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PLANT DISEASE RESISTANCE GENES

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ABSTRACT

In "gene-for-gene" interactions between plants and their pathogens, incompatibility (no disease) requires a dominant or semidominant resistance (*R*) gene in the plant, and a corresponding avirulence (*Avr*) gene in the pathogen. Many plant/pathogen interactions are of this type. *R* genes are presumed to (*a*) enable plants to detect *Avr*-gene-specified pathogen molecules, (*b*) initiate signal transduction to activate defenses, and (*c*) have the capacity to evolve new *R* gene specificities rapidly. Isolation of *R* genes has revealed four main classes of *R* gene sequences whose products appear to activate a similar range of defense mechanisms. Discovery of the structure of *R* genes and *R* gene loci provides insight into *R* gene function and evolution, and should lead to novel strategies for disease control.

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INTRODUCTION

Plants need to defend themselves against attack from viruses, microbes, invertebrates, and even other plants. Because plants lack a circulatory system, each plant cell must possess a preformed and/or inducible defense capability, so distinguishing plant defense from the vertebrate immune system (100). Following the rediscovery of Mendel's work, plant breeders recognized that resistance to disease was often inherited as a single dominant or semi-dominant gene (44). Considerable knowledge has since accumulated on the biochemical and genetic basis of disease resistance (27, 64, 73), while the use of resistant cultivars has become a valuable strategy to control crop disease (10). Only within the past four years have disease resistance (*R*) genes against distinct pathogen types been isolated. Intriguingly, the proteins encoded by *R* genes from different species against different pathogens have many features in common. Here we review this work and consider how *R* gene products may function and how the recognition of novel pathogen specificities could evolve. Several other recent reviews on isolated *R* genes are available (4, 37, 64, 87).

AN OVERVIEW OF PLANT-PATHOGEN ASSOCIATIONS AND THE GENETIC BASIS OF PLANT DEFENSE

Pathogens deploy one of three main strategies to attack plants: necrotrophy, biotrophy, or hemibiotrophy. Necrotrophs first kill host cells and then metabolize their contents. Some have a broad host range, and cell death is often induced by toxins and/or enzymes targeted to specific substrates (101). *Pythium* and *Botrytis* species are examples of fungal necrotrophs. Other necrotrophs produce host-selective toxins that are effective over a very narrow range of plant species. For this class of pathogens, plant resistance can be achieved via the loss or alteration of the toxin's target or through detoxification. Pathogen virulence is dominant because of the need to produce a functional toxin and/or enzyme, whereas avirulence, the inability to cause disease, is inherited as a recessive trait (Figure 1*A*). The first *R* gene to be iso-

lated was *Hm1* from maize, which confers resistance to the leaf spot fungus *Cochliobolus carbonum. Hm1* codes for a reductase enzyme that detoxifies the *C. carbonum* HC-toxin. This toxin inhibits histone deacetylase activity (35, 101), and the *Hm1* gene product is thought to inactivate the toxin.

Biotrophic and hemibiotrophic pathogens invade living cells and subvert metabolism to favor their growth and reproduction (1). The frequent formation of "green-islands" on senescing leaves surrounding the biotrophic infection sites of fungal rusts and mildews attests to the importance of keeping host cells alive throughout this intimate association (1). Biotrophs tend to cause disease on only one or a few related plant species. In contrast, hemibiotrophic fungi such as *Phytophthora* and *Colletotrichum* kill surrounding host cells during the later stages of the infection. Due to the specialized nature of these plant-biotrophic/hemibiotrophic pathogen associations, it is not surprising that minor differences in either organism can upset the balance. Incompatibility frequently results in the activation of plant defense responses, including localized host cell death, the hypersensitive response (HR) (27).

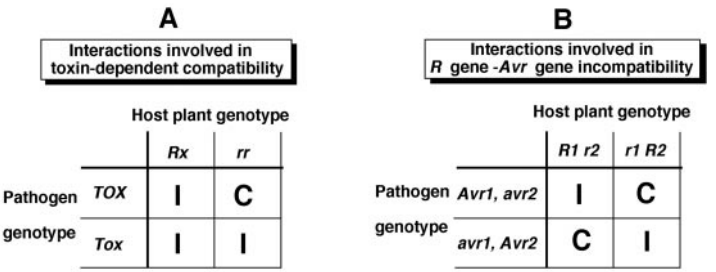


Figure 1 Various types of genetic interactions between plants and pathogenic microbes. In each panel, I denotes an incompatible interaction, where the plant is resistant to the pathogen, and C denotes a compatible interaction where the plant is susceptible to pathogen attack and disease occurs. (*A*) Interactions involved in toxin-dependent compatibility. The wild-type pathogen *TOX* gene is required for the synthesis of a toxin that is crucial for pathogenesis. *Tox* is the corresponding recessive, nonfunctional allele. The host *R* gene is required for detoxification, although resistance can also occur through expression of a toxin-insensitive form of the toxin target. Disease only occurs when the plant cannot detoxify the toxin produced by the pathogen. (*B*) Interactions involved in *R-Avr*-dependent incompatibility. *R1* and *R2* are two dominant plant resistance genes, where *r1* and *r2* are their respective recessive (nonfunctional) alleles. *R1* and *R2* confer recognition of pathogens carrying the corresponding pathogen avirulence genes, *Avr1* and *Avr2*, respectively, but not the respective recessive (nonfunctional) alleles, *avr1* and *avr2*. Disease (compatibility) occurs only in situations where either the resistance gene is absent or nonfunctional (*r1, r2*) or the pathogen lacks or has altered the corresponding avirulence gene (*avr1, avr2*). The interactions depicted in this panel are frequently called "the quadratic check" to indicate the presence of two independently acting *R-Avr* gene combinations (13, 19).

In the 1940s, using flax (*Linum usitatissimum*) and its fungal rust pathogen *Melampsora lini*, HH Flor studied the inheritance not only of plant resistance, but also of pathogen virulence (19). His work revealed the classic "gene-for-gene" model that proposes that for resistance to occur, complementary pairs of dominant genes, one in the host and the other in the pathogen, are required. A loss or alteration to either the plant resistance (*R*) gene or the pathogen avirulence (*Avr*) gene leads to disease (compatibility) (Figure 1*B*). This simple model holds true for most biotrophic pathogens, including fungi, viruses, bacteria, and nematodes (10, 44). The discovery that plants have centers of origin, where the greatest genetic diversity resides, and have co-evolved with pathogens, spurred a series of breeding programs to identify resistant germplasm in wild relatives of crop species and then introgress this for agricultural benefit (55). The spin-off for plant pathology was the development of several model "gene-for-gene" systems, ideal for intensive scrutiny because resistant and susceptible near-isogenic lines were available to minimize experimental differences due to background genetic variation. It is from these interactions that some of the first *R* genes and *Avr* genes have been isolated. The other *R* genes have been isolated from *Arabidopsis thaliana*, which has emerged in the past eight years as an excellent model system for plant-pathogen interaction studies (51).

Pathogen Avirulence (Avr) Genes

Although identified as the genetic determinants of incompatibility toward specific plant genotypes, the function of avirulence genes for the pathogen remains obscure. Plant viruses provide the only exception, where genes encoding either the coat protein, replicase, or movement protein have been demonstrated as the Avr determinant (62, 71, 93). Viral Avr specificity is altered by amino acid substitutions that do not significantly compromise the protein's function in pathogenesis. For the other microbial types, there often appears to be a fitness penalty associated with mutations from avirulence to virulence, and this suggests that the gene products have important roles for pathogenicity (11, 58). This view is reinforced by the fact that some *Avr* genes are always maintained within a pathogen population.

The molecular identities of a few fungal and bacterial Avr-generated signals are known. For fungi whose colonization is restricted to the plant's intercellular spaces (apoplast), small secreted peptides can elicit *R*-dependent defense responses in the pathogen's absence, e.g. Avr9 and Avr4 of *Cladosporium fulvum* and NIP1 of *Rhynchosporium secalis* (46). However, for biotrophic fungi that form intracellular haustoria, the nature of the Avr-derived signal is unknown.

For pathogenic bacteria, two distinct types of Avr-generated signals now appear to exist. Exported syringolides (C-glucosides with a novel tricyclic ring) are produced by enzymes encoded by the *avrD* locus of *Pseudomonas syringae* pv. *glycinea*, and these induce an HR on soybean cultivars that carry the *Rpg4* resistance gene (45). For other bacterial species, the Avr protein itself is now thought to be the signal. These *avr* gene products have no signal peptide, and yet they are recognized by *R* gene products that are likely to be cytoplasmic (see below). How do Avr products get into the plant cell? Bacterial *hrp* genes are required for both *h*ypersensitive *r*esponse induction and *p*athogenesis. *Hrp* genes code for a protein complex with strong homologies to the type III secretory system that is known to be used by some bacterial pathogens of mammalian cells (11). Recent work has conclusively shown that for the HR conditioned by genes for *Pseudomonas* resistance, i.e. *Pto, RPS2,* and *RPM1,* the corresponding Avr proteins must be delivered directly into the plant cell cytoplasm (22, 54a, 92). Although the *Xanthomonas avrBs3* family of *Avr* genes is very different in sequence from *Pseudomonas Avr* genes, delivery of the *avr* gene product into plant cells also appears necessary for their function. Members of the *avrBs3* family encode proteins with a highly reiterated internal motif of 34 amino acids in length, for example, *avrBs3* of *X. campestris* pv. *vesicatoria* and *avrXa10* of *X. oryzae* pv. *oryzae* (30, 32). By altering the number of repeats in *avrBs3* or the sequence within these repeats, both bacterial host range and *R*-mediated specificity was altered (30). Because *hrp* genes are essential for HR induction, and nuclear localization signals have been identified in the *avr* gene sequences, this indicates that gene product targeting to the plant cell nucleus may also be required for function (99a, 108).

