Molecular Genetics of Plant Disease Resistance

Brian J. Staskawicz,* Frederick M. Ausubel, Barbara J. Baker,
Jeffrey G. Ellis, Jonathan D. G. Jones

Plant breeders have used disease resistance genes (*R* genes) to control plant disease since the turn of the century. Molecular cloning of *R* genes that enable plants to resist a diverse range of pathogens has revealed that the proteins encoded by these genes have several features in common. These findings suggest that plants may have evolved common signal transduction mechanisms for the expression of resistance to a wide range of unrelated pathogens. Characterization of the molecular signals involved in pathogen recognition and of the molecular events that specify the expression of resistance may lead to novel strategies for plant disease control.

Plants, like animals, are continually exposed to pathogen attack. Because plants lack a circulatory system and antibodies, they have evolved a defense mechanism that is distinct from the vertebrate immune system (*1*). In contrast to animal cells, each plant cell is capable of defending itself by means of a combination of constitutive and induced defenses (*2*). Knowledge about the genetic and biochemical basis of plant disease resistance has accumulated since the turn of the century, when plant breeders first recognized that resistance was often controlled by Mendelian genes (*3*). The demonstration that plants have geographical centers of origin (*4*) and have co-evolved with their pathogens was a pivotal discovery for plant breeders and has led to the use of interspecific hybrids between crops and their wild relatives as sources of resistant germ plasm (*5*). Until 1992, however, no plant *R* gene had been cloned and characterized at the molecular level (*6*). Since then, *R* genes from several plant species have been cloned; this constitutes a major advance for molecular plant biology and may lead to the development of novel methods for disease control.

Plant Responses to Pathogen Attack

The range of phytopathogenic organisms that attack plants is diverse and includes viruses, mycoplasma, bacteria, fungi, nematodes, protozoa, and parasites (*7*). Each has a unique mode of pathogenicity. Despite the vast array of potential phytopathogens, resistance (lack of susceptibility) is the rule and susceptibility is the exception. Why one pathogen can cause disease in one plant but not in other plants—a phenomenon often termed nonhost resistance—remains an important unsolved problem in plant pathology.

Resistance to a pathogen is manifested in a variety of ways and is often correlated with a hypersensitive response (HR), localized induced cell death in the host plant at the site of infection (*8*). Although the molecular mechanism is obscure, HR is thought to be responsible for the limitation of pathogen growth. Resistance does not always involve visible HR, which may reflect either HR limited to individual plant cells or other uncharacterized defense mechanisms. Alternatively, the pathogen could lack a specific pathogenicity function required to cause disease in the host, or the host could lack a specific "susceptibility" factor. Although this review concerns the molecular basis of HR-mediated resistance, the elucidation of the mechanisms involved in nonhost resistance without HR may be an important component of future attempts to control plant disease.

The genetic basis of HR-mediated disease resistance was first clarified by Flor, who demonstrated that the resistance of flax to the fungal pathogen *Melampsora lini* was a consequence of the interaction of paired cognate genes in the host and the pathogen (*9*). His work provided the theoretical basis for the gene-for-gene hypothesis of plant-pathogen interactions and for the molecular cloning of pathogen avirulence (*avr*) genes and their corresponding plant *R* genes. An *avr* gene gives the pathogen an avirulent phenotype on a host plant that carries the corresponding *R* gene (Fig. 1) (*10*). In gene-for-gene interactions, the induction of the plant defense response that leads to HR is initiated by the plant's recognition of specific signal molecules (elicitors) produced by the pathogen; these elicitors are encoded directly or indirectly by avirulence genes, and *R* genes are thought to encode receptors for these elicitors. Elicitor recognition activates a cascade of host genes that leads to HR and inhibition of pathogen growth (*11*).

Gene-for-gene systems involving HR have been described for pathosystems involving intracellular obligate pathogens (vi-

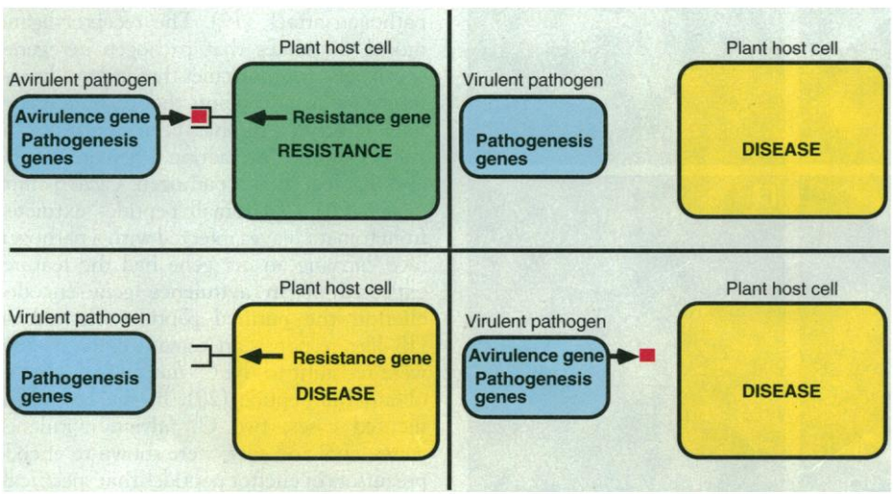

Fig. 1. Gene-for-gene interactions specify plant disease resistance. Resistance is only expressed when a plant that contains a specific *R* gene recognizes a pathogen that has the corresponding avirulence gene (upper left panel). All other combinations lead to lack of recognition by the host, and the result is disease. Green represents hypersensitive response; yellow represents susceptibility to disease.

