Signaling in Plant-Microbe Interactions

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Analysis of viral and bacterial pathogenesis has revealed common themes in the ways in which plants and animals respond to pathogenic agents. Pathogenic bacteria use macromolecule delivery systems (types III and IV) to deliver microbial avirulence proteins and transfer DNA-protein complexes directly into plant cells. The molecular events that constitute critical steps of plant-pathogen interactions seem to involve ligand-receptor mechanisms for pathogen recognition and the induction of signal transduction pathways in the plant that lead to defense responses. Unraveling the molecular basis of disease resistance pathways has laid a foundation for the rational design of crop protection strategies.

Since the onset of civilization, plant diseases have had catastrophic effects on crops and the well-being of human populations. For example, the fungus *Phytophthora infestans* caused the epidemic that triggered the Irish potato famine of the 1840s. Infectious plant diseases continue to cause human suffering and enormous economic losses. An increasing human population and decreasing amounts of land available for agriculture make all approaches to securing the world food supply critical. Protection of crops from disease can substantially improve agricultural production. Although pesticides have successfully controlled disease, their continued and increasing use will have harmful effects on our health and the environment. Use of high-yield crop varieties can also improve productivity, but carries a risk—such genetically uniform varieties cultivated over enormous areas are susceptible to devastating epidemics. Thoughtful application of the plant's own defense mechanisms, combined with understanding of the complex ecology of real-world disease processes, can lead to more effective protection against plant pathogens. In this review we analyze plant pathogen interactions, including microbial strategies for pathogenesis and key elements of host responses (Figs. 1 and 2). We focus on studies using the model plant *Arabidopsis thaliana*. Symbiotic interactions such as that of legume and *Rhizobium* are related and have been reviewed elsewhere (*1*, *2*).

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Common Defenses in Diverse Species

Plants are hosts to thousands of infectious diseases caused by a vast array of phytopathogenic fungi, bacteria, viruses, and nematodes (Fig. 1 and Table 1). A relatively small proportion of pathogens successfully invade the plant host and cause disease. Plants recognize and resist many invading phytopathogens by inducing a rapid defense response, termed the hypersensitive response (HR). The HR results in localized cell and tissue death at the site of infection, which constrains further spread of the infection (*3*) (Fig. 3A). This local response often triggers nonspecific resistance throughout the plant, a phenomenon known as systemic acquired resistance (SAR) (Fig. 3A) (*4*). Once triggered, SAR provides resistance to a wide range of pathogens for days. The HR and SAR depend on interaction between a dominant or semidominant resistance (*R*) gene product in the plant and a corresponding dominant phytopathogen avirulence (*Avr*) gene product, as predicted by Flor (*5*). It has been predicted that phytopathogen *Avr* products function as ligands and host *R* products function as receptors in an interaction leading to plant resistance to disease (*6*, *7*).

Genetic dissection of the basis of microbial pathogenicity and host resistance is made easier by *A. thaliana*, the botanic counterpart of geneticists' *Drosophila* (*8*–*11*). All classes of phytopathogens cause disease in *Arabidopsis* (Fig. 1 and Table 2),

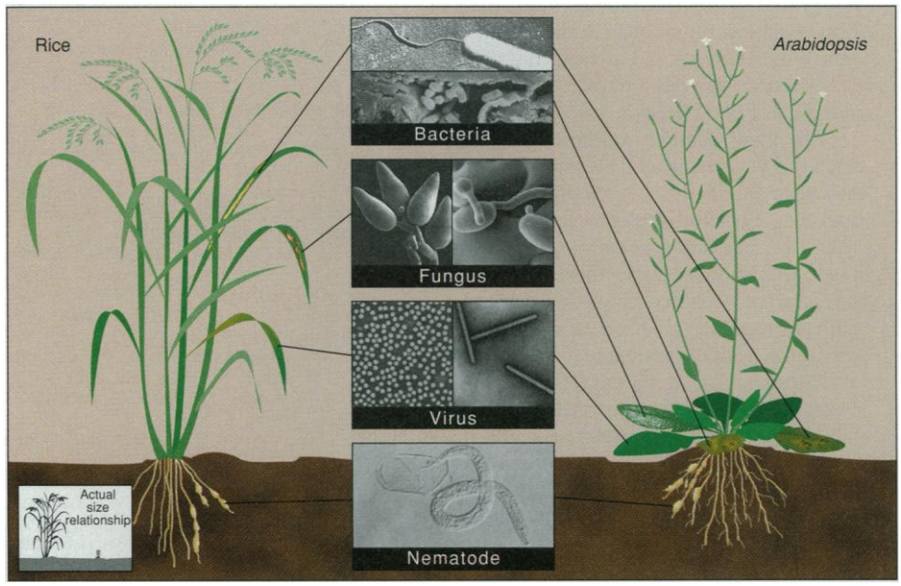

Fig. 1. A rice plant (left) and *Arabidopsis thaliana* (right), a model plant for host-pathogen interactions. The establishment of numerous pathosystems in the genetically tractable plant species *Arabidopsis* leads to rapid identification of components of host resistance and defense signaling pathways. Within each group, related bacterial, fungal, viral, and nematode pathogens cause diseases in both rice and *Arabidopsis*. Scanning electron micrographs (center panels) and disease reaction phenotypes of representative phytopathogens of *Oryza* and *Arabidopsis* are shown. The rice bacterial pathogen *X. oryzae* pv. *oryzae* causes chlorotic water-soaked stripes on rice leaves and lesions on *Arabidopsis* leaves. The bacterial pathogen *P. syringae* induces small water-soaked chlorotic lesions on *Arabidopsis*. The fungus *Erysiphe cihoracearum* causes powdery mildew disease on *Arabidopsis*. The most important fungal pathogen of rice is *Magnoportha grisea*, which produces gray necrotic lesions on all parts of the shoot. Tobacco mosaic virus infects and spreads throughout the *Arabidopsis* plant with few detectable symptoms. The spherical form of rice tungro virus causes yellow discoloration of the leaves. The plant parasitic nematode infects and causes disease in both rice and *Arabidopsis*.

