2
Plant Disease Resistance Genes

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1. INTRODUCTION

No adaptive immune system equivalent to the highly effective vertebrate immune system has been detected in plants. Nevertheless, the very existence of plants in the presence of many pathogens bears witness to the presence of highly effective systems for defense against pathogen invasion and disease. One system is based on disease resistance genes, which allow plants to detect pathogen infection and mount effective defense responses. These genes were first identified in the early years of the 20th century and were cloned and characterized more than 90 years later in the last decade of the century (*see* refs. *1* and *2* for reviews). Intense studies of these genes are now taking place in the present century to discern how their products function and how this knowledge can be applied to problems of disease resistance and food security.

2. RESISTANCE AND SUSCEPTIBILITY IN PLANTS

It is interesting to contemplate which of the two, plant diseases or human diseases, is the greater potential threat to human health. The human immune system, as effective as it is, cannot meet the challenge of plant disease-induced famine. Significant diseases of plants in agriculture are caused by diverse pathogens, including viruses, bacteria, fungi, and nematodes. These diseases cause various visible symptoms, and some result in death of the infected plant, but all important diseases result in reduced crop yields and quality and in some instances complete regional crop failures. In contrast to susceptibility, plant disease resistance is characterized by either partial or complete suppression of pathogen growth or replication at the site of infection. One of the most dramatic visible phenotypes that is frequently (but not always) associated with plant resistance is rapid localized cell death, the *hypersensitive response* (HR), at the site of infection, which is often compared with animal programmed cell death. This is an especially effective process in limiting pathogens that require living host cells. Other resistance responses include (but are not limited to) activation of defense gene expression, leading to production of antimicrobial proteins or low molecular weight antibiotics. In this respect, plant disease resistance has been likened to the innate immunity systems that have been described in insects and vertebrates. Most of the major plant pathogens mentioned above, with the exception of viruses, cause disease from the outside of plant cells. This includes patho-

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genic bacteria whose extracellular life styles in plants are in contrast to the intracellular life styles of many mammalian bacterial pathogens.

3. GENETIC STUDIES OF HOST PLANT RESISTANCE AND PATHOGEN VIRULENCE

When a single isolate of a plant pathogen species (e.g., the wheat rust fungus) is inoculated onto a collection of host (e.g., wheat) genotypes, it is common to find that some genotypes are resistant and others are susceptible to the pathogen. Thus the pathogen isolate distinguishes host plant variation that is manifested as clear differences in disease reaction. Furthermore, if several isolates of the rust fungus are inoculated separately onto a set of different wheat genotypes, the different rust isolates are frequently distinguished by their ability to infect different host genotypes. For example, rust isolate 1 may infect host genotype 1 but not host genotype 2, while rust isolate 2 infects wheat 2 but not wheat 1. These sorts of observations are general for many different host-pathogen interactions. The ability of the pathogen to infect and cause disease in its host plant is referred to as *virulence;* the inability of a pathogen isolate to infect a resistant genotype of its normal host is called *avirulence.* In these ways, pathogen isolates can be used to distinguish host genotypes and, conversely, host plant genotypes can be used to distinguish isolates of the pathogen.

The existence of polymorphism for resistance/susceptibility in the host plant and for virulence/avirulence in a pathogen was initially found in crop plant species and their pathogens and more recently in wild plant species such as the "model" plant *Arabidopsis thaliana* and its pathogens. These polymorphisms provide the opportunity to carry out simultaneous genetic analysis of the inheritance of resistance and susceptibility in host species and inheritance of virulence and avirulence in the pathogen species. The most extensive classical genetic analyses were carried out on the flax plant, a crop plant grown for linseed oil and linen fiber production, and the fungal pathogen, flax rust *(3).* This fungus grows at the expense of living plant tissue and produces masses of orange spores over the leaf surfaces of susceptible plants. These genetic experiments involved the analysis of sexual crosses between resistant and susceptible flax genotypes and also sexual crosses between different rust isolates that differed in their ability to infect the host genotypes. Particular rust strains were used to follow the segregation of resistance/susceptibility in the host plant and particular flax genotypes were used as hosts to follow the segregation of avirulence/virulence in the pathogen. The following points summarize the results of these experiments with flax and flax rust and with other host-pathogen systems, which have provided a genetic description of plant-pathogen interactions.

1. Resistance to a pathogen isolate can be determined by a single gene difference between the resistant and susceptible host genotype. This gene is referred to as a *resistance gene.*
2. Resistance is most commonly dominant to susceptibility.
3. Multiple resistance genes can occur in a single species. Each resistance gene frequently encodes resistance to some but not all isolates of a pathogen species. The resistance gene's ability or inability to determine resistance to different pathogen isolates can distinguish one resistance gene from another. This difference between resistance genes is referred to as *resistance gene specificity.* For example, 30 different rust resistance specificities have been identified in the flax genome.

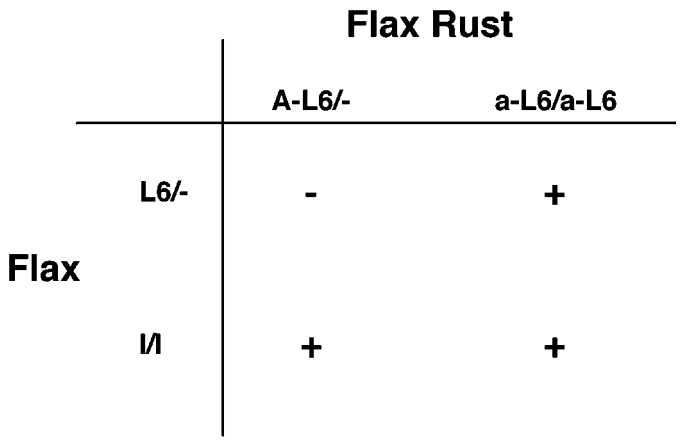

Fig. 1. Growth (+) or no growth (–) of the flax rust fungus on its host plant flax is determined by matching gene pairs at the host resistance locus and the pathogen avirulence locus. No growth (host resistance) occurs when the host carries one or more copies of the dominant resistance gene *L6* and the rust carries one or more copies of the corresponding avirulence gene *A-L6.*

4. Different resistance genes against a particular pathogen species can occur at several different loci in plant genomes, and in addition multiple resistance specificities often map to each genetic locus. In flax, for example, 30 rust resistance genes map to 5 loci with 11 specificities at the *L* locus.
5. Plant species are frequently highly polymorphic for resistance. Populations often contain individuals with different resistance specificities.