Three Predicted Properties of R Genes and Their Products

The dominant nature of *R* and *Avr* genes has led to the inference that *R* genes encode proteins that can recognize *Avr*-gene-dependent ligands. Following pathogen recognition, the R protein is presumed to activate signaling cascade(s) that coordinate the initial plant defense responses to impair pathogen ingress. Implicit in this view is the notion that R proteins would be expressed in healthy, unchallenged plants in readiness for the detection of attack. A third requirement of R proteins is the capacity for rapid evolution of specificity. Frequently new virulent races of pathogens regularly evolve that evade specific *R* gene–mediated resistance (10, 13, 44, 64). Thus a mechanism is required by which plants can rapidly evolve new *R* genes to resist virulent isolates.

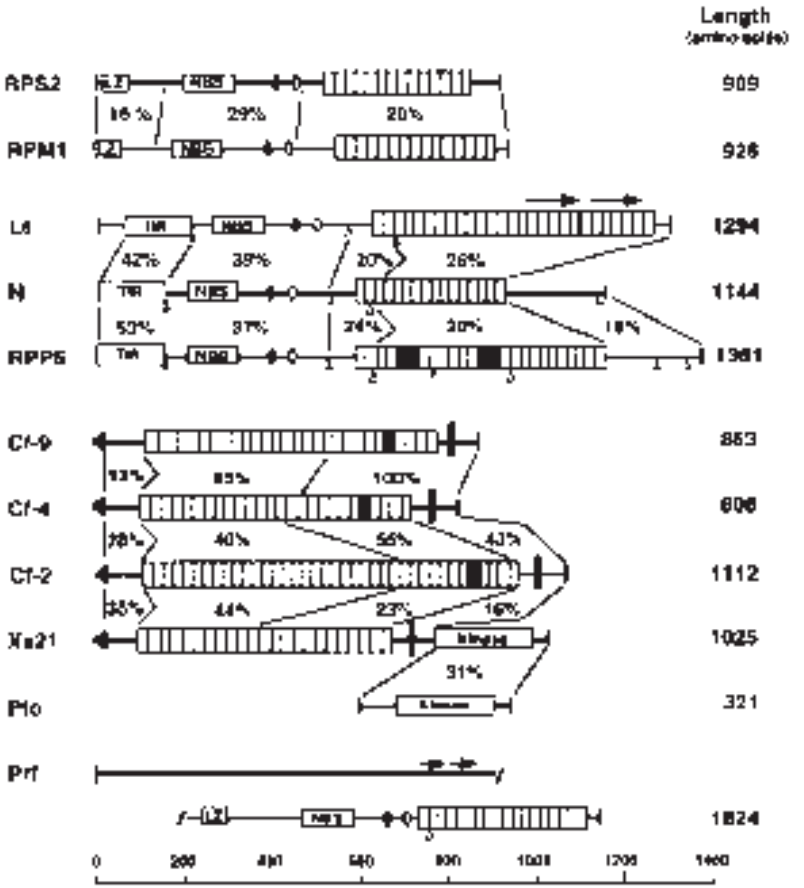
ISOLATED DISEASE RESISTANCE GENES

In the absence of a known biochemical role for R gene products, the *R* gene isolation strategies relied predominantly upon defining the gene's chromosomal location using segregating populations, and then identifying the correct sequence by either transposon insertion to destroy biological activity or cosmid complementation to restore the resistance phenotype. This technical challenge was solved simultaneously in several laboratories. A summary of the reported *R* genes is given in Table 1. Figure 2 provides an overview of the predicted structure of each R protein and their percent identity in specific regions. Figure 3 shows the alignment of amino acid sequences of particular common motifs and domains.

R Genes Predicted to Encode Cytoplasmic Proteins

ARABIDOPSIS *RPS2* AND *RPM1* GENES *RPS2* confers resistance to strains of *Pseudomonas syringae* bacteria that carry the plasmid-borne *avrRpt2* gene. *RPM1* provides resistance against *P. syringae* strains that express either of two nonhomologous *avr* genes, *avrB* or *avrRpm1* (5, 23, 65). The predicted gene products, 909 amino acids for RPS2 and 926 amino acids for RPM1, carry in their amino termini a possible leucine zipper region (LZ), a potential nucleotide binding site (NBS), and an internal hydrophobic domain. The carboxy-terminal halves are comprised of at least 14 imperfect leucine-rich repeats (LRRs). Overall, the two predicted sequences share 23% identity and 51% similarity (Figure 2). The LZs of RPS2 and RPM1 have 4 and 6 contiguous heptad sequences, respectively, that match the consensus sequence (I/R) XDLXXX (52). It is proposed that this domain facilitates the formation of a coiled-coil structure to promote either dimerization or specific interactions with other proteins. The NBS is found in numerous ATP- and GTP-binding proteins (98). The sequence GPGGVGKT of RPS2 matches the generalized consensus GXGXXG(R/k)V

Figure 2 Comparison of the predicted primary structure of R gene products and Prf (which is required for Pto function). Each protein has been drawn to scale, and the bar at the figure's foot indicates length in amino acids. Identified protein domains and motifs are shown either within boxes or as distinct shapes. Regions encoded by directly repeated DNA sequences in L6 and Prf are indicated by arrows above each protein. The percentage values placed between some R proteins reveal the amino acid sequence identity between either corresponding regions or exons, as determined by the GAP sequence alignment program (Genetics Computer Group, University of Wisconsin). For the comparison between RPS2 and RPM1, the regions aligned were 1-135 with 1-155, 135-418 with 155-442, and 418-909 with 442-926, respectively. For the comparisons between L6, N, and RPP5 the individual exons were aligned. The extracellular LRR proteins are divided into domains A to G for Cf proteins, and domains A to I for Xa-21 (38, 86). For Cf-9, domain A is the putative signal peptide; the domains B and D flank the LRRs that comprise domain C, and the



putative membrane anchor comprises domains E, F, and G. For the comparisons between the Cf-9, Cf-4, Cf-2, and Xa21, the regions aligned were grouped accordingly: domains A and B, the amino terminal 18 LRRs, 16 LRRs, 26 LRRs, and 11 LRRs, respectively; the carboxy terminal 12 LRRs, and domains D, E, F, and G, respectively. Full details of each gene product are given in the text. Abbreviations: LZ, Leucine zipper motif; NBS, Nucleotide binding site; TIR, Drosophila Toll/Human Interleukin-1 resistance gene cytoplasmic domain; ††††, Leucine-rich repeat domain, where the number of LRR motifs is indicated by the number of segments, and filled segments represent regions where the LRR motif is not conserved; Kinase, Serine/threonine kinase domain; filled circles, the GLPL(A/T)ax(V/S)aaG(S/G)aa motif, where a is an aliphatic amino acid; open circles, the L(R/K)xCFLY(C/I)(A/S)xF motif; +, transmembrane spanning region; ←, signal peptide; Δ, intron position.

for the kinase 1a, phosphate-binding loop (P-loop). This is followed by a kinase 2 domain, where an invariant aspartate is believed to coordinate the metal ion binding required for phospho-transfer reactions, and then a kinase 3a domain containing an arginine that in other proteins interacts with the purine base of ATP (98). These three domains have collectively been termed the NBS region in R proteins and are distinct from those found in protein kinases (94). The pres-

