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Plant–parasite interactions: has the gene-for-gene model become outdated?

Roger W. Innes

H.H. Flor's gene-for-gene model of plant–pathogen interactions has guided plant-pathology research for over 50 years^{1–3}. Based on his genetic analyses of the interactions of flax (*Linum usitatissimum*) and flax rust (*Melampsora lini*; an obligate biotrophic fungus), Flor postulated that 'for each gene conditioning rust reaction in the host there is a specific gene conditioning pathogenicity in the parasite'³. Flor found that the resistance of a given flax variety to a given flax-rust strain was invariably a dominant or semidominant trait that was inherited in a simple monogenic fashion. In the pathogen, avirulence was always dominant to virulence, and was also inherited as a simple monogenic trait. Thus, it was postulated that the interaction of a plant disease-resistance (*R*) gene and a corresponding pathogen avirulence (*avr*) gene somehow mediated a specific recognition event that was required to trigger plant disease-resistance responses. Flor

The detection of pathogens by plants is often described as a 'gene-for-gene' interaction.

However, recent work from several laboratories indicates that, in some instances, a single gene product in the plant can mediate the recognition of multiple pathogen signals, and that multiple plant genes are required for the recognition of, and response to, a single pathogen signal.

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likened this recognition event to antigen–antibody reactions in animal systems^{2,3}, but the molecular mechanism underlying gene-for-gene interactions is still unknown. Recent results from my group and from others suggest that the mechanism may not be as simple as the gene-for-gene model predicts.

Recognition of diverse pathogens

Flor's model has accurately described the interactions of plants with fungal, bacterial and viral pathogens⁴, which implies that plants must have a very large repertoire of *R* genes to allow the recognition of the multitude of potential pathogens likely to be encountered in nature. Mammals have solved the problem of pathogen recognition by the clever use of somatic recombination of immunoglobulin-encoding genes, which enables the generation of literally millions of different antibody molecules, each with a different specificity, from just a few hundred 'genes'. Plants do not generate antibodies; the lack of motile cells precludes the use of somatic recombination to generate diversity within an individual plant. Thus, there is a practical limit to how many *R* genes a plant genome can encode. The nuclear genome of *Arabidopsis thaliana* is approximately 100 000 kb, and is thought

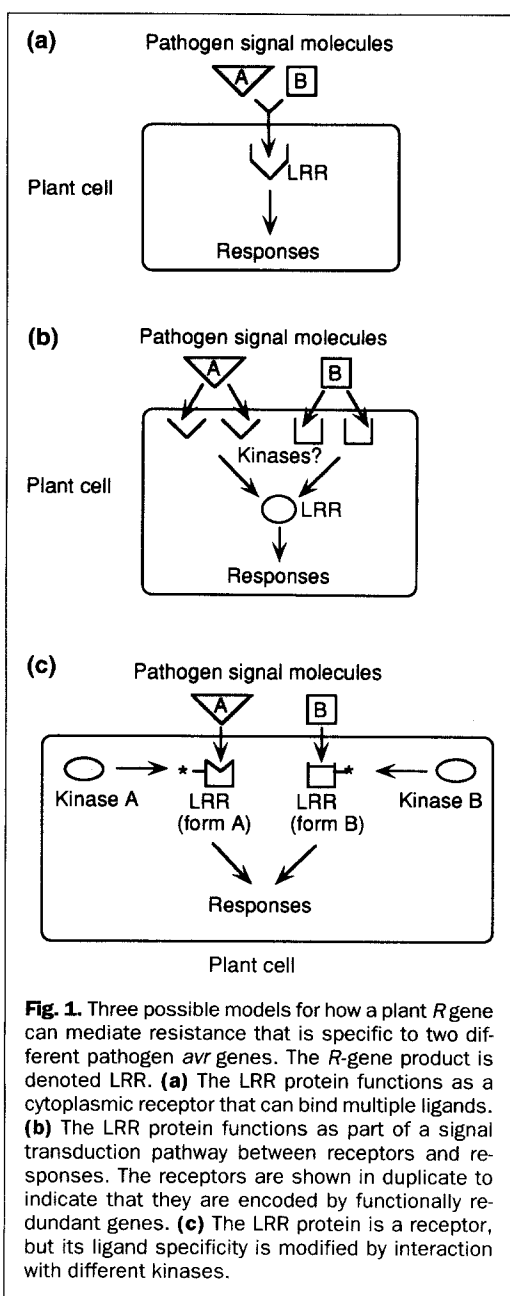


Fig. 1. Three possible models for how a plant *R* gene can mediate resistance that is specific to two different pathogen *avr* genes. The *R*-gene product is denoted LRR. **(a)** The LRR protein functions as a cytoplasmic receptor that can bind multiple ligands. **(b)** The LRR protein functions as part of a signal transduction pathway between receptors and responses. The receptors are shown in duplicate to indicate that they are encoded by functionally redundant genes. **(c)** The LRR protein is a receptor, but its ligand specificity is modified by interaction with different kinases.

