinases. In this class, both of these functional domains are encoded in the same protein. *Xa21* is the sole member of this class at present and is the only *Avr* gene–specific *R* gene from a monocotyledonous plant for which a sequence has been published (Song et al., 1995). *Xa21* encodes an apparent LRR receptor kinase in which the N-terminal LRRs are likely, by analogy to known proteins, to be arranged extracellularly. The external LRR domain is thought to be joined by a transmembrane region to a cytoplasmically oriented protein kinase domain. It is interesting to compare the protein encoded by *Xa21* with those encoded by *Pto* and *Prf* of tomato (Table 1). *Pto* (encoding a kinase) and *Prf* (encoding an NBS–LRR protein) are both required for resistance against the same avirulent pathogen, *P. s. tomato* expressing *avrPto*. Similarity between these two separate proteins and Xa21 suggests that the Pto and Prf proteins may interact closely to perceive and transduce the avirulence signal of the pathogen. This scenario is indicated as part of the model presented in Figure 2. The examples of *Pto*, *Prf*, and *Xa21* further suggest that functionally significant protein kinase partners are likely to exist for the gene products of *RPS2*, *Cf-9*, and those of many other LRR-containing *R* genes.

Similarity to Other Plant Proteins

Sequence similarities between *R* gene products and other plant proteins may provide further clues regarding the function of

these proteins in defense signal transduction. As an example, the kinase domains of Pto and Xa21 are highly similar to the *Brassica S*-receptor kinase (SRK; Nasrallah et al., 1994). The SRK protein is involved in pollen–stigma self-incompatibility, a system that is functionally quite analogous to the recognition–response activity triggered by *R* gene products. The extracellular domain of SRK in turn is highly similar to the *S*-linked glycoprotein (SLG), and these two proteins have been proposed to interact physically with each other as well as with a pollen-specific signal (Nasrallah et al., 1994). Cf-9 and Cf-2 appear to be analogous to SLG in that they lack an apparent signaling domain, and it has been suggested that Cf-9 and Cf-2 probably interact with an LRR receptor kinase or some other protein to trigger plant defense responses (Jones et al., 1994; Dixon et al., 1996). Progress may be forthcoming in identifying such interacting proteins, because additional tomato genes that are required for Cf-9 function have been identified by mutational analysis (Hammond-Kosack et al., 1994b; see also Hammond-Kosack and Jones, 1996, in this issue).

Intriguing analogies also exist between *R* gene products and other proteins that interact with pathogen-derived molecules. *PR5K* of Arabidopsis encodes an apparent transmembrane kinase in which the kinase domain is similar to Pto and Xa21 and the extracellular domain is similar to the well-known pathogenesis-related (PR) antifungal protein PR5 (Wang et al., 1996). Like SLG and SRK, PR5 and PR5K may interact with common or related targets. A second analogous system is illustrated by the high degree of sequence similarity between the LRRs of Cf-9 and Cf-2 and those of the antifungal polygalacturonase-inhibitor proteins (PGIPs; De Lorenzo et al., 1994). Fungal polygalacturonases are virulence factors that hydrolyze homogalacturonans in the plant cell wall, and PGIPs are specific high-affinity receptors that inhibit fungal polygalacturonases. A role for PGIPs in defense signaling has been suggested because the intermediate oligogalacturonide breakdown products (of chain lengths 10 to 14) that can accumulate upon incomplete inhibition of fungal polygalacturonase apparently serve as defense-inducing compounds (Cervone et al., 1989).

Promising conceptual and experimental leads may also emerge from the similarity of many *R* gene products to proteins of unknown function, such as the LRR receptor kinases TMK1 and RLK5 (Walker, 1994). For example, the recent discovery of interaction between RLK5 and the KAPP protein

