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Unraveling Disease Resistance Specificities

The cover image of this issue of THE PLANT CELL depicts the rust disease caused by the fungus *Melampsora lini* on its host plant, flax (*Linum usitatissiumum*). Experimental manipulations of the flax–rust system have long been influential in efforts to understand the molecular and genetic bases for plant disease resistance. Indeed, in the 1940s, Flor recognized that the outcome of the flax–rust interaction is determined by matched specificities at rust avirulence (*Avr*) loci and corresponding dominant or semidominant resistance (*R*) loci in the flax plant. If genes at both of these loci are expressed, the plant is able to react rapidly to repel the pathogen, often by inducing the suite of defense responses now known as the hypersensitive response (HR; Dangl et al., 1996; Hammond-Kosack and Jones, 1996). If either member of the gene pair (or both) is absent, there is no concerted defense response and disease generally ensues.

The simplest molecular explanation for these genetic data, upon which Flor based his "gene-for-gene" hypothesis (Flor, 1971), is that *R* and *Avr* gene products interact directly as a receptor and ligand, respectively. Although such direct interactions have been demonstrated recently for specific *R–Avr* pairs (e.g., Gopalan et al., 1996; Scofield et al., 1996; Tang et al., 1996), it is not clear whether the same holds for each genetically defined *R–Avr* interaction. Therefore, identifying the domains within *R* gene products that mediate pathogen recognition specificity is one of the more pressing objectives facing researchers working on disease resistance responses in plants.

This question is addressed in two papers in this issue of THE PLANT CELL. **On pages 641–651** (and also highlighted on the cover), **Anderson et al.** describe their cloning and characterization of the *M* gene at the complex *M* locus in flax, which confers resistance to *M. lini* pathotypes carrying the *A-M Avr* gene, and **on pages 521–532, Ori et al.** present their data showing that the *I2C-1* gene at the tomato locus *I2* confers resistance to race 2 of the vascular wilt fungus *Fusarium oxysporum* f sp *lycopersici*.

In addition to *M* and *I2C-1*, a number of *R* genes triggering gene-for-gene–mediated defense responses have now been identified and characterized. Their deduced amino acid sequences have been used to classify them into a number of groups, the most extensive of which comprises proteins with a large leucine-rich repeat (LRR) domain and a putative nucleotide binding site (NBS; see Bent, 1996, for a detailed discussion).

LRR domains, which have been identified in a large number of eukaryotic proteins that perform a diverse range of functions, are thought to mediate protein–protein interactions (Kobe and Deisenhofer, 1995). It has, therefore, been proposed that the role of these domains in *R*-gene products may be to confer pathogen recognition specificity via interactions with *Avr*-gene products (Staskawicz et al., 1995; Bent, 1996; Jones and Jones, 1997). If this is true, LRR domains must be extremely versatile in their recognition capabilities—collectively, LRR-containing *R*-gene products are known to confer resistance to viral, bacterial, and fungal pathogens that cause foliar diseases in both dicots and monocots (Bent, 1996).

Anderson et al. identified the *M* locus, which includes ~15 related genes, by using map-based and transposon-tagging approaches. The authors were able to confirm the identity of the *M* gene within this complex locus by sequencing a number of mutant alleles that fail to mediate the detection of rust fungi carrying the matching *A-M Avr* gene. Anderson et al. show that *M* encodes an NBS–LRR protein and go on to determine that several of the loss-of-function mutations fall within the LRR-encoding region (see below). These data provide strong evidence that the LRR domain in M is involved either in pathogen recognition or the transduction of the *A-M*–derived signal.

M and most of the other *R* genes that have been cloned and sequenced to date mediate resistance to foliar pathogens by triggering defense responses typified by the HR. Recently, however, it is has been recognized that the types of pathogen recognized by LRR-containing *R*-gene products and the nature of the defense responses that are thereby triggered are not restricted to foliar diseases and the HR.

For example, the sugar beet $Hs1^{pro-1}$ gene, which confers resistance to the beet cyst nematode *Heterodera schachtii*, is now known to encode an LRR-containing protein (Cai et al., 1997). Although the molecular details are not clear, $Hs1^{pro-1}$ expression in beet roots leads to the developmental arrest of nematodes attempting to feed on beets and the eventual breakdown of their feeding structures (Cai et al. 1997; see Williamson and Hussey, 1996, for an overview of nematode pathogenesis).

The vascular wilt pathogen *F.o.* f sp *lycopersici* also triggers *R* gene–mediated defense responses that are very different from the HR. In the absence of defense responses, these soil-borne fungi cause serious diseases that can kill crops very quickly via blockages in the xylem vessels above the point of infection. The resistance reaction is characterized by deposition of callose and gels and outgrowths from xylem parenchyma cells, termed tyloses, that col-

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lectively serve to restrict the spread of the fungus through the vasculature. Although similar responses also occur in compatible (i.e., disease-causing) interactions, their induction is slower and they do not occur as extensively.