B. J. Staskawicz is in the Department of Plant Biology, University of California, Berkeley, CA 94720, USA. F. M. Ausubel is in the Department of Genetics, Harvard Medical School, Boston, MA 02115, USA, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA. B. J. Baker is at the Plant Gene Expression Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, CA 94710, USA, and Department of Plant Biology, University of California, Berkeley, CA 94720, USA. J. G. Ellis is at the CSIRO Division of Plant Industry and Cooperative Research Centre for Plant Science, Post Office Box 1600, Canberra, A.C.T., Australia. J. D. G. Jones is at the Sainsbury Laboratory, John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK.

*To whom correspondence should be addressed. E-mail: stask@garnet.berkeley.edu

ruses and mycoplasmas) as well as for intercellular facultative and obligate pathogens (bacteria, fungi, and nematodes). These findings suggest that common or similar recognition and signal transduction mechanisms are involved in different gene-for-gene signaling pathways (*2*, *11*). Physiological features of HR common to a plant's response to different pathogens include a rapid oxidative burst, ion fluxes characterized by K^+-H^+ exchange, cellular decompartmentalization, cross-linking and strengthening of plant cell wall, production of antimicrobial compounds (phytoalexins), and induction of pathogenesis-related (PR) proteins such as chitinases and glucanases (*2*). These events characterize a plant's defense response irrespective of the pathogen, although any particular defense response can vary with respect to timing, cell autonomy, or intensity. The mechanism by which these events limit the growth of specific pathogens remains unknown.

HR (*12*) and other necrotic reactions are hypothesized to trigger a subsequent response, referred to as systemic acquired resistance (SAR), that acts nonspecifically throughout the plant: SAR reduces the severity of disease caused by all classes of pathogens, including normally virulent pathogens (*13*). Experimental evidence suggests that HR induces an unidentified diffusible signal; salicylic acid is known to be involved in both HR and SAR, but may not participate in the systemic signaling pathway that induces SAR (*14*–*16*). SAR may be involved in general resistance in field situations where plants undergo HR. Manipulation of SAR by chemical inducers or by genetic engineering may aid disease control.

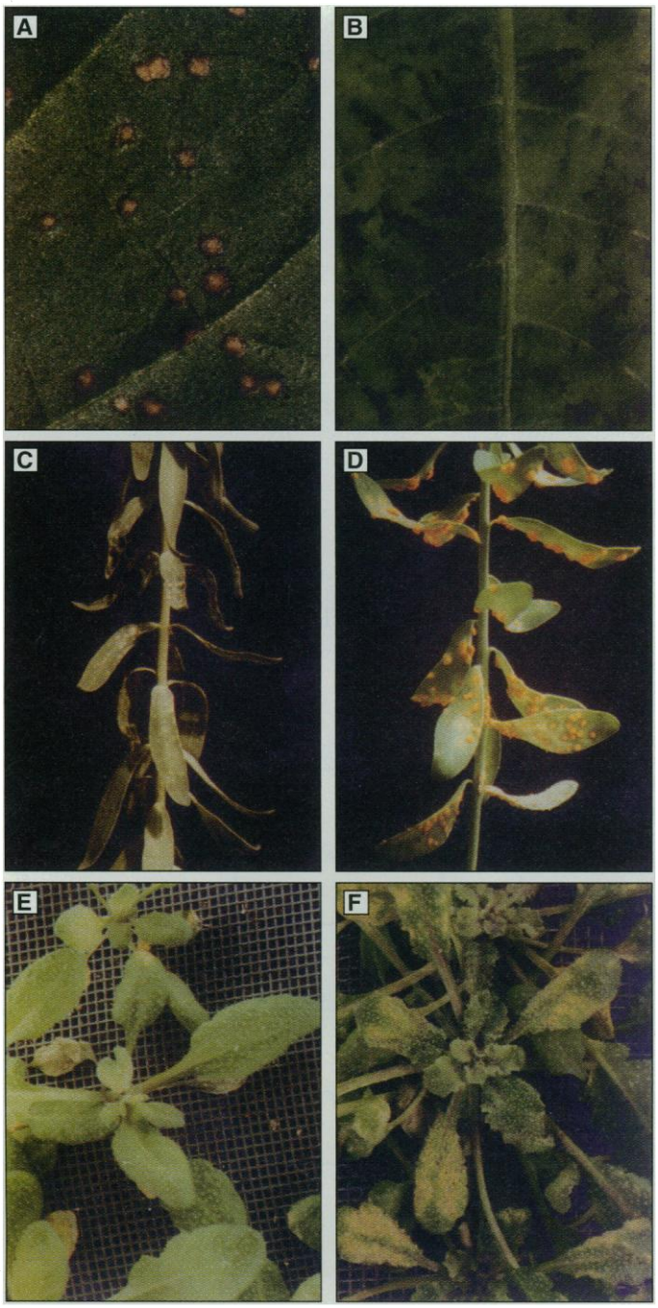

Fig. 2. Disease-resistant and -susceptible phenotypes of TMV, *M. lini*, and *P. syringae* inoculated on their respective hosts. TMV was inoculated on a resistant *NN* tobacco plant (**A**) and on a susceptible *nn* tobacco plant (**B**). *Melampsora lini* was inoculated on a resistant L^6 L^6 flax plant (**C**) and on a susceptible l^6 l^6 flax plant (**D**). *Pseudomonas syringae* was inoculated on a resistant *RPS2 RPS2 Arabidopsis* plant (**E**) and on a susceptible *rps2 rps2 Arabidopsis* plant (**F**).