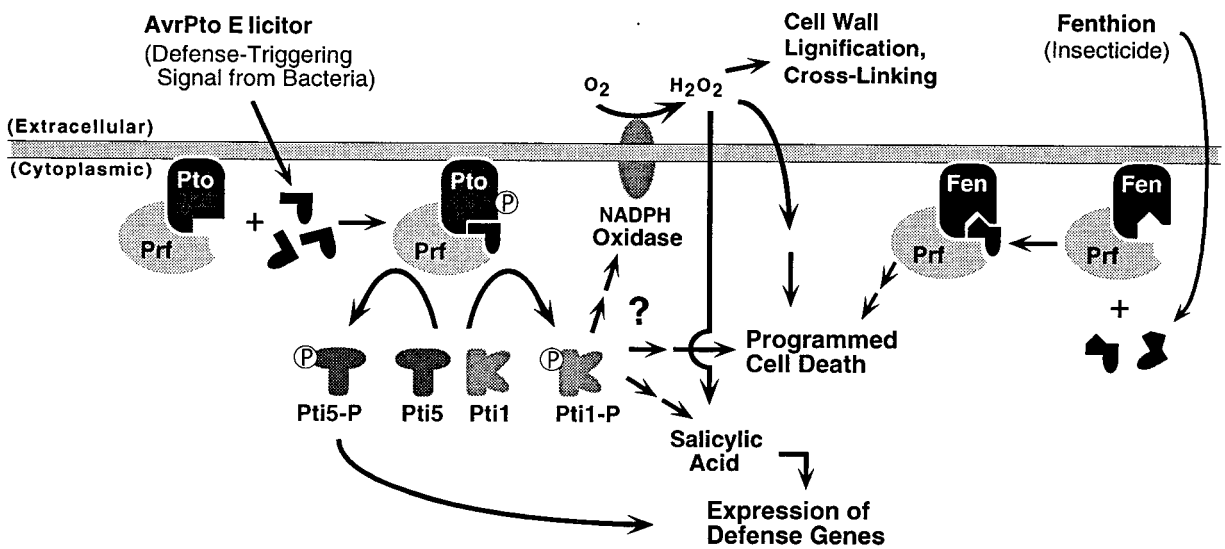

Figure 2. Hypothetical Model for Defense Signal Transduction.

In this model, the *R* gene products Pto and Prf associate at the inner face of the plasma membrane. Upon binding the *avrPto* elicitor, Pto autophosphorylates and then phosphorylates Pti1, a second kinase in the Pto kinase cascade. Pti1 then directly or indirectly activates intermediate signal transduction components, such as an NADPH oxidase enzyme complex (which generates an oxidative burst) and release of salicylic acid. These factors stimulate defense responses such as cell wall cross-linking and the expression of genes for defense proteins, such as β-1,3-glucanase and phenylalanine ammonia-lyase. Other responses activated by elicitation include ion channel gating and the induction of programmed cell death (the HR). A second signaling pathway leads from Pto directly to activation of the putative transcription factor Pti5, which promotes expression of defense genes. The Pto homolog Fen is involved in perception of a different ligand, the insecticide fenthion, and stimulates different but overlapping signal transduction cascades. This is one of many possible models, and models for the activity of other *R* gene products differ in many respects. For additional discussion, see the text and Dangl et al. (1996), Hammond-Kosack and Jones (1996), and Ryals et al. (1996), in this issue.

phosphatase (Stone et al., 1994) may foreshadow the discovery of protein phosphatases that are involved in defense signal transduction.

RESISTANCE GENES AND DEFENSE SIGNAL TRANSDUCTION

The *P. syringae*–tomato interaction involving *avrPto*, *Pto*, and *Prf* is one of the better developed examples of a gene-for-gene interaction, and this system has furnished a number of clues about the nature of signal transduction in plant defense. One such clue was the identification of *Fen*, a *Pto R* gene homolog that confers sensitivity to the insecticide fenthion (Martin et al., 1994; Rommens et al., 1995a). When the *Pto* gene was originally bred into tomato varieties from the wild relative *Lycopersicon pimpinellifolium*, a second trait, sensitivity to the insecticide fenthion, was accidentally cointrogressed. The traits showed complete linkage, but mutational studies demonstrated that *Pto* function (i.e., *avrPto*-specific resistance) is separable from fenthion sensitivity (Salmeron et al., 1994). After the *Pto* kinase gene had been cloned, it was shown to be part of a tightly clustered multigene family, and subsequent work showed that one of these homologs is *Fen* (Martin et al., 1994; Rommens et al. 1995a). Fen and Pto are 87% similar in amino acid sequence, and the identification of Fen as a functioning homolog of Pto raises the question as to the roles of the other Pto homologs encoded in this gene cluster.

The striking significance of this work emerged with the identification of *Prf*, an LZ–NBS–LRR *R* gene that is required, along with the Pto kinase, for resistance to *P. syringae* expressing *avrPto* (Table 1). In fact, it turns out that *Prf* is required for both *avrPto*-specific resistance and fenthion sensitivity (Salmeron et al., 1994). A simple linear model of defense signal transduction might place an LRR protein (a candidate receptor of the avirulence signal) upstream of the kinase signal generator. With *Prf*/*Pto*/*Fen*, however, specificity (in this case, distinction between fenthion and the *avrPto* signal) is conferred by the distinct kinase proteins and not by the LRR protein. A more likely model for this system might postulate physical interaction of Prf with either Pto or Fen to form alternative receptor complexes with different ligand specificities. This model is presented in Figure 2.