to encode roughly 20 000 genes⁵. What proportion of these genes is devoted to pathogen recognition? Even an arbitrarily generous estimate of 10% would allow for only 2000 *R* genes, which seems insufficient for the diversity in potential pathogens. How then do plants detect so many pathogen genotypes?

Gene-for-genes and genes-for-genes interactions

Two recent publications have demonstrated that a single plant *R* gene can mediate the recognition of two or more pathogen *avr* genes^{6,7}; thus,

one strategy used by plants is to make their *R* genes do double duty. Both publications describe the isolation of plant mutants that have become susceptible to specific strains of the bacterial pathogen *Pseudomonas syringae* pv. *tomato* expressing either of two different *avr* genes. Bisgrove *et al.* show that mutations in the *RPM1* disease-resistance gene of *Arabidopsis* lead to a loss of recognition of both *avrB* and *avrRpm1* (Ref. 7). More recently, the molecular isolation of *RPM1* has shown that it encodes a protein containing a potential nucleotide-binding site and 14 leucine-rich repeats (LRRs)⁸, which are motifs found in three other recently isolated *R* genes⁹⁻¹² (one of which is the flax *L6* gene defined by Flor¹³). The LRR motif has been implicated in protein-protein interactions and in ligand binding by receptors¹⁴. The simplest interpretation of these results is that *RPM1* encodes a receptor that can bind multiple ligands, as indicated in Fig. 1a. However, *RPM1* does not encode an obvious signal peptide or transmembrane domain; thus, if its product functions as a receptor, then it is probably a cytoplasmic one. This suggests either that the signal from the bacterium is getting inside the plant cell, or that a specific secondary signal is transduced.

The results of Salmeron *et al.*⁶ indicate that the model in Fig. 1a may be an oversimplification. Salmeron and colleagues have found that mutations in either of two closely linked tomato genes (*PRF* and *PTO*) result in the loss of recognition of *P. syringae* pv. *tomato* strains that express *avrPto*. Mutations in these genes also blocked the recognition of an unidentified second avirulence gene, as an avirulent strain that lacked *avrPto* also became virulent on the mutant tomato lines. Thus, both *PRF* and *PTO* are required for the recognition of two different *P. syringae* avirulence genes. Interestingly, *PTO* encodes a protein kinase and is part of a tightly clustered multigene family¹⁵. One of these *PTO* homologs is *FEN*, which confers sensitivity to the organophosphorous insecticide fenthion^{16,17}. Mutations in *PRF*, but not in *PTO*, also abolish sensitivity to fenthion. *PRF*, therefore, mediates responses

to at least three different molecules, whereas *PTO* and *FEN* appear to allow the plant to distinguish among these molecules. The genetic data appear to place *PTO* and *FEN* upstream of *PRF* in a signal transduction pathway, perhaps functioning as specific receptors (Fig. 1b). However, the molecular data do not agree with this picture, as *PTO* and *FEN* appear to contain little more than a kinase domain^{15,16}, whereas *PRF* encodes a protein similar to *RPM1*, containing a nucleotide-binding site and LRRs (J. Salmeron and B. Staskawicz, pers. commun.), and is seemingly a better candidate for a receptor.

How, then, can the genetic and molecular data be reconciled, and is there any similarity with the *RPM1* story? The model shown in Fig. 1c is one possible scenario. In this model, the *PRF* protein, which contains LRRs, functions as a receptor that can be modified by interaction with the kinases encoded by *PTO* and *FEN*, either by direct phosphorylation or by protein-protein interactions. Depending on its phosphorylation and/or protein-complex state, *PRF* then binds either fenthion or the *P. syringae*-derived signal molecules. By analogy, *RPM1* could be functioning as a receptor the specificity of which for either *avrB* or *avrRpm1* is determined by interaction with unidentified kinases. This scenario requires that these unidentified kinases are functionally redundant, as an extensive search of mutants yielded a dozen *RPM1* alleles, but no mutations in other genes⁷. However, this is not unreasonable, as mutations in the *PTO* gene in tomato typically confer only partial susceptibility⁶, suggesting that *PTO* function may be partially redundant in tomato¹⁵.

