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UNDERSTANDING THE FUNCTIONS OF PLANT DISEASE RESISTANCE PROTEINS

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■ **Abstract** Many disease resistance (R) proteins of plants detect the presence of disease-causing bacteria, viruses, or fungi by recognizing specific pathogen effector molecules that are produced during the infection process. Effectors are often pathogen proteins that probably evolved to subvert various host processes for promotion of the pathogen life cycle. Five classes of effector-specific R proteins are known, and their sequences suggest roles in both effector recognition and signal transduction. Although some R proteins may act as primary receptors of pathogen effector proteins, most appear to play indirect roles in this process. The functions of various R proteins require phosphorylation, protein degradation, or specific localization within the host cell. Some signaling components are shared by many *R* gene pathways whereas others appear to be pathway specific. New technologies arising from the genomics and proteomics revolution will greatly expand our ability to investigate the role of R proteins in plant disease resistance.

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INTRODUCTION

Plants are attacked by many disease-causing organisms including bacteria, fungi, viruses, and nematodes. These pathogens cause large crop losses and probably since the beginning of agriculture have contributed to human hunger and malnutrition. The control of plant diseases is thus of fundamental importance and is a major objective of plant-breeding and pathology programs and the agricultural chemical industry. Plants resist pathogen attacks both with preformed defenses such as antimicrobial secondary compounds and by inducing defense responses (72, 74). Inducible defenses can be activated upon recognition of general elicitors such as bacterial flagellin and even host cell fragments released by pathogen damage (65, 72). However, plants have also evolved sophisticated recognition systems to detect proteins produced during infection by specific races of pathogens. These proteins, referred to here as effectors, are recognized by plant disease resistance (R) proteins in a highly specific manner first described genetically as the gene-for-gene interaction (59). The identification of many *R* genes, and in many cases their corresponding effector proteins, has accelerated research into the molecular basis of gene-for-gene disease resistance (38, 112).

Classes of Disease Resistance (R) Proteins

The majority of R proteins that are activated upon effector recognition fall into five classes based primarily upon their combination of a limited number of structural motifs (Table 1). Class 1 consists of just one member, Pto from tomato, which has a serine/threonine kinase catalytic region and a myristylation motif at its N terminus (104, 113). Class 2 comprises a large number of proteins having a region of leucine-rich repeats (LRRs), a putative nucleotide binding site (NBS), and an N-terminal putative leucine-zipper (LZ) or other coiled-coil (CC) sequence. Class 3 is similar to class 2 but instead of the CC sequence these proteins have a region with similarity to the N terminus of the Toll and Interleukin 1 receptor (IL-1R) proteins that is

TABLE 1 Plant disease resistance (R) proteins

Class	*R Protein	Plant	Pathogen(s) or Pest(s)	Effector(s)	Reference(s)
1	Pto	Tomato	*Pseudomonas syringae* (B)	AvrPto, AvrPtoB	(91, 113, 151)
2	Bs2	Pepper	*Xanthomonas campestris* (B)	AvrBs2	(120, 172)
	Dm3	Lettuce	*Bremia lactucae* (F)		(117)
	Gpa2[a]	Potato	*Globodera pallida* (N)		(190)
	Hero	Potato	*G. rostochiensis, G. pallida* (N)		(56)
	HRT[b]	*Arabidopsis*	Turnip Crinkle Virus	Coat Protein	(41)
	I2	Tomato	*Fusarium oxysporum* (F)		(127, 167)
	Mi	Tomato	*Meloidogyne incognita* (N)		(118)
	Mi	Tomato	*Macrosiphum euphorbiae* (I)		(152, 192)
	Mla	Barley	*Blumeria graminis* (F)		(210)
	Pib	Rice	*Magnaporthe grisea* (F)		(194)
	Pi-ta	Rice	*M. grisea* (F)	AVR-Pita	(26, 126)
	R1	Potato	*Phytophthora infestans* (O)		(8)
	Rp1	Maize	*Puccinia sorghi* (F)		(39)
	RPM1	*Arabidopsis*	*P. syringae* (B)	AvrRpm1, AvrB	(44, 68, 174)
	RPP8[b]	*Arabidopsis*	*Peronospora parasitica* (O)		(115)
	RPP13	*Arabidopsis*	*P. parasitica* (O)		(16)
	RPS2	*Arabidopsis*	*P. syringae* (B)	AvrRpt2	(12, 119, 197)
	RPS5	*Arabidopsis*	*P. syringae* (B)	AvrPphB	(82, 195)
	Rx1[a]	Potato	Potato Virus X	Coat Protein	(10)
	Rx2	Potato	Potato Virus X	Coat Protein	(10, 11, 138)
	Sw-5	Tomato	Tomato Spotted Wilt Virus		(24)
	Xa1	Rice	*X. oryzae* (B)		(203)
3	L	Flax	*Melampsora lini* (F)		(97)
	M	Flax	*M. lini* (F)		
	N	Tobacco	Tobacco Mosaic Virus	Helicase	
	P	Flax	*M. lini* (F)		(51)
	RPP1	*Arabidopsis*	*P. parasitica* (O)		(22)
	RPP4	*Arabidopsis*	*P. parasitica* (O)		(183)
	RPP5	*Arabidopsis*	*P. parasitica* (O)		(130)
	RPS4	*Arabidopsis*	*P. syringae* (B)	AvrRps4	(62, 76)
4	Cf-2[c]	Tomato	*Cladosporium fulvum* (F)	Avr2	(49, 106)
	Cf-4[d]	Tomato	*C. fulvum* (F)	Avr4	(88, 177)
	Cf-5[c]	Tomato	*C. fulvum* (F)		(49)
	Cf-9[d]	Tomato	*C. fulvum* (F)	Avr9	(87)
5	Xa21	Rice	*Xanthomonas oryzae* (B)		(168)

(*Continued*)

TABLE 1 (*Continued*)

Class	*R Protein	Plant	Pathogen(s) or Pest(s)	Effector(s)	Reference(s)
6	Hm1	Maize	*Cochliobolus carbonum* (F)		(86)
	$HS1^{pro-1}$	Beet	*Heterodera schachtii* (N)		(28)
	mlo	Barley	*B. graminis* (F)		(27)
	Rpg1	Barley	*Puccinia graminis* (F)		(25)
	RPW8	*Arabidopsis*	*Erisyphe chicoracearum* (F)		(200)
	RRS1-R	*Arabidopsis*	*Ralstonia solanacearum* (B)		(46)
	RTM1	*Arabidopsis*	Tobacco Etch Virus		(36)
	RTM2	*Arabidopsis*	Tobacco Etch Virus		(199)
	$Ve1^{e}$, $Ve2^{e}$	Tomato	*Verticillium alboatrum* (F)		(90)

*Shown are R proteins characterized to date, sorted by structural class (see text). The host plant and corresponding pathogen(s) or pest(s) and effector proteins, where known, are given for each. Except for viruses, pathogen or pest type is indicated in parentheses, abbreviated as: B, bacterium; F, fungus; I, insect; N, nematode, O, oomycete. The bottommost proteins do not fit in any of the designated structural classes and are discussed briefly in the text. Highly similar members of the same gene cluster are identified by matching superscript letters.

therefore referred to as the TIR region. The R proteins belonging to the first three classes lack transmembrane (TM) domains and all are thought to be localized intracellularly; this has implications, which are discussed below, for how pathogen effector proteins are delivered to the plant cell and where R proteins act to intercept these proteins. The Cf proteins from tomato form class 4. They lack an NBS and instead have a TM and an extracellular LRR, and a small putatively cytoplasmic tail without obvious motifs (50, 87). Finally, class 5 consists of just the Xa21 protein from rice, which, in addition to an extracellular LRR and a TM, has a cytoplasmic serine/threonine kinase region (168). The functions of R protein structural motifs have been investigated to various degrees and are discussed below.

A few R proteins do not fit into these five classes (Table 1). Hm1 is a toxin reductase that confers resistance to a fungal pathogen of maize (86). Mlo in barley is an apparent membrane protein for which recessive mutant alleles confer resistance to powdery mildew, and may be a negative regulator of defense responses (27). RPW8 confers resistance in *Arabidopsis* to powdery mildew in a non-race-specific way (200). Other R proteins may act in specific recognition, but have novel structures. For example, $Hs1^{pro1}$ for resistance to a sugar beet nematode has a structure without obvious protein interaction domains (28). The Ve proteins for resistance to *Verticillium* in tomato are putative cell-surface glycoproteins with receptor-mediated endocytosis-like signals (90). RTM1 and RTM2 restrict systemic movement of tobacco etch potyvirus in resistant *Arabidopsis* ecotypes and display jacalin-like sequences and similarity to a small heat shock–like protein, respectively (36, 199). RRS1-R is a TIR-NBS-LRR protein, but is unusual in that it provides strain-nonspecific resistance to *Ralstonia solanacearum*, is recessive genetically, and contains in its C terminus a putative nuclear localization signal and

a 60–amino acid motif characteristic of the WRKY family of plant transcriptional activator proteins (46). Recently, the Rpg1 protein for resistance to barley stem rust was found to contain two tandem protein kinase domains and a predicted weak transmembrane domain (25). In this chapter, we generally limit our discussion to members of the five well-characterized classes described above.

Model Plant-Pathogen Systems for Studying R Protein Function

The use of model plant-pathogen systems has greatly accelerated the cloning and characterization of *R* genes. Because of the experimental tractability of *Arabidopsis*, the majority of *R* genes has been isolated from that species (Table 1). However, many *R* genes also have been isolated from solanaceous species (tomato, potato, pepper, and tobacco), and from barley, rice, and flax. These species have long histories of research on the genetics of their pathogen interactions, which has facilitated the cloning of their *R* genes. The importance of using a broad range of plant species for this research is evident from the fact that, to date, no *R* genes have been cloned from *Arabidopsis* that fall into the Pto, Cf, or Xa21 classes. Clear homologs of each of these genes are present in *Arabidopsis*, however, and it is possible that some will be found to function in resistance [e.g., FLS2 from *Arabidopsis*, although not an R protein, resembles Xa21 and plays a role in recognition of bacterial flagellin (64)]. There has been a clear bias toward dicot species and only a few *R* genes have been cloned from monocots [*Xa21*, *Rpg1*, *Mla* series (25, 71, 168, 207, 209)]. Interestingly, analysis of plant EST databases revealed that monocots do not have obvious TIR-NBS-LRR-like proteins (116, 129) and, if true (see below), this suggests there might be some fundamental differences in resistance mechanisms between dicots and monocots. In the future, studies using different plant species to compare R proteins and the components of their signaling pathways are likely to play an even greater role in understanding disease resistance. In addition, because structural similarities exist among R proteins and certain animal proteins with roles in immunity or development there is likely to be cross-feeding of knowledge gained from nonplant systems (38, 77, 124, 169).

R genes appear to have evolved under diversifying selection by a variety of genetic mechanisms. These have been reviewed recently (13, 53, 78, 187, 204). For several studied R loci, greater similarity among orthologs in different species than among paralogs at the same locus in one species suggests an ancient origin (13, 143, 188). Several *R* genes tested function transgenically within plant families, but only rarely across major taxonomic groups (176, 189). Analysis of *R* gene–like sequences in the *Arabidopsis* genome indicates that they are abundant, comprising an estimated 1% of the total genes (116), although the total number still is presumably far smaller than the number of potential pathogens. This observation suggests that the *R* genes may represent a pool for rapid development of new specificities, or that some have multiple specificities (see below).

In the past ten years over 40 *R* genes have been cloned, and many labs are now focused on functional characterization of R proteins. In this review, we discuss

recent developments in understanding the roles of R proteins in pathogen recognition and signal transduction. We begin with a brief discussion of the revealing characteristics of pathogen effector proteins that R proteins recognize.

PATHOGEN EFFECTOR PROTEINS

Effectors as Virulence and Avirulence Proteins

Features of pathogen effector proteins can provide clues to understanding the functions of R proteins. We use the term effector here to refer to pathogen proteins that are presented to the plant cell during infection. These proteins probably evolved to facilitate the pathogen life cycle and are thus virulence factors. In those cases where expression of a particular effector in a normally virulent pathogen strain causes the pathogen to be recognized by a host R protein the effector is referred to as an avirulence protein (107). Excellent recent reviews discussing general aspects of pathogen effectors are available (21, 40, 96, 198). Here we focus on aspects of the proteins that shed light on R protein function.

Effector Diversity

Single peptides, and in some cases subregions of those peptides, which play a role in recognition by R proteins, have been characterized in pathogenic viruses, bacteria, and fungi and their presence is suspected in nematodes and even some insects. Based on their sequences, pathogen effectors are extremely diverse and defy any simple classification scheme. For example, in different viruses either the replicase, the coat protein, or the movement protein have been found to function as recognition determinants for R proteins (55, 111, 128, 173). In fungi, diverse effectors have been identified from several species and, although none have proven biochemical activities, AVR-Pita from *Magnaporthe grisea* is a putative metalloprotease (126). Known fungal effectors include a large number of sequence-unrelated proteins from the tomato pathogen *Cladosporium fulvum* (106). Each *Cladosporium* protein, however, has an even number of cysteines of which some might form disulfide bridges to stabilize their structure and allow recognition by the corresponding R protein (106). Among bacteria, there is a growing list of over 40 diverse effectors identified both from screens for proteins that are delivered by the type III secretion system (TTSS) and from bioinformatics approaches relying on their characteristic "hrp box" promoter element or their putative secretion signal (60, 70, 134). Most bacterial effectors have no obvious biochemical activity or phenotypic effects on plants lacking a corresponding R protein. R proteins, therefore, collectively recognize an extraordinarily diverse array of pathogen effector proteins.

Functional Studies of Bacterial Effectors

The fact that pathogens betray their presence to the plant by expressing avirulence proteins was always puzzling but now many bacterial effectors are known to have a

(probably primary) role in promoting virulence (7, 19, 32, 34, 162, 182, 198). This virulence activity for most bacterial effectors is likely exerted from within the plant cell (35) and might involve several, not mutually exclusive, mechanisms including inhibition of host defense responses, masking of other avr genes, or increasing access to host nutrients (2, 35, 80, 110, 182).