The *Pto* gene was recently used in yeast two-hybrid interaction cloning (Fields and Song, 1989) to identify Pti1, a second serine–threonine protein kinase that participates in the *avrPto*-specific defense response (Zhou et al., 1995). In studies with fusion proteins expressed from *Escherichia coli*, Pti1 was found to be a substrate for autophosphorylation and for phosphorylation by Pto. Pto was not a substrate for phosphorylation by Pti1. These data suggest that Pti1 acts downstream of Pto in a protein kinase cascade (see model in Figure 2). No phosphorylation was observed between Pti1 and Fen, suggesting that Pti1 is part of a kinase cascade that is specific to *avrPto–Pto* signaling. A functional role for Pti1 in defense seems likely because expression of *Pti1* in transgenic tobacco enhances the HR of these plants in response to *P. syringae* expressing *avrPto* (Zhou et al., 1995). This important work not only provides strong evidence for signal transduction via a kinase cascade in this particular defense response, but it also suggests that similar kinase cascades may be operative in a number of other gene-for-gene systems.

A number of mutant alleles of *Pto* have been isolated, and many of these alleles confer a level of resistance that is intermediate between that observed in *Pto*/*Pto* (i.e., wild-type) plants and plants entirely lacking the *Pto* gene (Salmeron et al., 1994). This result points to the quantitative nature of the defense response triggered by many gene-for-gene systems. Other biological systems that utilize protein kinase cascades often show a similar capacity for gain modulation of the transduced signal (e.g., see Hafen et al., 1994). The intensity of the plant defense response can be modulated by numerous components ultimately involved in a single gene-for-gene defense system (see also Crute and Pink, 1996; Dangl et al., 1996; Hammond-Kosack and Jones, 1996; and Ryals et al., 1996, in this issue), and the characterization of genes known only as modifiers of resistance intensity is likely to become an important area for future research.

Further information about defense signal transduction has come from study of the *avrPto–Pto* system with the recent identification of Pto-interacting proteins that have similarity to transcription factors (G. Martin, personal communication). The Pto-interacting proteins, termed Pti4, Pti5, and Pti6, strongly resemble ethylene-responsive element binding proteins of tobacco. As transcription factors, these tobacco proteins bind to a PR-box DNA sequence that is conserved within the promoters of the genes for many PR proteins. Expression of PR proteins is a common response to infection in the interaction of a wide variety of plants and microbes (Dixon and Lamb, 1990; Dixon et al., 1994), including interactions involving *avrPto* and *Pto* (G. Martin, personal communication). Binding to a PR-box sequence has been experimentally confirmed for Pti5 and Pti6. The identification of these putative transcription factors preliminarily suggests a direct mechanism linking perception of an avirulent pathogen to the expression of one segment of the plant defense response (see model in Figure 2).

The above work and other studies with the tomato *Pto* gene in tobacco also provide the first published examples of transfer of disease resistance genes from one plant species to another (Rommens et al., 1995b; Thilmony et al., 1995). This topic, which is taken up in later sections, is of tremendous interest because of the potential for engineering resistance to disease in crop plants.

Avirulence Genes and Production of the Ligand

The perceptive reader will have noticed the one-sidedness of the above discussion, in which little attention is given to the

avirulence signal of the pathogen. What are the ligands that trigger the HR? Functionally, the products of some *Avr* genes play a disease-causing role (De Wit, 1992; Van Gijsegem et al., 1995; see also Alfano and Collmer, 1996; and Knogge, 1996, in this issue). This helps to explain why a pathogen would persist in producing a defense-eliciting signal. For many *Avr* genes, however, no obvious role in virulence can be detected.

The first pathogen *Avr* gene was cloned more than a decade ago, and numerous *Avr* genes have been isolated since that time (De Wit, 1992; Van Gijsegem et al., 1995). In most instances, the nucleotide and derived amino acid sequences of these genes have revealed very little about biochemical function. However, a few carefully studied *Avr* gene products have provided fascinating information about pathogen biology and the molecular basis of avirulence. One well-supported concept is that the protein encoded by an *Avr* gene can directly serve as the defense-eliciting ligand. Particularly extensive study has been made of two classes of avirulence protein: the coat protein of tobacco mosaic virus (TMV) (which triggers resistance in tobacco plants expressing the *N'* resistance gene; Taraporewala and Culver, 1996), and Avr9 and similar peptides of *C. fulvum* (which are recognized by tomato plants expressing *Cf-9* and similar *R* genes; van Kan et al., 1991). Impressive structure–function data relevant to defense elicitation have been gathered for the TMV coat protein, but the corresponding plant receptor has not been identified. *Avr9* is of interest because the elicitor has been isolated and a corresponding *R* gene has been cloned. The actual Avr9 elicitor is a small 28–amino acid peptide derived from a large Avr9 translation product by proteolytic cleavage (van Kan et al., 1991). Interestingly, both resistant tomato lines and lines without the *Cf-9* gene express a high-affinity binding site for the Avr9 elicitor (Kooman-Gersmann et al., 1996), and direct binding between Avr9 and Cf-9 has not been reported to date. The cysteine-rich NIP1 protein of the barley pathogen *Rhynchosporium secalis* and the coat protein of potato virus X are additional examples of known *Avr* gene protein products that serve as *R* gene–specific elicitors of defense (Bendahmane et al., 1995; Rohe et al., 1996).