Plants that are susceptible to a given pathogen still attempt to defend themselves. Indeed, many of the defense responses observed in resistant plants, with the exception of HR, are also observed in susceptible plants, although usually later after the infection. For example, even disease lesions in susceptible plants frequently have a defined and delimited shape, suggesting that the host is limiting the growth of the pathogen. By contrast, *Arabidopsis thaliana* mutants with decreased ability to synthesize phytoalexins (*17*) or to induce SAR (*18*), and *Arabidopsis* and tobacco plants engineered to degrade salicylic acid (*15*), develop larger lesions, are susceptible to very small amounts of pathogen infiltration, and allow the pathogen to grow to high titers.

Role of Pathogen Avirulence Genes in Triggering the Plant Defense Response

Genetic, biochemical, and physiological techniques have been used to study responses to pathogen attack in heterozygous populations of crop plants such as maize, soybean, bean, parsley, tomato, potato, and barley. Because plant defense responses are similar irrespective of the pathogen, it has been difficult to provide compelling evidence for the significance of particular responses in conferring specific resistance. Thus, tomato or the crucifer *A. thaliana* have been used as model hosts to study plant responses to pathogen attack (*19*). The receptor-ligand model postulates that pathogen *avr* genes specify elicitor molecules that induce disease resistance in host plants that contain a cognate *R* gene. This has been confirmed in studies of the interaction between tomato and its leaf mold pathogen *Cladosporium fulvum* (*20*, *21*). Small peptides extracted from tomato leaves infected with a pathogen race carrying an *avr* gene had the features expected of an avirulence gene–encoded elicitor; the purified peptides elicited an HR-like response on tomato cultivars that were resistant to the C. *fulvum* race used to obtain the peptide (*20*). In the best-documented cases, two C. *fulvum* avirulence genes, *avr9* and *avr4*, were shown to encode precursors of elicitor peptides that specifically elicited HR in tomato plants that harbored the corresponding *R* genes *Cf-9* and *Cf-4*, respectively (*22*).

The only bacterial avirulence gene in

which the *avr*-generated signal has been definitively identified is the *Pseudomonas syringae* pv. *glycinea avrD* locus. This locus encodes enzymes involved in the synthesis of exported syringolides that elicit HR in soybean cultivars carrying the *R* gene *Rpg4* (*23*). In the case of tobacco mosaic virus (TMV), the viral-encoded coat protein appears to function as a specific intracellular elicitor that activates HR in *Nicotiana sylvestris* cultivars that carry the *R* gene *N'* (*24*). Uncovering the molecular basis of how these genes and other cloned avirulence genes elicit a plant defense response will ultimately be necessary for a complete molecular understanding of host-pathogen specificity.

Why do pathogens contain avirulence genes? The *avr* genes may encode pathogenicity factors that confer a selective advantage for the pathogen, as has been shown for several bacterial avirulence genes that confer enhanced virulence on susceptible hosts, that is, on hosts that do not carry a cognate *R* gene (*10*). In the case of fungal pathogens, no role in pathogenicity for *avr*-encoded elicitor peptides has yet been established.

Cloning and Characterization of Plant *R* Genes

The cloning of several *R* genes since 1992 reflects, in part, the simultaneous development of the infrastructures required for insertional mutagenesis and positional cloning in several plant species. For many years, transposons have been exploited as insertional mutagens for efficient identification and isolation of genes (a process termed transposon tagging) in a wide range of organisms, including plants. The *Tam* elements of snapdragon and the *Ac/Ds*, *Spm*, and *Mu* elements of maize have been used for the isolation of a variety of genes. Fortunately, members of the maize *Ac* and *Spm* transposon families function when transferred into heterologous plant species; this attribute permits the engineering of efficient gene tagging systems in a variety of plant species (*25*). Transposon-based gene tagging systems have been used to clone *R* genes from maize, tobacco, tomato, and flax (*26*–*29*).

Map-based positional cloning of tomato and *Arabidopsis* genes has become feasible with the development of high-density physical-genetic maps for these two species (*30*). The small genome size of *Arabidopsis* (~150 Mb) and the relatively small genome size of tomato (~950 Mb), and the relatively small number of repeated sequences in these species, have facilitated the successful positional cloning of two *R* genes in these species (*31*, *32*).

The first plant *R* gene to be cloned was the maize *Hm1* gene (*26*). This gene, which controls resistance to race 1 isolates of *Cochliobolus carbonum*, was identified by transposon tagging with the maize (*Mu*) transposon. *Hm1* encodes a reduced nicotinamide adenine dinucleotide phosphate (NADPH)–dependent HC-toxin reductase, HTRC. HTRC inactivates HC-toxin, a pathogenicity factor produced by the fungus C. *carbonum* Nelson race 1 that permits the fungus to infect certain genotypes of maize (*26*, *33*). The genetics of the interaction between maize and C. *carbonum* differ from those of gene-for-gene systems because toxin-deficient C. *carbonum* strains lose their ability to cause disease in maize cultivars that do not carry *Hm1*.