The results of the genetic analysis of pathogenicity of flax rust and other pathogens are summarized as follows:

1. Inability of a pathogen isolate to infect a resistant plant (referred to as *avirulence*) can be determined by a single gene difference between the virulent and avirulent isolate.
2. Avirulence is most commonly dominant to virulence.
3. Pathogen species often carry multiple avirulence genes and are often highly polymorphic for these genes.

The joint analysis of genetic segregation data for resistance of the host on one hand and rust avirulence on the other demonstrated that for each resistance gene specificity that is identified in the flax plant, a single corresponding avirulence gene is identified in the rust. For instance, flax plants carrying only the *L6* rust resistance gene are resistant to rusts carrying the *A-L6* avirulence gene but are susceptible to those strains without this avirulence gene (Fig. 1). This one-for-one or gene-for-gene relationship has been observed in many different host-pathogen interactions and has been used to propose a receptor-ligand model (see below). Under this model, it is postulated that the resistance gene encodes a receptor that perceives the direct (protein) or indirect (enzymatic) product of the corresponding avirulence gene in the pathogen. The cloning of host resistance genes and corresponding plant pathogen avirulence genes is beginning to provide the opportunity to test this model directly. As will be seen later, physical proof of a simple receptor-ligand pair in general has been difficult to obtain and (as discussed below), this model, based on genetic data, may be an oversimplification.

4. MOLECULAR NATURE OF AVIRULENCE GENES

Avirulence genes in pathogens are those genes that confer the ability to be recognized by a resistant host plant. This concept is one that is often problematic to animal pathologists. The simplest analogy is probably with genes encoding antigens in animal pathogens that are recognized by the mammalian immune system. Clearly, the principal function of these sorts of pathogen genes is not to trigger recognition by the host resistance mechanism. However, ultimately, in the presence of the appropriate host receptor, they are determinants of avirulence.

A number of avirulence genes have been cloned from plant pathogens, particularly viral and bacterial avirulence genes *(4).* Viral avirulence genes encode a range of functions including capsid proteins and replicase proteins. Comparisons of the gene products of bacterial avirulence genes show that they are mostly unrelated and their function is largely unknown. There is now evidence that the bacterial avirulence gene products are introduced into plant cells by a type III secretion mechanism *(5).* Further evidence suggests that these gene products are involved in enhancement of bacterial virulence (in the absence of the corresponding host resistance gene) and so are analogous to the virulence effector proteins delivered to animal cells by mammalian bacterial pathogens *(6).* Only a few fungal avirulence genes have been cloned owing to the more complex genomes of fungi. The products of these genes include small, secreted proteins of unknown function *(4).* In one case, a fungal avirulence protein from the rice blast fungus has similarity to a zinc protease *(7).*

5. MOLECULAR NATURE OF RESISTANCE GENES

A growing number of resistance genes that recognize viral, bacterial, fungal, nematode, and insect pathogens have been cloned from both crop plants and the model plant *Arabidopsis.* Most of these genes are predicted to encode proteins with at least three core domains, a C-terminal leucine-rich repeat (LRR) domain, a central nucleotide binding site (NBS) domain, and an N-terminal domain that either contains homology to cytosolic domains of the *Drosophila* Toll or animal interleukin-1 receptors (TIR) or a potential coiled-coil (CC) domain (TIR-NBS-LRR or CC-NBS-LRR) *(8)* (Fig. 2). Two subclasses of CC domains have been described *(8),* but there are probably at least three subclasses of CC domains in the CC-NBS-LRR class of resistance proteins. The tripartite structure of the NBS-LRR resistance proteins resembles the tripartite structure of CARD-NBS-WD40 [caspase recruitment domain-NBS-tryptophan aspartic acid (WD) repeat, with a periodicity of approx. 40 amino acids], CARD-NBS-LRR, AT-NBS-LRR (acetyl transferase-NBS-LRR), and BIR-NBS-LRR (baculovirus inhibition of apoptosis repeat-NBS-LRR) proteins controlling either apoptosis or the activation of cellular defenses or both in animals.

Simplistically, in NBS-LRR plant resistance proteins, the C-terminal domain is thought to be a receptor domain involved primarily in recognition of the avirulence ligand, the central NBS domain a regulatory domain, and the N-terminal TIR or CC domain an effector domain; however, biochemical evidence is still lacking. Similarly, in the human apoptotic protease-activating factor-1 (Apaf-1) CARD-NBS-WD40 apoptosis protein, the C-terminal WD40 domain is thought to be involved in the recognition of cytochrome *c* released from the mitochondria following an apoptotic stimulus, the central NBS domain in the regulatory binding of ATP or dATP,