Studies of effector virulence activity are apt to shed light on the function of R proteins because protein motifs required for virulence are often also involved in avirulence. For example, myristylation of AvrPto and AvrRpm1 targets these proteins to the plasma membrane and this localization is required for both avirulence and virulence (123, 163). Thus, it is likely that recognition of these effectors by their matching R proteins, Pto and RPM1, respectively, occurs at the plasma membrane. Similarly, cysteine protease activity of different members of the AvrPphB/YopT family is required, in one instance, for recognition by a cognate R protein (RPS5) in plants and, in another, for virulence in a mammalian system (164). Thus, AvrPphB-mediated proteolysis of the RPS5 protein or, more likely, the PBS1 protein kinase that is required for RPS5 resistance may be involved in effector recognition. For members of the AvrBs3 family, both avirulence and virulence activity depend on a nuclear localization sequence and a transcriptional activation domain (171, 201). In addition, AvrXa7, an AvrBs3 family member, binds double-stranded DNA. These observations suggest that recognition of these effectors by cognate R proteins occurs in the plant nucleus and may involve interaction with host transcriptional activation machinery. The many observations that common motifs are involved in both avirulence and virulence activity suggest that R proteins have evolved to recognize regions of effectors in which mutations might lower their contribution to pathogen fitness. In fact, a recent study supports the notion that the degree of fitness loss due to mutations in avirulence proteins can be used to predict the durability of *R* genes (7, 98, 191). An exception to the correlation of virulence and avirulence activities has been observed with AvrPto. Mutations that disrupt recognition of this protein by Pto do not affect its virulence activity in laboratory and greenhouse studies (162). *Pto* has been exceptionally durable, however, and it is possible that such mutations in AvrPto are deleterious under field conditions. To date, resistance-breaking strains characterized have included only those having undergone complete deletion of AvrPto (S. Hirano, personal communication).

ROLE OF R PROTEINS IN EFFECTOR RECOGNITION

Structural Motifs in R Proteins for Effector Recognition

Table 1 lists known R proteins grouped into the major classes described above. The great majority are intracellular NBS-LRR proteins, with either a CC (class 2) or TIR (class 3) domain at the N terminus. The few membrane-spanning proteins have extracellular LRR domains, no NBS, TIR, or CC motifs, and either a short intracellular region without known motifs(class 4) or an intracellular serine/threonine

protein kinase (STK) domain (Xa21, class 5). Pto (class 1), an intracellular STK, solely represents the only well-studied class that lacks an LRR, and its function requires the CC-NBS-LRR protein Prf (155). Thus, R proteins in the five major classes rely on a limited number of structural and functional domains, of which the LRR appears to play a central role.

THE LRR DOMAIN Present in many proteins of diverse function, the LRR is implicated in protein-protein interactions. A short stretch of amino acid residues with leucine at every second or third position is repeated to form a flexible, solvent-exposed, parallel beta-sheet. Domain swaps among alleles of the *L* and *P* genes in flax support a role for the LRR as a major determinant of recognition specificity (51, 54), and an LRR-like domain of at least one R protein interacts directly with its cognate effector (83). But TIR and NBS comparisons and domain swaps among alleles of L showed that these regions also contribute to specificity, suggesting that interactions of the LRR and these regions might be involved (105). Xa21D is an Xa21 family member that lacks the transmembrane and kinase domains. Although it confers a lower level of resistance, it retains the functional specificity of Xa21 (193). Potentially secreted, it may participate in an effector-dependent heterodimer with another, membrane-spanning Xa21 family member via conserved residues in the LRR, a model similar to that proposed for the Cf proteins, which lack obvious intracellular signaling domains (50). Replacing the extracellular LRR of Xa21 with that of BRI1, a receptor-like kinase involved in brassinosteroid perception, yielded a brassinosteroid-inducible plant defense response in rice cells, further supporting a role for the LRR in signal recognition (73). Extensive mutational analyses of a few R proteins identified several essential residues in the LRR, but also overall a high degree of tolerance for substitutions. This observation is consistent with a role for the LRR in recognition, as this property would be important for the evolution of new specificities (5, 47, 179). In addition to recognition, several studies suggest roles for the LRR in signaling. A point mutation in the LRR of RPS5 compromises the function of different, structurally related R proteins, suggesting a dominant negative interaction with a shared signaling component (195). The function of alleles of RPS2 from different ecotypes depends on the genetic background in which they are expressed, and this dependence is determined by polymorphism at six residues in the LRR, suggesting that the LRR may interact with other host factors that are also polymorphic among ecotypes (9). Reciprocal swaps between Mi-1 and a paralogous gene, involving the LRR domain and a 161–amino acid N-terminal region, resulted in loss of function or lethality in different chimeras. Lethality of an Mi-1 chimera containing the paralogous LRR was suppressed (in a transient assay) by coexpression of the 161–amino acid N terminus of Mi-1. This study suggests a role for the LRR in signaling cell death that is controlled by an intramolecular interaction with the N terminus (79).

THE NBS REGION Mutational analyses indicate a critical role for the NBS region (5, 47, 155, 179). Present in several protein families, including ATPases and

G proteins, the NBS may affect R protein function through nucleotide binding or hydrolysis, although to date these properties have not been reported for any R protein. Several R proteins align over a roughly 320–amino acid region comprising the NBS, with the APAF-1 and CED-4 proteins involved in regulating programmed cell death in animals (184). In addition to the kinase 1a, 2, and 3a domains that make up the NBS, the alignment contains five other short motifs of undefined function and was designated the NB-ARC (nucleotide binding in APAF-1, R gene products, and CED-4) domain. The functional relevance of the alignment has not yet been determined, but it was suggested that R proteins may control plant cell death by virtue of the NB-ARC domain, activated via LRR-dependent recognition of the pathogen (184). Structure predictions based on threading onto known structures suggest that the NB-ARC domain might be involved in ATP-dependent oligomerization (81) or, surprisingly, histidine-aspartic acid phosphotransfer without nucleotide binding (144).

THE CC MOTIF The CC structure is a repeated heptad sequence with interspersed hydrophobic amino acid residues, of which the leucine zipper is one example. It consists of two or more alpha helices that interact to form a supercoil, is found in a variety of proteins involved in different biological processes, and is implicated in protein-protein interactions, including oligomerization, and oligomerization-dependent nucleic acid binding. The role(s) of the CC domain in resistance remains to be unraveled, but general dependence of CC-containing R proteins in *Arabidopsis* on downstream signaling components distinct from those required for TIR-NBS-LRR proteins (see below) suggests that this domain may be involved in signaling rather than in recognition (1, 183, 196).

THE TIR DOMAIN The TIR domain is implicated in signaling by its similarity to the cytoplasmic domain of Toll and IL-1R, and by the requirement for distinct downstream components cited above for the CC motif. Also, amino acid residues conserved among the animal and plant protein domains and essential for Toll and IL-1R signaling are also critical for the function of the *N* gene; deletion and point mutations lead to partial loss-of-function alleles or dominant negative alleles (47). In addition to signaling, the TIR domain can play a role in pathogen recognition as well, as shown by comparisons and domain swaps among alleles at the *L* locus, cited above. Initial searches of plant EST databases suggested that monocots do not have TIR-NBS-LRR-like proteins (116, 129). However, a recent search using a modified hidden Markov model has yielded a candidate TIR-domain-containing protein on chromosome 1 of rice. The protein has an NB-ARC domain, but lacks a typical LRR. It is located in a region containing a number of R gene homologs and near a known R locus, but whether it functions in disease resistance is not yet known. The protein may represent a previously undetected subfamily shared by monocots and dicots, or an ancient fold that has diverged significantly in the monocots (K. Sjolander, personal communication).

THE STK DOMAIN Both Pto and the kinase domain of Xa21 are functional STKs (101, 159). Activity and specifically autophosphorylation of Pto is required for resistance (141, 159). Pto interacts directly with its cognate effector, AvrPto (158, 175), and with several plant proteins, of which some are also substrates of Pto, including another STK and a family of transcriptional activators (20, 69, 208, 209). The biochemistry, cell biology, and evolution of Pto recognition of AvrPto and initiation of defense responses have been studied extensively and reviewed recently (18, 161). The discussion here is limited to a few key findings and some recent results. Further discussion is included in the second half of this review. T204 in the activation domain of Pto is required for specific interaction with AvrPto. This residue is conserved in a number of STKs, but is absent from nonfunctional pto alleles or closely related family members, and is not subject to autophosphorylation in vitro (159). A substitution of aspartic acid at residue 207 yields a constitutively active allele, Pto(Y207D), that is dependent on Prf, but not AvrPto (141).

Pto is a member of a small family of kinases in tomato, of which some beside Pto are active and may function as R proteins. At least one of these interacts functionally with AvrPto when transiently overexpressed and may confer a weak resistance that is observed in a "susceptible" haplotype, relative to the complete susceptibility of a *prf* null plant (33). Several family members show a Pto(Y207D)-like gain-of-function (cell death) phenotype when the Y207D equivalent mutation is introduced, and this gain of function is dependent on Prf, indicating a shared signaling mechanism. Pto overexpression also triggers defense responses independent of AvrPto, but dependent on Prf (X. Tang, personal communication). The overexpression and Pto(Y207D) phenotypes shed light on the possible molecular mechanisms by which Pto recognizes and signals response to AvrPto, and these are discussed further below. Pto was recently shown also to interact functionally with another bacterial effector, distinct from AvrPto (91).

OTHER MOTIFS AND IMPORTANT STRUCTURES Pto contains a myristylation motif that is not required for AvrPto recognition when Pto is expressed from a strong promoter in transgenic plants (104), but is required for the AvrPto-independent Pto(Y207D) and Pto overexpression phenotypes (X. Tang, L. Shan, B. Riely & G.B.M., unpublished results). Covalent attachment of myristic acid to the N-terminal motif targets a protein to the membrane. It has not been determined whether Pto is myristylated or membrane localized during recognition, but AvrPto shares and requires the myristylation motif, localizes to the membrane, and is post-translationally myristylated in vitro (163) (A.J.B. & G.B.M., unpublished results). Several other bacterial effector proteins also appear to depend on myristylation in the plant cell for membrane localization and function (123) and notably, the cognate R protein of two of these, RPM1, also localizes to the membrane (23). These observations suggest that at least for some bacterial effectors and their cognate R proteins, recognition and signaling may occur at the plant plasma membrane, and the myristylation motif may play an important role in localization of one or more of the proteins involved. A roughly 150–amino acid region of RPM1 between

the CC and NBS domains, without similarity to known motifs, is essential for its interaction with the RIN4 protein (110). Also, the Hero protein contains an acidic CC that may contribute to recognition. This motif is encoded by an unusual hexanucleotide microsatellite between two repeats of its LRR coding region (56). One of the short motifs within the NB-ARC domain, GLPLAL, is particularly highly conserved among R proteins and is used often to PCR amplify R gene homologs, but its function is unknown.

Multiple Recognition Specificities of Some R Proteins

The most straightforward prediction of the gene-for-gene model is that single R proteins recognize single pathogen avirulence proteins. Some R proteins, however, recognize more than one pathogen signal. RPM1 and Pto each recognize different pairs of structurally distinct effectors from the same or related bacteria. RPM1 recognizes AvrB and AvrRpm1 (14). This recognition probably involves a third protein (possibly RIN4, 110), because no direct interaction of RPM1 and either effector protein has been observed. Pto recognizes AvrPto and a newly discovered protein from the same pathogen, AvrPtoB, which is three times the mass of AvrPto (91). The effectors in this case have very short regions of similar sequence and at least one of these appears to play a role in interaction with Pto. AvrPtoB also interacts with an AvrPto-interacting member of the Pto family isolated from a wild species of tomato, *Lycopersicon hirsutum*. These observations suggest that there has been selection in *Lycopersicon* spp. over a long period of time for Pto-like kinases that specifically recognize a conserved feature present in both the AvrPto and AvrPtoB proteins (143).

Mi-1 confers resistance to a nematode and to an aphid pest, indicating that it recognizes a signal from each. Some R loci show dual specificity accounted for by tightly linked, nearly identical genes. Examples include *HRT* and *RPP8* of *Arabidopsis*, and *Gpa2* and *Rx1* of potato (see Table 1). Nevertheless, dual (and perhaps even multiple) recognition specificity for single R proteins may prove to be common. It would provide some genomic and physiological economy for plants, which are faced with perhaps thousands of potential pathogens. Supporting this idea is the observation that the *Arabidopsis* genome encodes only a few hundred potential members of the five major classes of R proteins (85 TIR-NBS-LRR proteins, 36 CC-NBS-LRR proteins, 15 Ser/Thr kinases with $>50\%$ identity to Pto, 174 Xa21-like proteins, and 30 Cf-like proteins) (3). The studies of AvrPto and AvrPtoB suggest that common structural motifs embedded within diverse pathogen proteins, likely related to their virulence activity, might account for the limited number of *R* genes.

R Proteins as Members of Multiprotein Recognition Complexes

Physical interaction between R protein and effector has been demonstrated only for Pto with AvrPto or AvrPtoB (91, 158, 175), Pi-ta with AVR-Pita (83), and RPS2

with AvrRpt2 or, puzzlingly, with the noncognate effector AvrB (99). Due largely to the research community's failure to detect direct interaction with effectors for most R proteins, multiprotein recognition complexes were suspected. This idea is supported not only by the dual requirement of Prf and Pto for AvrPto recognition, but also by the recent identification of other likely participants in recognition complexes, including other kinases.

There is no evidence for a direct Prf-AvrPto interaction that would indicate its participation in a recognition complex, but recent evidence places Prf very early in the Pto-mediated resistance pathway. *Prf* overexpression activates defense responses in the absence of AvrPto and this activity requires Pto (125; G. Oldroyd & B. Staskawicz, personal communication). Overexpression of Pto (100; X. Tang, personal communication), or expression of Pto (Y207D) (141), similarly results in AvrPto-independent defense and requires Prf. These observations are consistent with a model (among others, see below) in which a Prf and Pto interaction, enhanced by AvrPto in a recognition complex, drives defense. Overexpression of either could obviate the need for AvrPto. Gene-expression profiling has revealed that regulation of over 95% of more than 400 genes differentially expressed within four hours following inoculation with a bacterial strain expressing AvrPto required both Pto and Prf, further supporting an early role for Prf (122).

Xa21 combines an LRR with a kinase domain similar to that of Pto (168), suggesting that NBS-LRR proteins might each physically associate with a Pto-like kinase (or redundant kinases, which would explain why so few have been identified in genetic screens). RPS5 requires the PBS1 kinase (170), but an interaction has not been demonstrated, and unlike Pto, PBS1 has not been observed to interact with the cognate effector, AvrPphB. PBS1 belongs to a subfamily of kinases distinct from Pto, with no other members of known function, and may function differently from Pto in pathogen recognition. RIN4 (110), a protein with no known motifs that is required for RPM1 function, is phosphorylated following delivery of AvrB or AvrRpm1 into the plant cell. RIN4 interacts with RPM1 and with either of its cognate effectors, AvrB and AvrRpm1. All of these proteins localize to the membrane fraction (23, 110, 123). RIN4 therefore may participate in recognition complexes involving RPM1, which might assemble due to, or might include, a kinase. Although these observations point to important roles for kinases in pathogen recognition, the functional connection(s) between NBS-LRR proteins and kinases remain to be discerned.