TABLE 1 THE FIVE CLASSES OF CLONED PLANT DISEASE RESISTANCE GENES

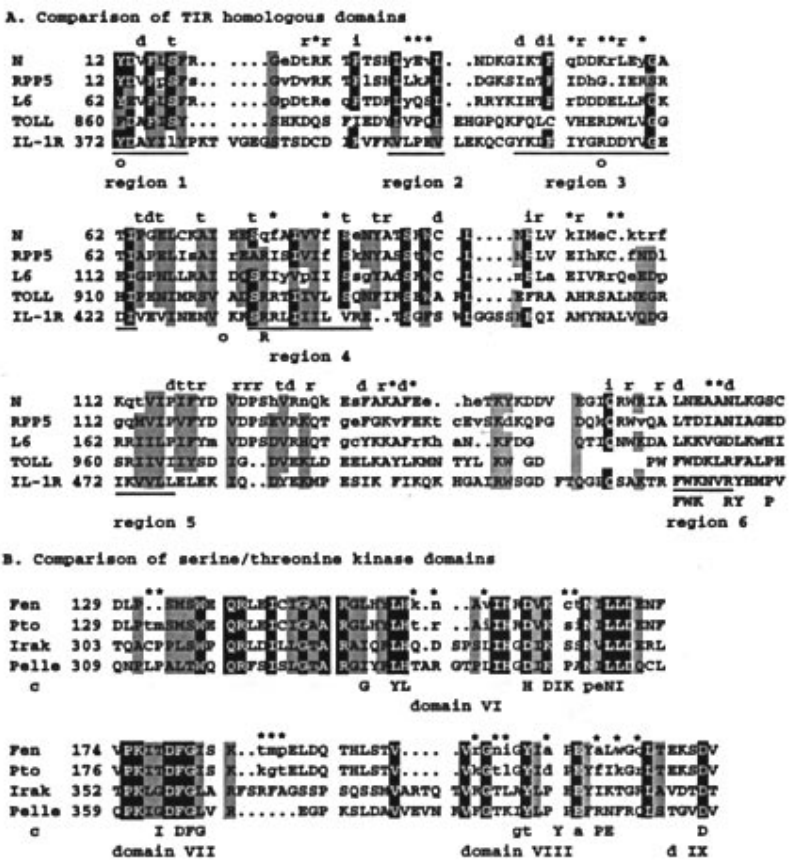
Class	Gene	Plant	Pathogen	Infection type/ organ attacked	Predicted Features of R protein	Reference
1.	*Hm1*	Maize	*Helminthosporium maydis* (race 1)	Fungal necrotroph / leaf	Detoxifying enzyme HC-toxin reductase	[35]
2.	*Pto*	Tomato	*Pseudomonas syringae* p.v. *tomato* (*avrPto*)	Extracellular bacteria / leaf	Intracellular serine/ threonine protein kinase	[59]
3a	*RPS2*	Arabidopsis	*Pseudomonas syringae* p.v. *tomato* (*avrRpt2*)	Extracellular bacteria / leaf	L. Zip / NBS / LRR	[5] [65]
	RPM1	Arabidopsis	*Pseudomonas syringae* p.v. *maculicola* (*avrRpm1/ avrB*)	Extracellular bacteria / leaf	Intracellular protein with amino terminal leucine zipper domain , and nucleotide binding site (NBS) and leucine rich repeat (LRR) domains	[23]
	I_2	Tomato	*Fusarium oxysporium* f.sp. *lycopersicon*	Necrotrophic fungus/root and vascular tissue		[A]
	[B]					
3b	*N*	Tobacco	Mosaic virus	Intracellular virus / leaf and phloem	Toll / NBS / LRR	[105]
	L6	Flax	*Melampsora lini* (*AL6, AM*)	Biotrophic fungal rust with haustoria / leaf	Intracellular protein with amino terminal domain homology with Drosophila Toll protein, and NBS and LRR domains	[53] [C]
	RPP5	Arabidopsis	*Peronospora parasitica*	Biotrophic downy mildew fungus with haustoria / leaf		[D]
4	*Cf-9, Cf-2, Cf-4 Cf-5*	Tomato	*Cladosporium fulvum* (*Avr9, Avr2, Avr4 Avr5*)	Biotrophic extracellular fungus without haustoria / leaf	Extracellular LRR protein with single membrane spanning region and short cytoplasmic carboxyl terminus	[38] [14] [41] [E]
5.	*Xa-21*	Rice	*Xanthomonas oryzae* pv. *oryzae* (all races)	Extracellular bacteria / leaf	Extracellular LRR protein with single membrane spanning region and cytoplasmic kinase domain	[86]

[A] G. Simons and R. Fluhr, pers. comm.; [B] a very tightly linked marker to the wheat *Cre3* gene that confers resistance to the root invading cereal cyst nematode *Heterodera avenae* is highly homologous to this *R* gene class, E. Lagudah and S. Anderson, pers. comm.; [C] P. Anderson, G. Lawrence and J. Ellis, pers. comm.; [D] J. Parker, M. Coleman, V. Szabo, M. Daniels and J. Jones, unpublished; [E] M. Dixon, K. Hatzixanthis and J. Jones, unpublished.

ence of the NBS suggests possible activation of a kinase or a role as a G-protein, though no biochemical evidence shows that the NBS actually binds ATP or GTP. The LRRs with an average repeat unit length of 23 amino acids show a good match to the cytoplasmic LRR consensus (LxxLxxLxxLxLxx(N/C/T) x(x)LxxIPxx) (37). LRRs have been implicated in protein-protein interactions and ligand binding in a diverse array of proteins (48). Collectively, the above features suggest both the *RPS2* and *RPM1* genes code for cytoplasmically localized proteins. This is intriguing because bacterial colonization is exclusively extracellular, but as stated above the Avr gene product may be delivered into plant cells via the bacterial Hrp secretory system. Comparison of the *avrRpt2*, *avrRpm1*, and *avrB* gene sequences reveals only minimal homology between them (11).

TOBACCO *N*, FLAX *L6*, AND ARABIDOPSIS *RPP5* GENES The tobacco (*Nicotiana tabacum*) *N* gene was originally introgressed from *N. glutinosa* and confers resistance to most strains of tobacco mosaic virus. Alternative splicing of the *N* gene transcript gives rise to two sizes of mRNA (105). The larger transcript codes for a 1144–amino acid protein (N), with an NBS, an internal hydrophobic domain, and 14 LRRs (23 amino acid type) present in the carboxyl terminal half. The less abundant truncated transcript codes for a 652–amino acid protein (N^{tr}) that possesses the amino terminal 616 amino acids of N including the NBS, the hydrophobic domain, and the first 1.5 LRRs followed by 36 amino acids. Although N shows similar structural organization to RPS2 and RPM1, the amino terminal domain of N is distinct, exhibiting homology with the cytoplasmic domains of the Drosophila Toll protein and the mammalian interleukin-1 receptor (IL-R) protein (Figure 3*A*) (20% and 16% amino acid identity and 42% and 41% amino acid similarity, respectively) (28, 85), and by inference another seven members of the growing Toll/IL-1R superfamily (66). This region in plant *R* genes has been designated the TIR (*T*oll/*I*nterleukin-1 *R*esistance) domain (B Baker, personal communication). Because the amino terminal domain of the N protein has homology to the cytoplasmic signaling domains of these receptors, it is probably involved in signaling and not ligand binding. Direct interaction between the tobacco N protein and the probable viral avr determinant, the replicase protein (71), is plausible because TMV replication is exclusively intracellular.

The flax (*Linum usitatissimum*) *L6* gene confers resistance to strains of the rust fungus *Melampsora lini* that carry the *AL6* avirulence gene (53). Like *N*, the *L6* gene gives rise to two mRNAs via alternative splicing. The larger and predominant transcript codes for a 1294–amino acid protein that like N contains within its amino terminus a TIR domain with homology to the cytoplasmic domains of Toll and IL-1R (21% and 16% amino acid identity and 50%


A. Comparison of TIR homologous domains
region 1
region 2
region 3
region 4
region 5
region 6
B. Comparison of serine/threonine kinase domains
domain VI
domain VII
domain VIII
C. Comparison of other conserved motifs in NBS/LRR proteins
Motif 3
HD

and 41% amino acid similarity, respectively). This TIR domain is followed by an NBS, a hydrophobic domain, and 27 LRRs that fit the 23–amino acid consensus but are highly imperfect in length (37). The carboxyl terminal 40% of the leucine-rich region is encoded by two directly repeated DNA sequences of 438 and 447 base pairs. L6 also possesses at its extreme amino terminus an additional 60 amino acids, which includes a potential signal anchor sequence, suggesting the protein might enter, but not pass through, the secretory pathway. The smaller transcript that arises via alternative splicing, codes for a 705–amino acid protein ($L6^{tr}$) that is identical to L6 for the first 676 amino acids but has a novel sequence of 29 amino acids and loses most of the LRR domain. The first 18 amino acids of the 29–amino acid C-terminal extension of $L6^{tr}$ is predicted to be a possible membrane-spanning region. Although the N/N^{tr} and $L6/L6^{tr}$ proteins appear structurally similar they may located in distinct subcellular compartments. Different cellular locations for these R proteins would fit well with the distinct biology of the two pathogens and the potential perception of the Avr ligand. The penetration peg