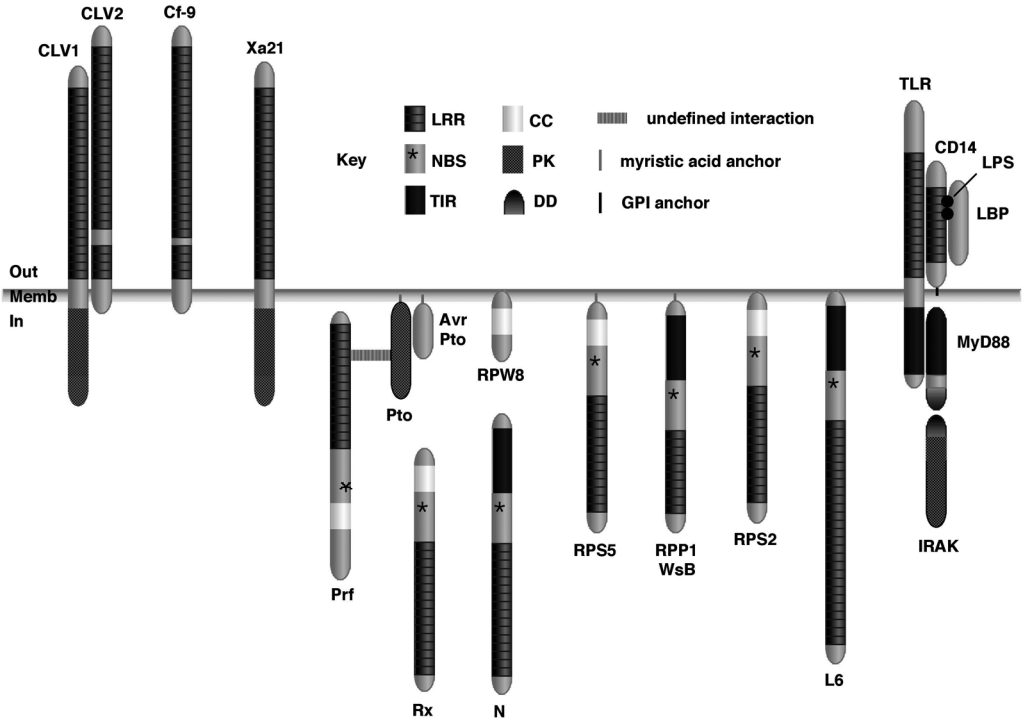

Fig. 2. Domain structures and predicted membrane topology of representative plant disease resistance proteins in comparison with one another and with similar proteins involved in plant development or animal innate immunity. All plant proteins have been described in the text apart from Rx, which is a potato gene for resistance to potato virus X *(27).* CD14 is a glycophosphoinositol (GPI)-anchored LRR protein, and LBP is a lipopolysaccharide binding protein that form part of the TLR complex recognizing bacterial lipopolysaccharides. MyD88 is an adaptor protein with TIR and DD domains that link signal transduction from the TLR complex to the serine/threonine protein kinase IRAK. The nature and function of these and other protein components involved in TLR signaling are described elsewhere in this book. The horizontal bar depicts a cell membrane with the cytosol below. All proteins except Prf are shown with the N terminus at the top. *Abbreviations:* LRR, leucine-rich repeat; PM, plasma membrane; NBS, nucleotide binding site; TIR, Toll/interleukin-1 receptor domain; CC, coiled-coil; PK, serine/threonine protein kinase; DD, death domain; TLR, Toll-like receptor; LPS, lipopolysaccharide; IRAK, interleukin-1 receptor-associated kinase. (Adapted from ref. *8*.)

and the N-terminal CARD in the triggering of the caspase cascade that effects apoptosis *(9–11).*

Initially, the NBS domain was defined in plant resistance proteins by the presence of kinase 1a (P-loop), kinase 2, and kinase 3a motifs likely to be involved in ATP/GTP or dATP/dGTP binding. As more resistance genes were cloned, at least five additional motifs were recognized, not only among resistance proteins, but also among the animal Apaf-1 *(10),* CED-4 (*Caenorhabditis elegans* death-regulating CARD-NBS protein) *(12),* and Ark (*Drosophila* Apaf-1-related killer CARD-NBS-WD-40 protein) *(13)* apoptosis proteins *(8,14,15)* (Fig. 3), leading to the redesignation of the NBS domain as an NB-ARC domain (nucleotide binding domain shared by Apaf-1, plant resistance proteins, and CED-4) *(15).* The additional motifs present in the NB-ARC domain do