Building on their earlier mapping efforts (Ori et al., 1994), Ori et al. report that, like *M*, the *I2* locus also contains a number of related NBS–LRR—encoding genes. The one that mediates recognition of the *F.o.* f sp *lycopersici* race 2 pathogen, which the authors named *I2C-1*, was identified from within this complex in part via the first successful use of an antisense strategy for the analysis of *R*-gene structure and function.

The authors show that transgenic *I2* plants expressing a portion of the *I2C-1* gene in an antisense orientation were susceptible to infection by *F.o.* f sp *lycopersici* race 2 fungi. Recognition of the race 1 fungus (a trait that maps to a different *R* locus) was unaffected, and untransformed controls remained resistant to the *F.o.* f sp *lycopersici* race 2 pathogen. This significant advance opens the possibility that the antisense approach may be broadly applicable in structure/function analyses of other *R–Avr* interactions.

Anderson et al. and Ori et al. are also beginning to address a second pressing issue in the study of gene-for-gene–mediated resistance—the molecular basis for the origin of novel pathogen recognition specificities. *R* loci show a variety of genomic arrangements in plants (Bent, 1996; Crute and Pink, 1996), and it seems likely that inter- or intragenic recombination events may facilitate the reassortment of *R* gene sequences and the eventual establishment of novel resistance capabilities.

Genomic rearrangements between closely related sequences within the complex *Rp1* locus of maize, which conditions resistance to the fungus *Puccinia sorghi*, have been associated with the acquisition of novel pathogen recognition specificities (Sudupak et al., 1993; Richter et al., 1995). However, the *Rp1* locus has not been cloned, which precludes the detailed characterization of the sequence rearrangements that may underlie the generation of these novel recognition specificities.

By analyzing the sequences of mutant and wild-type genes at the *M* and *I2* loci, Anderson et al. and Ori et al. also present experimental evidence pointing toward major genomic rearrangements within the LRR-encoding portion of the *M* and *I2C-1* genes as potential sources of novel resistance specificities. For example, within the LRR domain of M, there are two large direct repeats of ~150 amino acids that are over 75% identical at the amino acid level. Surprisingly, three independent *M* mutants exhibit the identical rearrangement within this region, each losing the majority of the first repeat and part of the second.

Anderson et al. point out that the boundaries of the deletions in these mutants fall between two stretches of identical, directly repeated nucleotides that are present in both repeats of the *M* gene. These data imply that intragenic recombination events within the LRR-encoding region may be important for the generation of structurally altered *R* genes and, possibly, new resistance specificities. However, despite testing with an isolate of *M. lini* that expresses many of the *Avr* genes for which *R* genes have been defined in flax, no new resistance specificities were detected in plants expressing the rearranged *M* gene.

Ori et al. also present sequence data suggesting that intragenic recombination events within the LRR-encoding region may trigger the rearrangement of genes at the *I2* locus as well. They are pursuing this observation by mapping the *I2C-1*–related sequences they have detected elsewhere in the tomato genome. Some of these sequences map close to previously identified *R* loci and may, therefore, function in distinct tomato–pathogen interactions.

Although the experiments of Ori et al. and Anderson et al. are very informative, a number of pressing questions remain. Clearly, further evidence for a role of the LRR in mediating Avr signal recognition is needed in these and many other plant–pathogen interactions. One promising approach is the comparative analysis of *R* genes and their relatives, which is one of the reasons Ori et al. and Anderson et al. have mapped sequences related to *I2C-1* and *M* in the tomato and flax genomes, respectively. The goal is to identify the most variable regions, which may be important in determining pathogen specificity, as well as repeated sequences that may be the engines for (or the results of) inter- or intragenic rearrangements. Conserved domains may be involved in initiating or propagating the Avr-triggered signal transduction cascade.

A number of comparative analyses are already in progress. For example, Jia et al. (1997) have exploited the fact that, like *R* genes in many other crops, the *Pto* and *Fen* genes (which mediate recognition of *Pseudomonas syringae* pv *tomato* carrying the *avrPto* gene and sensitivity to the insecticide fenthion, respectively) were introgressed into tomato from *Lycopersicon peruvianum*, a wild tomato species that exhibits genome synteny with its cultivated relative.