Figure 3 Amino acid sequence alignments between specific regions of R proteins, and where appropriate, related proteins of known biological function. In each Prettybox alignment (UWGCG program), the amino acids shown in white on a black background indicate identical residues, whereas those shown in black on a grey background indicate similar residues. Amino acid similarities were defined as follows: I=V=L=M, D=E=Q=N, F=Y=W, H=K=R, and G=A=S=T=P (67). (*A*) Comparison of the TIR (Toll/IL-1 Receptor cytoplasmic) domain in the tobacco N, Arabidopsis RPP5, and flax L6 R proteins with the corresponding cytoplasmic domains in the Drosophila Toll receptor and the human interleukin-1 receptor (IL-1R) proteins (28, 85). The asterisks above the alignment indicate amino acids in the three R proteins that do not conform to the Toll/IL-1R consensus. r indicates the 14 conserved amino acids specific to the three R proteins where there is no conserved consensus between Toll and IL-1R. d indicates the 11 amino acids where the three R proteins have the same consensus and this differs from the Toll/IL-1R consensus. t indicates the eight amino acids specifically conserved between the R proteins and Toll. i indicates the four amino acids specifically conserved between IL-1R and the three R proteins. Below the sequence alignment the locations of six conserved regions identified in the Toll/IL-1R superfamily (66) are shown, and the single amino acids that when mutated compromise protein function are highlighted with o for Toll function and with individual letters for IL-1R function (29, 82). (*B*) Comparison of the portion of the serine/threonine protein kinase domains of tomato R protein Pto (59), which confers AvrPto specificity (92), with the same region in the tomato Fen protein that confers Fenthion insecticide sensitivity (60), the human interleukin-receptor associated kinase (IRAK) (84), and the Drosophila Pelle kinase (8). The asterisks above the alignment indicate the 17 amino acids that differ between Pto and Fen (conserved substitutions are also underlined). Below the alignment is the eukaryotic consensus sequence for serine-threonine kinases (98). (*C*) Comparison of a portion of the region between the NBS and LRR regions in the tobacco N, flax L6, tomato Prf, and Arabidopsis RPP5, RPM1, and RPS2 proteins. The four conserved motifs of unknown function indicated are the HD motif; GLPL(A/T)ax(V/S)aaG(S/G)aa, where a is an aliphatic amino acid; motif 1 with the consensus L(R/K)xCFLY(C/I)(A/S)xF; motif 2, consensus Lx(I/L/F)SYxxL(N/E)P; and motif 3 (L/F)ExaAxxaV.

of the rust fungus spore passes through the host cell wall, but the haustorium does not breach the plasma membrane and eventually becomes entirely surrounded by it (1). Membrane localization of the L6/L6^{tr} proteins would therefore facilitate interception of the fungal avr-derived signal.

The Arabidopsis *RPP5* gene was isolated from the resistant accession Landsberg-*erecta* and confers resistance to the biotrophic downy mildew fungus *Peronospora parasitica,* which is a natural pathogen of Arabidopsis (39). The predicted gene product of 1361 amino acids possesses a TIR domain at the amino terminus, followed by an NBS and 21 LRRs in the carboxyl terminus. Each LRR motif varies in length from 21 to 24 amino acids, but this domain also contains two regions with less homology to the LRR consensus. Because the RPP5 sequence predicts neither the presence of a signal peptide or membrane spanning region, the protein is probably cytoplasmically localized. The *RPP5* gene is more closely related to *N* and *L6* than to *RPS2* and *RPM1* (described above), because of the TIR domain and the similarity in the positions of the intron/exon splice junctions that give rise to exons 1, 2, and 3 (Figure 2).

TOMATO *Pto, Fen,* AND *Prf* GENES The tomato *Pto* gene confers resistance to races of *Pseudomonas syringae* pv. *tomato* that carry the *avrPto* gene. This was the first race-specific *R* gene to be isolated (59). *Pto* codes for a 321–amino acid protein and has been shown to be a serine/threonine-specific protein kinase, capable of autophosphorylation (57). Pto possesses 27 serine and 13 threonine residues, and is in the same protein kinase class as the cytoplasmic domain of the *Brassica* self-incompatibility gene *SRK,* the mammalian signaling factor Raf, the *Drosophila* pelle kinase, and the human IRAK kinase (Figure 3*A;* 7, 8, 84, 88). The protein does not possess an LRR domain or an NBS. Thus Pto appears to possess a signal transduction but no obvious recognition capacity. However, recent experiments using both *Pto* and *avrPto* sequences in a yeast 2-hybrid system indicate that the AvrPto and Pto proteins do directly interact (83, 92). Pto autophosphorylation is also required for the Pto-avrPto interaction to occur.

Pto is a member of a clustered family of five genes (60). One of the other family members is *Fen,* which specifies sensitivity to the insecticide fenthion and codes for a 318–amino acid serine/threonine protein kinase (60). The Fen protein shares 80% identity (87% similarity) with Pto but does not confer avrPto-dependent bacterial resistance. Both protein kinase activity and a putative N-terminal myristoylation site, proposed to be involved in membrane tareting, are required to confer fenthion sensitivity (77). The Pto myristoylation site is not required for resistance to *P. syringae.* The Fen kinase does not interact with avrPto in the yeast 2-hybrid system (92). The analysis of a series

of Pto/ Fen chimeric genes in both yeast and transgenic plants has identified a 95–amino acid stretch of Pto, between residues 129 and 224, that is required for interaction with avrPto and for disease resistance (92). Within these 95 amino acids, Fen and Pto differ by only 13 nonconservative changes (Figure 3*B*). The Fen specificity is localized to the carboxy terminal 186 amino acids (77).

Mutagenesis of *Pto*-containing tomato has revealed an additional gene, *Prf*, that is required for both *Pto* and *Fen* to function (80). *Prf* is located within the *Pto* gene family, 24 kb from the *Pto* gene but just 500 bp from the *Fen* gene (81). *Prf* encodes a 1824–amino acid protein with leucine zipper, NBS, and leucine-rich repeat motifs of the 23–amino acid type, which identifies it as a member of the resistance gene class that includes *RPS2* and *RPM1*, and is more distantly related to the *N* and *L6* genes. Prf also possesses a large amino-terminal region, 720 residues in length, with no homology to any known protein. At the end of this region are two direct repeats of 70 and 71 amino acids with 49% sequence identity. Because both *Pto* and *Prf* are essential for resistance, this demonstrates that both LRR-containing proteins and protein kinases can be components of the same signaling pathway. However, the functional relationship between Pto and Prf proteins is not yet known (see below).

R Genes Predicted to Encode Proteins with Extracytoplasmic Domains

TOMATO *Cf-9*, *Cf-2*, *Cf-4*, AND *Cf-5* GENES Resistance to the leaf mould pathogen *Cladosporium fulvum* is conferred by distinct *Cf* genes, which have been introgressed from various wild *Lycopersicon* species or land races into cultivated tomato *Lycopersicon esculentum* (27). Two *C. fulvum Avr* genes, *Avr9* and *Avr4*, that confer avirulence on *Cf-9* and *Cf-4* expressing tomato, respectively, have been cloned (43, 99). Their secreted cysteine-rich peptide products of 28 (Avr9) and 88 (Avr4) amino acids are potentially ligands for the Cf-9 and Cf-4 proteins. Four tomato *Cf* genes have been isolated.

Cf-9 encodes an 863–amino acid membrane-anchored, predominantly extracytoplasmic glycoprotein containing 27 imperfect LRRs with an average length of 24 amino acids. The LRRs show a good match to the extracytoplasmic LRR consensus of LxxLxxLxxLxLxxNxLxGxIPxx (37). The LRR domain is interrupted by a short region, originally designated as LRR 24, which has only minimal LRR homology. This domain, now designated C2, divides the LRR domain into 23 amino terminal LRRs (domain C1) and 4 carboxy terminal LRRs (domain C3). This C2 “loop out” domain appears to be absent from most other extracytoplasmic LRR proteins, except the other Cf proteins

described below. It could act as a molecular hinge that connects the C1 and C3 regions or as an extended loop that interacts with other proteins that participate in signal transduction. Flanking both ends of the LRR domain are two regions (domains B and D) that contain several cysteine residues, conserved in other LRR proteins, that may be important in maintaining the overall protein structure (37). The 21–amino acid cytoplasmic terminus of Cf-9 (domain G) concludes with the motif KKxx, which in mammals or yeast would be expected to localize the protein to the endoplasmic reticulum (97).

Cf-4, which is tightly linked to *Cf-9*, encodes an 806–amino acid protein very similar to that of Cf-9 (41). Cf-4 differs from Cf-9 by possessing two fewer LRRs and by having one other small deletion and a number of amino acid substitutions in the amino-terminal half of the protein (Figure 2). The carboxy-terminal halves of both proteins, from LRR 18 of Cf-9 onward, are identical, suggesting that resistance specificity resides in their amino-terminal portions whereas the carboxyl-terminal portion probably interacts with common signaling/regulatory component(s).

The *Cf-2* locus, unlinked to the *Cf-4/Cf-9* locus, contains two functional genes that each independently confer resistance. Each *Cf-2* gene encodes a 1112–amino acid protein, which has a similar overall structure to Cf-9 but possesses 37 LRRs (14). Both Cf-2s lack the KKxx motif of Cf-9, suggesting either a different cellular location for Cf-2, which might account for some of the differences between Cf-9- and Cf-2-mediated defense responses activated by their respective Avr gene product (27), or that this motif has no relevance to Cf protein function. The LRRs of Cf-2 are nearly all exactly 24 amino acids in length, and 20 of these have a highly conserved alternating repeat motif. A similar arrangement is not evident in the Cf-9 and Cf-4 proteins. However, like the other two Cf proteins, a short C2 domain divides the LRRs into an amino terminal block of 33 LRRs and a carboxyl terminal block of 4 LRRs. Like the other Cf proteins, both Cf-2s have many predicted NxS/T glycosylation sites. As the highest homology between the Cf-2 and Cf-9 and Cf-4 proteins resides in the carboxyl terminal 360 amino acids of Cf-2 (Figure 2), this again suggests this region plays a similar role in all three proteins.