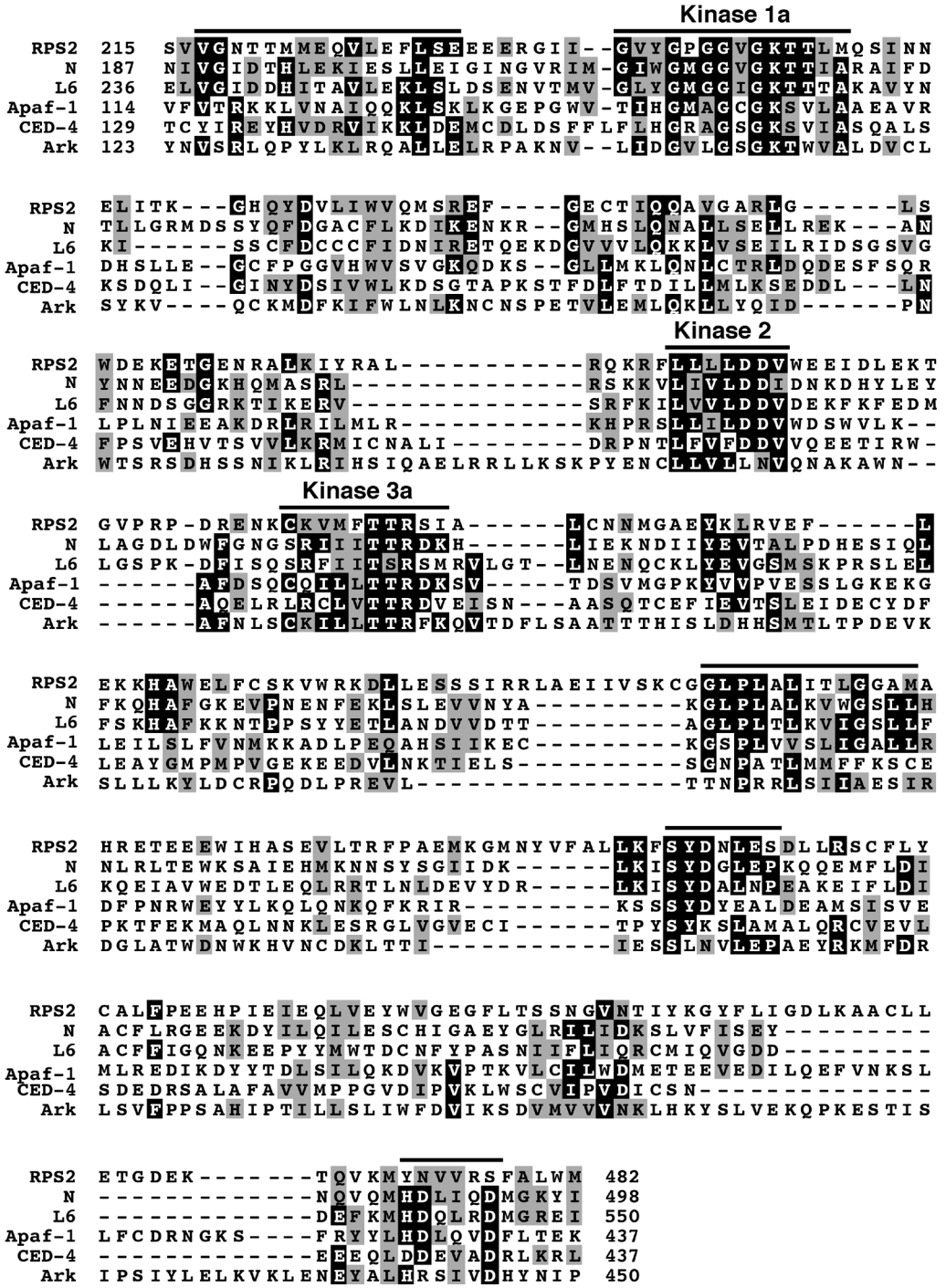

Fig. 3. Conserved motis in the NBS regions of the *Arabidopsis* RPS2 (Genbank accession U14158), tobacco N (U15605), and flax L6 (U27081) disease resistance proteins and the human Apaf-1 (AF013263), *Caenorhabditis elegans* CED-4 (X69016), and *Drosophila* Ark (AAF57916) apoptosis-activating proteins. Blocks of conserved motifs were aligned using Macaw *(71).* Amino acid identities are highlighted with black boxes and similarities with gray boxes. Conserved motifs are overlined, and the kinase 1a, 2, and 3a motifs of the NBS are indicated.

not seem to be present in the NBS domains of the human CIITA (MHC class II transcriptional activator) AT-NBS-LRR protein *(16,17),* Nod1 *(18,19),* Nod2 *(20),* and DEFCAP (death effector filament-forming CED-4-like apoptosis protein) *(21),* CARD-NBS-LRR proteins, or the BIR-NBS-LRR protein NIAP (neuronal apoptosis inhibitor protein) *(22,23).*

Superficially, the latter proteins resemble plant resistance proteins more than Apaf-1, CED-4, or Ark, because they share both NBS and LRR domains, but the resemblance is misleading because only the motifs directly involved in nucleotide binding are shared between the two types of NBS domain. This finding suggests that any other interactions mediated or regulated by the two types of NBS domain may differ. However, despite this difference, the proteins carrying this kind of NBS domain appear to be functionally more similar to plant disease resistance genes. Apaf-1, CED-4, and Ark are only involved in regulation of apoptosis, whereas CIITA, Nod1, Nod2, DEFCAP, and NIAP are involved in the regulation of apoptosis and/or cellular defenses *(16,19,20,21,24).* The latter function is more similar to that of plant disease resistance proteins, which regulate both cell death and pathogen-response protein expression, and in some cases plant disease resistance can be effected without cell death *(25–27).*

The remainder of the cloned resistance genes have been found in only one or a few kinds of plant-pathogen interactions. These comprise the rice *Xa-21* gene encoding an extracytosolic LRR receptor domain connected by a single transmembrane domain to a cytosolic serine/threonine protein kinase (LRR-TM-PK) and conferring resistance to the bacterial blight bacterium *Xanthomonas oryzae* pv. *oryzae (28);* the tomato *Pto* gene encoding a serine/threonine protein kinase (PK) and conferring resistance to the bacterial speck bacterium *Pseudomonas syringae* pv. *tomato (29);* the tomato *Cf-2, Cf-4, Cf-5, Cf-9,* and *Cf-ECP2* genes on the one hand and the tomato *Ve1* and *Ve2* genes on the other, encoding extracytosolic LRR receptor domains connected by a single transmembrane domain to a short cytosolic tail (LRR-TM) and conferring resistance to the leaf mold fungus *Cladosporium fulvum* or the vascular wilt fungus *Verticillium dahliae,* respectively *(30–35);* the *Arabidopsis RPW8.1* and *RPW8.2* genes encoding CC proteins conferring broad resistance to the powdery mildew fungi *Erysiphe cruciferarum* and *E. cichoracearum (36);* and the sugar beet *Hs1-pro* gene (possibly based on an incomplete clone) encoding a novel protein conferring resistance to the cyst nematode *Heterodera schachtii (37).*

LRR domains are common to both the NBS-LRR resistance proteins and a number of the non-NBS resistance proteins. However, the LRR domains of NBS-LRR proteins are predicted to be cytosolic and have slightly different and highly degenerate repeat motifs in terms of length and composition compared with the LRR domains of the LRR-TM or LRR-TM-PK proteins, which are predicted to be extracytosolic and have very regular and well-conserved repeat motifs *(38).* In fact, the LRRs of the NBS-LRR proteins are often difficult to discern, and assignments of LRRs are sometimes rather arbitrary as a consequence. A useful way to determine the presence of LRRs in a protein is to carry out similarity searches against the very large array of LRR proteins present in publicly available sequence databases. Such searches do not, for example, substantiate the original claim that Hs1-pro is an LRR protein. Despite the structural diversity of LRRs, their role as determinants of recognitional specificity in plant pathogen interactions is now well established (see below).

Besides LRR domains, the non-NBS-LRR proteins also show other intriguing relationships to one another, to NBS-LRR proteins, and to proteins involved in development and innate immunity in both plants and animals. The Xa-21 LRR-TM-PK protein bears an obvious resemblance to the Cf and Ve LRR-TM proteins and the Pto PK protein (Fig. 2), and it is not uncommon to find interacting protein domains that are separate in some organisms joined together in others *(39).* The Xa-21 protein is also structurally similar to a number of *Arabidopsis* LRR-TM-PK proteins including developmental proteins such as the CLV1 protein involved in shoot meristem development *(40)* (Fig. 2), the hormone receptor BRI protein involved in brassinosteriod perception *(41),* and the innate-immunity FLS2 protein involved in the sensing of bacterial flagellin and triggering of defense responses similar to those triggered by disease resistance proteins *(42).* Moreover, the CLV1 protein requires and interacts with CLV2, a LRR-TM protein structurally similar to the Cf and Ve proteins *(43)* (Fig. 2). The FLS2 protein is functionally analogous to the animal innate immunity Toll and Toll-like receptor (TLR) proteins involved in detection of pathogen-associated molecular profiles (PAMPs), such as bacterial lipopolysaccharides and proteoglycans (as described in detail elsewhere in this book).

