Plant pathology: **Many roads lead to resistance**

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Recent studies suggest that plant disease-resistance responses use multiple signaling pathways acting subsequent to pathogen recognition, and that phosphorylation cascades play a prominent role in the recognition and execution of foreign invaders.

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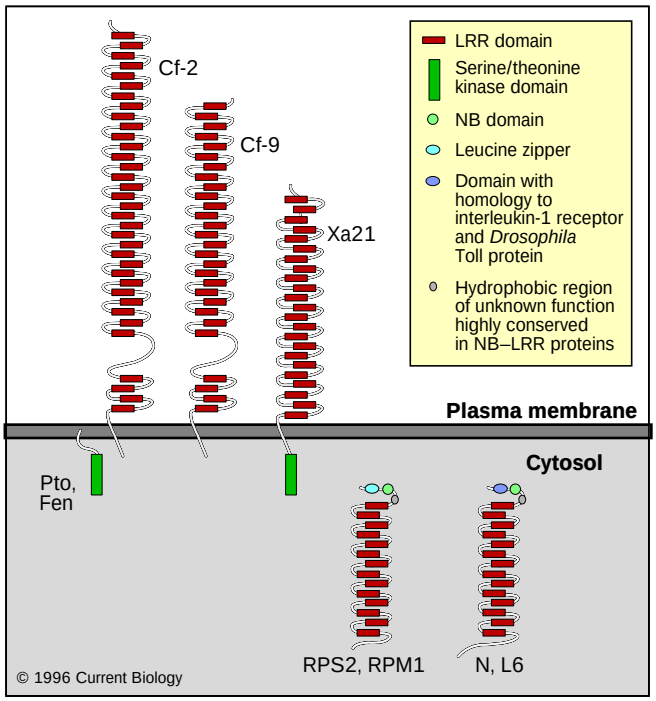
A plant's ability to resist infection by a potential pathogen often requires a single dominant, or semidominant, resistance (*R*) gene allele. The protein product of such a gene directly or indirectly 'recognizes' a signal generated via a corresponding avirulence (*avr*) gene product encoded by the pathogen. One model to explain the molecular basis of this 'gene-for-gene' recognition phenomenon states that the *R* gene product is a receptor that specifically binds the corresponding pathogen-derived, *avr*-dependent ligand [1]. The formation of this putative receptor–ligand complex is postulated to initiate a signaling cascade culminating in defense responses that halt the pathogen's progress. These are typified by rapid cell death at the site of infection (the hypersensitive response), an oxidative burst, cell-wall strengthening and the induction of defense gene expression [2,3].

Figure 1



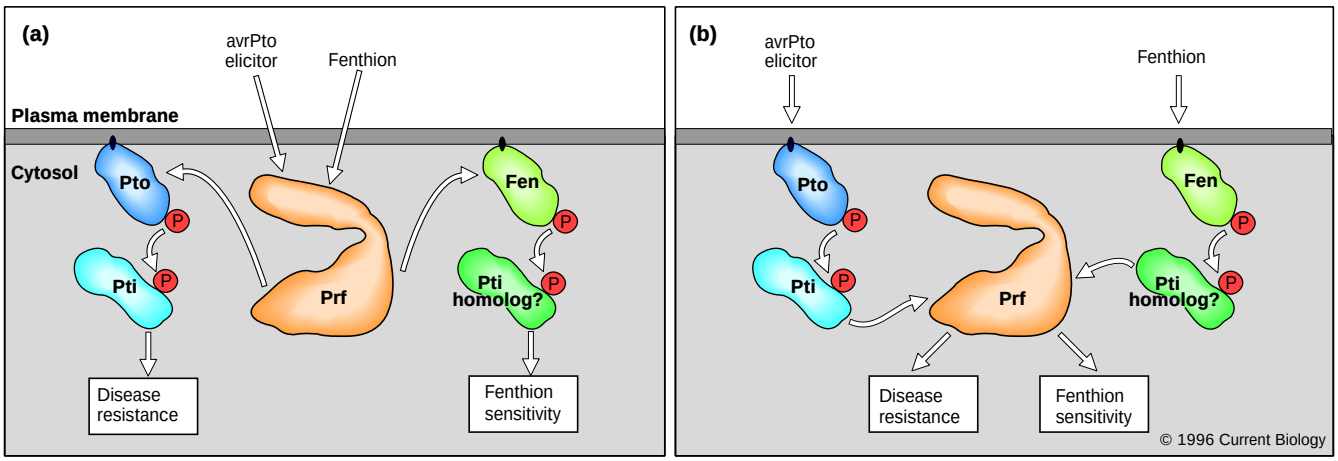
A comparison of proteins deduced from published *R* gene sequences. Pto and Fen contain consensus amino-terminal myristolation motifs and so are shown as membrane-associated. The leucine-rich domain of L6 does not conform to the canonical repeat characteristic of an LRR domain; furthermore L6 is made with a signal peptide, whereas N is predicted to be soluble.