The *Cf-5* gene, tightly linked to *Cf-2*, encodes a 968–amino acid protein very similar to that of *Cf-2* (Table 1). The two proteins differ by the exact deletion of six LRRs within the alternating repeat region of Cf-5 and by several amino acid changes in the amino terminal two thirds of each protein. The carboxyl terminal halves of Cf-2 and Cf-5 are also highly conserved.

RICE *Xa21* GENE *Xa21* confers resistance to over 30 distinct strains of the bacterium *Xanthomonas oryzae* pv. *oryzae*, which causes leaf blight in rice. *Xa21*

encodes a 1025–amino acid protein that possesses a putative signal peptide, 23 extracytoplasmic LRRs with numerous potential glycosylation sites, a single transmembrane domain, and an intracellular serine/threonine kinase domain (86). The LRR domain cannot be classified into C1, C2, and C3 domains, unlike in the Cf proteins. The Xa21 protein shows pronounced overall homology with the Arabidopsis receptor-like serine/threonine kinase RLK5, whose function is currently unknown (7). However, because Xa21 possesses both the LRR feature of the Cf-9 protein and a Pto-like serine/threonine kinase domain, this protein provides the first potential clue to the link between R proteins predicted to encode solely a receptor function and potential downstream signaling capacity.

It is somewhat surprising that resistance to strains of *Xanthomonas oryzae* pv. *oryzae* expressing *avrXa21* is conferred by an R protein structurally distinct from the other bacterial resistance proteins, i.e. RPS2, RPM1, or Pto. Although the Hrp secretory system is required by some *X. oryzae avr* genes to induce the resistance response, e.g. *avrXa10* on rice plants carrying *Xa10* (32), this has not yet been established for *avrXa21,* and neither has this gene been isoolated. The avrXa21-derived ligand might have a novel molecular identity, because *Xanthomonas oryzae* pv. *oryzae* is predominantly a xylem vessel colonizing bacterium. Conceivably, it is delivered extracellularly, unlike other bacterial Avr products, in which case the Xa21 LRRs might be involved in the recognition.

Are There Other R Gene Classes?

Various research groups have either isolated or are at the final stages of isolating *R* genes to a diverse array of additional microbes. These include the fungal resistance genes *I2* from tomato, the rust *M* gene from flax, and the cyst nematode resistance gene *Cre3* from wheat (Table 1). The majority of this second wave of isolated *R* genes can be recognized as highly related to members of existing *R* gene subclasses. In addition, based on conserved features found in the NBS/LRR class of R proteins (see below), candidate *R* genes of potato linked to the *Gro1* gene that confers resistance to the cyst nematode *Globodera rostochiensis* and the *R7* gene that gives resistance to the hemibiotrophic fungus *Phytophthora infestans,* have been identified (54). This suggests that perhaps plants use only a limited number of recognition/signal transduction systems to combat microbial attack. These new findings also clearly highlight the fact that R protein structure cannot be predicted from the nature of the pathogen or vice versa. It is possible (but not certain) that we have seen all the kinds of gene-for-gene *R* genes there are.

R PROTEIN MOTIFS AND THEIR POTENTIAL FUNCTION

Pathogen Recognition

Mechanistically, the simplest interpretation of Flor's gene-for-gene hypothesis is that the *Avr*-gene dependent ligand binds directly to the R gene product which then activates downstream signaling events to induce various defense responses (20). As the majority of the isolated *R* genes encode proteins that possess domains characteristic of authentic receptor proteins found in mammals, Drosophila, and yeast, a receptor-like function for plant R proteins appears likely. The most obvious candidate for providing the recognition specificity is the LRR domain. LRRs have been demonstrated to bind the corresponding ligand, for example in the porcine RNase inhibitor protein (PRI) and the receptors for gonadotropin and follicle-stimulating hormone (48, 69). Although the contact points between PRI and its RNase ligand have been accurately determined by co-crystallization studies (49), it is difficult to extrapolate these data to identify potential residues involved in protein binding in plant LRR proteins because of the unique nature of the PRI LRR motifs. PRI is comprised of alternating LRR motifs of 28 and 29 amino acids in length that form a horseshoe structure (49). The plant LRRs with motifs of 23 and 24 amino acids in length may form a β-helix, which is a more linear structure, as found in pectate lyase, P22 tailspike protein, and pertactin (18, 50, 89). For those R proteins predicted to be extracytoplasmic, where the LRR motifs and the integrity of the entire LRR domain are best conserved, a hydrophobic face could form to facilitate multiple interactions with other proteins or ligands. A key future goal is to elucidate the crystal structure of several plant LRR proteins.

If the tomato Cf proteins are localized to the plant plasma membrane, these could each directly bind a different extracellular peptide ligand derived from *Cladosporium fulvum.* The amino-terminal 18 LRR of Cf-9, 16 LRRs of Cf-4, and 28 LRRs of Cf-2 are likely to contain the binding region because these regions possess the greatest sequence divergence. However, it seems unlikely that all the LRRs would be involved in binding such a small ligand as Avr9, unless Avr9 multimers bind. Instead, some of the LRRs may be required to provide the correct structure surrounding the binding site. Alternatively, the Cf proteins may bind to a larger plant protein, and each Avr peptide may modulate this interaction by binding either to the Cf protein or the other protein. This model could also apply to the rice Xa21 protein if the avrXa21 gene product is secreted from bacteria but does not enter plant cells. For several extracellular LRR proteins, the glycosylation pattern within the LRR domain is crucial for ligand binding (109). Glycosylation sites are absent from the β-strand of the LRR motif in the C1 domain but are present

within the β-strand of the C3 LRRs (37). It remains to be established whether glycosylation patterns influence R protein function.

For the LRR proteins predicted to be cytoplasmically localized, it is unclear whether the function of the LRR domain is to confer the specificity of recognition. By default this domain is apparently accepted as the recognition domain, primarily because all the other motifs appear to have an obvious signaling capacity. However, the role of the LRR in these R proteins could be dimerization or interaction with either upstream or downstream signaling components. The LRR domain of yeast adenylate cyclase is required to interact with ras protein (91). Clearly the LRR domain is of functional importance, because some alleles of RPS2 and RPM1 with just single amino acid changes in the LRR domain do not confer resistance (5, 23, 65). Domain swap experiments between RPS2 and RPM1, or between different alleles of the *L* locus, should provide insight into the specificity domains of *R* gene products.

The tomato Pto protein binds directly to the *Pseudomonas syringae* avrPto gene product (83, 92). Although the actual amino acids specifying binding are undetermined, this interaction entirely conforms to the biochemical interpretation of Flor's gene-for-gene hypothesis.

Signal Transduction

For the NBS/LRR class of *R* genes, the nucleotide binding site and either the leucine zipper or TIR homologous domains are the most likely to be involved in signaling. The presence of the NBS, which is found in numerous ATP and GTP binding proteins (98), suggests that although these R proteins do not possess intrinsic kinase activity, they could activate kinases or G proteins. Mutagenesis of amino acids known to be required for NBS function destroys R protein and Prf biological activity (81). The NBS domains found in R proteins are most similar to those found in ras proteins, adenylate kinases, ATP synthase β-subunits, and ribosomal elongation factors (25, 98). An important future goal is to characterize the nature of the nucleotide triphosphate binding and its significance to R protein function.

The leucine zipper regions found in RPM1, RPS2, and Prf, which in each protein precede the NBS, potentially could facilitate homodimerization of the proteins themselves or heterodimerization with other proteins (52). R proteins could exist as monomers before pathogen challenge and then undergo dimerization or oligomerization upon activation. Alternatively, they could exist initially as dimers or multimers that disassociate upon activation. Computer data-base searches with these leucine zipper regions reveal the greatest similarity to the coiled-coil regions in myosin and paramyosin proteins (5, 52).