One might therefore predict that animal cells will recognize and respond to flagellins, perhaps via a TLR protein and, *vice versa,* that plant cells will respond to the PAMPs detected by Toll and the TLR proteins, perhaps by other as yet uncharacterized LRR-TM-PK proteins. Interestingly, Toll performs a dual function in both development and innate immunity, controlling development of the dorsal-ventral axis in the *Drosophila* embryo and innate immunity in the adult fly. Moreover, at least one TLR protein has been shown to have a role in the activation of apoptosis as well as cellular defenses *(44,45).* However, in addition to functional similarities, there are also structural similarities between the non-NBS-LRR plant disease resistance proteins and the Toll and TLR proteins besides that already noted for the TIR-NBS-LRR proteins. The Toll and TLR proteins are LRR-TM-TIR proteins with structural similarity to the LRR-TM domains of the Xa-21, Cf, and Ve proteins (Fig. 2). Moreover, the TIR domains of Toll, the interleukin-1 receptor, and the TLR proteins interact with serine/threonine protein kinases Pelle and interleukin-1 receptor-associated kinase (IRAK), which are similar to the PK domains of Xa-21 and Pto (Fig. 2). Nor does the web of intrigue end here. For functioning, Pto requires Prf, a CC-NBS-LRR protein *(46,47),* and the RPW8 proteins are similar to the amino-terminal CC domain of a subclass of CC-NBS-LRR proteins *(36),* suggesting a possible homotypic interaction.

Thus, there would seem to be at least two functional connections between the NBS-LRR and the non-NBS-LRR resistance proteins. A similar connection has also been made in animal cells with innate immunity to bacteria determined by both extracellular TLR4-mediated and intracellular Nod1-mediated recognition of bacterial lipopolysaccharides feeding into the same signaling pathway *(48).* However, owing to the extracellular life style of plant bacterial pathogens, it is unlikely that cytoplasmic plant NBS-LRR proteins would be involved in the recognition of the PAMPs associated with innate immunity in animals.

Proximity to cell membranes is another potential similarity between NBS-LRR proteins and other resistance proteins that needs to be explored. The LRR-TM and LRR-TM-PK proteins have obvious membrane associations. Pto has an N-terminal myristoylation site that is dispensable for Pto function *(49),* but its presence neverthe-

less suggests that Pto is membrane-associated. Furthermore, Fen, another member of the Pto family *(50,51),* and AvrPto, the bacterial avirulence ligand recognized by Pto *(52),* both have functionally-indispensable N-terminal myristoylation sites *(51,53),* with that of Avr-Pto processed in the plant, strengthening the argument that Pto functions at a membrane. RPW8.1 and RPW8.2 are predicted to be type Ib membrane proteins, i.e., anchored to the cytosolic face of the cell membrane by an N-terminal hydrophobic signal anchor domain that remains uncleaved (*36,* and our own analysis). The flax L6 *(54),* flax M *(55),* and *Arabidopsis* RPS2 *(56,57)* resistance proteins have N-terminal hydrophobic regions predicted to be potential translocation signal peptides (*54,* and our own analysis), but in light of the cytosolic composition of their LRR domains are more likely to function as type Ib signal anchors. Like RPW8, the tomato I2 *(58),* tomato Mi *(59,60),* lettuce Dm3 *(61,62),* and *Arabidopsis* RPP1-WsA *(63)* resistance proteins are also predicted to have N-terminal hydrophobic signal anchor domains that remain uncleaved (*63,* and our own analysis).

A number of other NBS LRR resistance proteins are not predicted to have translocation signal peptides or signal anchors at their N termini. The *Arabidopsis* RPM1 protein *(64)* is one of these, but nevertheless it has been shown experimentally to behave as a peripheral membrane protein *(65).* The *Arabidopsis* RPP1-WsB, RPP1-WsC *(66),* and RPS5 *(63)* NBS-LRR resistance proteins are also not predicted to have translocation signal peptides or signal anchors, but instead have predicted N-terminal myristoylation sites (our own analysis) and are therefore possibly associated with a cell membrane. Moreover, the bacterial avrB *(67)* and avrRpm1 *(68)* avirulence ligands recognized by RPM1 and the bacterial avrPphB *(69)* avirulence ligand recognized by RPS5 also have functionally indispensable plant-processed N-terminal myristoylation sites (revealed after removal of a propeptide in the case of AvrPphB), suggesting targeting to the host membrane *(70).*

Collectively, these examples might tend to point toward membrane association for the NBS-LRR proteins as a whole, however, it is possible that resistance proteins may be targeted to the same location as their cognate avirulence ligands, so only a subset of NBS-LRR resistance proteins recognizing membrane targeted avirulence ligands may themselves be membrane-associated. Thus, NBS-LRR proteins recognizing cytosolic viral or nuclear-targeted bacterial components may not have any functional association with membranes. Although perhaps indicative, type Ib membrane anchors and N-terminal myristoylation may be insufficient by themselves to ensure membrane localization, and other proteins may be required to stabilize any membrane associations. Moreover, it is possible that membrane associations provided by other proteins in a resistance complex could render those of the resistance protein redundant under some circumstances, as may be the case for Pto.