and over 150 wild isolates of *Arabidopsis* are available for genetic analysis of host-pathogen interactions (*9*, *11*). *Arabidopsis* is a useful model system because of its small size [~10 times smaller than rice (Fig. 1)], rapid generation time (6 to 12 weeks), and small genome size (120 Mb). Considerable information on its genetic map and genome sequence has been accumulated, and mutagenic and transgenic techniques have been developed. At least three *Arabidopsis R* genes have been isolated and more than 20 have been genetically mapped (*11*). Of 42 *Arabidopsis* expressed sequence tags showing homology to isolated *R* genes, several are near resistance loci (*12*). The *Arabidopsis R* genes *RPS2* and *RPM1* (Table 3 and Fig. 3B) have functional homologues in soybean, bean, and pea (*13*–*15*). Both *Arabidopsis* and mice respond to the same virulence factors for the opportunistic pathogen *Pseudomonas aeruginosa*, strain UCBPP-PA14 (*16*). Genetic approaches to elucidate the signal transduction pathways leading to resistance have identified numerous *Arabidopsis* mutants with altered resistance phenotypes. Four genes (*17*, *18*, *19*) corresponding to these mutations have been cloned.

Comprehensive genetic analysis of host-pathogen interactions is impractical in most crops such as rice because of their long generation times, large genomes, and scarcity of specific genetic knowledge. However, development of *Arabidopsis* pathosystems that reflect molecular interactions of crop species is facilitating the necessary rigorous genetic analysis of host defense responses. Because of the sequence conservation of *R* genes among evolutionarily diverse species (Table 3 and Fig. 3B) and the similarity of resistance and defense responses in plant species, it is anticipated that important components of host defense identified in *Arabidopsis* will have correlates in all important crop species.

Microbial Strategies for Attack

Most plant diseases are caused by viruses, bacteria, fungi, and nematodes. The molecular mechanisms involved in viral and bacterial pathogenicity are at this point better understood than are nematode and fungal pathogenecity. Bacteria use type III and IV secretion or transfer systems to deliver proteins or protein-DNA complexes into the plant host cell. The Avr-proteins from phytopathogenic Pseudomonads and Xanthomonads, Yop proteins from *Yersiniae*, Inv proteins from *Salmonella* and *Shigella*, and virulence factors from *Escherichia coli* are transferred by type III systems (*7*, *20*, *21*, *22*).

hrp *genes of phytopathogenic bacteria*. The identification of bacterial mutants that were simultaneously altered in their ability to cause disease and to induce an HR led to the discovery of *hrp* (hypersensitive response and pathogenicity) genes in *Ralstonia*, *Xanthomonas*, *Pseudomonas*, and *Erwinia* sp. (*23*, *24*). Cosmid clones containing multigenic *hrp* loci from *P. syringae* pv. *syringae*

Table 1. Examples of severe losses caused by plant diseases.*

Host/disease	Pathogen	Crop losses due to pathogens
Rice	All	15% ($33 billion) loss worldwide, 1988–1990
Rice blast	*Magnaporthe grisea*	11 to 30% (157 million tons) loss worldwide, 1975–1990
Rice sheath blight	*Rhizoctonia solani*	10 to 27% yield loss in India,
Bacterial blight of rice	*X. oryzae* pv. *oryzae*	1975–1988; up to 60% losses in India, 1979–1980
Rice tungro virus		15 to 30% losses in Asia, 1971–1981
Nematodes	*Meloidogyne* spp.	10 to 50% losses in China, 1984
Wheat	All	12.4% ($14 billion) loss, 1988–1990
Wheat rusts	*Puccinia* spp.	Up to 100% loss in Kazakhstan epidemic, 1980
Barley yellow dwarf virus		Up to 60% loss in the southern Ukraine, 1975
Barley		10.1% ($1.9 billion) loss worldwide, 1988–1990
Powdery mildew	*Erysiphe graminis*	Up to 8.7% loss in Great Britain and Ireland, 1977
Maize	All	10.9% ($7.8 billion) loss worldwide, 1988–1990
Southern corn leaf blight	*Cochliobolus heterostrophus*	$1 billion loss in U.S. epidemic, 1970
Potatoes	All	16.3% ($9.8 billion) loss worldwide, 1988–1990
Late blight of potato	*Phytophthora infestans*	5% loss ($368.8 million) in U.S. Midwest, 1986. Predicted 30 to 40% loss without fungicide treatment
Potato soft rot	*Erwinia carotovora*	30% loss in former East Germany, 1986
Potato virus X		10 to 75% yield loss in Austria, 1961–1980
Soybeans	All	9% ($3.2 billion) loss worldwide, 1988–1990
Cotton	All	10.5% ($4.3 billion) loss worldwide, 1988–1990
Coffee	All	14.8% ($2.8 billion) loss worldwide, 1988–1990
All major crops	All	13.3% ($76.9 billion) loss worldwide, 1988–1990

*Adapted from (*129*).

Table 2. *Arabidopsis* pathosystems.*

Disease	Pathogen
Fungal diseases	
1. Downy mildew	*Peronospora parasitica*
2. White blister	*Albugo candida*
3. Damping off	*Pythium* sp.
4. Dark leaf spot	*Alternaria brassicae*
5. Powdery mildew	*Erysiphe cruciferarum; E. cichoracearum*
6. Vascular wilt	*Fusarium oxysporum*
7. Leaf mold and leaf spot	*Cladosporium* sp.
8. Damping off or wire stem	*Thanatephorus cucumeris*
Bacterial diseases	
9. Black rot on crucifers	*Xanthomonas campestris* pv. *campestris*
10. Bacterial speck on crucifers	*Pseudomonas syringae* pv. *maculicola*; *P. syringae* pv. *tomato*
Viral diseases	
11. Mild stunting	Tobacco mosaic virus
12. Mild stunting and desiccation	Turnip crinkle virus
13. Vein clearing and chlorotic spots	Cauliflower mosaic virus
Nematode diseases	
14. Cyst nematode	*Heterodera schachtii*

*Adapted from (*10*).

