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Inside Avirulence

Plants have developed a plethora of overlapping strategies aimed at blocking infection by potential pathogens. For example, preexisting chemical and structural barriers may prevent a pathogen from ever gaining a foothold in the plant (Osbourn, 1996), whereas a host of induced defense responses often serve to restrict infection to the initial site of attack and/or to preclude the systemic spread of disease. One form of induced response is the hypersensitive response (HR; Goodman and Novacky, 1994), during which cells immediately surrounding the site of infection rapidly die. The mechanism by which this is achieved is largely unknown, but there is evidence that cell death is triggered ("programmed") by the plant and is not a direct consequence of pathogen toxicity (see Greenberg and Sussex, 1996; Jones and Dangl, 1996, for recent reviews on cell death in plants).

In many cases, genetic control of HR conforms to the gene-for-gene hypothesis (Keen, 1990), with a single gene (the resistance or *R* gene) in a host plant cultivar mediating the defense response elicited by a single gene (the avirulence or *avr* gene) in the corresponding pathogen race (Staskawicz et al., 1995). *avr* genes are thought to encode or control the production of race-specific signal molecules (elicitors), the recognition of which is mediated by the products of the *R* genes in the corresponding plant cultivar. During an interaction in which the plant expresses a specific *R* gene and the pathogen expresses the cognate *avr* gene, the HR is induced and there is no disease (an "incompatible" interaction). With any other combination of *R* and *avr* genes, the HR is not triggered and disease (a "compatible" interaction) ensues.

The fact that gene-for-gene interactions have been described in a diverse range of pathosystems (with specific pairs of *R* and *avr* genes operating in each system) would tend to suggest that the molecular mechanisms of pathogen recognition controlled by these genes are broadly conserved (Staskawicz et al., 1995). The recent cloning and sequencing of a number of *R* genes have provided additional evidence that this may be the case: All but one contain leucine-rich repeat motifs that have been shown to mediate molecular recognition events in other systems (reviewed by Dangl, 1995; the exception is the tomato *Pto* gene, which encodes a protein kinase [Martin et al., 1993; Loh and Martin, 1995]).

In contrast, the lack of any obvious conservation among the more than 30 reported bacterial *avr* gene sequences suggests that the details of *R* gene–mediated pathogen recognition are likely to be different for each pathosystem (Dangl, 1994). The variability in *avr* gene sequences presumably reflects the fact that invading pathogens express a large number of genes, the products of any one of which may be targeted for specific recognition by the host plant.

To manifest the specificity conferred by their *avr* genes in incompatible interactions (and to successfully infect compatible plant cultivars), *Pseudomonas syringae* and many other Gram-negative plant pathogens must also express a suite of genes known as the HR and pathogenicity (*hrp*) genes (Bonas, 1994). Remarkably, the heterologous expression of the *hrp* genes in saprophytic relatives of *P. syringae*, such as *P. fluorescens* and even *Escherichia coli*, confers on these otherwise nonpathogenic species the ability to induce HR-like symptoms in tobacco and several other plant species (e.g., Huang et al., 1988). Informative model systems have been developed in these saprophytic species, and it has recently been shown that saprophytes coordinately expressing the *hrp* genes and the appropriate *avr* gene are capable of eliciting an *R* genotype-specific HR (Collmer et al., 1996; Hutcheson et al., 1996; Pirhonen et al., 1996).

The *hrp* genes encode components of the so-called type III protein secretion system that is also required for pathogenicity of a number of Gram-negative animal pathogens (Van Gijsegem et al., 1993; Bonas, 1994). Interestingly, some of the Hrp proteins are related to proteins involved in the construction of bacterial flagellae, raising the possibility that the Hrp proteins (and their animal pathogen homologs) may form specialized infection structures capable of directly injecting bacterial virulence factors into host cells (Rosqvist et al., 1995; see Barinaga, 1996, for a recent discussion of the function and evolution of the type III protein secretion system).

Collectively, these data suggest that the Hrp-controlled secretion of bacterial Avr proteins may be required to trigger the HR in incompatible (resistant) plant cultivars. Other possibilities are that the Hrp proteins modify the Avr proteins in the bacterial cell or in the plant apoplast. However, there are no reports of bacterial Avr proteins inducing HR when infiltrated into resistant plant leaves, and Avr proteins have not been seen outside of bacterial cells (reviewed by Dangl, 1994). Thus, it is still not clear where the *R* and *avr* gene-mediated recognition event occurs, or even whether or not the R and Avr proteins interact directly. Some *R* gene products are predicted to reside in the plant cell cytoplasm, whereas others may be membrane-associated or transmembrane proteins (Dangl, 1995). Are the corresponding Avr proteins targeted to the same cellular compartments as their cognate *R* gene products? Are the Hrp proteins involved in this targeting process?

On pages 1095–1105 of this issue, Gopalan et al. show that *avr* genes must be expressed in the same bacterial cell

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as a functional *hrp* secretion system to elicit a genotype-specific HR. These results imply that the Hrp proteins are directly involved in the transmission of the Avr signal. Moreover, the authors provide compelling evidence that the products of the *avr* genes can trigger genotype-specific resistance responses from inside the host plant cell, the predicted location of the corresponding plant R proteins.

