Two modes of pathogen recognition by plants

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M ore than 50 years ago, J. B. S. Haldane (1) pre-
sciently predicted that polymorphism in proteins governing host–pathogen interactions was driven by the rapid rates of micro-
bial evolution. He suggested that slowly evolving host species could only manage to dodge microbial infection by generating high levels of polymorphism in the relevant immune system genes. These ideas were in part inspired by observations of variability in resistance of wheat to the wheat rust pathogen *Puccinia graminis*. Soon thereafter, H. H. Flor (2) published seminal studies delineating the genetic basis of resistance in flax to the rust fungus *Melampsora lini*. Flor documented genetic polymorphism and recognition specificity. Flor (3) also proposed the widely accepted “gene-for-gene” theory of disease resistance, which predicted that successful disease resistance is triggered only if a resistance (R) gene product in the plant recognizes a specific “avirulence” (Avr) gene product from the pathogen. An evolutionary corollary of this model is that pathogens are under evolutionary pressure to evolve diverse Avr proteins that no longer interact genetically with the cognate R proteins but presumably maintain their core function in the furtherance of a successful parasite life cycle. Conversely, natural selection favors evolution and maintenance of allelic diversity at plant R loci to expand recognition specificity.

In this issue of PNAS, Dodds et al. (4) provide evidence for direct, allele-specific interaction between alleles of a particular R protein and the corresponding pathogen-encoded Avr proteins. In a remarkable arc of history, they do so by using cloned alleles from flax and flax rust loci that were defined genetically five decades ago by Flor (2, 3, 5, 6). Combined with other recent breakthroughs, this work suggests that plant R proteins can function either by directly detecting the corresponding Avr protein (the “receptor–ligand” model) or by perceiving alterations in plant machines that are targets of Avr protein action in the promotion of pathogen virulence [the “guard hypothesis” (7–9)]. Pathogen Avr proteins can be “effectors” that promote pathogen virulence on plant hosts incapable of recognizing that pathogen. Plant pathogens have independently evolved mechanisms to deliver effectors into plant cells. For example, bacteria export effectors to plant cells through a type III secretion plus (bacterial pathogens of animals also use this strategy). Nematodes and aphids deliver effectors by poking a feeding styler directly into a single plant cell. Fungi and oomycetes often invaginate a feeding organ, called a haustorium, into a host cell. This angioplasty balloon-like structure sets up a unique and intimate interface through which effectors likely travel by an as-yet-undefined mechanism. Despite differences in the mechanism of effector delivery, the end results are similar: suites of pathogen proteins are smuggled into plant cells, where they contribute to pathogen virulence by, at least in some cases, sabotage of basic host defense responses.

Detection of an effector by an R protein triggers rapid activation of a very effective defense.

In response, plants evolved specific R protein alleles to detect specific effectors acting as saboteurs. Detection of an effector by an R protein triggers rapid activation of a very effective defense. Hence, any single effector protein recognized by an R protein qualifies as an avirulence factor. Different effector proteins, as a rule, share no common sequence features and presumably perform diverse functions inside the host cell. The majority of plant R genes encode NBS-LRR proteins (nucleotide-binding site, leucine-rich-repeat) that are structurally reminiscent of animal CATERPILLER/NOD/NLR proteins (10). NBS-LRR proteins are likely molded and held in a signal competent state by cytosolic HSP90 and various other receptor cochaperones (11). Intramolecular and intermolecular interactions likely keep NBS-LRR proteins inactive until they are activated by the presence a specific effector (12). Comparative genomics has demonstrated that all plants maintain large collections of NBS-LRR genes, many of which may have alternate alleles across genotypes (13). Although each individual NBS-LRR allele typically provides resistance to a single pathogen isolate, the collective surveillance capability of a population’s NBS-LRRs appears quite broad. How, then, is R-dependent recognition initiated at the molecular level?

Direct Objects of Virulence

The simplest mechanistic explanation for specific recognition of pathogen Avr proteins by plant NBS-LRR proteins is that the former are ligands for the latter. Dodds et al. (4) show this to be true in the case they studied. The flax L locus is polymorphic; there are at least 13 known alleles (14). Diversifying selection is observed across the LRR domains of the L proteins, as is the case for essentially all polymorphic NBS-LRR proteins analyzed to date. Previous work from this group established that a small family of polymorphic Avr proteins, encoded at a locus called *AvrL567*, trigger specifically the action of the flax L5, L6, and L7 NBS-LRR proteins when expressed from inside plant cell (using plant transcriptional regulatory sequences and transient gene expression) (6). These Avr proteins are small (150 aa) and contain a signal sequence that is removed (giving rise to a 127-aa mature peptide). They are presumably delivered across the haustorial membrane, through the extrahaustorial matrix, and into the plant cell (15, 16). Importantly, *AvrL567* proteins contain particular amino acid residues that are under selection for sequence divergence.

