Plant Response to Bacterial Pathogens. Overlap between Innate and Gene-for-Gene Defense Response

The immune system's role as defense from opportunistic microbes also necessitated the ability to distinguish between ''self'' and ''non-self'' to ensure that an immune response is not mounted against the organism's own tissues. This discrimination is partially achieved by the recognition of an invader's chemical motifs by host surface receptors. Consequently, potential pathogens have adapted numerous ways to overcome host immune systems while the hosts respond with new defenses. In plants, this ongoing ''battle'' against pathogens has led to two types of immune responses: an older, basal response to pathogen-associated molecular patterns (PAMPs) and a gene-for-gene response specific to a pathogen. This month's selection for *High Impact* examines the immune response of Arabidopsis (*Arabidopsis thaliana*) to one such PAMP and the interaction between the two immune responses. The article is ''The Transcriptional Innate Immune Response to flg22. Interplay and Overlap with Avr Gene-Dependent Defense Responses and Bacterial Pathogenesis'' by Lionel Navarro, Cyril Zipfel, Owen Rowland, Ingo Keller, Silke Robatzek, Thomas Boller, and Jonathan D.G. Jones. It appeared in our June 2004 issue in the section ''Plants Interacting with Other Organisms.'' As of September 2006, it had been cited 35 times according to Thompson ISI (Thompson ISI Web of Science, http://www.isinet.com).

BACKGROUND

Plants have evolved multiple defense strategies for combating invading pathogens. The exterior surfaces of plants have waxy cuticles and preformed antimicrobials to prevent the entry of many would-be invaders. Cell walls provide an effective second barrier to any invaders that are able to gain access to interior spaces. Any invaders that overcome both barriers must still face the formidable task of overcoming the plant immune response. Plant immunity can be broken down into two components operating on different time scales. The basal defense system appears early in pathogen interaction, while the resistance (*R*) gene-mediated defense operates on the time scale of hours.

The early basal response is mediated by PAMPs, which include lipopolysaccharide, peptidoglycan, bacterial flagellin, and mannans of yeast (for review, see Nurnberger et al., 2004). The bacterial elongation factor EFTu has also been found to induce the innate immune response (Kunze et al., 2004). Since these compounds are not just associated with pathogens but also found in nonpathogens as well, ''pathogen associated'' is a bit of a misnomer (Ausubel, 2005). PAMPs are recognized by receptors located in the plasma membrane, activating a phosphorylation cascade upon binding, leading to the induction of early basal resistance (Gómez-Gómez and Boller, 2002) that plays a role in preventing colonization by nonpathogenic bacteria. Typically, this PAMP-triggered immunity is enough to halt infection before the microbe becomes established (for review, see Chisholm et al., 2006). Indeed, a connection between curbing of pathogen growth and the recognition of the PAMP flagellin by the receptor FLS2 has been demonstrated (Zipfel et al., 2004).

Flagellin, the major protein of flagella as well as a well characterized PAMP, has been shown to be recognized by the Leu-rich repeat receptor kinase FLS2 in Arabidopsis. FLS2, located in the plasma membrane, is believed to be involved in early bacterial-plant interaction by recognizing and binding flagellin. Bacterial effector proteins are an array of bacterial proteins shown to be involved in overcoming of host defense systems. Included among bacterial effector proteins are avirulence (Avr) factors that can interact with host R proteins, if present, as part of the gene-for-gene interaction (Dangl and McDowell, 2006; for review, see Chisholm et al., 2006).

An effective virulence strategy of plant (and animal) pathogens is to secrete effector proteins or DNA into the host cell to attempt to overcome plant defense systems. In phytopathogenic bacteria, there are three types of secretion systems. Type II, found in the genus Erwinia, is used for the secretion of cell wall-degrading enzymes causing soft-rot, while type IV transfers proteins and DNA of Agrobacterium. A third type, type III (T3SS), typical of Pseudomonas pathovars, secretes effector proteins into the plant cell (for review, see Abramovitch et al., 2006). T3SS is a multiprotein complex related to bacterial flagellum. In T3SS, bacteria in the apoplastic space form a pilus to inject the effector proteins into the plant cell. Fungal pathogens also secrete effector proteins, possibly via haustoria, but into the apoplast, not into the cell.

Gene-for-gene-mediated defense is inherited and is specific to a particular pathogen. Plants have dominant *R* genes whose products recognize those of the pathogen's complementary *Avr* alleles. Avr proteins are effector proteins secreted into the plant cell to promote pathogen virulence and to overcome host defenses. Localized programmed cell death, the hypersensitive response, is a hallmark of *R* gene-mediated defense and also a target of effector proteins.