The TIR domain in N, L6, and RPP5, though exhibiting only moderate homology to the Toll/IL-R cytoplasmic domain (Figure 3*A*), is tantalizing. The Drosophila Toll receptor protein, which also has an extracytoplasmic LRR domain, controls dorsal-ventral polarity in embryos (21, 28, 68). Toll is activated by a processed small extracytoplasmic protein ligand, spätzle, that has a cysteine-knot structure. Binding of spätzle to Toll may lead to the activation of a cytoplasmic protein tube, which in turn activates the serine/threonine kinase, pelle, by recruiting it to the plasma membrane. Pelle then phosphorylates the inhibitory protein cactus that is complexed with the transcription factor dorsal. Phosphorylation of cactus leads to its own degradation, and this permits dorsal to relocate to the nucleus and activate genes controlling ventralization. Dorsal is a member of the rel/NF-κB family of proteins (102). Another Drosophila protein highly related to dorsal is Dif (*d*orsal related *i*mmunity *f*actor), which is involved in activating the defense response in fat bodies (72). Mutation studies suggest that Toll may also play a role in the nuclear localization of Dif (79). The human interleukin-1/interleukin-1 receptor protein system is involved in both the inflammatory and immune responses (70, 85). IL-1R activates the transcription factor NF-κB by releasing it from a cytoplasmically localized complex with the inhibitor protein (IκB) and requires the protein kinase (IRAK), which has high homology to pelle (30.5% amino acid identity) and Pto (34% identity) (Figure 3*B;* 102). Considering both the sequence homology and related functions of these vertebrate, insect, and plant proteins, the N, L6, and RPP5 proteins could function in an analogous manner. This view is reinforced because one of the fastest recognized components of the *N*-mediated defense response is the generation of reactive oxygen species, ROS (15). During the mammalian innate immune response in macrophages, activation of NF-κB is also redox regulated (63). In both situations, once defense is activated rapid amplification of the initial response could be achieved by a positive feedback loop involving ROS. The induced assembly of a multisubunit, membrane-localized, NADPH-oxidase complex, is required for generation of ROS (42). In plants, a similar complex appears to be needed for rapid ROS generation during defense (27). The sequence alignments of the TIR domains (Figure 3*A*) indicate that the R proteins are more closely related to Toll than IL-1R, although distributed throughout are either R protein–specific or Toll/IL-1R–specific amino acids (indicated with r, d, and * in Figure 3*A*). Overall 25% of the amino acid sequence compared falls in this category. In addition, the homology at the C-terminal end of the Toll/IL-1R superfamily consensus (66), a region required for IL-1R function (29), is absent in the three R proteins. These findings suggest the TIR domain of R proteins may provide a novel function.

The serine/threonine kinase capacity possessed by Pto and Xa21 could clearly facilitate downstream signaling. Because Pto is highly homologous to the Drosophila protein pelle required for Toll mediated signaling (described above), Pto kinase may serve a similar function, i.e. transcription factor activation. When Pto was used as the "bait" in a yeast two hybrid system, a number of interacting gene products were identified (110). These included sequences with homology to transcription factors as well as another protein kinase called Pti1 (*Pto*-interacting gene*1*), but not Prf. Pto may simultaneously activate several distinct signaling pathways. Because the Pti1 gene product can be phosphorylated by Pto and is capable of autophosphorylation but cannot phosphorylate Pto, a protein kinase cascade initiated by Pti phosphorylation by Pto may be one of these downstream signaling pathways. The kinase domain of the rice Xa21 gene product is most homologous to that of the Arabidopsis protein RLK5. When RLK5 was used in an interaction cloning system, a type 2C phosphatase was identified (90). Moreover, for many gene–mediated resistances, the addition of either kinase or phosphatase inhibitors significantly blocked the induction of rapid defense responses (16, 56). It appears likely that both kinases and phosphatases are involved in downstream R protein–mediated signaling events.

For the tomato Cf proteins, in the absence of any obvious signaling domains, the molecular identity of the signaling partners remains enigmatic. Possibly the short cytoplasmic domains might interact with a protein kinase. For the membrane-anchored CD14 receptor of T-cells, activation of the downstream kinase $p56^{Lck}$ requires only a short cytoplasmic domain (103). Alternatively, Cf proteins may already exist in or become associated with a membrane receptor complex involving transmembrane LRR-kinase proteins analogous to TMK1, RLK5, or Xa21 or some of the other five classes of transmembrane kinase (3, 7). Such a mechanism would recruit a kinase domain for intracellular signaling. The carboxyl terminal 10 LRRs are the most likely to interact with either a common or conserved signaling partner(s) because of their high sequence conservation (Figure 2). The binding of the Avr ligands, either directly or in association with another protein(s), may lead to a conformational change to the Cf protein and cause these domains to activate signaling partner(s).

The alternate splicing products expressed from the wild-type *N* and *L6* genes lead to the synthesis of both full-length R protein and a shorter protein that lacks most of the LRR domain. Several mammalian cell-surface receptors, e.g. growth-factor and cytokine receptors, also exist as soluble truncated proteins. These truncated proteins, although incapable of signal activation themselves, compete with the full-length forms for ligand binding and

thereby tightly control the concentrations of ligand available for signaling (70). Alternatively, these truncated forms have been shown to bind to the intact receptor and modulate its function (82, 107). A defined role for the truncated forms of L6 and N in resistance has yet to be established.

Other Shared Motifs of Unknown Function

Two short sequence motifs are found in the majority of NBS/LRR R proteins and Prf (Figures 2 and 3*C*) between the NBS and LRR domains. One, designated conserved domain 2 (23), encodes a hydrophobic domain (HD) with the consensus GLPL(A/T)ax(V/S)aaG(S/G)aa, where a is an aliphatic amino acid. The other, designated conserved domain 3 (23), is situated 50–70 amino acids carboxyl to the first and has the consensus L(R/K)xCFLY(C/I)(A/S)xF. There are also two other slightly less well conserved short domains in this region (Figure 3*C*). Computer data-base searches have revealed these domains to be unique to R proteins and Prf, with the exception of Genbank accession No. U19616 and several ESTs, which probably represent orphan *R* genes because of their high overall similarity to *RPM1* and *RPS2*. The function of these motifs is not known. The high amino acid sequence conservation and fixed location of domains 2 and 3 has permitted oligonucleotide primers to be designed that can specifically amplify *R* gene–related sequences in many plant species (54).

A MODEL FOR R PROTEIN ACTION

In Figure 4 a model for R protein function incorporating the current knowledge and predictions about R and Avr gene products is depicted. We anticipate that R proteins will activate multiple signaling pathways simultaneously. For numerous mammalian receptor proteins, this scenario is increasingly evident (70). Such a pivotal role for plant R proteins in resistance would ensure that a wide repertoire of potential defense responses are rapidly and coordinately induced in various cellular compartments. Immediate downstream signaling components will include kinase and phosphatase cascades, transcription factors, and reactive oxygen species. It is also likely that the plant's defense strategy relies on the induction of responses that are directed against pathogen attack in general and are not pathogen specific. This is suggested because R protein structure cannot be predicted from pathogen types (Table 1) and a similar array of defense responses are induced by unrelated organisms. For example, callose and lignin deposition, antimicrobial phytoalexin and salicylic acid synthesis, pathogenesis-related gene induction, and the hypersensitive cell death responses are frequent components of the local resis-

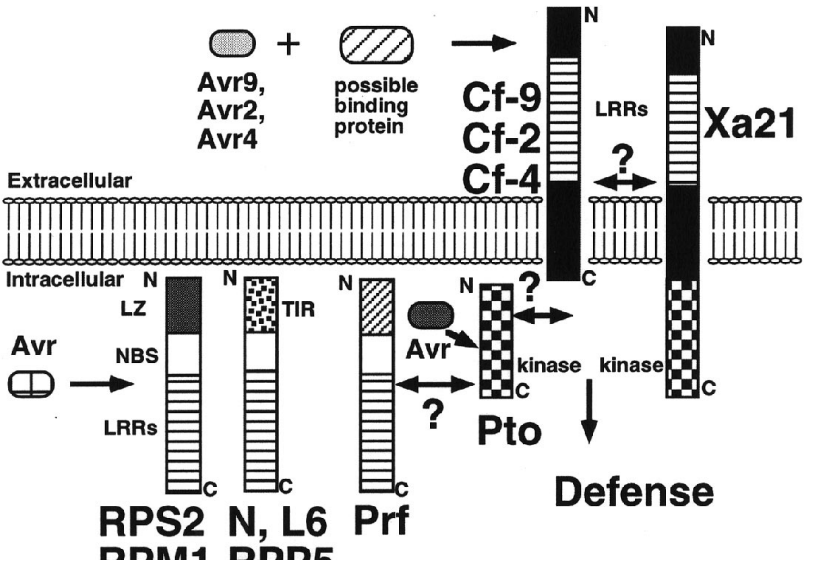


Figure 4 Representation of predicted R gene product structures and a model coupling the recognition of microbial Avr-dependent ligand and activation of plant defense. Pto can directly bind AvrPto (83, 92). The other R proteins probably bind the corresponding Avr gene products, either directly or in association with a binding protein. Both Pto and Xa21 have a protein kinase domain. It is likely that RPM1, RPS2, N, L6, and RPP5 and the Cf proteins also activate defense through a protein kinase, but the mechanism for this is not known. For example, the Cf proteins could interact with either an Xa21-like protein or a Pto-like protein to activate a protein kinase cascade. Prf is required for Pto-mediated resistance (80), but it is not understood why. Speculative interactions are indicated with a question mark. Abbreviations: LZ, putative leucine zipper region; TIR, region with homology to the cytoplasmic domain of the Drosophila Toll and human interleukin-1 receptors (see Figure 3*A*); LRR, leucine-rich repeat motifs; N, amino terminus; C, carboxyl terminus.

tance response activated by many different avirulent microbes (27). Because the predicted R proteins have many similar features, it is likely that several of the downstream signaling cascades activated by distinct subclasses of R proteins will rapidly converge to execute this common defense response. The identification of the Arabidopsis *Ndr* mutation that compromises *RPM1, RPS2,* and some *RPP* gene-mediated resistances to *P. parasitica* establishes that resistances involving different R protein classes can have common component(s) (9). In addition, because the slow Arabidopsis HR phenotype mediated by the gene combination *avrRpm1/RPM1* can interfere with the fast resistance mediated by *avrRpt2/RPS2* genes (74, 76), some initial steps in the signal transduction pathway may be triggered by both R proteins.