More recently, a number of laboratories have obtained evidence indicating that the protein products of *Avr* genes such as *avrPto*, *avrB*, and *avrBs3* from *P. syringae* and *Xanthomonas campestris* pathogens can also directly elicit *R* gene–dependent plant defenses (see Alfano and Collmer, 1996, in this issue). Elicitation of a plant defense response by these bacterial *Avr* gene products apparently requires delivery into the plant cell via a bacterial type III protein secretion apparatus (Alfano and Collmer, 1996, in this issue). In the case of AvrPto, direct physical interaction with the Pto serine–threonine kinase is suggested by yeast two-hybrid analyses (G. Martin, personal communication; S. Scofield, personal communication). The *avrBs3* example is particularly provocative because this family of *Avr* genes has been shown to encode proteins with functional plant nuclear localization signals (Y.N. Yang and Gabriel, 1995).

Avr genes can also play an indirect role in the production of *R* gene–specific elicitors. For example, *P. s. tomato* strains that express *avrD* produce a family of chemically similar elicitors that are low-molecular-weight organic compounds known as syringolides (Midland et al., 1993). The *avrD* gene apparently does not encode a proteinaceous elicitor but is instead postulated to encode an enzyme involved in the synthesis of these syringolides. For the *avrD* system and for all of the other gene-for-gene systems under study, an important future goal is to document and study the physical interaction between race-specific elicitors and their receptors.

The term gene-for-gene was coined to signify the matching specificity between *Avr* and *R* genes, but it need not suggest that one and only one *R* gene exists for a given *Avr* gene. The *Pto* and *Prf* genes, which encode biochemically distinct components of the same pathway (Table 1), provide only one of many examples of multiple *R* genes involved in the detection of the same *Avr* genotype. A conceptually distinct example of this is the two genes at the *Cf-2* locus (Table 1); in this case, the two separate *R* genes furnish essentially identical functions (Dixon et al., 1996). A third type of deviation from narrow interpretation of the term gene-for-gene has also been documented in which one *R* gene confers specificity for what are likely to be two different ligands. The Arabidopsis *R* gene *RPM1* confers resistance against *P. syringae* expressing either *avrB* or *avrRpm1*, two well-characterized and entirely dissimilar *Avr* genes (Bisgrove et al., 1994). Although their molecular structures have not been determined, the *avrB*-encoded elicitor is likely to be distinct from that encoded by *avrRpm1*; soybean individuals have been identified that are resistant to pathogens expressing *avrB* but susceptible to pathogens expressing *avrRpm1* and vice versa (Ashfield et al., 1994). As discussed above, specificity for multiple ligands has also been documented for *Prf* (Salmeron et al., 1994).

In considering potential receptors for the elicitors produced as a result of *Avr* gene expression, the geography of elicitor presentation and the subcellular location of *R* gene products are important to consider. *C. fulvum*, for example, grows as an extracellular pathogen, whereas TMV propagates within the plant cell cytoplasm. The apparent extracellular orientation of most of the Cf-9 protein and the probable intracellular location of the *N* gene product seem logical in this regard (Jones et al., 1994; Whitham et al., 1994). *P. syringae* pv *tomato* and pv *maculicola* are extracellular bacterial pathogens, yet the products of the *Pto*, *Prf*, *RPS2*, and *RPM1* *R* genes appear from their amino acid sequences to be cytoplasmic. An explanation for this apparent inconsistency is provided by the postulated secretion of the corresponding *Avr* gene products into the plant cell (discussed above). The rice *Xa21* gene apparently encodes a transmembrane receptor kinase (see Table 1 and above), and it will be interesting to learn whether the corresponding avirulence signal from *X. campestris* pv *oryzae* is presented to the plant without transmission through the type III secretion apparatus used by related bacterial pathogens.

