

Unique characteristics of *Xanthomonas oryzae* pv. *oryzae* AvrXa21 and implications for plant innate immunity

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This article provides a brief overview of some of the major concepts and molecular features of plant and animal innate immune systems. The rice pathogen recognition receptor, XA21, confers resistance to *Xanthomonas oryzae* pv. *oryzae* strains producing the AvrXa21 elicitor. Xa21 codes for a receptor-like kinase consisting of an extracellular leucine-rich repeat domain, a transmembrane domain, and a cytoplasmic kinase domain. We show that AvrXa21 activity requires the presence of *rax* (required for AvrXa21) A, *raxB*, and *raxC* genes that encode components of a type one secretion system. In contrast, an *hrcC*⁻ strain deficient in type three secretion maintains AvrXa21 activity. *Xanthomonas campestris* pv. *campestris* can express AvrXa21 activity if *raxST*, encoding a putative sulfotransferase, and *raxA* are provided in trans. Expression of *rax* genes depends on population density and other functioning *rax* genes. This and other data suggest that the AvrXa21 pathogen-associated molecule is involved in quorum sensing. Together these data suggest that AvrXa21 represents a previously uncharacterized class of Gram-negative bacterial signaling molecules. These results from our studies of the XA21/AvrXa21 interaction call for some modifications in the way we think about innate immunity strategies.

XA21 | pathogen-associated molecule pattern | type I secretion | quorum sensing | rice

Animals and plants both have well developed immune systems for protection against pathogen challenges. Adaptive immunity, specific to animals, depends on somatic gene rearrangements for generation of antigen receptors with random specificities. In contrast, innate immunity is common to metazoans and plants and involves perception of pathogen-associated molecular patterns (PAMPs) by pathogen recognition receptors (PRRs) (1). PAMPs have been defined as microbe-associated molecules that are conserved among diverse species and required for the microbe's lifecycle (2, 3). Representative PAMPs recognized by plants and/or animals that have been identified to date are flagellin, a proteinaceous component of bacterial polar flagella (4), the peptidoglycan of Gram-positive bacteria (5), lipopolysaccharide of Gram-negative bacteria (6), single-stranded viral RNA (7), and oomycete transglutaminase (8).

Detection of Pathogens by Plant and Animal Hosts

In animals, recognition of PAMPs in extracellular compartments largely is carried out by the Toll-like receptor (TLR) family, which contains extracellular leucine-rich repeats (LRRs) that act in ligand recognition and an intracellular Toll-interleukin 1 (TIR) domain (9). Although TLRs recognize diverse molecules, they activate a common signaling pathway to induce a core set of defense responses (10). Intracellular recognition largely is carried out by the cytoplasmic nucleotide-binding oligomerization domain (NOD) protein family. The NOD family contains a large number of proteins from animals, plants, fungi, and bacteria (11).

Evidence has accumulated that plants also detect PAMPs but, unlike animal systems, which have PRRs for cytoplasmic and extracellular perception of PAMPs, all biochemically character-

ized phytopathogen PAMPs are active at the cell surface (12). With notable exceptions, another general characteristic of PAMP recognition by plants is that, although it promotes expression of pathogenesis-related proteins and other characteristics of pathogen response, it does not lead to clear disease resistance or a related hypersensitive response, which includes localized plant cell death. For example, treatment of tobacco with lipopolysaccharide leads to increases in expression of genes associated with defense response but has no immediate effect on infection with virulent strains (6).

Surprisingly little is known about PAMP receptors in plants. The best characterized plant PRR for a PAMP is the *Arabidopsis thaliana* receptor-like kinase (RLK), FLS2 (flagellin sensing 2), which includes an extracellular LRR ligand-binding domain and an intracellular serine/threonine kinase and directly recognizes a conserved N-terminal fragment of bacterial flagellin (13, 14). Stimulation of FLS2 by flagellin activates pathogenesis-related gene expression, and pretreatment with it leads to resistance (15, 16). Recent studies have shown that the presence of FLS2 decreases host susceptibility to a pathovar of *Pseudomonas syringae* when the pathogen is applied to leaves by spraying but not infiltration (16, 17). This finding demonstrates a direct link between disease resistance and PAMP perception that previously had been lacking in plants. Another recently characterized plant receptor that perceives a PAMP is EFR (elongation factor Tu receptor) (18), which recognizes elf18, consisting of the first 18 aa of EF-Tu (elongation factor Tu) (19).