The phenotype of Prf mutants demonstrates that Prf is required for Pto function. However, because avrPto and Pto directly interact in yeast, this leaves the precise function of Prf unclear. The LRR motif in Prf is unlikely to be directly involved in avr ligand recognition. Possibly Prf acts as an anchor protein that localizes the Pto kinase, or it might participate in a protein complex engaged by avrPto-Pto to stimulate additional signaling pathways. Alternatively, F Katagiri (personal communication) has proposed that Prf could recognize the phosphorylated form of a plant-encoded protein that is an avrPto-dependent substrate of Pto. Whatever the mechanism, the identification of Prf as a participating protein in Pto-mediated resistance provides a link between the NBS/LRR R proteins and protein kinases. This raises the possiblity that all cytoplasmic-located R proteins with NBS/LRR domains will interact with a kinase(s) to activate downstream signaling events, and vice versa.

ADDITIONAL FEATURES OF *R* GENES

R Gene Function in Heterologous Plant Species

Several of the isolated *R* genes have been introduced, via *Agrobacterium*-mediated transformation, into other plant species, and have been demonstrated to retain biological activity. The tomato *Pto* gene functions in *N. tabacum* and *N. benthamiana*, the tobacco *N* gene is active in tomato where the N resistance response retains temperature sensitivity, and the tomato *Cf-9* gene mediates recognition of Avr9 peptide and necrosis formation in tobacco and potato (40, 78, 95, 106). Thus Avr-dependent R protein–triggered signaling cascades are conserved between plant species. However, when *Pto* or Fen is expressed at high levels in *Nicotiana clevelandii* by using a potato virus X vector system, necrotic symptoms develop in the absence of pathogen challenge (77; K Swords & B Staskawicz, unpublished data). These data highlight how finely tuned the relationship is between R proteins and signaling partners in their native plant species. Because sequences homologous to the introduced *R* genes can be detected in these other plant species, it is tempting to speculate that these may also function as *R* genes. We envision that *R* gene evolution is constrained not only by selection for pathogen recognition but also by selection against recognition of endogenous plant proteins, and that some other transgenic *R* gene transfer experiments may therefore lead to necrosis.

R Gene Expression

For plants to respond rapidly to microbial attack it was anticipated that R proteins should be present in healthy plants throughout life. RNA gel blot analyzes using *RPS2, RPM1, Pto, Cf-9,* and *Cf-2* as gene probes have revealed

the presence of low abundance transcripts in unchallenged plants, indicating that at least the *RPM1* gene and some members of multigenic *R* families are expressed in the absence of the corresponding *Avr*-expressing pathogen (14, 23, 38, 59, 65). It is not yet known whether the levels of *R* gene expression increase at the site of microbial infection. However, this may not be a prerequisite because for the human immune response to be fully activated by the interleukin-1 receptor only 10 receptor molecules are required per cell to initiate the full response to ligand challenge (70).

R GENE ORIGIN AND EVOLUTION

From What Did R Genes Evolve?

The most likely ancestors of *R* genes probably coded for proteins involved in endogenous recognition/signaling systems required for the plant's normal growth or development, because a significant number of the mammalian, yeast, and insect proteins related to plant R proteins control endogenous signaling, development, and/or cell-to-cell adhesion. Two plant proteins similar to the extracytoplasmic LRR R protein, Xa21, are encoded by the Arabidopsis *erecta* and *clavata* genes that determine floral organ shape and size (96) (S Clark & E Meyerowitz, personal communication). Both the Erecta and Clavata proteins are thought to be involved in cell-to-cell communication events utilizing an extracellular ligand. Plant pathogens may have enhanced their own pathogenic potential by evolving a signaling capacity that could modify endogenous plant signaling systems. An alternative explanation for *R* gene evolution is that genes involved in multicellularity evolved from progenitor "*R*" genes involved in pathogen recognition by their unicellular ancestors; this seems less likely. The considerable structural homology between the NBS/LRR class of R proteins and the human major histocompatibility complex (MHC) class II transcription activator (CIITA), and between the exLRR class of R proteins and the mouse RP105 protein involved in B cell proliferation and protection against programmed cell death (see 37), suggests plant *R* genes and genes involved in mammalian immunity may have a common evolutionary origin.

Resistance to the same bacterial *Avr* genes has been observed in taxonomically distinct plant species (47, 104). This suggests that either there has been preservation of an ancient specificity or the same recognitional specificity evolved multiple times to a prevalent pathogen ligand. As additional plant *R* genes are isolated and related gene families recognized, it may be possible to determine which of these evolutionary scenarios is the more likely.

Generation of Evolutionary Novelty at R Gene Loci

Many plant pathogens exhibit a high mutation rate from avirulence to virulence that renders obsolete the effectiveness of individual *R* genes (10, 13, 44, 73). Because natural selection would favor the multiplication of these virulent races, plants must evolve novel R protein variants that can detect either the modified Avr determinant or another component of the pathogen. The most informative clue to the evolution of *R* gene diversity is their genomic organization. Different *R* loci can exist in one of four arrangements: They can consist of a single gene with an array of distinct alleles, each providing a different recognition specificity. The flax *L* locus is organized in this manner, so that only one of the 13 or more *L* specificities to *M. lini* are present within a pure breeding line (73). The *R* gene may exist as a single copy gene that is present in resistant lines but absent from susceptible lines. The Arabidopsis *RPM1* gene is of this type. For many *R* genes, the *R* locus is comprised of tandem arrays of closely linked *R* gene homologues with differing specificities. Examples of these "complex loci" include the *M* locus in flax, with 7 specificities to *M. lini* (73). Of the *R* genes isolated, *Cf-9, Cf-4, Cf-2, Cf-5, N, Pto,* and *Xa21* have been revealed by DNA gel blot analyses to reside within a linked cluster of related gene sequences (14, 38, 41, 59, 86, 105). Based on genetic analyses the *Rp1* locus of maize with 14 specificities to the rust *Puccinia sorghi* will likely also be of this type (34). Finally, in particular genomic regions, *R* genes to viral, bacterial, and fungal pathogens are loosely clustered, i.e. 1–2 cm apart (14, 51, 64). In Arabidopsis, five of these *R* gene–rich regions are now recognized and have been designated as "major resistance complexes" (MRCs) (31). It remains to be seen whether the *R* gene specificities identified in these "complexes" show enough relatedness to have had a common evolutionary origin. Tight clustering of *R* genes probably arose because of an initial duplication of the genomic segment that carried the ancestral gene. This was achieved by a rare crossing-over event, between homologous sequences at nonhomologous locations, possibly facilitated by the existence of linked repetitive elements (73). Because highly homologous gene sequences exist at the flax *L* and *M* loci, it is likely that repeated DNA structures surrounding the *M* locus but absent from the *L* region assisted the duplication of the intervening *M* genes (17).

At complex *R* loci, unequal cross-over via meiotic mispairing between different genes is currently thought to be the major way in which novel resistance specificities are generated and new combinations of parental *R* genes are created (Figure 5). Detailed genetic analysis of the highly unstable *Rp1* complex of maize, using DNA flanking markers to analyze the types of re-

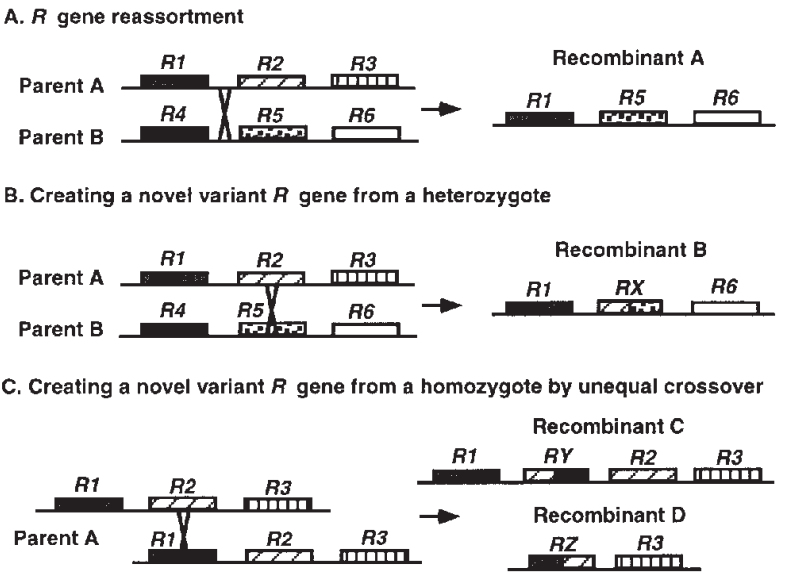
combination events occurring, indicates that diversity has arisen via cross-overs and also to a lesser extent by gene conversion (34, 75). One example of an unequal cross-over at a cloned *R* gene locus has been molecularly characterized. The tomato *Cf-2* and *Cf-5* genes are essentially allelic. A *Cf-2/Cf-5* trans-heterozygote was testcrossed and progeny were screened for individuals that carried neither *Cf-2* nor *Cf-5;* one was recovered in 12,000. In this disease-susceptible individual, *Cf-2* and *Cf-5* homologue copy number was reduced from 3 (in *Cf-2*) or 4 (in *Cf-5*) to 2 (14). Further analysis has revealed this recombination event took place within a *Cf-2* reading frame (M Dixon, personal communication). Overall, plants may not have devised a specialized mechanism to promote rapid *R* gene evolution (Figure 5). This contrasts strongly with the mechanism in mammals required for the recognition of nonself, where somatic events generate antibody diversity. An interesting feature of the plant mechanism to generate *R* gene diversity is that not all copies within the tandemly arranged *R* gene homologues would need to be expressed or functional.