An *Avr* gene is most likely to provide a stable target for plant disease resistance if loss of that gene carries some fitness penalty. As was stated above, some *Avr* genes can be lost with

no obvious penalty, whereas others contribute to the virulence of the pathogen on plants lacking resistance. Loss of elicitor production by the pathogen can have serious ramifications in agricultural situations: the effectiveness of single *R* genes in preventing disease is often compromised by shifts in pathogen populations toward individuals lacking avirulence (Agrios, 1988; Keen et al., 1993). One mechanism for loss of avirulence can be through subtle changes in the structure of the elicitor that presumably alter binding by the plant receptor. The TMV coat protein is an example of an *Avr* gene that is essential for virulence but for which variant forms have been observed in which single amino acid changes result in loss of avirulence (Taraporewala and Culver, 1996). Single amino acid changes that eliminate avirulence have also been observed in *C. fulvum* Avr peptides (Joosten et al., 1994). For other bacterial and fungal *Avr* genes, complete loss of avirulence caused by major gene rearrangements, insertion of cryptic transposons, chromosomal deletion, or loss of extrachromosomal plasmids has been observed (De Wit, 1992; Van Gijsegem et al., 1995). One particularly interesting example of *Avr* gene evolution involves the *avrBs3* family of bacterial *Avr* genes (Herbers et al., 1992). These genes encode variable numbers of serially repeated, highly similar 34–amino acid repeats. Manipulation of the number of these repeats or the sequence within individual repeats cannot only cause loss of original avirulence (with respect to a particular *R* gene) but can also generate new *Avr* gene alleles that reveal previously unknown plant resistance specificities (Herbers et al., 1992; Y.O. Yang and Gabriel, 1995).

Downstream Events in Defense Signal Transduction

After an avirulent pathogen has generated a defense-eliciting signal and the plant has perceived it, what downstream events are stimulated? Gene-for-gene interactions can induce signaling responses, such as activation of protein kinases, stimulation of an oxidative burst, and induction of ion fluxes across the cellular membrane. These and other events in turn activate defense responses, such as cell wall cross-linking and the expression of defense-related genes (Figure 2; Dixon et al., 1994; Levine et al., 1994; see also Dangl et al., 1996; Hammond-Kosack and Jones, 1996; and Ryals et al., 1996, in this issue). A key question at this time concerns how these downstream events are activated by *R* gene products. For Xa21 and Prf–Pto, it is likely that protein kinase activity follows immediately from pathogen recognition, but the signals generated by other *R* gene products and the downstream moieties that receive these signals are not known.

A variety of biochemical, genetic, and molecular biological approaches are likely to be productive in dissecting *R* gene–mediated defense signal transduction. The importance of downstream events, such as the oxidative burst, has led researchers to focus on the cellular components that perform these processes (e.g., Dwyer et al., 1995; Desikan et al., 1996; see also Hammond-Kosack and Jones, 1996, in this issue). Observation that a biochemical stimulus can elicit a defense-related response does not, however, demonstrate a causal requirement for that process during defense. For example, ethylene is a known inducer of PR gene expression, but responsiveness to ethylene is not required for effective expression of gene-for-gene disease resistance (Bent et al., 1992). Interaction cloning, in which one protein is used to identify a physically interacting protein and its corresponding gene (Guarente, 1993), offers a promising avenue for research because one can document direct physical involvement with a known component of a gene-for-gene system. Pti1 is an example of an apparent defense protein that was identified by interaction cloning (Zhou et al., 1995). However, it is important to document a biological role for candidate signal transduction components such as Pti1; this is often achieved using antisense suppression or transgenic overexpression of the gene for the candidate compound. Mutational analysis will continue to be an extremely reliable method to identify genes likely to control downstream events in gene-for-gene pathways (e.g., Freialdenhoven et al., 1994; Hammond-Kosack et al., 1994b; Century et al., 1995). Study of quantitative trait loci that affect gene-for-gene resistance (Michelmore, 1995) may also contribute to progress in this area.

One downstream component of defense signal transduction that deserves particular emphasis is salicylic acid. Salicylic acid is a key signaling intermediary that triggers systemic acquired resistance, a process in which localized infections induce a quantitative enhancement of resistance both locally and in tissues distant from the site of original infection (see Ryals et al., 1996, in this issue). Salicylic acid had previously been shown to induce responses that lessen the damage caused by virulent pathogens, but recent work suggests that salicylic acid is also an important mediator of defense responses critical to gene-for-gene resistance against avirulent pathogens (Figure 2; Cao et al., 1994; Delaney et al., 1994, 1995; Ryals et al., 1996, in this issue).