Other putative components of recognition complexes have been suggested by several studies. The Pi-ta and AVR-Pita interaction demonstrated using the LRR-like domain in the yeast two-hybrid system loses specificity when tested with full-length protein in vitro, such that Pi-ta interacts also with a nonfunctional AVR-Pita allele from a virulent strain of the pathogen. This in vitro interaction suggests that specificity in the plant cell might be maintained through interaction of the N-terminal portion of Pi-ta with another protein that would preclude interaction with the virulent AVR-Pita$_{176}$ allele. RPS2 interaction with AvrRpt2 was demonstrated by coimmunoprecipitation from protoplasts, but could not be demonstrated

in vitro using purified proteins, suggesting that one or more other binding partners present in the cell is required. Curiously, RPS2 coimmunoprecipitates also with the noncognate effector AvrB, demonstrating that R-Avr protein interaction alone does not account for specificity in this system, and again suggesting a role for at least one additional protein. P75, a protein that coprecipitates with RPS2 irrespective of the presence of an effector, is an important candidate that awaits further characterization (99). In HRT-mediated resistance to *Turnip crinkle virus* (TCV), the viral coat protein (CP) is the effector and appears to require interaction with TIP (TCV-interacting protein), a member of the NAC-family of proteins that are important in development. TIP message, however, is identical and expressed the same in both resistant and susceptible lines, supporting the idea that interaction of the CP-TIP complex with yet another protein, presumably HRT, triggers resistance (142). Cf-9 and Avr9 were shown not to interact in a variety of sophisticated binding assays (108). A high-affinity binding site (HABS) for Avr9 exists, however, in near-isogenic lines both with and without Cf-9, as well as in tobacco and other solanaceous plants (93). The affinity of HABS for various Avr9 alleles correlated with allele function, suggesting a required role in Cf-9-mediated recognition (94). A yeast three-hybrid screen identified candidate proteins involved in recognition of AvrPto (20). These AvrPto-dependent Pto interactors (Adis) interact with Pto only in the presence of AvrPto. This property suggests a potentially direct role in recognition, but confirmation has not yet been obtained. Affinity purification and gel filtration revealed participation of Cf-4 and Cf-9 in 400-kD and 420-kD protein complexes, respectively, that appear to contain other glycoproteins (145, 146).

Mutant screens for suppressors of R gene function have revealed additional genes whose products might participate in recognition. The *Rcr3* gene product (95) is required specifically for Cf-2 (and not Cf-9) function. It is a secreted cysteine protease. A Cf-2 and Cf-9 chimera containing the extracellular LRR region only of Cf-2 requires Rcr3, indicating that the requirement involves the LRR. An attractive hypothesis is that Cf-2 recognizes a complex of Avr2 and Rcr3. *Rcr3* was also determined to be the *Ne* gene, a suppressor for Cf-2-dependent autonecrosis cointrogressed from wild tomato, and its corresponding allele in cultivated tomato (Rcr^{esc}) leads to Avr2-independent, Cf-2-dependent autonecrosis when its product, which is developmentally regulated, accumulates in mature plants. This observation is reminiscent of Pto(Y207D)-mediated AvrPto-independent, Prf-dependent autonecrosis and may indicate that Rcr^{esc} mimics an Avr2-dependent conformation of Rcr3/Ne that is recognized by Cf-2. Recognition may require processing of Avr2, Cf-2, or another protein by Rcr3. Processing of Avr2 alone would not likely be the only role for Rcr3, however, because Avr2 isolated from infected plants (and presumably processed) does not elicit defense in an rcr3 mutant (95). Mutations at the *Rme* locus of tomato (43) specifically suppress Mi-1 function both in nematode and potato aphid resistance, indicating that *Rme* encodes a shared component of these Mi-1 mediated pathways. Mi-1 most closely resembles Prf. Whether Rme is akin to Pto awaits cloning of the gene.

R Protein Localization

In addition to the direct physical interactions that have been demonstrated (83, 91, 99, 158, 175), and the observation that RPM1 and its cognate effectors all associate with the plasma membrane (123), several considerations suggest that R proteins in general colocalize with pathogen effectors. Viral effectors are present inside the plant cell, and the predicted structures of all known R proteins against viruses indicate that they are also intracellular. Avr9 and other *Cladosporium fulvum* effector proteins are found in the plant extracellular space (96), and the LRRs of the Cf proteins are predicted to be extracellular. The HABS for Avr9 is located on the plasma membrane, and as discussed above, recognition of Avr9 may involve a three-way interaction on the plant cell surface among Avr9, the HABS, and Cf-9 (93). The AVR-Pita protein of *Magnaporthe grisea*, an extracellular pathogen, was shown to function when expressed in the plant cell, suggesting that it may be delivered into the plant cell during infection (83). Concordantly, Pi-ta, with which it is known to interact physically, is a class 2 (intracellular) R protein. All bacteria-directed R proteins appear to be intracellular, except Xa21. A number of bacterial effector proteins were shown to function when expressed within the plant cell, and the predicted localization of the corresponding R proteins lent weight to the conclusion, now cemented with direct evidence (29, 84, 171a), that these proteins localize inside plant cells following secretion through the bacterial type III secretion system–encoded Hrp pilus. The effector corresponding to Xa21 has not yet been identified, although it appears that it might be a sulphated protein secreted to the apoplast through a type II secretion system, where it could interact with the extracellular LRR portion of Xa21 (165).

Given these observations, determining the virulence targets of effectors may in many cases be an important first step toward localizing corresponding R proteins. This concept is supported by examples discussed earlier, i.e., the requirement for AvrPto and AvrRpm1 to localize to the plasma membrane for both virulence and avirulence activity, and observations suggesting that AvrXa7 virulence activity and Xa7-mediated recognition both take place in the nucleus. It is important to consider also that localization of the R protein could depend on the effector, such that R proteins that recognize more than one effector may localize to more than one subcellular location. This may be the case for Pto, which interacts with AvrPto and AvrPtoB. Unlike AvrPto, AvrPtoB lacks a myristylation motif and is predicted to be in the cytoplasm. Pto may be recruited to the membrane when it interacts with AvrPto, or localize in the cytoplasm upon interaction with AvrPtoB. Translocation of some R proteins during signaling might take place as well. Further biochemical and histochemical studies are required to explore these possibilities.

Models of R Protein Recognition Complexes

Despite some key breakthroughs, for several reasons progress toward understanding how R proteins function has been slower than might have been anticipated.

Because of their low abundance in the plant cell [and in at least some cases rapid degradation upon effector recognition (23)] many R proteins have proven difficult to study biochemically. Perhaps because of their structural complexity, LRR proteins have been recalcitrant to yeast two-hybrid analyses and other protein-protein interaction screens and this has limited the number of potential partner proteins that have been identified (77, 110). The lack of direct interaction between R proteins and most of their corresponding effectors has made it apparent that multiprotein receptor complexes are probably involved in pathogen recognition, and sophisticated biochemical analyses will be needed to characterize these complexes [e.g., (108)]. Some biochemical studies of R proteins are being reported and are allowing characterization of new components required for R protein function (23, 77, 99, 110). The patterns of protein-protein interactions that occur during R protein–mediated recognition of effectors, however, remain largely unknown.

An important and influential model for these interactions was conceived based on the dual requirement of Prf and Pto for AvrPto-triggered resistance, and was designated as the "guard" hypothesis (185). In this model, the effector (AvrPto) targets a plant protein (Pto) to promote disease (it was proposed that binding of AvrPto disrupts an interaction of Pto with other proteins that normally maintains a basal level of defense), and the R protein (Prf) guards against effector attack by recognizing the effector-target complex and activating defense responses. A variation was proposed in which recognition occurs when an effector-induced conformational change in the target protein disrupts a constitutive interaction of that protein with the guard (42). Recent observations have suggested yet other versions, including recognition of effector and target interaction following effector-dependent phosphorylation of the target (110), or following proteolytic processing of the target by the effector (164). The essential concept behind the several mechanistic variations of the guard model is that recognition of an effector takes place indirectly as recognition of an interaction of that effector with a target of its virulence function. In the absence of the guard, interaction of the effector and its target in some way downregulates plant defense, aids in releasing nutrients to the apoplast, or otherwise contributes to pathogenesis. In those plant-pathogen systems where evidence suggests a guard model might apply, therefore, the identification of host targets of virulence factors may uncover new members of R protein recognition complexes (20, 77, 99, 110).

For Pto-mediated resistance, evidence has accrued indicating that the guard model originally proposed does not apply, and that the interaction of AvrPto with Pto is directly involved in defense response elicitation and distinct from AvrPto virulence activity [for a detailed discussion, see (17)]. This evidence includes the observations, among others, that AvrPto virulence function is evident even in the absence of Pto (32, 162), that some AvrPto mutants that do not interact with Pto fail to elicit defense but retain virulence function (162), that AvrPto interacts with potential virulence targets distinct from Pto (20), and that some candidate downstream signaling components interact with Pto only in the presence of AvrPto (20). Participation of Prf in a receptor complex with Pto and AvrPto is possible,

even likely (see below), but Pto does not appear to be a virulence target of AvrPto guarded by Prf.

Figure 1 depicts several possible patterns of protein-protein interactions that could account for Pto or other R protein–mediated recognition of effector proteins. Some of these models are consistent with a role for R proteins as guards; others clearly are not. Evidence for and against each model is given below.

RECEPTOR-LIGAND In this model, which is the simplest interpretation of the gene-for-gene hypothesis, R protein and Avr protein interact directly and activate defense. In support of this model, Pto and Pi-ta interact directly with their respective effectors, and there are several examples of additional plant proteins required for resistance that may function downstream of recognition (110, 125, 170). However, interaction with an effector has not been demonstrable for the majority of R proteins. In at least one exhaustive study, the lack of evidence for an interaction seems convincing enough to reject this model for that system (108). Applicability of this model to a system would depend on some evidence, either biochemical or genetic (e.g., through identification of correlative suppressor mutations), that the R and Avr proteins interact directly, and that other required proteins function downstream. The model could be consistent with the guard hypothesis if the guard detects a cellular perturbation downstream of the interaction of effector and target.

BRIDGE In this model, the effector binds independently to the R protein and to a third protein, recruiting one to the other. The effector-dependent interaction of these two proteins activates downstream signaling for defense. In support of this model are the R proteins that have been shown to interact directly with effector(s) (Pto, Pi-ta, RPS2), and the growing number of examples of third proteins required for resistance (Prf, PBS1, TIP, RIN4, Rcr3, and possibly Rme and p75). Countering this model at least for Pto-mediated resistance are the observations that expression of the constitutively active mutant Pto(Y207D) or overexpression of Pto activates defense in a Prf-dependent fashion, in the absence of AvrPto. Likewise, Prf overexpression confers resistance dependent on Pto, but independent of AvrPto (G. Oldroyd & B. Staskawicz, personal communication). AvrPto, therefore, likely does not play the role of a bridge between Pto and Prf. For confirmation of this model in a recognition system, evidence is needed to show that the effector interacts with both plant proteins by means of distinct domains. In the absence of a demonstrable interaction for one or the other, identification of mutations in the effector that do not affect the observable interaction yet destroy avirulence function would support this model. The model, strictly speaking, is conceptually distinct from a guard model because both plant proteins interact directly and independently with the effector and interact only indirectly (or perhaps not at all) with each other.

MATCHMAKER In this model, the effector induces a direct interaction between the R protein and a third protein by causing a conformational change in one or the other, or both. The effector may or may not remain associated with the

complex following binding of the two plant proteins. In favor of this model is the evidence cited above for the bridge model. Also consistent is the observation that Pto(Y207D) activates defense, as it could be mimicking an AvrPto-induced conformation. The identification of Rcr^{esc} as *ne* could also support this model, assuming its conformation mimics that of an effector-modulated Rcr3 protein, as discussed above. The AvrPto-independent overexpression phenotypes for Prf and Pto are not consistent with this model, because the model proposes a requirement of AvrPto for interaction of the two plant proteins. Biochemical and genetic (through mutational analysis) evidence of an effector-dependent association of the two plant proteins would be needed to support this model for a particular system. This pattern of interactions could be a guard model if the third protein were a virulence target of the effector.

AFFINITY ENHANCEMENT In this model, interaction of the effector with the R protein, a third protein, or both, stabilizes a pre-existing, weak interaction between the two plant proteins such that abundance of the complex increases and drives downstream signaling to activate the induced defense response. Steady-state levels of interaction between the two plant proteins may function to maintain basal defense. Evidence cited in support of the bridge and matchmaker models also supports this model. Significantly, Pto and Prf overexpression phenotypes are consistent with this model, as increased concentrations of one or the other protein would drive formation of the complex independent of AvrPto. Pto(Y207D) might mimic an AvrPto-induced conformation of Pto, resulting in enhanced Pto-Prf interaction. To date, however, no interaction whatsoever between Prf and Pto has been reported. In RPM1-mediated defense, RIN4 interacts with RPM1, and with both cognate effectors, yet the observation that RIN4 functions genetically as a negative regulator of basal defense suggests that the affinity enhancement model does not apply to this system. Demonstration of interaction between the two plant proteins in the absence of the effector, and of enhancement of that interaction by the effector, perhaps by carefully controlled coimmunoprecipitation experiments, would be required to ascribe this model for recognition. The affinity enhancement pattern of protein-protein interactions could be a guard model if one or the other plant protein is a virulence target of the effector. Given the high degree of conservation of Prf across species relative to Pto, and its role in both the Pto and Fen (Fen is a protein kinase closely related to Pto) signaling pathways (154, 155), it is conceivable that Prf is a target of AvrPto guarded by Pto. It could not be the only target, however, because AvrPto virulence activity is quantifiable in the absence of functional Prf (32).

DEREPRESSION In this model, the effector derepresses defense responses by disrupting an interaction of the R protein and a third protein that negatively regulates activity of the R protein. Knowledge so far of RPM1-mediated resistance, in which antisense suppression of the RPM1 interactor RIN4 results in enhanced constitutive defense, provides an example consistent with this model. AvrB or AvrRpm1 could disrupt the interaction between RIN4 and RPM1 and derepress

RPM1 activity. In the Pto system, Pto(Y207D) could fail to interact with Prf in the same way as an AvrPto-modulated Pto. So far, however, as mentioned, no binding has been demonstrated between Prf and Pto, or between RPS5 and PBS1, Cf-2 and Rcr3, or HRT and TIP. And, of course, for many of these pairs, both proteins are required for resistance, precluding negative regulatory roles. The constitutive disease resistance conferred by overexpression of Pto or Prf also speaks against this model. For this model to apply to a given system, the R protein and third protein must interact, and downregulation or mutagenesis of the third protein would be expected to activate defense in the absence of the effector. As is true for the matchmaker and affinity enhancement models, this could be a guard model if the third protein were a target of the virulence function of the effector.