61 and *Erwinia amylovora* Ea321 confer on certain plant hosts the ability to elicit an HR to the nonpathogens *P. fluorescens* and *E. coli* but do not render them pathogenic (*25*, *26*) (Fig. 2A). Several of the *hrp* genes, designated *hrc* (*hrp* conserved), encode membrane-associated proteins that form portions of a type III secretory pathway that is active during infection of the plant.

The type III secretory pathway has been best characterized in mammalian pathogens (*20*, *27*). Transfer of two proteins, YopE and YopH, from *Yersinia enterocolitica* directly into the host cell requires both chaperone-like proteins and a secretory apparatus (*22*, *28*). Transfer in both cases is polar and contact-dependent, occurring only at the closely apposed region of pathogen and host (*29*, *30*). *Shigella flexneri* has a similar set of proteins that are also secreted upon host cell contact and require the same transport mechanisms. These proteins facilitate internalization of *Shigella* (*31*).

A similar contact-dependent transfer mechanism may act to deliver Avr proteins into plant cells. Thus, the barrier to direct plant-bacterial cell contact that is presented by the plant cell wall needs to be overcome either by physical disruption or by direct transport across it. Translocation to allow bacterial proteins to cross the plant cell membrane then may follow as in mammalian pathogenesis.

Transkingdom conjugal transfer. The genetic transformation of plant cells by *Agrobacterium* through transfer of its own DNA is a striking example of the evolutionary economy of the microbial world (*32*). The first hint of this strategy was the discovery that *Agrobacterium* produced a single-stranded DNA transfer intermediate, the T strand. The initiation and termination sites for synthesis of the T strand—the transfer DNA (T-DNA) borders—are homologous to sites used for transfer of DNA between conjugating bacteria. T-DNA borders are recognized by an endonuclease that is homologous to enzymes used for bacterial transfer, and the *Agrobacterium VirB* operon required for transfer encodes 11 membrane-associated proteins that are homologous to bacterial proteins required for conjugal plasmid transfer or toxin secretion (*33*). Research is now focused on determining the structure of the putative conjugal channel in *Agrobacterium* for comparison with other type IV secretion systems (Fig. 2B).

The actual transfer intermediate contains the T strand, VirD2 protein at the 5′ end, and the cooperative single-stranded DNA binding protein VirE2 along its length (Fig. 2B). The protein components presumably provide signal sequences for T-strand transit. Indeed, the fact that the bacterial proteins VirD2 and VirE2 contain functional plant nuclear localization signal sequences explains how this complex targets to the plant cell nucleus. However, it remains a challenge to uncover how an elongated protein nucleic acid complex is transported through the wall and two membranes of the bacterium, as well as the plant cytoplasmic membrane. The pili produced by *Agrobacterium*, which are essential for plant cell transformation, may facilitate the initial docking of the transfer complex at the plant cell surface (*34*, *35*). It will be interesting to investigate parallels between this type of contact with plant cells and conjugal transfer of DNA between bacteria.

Viruses and plasmodesmata. Plant microbes can be exploited to uncover fundamental cellular processes, such as intercellular communication. Plants have evolved cytoplasmic channels, called plasmodesmata (PD), to span the relatively thick cell walls that form between contiguous cells (*36*). PD were originally thought to have a passive role in creating and maintaining cytoplasmic continuity, allowing diffusion of small molecules from cell to cell. The discovery that plant viruses pirate PD for cell-to-cell movement of their genomes