The first plant *R* gene to be cloned that conforms to a classic gene-for-gene relation was the tomato *PTO* gene (*32*). The *PTO* locus confers resistance to strains of *P. syringae* pv. *tomato* (*Pst*) carrying the avirulence gene *avrPto* (*34*). A yeast artificial chromosome (YAC) clone that spanned the *PTO* region was identified with the use of a map-based cloning strategy and a restriction fragment length polymorphism (RFLP) marker tightly linked to *PTO*. This YAC clone was then used to isolate complementary DNAs (cDNAs) corresponding to the *PTO* region, and subsequent genetic complementation tests identified a cDNA clone corresponding to *PTO*. The translation product of *PTO* predicts that it encodes a serine-threonine protein kinase; hence, this product may play a role in signal transduction.

Interestingly, *PTO* appears to be part of a complex locus that consists of a cluster of five to seven genes, all homologous to *PTO*. One of these *PTO* homologs, *FEN*, confers sensitivity to the organophosphorous insecticide fenthion. The evidence that *FEN* is a separate gene comes from mutational analyses (*35*) and from the demonstration that a cDNA clone with ~80% homology to *PTO* confers sensitivity to the insecticide (*36*). A third gene, involved in both *PTO* resistance and fenthion sensitivity, was identified by the isolation of tomato mutants that were simultaneously altered in both bacterial resistance and fenthion sensitivity. These plants carry mutations in a new locus, designated *PRF* (to indicate *Pseudomonas* resistance and fenthion sensitivity), which is tightly linked to *PTO* (*35*). Apparently, *PRF* is part of the signal transduction pathway that includes *PTO* and *FEN*. Because many tools are available to dissect the *PTO* signal transduction pathway, this system is a promising area for future research.

Four additional plant *R* genes that conform to classical gene-for-gene relations have been cloned. The *Arabidopsis RPS2* gene, which confers resistance to the bacterial pathogen *P. syringae* pvs. *tomato* and *maculicola* expressing the avirulence gene *avrRpt2*, was identified by isolation of *Arabidopsis* mutants that did not exhibit HR in response to *P. syringae* strains carrying *avrRpt2*. The *RPS2* gene was then cloned by means of a map-based strategy similar in concept to the method used to identify the tomato *PTO* gene (*31*).

The tobacco *N* gene, which confers resistance to TMV, was isolated by transposon tagging with the autonomous maize transposon *Ac* (*28*). At elevated temperatures, *N* does not mediate HR after TMV infection, but if the temperature is lowered after TMV infection of seedlings carrying the *N* gene, the seedlings become necrotic and die. Some survivors contained *Ac*-tagged mutations at the *N* locus.

The tomato *Cf-9* gene, which confers resistance to the fungal pathogen C. *fulvum* expressing the avirulence gene *avr9*, was tagged by a maize *Ds* transposable element (*27*). A tomato line lacking *Cf-9* was engineered that expressed the C. *fulvum avr9* gene under the control of a plant gene promoter (*37*). When this line was crossed with a line containing both *Cf-9* and a *Ds* element, most of the progeny died because the interaction of the *avr9* gene product with the *Cf-9* gene product resulted in the elicitation of systemic HR. However, mutants carrying a *Ds*-inactivated tagged *Cf-9* gene survived.

The flax L^6 gene, which confers resistance to the fungal pathogen M. *lini*, was also identified by transposon tagging with the maize transposon *Ac*. However, because no selection for L^6 mutations was available, mutants were identified by visual inspection of thousands of flax plants containing putative transpositions of *Ac* into the L^6 gene (*38*). The phenotypes of TMV, M. *lini*, and *P. syringae* inoculated on both resistant and susceptible hosts are shown in Fig. 2.

Although the *RPS2*, *N*, *Cf-9*, and L^6 genes confer resistance to bacterial, viral, and fungal pathogens, DNA sequence analysis revealed that all four genes encode proteins that contain leucine-rich repeats (LRRs). LRR motifs are found in many plant and animal proteins and are usually involved in protein-protein interactions (*39*). Moreover, all four of these genes are fundamentally different from both the maize *Hm1* and the tomato *PTO* *R* genes. A comparison of the sequences of the RPS2, N, CF-9, and L^6 proteins reveals that RPS2, N, and L^6 share significant homology, whereas CF-9 appears to belong to a separate class (Fig. 3). RPS2, N, and L^6 all contain a conserved nucleotide binding site (NBS) in addition to the LRRs. Further inspection of these three proteins reveals that N and L^6 are more closely related to each other than to RPS2. The NH_2-terminus of RPS2 contains a leucine zipper, which might be involved in protein dimer-

ization, whereas the NH_2-terminus of N shares homology with the Toll protein of *Drosophila* and the mammalian interleukin-1 receptor (IL-1R). Preliminary mutagenesis studies and sequence analysis indicate that the RPS2 and N proteins, which lack a leader peptide, are most likely cytoplasmic and probably recognize intracellular ligands, whereas the L^6 protein may attach to the cell membrane by means of a signal anchor.