Dodds et al. (4) add several vital new facts. First, they sampled geographically diverse flax rust strains to establish that diversifying selection contributes to the high polymorphism of *AvrL567* proteins. Second, they used transient expression assays to demonstrate that six of the seven new *AvrL567* alleles encode specific avirulence activity on L5, L6,
and/or L7 flax plants. Third, they took advantage of an L6L11 protein chimera to show that L6 function tracks with 11-aa differences in the C-terminal LRRs. Fourth, and most importantly, they demonstrated that direct Avr–NBS-LRR interaction is strictly correlated with their functional results.

Dodds et al. (4) further showed that a very limited number of informative polymorphic sites across the AvrLS67 defined the amino acid changes that account for specificity. Secondary structure predictions placed these residues on the proposed solvent-exposed face of AvrLS67, in position to interact with the NBS-LRR protein. Circular dichroism and limited proteolysis analyses demonstrated that, despite the high sequence polymorphism, at least three of the AvrLS67 alleles are likely to adopt the same simple structure. The authors (4) propose that selection has favored substitutions in surface residues that preserve the core AvrLS67 structure. Maintenance of AvrLS67 diversity suggests that this locus contributes to colonization of susceptible flax genotypes; otherwise, selection would likely favor fixation of null AvrLS67 alleles to dodge L-dependent recognition.

Thus, AvrLS67 variants trigger disease resistance by means of specific physical interaction with the corresponding L-encoded NBS-LRR protein. Dodds et al. (4) do not present the first demonstration of direct R–Avr interaction. Their work is, however, particularly compelling because of the correlation between functional allelic diversity and protein–protein interaction specificity driven by diversifying selection and consequent dynamic coevolution in both host and pathogen. As noted above, there are other cases demonstrating that pathogen Avr proteins are recognized indirectly, through their action on a plant protein whose structural integrity is monitored by an associated NBS-LRR protein. These cases are consistent with the “guard hypothesis” (8, 9), where the NBS-LRR proteins in question are activated as sensors of “pathogen-induced,” or “altered self,” molecular patterns.

**Indirect Objects of Virulence**

In interesting contrast to L, allelic diversity at R loci that encode “guards” is very limited; functional alleles are ancient, and they are maintained by balancing selection (17, 18). Moreover, the functional R alleles thus far shown to function by indirect recognition are not under diversifying selection, perhaps because they have evolved a stable association with a target of effector action that precludes further diversification of the NBS-LRR sequence. On the pathogen side, virulence effectors that trigger indirect recognition are often found as presence–absence alleles across a pathogen species.

Direct and indirect recognition might confer distinct advantages. NBS-LRR proteins that guard host molecular machinery would impose selection favoring complete nullification of the corresponding effector Avr gene (e.g., the mode of action of a protease effector cannot be altered without fundamental loss of virulence function; hence, the gene is jettisoned from the pathogen). But because pathogens evolve faster, and because at least bacterial pathogens can acquire effectors through horizontal gene transfer, they can “retarget” the same host machine with a new virulence factor. Indirect NBS-LRR activation, then, provides a mechanism for the plant to perceive the multiple effectors that might converge on the same target. In addition, multiple NBS-LRR proteins might sense alterations of the same host protein mediated by different effectors. These cases have indeed been documented (9). In contrast, direct interaction provides a mechanism for the host to recognize effectors that do not necessarily provoke structural alterations in their targets. The two modes of pathogen recognition may therefore be complementary.

Dodds et al. (4) speculate that adoption of one recognition mode or the other may be driven by the life history of the corresponding pathogen or the functionality of the effector. Pathogens with relatively broad host ranges and/or capacity to survive apart from plants (like plant-pathogenic bacteria) might deploy effectors with dramatic effects on multiple hosts. In contrast, pathogens with narrow host ranges and an inability to survive apart from their hosts (obligate biotrophs like flax rust) may have been driven toward a preponderance of virulence effectors with subtle effects that do not result in altered, or modified, host machinery.

Embellishment of these first few mechanistic paradigms will require surveys of polymorphism and allelic diversity across all NBS-LRR loci in at least one reference genome, likely *Arabidopsis*, to determine whether general mechanistic distinctions can be inferred between fast- and slow-evolving NBS-LRR classes (19, 20). It is exciting to contemplate the development of new predictive molecular and genetic tools that could identify durable R genes that guard critical virulence targets or directly interact with key pathogen effectors. These could be of use for disease control in crops. In this regard, molecular and evolutionary biologists might discharge the debt that they owe plant breeders for laying a solid genetic foundation that underpins a fascinating area of fundamental inquiry.