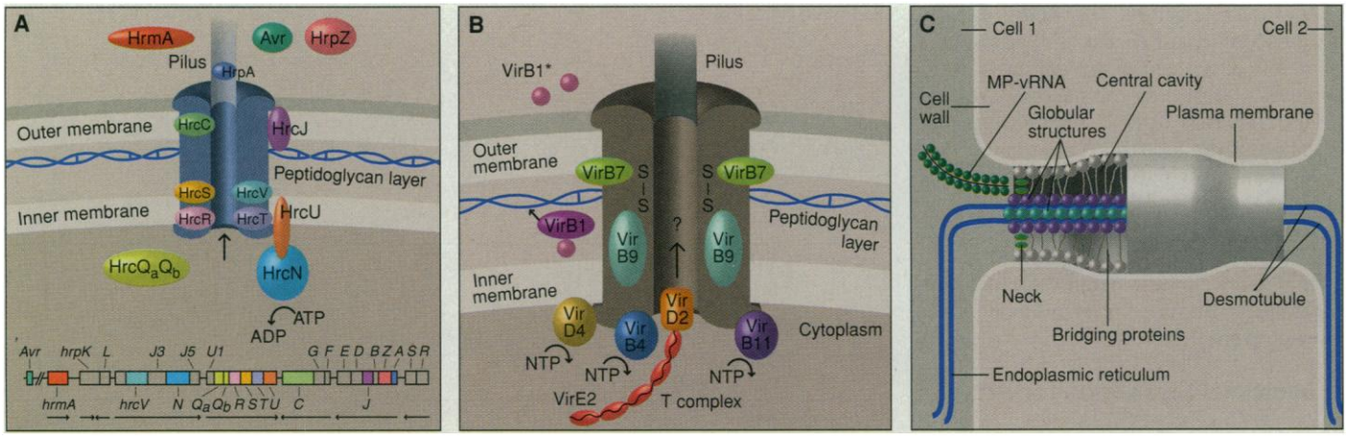

Fig. 2. (**A**) The Hrp secretory apparatus of *P. syringae* pv. *syringae* 61. The model depicts the hypothetical structures involved in the delivery of bacterial proteins from *P. syringae* into the plant cell cytoplasm via a type III secretion apparatus. The genomic organization of the *hrp/hrc* genes is presented in the lower section of the panel. The designation of the genes and the putative cellular location of their products are derived from (*127*, *128*). It is hypothesized that the genes in the Hrp regulon encode genes that are involved in the secretion of HrmA, Avr, and HrpZ out of the bacterial cell. HrcN has a conserved ATPase domain, which suggests that it may play a role in providing the energy needed to actively transport these proteins into the plant cell. (**B**) A model for T-complex transfer through the transmembrane VirB channel from *A. tumefaciens* to host plant cells (*32*, *35*). The T strand is covalently attached to VirD2 at the 5′ end and coated along its entire length with the single-stranded DNA binding protein VirE2. Eleven VirB proteins and VirD4 are required to transfer the T complex through the bacterial inner and outer membrane into the host plant cell's cytoplasm. VirB1 may be involved in assembly of the transmembrane complex by local lysis of the peptidoglycan layer. VirB*, the proteolytically processed extracellular secreted VirB1 product, may be a pilus component. The disulfide-linked VirB7-VirB9 heterodimer stabilizes the transmembrane VirB channel. The energy required for the assembly or transfer (or both) of the T complex may be provided by nucleotide triphosphatase activities of VirD4, VirB4, and VirB11. (**C**) The structure of the plasmodesmatal channel and transport complexes of the movement protein–viral RNA (MP-vRNA) genome (*36*, *37*). The plasmodesmatal channels connect the cytoplasms of neighboring cells and facilitate cell-to-cell communication and transport of vRNA. The plasma membrane adjacent to the cell wall forms an outer boundary and is contiguous between two cells. The desmotubule—a tube of appressed endoplasmic reticulum—is located in the center of the plasmodesma. Between the desmotubule and the plasma membrane is a central cavity. MP-vRNA complexes are transported along microtubules to reach the plasmodesma. At the plasmodesma, the MP may interact with actin for gating or for active transport. The gating of the plasmodesma along its entire length would provide easy access to the transport channel, facilitating diffusion of MP-vRNA complexes.

during infection provided compelling evidence that PD are inherently dynamic and can be stimulated to transport large molecules. Consequently, plant viral movement proteins (MPs) have been used to probe the regulation and function of PD (*37*, *38*) (Fig. 2C). Microinjection experiments show that MPs alone or complexed with nucleic acid can be transported by leaf PD (*39*–*42*). A variety of nucleic acids can be transported in association with MPs, which suggests that movement is likely to be conferred by the supporting MP. The nucleic acid binding activity of the MPs presumably shape viral genomes into a thin structure that is compatible with the narrow dimensions of PD channels (*43*, *44*).

The viral MP paradigm recently provided insight into how molecules move intracellularly to reach PD. Transiently expressed tobacco mosaic virus (TMV) MP appears as filaments that colocalize primarily with microtubules (*45*, *46*), and to a lesser extent with actin filaments (*46*). Whether this cytoskeletal association plays a role in active intracellular transport of the MP (or MP-viral genome complexes) or in anchoring the MP in the cytoplasm for nucleoprotein complex formation remains to be determined. Cytoskeleton-mediated transport of viral nucleoprotein complexes to PD would be more effective than diffusion of such elongated complexes through viscous cytoplasm.

Little attention has been paid to the role of intercellular transport in signal transduction pathways of plant defense responses—we assume that signaling molecules move. Hypothetically, pathogenesis-related (PR) proteins induced in response to pathogen attack actively alter symplastic transport by regulating PD function or structure. In fact, a maize PR protein has recently been localized to PD (*47*). The systemic acquired resistance (SAR) defense response also may be dependent on intercellular transport through PD. Finally, a wound-inducible signaling molecule, the 18–amino acid systemin, moves through the phloem to eventually promote the induction of proteinase inhibitors (which interfere with the digestion process of insects) at remote wound sites (*48*). Whether systemin moves symplastically through PD has not been investigated.