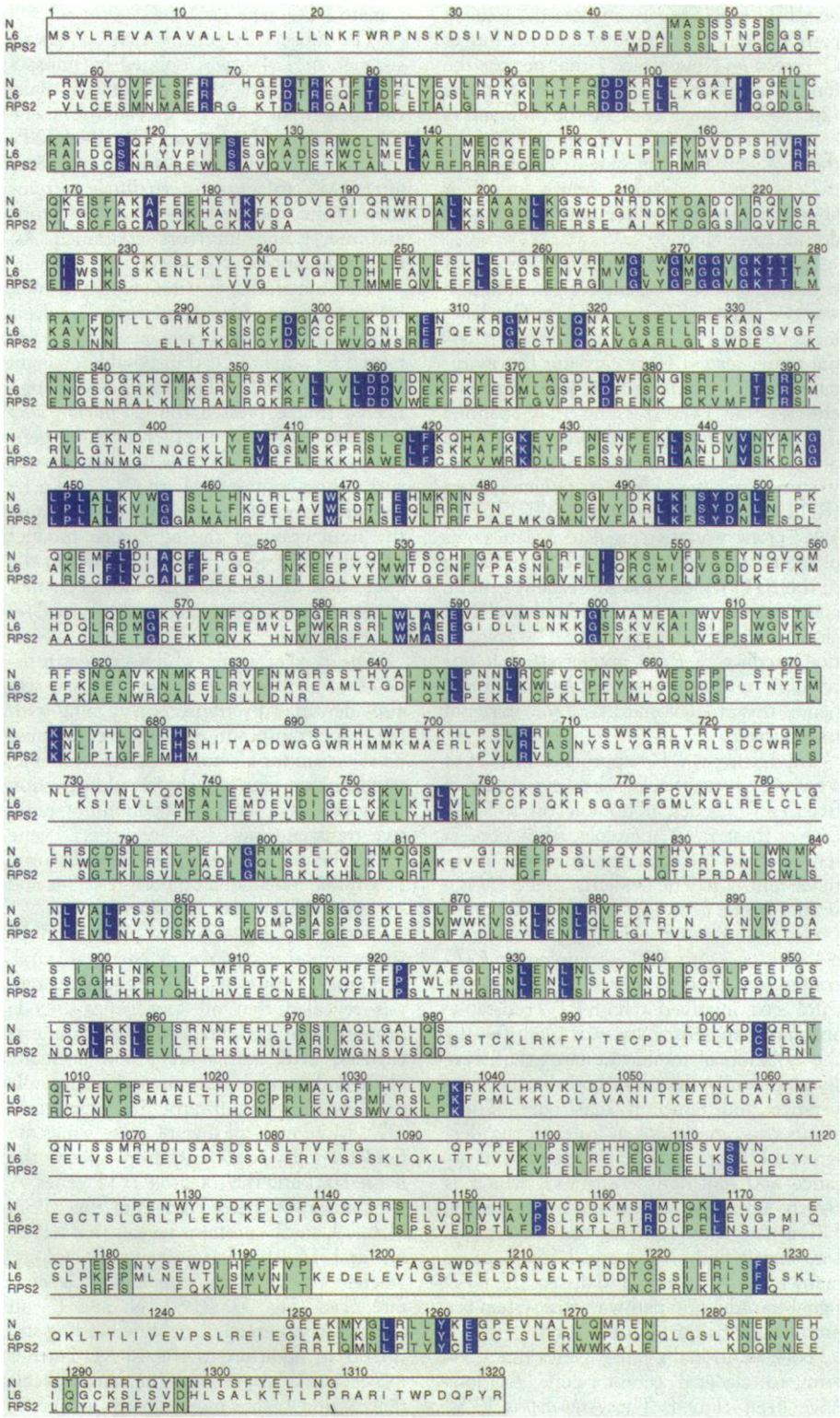

Fig. 3. Amino acid alignment of the proteins expressed by the *R* genes *N*, *L*^{6}, and *RPS2*. The sequences for N (*28*), L^6 (*29*), and RPS2 (*31*) were aligned with the use of the programs AMAS (*62*) and Clustal W (*63*). Identical matches are shown in blue, and similarities are shown in green.

The CF-9 protein appears to consist primarily of extracytoplasmic LRRs, with a COOH-terminal membrane anchor. This structure suggests that the CF-9 protein is a receptor for the extracellular ligand provided by the Avr9 elicitor peptide. Whether a direct interaction occurs between CF-9 and the Avr9 peptide is unknown. Membrane protein preparations from leaves of plants that express *Cf-0* and *Cf-9* bound the Avr9 peptide with almost equal affinity, whereas the intact leaves of the *Cf-0*–expressing plants did not respond to the Avr9 peptide. Thus, although CF-9 protein binds the Avr9 peptide, other plant proteins (perhaps expressed by other members of the *Cf-9* multigene family) also bind the Avr9 peptide (*40*).

Additional *R* genes will likely be isolated by positional cloning and transposon tagging. Transposon tagging may be a more general method for *R* gene isolation from a wide variety of plant species. Because four of the six *R* genes cloned to date have been found to encode products with strikingly similar sequences and structural features, it is likely that other *R* genes will be isolated on the basis of homology to the known *R* genes. Indeed, homologs of *RPS2*, *N*, *L*^{6}, and *Cf-9* exist in a variety of species. The *L*^{6} gene hybridizes to RFLPs linked to the unlinked rust resistance genes at the flax M resistance locus (*38*).