Combinatorial possibilities

The notion that *R*-gene specificity is conferred by an interaction between an LRR-containing receptor and a kinase suggests that a single receptor may adopt multiple specificities depending on the specific kinase with which it interacts. For example, if each receptor can interact with ten different kinases and each kinase can interact with 100

different receptors, 1000 different specificities can be generated from only 110 genes. Although there is no direct evidence yet to support this model, the recent isolation of several LRR-containing *R* genes, including *PRF*, should allow this model to be tested directly in the near future.

What about Flor?

How can the model in Fig. 1c be reconciled with the vast amounts of genetic data that demonstrate gene-for-gene interactions occurring between plants and pathogens? If both a kinase and an LRR-containing protein are required to produce a functional receptor, why have more examples not been found where two plant genes are required to confer resistance to a specific pathogen? The answer is probably that we have not looked in the correct manner. The majority of the gene-for-gene-type interactions that have been described are based on naturally occurring variation in the plant host and in the pathogen. When two plant varieties are crossed and resistance segregates in a 3:1 ratio in the F_2 generation, it indicates only that the two varieties differ at a single locus; it does not indicate that only a single gene is required for specificity. In the context of Fig. 1c, it is easy to imagine that the kinases are conserved between plant varieties and that natural variation occurs in the LRR-containing protein, although the reverse is also possible. Another confounding factor is that the genes encoding the LRR-containing protein and the kinase might be closely linked, as illustrated by *PRF* and *PTO* (Ref. 6). In such cases, a cross between a plant variety lacking both genes and a variety containing both genes would still produce an F_2 population with approximately 3:1 segregation, and resistance would be assumed to be under the control of a single gene, rather than two.

However, there are several examples in the older literature that show that when two susceptible plant lines are crossed they produce resistant progeny. Such observations are consistent with each parent lacking a different gene function so that they can complement each

other in the F_1 plant to produce a functional *R*-gene product. For example, in wheat, resistance to strain 10-1,2,3,4 of *Puccinia recondita* f. sp. *tritici* (seedling leaf rust) requires both the *Lr27* and *Lr31* genes¹⁸.

Rather than rely on natural variation, many investigators are now dissecting gene-for-gene interactions by mutagenesis. It is this approach that revealed the existence of *PRF* and demonstrated that *RPM1* mediates resistance to two different avirulence genes. Similarly, a screen for mutants in tomato revealed that resistance to race 9 of the fungal pathogen *Cladosporium fulvum* requires three genes, the classically defined *R* gene *Cf9*, and two previously unidentified loci designated *Rcr1* and *Rcr2* (Ref. 19). Mutations in *Rcr1* and *Rcr2* produce a partially susceptible phenotype, suggesting that their functions may be partially redundant to each other, or to other unidentified genes. Similar results have also been reported in barley for resistance to powdery mildew (*Erysiphe graminis*); resistance mediated by the *Mla₁₂* gene requires two additional genes, *Nar1* and *Nar2* (Refs 20,21). These mutations do not affect resistance mediated by the *Mlg* or *mlo* resistance genes, suggesting that the *Nar1* and *Nar2* proteins might interact directly with *Mla₁₂*.

Is Flor's gene-for-gene model therefore outdated? Yes, in the sense that it is now clear that the interaction between plants and parasites probably involves much more than a single gene in a plant and a single gene in the parasite. However, Flor never claimed otherwise. His model simply points out that the difference between a resistant and a susceptible plant variety usually can be attributed to a single gene, which still holds true. It remains to be determined how these single genes

Questions to be answered

- How many different avirulence genes are there in a given pathogen species?
- How many disease-resistance genes does a single plant have?
- Does the leucine-rich repeat motif in *R*-gene products interact directly with pathogen signal molecules?
- Will it be possible to construct 'designer' *R* genes that enable recognition of new pathogen signals?
- How do *R*-gene products turn on defense responses?

contribute to specificity in disease resistance.

Acknowledgements

I thank John Salmeron and Brian Staskawicz for sharing data before publication, and the members of my laboratory for comments on the manuscript. Work from my laboratory was supported by NIH grant no. R29 GM 46451 and USDA grant no. 93-37303-9136.

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