Large numbers of NBS-LRR, LRR-TM-PK, and LRR-TM genes have been revealed in the sequence of the *Arabidopsis* genome. There are at least 135 NBS-LRR genes distributed somewhat unevenly over the five *Arabidopsis* chromosomes and at least 208 LRR-TM-PK genes distributed more evenly (Table 1), although genes of both types show a degree of clustering within each chromosome. Despite the large numbers of NBS-LRR sequences, of which several have been shown to function as disease resistance genes, none as yet have been shown to be involved in any other function, whereas LRR-TM-PK and LRR-TM genes have been shown to be involved in a number of other

Table 1
Number and Chromosomal Distribution of NBS-LRR and LRR-TM-PK Genes in the *Arabidopsis* Genome[a]

Chromosome	NBS-LRRs	LRR-TM-PKs
1	33	66
5	55	53
3	15	33
2	4	34
4	28	22
Total	135	208

[a] Chromosomes are listed in descending size.
Adapted from http://www.niblrrs.ucdavis.edu, May 2001.

functions including development, disease resistance, and innate immunity. It would therefore seem that NBS-LRR genes are dedicated plant disease resistance genes, whereas the LRR-TM-PK and LRR-TM genes seem able to diversify, and part of this diversification may be recruited into or from a disease resistance function.

6. COMPLEX AND SIMPLE GENETIC LOCI

Different disease resistance specificities often map to tightly linked regions of plant genomes, referred to as complex loci. Recent molecular analysis of these loci show that they consist of several genes closely related in DNA sequence (called *paralogs*) that occur as tandem direct repeats *(71–74).* Any particular complex locus can differ between genotypes of particular plant species in the absolute number of genes, the DNA sequence of the genes, and the resistance specificities encoded. A particular complement of related resistance genes at a complex locus in an individual genotype is referred to as a resistance gene *haplotype.* For example, 14 different resistance specificities (*Rp1-A, -B,* and so on) for common maize rust that map to the *Rp1* locus of maize have been identified in the maize gene pool, and these occur in different resistance gene haplotypes *(75).* The *Rp1* haplotype contains a family of nine CC-NBS-LRR genes, five of which are transcribed and only one of which encodes an identified resistance specificity (the Rp1-D specificity) *(75).* In contrast, only a single gene occurs in the naturally occurring Rp1-D haplotype of the maize line A188, and this gene encodes no known resistance specificity (A. Pryor, personal communication).

Locus expansion and contraction probably occur by unequal crossing over events at meiosis. In *Arabidopsis,* extensive genome sequence data provide further insight into the molecular complexity of resistance gene haplotypes. For example, nine TIR-NBS-LRR genes occur at the RPP5 locus of the *Landsberg erecta* ecotype *(73).* One of these genes encodes the RPP5 resistance specificity (resistance to *Peronospora parasitica*), whereas the eight other genes in this haplotype contain stop codons or insertions of transposable elements in their coding regions. Two further well-studied complex resistance gene loci are *Cf-4/Cf-9 (72)* and *Cf-2/Cf-5 (31,32),* which occur on tomato chromosomes 1 and 6, respectively. These loci contain genes for resistance to the fungus *Cladosporium fulvum.* The occurrence of multiple repeated related genes at these loci indicates several episodes of gene duplication during their evolution.

Resistance genes can also occur in simple loci apparently containing only a single gene. Multiple resistance specificities can be encoded by different allelic variants of a single gene. For example, 11 rust resistance specificities map to the *L* locus in flax, which contains a single TIR-NBS-LRR gene *(76).*

7. MOLECULAR BASIS FOR RESISTANCE GENE SPECIFICITY

The receptor-ligand model postulates that specificity differences are caused by different ligand recognition capacities. The most informative analyses of the molecular basis of gene-for-gene specificity have been generated using sequence information from either multiple alleles of a simple locus or several closely related resistance genes from a single complex locus. Examples of these situations are the 11 alleles at the *L* locus of flax *(76),* the 30 specificities (two cloned; *77,78*) at the *Mla* locus in barley and the 2 specificities at each of the *Cf-4/Cf-9 (30,33)* and *Cf-2/Cf-5 (31,32)* loci in tomato. The experimental approach has been to compare the sequences of closely related genes and their protein products and attempt to correlate sequence differences with specificity differences. The correlations can then be tested by making in vitro exchanges between genes encoding different specificities and testing the function and specificity of the recombinant genes in transgenic plants using discriminating isolates of the appropriate pathogen species. The most extensive studies have been carried out using the multiple allelic resistance specificities at the flax *L* locus *(76)* and two resistance specificities at the tomato *Cf-4/Cf-9* locus *(79,80).*

The common structural domain in several different classes of plant disease resistance proteins, and originally proposed as a specificity determinant, is the leucine-rich repeat (LRR). Although no crystal structure of the LRR region of a plant disease resistance protein has been reported, structures are known for the human and porcine ribonuclease LRR proteins either alone or in complex with their ligands *(81).* These proteins adopt nonglobular, horseshoe-shaped, α/β-helical structures. Each repeat unit includes a short β-strand/β-turn region with consensus xxLxLxx (where x is any amino acid and L is a leucine or other aliphatic residue buried in the hydrophobic core of the protein helix; Fig. 4A); most of the ligand contact points involve the variable x residues in this motif. Among products of resistance gene alleles or paralogs from complex loci, extremely high levels of polymorphism frequently occur in the LRR sequences, and particularly in the analogous xxLxLxx motif (Fig. 4B). This motif, which is found in many proteins, is involved in protein-protein interactions.