DUAL RECOGNITION This model, in which independent interactions between the effector and the *R* gene and the effector and a third protein are both required for resistance, is a formal possibility, supported at the very least by the dual requirement of Pto and Prf, and the interaction of AvrPto and Pto. This type of mechanism would be costly both in terms of structural evolutionary constraints and physiology, and seems unlikely to be maintained without significant, frequent disease pressure. Again, AvrPto and Prf interaction has not been demonstrated, and the observed AvrPto-independent resistance resulting from overexpression of either Prf or Pto would not be predicted from this model. If both plant proteins in this model are strictly required, the signaling pathways originating from these interactions would have to converge at some point downstream rather than each contributing quantitatively to resistance. Demonstration of the interactions, and genetic characterization of shared downstream components would be necessary to adopt this model. This model is not a guard model.

Just as there is a level of structural diversity among R proteins, there is likely to be diversity among the patterns of protein-protein interactions for recognition in different systems. Multiple models almost certainly will apply, and different mechanisms to bring about the plant protein-protein interactions are likely as well. Some of these have been mentioned, and they might include effector-binding induced conformational changes, effector-dependent phosphorylation, effector-driven translocation or targeting, effector-dependent degradation (in the derepression model, for example), or proteolytic processing by or due to the effector. Downstream signaling components will be distinct for many systems as well. Several characterized examples of these are discussed in the following section.

SIGNAL TRANSDUCTION EVENTS MEDIATED BY R PROTEINS

Loci Required for *R* Gene–Mediated Signaling

In the search for signaling components acting downstream of *R* genes, extensive genetic screens for mutants impaired in *R* gene–mediated resistance have been

employed in several dicot and monocot species [e.g., (30, 43, 89, 121, 154, 180, 196)]. These screens have identified a limited number of loci required for *R* gene signaling and in several instances led to the identification of the corresponding genes (Table 2). Lethality, redundancy, and the existence of parallel and additive pathways could account for the limited number of signaling components identified to date. *R* signaling components differ in their capability to affect single or multiple *R* gene–mediated cascades. Genes required for the function of individual *R* genes may encode proteins that fulfill tasks in early events of pathogen recognition or in signal transduction upstream or immediately downstream of pathogen recognition. Representative of this group are the tomato *Prf*, *Rcr3*, and *Rme1* genes (43, 95, 155), and the *Arabidopsis PBS1* and *RIN4* genes (110, 170).

As discussed above, the tomato Prf protein appears to act in conjunction with Pto in early signaling events of speck disease resistance (155). However, its function in signal transduction is still unclear. A signaling role upstream of R-Avr recognition has been proposed for the Rcr3 protease, which is specifically required for resistance of tomato expressing the *Cf-2* gene to *Cladosporium fulvum* expressing *avr2* (95). Its extracellular localization and enzymatic activity suggest that Rcr3 is likely to function in processing of molecules such as Avr2, Cf-2, or other plant proteins before R-Avr protein recognition. The *Rme1* locus is required for *Mi-1*–mediated resistance against root-knot nematodes and potato aphids (43). The *Rme1* gene has not been isolated yet, however, and its potential role in signaling remains unexplored.

A signal transduction function for the *Arabidopsis PBS1* gene, which is specifically required for resistance to *P. syringae* expressing *avrPphB*, is indicated by the enzymatic properties of its encoded protein (196). PBS1 is a serine/threonine kinase and it might act immediately downstream of pathogen recognition, as has been proposed for the Pto interactor Pti1, which encodes a serine/threonine kinase showing 50% identity to the PBS1 catalytic domain (170). However, it is also possible that PBS1 is a virulence target for AvrPphB and the "guardee" of RPS5, or an RPS5-modifying protein (170).

In the search for *R* gene–signaling components, protein-protein interaction screens are particularly suited for the identification of proteins that might be essential for plant viability and cannot be isolated in suppressor screens (6, 77, 110, 208, 209). A yeast two-hybrid screen using as bait the *P. syringae* effector AvrB identified the *Arabidopsis* RIN4 protein, which is specifically required for *RPM1*-mediated resistance (110). However, genetic and biochemical evidence strongly suggests that RIN4 is involved in pathogen recognition rather than in signal transduction.

Some signaling components are required for resistance conferred by multiple *R* genes, and are thus proposed to function downstream of the initial pathogen recognition event (Table 2). These can be divided into groups that affect classes of *R* genes with common structural characteristics, including *EDS1* (57), *PAD4* (58), and *NDR1* (31), and those that are required for different classes of *R* genes, and include *PBS3* (196), *PBS2/RAR1* (89, 121, 166, 180), *Rar2* (89), and *SGT1* (4, 6, 178).

TABLE 2 Signaling components that affect multiple *R* gene–mediated pathways

***R* gene**	**EDS1**	**PAD4**	**NDR1**	**PBS2/RAR1**	**SGT1**	**NPR1/NIM1**	**PBS3**	**RAR2**
RPM1 (CC)	*No (1, 58)	No (58)	Yes (30)	Yes (121, 196)	No (4, 178)	No (140)	Yes (196)	
RPS2 (CC)	Y/N (1, 58)	N/Y (58)	Yes (30)	Yes (121)	No (4)	No (140)	Yes (196)	
RPS4 (TIR)	Yes (58)	Y/N (58)	No (1)	Yes (121)	No (4, 178)	No (140)	Yes (196)	
RPS5 (CC)	No (1)		Yes (30)	Yes (121, 196)	No (178)		Yes (196)	
RPP1A (TIR)	Yes (58)	Y/N (58)	No (52)	No (121)	No (4)			
RPP1B (TIR)	Yes (58)	Y/N (58)						
RPP1C (TIR)	Yes (58, 131)	Y/N (58)						
RPP2 (TIR)	Yes (1)		No (1)	No (121, 196)	Yes (4, 178)		No (196)	
RPP4 (TIR)	Yes (1, 183)	Yes (183)	Y/N (1, 183)	Yes (121, 183, 196)	Yes (4, 178)	Y/N (183)	Y/N (183, 196)	
RPP5 (TIR)	Yes (1, 58)	Y/N (58)	Y/N (1, 183)	Yes (121)	Yes (4)	Yes (140)		
RPP6				No (196)	Yes (178)		No (196)	
RPP7 (CC)	No (58, 114)	No (58)		No (121, 196)	Yes (4, 178)		No (196)	
RPP8 (CC)	No (1, 58, 114)	No (58)	No (1)	No (121)	No (4)	No (140)		
RPP10 (TIR)	Yes (131)		No (52)					
RPP12	Yes (131)							
RPP13 (CC)	No (15)	No (15)	No (15)	No (15)		No (15)		
RPP14 (TIR)	Yes (131)		No (52)					
RPP19				No (196)			No (196)	
RPP21	Yes (1, 58)	Y/N (58)	No (1)	Yes (121)	Y/N (4)			
RPW8 (CC-like)	Yes (200)		No (200)					
N (TIR)	Yes (102, 132)			Yes (102)	Yes (103)	Yes (102)		
**Mla1* (CC)				No (6, 166)	No (6)			No (166)
**Mla* 6 (CC)				Yes (6, 71)	Yes (6)			Yes (71)

*Yes = resistance mediated by the specified *R* gene pathway requires this signaling component; No = resistance mediated by the specified *R* gene pathway does not require this signaling component; Y/N = component plays a limited role in the *R* gene pathway specified (mutation of signaling component gives partial resistance); blank = role in the specified *R* gene pathway has not been reported. CC = coiled-coil class of NBS-LRR protein, TIR = Toll/Interleukin 1-receptor class of R protein. **Mla1* and *Mla6* genes represent groups of powdery-mildew *R* genes that differ in their requirement for *Rar1* and *Rar2* as reviewed in (156).

The *Arabidopsis EDS1* and *PAD4* genes are specifically required for resistance to *P. syringae* and *Peronospora parasitica* conditioned by the same spectrum of TIR-NBS-LRR *R* genes (58). These two genes, which encode lipase-like proteins (57, 85), probably participate in the same signaling pathway and fulfill different signal transduction functions (1, 58). Interestingly, a subset of TIR-NBS-LRR genes, including *RPP5*, *RPP1*, and *RPP4*, is also affected by mutations in the systemic acquired resistance (SAR)-associated gene *NPR1/NIM1* (139, 183). On the other hand, the *Arabidopsis NDR1* gene encodes a putative membrane-bound protein required for resistance specified by *R* genes of the CC-NBS-LRR class (1, 30).

In contrast to *EDS1*, *PAD4*, and *NDR1*, the *RAR1*, *Rar2*, *SGT1*, and *PBS3* loci are required by distinct classes of *R* genes. Barley *Rar1* and *Rar2* were identified in a mutational screen for suppressors of *Mla12*-mediated resistance to the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (89). The *Arabidopsis* ortholog of barley *Rar1* was identified in suppressor screens targeting the *RPM1*- and *RPP5*-mediated resistance (121, 180). Interestingly, the *rar1* mutation was found to be allelic to the previously described *pbs*2 mutation, which suppresses *RPS5* disease resistance to *P. syringae* (196).

Arabidopsis SGT1 was identified in mutational analysis for loss of *RPP7*- and *RPP5*-mediated resistance (4, 178), and in a yeast two-hybrid screen as an RAR1 interactor (6). The RAR1-SGT1 physical interaction, their structural properties and physical interactions with ubiquitination-related proteins, strongly suggest that the two proteins are signaling components that may act in concert and are involved in protein degradation processes (4, 6, 121, 166, 178, 180). *Arabidopsis PBS3* is an additional locus required for resistance to *P. syringae* and *P. parasitica* mediated by multiple *R* genes (196). However the *PBS3* gene has not yet been isolated and its role in signal transduction remains unknown.

Virus-induced gene silencing (VIGS) in *Nicotiana benthamiana* has been successfully used to test the involvement of several signaling components in *N*-gene and *Pto*-gene mediated disease resistance against TMV and *P. syringae* pv. *tomato*, respectively (102, 103, 132; O. del Pozo, S. Ekengren, J. Van Eck & G. Martin, unpublished data). In VIGS, a virus vector carrying a fragment of the host gene to be tested is targeted against the corresponding host RNA. If the gene is required for disease resistance the virus-infected plant becomes susceptible to the pathogen. By using VIGS systems, tobacco homologues of *Rar1*, *EDS1*, *NPR1/NIM1*, *SGT1*, *SKP1*, and subunits of the COP9 signalosome have been shown to be involved in *N*-specified resistance to TMV (102, 103, 132). VIGS thus represents an excellent system to assess the role of candidate signaling components in disease resistance of Solanaceae. Moreover, high-throughput VIGS screens using cDNA libraries in a virus vector will allow the identification of signaling components that, because of lethality or redundancy, may not be identified in classical forward genetic screens.

Multiple Parallel and Interacting Pathways in *R* Gene Signaling

Structurally different R proteins trigger defense responses that are common to a large array of plant-pathogen interactions. This observation raises the question of

whether defense responses are triggered by parallel or by converging signaling pathways originating from different recognition events. Alternatively, a network of multiple interconnected signaling pathways acting in parallel might propagate *R* gene–mediated signals.

The different requirements of *R* genes for downstream signaling components imply that there are distinct signaling pathways leading to disease resistance. For example, the structurally different RPM1 and RPS4 proteins, which confer resistance against different *P. syringae* strains, clearly require distinct downstream effectors (Table 2). In addition, even similar *R* genes were found to be dependent for their function on distinct downstream components. This is the case for the *Mla1* and *Mla6* barley genes, which, despite a high degree of homology and the capability to trigger a very similar resistance phenotype, show a differential requirement for the *Rar1*, *Rar2*, and *SGT1* signaling components (6, 71, 156, 207). Similarly, the function of the CC-NBS-TIR *RPP13* and *RPP8* is not *NDR1*-dependent, in contrast with resistance mediated by other known *RPP* genes of the CC-NBS-LRR class (15, 114). Additional evidence suggests that the same *R* gene can use multiple parallel signaling pathways. For instance, in the analysis of the contribution of *RAR1* and *NDR1* to disease resistance mediated by several *R* genes, an *rar1 ndr1* double mutant showed an additive phenotype with respect to *RPP7* function, strongly suggesting the existence of two pathways acting in parallel downstream of *RPP7* (180). Interestingly, *RPP4*-mediated resistance has a differential requirement for signaling components in true leaves and cotyledons, indicating that the preferential utilization of a certain pathway downstream to *RPP4* is developmentally regulated (183).

In the signaling networks used by *R* genes and their downstream components, there are pathways that converge into common elements, reinforcing the notion that common pathways, which are parallel and interconnected, function downstream of *R* genes. For example, *RPS2*, *RPP4*, and *RPP5* genes share a similar requirement for *EDS1*, *PAD4*, and *RAR1* (Table 2). However, *RPP4* and *RPP5* differ from *RPS2* in their requirement for *SGT1*, suggesting the existence of interconnected rather than linear pathways. The *NDR1* gene represents a convergence point for cascades specified by *R* genes of the CC-NBS-LRR class (1, 31), whereas the *EDS1* and *PAD4* genes are convergence points for pathways originating from *R* genes of the TIR-NBS-LRR class (1, 58). *EDS1* and *PAD4* function in close proximity in the signaling pathway, as the proteins they encode physically interact in vitro and coimmunoprecipitate from plant extracts (58). However, they fulfill distinct roles in resistance: *EDS1* is essential for the oxidative burst and HR elicitation, while *PAD4* is required for phytoalexin, PR1, and SA accumulation (147, 153, 211). The study of the requirement for *NDR1* and *EDS1* by the CC-NBS-LRR *R* genes *RPP7* and *RPP8* revealed that the utilization by a specific *R* gene of either an *EDS1/PAD4* or *NDR1* pathway is not mutually exclusive (114). In fact, resistance to *P. parasitica* conferred by *RPP7* or *RPP8* was not significantly suppressed by mutations in either *EDS1* or *NDR1*, and was only partially suppressed in *eds1/ndr1* double mutants (114). These results, together with the evidence that a slight reduction in resistance

is observed in an *ndr1* background for certain *EDS1*-dependent *R* genes (1, 183), suggest that certain pathways can operate additively through *EDS1*, *NDR1*, and additional unknown signaling components. Taken together, the emerging picture is that *R* genes relay their signal through a complex network of additive and interconnected pathways.