WHAT WAS SHOWN

Navarro et al. (2004) were interested in investigating the ''possible connections between innate immunity,

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race-specific, and nonhost types of resistance responses." Arabidopsis cell cultures (Landsberg *erecta* [L*er*]) and seedlings (Columbia [Col-0]) were treated with flg22, a 22-amino acid epitope of the flagellin protein, and the major protein component of bacteria flagella.

Transcripts isolated from both systems were used for microarray analysis using high-density oligonucleotide arrays. Expression of 3.0% of transcripts was found to be altered due to the exposure of the cell culture or seedling to flg22. The majority of these Flagellin Rapidly Elicited (*FLARE*) genes were up-regulated, and 80% encoded proteins of known or predicted function. Not surprisingly, included in the up-regulated genes were those involved in stopping pathogen growth. The *FLARE* genes were functionally classified as belonging to one of the following groups: signal transduction-related, signal perception-related, effector proteins, or others. Many of the signal transduction-related genes include transcription factors and those involved in regulation of protein turnover. Among the signal transduction-related genes was a *R* gene to *AvrRpt2* (a *Pseudomonas syringae* Avr protein) along with receptor-like kinases. One surprising result was the inclusion of many auxin signaling-related genes with the down-regulated genes. A recent study by Navarro et al. (2006) investigated this further and found the mRNA for three auxin receptors to be negatively regulated by microRNA induced by flagellin perception. The effect of this down-regulation was an increased resistance to the *P. syringae* infection.

Although there was significant overlap in gene expression between the cell culture and the seedlings, some differences were observed. This could potentially arise from the dissimilar concentrations of flg22 used, differential response of the ecotypes, or due to differences between the cell culture and seedling systems. To address these possibilities, cell cultures of Col-0 and L*er* were treated with the same concentration of flg22, followed by reverse transcription-PCR on three genes that were up-regulated in the L*er* cultures in the previous experiment. Both cell cultures had similar patterns of induction and transcript levels, suggesting the difference was due to experimental system used and not due to ecotype variation.

Among the signal transduction-related genes, many were found to encode transcription factors, including several WRKY transcription factors. The WRKY superfamily of transcription factors is unique to plants and is involved in regulating diverse plant functions, including pathogen defense, senescence, and trichome development (for review, see Eulgem et al., 2000). Nine WRKY proteins were identified in this study, six new ones and three previously found to increase resistance to bacterial and fungal pathogens when overexpressed (Asai et al., 2002). The promoter region of a group of progressively induced genes was found to contain the WRKY consensus binding site. This group of genes was selected due to their clustering with an Arabidopsis ortholog of a tobacco (*Nicotiana tabacum*) gene induced in response to Avr9, an Avr protein from *P. syringae*. The authors interpreted this finding as suggesting "common regulatory processes involved during early race-specific and innate immune response."

When tobacco cell cultures are exposed to the *Cladosporium fulvum* effector protein Avr9, there is a rapid induction of a group of genes called Avr9/Cf-9 rapidly elicited (*ACRE*; Durrant et al., 2000). Avr9 is an effector protein; thus, response would be part of the gene-for-gene defense system. Similar to what is found with *FLARE* genes, *ACRE* genes are rapidly induced in response to Avr9 exposure. A search for orthologs of tobacco *ACRE* genes in Arabidopsis uncovered 32 putative *AtACRE* candidates, 14 of which were present on the array. Induction of *AtACRE* genes in response to flg22 was similar in both suspension cell and seedlings, with 13 rapidly and transiently and five progressively induced. These results suggest an overlap between the basal and gene-for-gene response to pathogens in Arabidopsis.

The *FLARE* gene set was compared with genes induced by different pathovars of *P. syringae* from both the innate and gene-for-gene interaction genes. A modest overlap (12%) between *FLARE* and nonhost-specific genes was found 3 h postinfection that then increased to 34% after 6 h. Less overlap was observed between "compatible interactions" and *FLARE* genes. The authors hypothesized that, in this instance, the initial response to flagellin could have been repressed.

In Arabidopsis seedlings exposed to the antibiotic cycloheximide prior to flg22 treatment, a majority (70%) of *FLARE* genes had similar transcription changes. When the seedlings were treated with cycloheximide alone, 82% of *FLARE* genes were induced. Together, this suggested negative regulation of *FLARE* genes by rapidly turned-over repressor proteins.

