Plant–parasite interactions: has the gene-for-gene model become outdated?

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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.

Recognition of diverse pathogens
Flor's model has accurately described the interactions of plants with fungal, bacterial and viral pathogens, 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

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to encode roughly 20,000 genes. 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; 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)\(^\text{10}\), which are motifs found in three other recently isolated R genes\(^\text{9,12}\) (one of which is the flax \(L_r\) gene defined by Flor\(^\text{11}\)). The LRR motif has been implicated in protein–protein interactions and in ligand binding by receptors\(^\text{14}\). 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 Salmoner et al.\(^\text{4}\) indicate that the model in Fig. 1a may be an oversimplification. Salmoner 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 family\(^\text{15}\). One of these PTO homologs is FEN, which confers sensitivity to the organophosphorous insecticide fenthion\(^\text{16,17}\). 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\(^\text{12,13}\), whereas PRF encodes a protein similar to RPM1, containing a nucleotide-binding site and LRRs (J. Salmoner 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 function 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 genes. However, this is not unreasonable, as mutations in the PTO gene in tomato typically confer only partial susceptibility\(^\text{6}\), suggesting that PTO function may be partially redundant in tomato\(^\text{11}\).

**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, 1,000 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 F₁ 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 F₁ 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 F₁ 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 genes.

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 CPF, 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 MLA₁₁ 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 MLA₁₁.

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 contribute to specificity in disease resistance.

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