Signal Transduction Events and Expression of Disease Resistance

The mechanisms underlying gene-for-gene resistance probably involve specific recognition of a pathogen-generated ligand (produced by an *avr* gene) by a plant receptor encoded by an *R* gene (Fig. 4). The events that occur after recognition are a matter of speculation, but the domains in *R* gene proteins provide clues. For example, if CF-9 is a transmembrane receptor and its LRR region binds the Avr9 peptide directly, the cytoplasmic domain of CF-9 might directly activate a kinase such as that encoded by the tomato *R* gene *PTO*. This event would be analogous to the mechanism by which CD4, a membrane-anchored receptor on T cells, activates the tyrosine protein kinase p56^{Lck} (*41*). Alternatively, CF-9 might interact with other proteins, including transmembrane protein kinases that also carry extracellular LRRs (*42*) or secreted LRR-carrying proteins such as polygalacturonase-inhibiting proteins (PGIPs) (*43*). A genetic approach holds promise for the identification of genes required for *Cf-9*–dependent resistance (*44*).

The plant cellular defense responses activated by the N protein may be analogous

to "natural" or innate immunity in vertebrates and insects. In mammals, perception of signals produced by pathogens results in translocation of the Rel-related transcription factor NF-κB (*45*) from the cytoplasm to the nucleus, where it activates transcription of defense-related genes (*46*); in *Drosophila* the same process occurs with the Rel-related transcription factor Dif (*47*, *48*). In the mammalian immune system, the cytoplasmic domain of IL-1R is involved in the transduction of the signal required for the translocation of NF-κB; this domain has sequence and functional similarity to the cytoplasmic domain of the *Drosophila* Toll protein (*49*). In *Drosophila* development, the perception of an extracellular signal by Toll results in the translocation of Dorsal, a homolog of NF-κB (*50*). The presence of a domain in the NH_2-terminus of the N protein that is similar to the cytoplasmic domains of Toll and IL-1R suggests that this domain may trigger an intracellular signal transduction cascade in plants, analogous to the Toll and IL-1R pathways in animals. The N protein, and possibly other *R* gene–encoded proteins, may serve as receptors that activate a Rel-related transcription factor that induces the expression of genes responsible for HR. Unlike that of the N protein, the NH_2-terminal domain of the *Arabidopsis* RPS2 protein is not similar to those of Toll or IL-1R. However, RPS2 does contain a leucine zipper motif at the NH_2-terminus that may be involved in the formation of a heterodimer with a Toll-like protein.

The chain of events between pathogen infection in a plant and the onset of HR is not well defined. However, it has been widely observed that HR is preceded by a rapid outburst of the reactive oxygen intermediates (ROIs) O_2^-, H_2O_2, and $OH^·$ (*51*). A plasma membrane multisubunit NADPH oxidase complex, similar to the one found in mammalian phagocytes, might be involved in the release of ROIs in plants. If a rapid oxidative burst is crucial to HR, the activation of a protein kinase could lead to the activation of an NADPH oxidase rather than to transcriptional activation. In the mammalian innate immune response, ROIs have been shown to induce the expression of acute phase response genes by activating the transcription factors NF-κB (*52*) and AP-1 (*53*). In plants, *R* gene–mediated induction of intracellular ROIs suggests that a redox-regulated transcription factor may also be involved in the activation of HR.

Evolution of Plant Disease Resistance

The following scenario for the evolution of plant disease resistance has been proposed (*54*): The evolutionary ground state is considered to be a compatible interaction in which a pathogen has evolved to be virulent on a particular host plant. Selection favors the evolution and spread of host individuals that specifically recognize the pathogen and resist infection. For example, a receptor that evolved to activate defense responses to pathogens in general may be modified so that it specifically recognizes a particular pathogen product (an avirulence gene product). The pathogen responds by losing the avirulence gene by mutation. This phenomenon is absolutely essential for the survival of obligate parasites. The host is now susceptible, and again selection is brought to bear on new host *R* gene specificities. Consequently, the evolution of gene-for-gene interactions can be seen as a continuing step-by-step or move-countermove process, whose consequence in plant populations is a diversity of *R* genes in different individuals of a host species and a corresponding diversity of avirulence genes in different pathogen races.

The existing diversity of *R* genes is the product of an evolutionary process that appears to have proceeded along two major branches. On one branch, exemplified by the M rust resistance locus in flax, tandem arrays of related *R* genes with different specificities are found in the plant genome (*38*). The other evolutionary branch is exemplified by the flax *L* rust resistance locus; the specificities at this locus behave genetically as alleles of a single gene, and different specificities existing in heterozygotes cannot be recombined (*38*). The cloning of the L^6 allele of this locus supports the classical genetic interpretation of a simple *L* locus but has also provided some surprises (*29*). The genes at the genetically complex M locus are related in sequence to the unlinked *L* gene, with 70 to 90% nucleotide identity. The M locus appears to have evolved by local duplication and divergence from an *L*-like *R* gene progenitor, whereas the *L* locus appears to have evolved as a multiple allelic series, with only a single *L* specificity capable of existing in a homozygote. The contrasting evolution of two such closely related genes may be the result of a rare duplication event that occurred only at the M locus and then provided the opportunity for rapid amplification by unequal crossing over. Similarly, molecular analyses of the TMV resistance locus *N* in tobacco and of the *Cf-9* locus in tomato have revealed a clustered gene family (*27*, *28*).

Molecular Basis and Evolution of *R* Gene Specificity

R genes specifically distinguish isolates of a single pathogen species. The multiple resistance specificities encoded by the 13 alleles of the cloned *L* gene of flax provide an opportunity to study the molecular basis of this specificity. Three alleles, L^2, L^6, and L^{10}, have been cloned and partially characterized. Although L^6 and L^{10} are similar, L^2 has additional numbers of an LRR motif that occurs in the COOH-terminal region of the gene product. This region may determine ligand specificity.