The role of the LRR regions in resistance protein specificity has now been demonstrated experimentally *(76),* and the importance of variation in the β-strand region has now also been confirmed *(82).* For example, the alleles of the flax rust resistance gene *L,* encoding closely related, polymorphic TIR-NBS-LRR resistance proteins, control distinct rust resistance specificities. In most cases, the corresponding avirulence genes in the flax rust fungus map to unlinked loci. Comparison of 11 L protein sequences indicated that although sequence differences occur in all domains of the protein, the most polymorphic domain is indeed the LRR region. The importance of the LRR region in specificity is indicated by comparison of the L6 and L11 proteins. L6 and L11 are identical in the TIR and NBS domains and differ at 33 positions in the LRR region (Fig. 4B). Therefore one or more of these polymorphisms must differentiate L6 and L11 specificities. These LRR polymorphisms in the products of the *L* alleles occur pre-

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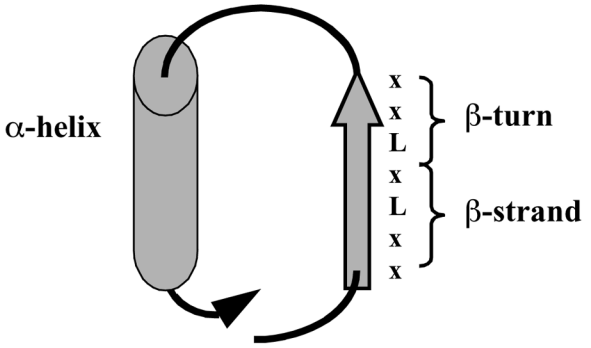

B

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L6  601  FLNLSELRYLHAREAMLTGDFNNLLPNLKWLELPFYKHGEDDPPLTNYTMKNLIIVILEH 660
L11 601  ............S................................F............ 660

L6  661  SHITADDWGGWRHMMKMAERLKVVRLASNYSLYGRRVRLSDCWRFPKSIEVLSMTAIEMD 720
L11 661  ..................................................... 720

L6  721  EVDIGELKKLKTLVLKFCPIQKISGGTFGMLKGLRELCLEFNWGTNLREVVADIGQLSSL 780
L11 721  ..................L.......................................... 780

L6  781  KVLKTTGAKEVEINEFPLGLKELSTSSRIPNLSQLLDLEVLKVYDCKDGFDMPPASPSED 840
L11 781  ...........................................A.................. 840

L6  841  ESSVWWKVSKLKSLQLEKTRINVNVVDDASSGGHLPRYLLPTSLTYLKIYQCTEPTWLPG 900
L11 841  ..................................................... 900

L6  901  IENLENLTSLEVNDIFQTLGGDLDGLQGLRSLEILRIRKVNGLARIKGLKDLLCSSTCKL 960
L11 901  ............K.....................................  960

L6  961  RKFYITECPDLIELLPCELGGQTVVVPSMAELTIRDCPRLEVGPMIRSLPKFPMLKKLDL 1020
L11 961  ..L..R.............R..........D.......................... 1020

L6  1021 AVANITKEEDLDAIGSLEELVSLELELDDTSSGIERIVSSSKLQKLTTLVVKVPSLREIE 1080
L11 1021 .........................K.............L................... 1080

L6  1081 GLEELKSLQDLYLEGCTSLGRLPLEKLKELDIGGCPDLTELVQTVVAVPSLRGLTIRDCP 1140
L11 1081 .........R.F.V.....................................VE...W... 1140

L6  1141 RLEVGPMIQSLPKFPMLNELTLSMVNITKEDELEVLGSLEELDSLELTLDDTCSSIERIS 1200
L11 1141 ............N.....................A...........V.K............ 1200

L6  1201 FLSKLQKLTTLIVEVPSLREIEGLAELKSLRILYLEGCTSLERLWPDQQQLGSLKNLNVL 1260
L11 1201 S........R.K..................YE...Q..................E...EI 1260

L6  1261 DIQGCKSLSVDHLSALKTTLPPRARITWPDQPYR 1294
L11 1261 N.R................P.............. 1294
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Fig. 4. **(A)** The LRR structural unit. L, leucine or another aliphatic amino acid; x, any amino acid. **(B)** Amino acid alignment of the LRR region of the flax rust resistance proteins L6 and L11. (Identical residues are indicated by dots.) These proteins are identical in the TIR and NBS regions (residues 1–600) and differ at 33 positions in the LRR region, principally in or close to the xxLxLxx motif (overlined) of the LRR units.

dominantly in the predicted solvent-exposed xxLxLxx motifs in several of the individual LRR units (Fig. 4B). Analysis of TIR-NBS-LRR flax rust resistance proteins encoded at the flax *P* locus was also informative. The difference between *P* and *P2* specificity is owing to at most six amino acid differences between the two proteins and these differences occur exclusively in the xxLxLxx motifs of four LRR units *(82).*

Other sequence comparisons and domain swaps indicate that specificity differences are not solely determined by the LRR. The N-terminal domain TIR domain can also affect specificity. For example, the L6 and L7 proteins, which have distinct resistance specificities, are identical in the NBS-LRR region and differ at 11 residues in the TIR region *(76).* Domain swaps implicate at most three polymorphisms as being sufficient for the specificity differences *(83).* Whether these residues, together with the LRR region, are involved in the postulated interactions with pathogen ligands is unknown.

Extensive analysis of the molecular basis of specificity has also been carried out with the TM-LRR resistance proteins Cf-4 and Cf-9, from tomato. More than 50% of the single-amino acid substitution polymorphisms between the two proteins occur in the nonleucine residues of the xxLxLxx motif. Domain swap and gene shuffling experiments between *Cf-4* and *Cf-9* have further refined the definition of critical polymorphisms and have shown that the Cf-4 protein can be converted to *Cf-9* specificity (and *vice versa*) by a limited number of sequence changes *(79,80).* It is important to stress that these sorts of sequence comparisons and domain swaps can only identify the regions that contribute to the *differences* in specificity between the genes under comparison. Conserved residues can also contribute to binding and thus to the overall specificity of recognition.

8. EVOLUTION OF DISEASE RESISTANCE SPECIFICITIES

When extensive comparisons have been made between closely related resistance genes from a single locus, it is evident that variation is generated by standard evolutionary processes, including point mutation, small deletions, insertions, and meiotic recombination. No evidence has been uncovered suggesting that specialized processes accelerate the evolution of resistance genes, such as site-directed recombination or mutation mechanisms. Although point mutations provide the source of new sequence variation in resistance gene evolution, much of the variability among resistance gene families appears to result from recombination, which shuffles polymorphic sites between individual genes. Patchworks of sequence similarities shared between alleles of a single gene and also between members of complex resistance gene haplotypes are frequently observed and provide evidence for past exchanges of blocks of sequence variation by recombination. There is also evidence from sequence comparisons that unequal exchanges can occur after mispairing of complex resistance loci; however, the extent of this sort of exchange appears to decrease as the sequence similarly diverges.