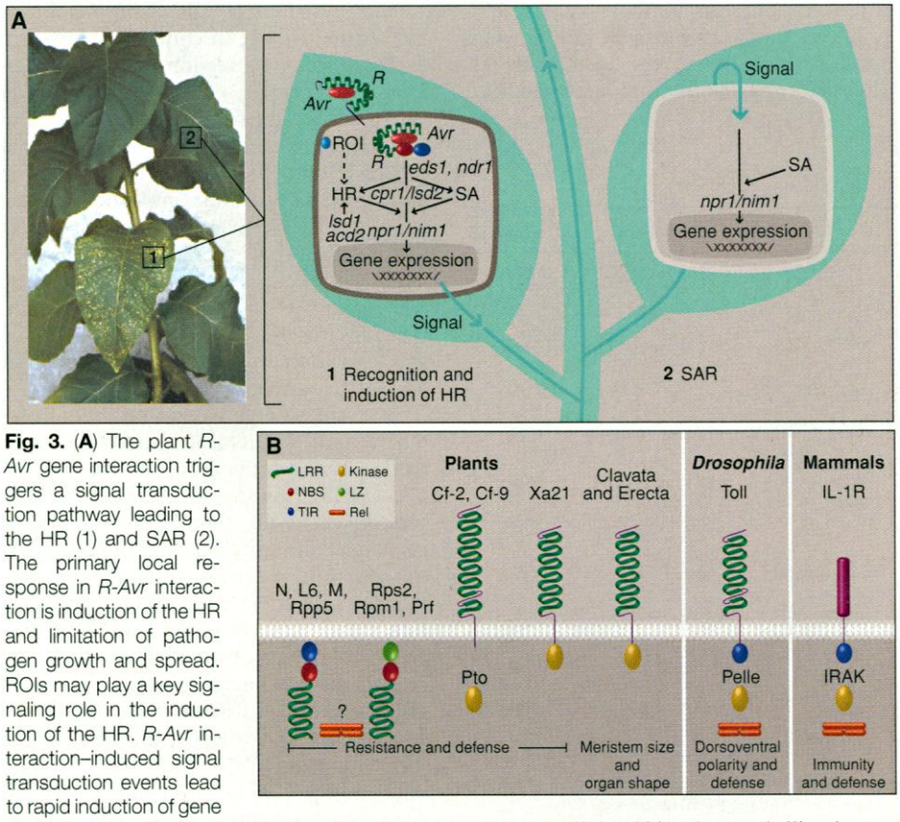

Fig. 3. (**A**) The plant *R-Avr* gene interaction triggers a signal transduction pathway leading to the HR (1) and SAR (2). The primary local response in *R-Avr* interaction is induction of the HR and limitation of pathogen growth and spread. ROIs may play a key signaling role in the induction of the HR. *R-Avr* interaction–induced signal transduction events lead to rapid induction of gene expression and defense responses. SA is a molecule that is involved in local (1) and systemic (2) resistance responses. The proposed placement of mutants in the pathway is based on results of genetic analysis in *Arabidopsis* (see text for details). (**B**) Shared structural domains among plant, insect, and mammalian defense and developmental pathway genes. TIR, Toll–IL-1R homology domain; kinase, serine-threonine kinase; LZ, leucine zipper; Rel, Rel-related transcription factors (Dorsal and Dif of *Drosophila* and NF-κB of mammals) and inhibitors (Cactus of *Drosophila* and IκB of mammals).

Strategies for Host Defense

R gene structure

Over the past 3 years, numerous *R* genes were cloned from several plant species. Although these genes confer resistance to diverse bacterial, fungal, viral, and nematode pathogens, their products share striking structural similarities, which suggests that certain signaling events are held in common in plant defense (Fig. 3, A and B) (*49*, *50*). *R* genes can be grouped into five classes (Table 3). Structural features shared by their products are a leucine-rich repeat (LRR) motif or a serine-threonine kinase domain. The first class encodes cytoplasmic receptor-like proteins that contain an LRR domain and a nucleotide binding site (NBS). The family of genes encoding proteins with the LRR-NBS motif includes *RPS2* and *RPM1* from *Arabidopsis* (conferring resistance to bacterial pathogens *P. syringae avrRpt2* and *avrRpm1*, respectively) (*51*–*53*); *Prf* from tomato (resistance to *P. syringae* pv. *tomato*) (*54*); *N* from tobacco (resistance to tobacco mosaic virus) (*55*); L^6 and M from flax (resistance to different races of *Melampsora lini* fungi) (*56*, *57*); *RPP5* from *Arabidopsis* (resistance to fungus *Peronospora parasitica*, Noco2) (*58*); and I_2 from tomato (resistance to *Fusarium oxysporium* f. sp. *lycopersicon*) (*59*). Besides LRR and NBS domains, *N*, L^6, and *RPP5* encode NH_2-terminal domains with homology to cytoplasmic domains of the *Drosophila* developmental gene *Toll* and the mammalian immune response gene encoding the interleukin-1 receptor (IL-1R) (TIR: Toll–IL-1R homology region) (*60*, *61*). *RPS2*, *RPM1*, and *Prf* encode proteins containing a putative leucine zipper motif at their NH_2-terminus. The second class of genes includes *Pto*, which confers resistance to the bacterial pathogen *P. syringae* pv. *tomato*, containing *avrPto* (*62*). *Pto* encodes a serine-threonine kinase with homology to mammalian Raf, IRAK, and *Drosophila* Pelle kinases. Interestingly, genetic analysis shows that an LRR-NBS–containing protein, Prf, is necessary for *Pto* function. The third class includes *Cf-2* and *Cf-9* from tomato and $HS1^{pro\text{-}1}$ from sugar beet. *Cf-2* (*63*) and *Cf-9* (*64*) encode putative transmembrane receptors with large extracytoplasmic LRR domains and confer resistance to different races of *Cladosporium fulvum*. $HS1^{pro\text{-}1}$ (*65*) encodes a transmembrane LRR protein that confers resistance to the beet cyst nematode *Heterodera schachtii*. The fourth class is represented by the rice gene *Xa21*, which confers resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (*66*). *Xa21* encodes a

putative transmembrane receptor with an extracellular LRR domain and an intracellular serine-threonine kinase domain. The *Xa21* structure suggests an evolutionary link between LRR protein (*Cf*) and the *Pto* kinase. The fifth class includes the *HM1* gene, which confers resistance to the fungal pathogen *Cochliobolus carbonum* race 1 (*67*). *HM1* encodes a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH)–dependent reductase that inactivates toxin produced by *C. carbonum* race 1. *HM1* is distinct from the above-mentioned *R* genes because an Avr component is not involved in toxin degradation by *HM1*.

Role of R gene products

Pathogen recognition. Plant *R* genes seem to encode receptors that interact directly or indirectly with elicitors (ligands) produced by pathogen *Avr* genes (*6*, *7*). Because of the structural similarities among many cloned *R* genes, a likely candidate motif for ligand binding is the LRR domain. LRRs have been implicated in protein-protein interactions and ligand binding in signal-transducing proteins of eukaryotes (*68*). However, sequence comparisons among the 30 corresponding bacterial *Avr* genes that have been isolated have provided no clues about the recognition process. The site of action of *Avr* determinants is apparently based on the location of the relevant *R* gene partners. For example, the *N* product, which is predicted to be intracellular, may interact with the TMV replicase product in the cytoplasm (*69*). As described previously, *Avr* gene products from extracellular pathogens are probably delivered directly into the plant cells through a hrp type III secretion pathway. In fact, *avrPto*, *avrRpt2*, and *avrB* of *P. syringae*, as well as *avrBs3* of *Xanthomonas campestris vesicatoria*, actively elicit an *R* gene–specific necrotic reaction when expressed within the plant (*21*, *70*–*73*). Further, the extracellular domain of Cf-9 may interact with the secreted, 28–amino acid, cysteine-rich peptide encoded by *Avr9* (*74*). Two fungal resistance genes, L^6 and *RPP5*, apparently encode putative cytoplasmic proteins, but how these fungi transport their elicitor molecules into plant cells is unknown.