Pathogen propagules increase to vast numbers in comparison with their hosts, and consequently there is a greater opportunity for virulent pathogen races to arise than for corresponding new host resistance specificities. Coupled with the differences in population size, pathogen evolution from

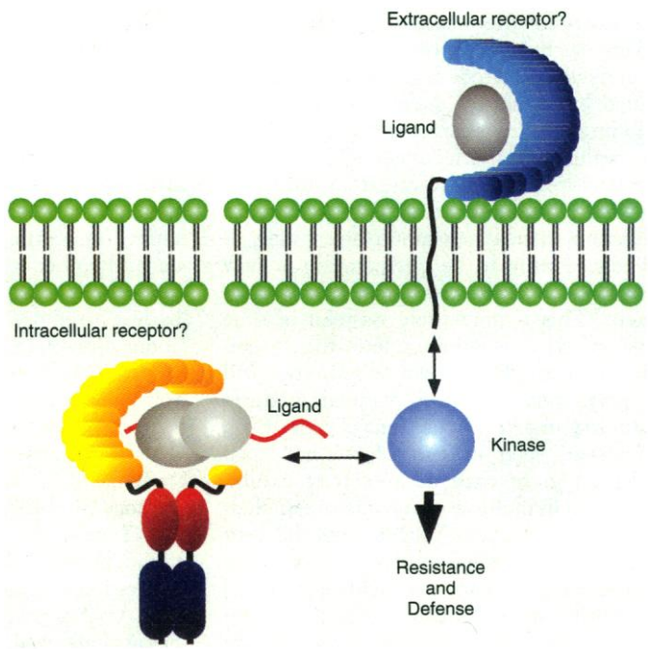

Fig. 4. Receptor-ligand model for the recognition and expression of plant disease resistance. In this hypothetical model, the *R* gene is thought to encode for either an extracellular receptor, such as the protein product of the *Cf-9* gene of tomato, or an intracellular receptor, such as the product of the *N* gene of tobacco. The ligand in this model may represent the direct or indirect product of the pathogen's avirulence gene. The specific recognition event triggers a signal transduction cascade that may involve protein kinases and may lead to the expression of plant disease resistance.

avirulence to virulence usually results from loss-of-function mutation. The corresponding gain of function (that is, resistance in the host) is unlikely to occur by simple mutation. Do plants have a mechanism to generate new *R* gene specificities and keep pace with the evolutionary progress of pathogens? Pryor, Hulbert, Bennetzen, and their colleagues have observed high-frequency loss of rust resistance in corn associated with unequal crossing over at the *Rp1* locus, and they have proposed that this shuffling of preexisting coding information may provide a means for plants to generate new specificities (*55*). The LRR domains, which are important in receptor selectivity, may be involved in defining the recognitional specificity of the pathogen (*56*).

Bioengineering for Novel and Stable Plant Disease Resistance

The isolation of plant *R* genes provides opportunities for producing crop plant varieties with increased disease resistance. Potential approaches can be subdivided into those that augment classical breeding techniques and those that involve direct engineering of crop plants.

The identification of a variety of *R* genes on the basis of amino acid sequence conservation will enable plant breeders to monitor *R* gene segregation using appropriate DNA probes instead of testing progeny for disease resistance and susceptibility. The same approach may greatly facilitate the identification and introgression of new resistances from wild species that either interbreed poorly with crop species or do not cross at all. For example, additional nematode resistances exist in wild accessions of *Lycopersicon peruvianum*, but the amount of work necessary for the recurrent introgression of many such new resistances is daunting. Candidate nematode *R* genes could be identified by homology, isolated by molecular cloning, and transformed into crop varieties to evaluate their effectiveness.

It is frequently the case that after protracted breeding efforts of 10 or more years' duration, a new resistant plant variety is produced only to be overcome by a new pathogen race within a few years of deployment. This is immensely wasteful of time and effort. Population genetic theory predicts that the breakdown of resistance will happen more slowly in varietal mixtures carrying an array of different *R* genes (*57*). However, such mixtures have not been adopted in practice because they exhibit variation in multiple characteristics, including time to harvest. With several different cloned *R* genes responsive to the same pathogen, plant varieties could be produced consisting of mixtures of lines that differ only in the *R* gene allele they carry. Such an environmentally and ecologically sound approach to disease control would find acceptance among consumers and growers.

One exception to the lack of durability of *R* gene–mediated resistance is the *BS2* gene of pepper, which confers resistance to strains of *Xanthomonas campestris* pv. *vesicatoria* that contain the *avrBs2* avirulence gene and is extremely effective in controlling the bacterial spot disease of pepper. The success of this gene seems to be related to the fact that all strains of the pathogen examined to date contain an active copy of the *avrBs2* gene, and if the gene is lost, the pathogen suffers a severe fitness penalty (*58*).

The approaches described above to facilitate the introduction of disease-resistant varieties all take advantage of cloned *R* genes. However, *R* genes appear to function at or near the beginning of a complex signal transduction cascade that leads to HR and ultimately to SAR. It would be desirable to directly manipulate HR and SAR by engineering the signal transduction pathways that lead to their activation. Genetic dissection of HR and SAR and their regulation is beginning in *Arabidopsis*.