The focus of the work reported by Gopalan et al. is race-specific resistance of soybean to *P. syringae* pv *glycinea*, which is mediated by the bacterial *avrB* gene and the plant *RPG1* gene (Staskawicz et al., 1987). In an interesting departure from the usual gene-for-gene scenario, *avrB* also induces the HR in Arabidopsis lines that express the *RPM1* resistance gene (originally defined through its interaction with a different *P. syringae* avirulence gene, *avrRpm1*; Bisgrove et al., 1994). This latter observation forms the basis for experiments, described below, in which *avrB* gene expression has been manipulated in transgenic Arabidopsis plants.

To detect bacterially produced AvrB in inoculated plants, Gopalan et al. first engineered *P. fluorescens* to express an epitope-tagged AvrB derivative, AvrB-FLAG. In control experiments, bacteria expressing AvrB-FLAG were capable of triggering the HR on incompatible Arabidopsis and soybean lines in an *hrp*-dependent manner. However, when either of two components of the Hrp system, HrpZ and HrcC, was mutated in the bacteria, no HR was seen on the plants despite the presence of detectable levels of AvrB-FLAG (presumably still inside the bacteria) in the infiltrated plant tissue.

In ingenious experiments designed to investigate the basis for *hrp* gene action, the authors next coinfiltrated plants with two different bacterial strains, one producing the elicitor AvrB-FLAG and the other expressing the *hrp* genes. Once again, high levels of AvrB-FLAG were present in the plant tissue (in the bacteria), yet the HR was not triggered. These data argue strongly for a direct role of the Hrp system in transmitting the Avr protein to plant cells and contradict theories postulating a role for the Hrp proteins in modifying the Avr signal either in the bacterial cell or the plant apoplast.

If the Hrp system is required to transmit an exogenous (i.e., bacterially produced) Avr protein into plant cells, what is the effect of producing the Avr signal inside the plant? Is the simultaneous presence of the *R* gene product required to induce the HR? To address these questions, Gopalan et al. attempted to transform an *RPM1* Arabidopsis line (which exhibits the HR in interactions with *avrB*-expressing bacteria) and an *rpm1* line (which does not) with the *avrB* gene.

The only transformants that could be recovered were those in which *avrB* was expressed in an *rpm1* background. Surprisingly, most of these *rpm1* (*avrB*) transgenics showed HR-like lesions, the severity of which correlated with the levels of *avrB* expression. One interpretation of this result is that the *rps3-1* allele of *rpm1* used in these experiments is not null and the mutant protein (which is truncated at amino acid 818; Grant et al., 1995) is capable of triggering the HR when relatively high levels of AvrB (produced endogenously, rather than by invading bacteria) are present in the cytoplasm. One *rpm1* (*avrB*) transgenic line that expresses very low levels of *avrB* and is free of HR-like symptoms, line 12, was recovered. To bring wild-type RPM1 and the AvrB protein together in the same plants, line 12 was crossed to the *RPM1*-expressing Arabidopsis line. F_1 seedlings from this cross developed spontaneous and extensive HR-like lesions and quickly died, demonstrating the lethal consequences of *avrB* and *RPM1* coexpression within the same plant cell.

To substantiate the conclusions drawn from their work with stably transformed Arabidopsis, Gopalan et al. have also explored RPM1–AvrB interactions using a transient expression system. In these experiments, *RPM1* and *rpm1* Arabidopsis leaves were cobombarded with *GUS*- and *avrB*-expressing plasmids. The authors report that no GUS activity was detectable in leaves of *RPM1* plants cobombarded with *GUS* and *avrB*, suggesting that an interaction between AvrB and RPM1 triggered the HR cell death in these leaves prior to the onset of *GUS* expression. In control experiments, the authors show that cobombardment of *rpm1* Arabidopsis leaves with *avrB* and *GUS* does not preclude GUS activity.

Where do we go from here? Although the evidence pointing toward an intracellular mode of action of AvrB in triggering the HR is strong, it is based in part on "negative data." Direct identification of AvrB inside plant cells would help to clinch the argument. The anti-FLAG epitope antibody could potentially be used to investigate the distribution of the AvrB-FLAG fusion protein in plants infiltrated with HR-inducing and control bacteria. Alternatively, bacteria could be engineered to express an AvrB–green fluorescent protein fusion construct that would be readily detectable in infiltrated tissue. The success of these approaches will hinge both on the capacity of the Hrp system to appropriately target the fusion proteins and on the stability of the fusion proteins in the plant cells.

The demonstration that the AvrB protein most likely functions inside plant cells will likely form the basis for a whole series of experiments aimed at elucidating the details of the molecular recognition event that triggers the HR. Modulating the expression of the *avrB* gene in transgenic Arabidopsis using tightly regulated promoters will facilitate an analysis of the events occurring in the plant cell immediately following the induction of *avrB* expression. Meanwhile, structure–function analyses of AvrB and RPM1 will help to define the domains in each protein that mediate their action in pathogenesis and defense. Ultimately, these approaches should combine to settle the fundamental question of whether the HR is initiated by a direct interaction between a bacterial Avr protein and the corresponding plant *R* gene product.

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