Roles of *R* Gene Signaling Components in Other Plant Processes

One possible explanation for the limited number of signaling components identified in mutational screens is that certain genes might be required for both disease resistance and other essential cellular processes. In several instances, morphological and developmental abnormalities are observed in plants having mutations in *R* gene signaling components. This is the case for the *Arabidopsis* RPM1- and RPP5-interacting protein TIP49a, which is homologous to animal proteins interacting with the TATA binding protein complex and modulating specific signaling pathways (77). TIP49a was shown to be a negative regulator of *RPP2*- and *RPP5*-mediated resistance and to be required for meristem establishment, as well as for sporophyte and female gametophyte viability (77). It is also possible that *R* gene–specified signaling pathways utilize cellular machinery shared by multiple unrelated signaling pathways. This hypothesis is supported by the finding of protein complexes including the RAR1 and SGT1 proteins and subunits of the COP9 signalosome, which is involved in protein degradation and in several different developmental processes (157).

Numerous *Arabidopsis* mutants showing activation of basal defense and morphological abnormalities have been identified (63, 110, 133). Similarly to TIP49a, the corresponding genes might function in both disease resistance and development. Alternatively, constitutive activation of defense responses in these mutants might shift cellular activity from rest to stress metabolism, causing impairment of cellular functions and growth. The existence of dual roles for certain *R* gene signaling components reinforces the need to adopt alternative and complementary strategies to mutational screens in the dissection of *R* gene–mediated pathways.

Protein Degradation in *R* Gene Signaling

An important role for protein degradation in *R* gene–mediated signaling is emerging from the characterization of the RAR1 and SGT1 proteins and their interaction with components of the SCF (Skp1, Cullin, F-box) E3 ubiquitin ligase complex and with subunits of the COP9 signalosome (4, 6, 103, 121, 166, 178, 180).

RAR1 encodes a cytoplasmic protein with two zinc-binding CHORDs (cysteine- and histidine-rich domains), which are conserved in sequence and tandem organization among all eukaryotic phyla examined (121, 166, 180). In contrast to plant RAR1, *Drosophila*, *Caenorhabditis elegans*, and human CHORD proteins contain a C-terminal extension with sequence similarity to the yeast protein SGT1,

which is essential for activation and assembly of the ubiquitin ligase SCF complex (92). This complex mediates degradation of proteins involved in diverse signaling pathways through a ubiquitin proteasome pathway (45). Interestingly, plant SGT1, which is essential for several *R* gene–mediated pathways, physically interacts with RAR1 in yeast two-hybrid screens and in plant extracts (4, 6, 103, 178). In addition, RAR1 and SGT1 from barley and *N. benthamiana* extracts coimmunoprecipitated with components of the SCF complex and with subunits of the COP9 signalosome (6, 103). The COP9 multiprotein complex is involved in protein degradation through the 26S proteasome, which directly interacts with SCF E3 ubiquitin ligases (109, 157). Remarkably, suppression of the COP9 subunits CSN3 and CSN8, and of the SCF subunit SKP1 leads to loss of *N*-mediated disease resistance in *N. benthamiana* (103). Despite the physical interaction observed for RAR1 and SGT1, the requirements of different *R* genes for these proteins only partially overlap (4) (Table 2), indicating that they have both combined and distinct functions in different *R* gene–mediated signaling pathways. Taken together, these observations point to a key involvement of protein degradation in *R* gene signaling pathways.

What is the role of ubiquitination and protein degradation in disease resistance? A possible role is removal of negative regulators of plant defense responses. Candidate targets for degradation are products of genes whose mutations fall into phenotypic classes of enhanced disease resistance (*edr1*), constitutive expression of defense genes (*cpr1*), or lesions simulating disease (*lsd*) (63). Alternatively, protein degradation might modulate R protein levels. In support of this possibility, *RPM1* is rapidly degraded following RPM1-mediated activation of signaling (23); however, the observation that RPM1 protein is undetectable in *rar1* mutants cannot be integrated into this scenario (179). Finally, the role of ubiquitination in disease resistance could be to modify signal proteins and regulate cellular processes such as transcription, protein trafficking, membrane transport, or activation of protein kinases (135).

Phosphorylation in *R* Gene Signaling

The *Pto*, *Xa21*, and *Rpg1 R* genes and several *R*-mediated signaling components encode protein kinases, suggesting a major role for phosphorylation in *R*-specified signaling. The role of phosphorylation in signal transduction by the tomato serine/threonine kinase Pto and the rice receptor-like kinase Xa21 has been extensively examined. Kinase activity is required for HR elicitation mediated not only by the AvrPto-Pto interaction but also by the constitutively active Pto mutant (PtoY207D) (141, 160). Pto in vitro autophosphorylation sites were identified, and among them Thr38 and Ser198 are essential for HR induction (159). However, the requirement of autophosphorylation for Pto functionality is still unclear, as autophosphorylation was not detected in vitro for certain functional Pto homologs or for Pto(Y207D) (33, 141). The Xa21 catalytic domain shares similar kinetic properties with Pto, but has a different pattern of phosphorylation sites (101). Function of the BRI::XA21 chimeric protein mentioned earlier was strictly dependent on kinase activity (73).

It will be interesting to determine functionality and requirement for stem rust resistance of the two tandem kinase domains, which are encoded by the recently isolated *Rpg1* barley *R* gene (25).

Phosphorylation-related events and protein kinases participate in *R* gene–mediated pathogen recognition and downstream signaling. RIN4 and PBS1 are important examples already discussed. Members of the calcium-dependent protein kinase (CDPK) family also participate in *R* gene–mediated disease resistance. Two tobacco-related CDPKs, NtCDPK2 and NtCDPK3, are rapidly phosphorylated and activated in cell cultures in a *Cf-9/Avr9*-dependent manner (148, 149). Moreover, VIGS in tobacco of the *NtCDPK2* gene family caused a reduced elicitation of the HR mediated by the *Cf-4* and *Cf-9 R* genes (148).

Recent findings clearly demonstrate the involvement of MAP kinase cascades in *R* gene–dependent signaling. SIPK and WIPK MAP kinases are activated during resistance responses mediated by the tobacco *N* and the tomato *Cf-9* genes (150, 205). In addition, overexpression of SIPK in tobacco plants induced the elicitation of the HR and additional defense responses (206). Moreover, a tobacco MAPK kinase (NtMEK2) acting upstream of SIPK and WIPK specifically phosphorylates and activates SIPK and WIPK, and induces activation of defense responses when expressed in a constitutively active form (202). MAP kinase cascades can also negatively regulate defense responses, as shown by the isolation of the *Arabidopsis* MAPKKK *EDR1* and MAPK *MPK4*, which encode negative regulators of SA-mediated response (61, 133).

Additional Signaling Events and Components in *R* Gene Function

Additional posttranslational modifications, such as glycosylation, and signaling events, including ion fluxes, production of reactive oxygen species (ROS), and nitric oxide (NO), appear to play important roles in signaling mediated by *R* genes. However, the regulation of these events and the molecular components involved remain largely unknown. Glycosylation may have roles in signaling mediated by the Ve protein and by proteins of the Cf family (90, 137). Although these proteins contain putative glycosylation sites (90, 137), and Cf-9 is glycosylated in planta, the involvement of glycosylation in their function has not yet been established (137).

The relation between R proteins and early signaling events in the plant defense response is beginning to be unraveled. For example, *RPM1*-dependent calcium fluxes have been observed in leaves by using an aequorin-mediated bioluminescence assay (67). Among the potential targets of elevated Ca^{2+} levels in the cytoplasm are CDPKs and calmodulin. Indeed, a tobacco CDPK is involved in *Cf-9* signaling (148), and specific calmodulin isoforms were found to be activated in a gene-for-gene–specific manner and to participate in Ca^{2+}-mediated induction of defense responses (75). Interestingly, in *dnd1* (defense, no death) mutants, a reduced ability to produce HR and constitutive resistance is activated by disruption

of the cyclic nucleotide-gated ion channel protein AtCNGC2, which has a putative calmodulin-binding domain and is Ca^{2+} permeable (37).

In some instances, an increase in cytosolic Ca^{2+} is necessary for the activation of the oxidative burst, which is engaged during HR formation (66, 67, 137). The isolation of several components of *R*-mediated signaling pathways has allowed the testing of their requirement for the production of ROS in incompatible interactions. For example, plants that are impaired in resistance by mutations in *EDS1*, *NDR1*, *RAR1*, and *SGT1b*, but not *PAD4*, are also inhibited in the production of ROS (121, 153, 166). Remarkably, *EDS1* encodes a protein with homology to phospholipases, which in mammals are involved in the activation of ROS-producing NADPH oxidases. In plants, pharmacological inhibitor studies indicated that phospholipase activity is an intermediate in the *Cf-9/Avr9*-dependent signaling pathway that leads to ROS production (136). In addition, *Arabidopsis* mutants missing functional NADPH oxidase catalytic subunits show inhibited ROS production and alterations in cell death in response to incompatible pathogens (181). Taken together, these studies establish a first linkage between *R* genes and early physiological events observed during incompatible plant-pathogen interactions. However, further investigation is required to unravel molecular components and mechanisms orchestrating these early signaling events of plant resistance.

FUTURE PERSPECTIVES

New Experimental Approaches to Understanding R Protein Function

Despite major advances in the field of plant disease resistance, the precise molecular mechanisms of plant-pathogen recognition and the detailed dissection of *R* gene–mediated signaling networks remain elusive. In the years ahead, new genomics and proteomics technologies will assist in the identification of signaling components and in the investigation of the biochemical functions of R proteins and other signaling molecules. The availability of the complete sequence of plant and microbial genomes and of large collections of expressed sequence tags for a number of plant species provide new opportunities to shed light on plant disease resistance. Pure computational analysis and database mining can lead to the identification of genes involved in plant-pathogen interactions (60, 129, 134, 186). In addition, the wealth of sequence information available in conjunction with powerful technologies, such as cDNA and oligonucleotide microarray, serial analysis of gene expression (SAGE), and cDNA AFLP, is starting to yield a detailed analysis of gene expression profiles during the plant defense response. A major output of these analyses is sets of genes representing putative candidates involved in early signal transduction pathways originating from the plant-pathogen recognition events, or in downstream defense responses. High-throughput functional analysis will assess the requirement and roles of these genes in plant disease resistance. Valuable tools in this analysis will be the rapidly growing number of gene

knockouts and activation-tagged lines that are becoming available for functional genomics in *Arabidopsis* and rice. In addition, sophisticated VIGS systems will assist in high-throughput functional analysis in plant species recalcitrant to other genetic manipulations. T-DNA populations, VIGS, and activation tagging also represent important tools for the design of efficient gain- or loss-of-function mutant screens for the identification of *R*-signaling components.

Beyond gene identification, proteomics approaches will provide insights into the biochemical properties of proteins involved in disease resistance. New advances in proteome analysis include the development of techniques for reproducible 2-D gel electrophoresis and for protein identification based on accurate mass spectrometry. These techniques will assist in the analysis of differential protein expression and posttranslational modification, such as phosphorylation and glycosylation, during plant-pathogen interactions. In addition, the introduction of protein microarrays will boost the study of protein-protein interactions, and screens for substrates of protein kinases and for targets of small molecules. Taken together, these investigations will significantly contribute to our understanding of the mechanisms of plant-pathogen recognition and of the complex signaling networks mediating the activation of defense responses.

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LITERATURE CITED

1. Aarts N, Metz M, Holub E, Staskawicz BJ, Daniels MJ, Parker JE. 1998. Different requirements for EDS1 and NDR1 by disease resistance genes define at least two *R* gene-mediated signaling pathways in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 95:10306–11
2. Abramovitch RB, Kim Y-J, Chen S, Dickman MB, Martin GB. 2003. *Pseudomonas* type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. *EMBO J.* In press
3. Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408:796–815
4. Austin MJ, Muskett P, Kahn K, Feys BJ, Jones JD, Parker JE. 2002. Regulatory role of SGT1 in early *R* gene-mediated plant defenses. *Science* 295:2077–80
5. Axtell MJ, McNellis TW, Mudgett MB, Hsu CS, Staskawicz BJ. 2001. Mutational analysis of the *Arabidopsis RPS2* disease resistance gene and the corresponding *Pseudomonas syringae avrRpt2* avirulence gene. *Mol. Plant-Microbe Interact.* 14:181–88
6. Azevedo C, Sadanandom A, Kitagawa K, Freialdenhoven A, Shirasu K,

Schulze-Lefert P. 2002. The RAR1 interactor SGT1, an essential component of *R* gene-triggered disease resistance. *Science* 295:2073–76

7. Bai J, Choi SH, Ponciano G, Leung H, Leach JE. 2000. *Xanthomonas oryzae* pv. *oryzae* avirulence genes contribute differently and specifically to pathogen aggressiveness. *Mol. Plant-Microbe Interact.* 13:1322–29
8. Ballvora A, Ercolano MR, Weiss J, Meksem K, Bormann CA, et al. 2002. The *R1* gene for potato resistance to late blight (*Phytophthora infestans*) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *Plant J.* 30:361–71
9. Banerjee D, Zhang X, Bent AF. 2001. The leucine-rich repeat domain can determine effective interaction between RPS2 and other host factors in *Arabidopsis* RPS2-mediated disease resistance. *Genetics* 158:439–50
10. Bendahmane A, Kohn BA, Dedi C, Baulcombe DC. 1995. The coat protein of potato virus X is a strain-specific elicitor of Rx1-mediated virus resistance in potato. *Plant J.* 8:933–41
11. Bendahmane A, Querci M, Kanyuka K, Baulcombe DC. 2000. *Agrobacterium* transient expression system as a tool for the isolation of disease resistance genes: application to the *Rx2* locus in potato. *Plant J.* 21:73–81
12. Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, et al. 1994. *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. *Science* 265:1856–60
13. Bergelson J, Kreitman M, Stahl EA, Tian D. 2001. Evolutionary dynamics of plant R genes. *Science* 292:2281–85
14. Bisgrove SR, Simonich MT, Smith NM, Sattler A, Innes RW. 1994. A disease resistance gene in *Arabidopsis* with specificity for two different pathogen avirulence genes. *Plant Cell* 6:927–33
15. Bittner-Eddy PD, Beynon JL. 2001. The *Arabidopsis* downy mildew resistance gene, *RPP13–Nd*, functions independently of NDR1 and EDS1 and does not require the accumulation of salicylic acid. *Mol. Plant-Microbe Interact.* 14: 416–21
16. Bittner-Eddy PD, Crute IR, Holub EB, Beynon JL. 2000. *RPP13* is a simple locus in *Arabidopsis thaliana* for alleles that specify downy mildew resistance to different avirulence determinants in *Peronospora parasitica*. *Plant J.* 21:177–88
17. Bogdanove A. 2002. Protein-protein interactions in pathogen recognition by plants. *Plant Mol. Biol.* 50:981–89
18. Bogdanove A. 2002. Pto update: recent progress on an ancient plant defence response signaling pathway. *Mol. Plant Pathol.* 3:283–88
19. Bogdanove AJ, Kim JF, Wei Z, Kolchinsky P, Charkowski AO, et al. 1998. Homology and functional similarity of an hrp-linked pathogenicity locus, *dspEF*, of *Erwinia amylovora* and the avirulence locus *avrE* of *Pseudomonas syringae* pathovar *tomato*. *Proc. Natl. Acad. Sci. USA* 95:1325–30
20. Bogdanove AJ, Martin GB. 2000. AvrPto-dependent Pto-interacting proteins and AvrPto-interacting proteins in tomato. *Proc. Natl. Acad. Sci. USA* 97: 8836–40
21. Bonas U, Van den Ackerveken G. 1999. Gene-for-gene interactions: bacterial avirulence proteins specify plant disease resistance. *Curr. Opin. Microbiol.* 2:94–98
22. Botella MA, Parker JE, Frost LN, Bittner-Eddy PD, Beynon JL, et al. 1998. Three genes of the *Arabidopsis RPP1* complex resistance locus recognize distinct *Peronospora parasitica* avirulence determinants. *Plant Cell* 10:1847–60
23. Boyes DC, Nam J, Dangl JL. 1998. The *Arabidopsis thaliana RPM1* disease resistance gene product is a peripheral

plasma membrane protein that is degraded coincident with the hypersensitive response. *Proc. Natl. Acad. Sci. USA* 95:15849–54