When plants are infected by different pathogens expressing different *Avr* genes, are the resulting downstream responses identical or divergent? From visual inspection of infected tissues, it has been clear for many years that different gene-for-gene pairings can trigger overlapping but distinct resistance responses (e.g., Minsavage et al., 1990; Holub et al., 1994). This was recently demonstrated at the level of induced gene expression by using an isogenic system in which Arabidopsis plants carrying *RPM1* and/or *RPS2* were infected with *P. syringae* pv *maculicola* expressing *avrRpm1* and/or *avrRpt2* (Reuber and Ausubel, 1996; Ritter and Dangl, 1996). Differences were observed both in the time of appearance of the HR and in the constellation of specific PR mRNAs that were induced. Surprisingly, the presence of one avirulent pathogen could block the plant responses characteristic of infection by the other pathogen. This interference was dose dependent in that it could be overcome by shifting the relative titers of the two pathogens (Reuber and Ausubel, 1996; Ritter and Dangl, 1996). The *avrRpm1* and *avrRpt2* elicitors seem unlikely to bind the same receptor because they exhibit

specificity for different *R* genes and trigger different defense responses. They may, however, compete for shared components of heterogeneous multisubunit receptors or for downstream components of the signaling pathway. An alternative hypothesis is that the two *Avr* gene products are enzymes that compete to modify related precursors of the final elicitor compounds.

In addition to studying the mechanisms that lead to the induction of defense responses, it is important to understand how plants prevent excessive propagation of those responses. One approach to examining this subject utilizes disease lesion-mimic mutants (see Dangl et al., 1996, in this issue). These mutant plants develop disease-like lesions in the absence of pathogens or in the presence of opportunistic saprophytes that do not normally cause disease. A wide variety of genetic lesions could cause lesion-mimic phenotypes. The expectation has been that some of these mutations will reveal genes important in disease development and resistance, however, and work with lesion-mimic mutants is turning out to overlap with the study of *R* gene–mediated defense in a variety of ways. For example, Johal and colleagues have used transposon mutagenesis to tag *Lls1* of maize, a gene that apparently plays a role in restricting or delimiting programmed cell death (G. Johal, personal communication). Plants with mutations in this gene develop spreading regions of dead tissue that expand well past infected areas and eventually consume entire leaves. Further analysis of genes such as this should lead to a greater appreciation for the homeostatic mechanisms that limit the severity and spatial extent of defense responses (see also Dangl et al., 1996, in this issue).

RESISTANCE GENE FAMILIES AND THE GENERATION OF NEW RESISTANCE SPECIFICITIES

How do new *R* genes arise? Most cloned *R* genes, despite gross similarities such as LRR-encoding sequences, are highly divergent at the DNA sequence level. Nevertheless, multigene families of relatively similar sequences have been detected for most cloned *R* genes. This often extends to the presence of similar DNA sequences in other plant species (see also references in Table 1). In a given plant line, members of these gene families can be tightly linked, entirely unlinked, or can occur in both linked and unlinked clusters in the genome. The demonstration of these gene families is not particularly surprising given that classical genetic studies have documented many instances in which *R* genes occur in tightly linked clusters (see Crute and Pink, 1996, in this issue). Different loci in these clusters often encode resistance against different races of the same pathogen species.

For both tightly linked and unlinked but related genes, gene duplication followed by divergence is the most likely source of new resistance specificities. For example, studies of the *Rp1* resistance locus of maize provide direct evidence for unequal crossing over within clusters of similar sequences as a mechanism that could accelerate the appearance of new *R* gene alleles (Sudapak et al., 1993). Recent studies with *Rp1* have confirmed these findings and have also drawn another link between *R* genes and lesion-mimic phenotypes: new lesion-mimic genes have been identified whose genesis can be attributed to unequal crossing over in the small chromosomal region carrying *Rp1* genes (Hu et al., 1996). In separate studies involving the *M* cluster of *R* genes in flax, unequal exchange between LRR-encoding sequences both within and between adjacent *M* genes has been observed (J. Ellis, personal communication). It is interesting to note the parallel between recombinational generation of new resistance specificity in plants and the mechanisms that generate immunological diversity in mammals. It is also worth noting that recombination can generate new avirulence specificities in pathogens, as has been described for the *avrBs3* family of *Avr* genes from *Xanthomonas* (Herbers et al., 1992; Y.O. Yang and Gabriel, 1995). On an entirely different note, alternative splicing of *R* gene transcripts bears mention as an additional mechanism that may supply a degree of resistance diversity (Whitham et al., 1994; Lawrence et al., 1995).