In recent years a number of plant *R* genes conferring resistance to viral, fungal and bacterial pathogens have been cloned [4–6]. They encode structurally related proteins, suggesting that they function in common signaling pathways culminating in disease resistance. Although some evidence has accumulated to support this hypothesis, recent genetic and molecular characterization of *R*-gene-mediated signaling pathways has revealed an unexpected level of divergence in the events associated with the activation of individual *R* genes. Here we shall provide an update of recently characterized *R* gene sequences and summarize some of the recent findings regarding *R*-gene-dependent signaling pathways.

The first cloned *R* genes have been the subject of previous reviews [4–6], so we shall limit our discussion to those that have been characterized most recently. Most of the *R* genes characterized to date encode proteins that contain a leucine-rich repeat (LRR) domain (Fig. 1). These domains are increasingly being discovered in diverse proteins, and function largely as sites of protein–protein interaction, peptide–ligand binding and protein–carbohydrate interaction [7]. LRR-containing *R* gene products can be classified according to the presence or absence of a conserved nucleotide-binding (NB) motif, and those *R* products that do contain an NB motif can be further sub-classified based on the nature of their amino-terminal domains (Fig. 1). A recent addition to this sub-class is the *Arabidopsis RPM1* gene. The RPM1 protein shows 51 % overall sequence similarity to the product of another *R* gene, *RPS2*, and both proteins are predicted to have leucine zippers at their amino termini [8]. While *RPS2* conditions resistance to *Pseudomonas syringae* isolates that express the *avrRpt2* gene, *RPM1* conditions resistance to *P. syringae* isolates that express either of two unrelated avirulence genes, *avrRpm1* or *avrB* [8,9]. This dual-specificity resistance is unique among characterized *R* genes and presents an interesting twist to the 'gene-for-gene' paradigm.

Although the RPM1 and RPS2 proteins are related structurally and condition resistance to different isolates of the

Figure 2



Two possible pathways that mediate *avrPto*-dependent resistance and fenthion sensitivity in tomato plants. (a) Prf may act as an upstream component (perhaps a receptor) that channels signals through Pto and Fen. (b) Alternatively, *Prf* may act as a downstream effector into which signals from the Pto and Fen phosphorylation cascades feed.

same pathogen, the extensive sequence divergence between them complicates armchair prediction of which domains may be responsible for 'effector' function and which may confer the specificity of *avr* signal recognition. However, comparison of the tomato *Cf-9* and *Cf-2* genes, which confer resistance to different *Cladosporum fulvum* isolates, has provided some insight into this question. The *Cf-2* locus was recently isolated by positional cloning and shown to comprise two nearly identical genes which can independently confer resistance on susceptible plants [10]. Each of the *Cf-2* genes and the previously characterized *Cf-9* gene [11] encodes a protein with a putative signal peptide at the amino terminus, followed by a number of LRRs and a carboxy-terminal transmembrane domain. Individual Cf-2 LRR units exhibit a higher degree of conservation than is seen among the LRR units of Cf-9 repeats.