24. Brommonschenkel SH, Frary A, Tanksley SD. 2000. The broad-spectrum tospovirus resistance gene *Sw-5* of tomato is a homolog of the root-knot nematode resistance gene *Mi*. *Mol. Plant-Microbe Interact.* 13:1130–38
25. Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, et al. 2002. The barley stem rust-resistance gene *Rpg1* is a novel disease-resistance gene with homology to receptor kinases. *Proc. Natl. Acad. Sci. USA* 99:9328–33
26. Bryan GT, Wu KS, Farrall L, Jia Y, Hershey HP, et al. 2000. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *Plant Cell* 12:2033–46
27. Buschges R, Hollricher K, Panstruga R, Simons G, Wolter M, et al. 1997. The barley *Mlo* gene: a novel control element of plant pathogen resistance. *Cell* 88:695–705
28. Cai D, Kleine M, Kifle S, Harloff HJ, Sandal NN, et al. 1997. Positional cloning of a gene for nematode resistance in sugar beet. *Science* 275:832–34
29. Casper-Lindley C, Dahlbeck D, Clark ET, Staskawicz BJ. 2002. Direct biochemical evidence for type III secretion-dependent translocation of the AvrBs2 effector protein into plant cells. *Proc. Natl. Acad. Sci. USA* 99:8336–41
30. Century KS, Holub EB, Staskawicz BJ. 1995. *NDR1*, a locus of *Arabidopsis thaliana* that is required for disease resistance to both a bacterial and a fungal pathogen. *Proc. Natl. Acad. Sci. USA* 92:6597–601
31. Century KS, Shapiro AD, Repetti PP, Dahlbeck D, Holub E, Staskawicz BJ. 1997. NDR1, a pathogen-induced component required for *Arabidopsis* disease resistance. *Science* 278:1963–65
32. Chang JH, Rathjen JP, Bernal AJ, Staskawicz BJ, Michelmore RW. 2000. AvrPto enhances growth and necrosis caused by *Pseudomonas syringae* pv. *tomato* in tomato lines lacking either *Pto* or *Prf*. *Mol. Plant-Microbe Interact.* 13:568–71
33. Chang JH, Tai YS, Bernal AJ, Lavelle DT, Staskawicz BJ, Michelmore RW. 2002. Functional analyses of the *Pto* resistance gene family in tomato and the identification of a minor resistance determinant in a susceptible haplotype. *Mol. Plant-Microbe Interact.* 15:281–91
34. Chang JH, Tobias CM, Staskawicz BJ, Michelmore RW. 2001. Functional studies of the bacterial avirulence protein AvrPto by mutational analysis. *Mol. Plant-Microbe Interact.* 14:451–59
35. Chen Z, Kloek AP, Boch J, Katagiri F, Kunkel BN. 2000. The *Pseudomonas syringae avrRpt2* gene product promotes pathogen virulence from inside plant cells. *Mol. Plant-Microbe Interact.* 13:1312–21
36. Chisholm ST, Mahajan SK, Whitham SA, Yamamoto ML, Carrington JC. 2000. Cloning of the *Arabidopsis RTM1* gene, which controls restriction of long-distance movement of tobacco etch virus. *Proc. Natl. Acad. Sci. USA* 97:489–94
37. Clough SJ, Fengler KA, Yu IC, Lippok B, Smith RK Jr, Bent AF. 2000. The *Arabidopsis dnd1* "defense, no death" gene encodes a mutated cyclic nucleotide-gated ion channel. *Proc. Natl. Acad. Sci. USA* 97:9323–28
38. Cohn J, Sessa G, Martin GB. 2001. Innate immunity in plants. *Curr. Opin. Immunol.* 13:55–62
39. Collins N, Drake J, Ayliffe M, Sun Q, Ellis J, et al. 1999. Molecular characterization of the maize *Rp1-D* rust resistance haplotype and its mutants. *Plant Cell* 11:1365–76

40. Collmer A. 1998. Determinants of pathogenicity and avirulence in plant pathogenic bacteria. *Curr. Opin. Plant Biol.* 1:329–35
41. Cooley MB, Pathirana S, Wu HJ, Kachroo P, Klessig DF. 2000. Members of the *Arabidopsis HRT/RPP8* family of resistance genes confer resistance to both viral and oomycete pathogens. *Plant Cell* 12:663–76
42. Dangl JL, Jones JD. 2001. Plant pathogens and integrated defence responses to infection. *Nature* 411:826–33
43. de Ilarduya OM, Moore AE, Kaloshian I. 2001. The tomato *Rme1* locus is required for *Mi-1*–mediated resistance to root-knot nematodes and the potato aphid. *Plant J.* 27:417–25
44. Debener T, Lehnackers H, Arnold M, Dangl JL. 1991. Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. *Plant J.* 1:289–302
45. Deshaies RJ. 1999. SCF and Cullin/Ring H2–based ubiquitin ligases. *Annu. Rev. Cell Dev. Biol.* 15:435–67
46. Deslandes L, Olivier J, Theulieres F, Hirsch J, Feng DX, et al. 2002. Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive *RRS1-R* gene, a member of a novel family of resistance genes. *Proc. Natl. Acad. Sci. USA* 99:2404–9
47. Dinesh-Kumar SP, Tham WH, Baker BJ. 2000. Structure-function analysis of the tobacco mosaic virus resistance gene *N*. *Proc. Natl. Acad. Sci. USA* 97:14789–94
48. Deleted in proof.
49. Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JD. 1998. The tomato *Cf-5* disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. *Plant Cell* 10:1915–25
50. Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JDG. 1996. The tomato *Cf-2* disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. *Cell* 84:451–59
51. Dodds P, Lawrence G, Ellis J. 2001. Six amino acid changes confined to the leucine-rich repeat beta-strand/beta-turn motif determine the difference between the P and P2 rust resistance specificities in flax. *Plant Cell* 13:163–78
52. Dodds PN, Schwechheimer C. 2002. A breakdown in defense signaling. *Plant Cell* 14:S5–8
53. Ellis J, Dodds P, Pryor T. 2000. Structure, function and evolution of plant disease resistance genes. *Curr. Opin. Plant Biol.* 3:278–84
54. Ellis JG, Lawrence GJ, Luck JE, Dodds PN. 1999. Identification of regions in alleles of the flax rust resistance gene *L* that determine differences in gene-for-gene specificity. *Plant Cell* 11:495–506
55. Erickson FL, Holzberg S, Calderon-Urrea A, Handley V, Axtell M, et al. 1999. The helicase domain of the TMV replicase proteins induces the N-mediated defence response in tobacco. *Plant J.* 18:67–75
56. Ernst K, Kumar A, Kriseleit D, Kloos DU, Phillips MS, Ganal MW. 2002. The broad-spectrum potato cyst nematode resistance gene (Hero) from tomato is the only member of a large gene family of NBS-LRR genes with an unusual amino acid repeat in the LRR region. *Plant J.* 31:127–36
57. Falk A, Feys BJ, Frost LN, Jones JD, Daniels MJ, Parker JE. 1999. EDS1, an essential component of R gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. *Proc. Natl. Acad. Sci. USA* 96:3292–97
58. Feys BJ, Moisan LJ, Newman MA, Parker JE. 2001. Direct interaction between the *Arabidopsis* disease resistance signaling proteins, EDS1 and PAD4. *EMBO J.* 20:5400–11
59. Flor HH. 1971. Current status of the

gene-for-gene concept. *Annu. Rev. Plant Pathol.* 9:275–96
60. Fouts DE, Abramovitch RB, Alfano JR, Baldo AM, Buell CR, et al. 2002. Genomewide identification of *Pseudomonas syringae* pv. *tomato* DC3000 promoters controlled by the HrpL alternative sigma factor. *Proc. Natl. Acad. Sci. USA* 99:2275–80
61. Frye CA, Tang D, Innes RW. 2001. Negative regulation of defense responses in plants by a conserved MAPKK kinase. *Proc. Natl. Acad. Sci. USA* 98:373–78
62. Gassmann W, Hinsch ME, Staskawicz BJ. 1999. The *Arabidopsis RPS4* bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. *Plant J.* 20:265–77
63. Glazebrook J. 2001. Genes controlling expression of defense responses in *Arabidopsis*–2001 status. *Curr. Opin. Plant Biol.* 4:301–8
64. Gomez-Gomez L, Boller T. 2000. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol. Cell* 5:1003–11
65. Gomez-Gomez L, Boller T. 2002. Flagellin perception: a paradigm for innate immunity. *Trends Plant Sci.* 7:251–56
66. Grant JJ, Loake GJ. 2000. Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. *Plant Physiol.* 124:21–29
67. Grant M, Brown I, Adams S, Knight M, Ainslie A, Mansfield J. 2000. The *RPM1* plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. *Plant J.* 23:441–50
68. Grant MR, Godiard L, Straube E, Ashfield T, Lewald J, et al. 1995. Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. *Science* 269:843–46
69. Gu YQ, Yang C, Thara VK, Zhou J, Martin GB. 2000. *Pti4* is induced by ethylene and salicylic acid, and its product is phosphorylated by the Pto kinase. *Plant Cell* 12:771–86
70. Guttman DS, Vinatzer BA, Sarkar SF, Ranall MV, Kettler G, Greenberg JT. 2002. A functional screen for the type III (Hrp) secretome of the plant pathogen *Pseudomonas syringae*. *Science* 295: 1722–26
71. Halterman D, Zhou F, Wei F, Wise RP, Schulze-Lefert P. 2001. The MLA6 coiled-coil, NBS-LRR protein confers AvrMla6-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat. *Plant J.* 25:335–48
72. Hammond-Kosack K, Jones JDG. 2000. Response to plant pathogens. In *Biochemistry and Molecular Biology of Plants*, ed. B Buchanan, D Gruissem, R Jones, pp. 1102–56. Rockville, MD: Am. Soc. Plant Physiol.
73. He Z, Wang ZY, Li J, Zhu Q, Lamb C, et al. 2000. Perception of brassinosteroids by the extracellular domain of the receptor kinase BRI1. *Science* 288: 2360–63
74. Heath MC. 2000. Nonhost resistance and nonspecific plant defenses. *Curr. Opin. Plant Biol.* 3:315–19
75. Heo WD, Lee SH, Kim MC, Kim JC, Chung WS, et al. 1999. Involvement of specific calmodulin isoforms in salicylic acid-independent activation of plant disease resistance responses. *Proc. Natl. Acad. Sci. USA* 96:766–71
76. Hinsch M, Staskawicz B. 1996. Identification of a new *Arabidopsis* disease resistance locus, *RPS4*, and cloning of the corresponding avirulence gene, *avrRps4*, from *Pseudomonas syringae* pv. *pisi*. *Mol. Plant-Microbe Interact.* 9:55–61
77. Holt BF, Boyes DC, Ellerstrom M, Siefers N, Wiig A, et al. 2002. An evolutionarily conserved mediator of plant disease resistance gene function is required for normal *Arabidopsis* development. *Dev. Cell* 2:807–17

78. Hulbert SH, Webb CA, Smith SM, Sun Q. 2001. Resistance gene complexes: evolution and utilization. *Annu. Rev. Phytopathol.* 39:285–312
79. Hwang CF, Bhakta AV, Truesdell GM, Pudlo WM, Williamson VM. 2000. Evidence for a role of the N terminus and leucine-rich repeat region of the *Mi* gene product in regulation of localized cell death. *Plant Cell* 12:1319–29
80. Jackson RW, Athanassopoulos E, Tsiamis G, Mansfield JW, Sesma A, et al. 1999. Identification of a pathogenicity island, which contains genes for virulence and avirulence, on a large native plasmid in the bean pathogen *Pseudomonas syringae* pathovar *phaseolicola*. *Proc. Natl. Acad. Sci. USA* 96:10875–80
81. Jaroszewski L, Rychlewski L, Reed JC, Godzik A. 2000. ATP-activated oligomerization as a mechanism for apoptosis regulation: fold and mechanism prediction for CED-4. *Proteins* 39:197–203
82. Jenner C, Hitchin E, Mansfield J, Walters K, Betteridge P, et al. 1991. Gene-for-gene interactions between *Pseudomonas syringae* pv. *phaseolicola* and *Phaseolus*. *Mol. Plant-Microbe Interact.* 4:553–62
83. Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B. 2000. Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO J.* 19:4004–14
84. Jin Q, He SY. 2001. Role of the Hrp pilus in type III protein secretion in *Pseudomonas syringae*. *Science* 294:2556–58
85. Jirage D, Tootle TL, Reuber TL, Frost LN, Feys BJ, et al. 1999. *Arabidopsis thaliana PAD4* encodes a lipase-like gene that is important for salicylic acid signaling. *Proc. Natl. Acad. Sci. USA* 96:13583–88
86. Johal GS, Briggs SP. 1992. Reductase activity encoded by the *HM1* disease resistance gene in maize. *Science* 258:958–87
87. Jones DA, Thomas CM, Hammond-Kosack KE, Balint -Kurti PJ, Jones JDG. 1994. Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. *Science* 266:789–93
88. Joosten MHAJ, Cozijnsen TJ, de Wit PJGM. 1994. Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. *Nature* 367:384–86
89. Jorgensen JH. 1996. Effect of three suppressors on the expression of powdery mildew resistance in barley. *Genome* 39:492–98
90. Kawchuk LM, Hachey J, Lynch DR, Kulcsar F, van Rooijen G, et al. 2001. Tomato *Ve* disease resistance genes encode cell surface-like receptors. *Proc. Natl. Acad. Sci. USA* 98:6511–15
91. Kim YJ, Lin NC, Martin GB. 2002. Two distinct *Pseudomonas* effector proteins interact with the Pto kinase and activate plant immunity. *Cell* 109:589–98
92. Kitagawa K, Hieter P. 2001. Evolutionary conservation between budding yeast and human kinetochores. *Nat. Rev. Mol. Cell. Biol.* 2:678–87
93. Kooman-Gersmann M, Honee G, Bonnema Wit PJGMd. 1996. A high-affinity binding site for the AVR9 peptide elicitor of *Cladosporium fulvum* is present on plasma membranes of tomato and other solanaceous plants. *Plant Cell* 8:929–38
94. Kooman-Gersmann M, Vogelsang R, Vossen P, van den Hooven HW, Mahe E, et al. 1998. Correlation between binding affinity and necrosis-inducing activity of mutant AVR9 peptide elicitors. *Plant Physiol.* 117:609–18
95. Kruger J, Thomas CM, Golstein C, Dion MS, Smoker M, et al. 2002. A tomato cysteine protease required for Cf-2-dependent disease resistance and suppression of autonecrosis. *Science* 296: 744–47
96. Lauge R, De Wit PJ. 1998. Fungal avirulence genes: structure and possible functions. *Fungal Genet. Biol.* 24:285–97