There is evidence not only for the conservation between species of highly similar *R* gene DNA sequences but also for functional conservation of resistance that is specific for a given *Avr* gene. An early and far-reaching example of this functional conservation came from studies with *X. campestris* pathogens expressing the *Avr* gene *avrRxv*, which was first isolated from the tomato pathogen *X. c. vesicatoria* (Whalen et al., 1988). Expression of cloned *avrRxv* in other originally virulent *X. campestris* strains that are pathogens of other plants (such as cabbage or corn) revealed the presence of corresponding resistance specificity in these plant species as well. Resistance specificity for the identical *Avr* gene in diverse plant species has now been shown in many other cases and has been observed using *avrRpt2*, *avrRpm1*, and *avrPto* (genes for which a corresponding *R* gene has been cloned; Van Gijsegem et al., 1995). The discovery of functionally similar resistance between Arabidopsis and major crop plants such as soybean had suggested that Arabidopsis could be used as a source of *R* genes for these crops (Whalen et al., 1991; Innes et al., 1993), but this concept has not been fully exploited to date. The molecular basis of functionally similar resistance is assumed to reside in structural similarity between the relevant *R* genes, but again this has not been experimentally demonstrated.

ENGINEERING OF DISEASE RESISTANCE

Plant breeders have for many years utilized wild relatives of crop plants as a source for new *R* genes, and they have become very adept at introgressing *R* genes into elite varieties. The availability of cloned *R* genes now opens up possibilities for addition of new *R* genes to a plant line by genetic transfor-

mation. Widespread use of this transformation approach will not occur unless it can match or exceed traditional plant breeding in reliability, versatility, and cost effectiveness. However, genetic transformation seems likely to be incorporated into resistance breeding programs in the near future, given the exciting and powerful new capability of transferring *R* genes across species barriers and of deploying *R* genes that have been modified by rational design.

R genes must be cloned and available before they can be used in plant transformation. An important new method for the isolation of these candidate *R* genes utilizes the polymerase chain reaction (PCR). Although the DNA sequences of the *R* genes listed in Table 1 are highly divergent overall, small regions of close similarity can often be identified in the derived amino acid sequences (discussed above; see also Grant et al., 1995; Staskawicz et al., 1995). PCR primers that have been designed to detect these highly conserved regions are being used in a number of laboratories to identify candidate *R* genes. Gebhardt and colleagues, for example, have used primers based on sequences that are highly conserved between *RPS2* and *N* to identify a number of PCR products, including a gene that is very closely linked to *Gro1* of potato (Leister et al., 1996). These results are particularly exciting because *Gro1* is a race-specific gene encoding resistance against root cyst nematode pathogens of potato and no published examples of a cloned nematode *R* gene are available. However, it is impossible at this time to use derived amino acid sequence data to confirm the *Avr* gene specificity of a given *R* gene homolog. Experiments to assess genetic linkage between a candidate DNA and a locus that encodes the relevant resistance phenotype are an important first test, but *R* genes often occur in tight clusters. Confirmation that a candidate *R* gene encodes the appropriate specificity typically requires functional complementation of a susceptible genotype by transformation.

Although the PCR approach discussed above will form an increasingly important method for the isolation of new *R* genes, all of the confirmed *R* genes that have been cloned to date were isolated using the methods of positional cloning or transposon tagging (see references in Table 1). Positional cloning and transposon tagging can be technically challenging, but improved protocols and biological resources are consistently being developed. Other methods to clone *R* genes may also become feasible as more is learned. One standard molecular biology method that is now applicable is the screening of genomic or cDNA libraries using *R* gene sequences as probes. It may also become possible to use antibodies raised against specific *R* genes to isolate clones for homologous genes. Computer data base searches to identify expressed sequence tag clones resembling known *R* genes are an additional possibility, and expression cloning (functional complementation by transient transformation with random genomic or cDNA library clones) may also become a feasible approach. All of the above methods have specific benefits and drawbacks, and our ability to execute these methods will have to improve if the cloning of *R* genes is to be made routine (Keen et al., 1993).

A landmark goal in the engineering of plants for improved disease resistance has been to transfer resistance between species by genetic transformation with an *R* gene. This has now been achieved with the transfer of *Pto* to tobacco (Rommens et al., 1995b; Thilmony et al., 1995). Clearly, the product of an *R* gene must be compatible with other components of the defense apparatus in order to function, and transfer of *R* genes between species cannot be expected to impart resistance in all cases. Transfers of *Pto* and *RPS2* between tomato and Arabidopsis, for example, have not yielded plants with expanded resistance. Tomato and tobacco are closely related, and success in transferring *Pto*-mediated resistance to tobacco has been mirrored by the successful transfer of *N* gene–mediated virus resistance from tobacco to tomato (Whitham et al., 1996). These results may foreshadow a general trend in which the expression of essentially unmodified *R* genes will be most successful in *R* gene transfers between closely related species.