A potential model of early signaling events in Arabidopsis bacterial response was suggested by the work from this study. In the model, the detection of bacterial PAMPs in the apoplastic space by receptors such as FLS2 elicit early defense response inducing the expression of *FLARE* genes, including those potentially involved in the "rapid and transient induction of signaling-related genes." This study indicated that this can be achieved by the degradation of highly turned-over negative regulators since treatment of seedling with cycloheximide induced expression of *FLARE* genes. To counter this defensive response, bacteria "inject" effector proteins into the host cell via the T3SS. Such effectors include Avr proteins as well as those that potentially interfere with the early defense-signaling pathway. If the plant contains the R proteins corresponding to the bacterial Avr protein, the host gene-for-gene defense pathway is activated. Both of these plant responses to pathogen attack include ion fluxes, production of reactive oxygen species, and activation of MAPKs and CDPKs. Bacterial effector proteins would also target the gene-for-gene defense pathway to "suppress this elicitation."

THE IMPACT

The activities of bacterial effector proteins are currently being elucidated, and studies in plants have

indicated they are able to allow a nonpathogen to overcome some host defense systems as well as possibly function both as suppressors and inducers of plant resistance to the pathogen, as recently shown in tomato (*Solanum lycopersicum*) and bean (*Phaseolus vulgaris*; de Torres et al., 2006).

The study by de Torres et al. (2006) demonstrated that the effector protein AvrPtoB from *P. syringae* is able to suppress basal defenses in Arabidopsis in an ecotype-specific manor. *P. syringae* pv. *phaseolicola* strain RW60, which contains a fully functional type-III secretion system, was used as the delivery system for the effector protein since it does not cause a hypersensitive response in Arabidopsis and is not able to overcome any basal defense. Thus, any (gained) ability of the pathogen to colonize Arabidopsis would be due to the presence of the inserted effector protein. Arabidopsis plants were inoculated with RW60 expressing or not expressing *avrPtoB*, and the responses were categorized as (1) forming lesions, (2) some symptoms, or (3) no symptoms. As expected, none of the accessions tested had symptoms to RW60 on its own, but there was a range in response when the effector protein was expressed. Wassilewskija was the most susceptible, L*er* and Col (Col-0 or Col-5) displayed some symptoms, and Niedersenz remained symptomless. Genetic crosses between the resistant Niedersenz and susceptible Wassilewskija cultivars revealed the difference to be the lack of a functional flagellin receptor *FLS2* allele in Wassilewskija, in which the *FLS2* allele contains a stop codon resulting in a nonfunctional protein. One copy of the Niedersenz *FLS2* allele was sufficient to confer resistance, as both heterozygous and homozygous plants were symptom-free. Expression of a functional *FLS2* in Wassilewskija resulted in plants that were resistant to RW60 + *avrPtoB*. This finding is interesting in that FLS2 is a receptor involved in the early, basal PAMP-triggered immunity, a defense that is different from the gene-for-gene response. The dominance of the *FLS2* gene is reminiscent of an *R* gene. de Torres et al. (2006) suggest that the effector AvrPtoB ''acts on a signaling pathway common to FLS2 and other PAMP-receptor-mediated defenses,'' supporting the suggestion by Navarro et al. (2006) of a common signaling pathway between these two defense systems.

What are the proteins involved in early basal resistance? It is known that membrane receptors are involved, but are there soluble factors? The apoplastic fluid of tobacco leaf parenchyma was sampled to isolate such soluble proteins potentially involved in early resistance to pathogens (Ott et al., 2006). Two novel chitinases were the most prominent proteins. The appearance of the chitinases in the intercellular wash was quickly induced both before and during early basal resistance by nonpathogenic, saprophytic, and avirulent bacteria and was not altered by light or temperature, two stresses which are known to affect pathogenesis-related proteins, or by the defense-signaling compounds salicylic acid, jasmonic acid, and ethylene. These finding suggest that there are soluble proteins involved in early basal resistance that are also induced by PAMPs.

CONCLUDING REMARKS

As more details of plant immunity become known, the contributions of basal and gene-for-gene-mediated responses to bacterial pathogens are slowly becoming elucidated, and the distinction between them is becoming more vague. Studies such as the one by Navarro et al. (2004) are lending support to the idea of overlap between the two responses. A recent review by Abramovitch et al. (2006) suggests that it might be useful to reclassify the two responses by their timing and location in the plant to ''early, extracellular defenses and later, intracellular defenses.''

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