With the isolation of *R* genes and their corresponding *Avr* genes, direct interactions of *R* and *Avr* products can be tested. Evidence for direct interaction between Pto and avrPto proteins has recently been reported (*71*, *72*). The Pto kinase belongs to a linked multigene family and shares 87% sequence similarity with Fen kinase, which confers sensitivity to the insecticide fenthion (*75*, *76*). Fenthion-induced cell death is similar to the HR induced by *avrPto*-containing bacteria. The *avrPto* product does not interact with the Fen kinase. Analyses of chimeric Pto-Fen proteins indicate that the Pto kinase subdomain VIII that specifies serine-threonine kinase activity is required for interaction with avrPto. The same region of Pto is also necessary to mount an HR in transgenic tomato plants when infected with bacteria containing *avrPto*.

The observed interaction between Pto and avrPto is somewhat surprising because cytoplasmic kinases are generally not known to function as receptors. Indeed, the Pto-avrPto studies extend the potential functional diversity of ligand-receptor interactions. Several questions remain: What is the function of the LRR-NBS–containing *Prf* gene product in the Pto-avrPto pathway? Does the *Prf* gene product interact with avrPto? Does Pto act alone in the pathogen recognition step? Do other cytoplasmic LRR-NBS–type *R* gene products require a Pto-like kinase in order to interact with their chosen elicitor?

Cf-like and Pto-like encoded products may constitute a two-component receptor system resembling the transmembrane LRR kinase Xa21. In contrast to the Pto-avrPto system, expression of the extracellular LRR domain of Xa21 alone in rice plants confers partial resistance to six races of *X. oryzae* (*77*), which suggests that the LRR domain of Xa21 is involved in pathogen recognition. Furthermore, domain swap experiments from *L* alleles of flax suggest that the LRR domain is a major component in providing pathogen recognition specificity (*78*). However, direct binding of Avr components of Xa21 and L to the corresponding LRR domains has yet to be demonstrated.

Signaling. Little is known about the function of different *R* gene domains in the resistance signaling pathway. The LRR, NBS, kinase, and TIR domains of *R* gene products are found in a number of eukaryotic proteins participating in signal transduction cascades. Preliminary data indicate that these domains are indispensable for *R* gene function. Although the primary function of the LRR is assumed to be Avr protein recognition, it is also possible that the LRR may participate in downstream signaling. LRRs are found in a variety of proteins that differ in their function and location in the cell and are implicated in protein-protein interactions, cell adhesion, and membrane association (*68*). The LRRs of the NBS-containing class of *R* gene products possess imperfect repeats that contain a consensus sequence similar to that of yeast adenylate cyclase

Table 3. Isolated plant resistance genes.

Class	*R* gene	Plant	Pathogen	*Avr* gene	Structure*	Reference
1	*RPS2*	*Arabidopsis*	*Pseudomonas syringae* pv. *tomato*	*avrRpt2*	LZ-NBS-LRR	(*51*, *52*)
	RPM1	*Arabidopsis*	*P. syringae* pv. *maculicola*	*avrRpm1*, *avrB*	LZ-NBS-LRR	(*53*)
	Prf	Tomato	*P. syringae* pv. *tomato*	*avrPto*	LZ-NBS-LRR	(*54*)
	N	Tobacco	Tobacco mosaic virus	TMV *Replicase?*	TIR-NBS-LRR	(*55*)
	L^6	Flax	*Melampsora lini*	AL^6	TIR-NBS-LRR	(*56*)
	M	Flax	*M. lini*	*AM*	TIR-NBS-LRR	(*57*)
	RPP5	*Arabidopsis*	*Peronospora parasitica*	*avrPp5*	TIR-NBS-LRR	(*58*)
	I_2	Tomato	*Fusarium oxysporurm*	Unknown	NBS-LRR	(*59*)
2	*Pto*	Tomato	*P. syringae* pv. *tomato*	*avrPto*	Protein kinase	(*62*)
3	*Cf-9*	Tomato	*Cladosporium fulvum*	*Avr9*	LRR-TM	(*64*)
	Cf-2	Tomato	*C. fulvum*	*Avr2*	LRR-TM	(*63*)
	$HS1^{pro-1}$	Sugar beet	*Heterodera schachtii*	Unknown	LRR-TM	(*65*)
4	*Xa21*	Rice	*Xanthomonas oryzae* pv. *oryzae*	Unknown	LRR, protein kinase	(*66*)
5	*Hm1*	Maize	*Cochliobolus carbonum*, race 1	None	Toxin reductase	(*67*)

*"Structure" refers to predicted protein domains of the listed genes: leucine zipper (LZ) (*30*), NBS (*31*), LRR (*66*), TIR (*132*), and transmembrane domain (TM) (*133*).

(79). In contrast, the LRRs of non–NBS-containing *R* gene products Cf-2, Cf-9, and Xa21 contain a conserved glycine that is characteristic of extracellular LRR domains. Single–amino acid changes in the LRR domain of Rps2, Rpm1, and N result in failure of the HR upon pathogen infection (*51*, *53*, *80*). This suggests that the function of the LRR domain can be easily disrupted by minor modifications. Mutations in the LRR may be particularly effective at disrupting the response to a pathogen, as the LRR may be required for interaction with the Avr component of the pathogen or with a component that mediates downstream signal transduction. Future identification of proteins that interact with the LRR domain and determination of LRR structure will clarify the role of *R* genes in the induction of defense responses.