Computer models suggest that the LRRs form an extracellular rod which may interact with extracellular elicitors. The carboxy-terminal LRRs and transmembrane domains are very highly conserved between Cf-2 and Cf-9, and potentially represent the 'effector' portion of these molecules. Domain-swaps between these two proteins can be used to define the functions of these conserved regions and may also provide insights into structure–function relationships of less closely related NB–LRR class *R* gene products. The Cf-2/Cf-9 sequence comparison also suggests that intragenic or intergenic recombination in the LRR-encoding regions could be a potent source of resistance genes with novel recognition capabilities. Interestingly, each of the three nucleotide substitutions that differentiate the two *Cf-2* copies causes an amino-acid substitution [10], suggesting that positive selection for point mutations, as well as recombination, is a significant factor in *R* gene evolution. Further characterization of the *Cf* gene clusters will undoubtedly facilitate experimental testing of these ideas.

Another *R* gene class is defined by the rice *Xa21* gene, which confers resistance to *Xanthomonas oryzae* pv. *oryzae* race 6 and encodes a protein with characteristics of a transmembrane receptor-like kinase [12]. The predicted extracellular domain of Xa21 is 54.9 % similar to Cf-9, and the kinase domain is 56.5 % similar to the product of the *R* gene *Pto*, which is required for resistance to *P. syringae* isolates that express *avrPto* [13]. These striking similarities suggest that signaling through Cf-type proteins, which lack an apparent signal transmission domain, may occur through a serine/threonine kinase, either *via* carboxy-terminal interaction with a Pto-like molecule, or perhaps by dimerization with an Xa21-like molecule.

A second gene required for *Pto* function is *Prf* [14], shown recently to encode a NB–LRR type protein (J. Salmeron and B. Staskawicz, personal communication). *Prf* is also required for sensitivity to the insecticide fenthion [14], as is the *Fen* gene, which encodes a serine/threonine kinase that is closely related to the *Pto* gene product [15,16]. Fenthion sensitivity is manifested as the development of hypersensitive-response-like lesions, and is consequently thought to be mediated by a signaling mechanism closely related to that used for disease resistance. It is likely that *Pto* and *Fen* have analogous functions in two parallel pathways. However, it is currently unclear whether Prf is positioned upstream or downstream of Pto and Fen in the signaling pathway (Fig. 2).

A gene for a third component of the *avrPto*-specific resistance response pathway, *Pti*, was isolated recently using

the yeast two-hybrid system to identify proteins that interact with Pto [17]. Pti1 is also a serine/threonine protein kinase, and associates specifically with the phosphorylated form of Pto. *In vitro* assays were used to demonstrate that although Pto is able to phosphorylate Pti1, the reverse reaction did not occur. Thus, recognition of the *avrPto* elicitor is probably amplified by the activation of a phosphorylation cascade, with Pti1 acting as a downstream effector of Pto. Pti1 does not interact with Fen, consistent with the notion of separate but analogous pathways for signaling recognition of the *avrPto* elicitor and fenthion. The tomato genome encodes a number a Pti1 homologs, and these are potential candidates to fulfill a similar Fen-specific effector role. Although the involvement of parallel phosphorylation cascades in the *avrPto* and fenthion response pathways is somewhat unexpected, examples of similar pathway complexities are well documented in the animal literature [18].

Another emerging and somewhat unexpected theme in *R*-gene signal transduction is that different pathogen signals can trigger different defense responses, and that these responses may be part of complex pathways that can branch and possibly reticulate. For example, the *ndr-1* (non-specific disease resistance) mutation in *Arabidopsis* defines a common step for resistance to *P. syringae* and the fungal pathogen *Peronospora parasitica* [19]. However, the loss of some *Peronospora* resistance specificities is not complete in *ndr-1* plants, suggesting that more than one pathway is involved in *Peronospora* resistance. In addition, *ndr-1* mutants support high levels of *P. syringae* growth in leaves, but the hypersensitive response still occurs in response to three of the four *P. syringae avr* genes assayed. It appears that different pathways can trigger a hypersensitive response, and that the hypersensitive response is not sufficient for resistance to *P. syringae*.

