Nonhost resistance and nonspecific plant defenses

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In the past year, most of the advances in our understanding of nonhost resistance to plant pathogens have been incremental. Highlights include the discovery of a general bacterial elicitor of plant defenses, the description of more similarities between the hypersensitive response and animal programmed cell death, and a growing appreciation of the cell wall as the site of initiation and expression of nonhost resistance towards fungi.

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Abbreviations

avr	*avirulence*
HR	hypersensitive response
PCD	programmed cell death
PR1	pathogenesis-related 1
pth	*pathogenicity*
R	*resistance*
ROS	reactive oxygen species
TIR	Toll/Interleukin-1 receptor

Introduction

Resistance shown by an entire plant species to a specific parasite or pathogen is known as nonhost resistance, and is expressed by every plant towards the majority of potentially pathogenic microbes [1]. Nonhost resistance, therefore, is the most common form of disease resistance exhibited by plants. Nonhost resistance to fungi, at least, generally seems be under complex genetic control and can involve a mutiplicity of defense factors that, individually, may segregate within the species without compromising overall resistance [1,2]. Such resistance contrasts with host resistance, which is expressed by plant genotypes within an otherwise susceptible host species. Host resistance is usually parasite-specific in that it is restricted to a particular pathogen species, and commonly is expressed against specific pathogen genotypes. Variation in host resistance is often controlled by the segregation of single *resistance* (*R*) genes, the products of which directly or indirectly interact with 'specific elicitors' produced by the pathogen and coded for by *avirulence* (*avr*) genes [3].

It has been appreciated for over 70 years that plants have constitutive and inducible defenses that are potentially antimicrobial and that do not require the known *R* genes for their presence or activation. Interest has generally focused on inducible responses, which in the past few years have been shown to be part of a diverse, complex, and interrelated array of stress responses that can be elicited by many stimuli, as well as by microbial attack. Many inducible defense responses are involved in the expression of both host and nonhost resistance, suggesting that they can be elicited by both parasite-specific and -nonspecific signals.

Because a potential defense mechanism is only an actual defense mechanism if the potential pathogen is deleteriously affected by it, different defensive features may be important in different plant–microbe interactions [2]. A recent report [4•] has amply demonstrated that the *Arabidopsis* mutant *pad3-1* (*phytoalexin-deficient 3-1*), which is deficient in the production of an antimicrobial phytoalexin, displays similar levels of susceptibility to the bacterium *Pseudomonas syringae*, the biotrophic oomycete *Peronospora parasitica*, the biotrophic fungus *Erysiphe orontii*, and the necrotrophic fungus *Botrytis cinerea* as do wild-type plants. The *pad3-1* mutant is, however, more susceptible than wild-type plants to another necrotroph, *Alternaria brassicicola*. This review focuses on information published within the past year that has directly or indirectly enhanced our understanding of nonhost resistance to bacterial or fungal pathogens.

Preformed defenses

Despite the recent focus on inducible defensive responses in plants, there is considerable evidence that preformed defenses are a major component of nonhost resistance, particularly in non-domesticated plants [2]. Plants contain preformed peptides [5], proteins, and non-proteinaceous secondary metabolites that are potential deterrents against herbivory and/or microbial infection, and which may determine the host range of some fungal pathogens [6]. Although protease inhibitors were originally recognized as a defense against insects, some also have anti-fungal properties. The recent discovery that a cysteine protease inhibitor from pearl millet has its anti-fungal and anti-feedent activities at different reactive sites [7•] supports the idea that some nonspecific, broad-spectrum defenses in plants have evolved as the result of the collective selective pressure exerted by a number of parasites, herbivores, and abiotic stresses [2]. The number of identified preformed antimicrobial compounds is growing, as witnessed by last year's report of a new type of antimicrobial compound from strawberry that has particularly high activity against bacteria [8]; this finding is of interest as many plant toxins appear to have greater activity against fungi than bacteria (e.g. see [5]).

Elicitors of inducible defense responses

It is well established that a variety of fungal products can elicit inducible defensive plant responses in both host and nonhost plants, and that such responses can also be triggered by plant products released during cell-wall degradation. Indeed, it appears that sensitivity to oligosaccharide signals may have evolved before plants moved from water to land [9]. It seems a reasonable assumption, therefore, that these 'nonspecific elicitors' are the prime

inducers of defense responses in nonhost plant–pathogen interactions. New experimental evidence suggests that cryptogein, one of a family of proteinaceous elicitors produced by *Phytophthora* species, has binding sites on cells from both plant species that do and that do not defensively respond to the elicitor; this raises important questions about receptor and signal pathway differences between species [10••]. In contrast, a recent study involving elicitors from yeast and commercial enzyme preparations used to make plant protoplasts suggests that these elicitors directly interacted with lipid bilayers and formed large-conductance pores [11•]. The fact that some elicitors may not require a receptor-based mechanism for their activity is an important revelation that should influence subsequent studies of elicitor signaling.