Intragenic unequal sequence exchanges between repeated sequences within LRR-encoding regions also appear to be an important source of variation between resistance gene homologs. Unequal exchange can delete and duplicate sequence information that could form new ligand binding surfaces and alter spatial arrangements between critical residues involved in ligand binding. These events could alter or optimize specific ligand interactions. Examples of this type of variation are found among the *L* alleles of flax, which contain one, two or four copies of a 450-bp DNA sequence encoding six

LRR units (where one unit is approx 24 residues) *(76).* A second example is provided by the *RPP5* locus of *Arabidopsis. RPP5* homologs, with variable numbers of direct DNA repeats, encode proteins with 13, 17, 21, or 25 LRR units *(74).* Homologs from the *Cf-2/Cf-5* locus of tomato also differ widely in the number of LRR units, which result from deletion/duplication of individual LRR units *(32).*

When the individual LRR units of a resistance protein are aligned with those of its homologs or allelic variants, it is apparent that the leucine or other hydrophobic residues that form the backbone of the repeats are highly conserved, whereas the intervening residues are more variable *(72,76).* Variation is particularly evident in the x residues of the xxLxLxx motif within each repeat. Analysis of DNA sequence variation using approaches pioneered for the analysis of variation in the human MHC genes *(84)* has been applied to studies of plant disease resistance gene variation *(62,72,82,85).* Analysis of the rates of synonymous (non-amino acid altering) and nonsynonymous (amino acid altering) nucleotide substitution rates (Ks and Ka, respectively) in closely related resistance gene sequences has been particularly informative.

Most genes are subject to conservative selection because most amino acid changes to proteins are deleterious, or at best neutral, and hence allelic gene comparisons find Ka < Ks. However, in resistance genes it has generally been found that Ka < Ks for non-LRR coding regions, the LRR coding regions showing Ka > Ks. This is particularly evident in the codons for the x residues in the xxLxLxx motif. The result indicates that selection favors amino acid variation at these sites, presumably because such changes can introduce new or more efficacious recognition specificities. These molecular evolutionary analyses further support the view that this region is involved in binding pathogen-derived ligands and thus specificity of recognition. Similarly, diversifying selection has been detected in the TIR-encoding region of *L* alleles of flax, which also contributes to the specificity of these resistance proteins *(83).*

9. THE RECEPTOR-LIGAND MODEL AND THE GUARD HYPOTHESIS

Although direct interaction between a resistance protein receptor and the corresponding avirulence gene product has been predicted on the basis of gene-for-gene interactions, little direct evidence exits from biochemical analysis. Furthermore, apart from viruses, the other major groups of plant pathogens are extracellular, and the largest class of resistance proteins, the NBS-LRR group, is probably located in the cytoplasm. Bacterial avirulence proteins and resistance proteins are probably brought together via the bacterial type III secretion system *(5),* and although no evidence has yet been reported, uptake of proteins secreted by pathogenic fungi at the host-pathogen interface is also likely. Direct interaction in a yeast two-hybrid system has been demonstrated between the cytoplasmic serine/threonine protein kinase Pto (a resistance protein from tomato) and AvrPto (the corresponding bacterial avirulence protein) *(86,87).* Although there is a strong correlation between the ability of mutant forms of Pto to bind AvrPto in the yeast test system and the ability to function as a resistance protein in vivo, no direct evidence for in vivo binding has been reported. Direct interaction between the NBS-LRR resistance protein Pi-ta and its corresponding avr protein has been reported from in vitro but not in vivo experiments *(88).* Failure to detect direct interaction between several other resistance proteins and their corresponding Avr proteins has also been reported in meetings, but not published. Although these negative

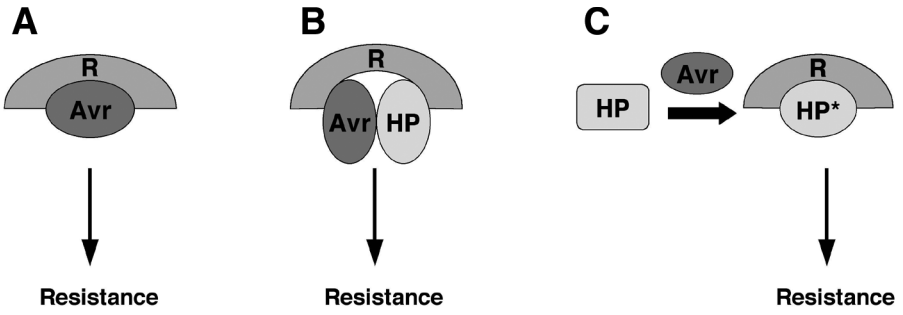

Fig. 5. The guard model. **(A)** Data supporting the simple direct binary interaction of resistance proteins (R) and avirulence proteins (Avr) have been difficult to obtain, and no demonstration of in vivo interactions is reported. **(B)** Higher order interactions have been proposed whereby the interaction between Avr and one or more host proteins (HP), potentially involved in enhancing virulence in susceptible plants, is detected in resistant plants by the resistance protein. **(C)** Alternatively, the HP may be enzymatically modified to HP* by Avr, for instance for repression of a defense activity, and the HP* may act as a ligand for R. (Reprint with permission from Trends in Plant Science vol 9, 2000 p. 373–379. Ellis J, Dodds P, and Pryor T. The generation of plant disease resistance gene specifications.)

data may reflect technical difficulties and/or low affinity between the receptor-ligand pairs, the possibility that simple binary interactions may not generally occur is now being seriously considered. Recently, the first documentation of resistance proteins and Avr proteins being involved in higher order complexes has been reported *(89)*. Furthermore, mutation experiments have identified other plant genes necessary for activity of specific resistance genes *(46,47)*. A new scenario is therefore being considered and examined in experimental systems. Avirulence proteins are postulated to have a primary role as virulence determinants through interaction with host proteins, similar to the type III effector proteins of bacterial pathogens of animals. Resistance proteins, it is further postulated, have a role in guarding host cellular proteins from recruitment by pathogen avirulence proteins *(90)*. Models for this so-called guard hypothesis are presented in Fig. 5.

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