Two other recent papers demonstrate that different *avr* genes trigger distinct downstream responses [20,21]. Both studies compared responses in *Arabidopsis* to the *avrRpm1* and *avrRpt2* avirulence genes of *P. syringae*. As described above, the corresponding *R* genes — *RPM1* and *RPS2* — encode related proteins. However, the timing of their resistance reactions differs. Reuber and Ausubel [20] isolated two genes, *AIG1* and *AIG2*, which are induced specifically in response to *avrRpt2*, but not *avrRpm1*. Conversely, they show that the previously isolated *ELI3* gene [22] is induced by *avrRpm1*, but not *avrRpt2*. Thus, the two resistance reactions are qualitatively different and may employ distinct signaling pathways.

Interestingly, Ritter and one of us (JLD) [21] have found that, in response to infection by bacteria which express both *avr* genes, the slower *RPS2* reaction is 'epistatic' to that of *RPM1* (as judged by hypersensitive response timing, *in planta* bacterial growth, and induction of *AIG1* and *ELI3*) [21]. This interference occurs outside the bacteria and can be overcome by a numerical excess of *avrRpm1*-expressing bacteria. This implies that the two *avr* genes compete at some step in signal processing and/or transduction, and that the *RPM1* and *RPS2* pathways may connect at some point.

An extremely interesting *Arabidopsis* mutant, *eds-1*, clearly separates *R*-gene-dependent responses to different *Peronospora* strains (Jane Parker, personal communication). The *eds-1* mutant was named because of its enhanced disease susceptibility to downy mildew strains which are otherwise avirulent on the parental *Arabidopsis* plants. This mutant does not, however, abolish resistance to all avirulent *Peronospora* strains, demonstrating that more than one *Arabidopsis* pathway can function subsequent to downy mildew recognition. The most interesting feature of the *eds-1* mutant is that it can also be parasitized by *Peronospora* strains that normally do not infect *Arabidopsis* at all, suggesting an analysis of this mutant's reactions to a wider variety of pathogens may provide insight into 'non-host' resistance.

Other enticing examples strengthen the idea that signaling subsequent to engagement of an *R* gene product is complex and can contain steps unique to the *R* gene in question. Mutations in barley define two loci specifically required for function of the race-specific *Mla-12* resistance gene. These mutations do not adversely affect function of the race-non-specific *mlo R* gene. The recent identification of two new loci required for *mlo* function will allow analysis of their role in race-specific resistance [23,24]. Two tomato loci required for *Cf-9* gene function have also been identified [25], and similar analyses will address whether they function in *Cf-9*-specific signaling steps, or are common mediators of *Cf* gene function. Interestingly, these mutations all result in incomplete loss of *R* gene function, suggesting either that all available alleles are weak, or that interdigitating response pathways may be responsible for residual activity. Positional cloning of these important genes proceeds apace.

Taken together, these recent results strongly suggest that, although plants may use similar molecules to recognize pathogen signals, they may not recruit a 'unified' response pathway [4]. Different response mechanisms can be employed for different pathogens, and possibly for different strains of the same pathogen. This supports the idea of layered levels of functionally interacting polymorphic molecules, as described for the Pto and Fen pathways. Signaling diversification may be driven by an adaptive imperative to recognize different signal molecules and cope with an ever-changing array of pathogens.

Identification of the microbial elicitors of resistance pathways, the precise definition of the *R*-gene functions, and

molecular characterization of the proteins defined by the new signal transduction mutations mentioned here will represent the next major advances in our understanding of microbial perception in plants. In addition to saturating genetic screens, the isolation of candidate protein partners *via* the yeast two-hybrid screen and unraveling of their mutant phenotypes by screening for insertion alleles will undoubtedly reveal other important players in this game of host–pathogen tug-of-war.

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