Specific and nonspecific elicitors seem to trigger signal transduction cascades involving protein kinases, elements of the mitogen-activated protein (MAP) kinase pathway, and protein phosphatases [12,13]. Details of how elicitor signaling may lead to defense gene activation in nonhosts has recently been demonstrated for the oligopeptide elicitor from *Phytophthora sojae* interacting with parsley leaves and cells. Eulgem *et al.* [14•] report the cloning of *WRKY1*, a gene encoding a nucleus-located zinc-finger-type transcription factor, and show that a novel arrangement of palindromically positioned W boxes functions as a rapid-acting elicitor-response element that results in the expression of the gene. The WRKY1 transcription factor, perhaps activated by phosphorylation, binds to the W boxes within promotors of both its own and the PR1 (pathogenesis-related 1) target gene.

Nonspecific elicitors have been isolated with ease from fungi. Until recently, however, the only known equivalent elicitors from bacteria have been the cell-death-eliciting harpins, heat-stable proteins encoded by members of the *hrp* (*hypersensitive response pathogenicity*) gene cluster of some Gram-negative bacteria [13]. An exciting new discovery, therefore, is that some plant cell cultures respond to a conserved domain of flagellin, a component of the bacterial flagellum, with ion fluxes and other defensive responses [15••]. This represents the first example of a general bacterial elicitor, and its role in host-species specificity is further indicated by the fact that the corresponding flagellin domains of bacteria that have highly intimate associations with plants (e.g. *Agrobacterium tumefaciens* and *Rhizobium meliloti*) are inactive. Interestingly, one *Arabidopsis* ecotype, Ws-0, is insensitive to flagellin. Crosses involving this ecotype have revealed a dominant locus, *FLS-1* (*flagellin sensitive-1*) that is important for the perception of the flagellin signal [16••].

The hypersensitive response

The most common expression of host resistance, and a frequent expression of nonhost resistance, is the hypersensitive response (HR), a rapid death of cells at the infection site that is associated with pathogen limitation as well as with defense gene activation [17]. Some of the *avr* genes that control the HR response to bacterial pathogens in resistant hosts also seem to act as *pathogenicity* (*pth*) genes in susceptible plants. Gabriel [18•] has recently argued that, because of horizontal gene transfer between bacteria, some *avr* genes may be maladapted *pth* genes that 'inadvertently' elicit an HR in host and nonhost plants. Such a situation seems particularly likely if the nonhosts have *R*-gene products with which *avr*-gene products can interact [19]. Whether all examples of the HR in nonhost interactions with bacteria are caused by *avr* gene action is uncertain, and the roles of harpins and bacterial flagellin as nonspecific elicitors of the HR remain to be determined.

Plant cells commonly (but not always) respond to elicitors or microbial pathogens with an 'oxidative burst' during which reactive oxygen species (ROS) are generated, usually extracellularly [20]. Although the common assumption that ROS universally mediate hypersensitive cell death is beginning to be questioned [20,21], there is new evidence for the role of ROS in the hypersensitive cell death of plants showing nonhost resistance to bacteria. Mittler *et al.* [22•] found that transgenic tobacco plants expressing antisense RNA for the ROS scavengers cytosolic ascorbate peroxidase or catalase exhibited ion leakage in response to smaller numbers of a bacterial pathogen of bean than did control plants; enhancing or suppressing ROS-scavenging mechanisms using high oxygen pressure (either before or after inoculation) correspondingly reduced or enhanced cell death.

Hypersensitive cell death is now almost universally accepted as a form of programmed cell death (PCD), and the past few years have seen a plethora of studies investigating PCD in plants. Although key genes regulating apoptosis (a form of mammalian PCD) do not appear to have homologs in plants [21], there appear to be a growing number of functional similarities between animal PCD, plant PCD, and hypersensitive cell death (e.g. see [23,24]). Recent papers of special interest report that the overexpression of mammalian and nematode PCD suppressor genes in tobacco plants reduced host hypersensitive cell death induced by viral infection [25•]; likewise a death-promoting member of the same gene family triggered cell death when expressed in plants using a tobacco mosaic virus vector [26•]. The release of mitochondrial cytochrome *c* is critical for some forms of animal apoptosis, and recent papers have demonstrated apoptosis-like effects of cytochrome *c* both in plant protoplasts [27] and in mouse liver nuclei bathed in the cytosol of carrot cells [28]. The release of cytochrome *c* during heat-induced PCD in cucumber plants has also been reported [29]. It may, therefore, be significant that the bacterial death-elicitor harpin can reduce the capacity of mitochondrial cytochrome pathway electron transport [30].

Genes and genetic engineering

The common themes found in defense signaling in plants were illustrated recently by the finding that a gene

expressed in nonhost resistant marigold roots that were exposed to the vascular plant parasite *Striga asiatica* encodes a predicted cytoplasmic protein containing a TIR (Toll/Interleukin-1 receptor)-like domain. This domain is found in some *R* genes that control host resistance to microbial pathogens, although the marigold protein lacks a nucleotide-binding sequence that is found in such genes [31]. The TIR motif appears to be evolutionarily ancient [32] and, as some *R* genes of flax differ only in their TIR region, may be involved in pathogen recognition [33].

