Primer

Recognition of pathogens by plants

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Plants must respond appropriately to a broad array of abiotic and biotic factors in the attempt to accumulate nutrient reserves and reach reproductive maturity. To complicate matters, their stockpile of nutrients makes them prime targets for pathogen attack. Phytopathogenic bacteria, fungi, and viruses all make raids on the stockpile, yet most plants are able to remain disease free.

Plants have many preformed mechanisms for preventing pathogen ingress, such as waxy cuticles and constitutively produced toxic compounds. Additionally, most pathogens are highly specialized and only cause disease on a narrow host range. Nevertheless, many pathogens are capable of circumventing preformed defenses, and plants must prevent disease by recognizing these invaders and halting their growth.

Gene-for-gene resistance

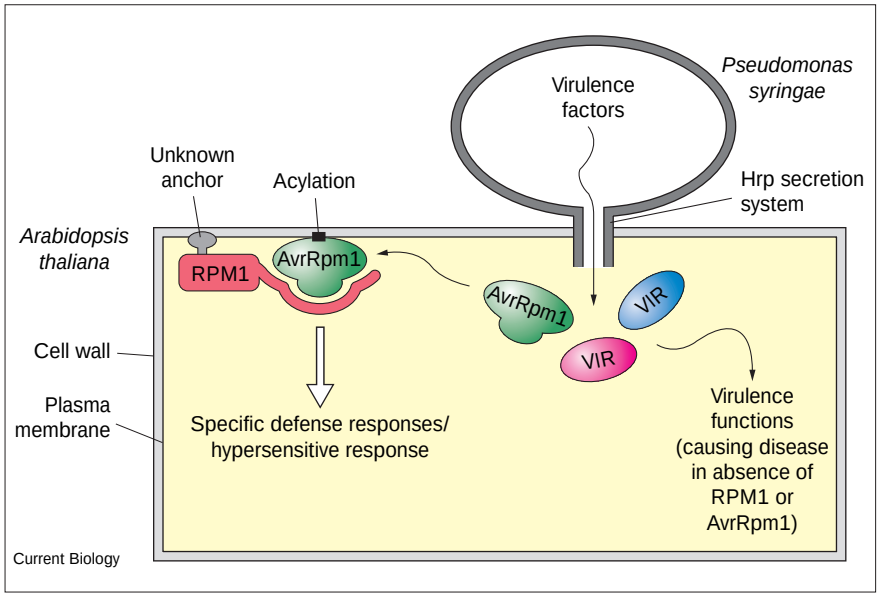
So how do plants recognize and respond to specific pathogens? Unlike animals, plants do not have the luxury of a circulating immune system capable of quickly recognizing non-self invaders and somatically generating new recognition specificities. Because of this limitation, essentially all plant cells are individually capable of recognizing pathogens and turning on an effective defense system. This type of defense response is achieved through the interaction of a putative plant-derived receptor and a corresponding pathogen molecule called an 'elicitor'. Elicitor production is dependent on so-called 'avirulence' (*avr*) genes. It is still unclear for most *avr* genes whether the final elicitor is the actual Avr protein or an Avr-dependent by-product. The products of plant resistance genes (known as *R* genes) are hypothesized to be the receptor molecules that recognize specific elicitors. This *R–avr* interaction initiates what is referred to as gene-for-gene resistance.

One well-studied example is resistance to bacteria of the *avrRpm1* genotype mediated by the *RPM1* (resistance to *Pseudomonas syringae pv maculicola*) resistance gene. When this pathogen gains access to the mesophyll tissue of an *Arabidopsis thaliana* leaf (typically, via a stomatal opening or a wound surface), it begins to secrete specialized molecules (including virulence factors) necessary for the utilization of host resources (see Figure 1). These molecules, which include AvrRpm1, are probably delivered directly into the cytoplasm of the host by the Type III/Hrp (hypersensitive response and pathogenicity) secretion system. If the plant cells contain RPM1, the pathogen is recognized and the outcome is resistance. If either RPM1 or AvrRpm1 is absent from the interaction, disease is the result.