97. Lawrence GJ, Finnegan EJ, Ayliffe MA, Ellis JG. 1995. The *L6* gene for flax rust resistance is related to the *Arabidopsis* bacterial resistance gene *RPS2* and tobacco viral resistance gene *N*. *Plant Cell* 7:1195–206
98. Leach JE, Vera Cruz CM, Bai J, Leung H. 2001. Pathogen fitness penalty as a predictor of durability of disease resistance genes. *Annu. Rev. Phytopathol.* 39:187–224
99. Leister RT, Katagiri F. 2000. A resistance gene product of the nucleotide binding site—leucine rich repeats class can form a complex with bacterial avirulence proteins in vivo. *Plant J.* 22:345–54
100. Li J, Shan L, Zhou JM, Tang X. 2002. Overexpression of Pto induces a salicylate-independent cell death but inhibits necrotic lesions caused by salicylate-deficiency in tomato plants. *Mol. Plant-Microbe Interact.* 15:654–61
101. Liu GZ, Pi LY, Walker JC, Ronald PC, Song WY. 2002. Biochemical characterization of the kinase domain of the rice disease resistance receptor-like kinase XA21. *J. Biol. Chem.* 277:20264–69
102. Liu Y, Schiff M, Marathe R, Dinesh-Kumar SP. 2002. Tobacco *Rar1*, *EDS1* and *NPR1/NIM1* like genes are required for *N*-mediated resistance to tobacco mosaic virus. *Plant J.* 30:415–29
103. Liu Y, Schiff M, Serino G, Deng X-W, Dinesh-Kumar SP. 2002. Role of SCF ubiquitin-ligase and the COP9 signalosome in the *N* gene-mediated resistance response to tobacco mosaic virus. *Plant Cell* 14:1483–96
104. Loh YT, Zhou J, Martin GB. 1998. The myristylation motif of Pto is not required for disease resistance. *Mol. Plant-Microbe Interact.* 11:572–76
105. Luck JE, Lawrence GJ, Dodds PN, Shepherd KW, Ellis JG. 2000. Regions outside of the leucine-rich repeats of flax rust resistance proteins play a role in specificity determination. *Plant Cell* 12:1367–77
106. Luderer R, de Kock MJD, Dees RHL, de Wit PJGM, Joosten MHAJ. 2002. Functional anlaysis of cysteine residues of ECP elicitor proteins of the fungal tomato pathogen *Cladopsorium fulvum*. *Mol. Plant Pathol.* 3:91–95
107. Luderer R, Joosten MH. 2001. Avirulence proteins of plant pathogens: determinants of victory and defeat. *Mol. Plant Pathol.* 2:355–64
108. Luderer R, Rivas S, Nurnberger T, Mattei B, Van den Hooven HW, et al. 2001. No evidence for binding between resistance gene product Cf-9 of tomato and avirulence gene product AVR9 of *Cladosporium fulvum*. *Mol. Plant-Microbe Interact.* 14:867–76
109. Lyapina S, Cope G, Shevchenko A, Serino G, Tsuge T, et al. 2001. Promotion of NEDD-CUL1 conjugate cleavage by COP9 signalosome. *Science* 292:1382–85
110. Mackey D, Holt BF, Wiig A, Dangl JL. 2002. RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis*. *Cell* 108:743–54
111. Malcuit I, de Jong W, Baulcombe DC, Shields DC, Kavanagh TA. 2000. Acquisition of multiple virulence/avirulence determinants by potato virus X (PVX) has occurred through convergent evolution rather than through recombination. *Virus Genes* 20:165–72
112. Martin GB. 1999. Functional analysis of plant disease resistance genes and their downstream effectors. *Curr. Opin. Plant Biol.* 2:273–79
113. Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, et al. 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science* 262:1432–36
114. McDowell JM, Cuzick A, Can C, Beynon J, Dangl JL, Holub EB. 2000.

Downy mildew (*Peronospora parasitica*) resistance genes in *Arabidopsis* vary in functional requirements for NDR1, EDS1, NPR1 and salicylic acid accumulation. *Plant J.* 22:523–29

115. McDowell JM, Dhandaydham M, Long TA, Aarts MG, Goff S, et al. 1998. Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the *RPP8* locus of *Arabidopsis*. *Plant Cell* 10:1861–74
116. Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S, Sobral BW, Young ND. 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. *Plant J.* 20:317–32
117. Meyers BC, Shen KA, Rohani P, Gaut BS, Michelmore RW. 1998. Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. *Plant Cell* 10:1833–46
118. Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM. 1998. The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. *Plant Cell* 10:1307–19
119. Mindrinos M, Katagiri F, Yu G-L, Ausubel FM. 1994. The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. *Cell* 78:1089–99
120. Minsavage GV, Dahlbeck D, Whalen MC, Kearney B, Bonas U, et al. 1990. Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*-pepper interactions. *Mol. Plant-Microbe Interact.* 3: 41–47
121. Muskett PR, Kahn K, Austin MJ, Moisan LJ, Sadanandom A, et al. 2002. *Arabidopsis* RAR1 exerts rate-limiting control of *R* gene-mediated defenses against multiple pathogens. *Plant Cell* 14:979–92
122. Mysore KS, Crasta OR, Tuori RP, Folkerts O, Swirsky PB, Martin GB. 2002. Comprehensive transcript profiling of *Pto*- and *Prf*-mediated host defense responses to infection by *Pseudomonas syringae* pv. *tomato*. *Plant J.* 32:299–315
123. Nimchuk Z, Marois E, Kjemtrup S, Leister RT, Katagiri F, Dangl JL. 2000. Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. *Cell* 101:353–63
124. O'Neill L. 2000. The Toll/interleukin-1 receptor domain: a molecular switch for inflammation and host defence. *Biochem. Soc. Trans.* 28:557–63
125. Oldroyd GED, Staskawicz BJ. 1998. Genetically engineered broad-spectrum disease resistance in tomato. *Proc. Natl. Acad. Sci. USA* 95:10300–5
126. Orbach MJ, Farrall L, Sweigard JA, Chumley FG, Valent B. 2000. A telomeric avirulence gene determines efficacy for the rice blast resistance gene *Pita*. *Plant Cell* 12:2019–32
127. Ori N, Eshed Y, Paran I, Presting G, Aviv D, et al. 1997. The *I2C* family from the wilt disease resistance locus *I2* belongs to the nucleotide binding leucine-rich repeat superfamily of plant resistance genes. *Plant Cell* 9:521–32
128. Padgett HS, Beachy RN. 1993. Analysis of a tobacco mosaic virus strain capable of overcoming N gene-mediated resistance. *Plant Cell* 5:577–86
129. Pan Q, Wendel J, Fluhr R. 2000. Divergent evolution of plant NBS-LRR resistance gene homologues in dicot and cereal genomes. *J. Mol. Evol.* 50:203–13
130. Parker JE, Coleman MJ, Dean C, Jones JDG. 1997. The *Arabidopsis* downy mildew resistance gene *RPP5* shares similarity to the Toll and

Interleukin-1 receptors with N and L6. *Plant Cell* 9:879–94
131. Parker JE, Holub EB, Frost LN, Falk A, Gunn ND, Daniels MJ. 1996. Characterization of *eds1*, a mutation in *Arabidopsis* suppressing resistance to *Peronospora parasitica* specified by several different *RPP* genes. *Plant Cell* 8:2033–46
132. Peart JR, Cook G, Feys BJ, Parker JE, Baulcombe DC. 2002. An EDS1 orthologue is required for N-mediated resistance against tobacco mosaic virus. *Plant J.* 29:569–79
133. Petersen M, Brodersen P, Naested H, Andreasson E, Lindhart U, et al. 2000. *Arabidopsis* map kinase 4 negatively regulates systemic acquired resistance. *Cell* 103:1111–20
134. Petnicki-Ocwieja T, Schneider DJ, Tam VC, Chancey ST, Shan L, et al. 2002. Genomewide identification of proteins secreted by the Hrp type III protein secretion system of *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl. Acad. Sci. USA* 99:7652–57
135. Pickart CM. 2001. Ubiquitin enters the new millennium. *Mol. Cell* 8:499–504
136. Piedras P, Hammond-Kosack KE, Harrison K, Jones JDJ. 1998. Rapid, Cf-9- and Avr9-dependent production of active oxygen species in tobacco suspension cultures. *Mol. Plant-Microbe Interact.* 11:1155–66
137. Piedras P, Rivas S, Droge S, Hillmer S, Jones JD. 2000. Functional, c-myc-tagged Cf-9 resistance gene products are plasma-membrane localized and glycosylated. *Plant J.* 21:529–36
138. Querci M, Baulcombe DC, Goldbach RW, Salazar LF. 1995. Analysis of the resistance-breaking determinants of potato virus X (PVX) strain HB on different potato genotypes expressing extreme resistance to PVX. *Phytopathology* 85:1003–10
139. Rairdan GJ, Delaney TP. 2002. Role of salicylic acid and NIM1/NPR1 in race-specific resistance in *Arabidopsis*. *Genetics* 161:803–11
140. Rairdan GJ, Donofrio NM, Delaney TP. 2001. Salicylic acid and NIM1/NPR1-independent gene induction by incompatible *Peronospora parasitica* in *Arabidopsis*. *Mol. Plant-Microbe Interact.* 14:1235–46
141. Rathjen JP, Chang JH, Staskawicz BJ, Michelmore RW. 1999. Constitutively active Pto induces a Prf-dependent hypersensitive response in the absence of AvrPto. *EMBO J.* 18:3232–40
142. Ren T, Qu F, Morris TJ. 2000. *HRT* gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to turnip crinkle virus. *Plant Cell* 12:1917–26
143. Riely BK, Martin GB. 2001. Ancient origin of pathogen recognition specificity conferred by the tomato disease resistance gene *Pto*. *Proc. Natl. Acad. Sci. USA* 98:2059–64
144. Rigden DJ, Mello LV, Bertioli DJ. 2000. Structural modeling of a plant disease resistance gene product domain. *Proteins* 41:133–43
145. Rivas S, Mucyn T, van den Burg HA, Vervoort J, Jones JD. 2002. An approximately 400 kDa membrane-associated complex that contains one molecule of the resistance protein Cf-4. *Plant J.* 29:783–96
146. Rivas S, Romeis T, Jones JD. 2002. The Cf-9 disease resistance protein is present in an approximately 420-kilodalton heteromultimeric membrane-associated complex at one molecule per complex. *Plant Cell* 14:689–702
147. Rogers EE, Ausubel FM. 1997. *Arabidopsis* enhanced disease susceptibility mutants exhibit enhanced susceptibility to several bacterial pathogens and alterations in *PR-1* gene expression. *Plant Cell* 9:305–16
148. Romeis T, Ludwig AA, Martin R, Jones JD. 2001. Calcium-dependent protein

kinases play an essential role in a plant defence response. *EMBO J.* 20:5556–67
149. Romeis T, Piedras P, Jones JD. 2000. Resistance gene-dependent activation of a calcium-dependent protein kinase in the plant defense response. *Plant Cell* 12:803–16
150. Romeis T, Piedras P, Zhang S, Klessig DF, Hirt H, Jones JD. 1999. Rapid Avr9- and Cf-9-dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. *Plant Cell* 11:273–87
151. Ronald PC, Salmeron JM, Carland FM, Staskawicz BJ. 1992. The cloned avirulence gene *avrPto* induces disease resistance in tomato cultivars containing the *Pto* resistance gene. *J. Bacteriol.* 174:1604–11
152. Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM. 1998. The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proc. Natl. Acad. Sci. USA* 95:9750–54
153. Rusterucci C, Aviv DH, Holt BF, Dangl JL, Parker JE. 2001. The disease resistance signaling components EDS1 and PAD4 are essential regulators of the cell death pathway controlled by LSD1 in *Arabidopsis*. *Plant Cell* 13:2211–24
154. Salmeron JM, Barker SJ, Carland FM, Mehta AY, Staskawicz BJ. 1994. Tomato mutants altered in bacterial disease resistance provide evidence for a new locus controlling pathogen recognition. *Plant Cell* 6:511–20
155. Salmeron JM, Oldroyd GED, Rommens CMT, Scofield SR, Kim H-S, et al. 1996. Tomato *Prf* is a member of the leucine-rich repeat class of plant disease resistance genes and lies embedded within the *Pto* kinase gene cluster. *Cell* 86:123–33
156. Schulze-Lefert P, Vogel J. 2000. Closing the ranks to attack by powdery mildew. *Trends Plant Sci.* 5:343–44
157. Schwechheimer C, Serino G, Callis J, Crosby WL, Lyapina S, et al. 2001. Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCFTIRI in mediating auxin response. *Science* 292:1379–82
158. Scofield SR, Tobias CM, Rathjen JP, Chang JH, Lavelle DT, et al. 1996. Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science* 274:2063–65
159. Sessa G, D'Ascenzo M, Martin GB. 2000. Thr38 and Ser198 are Pto autophosphorylation sites required for the AvrPto-Pto-mediated hypersensitive response. *EMBO J.* 19:2257–69
160. Sessa G, Martin GB. 2000. Protein kinases in the plant defense response. In *Advances in Botanical Research*, ed. M Kreis, JC Walker; Series ed. JA Callow, pp. 379–404. London: Academic
161. Sessa G, Martin GB. 2000. Signal recognition and transduction mediated by the tomato Pto kinase: a paradigm of innate immunity in plants. *Microbes Infect.* 2:1591–97
162. Shan L, He P, Zhou JM, Tang X. 2000. A cluster of mutations disrupt the avirulence but not the virulence function of AvrPto. *Mol. Plant-Microbe Interact.* 13:592–98
163. Shan L, Thara VK, Martin GB, Zhou JM, Tang X. 2000. The *Pseudomonas* AvrPto protein is differentially recognized by tomato and tobacco and is localized to the plant plasma membrane. *Plant Cell* 12:2323–38
164. Shao F, Merritt PM, Bao Z, Innes RW, Dixon JE. 2002. A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis. *Cell* 109:575–88
165. Shen Y, Sharma P, Silva FG, Ronald P. 2002. The *Xanthomonas oryzae* pv. *lozengeoryzae raxP* and *raxQ* genes encode an ATP sulphurylase and adenosine-5′-phosphosulphate kinase that are