Because of the durability of nonhost resistance over time (pathogens have rarely altered their host species range over recorded history), it is commonly speculated that nonhost resistance could be exploited by plants breeders seeking to improve disease resistance within host species [1]. The overproduction of single nonspecific defense components in transgenic plants has been attempted with various degrees of success [34]. In one of the newest approaches, broad-spectrum disease resistance has been generated in transgenic tobacco by the introduction of an elicitin gene coupled to a pathogen-inducible promoter [35].

Resistance mechanisms

Despite all the information described above, research in the past year has added very little to our sparse knowledge of the actual mechanisms of nonhost resistance. For bacteria that are experimentally introduced into plant tissue or added to cell cultures, data generally suggest that defense gene activation may be more important than hypersensitive cell death in inhibiting pathogen growth (e.g. see [36]). This conclusion is supported by a recent study of nonhost resistance expressed prior to plant cell death [37]. Unlike bacteria, fungal attack is usually initiated from spores on external plant surfaces. Despite the apparent importance of the HR in the nonhost resistance of *Nicotiana* spp. to the oomycete *Phytophthora* [38] and the fact that successful fungal penetration of nonhost cells always induces an HR, nonhost resistance to fungi often does not involve cell death. Instead, nonhost resistance can be expressed prior to fungal entry into cells as mis-cues from the plant surface, or as inhibition within intercellular spaces following stomatal entry [39], or as growth restriction within cell walls [40].

For fungi that try to penetrate directly into epidermal cells, considerable evidence points to the cell wall as a primary site of nonhost resistance expression [41]. Indeed, recent studies suggest that the infection outcome in both host and nonhost plants may be determined while the fungus is growing within the cell wall [42,43]. Wall-associated defenses include the peroxidative cross-linking of phenolic compounds fueled by ROS generation [44], and the deposition of other substances such as silica [40] and callose-containing papillae, which combine to produce physical barriers to infection. The elicitors of these responses may be cell-wall components that are released as the fungus digests its way through the wall [45] and/or physical damage [46]. A new example of nonhost resistance expressed during wall penetration comes from a comparison of host and nonhost resistance in sorghum [47•]. In the nonhost interaction, the fungus does not successfully breach the cell wall apparently because of the earlier transcriptional activation of PR-10 and chalcone synthase, and the earlier accumulation of phytoalexins, than is the case in the host interaction.

There is plenty of evidence to show that unless a pathogen suppresses them, non-specific wall-associated defense responses are triggered in host as well as nonhost plants. In red onion epidermal cells resisting infection by *Botrytis allii*, phenolic compounds and peroxidase activity increase at infection sites in association with polarization of the actin cytoskeleton [48•]. The latter observation supports a general role for the actin cytoskeleton in the expression of wall-associated defense responses. Additional evidence of this role is provided by the increase in successful wall penetration caused by antimicrofilament agents in a number of nonhost interactions [49].

An important new revelation is that nonhost wall-associated responses to the fungi *Uromyces vignae* and *Erysiphe cichoracearum* are dependent not only on the actin cytoskeleton but also on the adhesion of the plasma membrane to the plant cell wall (DG Mellersh, MC Heath, unpublished data). This adhesion can be eliminated by peptides containing an RGD (i.e. Arg–Gly–Asp) motif, indicating some similarity between plant plasma-membrane–cell-wall interactions and those involved in the animal integrin-mediated communication system between the cytoskeleton and the extracellular matrix. *E. cichoracearum* also induces an RGD-insensitive increase in plasma-membrane–cell-wall adhesion in nonhost plants (DG Mellersh, MC Heath, unpublished data), suggesting the existence of multiple plasma-membrane–cell-wall interacting molecules of different functions in plants. Significantly, a cytoplasmic serine/threonine kinase that spans the plasma membrane and binds tightly to the cell wall has been reported and is encoded by the gene *Wak1* (*wall-associated receptor kinase 1*) in *Arabidopsis*. The expression of this gene is induced by compatible bacterial infection [50]. The data from *U. vignae* suggests that this fungus locally eliminates the adhesion of the plasma membrane and the cell wall in its host plant as a mechanism of eliminating wall-associated defenses (DG Mellersh, MC Heath, unpublished data).

Conclusions

The past year has seen a steady increase in our knowledge of the nature, elicitation, and regulation of microbial defenses in plants, and much of it is applicable to our understanding of nonhost resistance. Nevertheless, unequivocal identification of features that actually stop pathogen growth in a given nonhost plant is still rare (as it is for host resistance). For fungal pathogens that attempt to penetrate cells, recent data support the idea that highly localized responses within the cell wall and early signaling events play a primary role in nonhost resistance.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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