The defense response

When a specific *R–avr* interaction takes place, massive intracellular and intercellular changes occur. Cells in and around the recognition site undergo changes of phosphorylation state and experience large ion fluxes (especially of Ca^{2+}) as part of early signal transduction events. In addition, salicylic acid, a signaling molecule for subsequent systemic plant defenses, is rapidly induced. Reactive oxygen intermediates and nitric oxide are rapidly induced and act synergistically to promote the hypersensitive response, which is a type of rapid and localized programmed cell death. The hypersensitive response serves to

Figure 1



The interaction between the RPM1 resistance protein (red) of *Arabidopsis thaliana* and the AvrRpm1 avirulence factor (green) of *Pseudomonas syringae*, that leads to defense responses in the plant. The virulence factors enter the plant cell by means of the Hrp secretion system. Other virulence factors (pink and blue) would cause disease in the absence of RPM1 or AvrRpm1.

isolate the pathogen while cell walls surrounding the lesion site are reinforced (with callose and lignins) against further invasion. Some reactive oxygen intermediates may also be toxic to invading pathogens and this toxicity is enhanced by a variety of antimicrobial compounds (for example, phytoalexins) and digestive enzymes (for example, chitinases and glucanases). The overall effect is to isolate the pathogen and present it with a suite of defense mechanisms that limit its ability to cause disease.

Virulence and avirulence

It is reasonable to ask why a pathogen makes avirulence proteins — why would a pathogen produce the very signal that limits its growth? The simple answer is that many avirulence proteins may also have a virulence function (their confusing name is a legacy of the fact that these factors were cloned on the basis of their avirulence functions). For example, AvrRpm1 only acts as an avirulence protein on plants expressing RPM1. Where RPM1 is absent in the host, AvrRpm1 increases the ability of the pathogen to sustain growth and promote plant disease (as measured comparatively between isogenic bacterial strains with mutant or wild-type *avrRpm1* genes). Therefore, loss of an avirulence protein can reduce fitness of the pathogen. In other cases, the redundant nature of pathogen virulence factors might make them more expendable.

Specificity of the resistance response

Because they use avirulence factors as a means of resisting disease, plants exert selective pressure on pathogens towards the loss and/or mutation of avirulence genes; and, in fact, the cloned products of these avirulence genes have highly diverse amino acid sequences. With this insight, what would be the structural and functional requirements for the system by which plants recognize pathogen elicitors? The recognition components must act like sentries, constantly on guard and prepared at any moment to signal the troops. Individual sentries recognize specific enemies; however, pathogens can avoid detection by discarding or altering the molecules that betray their presence. To remain vigilant, plants need a genetically adaptable and flexible mechanism for generating new recognition specificities.

A large number of *R* genes have been cloned from many plant species and nearly all contain a sequence motif called leucine-rich repeats (LRRs). Because LRRs are known to mediate protein–protein interactions, it is thought that the LRRs of R proteins serve as the specificity determinants (receptors) for elicitors of pathogens. Analysis of structure and function of the proteins encoded by *R* genes supports this hypothesis. There are two broad classes of R proteins: those with extracellular LRRs and those with cytoplasmic LRRs. R proteins of the first class contain extracellular domains composed almost entirely of LRRs, a transmembrane domain, and a cytoplasmic domain that in one case (Xa-21 of rice) is a kinase, but in most cases is a short sequence of unknown function. The structure of these R proteins indicates that they are extracellular receptors whose LRRs detect *avr*-dependent elicitors of pathogens.

Members of the cytoplasmic class of R proteins contain a nucleotide binding site domain in addition to LRRs. The nucleotide binding site is thought to engage signaling molecules activated in response to pathogen recognition. Although these R proteins are predicted to be cytoplasmic, at least one of them, RPM1, associates peripherally with the inner surface of the plasma membrane, possibly by association with another protein. Several Avr proteins of *P. syringae* that gain access to the plant's cytoplasm contain consensus sequences for acylation at their amino termini, modifications that would direct them to the inner surface of the plasma membrane. The acylation sites are required for plasma membrane association of AvrRpm1, and for its efficient detection by RPM1. This places RPM1 and AvrRpm1 at the same sub-cellular location, supporting the hypothesis that LRRs are cellular receptors for Avr proteins.

Studies on the structure of LRRs indicate that these domains of the R proteins are ideally suited for pathogen recognition. The crystal structure of a protein containing LRRs — ribonuclease inhibitor — in a complex with ribonuclease A has been determined. Ribonuclease A binds to the horseshoe-shaped pocket (see Figure 2) of the ribonuclease inhibitor, changing the pocket to a more 'open' conformation. This structural plasticity of the LRRs allows them potentially to bind to a diverse collection of ligands. Ribonuclease inhibitor efficiently inhibits a wide variety of ribonucleases with sequence identity as low as 24%. The ability to bind tightly to diverse sequences would make LRRs well suited to their proposed role as surveillance molecules of the plant immune system.