required for AvrXa21 avirulence activity. *Mol. Microbiol.* 44:37–48
166. Shirasu K, Lahaye T, Tan MW, Zhou F, Azevedo C, Schulze-Lefert P. 1999. A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in *C. elegans*. *Cell* 99:355–66
167. Simons G, Groenendijk J, Wijbrandi J, Reijans M, Groenen J, et al. 1998. Dissection of the *Fusarium I2* gene cluster in tomato reveals six homologs and one active gene copy. *Plant Cell* 10:1055–68
168. Song WY, Wang G-L, Chen L-L, Kim H-S, Pi L-Y, et al. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science* 270:1804–6
169. Staskawicz BJ, Mudgett MB, Dangl JL, Galan JE. 2001. Common and contrasting themes of plant and animal diseases. *Science* 292:2285–89
170. Swiderski MR, Innes RW. 2001. The *Arabidopsis PBS1* resistance gene encodes a member of a novel protein kinase subfamily. *Plant J.* 26:101–12
171. Szurek B, Marois E, Bonas U, Van den Ackerveken G. 2001. Eukaryotic features of the *Xanthomonas* type III effector AvrBs3: protein domains involved in transcriptional activation and the interaction with nuclear import receptors from pepper. *Plant J.* 26:52–34
171a. Szurek B, Rossier O, Hause G, Bonas U. 2002. Type III-dependent translocation of the *Xanthomonas* AvrBs3 protein into the plant cell. *Mol. Microbiol.* 46:13–23
172. Tai TH, Dahlbeck D, Clark ET, Gajiwala P, Pasion R, et al. 1999. Expression of the *Bs2* pepper gene confers resistance to bacterial spot disease in tomato. *Proc. Natl. Acad. Sci. USA* 96:14153–58
173. Takahashi H, Suzuki M, Natsuaki K, Shigyo T, Hino K, et al. 2001. Mapping the virus and host genes involved in the resistance response in cucumber mosaic virus-infected *Arabidopsis thaliana*. *Plant Cell Physiol.* 42:340–47
174. Tamaki S, Dahlbeck D, Staskawicz B, Keen NT. 1988. Characterization and expression of two avirulence genes cloned from *Pseudomonas syringae* pv. *glycinea*. *J. Bacteriol.* 170:4846–54
175. Tang X, Frederick RD, Zhou J, Halterman DA, Jia Y, Martin GB. 1996. Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. *Science* 274:2060–63
176. Thilmony RL, Chen Z, Bressan RA, Martin GB. 1995. Expression of the tomato *Pto* gene in tobacco enhances resistance to *Pseudomonas syringae* pv. *tabaci* expressing *avrPto*. *Plant Cell* 7: 1529–36
177. Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, et al. 1997. Characterization of the tomato *Cf-4* gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognitional specificity in Cf-4 and Cf-9. *Plant Cell* 9:2209–24
178. Tor M, Gordon P, Cuzick A, Eulgem T, Sinapidou E, et al. 2002. *Arabidopsis* SGT1b is required for defense signaling conferred by several downy mildew resistance genes. *Plant Cell* 14:993–1003
179. Tornero P, Chao RA, Luthin WN, Goff SA, Dangl JL. 2002. Large-scale structure-function analysis of the *Arabidopsis* RPM1 disease resistance protein. *Plant Cell* 14:435–50
180. Tornero P, Merritt P, Sadanandom A, Shirasu K, Innes RW, Dangl JL. 2002. RAR1 and NDR1 contribute quantitatively to disease resistance in *Arabidopsis*, and their relative contributions are dependent on the *R* gene assayed. *Plant Cell* 14:1005–15
181. Torres MA, Dangl JL, Jones JD. 2002. *Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. *Proc. Natl. Acad. Sci. USA* 99:517–22
182. Tsiamis G, Mansfield JW, Hockenhull R, Jackson RW, Sesma A, et al. 2000.

Cultivar-specific avirulence and virulence functions assigned to *avrPphF* in *Pseudomonas syringae* pv. *phaseolicola*, the cause of bean halo-blight disease. *EMBO J.* 19:3204–14

183. van der Biezen EA, Freddie CT, Kahn K, Parker JE, Jones JD. 2002. *Arabidopsis RPP4* is a member of the *RPP5* multigene family of TIR-NB-LRR genes and confers downy mildew resistance through multiple signalling components. *Plant J.* 29:439–51
184. Van der Biezen EA, Jones JDG. 1998. The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals. *Curr. Biol.* 8:R226–27
185. Van der Biezen EA, Jones JDG. 1998. Plant disease-resistance proteins and the gene-for-gene concept. *Trends Biochem. Sci.* 23:454–56
186. Van der Hoeven R, Ronning C, Giovannoni J, Martin G, Tanksley S. 2002. Deductions about the number, organization, and evolution of genes in the tomato genome based on analysis of a large expressed sequence tag collection and selective genomic sequencing. *Plant Cell* 14:1441–56
187. Van der Hoorn RA, De Wit PJ, Joosten MH. 2002. Balancing selection favors guarding resistance proteins. *Trends Plant Sci.* 7:67–71
188. Van der Hoorn RA, Kruijt M, Roth R, Brandwagt BF, Joosten MH, De Wit PJ. 2001. Intragenic recombination generated two distinct *Cf* genes that mediate AVR9 recognition in the natural population of *Lycopersicon pimpinellifolium*. *Proc. Natl. Acad. Sci. USA* 98:10493–98
189. Van der Hoorn RA, Laurent F, Roth R, De Wit PJ. 2000. Agroinfiltration is a versatile tool that facilitates comparative analyses of Avr9/Cf-9-induced and Avr4/Cf-4-induced necrosis. *Mol. Plant-Microbe Interact.* 13:439–46
190. Van der Vossen EA, van der Voort JN, Kanyuka K, Bendahmane A, Sandbrink H, et al. 2000. Homologues of a single resistance-gene cluster in potato confer resistance to distinct pathogens: a virus and a nematode. *Plant J.* 23:567–76
191. Vera Cruz CM, Bai J, Ona I, Leung H, Nelson RJ, et al. 2000. Predicting durability of a disease resistance gene based on an assessment of the fitness loss and epidemiological consequences of avirulence gene mutation. *Proc. Natl. Acad. Sci. USA* 97:13500–5
192. Vos P, Simons G, Jesse T, Wijbrandi J, Heinen L, et al. 1998. The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. *Nat. Biotechnol.* 16:1365–69
193. Wang GL, Ruan DL, Song WY, Sideris S, Chen L, et al. 1998. *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. *Plant Cell* 10:765–79
194. Wang ZX, Yano M, Yamanouchi U, Iwamoto M, Monna L, et al. 1999. The *Pib* gene for rice blast resistance belongs to the nucleotide binding and leucine-rich repeat class of plant disease resistance genes. *Plant J.* 19:55–64
195. Warren RF, Henk A, Mowery P, Holub E, Innes RW. 1998. A mutation within the leucine-rich repeat domain of the *Arabidopsis* disease resistance gene *RPS5* partially suppresses multiple bacterial and downy mildew resistance genes. *Plant Cell* 10:1439–52
196. Warren RF, Merritt PM, Holub E, Innes RW. 1999. Identification of three putative signal transduction genes involved in *R* gene-specified disease resistance in *Arabidopsis*. *Genetics* 152:401–12
197. Whalen MC, Innes RW, Bent AF, Staskawicz BJ. 1991. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *Plant Cell* 3:49–59

198. White FF, Yang B, Johnson LB. 2000. Prospects for understanding avirulence gene function. *Curr. Opin. Plant Biol.* 3: 291–98
199. Whitham SA, Anderberg RJ, Chisholm ST, Carrington JC. 2000. *Arabidopsis RTM2* gene is necessary for specific restriction of tobacco etch virus and encodes an unusual small heat shock-like protein. *Plant Cell* 12:569–82
200. Xiao S, Ellwood S, Calis O, Patrick E, Li T, et al. 2001. Broad-spectrum mildew resistance in *Arabidopsis thaliana* mediated by *RPW8*. *Science* 291:118–20
201. Yang B, Zhu W, Johnson LB, White FF. 2000. The virulence factor AvrXa7 of *Xanthomonas oryzae* pv. *oryzae* is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein. *Proc. Natl. Acad. Sci. USA* 97:9807–12
202. Yang KY, Liu Y, Zhang S. 2001. Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. *Proc. Natl. Acad. Sci. USA* 98:741–46
203. Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, et al. 1998. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc. Natl. Acad. Sci. USA* 95: 1663–68
204. Young ND. 2000. The genetic architecture of resistance. *Curr. Opin. Plant Biol.* 3:285–90
205. Zhang S, Klessig DF. 1998. Resistance gene *N*-mediated de novo synthesis and activation of a tobacco mitogen-activated protein kinase by tobacco mosaic virus infection. *Proc. Natl. Acad. Sci. USA* 95: 7433–38
206. Zhang S, Liu Y. 2001. Activation of salicylic acid-induced protein kinase, a mitogen-activated protein kinase, induces multiple defense responses in tobacco. *Plant Cell* 13:1877–89
207. Zhou F, Kurth J, Wei F, Elliott C, Vale G, et al. 2001. Cell-autonomous expression of barley Mla1 confers race-specific resistance to the powdery mildew fungus via a Rar1–independent signaling pathway. *Plant Cell* 13:337–50
208. Zhou J, Tang X, Martin GB. 1997. The Pto kinase conferring resistance to tomato bacterial speck disease interacts with proteins that bind a *cis*-element of pathogenesis-related genes. *EMBO J.* 16:3207–18
209. Zhou J-M, Loh Y-T, Bressan RA, Martin GB. 1995. The tomato gene *Pti1* encodes a serine-threonine kinase that is phosphorylated by Pto and is involved in the hypersensitive response. *Cell* 83:925–35
210. Zhou J-M, Trifa Y, Silva H, Pontier D, Lam E, et al. 2000. NPR1 differentially interacts with members of the TGA/OBF family of transcription factors that bind an element of the *PR-1* gene required for induction by salicylic acid. *Mol. Plant-Microbe Interact.* 13:191–202
211. Zhou N, Tootle TL, Tsui F, Klessig DF, Glazebrook J. 1998. PAD4 functions upstream from salicylic acid to control defense responses in *Arabidopsis*. *Plant Cell* 10:1021–30

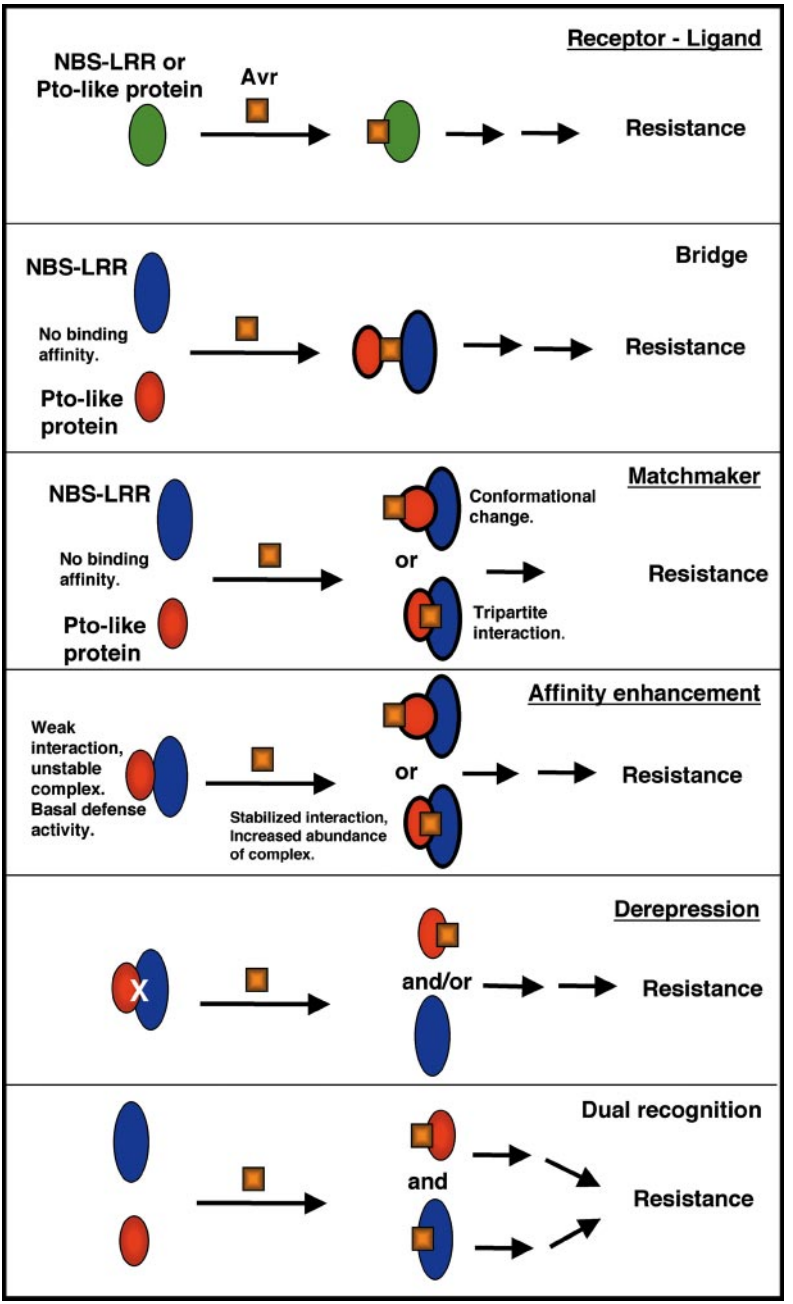

Figure 1 Models for protein-protein interactions that might underlie plant-pathogen "gene-for-gene" recognition. Models that encompass interactions that could be consistent with the "guard" hypothesis are underlined. See text for a discussion of these models.