NBSs are found in many families of proteins, including the RAS group, adenosine triphosphatases (ATPases), elongation factors, and heterotrimeric GTP-binding proteins (G proteins) (*81*). These proteins are critical for numerous fundamental eukaryotic cellular events such as cell growth, differentiation, cytoskeletal organization, vesicle transport, and defense (*82*). The presence of an NBS in some of the predicted *R* gene products suggests that nucleotide binding is necessary for *R* gene function. Site-directed mutagenesis of key residues implicated in nucleotide binding abolishes the capacity of N and Rps2 to induce HR upon pathogen infection (*80*, *83*). Although many mutations in the NBS region of N result in the loss of function, some mutations in the putative Mg^{2+} binding site in the P loop lead to a partial loss of function or dominant change of function (*80*). Analogous mutations in RAS and stimulatory G proteins also interfere with endogenous protein function (*84*, *85*). To understand the role of NBS domains in *R* gene function, characterization of the nucleotide interaction and identification of any accessory effector molecules are necessary.

The plant's defense signal transduction pathway

The HR, which is the primary local response in gene-for-gene–type resistance, results in cell death (Fig. 3A). It is not yet clear whether this cell death is the direct consequence of biochemical and physiological changes induced by the *R-Avr* interaction. Other aspects of the defense responses include an oxidative burst leading to production of reactive oxygen intermediates (ROIs), expression of defense-related genes, alteration of membrane potentials, an increase in lipoxygenase activity, cell wall modifications, lignin deposition, and production of antimicrobial compounds such as phytoalexins (*3*). ROIs are a key signal in plant defense. Generation of ROIs, which in some cases requires activation of Ca^{2+} and anion channels, occurs within minutes after *R-Avr* interactions (*86*–*88*). ROIs may directly trigger the HR or cell death and the subsequent induction of defense-related genes (*86*, *88*). ROI generation and defense responses can be blocked by diphenylene iodonium, an inhibitor of mammalian NADPH oxidase, which suggests that a similar system is required in plants (*89*, *90*). Furthermore, antibodies to various mammalian NADPH oxidase components cross-react with plant proteins of similar size (*89*, *90*). The *rbohA* gene product from rice is a homolog of the gp91phox component of NADPH oxidase (*91*).

Cell death mutants. The HR in plants is under genetic control. Mutants showing spontaneous HR lesions in the absence of pathogen infection are found in several plant species (*92*). *Arabidopsis* plants mutated at the *lsd1* (lesions simulating disease) (*93*) and *acd2* (accelerated cell death) (*94*) loci develop self-propagating lesions, express PR proteins, and produce salicylic acid (SA) even in the absence of a pathogen. The wild-type genes presumably encode a repressor of the HR.

The *rp1* and *mlo* mutations from maize and barley, respectively, also mimic disease lesions. The *Rp1* locus of maize confers resistance to the fungal rust *Puccinia sorghi*. Genetic studies indicate that certain derivatives of the *Rp1* locus show a constitutive lesion phenotype (*95*). In barley, a recessive allele *mlo* confers resistance to all races of the powdery mildew fungus *Erysiphe graminis* f. sp. *hordei*. These *mlo* plants also show spontaneous lesions without pathogen infection (*96*). In contrast to *lsd1* and *acd2* lesion phenotypes, *mlo*-induced lesions are discrete and contained. Plants carrying *lsd1* and *mlo* show enhanced resistance to pathogens. *LSD1* encodes a zinc finger protein that may regulate transcription of death-response genes (*18*). *Mlo* encodes a novel protein without homology to any known sequence (*96*). Mlo protein possesses at least six transmembrane helices and a putative nuclear localization signal. Further molecular and biochemical characterization of these proteins and cloning of other cell death–related genes will provide an opportunity to study signaling mechanisms underlying cell death and defense responses in plants.

SAR mutants. SAR is associated with the elevated production of SA and the expression of PR proteins (*4*). SA is required for SAR and PR gene expression (*97*, *98*). Whether SA acts as a long-distance systemic signal for SAR induction in plants remains unclear (*99*, *100*). Several *Arabidopsis* mutants provide insights into signaling mechanisms leading to SAR (Fig. 3A). *cpr1* (*101*) and *lsd2* (*93*) mutants show increased levels of SA and constitutive expression of PR genes, as well as enhanced resistance to virulent bacterial and fungal pathogens. *lsd2* plants show a constitutive lesion phenotype, which suggests that *LSD2* encodes a negative regulator acting upstream of SA synthesis or perception but downstream of the HR. Another class of mutant loci, including *npr1* (*102*) and *nim1* (*103*), induces a normal HR and SA accumulation in response to pathogen infection but fails to express PR genes upon treatment with chemical inducers such as SA. Thus, *NPR1* probably functions downstream of SA accumulation. *NPR1* encodes a novel protein containing ankyrin repeats (*17*), which are found in many eukaryotic proteins with diverse functions mediated by protein-protein interactions. NPR1 is most similar to mammalian Ankyrin 3 and Iκ B, an inhibitor of the nuclear factor kappa B (NF-κ B) transcription factor. On the basis of these homologies, NPR1 may act as a transcriptional regulator of PR gene expression.

Common signaling pathway mutants. The *Arabidopsis* mutants *ndr1* (*104*) and *eds1* (*105*) provide evidence for convergence of signals downstream of different *R-Avr* interacting partners into a single signaling pathway (Fig. 3A). The *ndr1* mutant suppresses resistance to a bacterial pathogen, *P. syringae*, expressing any one of the avirulence genes *avrB*, *avrRpm1*, *avrRpt2*, or *avrPph3* and to the fungal pathogen *Peronospora parasitica*. In contrast, the *eds1* mutant suppresses resistance to different isolates of the fungal pathogen *P. parasitica* but not to the bacterial pathogen *P. syringae* expressing *avrB*. These mutants differ from *nim1* and *npr1* because they retain the ability to induce SAR. However, SA levels were not determined in these mutants. The *EDS1* and *NDR1* products may act upstream of SA accumulation but downstream of the initial recognition step. *EDS1* may act upstream of *NDR1*.