Diversification of R proteins

Selective pressure resulting from pathogen evolution would drive diversification of the plant component involved in recognition. The specificity of *R* genes evolves by means of diversifying selection. Most *R* genes occur in clusters of homologous genes and pseudogenes. Often, individual genes of the cluster specify resistance to distinct pathogens. Numerous studies of these clusters have shown that the rate of non-synonymous changes is high within the β sheets that comprise the putative binding pocket of the LRRs. Non-synonymous (K_a) and

Figure 2

Ribbon diagram of the crystal structure of ribonuclease inhibitor, a protein that contains leucine-rich repeats (LRRs). Each LRR is an iterative unit composed of a β sheet (yellow arrows) and a roughly parallel α helix (purple coils) joined by a short loop. The α helices form the outer surface of the structure; the concave, inner surface is made up of β sheets. Because the α helices pack less tightly, the molecule forms a horseshoe-shaped structure. Ribonuclease A (not shown) binds primarily to the inner surface of the ribonuclease inhibitor, changing the pocket to a more open conformation.

synonymous (K_s) base changes result in codons that encode different or identical amino acids, respectively. The K_a:K_s ratio in genes on which no selective pressure is acting is typically about one. The K_a:K_s ratio within the pocket of the LRRs of all *R* genes studied has been greater than one, indicating that diversifying selection acts on this portion of the LRRs. The diversity observed in the pocket is entirely consistent with the hypothesis that this domain specifies pathogen recognition. Resistance to *Cladosporium fulvum* mediated by the *Cf* genes of tomato provides an example where the sequence of the LRRs clearly determines specificity. *Cf* genes whose amino acid sequences differ only within the LRRs specify resistance to races of *C. fulvum* with different *avr* genes. Therefore, the *avr*-dependent elicitor detected by a given *Cf* gene is specified solely by the sequence of its LRRs.

Many of the pathogens that besiege plants are single-cell organisms that can evolve rapidly. Plants have much longer generation times than these pathogens and therefore the regions of *R* genes that specify recognition of elicitors must evolve efficiently. The rate-limiting step for diversification of a protein domain is the accumulation of mutations. Two features of LRRs maximize the diversity created by mutations within them. First, the target size for mutation is large. Ligands of LRRs may interact with a large surface area; therefore, changes to any of a large number of amino acids may alter specificity. Second, the iterative nature of LRRs promotes recombination between different alleles of the same gene and gene conversion events that result in increased diversity.

The grouping of *R* genes into clusters also permits recombination between homologous genes. When clusters of *R* genes are compared between different genotypes, orthologs (genes occupying the same locus in distinct genotypes) show a greater degree of homology than do paralogs (homologous genes within a genotype). This indicates that interallelic recombination is more common than intergenic recombination within the cluster. If intergenic recombination predominated, the gene clusters would become homogenized. Rather, individual alleles evolve independently, through primary mutations and interallelic recombination. Thus, plants efficiently generate and maintain a diverse set of recognition specificities within the LRRs of their *R* genes.

It is widely hypothesized that the LRRs of proteins encoded by *R* genes are specificity determinants that allow the plant immune system to recognize pathogens. The LRRs are thought to bind either Avr-dependent elicitors or Avr proteins. We have presented several lines of evidence in support of this general hypothesis; however, generalized interactions between LRRs and Avr proteins have yet to be demonstrated. Understanding how plants recognize pathogens at the molecular level will greatly enhance our understanding of disease resistance in plants.

Key references

Biotic interactions. *Curr Opin Plant Biol* 1999, 2:255-338.

Galán JE, Collmer A: Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* 1999, 284:1322-1328.

Jones DA, Jones JDG: The role of leucine-rich repeat proteins in plant defences. *Adv Bot Res* 1997, 24:89-167.

Kobe B, Deisenhofer J: Mechanism of ribonuclease inhibition by ribonuclease inhibitor protein based on the crystal structure of its complex with ribonuclease A. *J Mol Biol* 1996, 264:1028-1043.

Kajava AV: Structural diversity of leucine-rich repeat proteins. *J Mol Biol* 1998, 277:519-527.

Michelmore RW, Meyers BC: Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. *Genome Res* 1998, 8:1113-1130.

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