The engineering of HR and SAR cannot take place unless certain problems are addressed, such as the lethality of a constitutively activated defense response (*37*). To circumvent this problem, alleles of *R* genes, or of genes that encode the products with which the products of *R* genes interact, could be found that would partially activate the defense response. The result would be a phenotype analogous to SAR that confers some degree of resistance but does not kill the plant. Mutations of this sort are likely to be selected against in natural populations because they would likely partially cripple the host in the absence of severe pathogen attack. In agricultural settings, however, they could be advantageous, even though they might be associated with yield penalties. Dominant mutations at *R* gene loci and recessive mutations at some other loci might be expected to result in partial constitutive expression of the defense response. Some necrotic or disease lesion mimic mutations may arise in this manner (*59*). Indeed, the phenotype of the recessive barley mutant *mlo*, which has been widely used in barley breeding, can be phenocopied by application of 2,6-dichloroisonicotinic acid, a chemical that elicits a SAR-like response (*60*). Thus, in some circumstances, useful mutations can be identified in which the defense response has been "primed."

It has been suggested that if a suitable pathogen-inducible promoter, such as the *prp1-1* promoter of potato (*61*), could be found, it would be possible to induce the expression of a race-specific elicitor such as the Avr9 peptide only in cells that are being challenged by a compatible pathogen (*20*). If this engineered plant also contained a functional *Cf-9* gene, then a previously compatible pathogen would now elicit HR. Because potato and tomato are so closely related, *Cf-9* seems likely to function in potato, and this system offers real potential for increasing resistance to potato late blight caused by *Phytophthora infestans*.

In the course of transposon tagging of the tomato *Cf-9* gene, alleles have been generated in which the *Ds* transposon somatically excises from *Cf-9* and thus restores function. In the presence of Avr9, this excision results in the formation of localized necrotic sectors in which both Avr9 and CF-9 are active (*27*). Preliminary experiments indicate that plants with this phenotype show some characteristics of SAR, including enhanced resistance to pathogens that would otherwise be compatible. This phenomenon has been designated genetic acquired resistance (GAR) to indicate that it is a genetically imposed SAR. Fine tuning of the system will undoubtedly be required to achieve the optimum balance between activation of the defense response and crop yield.

Summary

This is an extremely exciting time for the field of plant pathology. The cloning and characterization of several plant *R* genes constitutes a major breakthrough in the elucidation of the molecular basis of disease resistance to a wide range of phytopathogens. As a result, we are finally in a position to determine the molecular basis of plant-pathogen specificity and expression of disease resistance. Future research challenges include the determination of the mechanisms by which *R* gene products recognize pathogen elicitors and the plant defense response blocks pathogen growth. The basic knowledge obtained from this research will undoubtedly help to produce novel forms of durable disease resistance and will lead to a decline in the use of environmentally damaging pesticides.

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The Ethylene Signal Transduction Pathway in Plants

Joseph R. Ecker

Ethylene (C_2H_4), the chemically simplest plant hormone, is among the best-characterized plant growth regulators. It participates in a variety of stress responses and developmental processes. Genetic studies in *Arabidopsis* have defined a number of genes in the ethylene signal transduction pathway. Isolation of two of these genes has revealed that plants sense this gas through a combination of proteins that resemble both prokaryotic and eukaryotic signaling proteins. Ethylene signaling components are likely conserved for responses as diverse as cell elongation, cell fate patterning in the root epidermis, and fruit ripening. Genetic manipulation of these genes will provide agriculture with new tools to prevent or modify ethylene responses in a variety of plants.

The simple gas ethylene is an endogenous regulator of developmental adaptations in higher plants (*1*). Exposure to ethylene can produce a myriad of effects on plant growth, development, and physiology, most notably the ripening of fruits, inhibition of stem and root elongation, promotion of seed germination and flowering, senescence of leaves and flowers, and sex determination. How this simple olefin evokes such a diverse array of physiological processes has been a central question in ethylene research.

The biosynthesis of ethylene is stimulated prior to several developmentally programmed senescence processes and in response to environmental insults such as mechanical trauma and pathogen infection (*2*, *3*). As a result of biochemical analysis, the route of ethylene synthesis (the Yang Cycle) is now largely understood (*4*, *5*). The rate-limiting step is the conversion of *S*-adenosyl-L-methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC), which is catalyzed by ACC synthase. The enzyme ACC oxidase converts ACC to ethylene, carbon dioxide, and cyanide. ACC oxidase is constitutively present in most tissues, but its synthesis is increased during fruit ripening in tomato. The genes that encode ACC synthase and ACC oxidase have been cloned and characterized from many plant species (*5*, *6*). ACC synthase is encoded by multigene families in all species examined, and individual gene family members are transcriptionally activated by a variety of inducers. Environmental stresses (physical, chemical, and biological) and hormonal signals, such as auxin, cytokininin, and even ethylene itself, stimulate synthesis of the ACC synthase enzyme, thereby providing a means for autoregulation of its production. Although tremendous progress has been made since 1989, questions still remain regarding the complex regulation of ethylene biosynthetic genes. However, it is clear that genetic manipulation of the ACC synthase and ACC oxidase genes by expression of antisense RNA (*7*) will provide a simple means to control the ripening of fruits in a variety of plants (*4*, *8*).

By contrast, biochemical approaches toward dissection of the mechanisms by

The author is in the Plant Science Institute, Department of Biology, University of Pennsylvania, Philadelphia, PA 19104–6018, USA.