A more straightforward and possibly more common use of cloned *R* genes will be in the transformation of different varieties within the original source species. Indeed, same-species addition of new resistance has already been successfully demonstrated in a number of cases (see references in Table 1). Based on the long history of success in adding *R* genes through traditional plant breeding, same-species transfers of *R* genes are likely to succeed in most cases. As noted above, however, use of genetic transformation to deploy *R* genes will only be preferable over traditional breeding when substantial savings can be realized in terms of time, labor, expense, effectiveness, or as a means to avoid disruption of highly developed and commercially desirable genotypes.

The most exciting approach toward engineering improved resistance to disease may be the creation of new *R* genes in the laboratory. Initially, it may be most effective to swap domains between *R* genes that are otherwise unmodified. This approach was recently demonstrated with the highly similar *Fen* and *Pto* genes, when genes with the opposite ligand specificity were created by domain swapping (Rommens et al., 1995a). More refined rational design strategies are likely to become prominent as more is learned about the molecular basis of pathogen recognition and the subsequent induction of defense cascades. The challenge here will be to not simply generate new resistance specificities but to synthesize *R* genes that detect a broad range of pathogen genotypes. The new *R* genes should detect a high percentage of the individuals in the pathogen population and should be targeted toward durable pathogen traits that are likely to remain present even in the face of strong selection for isolates that do not exhibit avirulence.

Two strategies in particular are being pursued that use *R* genes to engineer broader spectrum and more durable disease resistance. One is to identify pathogen traits that contribute to virulence (e.g., Kearney and Staskawicz, 1990) and then to focus on the cloning or design of *R* genes that confer recognition based on those traits. A second, more

complex strategy involves the generation of plants that express a pathogen *Avr* gene (such as *Avr9*) under the control of a heterologous, infection-inducible promoter (De Wit, 1992; Hammond-Kosack et al., 1994a). If the plant also carries the corresponding *R* gene (such as *Cf-9*), it will respond with an HR in the region in which expression of the *Avr* gene has been triggered. This strategy has been demonstrated experimentally, and it has several advantages (Hammond-Kosack et al., 1994a). First, pathogen specificity is very broad (resistance is effective against any pathogen that induces expression of the chosen promoter). Second, the multifactorial defense response mediated by the *R* gene is more effective than is the expression of a single defense gene such as chitinase. Third, defense is theoretically expressed only in infected tissues. This last point represents an unsolved challenge, however. It is essential to utilize an infection-specific promoter that is very tightly regulated, or else the defense response will be induced in inappropriate tissues or at inappropriate developmental stages.

As newly designed *R* genes are released into the field, it will be important to consider older plant breeding and cultivar release strategies that could enhance the durability of disease control (see Crute and Pink, 1996, in this issue). These include the pyramiding in a single plant of multiple *R* genes with specificity for a given pathogen (to exploit the minimal likelihood that a single pathogen would simultaneously lose the corresponding *Avr* genes), release of varieties with different *R* genes in alternate growing seasons, or the planting of mixed resistant and susceptible seed or susceptible refuge plots so that avirulent pathogen genotypes remain predominant over rare virulent strains in the local pathogen population.

CONCLUSION

The isolation and preliminary characterization of *R* genes has been achieved, and a number of more precisely defined challenges now present themselves as we seek to understand the molecular basis of gene-for-gene plant disease resistance. Where, for instance, do *R* gene products localize in the cell? Information to date has been based almost exclusively on comparative sequence analysis. Do *R* gene products truly serve as the elicitor-binding receptors, and if they do not, then what does? There are very exciting possibilities for structure–function research into the mechanisms of pathogen detection and signaling initiation, ranging from sequence comparison, domain swapping, and site-specific mutagenesis to determination of crystal structures. Efforts to define how *R* gene products interface with downstream (or upstream) components of defense signal transduction will be very important. Diverse approaches, such as mutational analysis, interaction cloning, analysis of quantitative trait loci, microscopy, enzyme biochemistry, and electrophysiology, are all likely to come into play in this regard. As the other reviews in this issue demonstrate, *R* genes are only one of many components of the plant defense machinery that merit further study. In gene-for-gene systems, function (i.e., the resistance phenotype) has met structure with the isolation of *R* genes. We now turn to functional studies at the cellular and molecular level as we seek to understand the powerful phenotype conferred by plant disease resistance genes.

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