Suppressor screening strategies have been used to isolate mutants in race-specific *R* gene signaling pathways. In barley, *Rar1* and *Rar2* are required for *mlo*-dependent resistance to the fungus *E. graminis* f. sp. *hordei* (*106*). In tomato, *Rcr1* and *Rcr2* are necessary for *Cf-9*–dependent resistance to the fungal pathogen *C. fulvum* (*107*).

Phytoalexin mutants. Phytoalexins are compounds that restrict pathogen growth.

In *Arabidopsis*, camalexin accumulates in response to both virulent and avirulent bacterial pathogens (*108*). In plants with phytoalexin-deficient (*pad*) (*109*) mutations, growth of the avirulent bacteria was not compromised and expression of SAR genes was not affected. Thus, phytoalexins may not play a significant role in gene-for-gene–type resistance. However, the *pad1* and *pad2* mutants were more susceptible to virulent bacteria than were wild-type plants.

Evolution of Host-Phytopathogenic Interactions

Most *R* genes, which are members of multigene families, are arranged in large arrays forming complex loci. Such arrays provide substrates for frequent recombination events leading to the evolution of novel specificities through mispairing, intra/intergenic recombination, and gene duplication. It is predicted that the outcomes of recombination events give plants a selective advantage in the face of rapidly evolving pathogen populations; indeed, novel discriminating capabilities can be generated at these *R* loci by recombination or gene conversion events (*57*, *110*, *111*). Genetic analysis of the maize *Rp1* locus reveals that new resistance specificities are associated with recombination of flanking marker genes (*112*). Sequence analysis indicates that evolution of an *Xa21* gene family followed precise recombination, duplication, and transposition events (*111*). The *Cf-9* (*64*) and *Cf-2* (*63*) loci of tomato are composed of arrays of five or more related genes; two nearly identical *Cf-2* genes encode *Cf-2* resistance specificity. Molecular analysis of the TMV resistance gene suggests the presence of an array of related genes at the *N* locus in tobacco (55). An *N*-like cluster has also been identified in tomato, which suggests that this complex locus arose in a progenitor species (*113*). The *Pto* locus of tomato contains five *Pto*-homologous genes and an LRR-NBS gene, *Prf*, which is necessary for *Pto*-mediated resistance. (*54*). The unlinked *L* and M loci of flax are composed of highly related genes of the TIR-NBS-LRR class of resistance genes. *L* is a single-copy locus with 13 different allelic versions, and M is a complex locus composed of approximately 15 linked members. Characterization of three spontaneous M mutations suggests that these mutations arose by recombination within the repeated LRR motifs (*57*).

Transkingdom Parallels

Increasing evidence suggests that plant cellular defense responses may be analogous to the "natural" or "innate" immune responses of vertebrates and insects (Fig. 3B). In mammals and *Drosophila*, binding of ligands (IL-1 and Spatzle) to receptors (IL-1R and Toll) results in the translocation of Rel-related transcription factors (NF-κ B and Dorsal) from the cytoplasm to the nucleus (Fig. 3B) (*114*, *115*). In the nucleus, NF-κ B and Dorsal bind to cognate promoters with κ B-like motifs and induce immune and zygotic gene transcription, respectively. These factors are retained in the cytoplasm by ankyrin repeat–containing inhibitory proteins (Iκ B and Cactus) (*114*, *115*). Nuclear import of Rel factors requires a phosphorylation cascade involving serine-threonine kinases (IRAK and Pelle) (*116*, *117*). In *Drosophila*, the Toll receptor is also involved in the induction of acute immune responses to bacterial infection through a Dorsal-related immunity factor called Dif (*118*), and mutations in the Toll pathway impair resistance to fungal infection in *Drosophila* (*119*). These striking parallels between mammalian immune and *Drosophila* developmental responses can extend to plant defense and development pathways (Fig. 3B). The NH_2-terminal domains of N, L6, and Rpp5 share homology with the cytoplasmic domains of the Toll and IL-1 receptors, which suggests that they trigger an intracellular signal transduction cascade related to the Toll and IL-1R pathways (*119*, *120*). Similarities also extend to downstream signaling components (Fig. 3B). For example, the SAR signaling gene *NPR1* encodes a protein with ankyrin repeats that is similar to Iκ B and Cactus. Also, the *R* genes *Pto* and *Xa21* and the plant developmental genes *CLAVATA1* (*121*) and *ERECTA* (*122*) share homology with kinase domains of *Drosophila pelle* and *IRAK*.

In addition to structural similarities, plant and mammalian defense responses share functional similarities. In mammals, innate immunity is characterized by the rapid induction of gene expression after microbial infection. A characteristic feature of plant disease resistance is rapid induction of the HR. In the mammalian immune responses, ROIs induce acute-phase response genes by activating the transcription factors NF-κ B and AP-1 (*123*, *124*), and SA may play a role in NF-κ B–related transcription (*125*). In plants, ROIs and SA regulate *R* gene–mediated defense responses, implying the involvement of Rel-related transcription factors. These functional and structural similarities among evolutionarily divergent organisms suggest that the mammalian immune response and the plant pathogen defense pathways may be inherited from a common ancestor.

Summary

Plant and animal pathogens have features that appear to be common to higher eukaryotic bacterial pathogens, including conserved systems for deploying virulence proteins and convergent pathogenic strategies (*24*, *126*). Studies on the mechanism of viral pathogenicity have revealed how these microbes have pirated fundamental strategies for intercellular communication.

The discovery of structurally similar host *R* genes from evolutionarily diverse plant species encoding resistance to viral, bacterial, fungal, and nematode pathogens suggests that conserved resistance mechanisms exist among plants. These findings highlight the utility of the genetically tractable plant *Arabidopsis* for rigorous genetic dissection of fundamental components of host defense pathways.

Structure-function studies of *R* genes, potential ligand-receptor interactions between pathogen Avr proteins and plant R proteins, and genetic dissection of *R* gene–mediated induction of the HR and SAR host defense are providing information that can be used to engineer future crops so that they will be resistant to a broad spectrum of pathogens.

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