At "simple" resistance loci, cross-over and gene conversion events probably play a similar role in generating *R* gene diversity. However, the number of distinct *R* gene sequences that can pair and recombine will be more limited, particularly in diploid self-fertile plant species. It is therefore likely that unequal intragenic recombination or slipped alignment during replication will play a significant role in modifying *R* gene product function. The sequence of the flax *L6* gene has revealed how this could occur. The C-terminal half of the LRR domain comprises two direct repeats of 480 bp with 85% identity (53), and comparison of the *L6*, *L2*, and *L10* alleles reveals variation in both LRR number and sequence in this region (17). In addition, at the flax *M* locus, loss of gene function is associated with the loss of one of these repeat units (P Anderson, J Ellis & G Lawrence, personal communication). Amplification or reduction in the number of LRR blocks within a single R protein may modify recognition specificity (87). However, none of the three mutant flax *M* alleles that have lost approximately six LRRs appears to confer novel resistance specificity (P Anderson, J Ellis & G Lawrence, personal communication). How the single copy Arabidopsis *RPM1* gene evolved to provide dual specificity of recognition of avrB and avrRpm1 is currently unclear. In soybean the recognition mediated by these two sequence-unrelated *avr* genes involves either two different alleles of the *RPG1* resistance gene or a second closely linked *R* gene (2).

For the *N*, *L6*, and *RPP5* genes the recognized structural domains of these proteins are located within different exons: The TIR homology domain resides within exon 1, the NBS and HD in exon 2, and the LRR domain pre-

dominantly in exon 4 and subsequent exons (Figure 2). Because these intron locations are conserved in other members of each gene family, exon shuffling resulting in protein domain replacement is another potential mechanism that could facilitate the creation of novel R protein variants, at both simple and complex *R* loci. Such a mechanism has been proposed for other genes with this type of structural organization (61).

Several novel *R* gene variants have arisen at the meiotically unstable maize *Rp1* locus that have lost *R-Avr* specificity. These *R* alleles confer resis-



D. Resistance specificity

	Pathogen race								
	A1	A2	A3	A4	A5	A6	AX	AY	AZ
Parent A	I	I	I	C	C	C	C	C	C
Parent B	C	C	C	I	I	I	C	C	C
Recombinant A	I	C	C	C	I	I	C	C	C
Recombinant B	I	?	C	C	?	I	I	C	C
Recombinant C	I	?	I	C	C	C	C	I	C
Recombinant D	?	?	I	C	C	C	C	C	I

tance to all *Puccinia sorghi* pathotypes tested and even other rust species, e.g. *P. polysora* (33, 34). The resistance is often weak and is invariably associated with a visible necrotic reaction. In the absence of pathogen challenge, plants with some of these variant *R* alleles still develop necrotic spotting (34). Other so-called "disease lesion mimics" are well known in maize, barley, and tomato (12, 36). A characteristic feature of many but not all, including the *Rp1* variant alleles, is that the necrotic spotting does not occur when the plants are grown under sterile conditions. Therefore a biotic stimulus (e.g. saprophytic microbe) is often required for phenotype expression. Possibly these variant R proteins are affected in the ligand binding/recognition domain, so that they are able to recognize a broad array of microbe/interaction-derived products. Alternatively, modifications to the signaling domain might constitutively activate defense signaling cascades, or provide a "hair trigger" that even weak Avr recognition events activate. Clearly, these novel *R* alleles are interesting to disease control because of the nonpathogen-specific nature of the resistance response. Their isolation is keenly awaited.

CONCLUDING REMARKS AND FUTURE PROSPECTS

With the isolation of the first few plant *R* genes, immense opportunities now unfold for protein biochemists, biologists, physiologists, and geneticists alike to elucidate how these gene products function and the gene families evolve.

Figure 5 Models for the creation of novel variant *R* genes. The lines and boxes represent contiguous regions of paired chromosomes during meiosis of either an F_1 hybrid between two different parental lines A and B that each carry three closely linked different *R* genes (*boxes*) (*panels A and B*) or after self-fertilization of the parent line A (*panel C*). Three different cross-over events and some of the different genetic outcomes are illustrated. (*A*) Crossover (X) in the intergenic region of correctly aligned genes creates Recombinant A, which carries a novel combination of *R* genes. The other recombinant chromosome not drawn would be *R4, R2, R3.* (*B*) Intragenic cross-over between two different *R* genes creates a novel *R* gene (*RX*) in Recombinant B capable of recognizing a different pathogen avirulence gene (*AX*). The other recombinant chromosome not drawn would be *R4, RW, R3.* (*C*) Misalignment during chromosome pairing caused by the high sequence relatedness of the clustered *R* genes permits unequal cross-over to create the novel genes, *RY* and *RZ* in Recombinant C and D, respectively, capable of recognizing additional pathogen avirulence genes *AY* and *AZ*, respectively. After an unequal cross-over event within a complex *R* locus, the number of homologues inherited will be either expanded or contracted. (*D*) Responses of the two parental lines and four recombinants to nine different pathogen races. I, incompatible interaction; C, compatible interaction. Recombinant A is resistant to the same assortment of the pathogen races as each parental line, but to no additional races. Recombinant B is resistant to one pathogen race that carries the *AX* gene, to which both parents were susceptible. Recombinants C and D are resistant to two different pathogen races that carry the *AY* or *AZ* gene, respectively, to which the parent plant was susceptible. The ? indicates that it cannot be predicted whether the recombinant gene will retain the recognition specified of the original *R* genes as well as confer a novel recognition function.

Key unresolved questions include: What are the domain(s) or residues within each R protein that confer the specificity of microbial recognition? Do the Avr gene products always interact directly with R proteins? What are the immediate downstream signaling components and how do these activate multiple defenses? Which induced defense responses are crucial for conferring resistance against each microbial type? What roles do the gene products of other loci already identified by mutation analysis as required for *R-Avr* gene-mediated disease resistance, the *rdr* loci (9, 27, 51), play in defense? How are novel variant *R* genes naturally generated? Solving these questions is also likely to reveal new insight into the processes underlying normal plant growth and development and plant genome organization.

The identification of so few distinct classes of *R* genes is most intriguing. This suggests that plants have evolved only a limited number of mechanisms to defend themselves against microbial attack. Therefore, will nonrace-specific *R* genes, like the barley powdery mildew resistance gene *mlo*, code for a distinct R protein class? Likewise will the receptors for nonspecific microbial elicitors, for example chitin fragments, heptaglucosides, and the PEP-13 ligand from the fungus *Phytophthora sojae* (6, 24), which each confer defense response activation, be encoded by genes with homology to *R* genes? When resistance is inherited polygenically, will the quantitative trait loci (*QTL*s) that do not map to *R* gene clusters (64) identify other *R* gene classes, or will these *QTL* genes code for signaling or defense components? Also, for numerous pathogens, where completion of their life cycles involves two alternate hosts (1), for example the fungal rusts that require both wheat plants and barberry bushes, will the resistance manifested by these distinct plant species be mediated by related *R* genes or not?

The initial interest by plant breeders in resistant plant germplasm arose because it provided the possibility of a cheap solution to disease control in crops. Unfortunately, expectations of success for the new elite resistant cultivars were rarely achieved and the "Boom and Bust" cycle of disease control has prevailed in many crops for almost 50 years (1). Cloned *R* genes now provide novel tools for plant breeders to improve the efficiency of plant breeding strategies, via marker assisted breeding, and by using transformation for accelerating the introgression of useful *R* genes from related species (10, 64). It will also be possible to isolate and transfer homologous *R* genes between different plant species, e.g. wheat and barley, and therefore determine whether the nonhost status of individual plant species to *formae speciales* of a pathogen like *Erisyphe graminis* is caused by particular homologues of known *R* genes. Plant biotechnologists can attempt to manipulate both *Avr* and *R* gene sequences to provide broad-spectrum and durable disease control (26, 87).

Hopefully, a combination of strategies will reduce the requirement for agrochemicals to control crop diseases and will accelerate effective retrieval and deployment of the natural variation in *R* genes of wild plant species.

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