Jia et al. (1997) cloned and sequenced *pto* and *fen* "alleles" at the orthologous positions in the genome of an unmodified, disease-susceptible cultivated tomato variety. Interestingly, these alleles are closely related to their functional *L. peruvianum*-derived counterparts, yet the corresponding proteins do not appear to play a role in the detection of the *avrPto* or fenthion signals. By pursuing the minor amino acid sequence differences between these allelic proteins, it should be possible to define functionally important domains in Pto and Fen.

In addition to this work on Fen and Pto, which, as serine/threonine protein kinases are members of a distinct *R*-gene class, several additional candidate members of the NBS–LRR class have been

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cloned recently from potato (Leister et al., 1996) and soybean (Kanazin et al., 1996; Yu et al., 1996) by using the polymerase chain reaction. "Domain swapping" experiments between *M* and another flax rust resistance gene, *L6*, will also facilitate comparative analyses of *R* gene sequences (Lawrence et al., 1995; Anderson et al., 1997).

Nevertheless, primary amino acid sequence may not be the only determinant of pathogen recognition specificity. Indeed, the LRR domain of the tomato Cf-2 protein (which mediates resistance to the fungus *Cladosporium fulvum* carrying the *Avr-2* gene) includes numerous potential N-glycosylation sites (Dixon et al., 1996). Moreover, it has been shown that transcripts of the tobacco mosaic virus *R* gene, *N* (Whitham et al., 1994), and the *L6* gene in flax (Lawrence et al., 1995) can be differentially spliced to encode truncated protein products. Whether these shorter *R*-gene products mediate interactions with the same pathogen varieties as the full-length proteins or trigger the recognition of distinct pathotypes is unknown.

Even if the mechanisms that generate novel resistance specificities are elucidated, it will still be necessary to determine whether plants possess specialized mechanisms that allow them to accumulate and maintain (i.e., "bank") rearranged *R* genes in the population. If so, is there a constitutive process for generating variation, or could pathogen attack actually increase the rate at which rearrangements in *R* gene structure occur?

It is also important to continue analysis of the other partner in these interactions—the *Avr* gene products. Although ~30 *Avr* genes have been sequenced to date (Dangl, 1994), many share little sequence similarity. This sequence diversity has contributed to difficulties in determining whether rearranged *R* gene products are capable of mediating defense responses triggered by pathogens carrying different *Avr* genes (see above). Identifying the regions within *Avr*-gene products that facilitate their presumed interactions with *R*-gene products would, therefore, be most informative. Interestingly, Joosten et al. (1997) have recently shown that mutations in *Avr* genes may affect pathogen recognition specificity indirectly by destabilizing the *Avr*-gene products.

The *R–Avr* interaction is only the first step in a complicated web of responses that collectively confer resistance on the plant. Although considerable progress is being made in our understanding of the HR and the events that trigger systemic acquired resistance (SAR; Dangl et al., 1996; Ryals et al., 1996), the differences among the signal transduction cascades that lead to responses as diverse as the HR, the degradation of nematode feeding structures, and the obstruction of vascular wilt fungi are largely unknown.

One of the most promising approaches toward the identification of links in this network has been the identification of mutants that are affected in various "downstream" defense responses. Such mutations may define genes whose products interact, either directly or indirectly, with those of the *R* genes, or function elsewhere in the transduction pathway. Examples include the *RCR* loci, which are required for *Cladosporium* resistance in tomato (see Hammond-Kosack and Jones, 1996, for a review); the *EDS* (enhanced disease susceptibility) loci in Arabidopsis, which increase the susceptibility of mutant plants to several fungal and bacterial pathogens (Parker et al., 1996; Rogers and Ausubel, 1997); and the Arabidopsis *LSD* (lesions simulating disease) loci (Dangl et al., 1996; Dietrich et al., 1997). Moreover, the recent cloning and characterization of the Arabidopsis *NIM1/NPR1* (noninducible immunity/nonexpresser of PR genes) gene, which defines an important control point in the SAR pathway, points toward potentially intriguing parallels between defense responses in plants and animals—the gene encodes an ankyrin repeat–containing protein with some similarity to the mammalian transcriptional inhibitor IκB (Cao et al., 1997; Ryals et al., 1997).

The introgression of *R* genes into crop species has long provided the most efficient, cost-effective, and environmentally sound form of disease protection (Bent, 1996), but the process is complicated and time consuming. If molecular genetic approaches are to live up to their promise in enormously accelerating the rate at which novel and durable resistance specificities can be added to crop species (Staskawicz et al., 1995; Bent, 1996; Crute and Pink, 1996), it is imperative to develop a thorough understanding of the molecular mechanisms underlying both the interaction between *R*- and *Avr*-gene products and the generation of novel *R*-gene specificities. Clearly, the exciting findings reported by Anderson et al. and Ori et al. in this issue of THE PLANT CELL contribute significantly to these important goals.

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