In addition to recognition of conserved PAMPs, plant PRRs also recognize strain-specific molecules produced by phytopathogens, termed pathogen avirulence factors (20, 21). This specific recognition generally triggers a strong defense response, often including the hypersensitive response. To explain the observation of interactions between dominant PRR genes and bacterial *avr* genes, H. H. Flor proposed the gene-for-gene hypothesis in which a single plant-gene product recognizes a single bacterial *avr* gene product and blocks disease formation (22). Twenty-one rice loci that confer genetically dominant

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Abbreviations: LRR, leucine-rich repeat; PAMP, pathogen-associated molecular pattern; PRR, pathogen recognition receptor; RLK, receptor-like kinase; *Xoo*, *Xanthomonas oryzae* pv. *oryzae*; NBS, nucleotide-binding site; TTSS, type three secretion system; TOSS, type one secretion system; R, arginine; D, aspartate; IRAK, interleukin 1 receptor-associated kinase; *Xcc*, *X. campestris* pv. *campestris*; QS, quorum sensing.

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resistance to bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) have been identified, and four of these genes have been cloned (reviewed in ref. 23).

The majority of plant PRR genes cloned to date code for cytoplasmically localized NOD family members, with a domain that contains a nucleotide-binding site (NBS) and a domain with LRRs (NBS-LRRs). All characterized NBS-LRRs that block bacterial pathogenesis recognize Avr proteins from pathogenic bacteria that are secreted through a large bacterial complex called a type three secretion system (TTSS). The TTSS is thought to function by transporting proteins, called effectors, directly into the cytoplasm of the host cell, and it is known to be an essential transport system for disease development and bacterial multiplication (24, 25). As recently reviewed in refs. 26 and 27, many Avr molecules rely on the TTSS for secretion and act as type III effectors. These molecules include AvrRpt2, AvrB, AvrRpm1, and AvrPto from *P. syringae*; XopD and AvrBsT from *Xanthomonas campestris*; and the three cloned Avr factors from *Xoo*, AvrXa7, AvrXa10, and AvrXa27. However, only a few type III effectors have known biochemical functions. Of those effectors that have been characterized, several are enzymes, such as proteases and phosphatases, that act on host protein substrates to interfere with, suppress, and manipulate host defense and signaling by defense hormones such as salicylic and jasmonic acid (28). A recent elegant example of this comes from the human pathogen *Yersinia*, in which the type III effector, YopJ, acetylates a mitogen-activated protein kinase (MAPK) kinase, blocking the site of its activation by phosphorylation (29). Others encode proteins with a nuclear localization signal and more directly modulate host transcription. For example, the AvrBs3/PthA family has a nuclear localization signal and an acidic transcription activation domain that is required for AvrBs3-dependent hypersensitive response (30). Few direct interactions have been reported between NBS-LRR PRRs and their corresponding effectors (31). Instead, the defense response often is triggered by interaction between the NBS-LRR and another plant protein, which is targeted or modified by the type III effector (32).

In addition to the cytoplasmic NBS-LRRs, two other classes of plant PRR that recognize Avr proteins have been described. These are the RLKs, composed of various putative ligand-binding extracellular domains and an intracellular kinase, and receptor-like proteins (RLPs), composed only of a membrane-anchored extracellular domain (e.g., tomato CF9) or a presumed secreted extracellular domain (e.g., rice XA21D) (33, 34). Relatively few plant RLKs and RLPs have been cloned and characterized to date, although, like NBS-LRRs, sequence analyses indicate that there are a large number of genes of these classes in plants (33). The best studied PRR RLK that confers a race-specific response is the rice XA21 protein, which recognizes *Xoo* strains carrying AvrXa21 activity (35). The three other dominant RLKs cloned and characterized to date are the rice Xa26 and Pi-d2 proteins and barley RPG1 (36–38). The potential ligand-binding capabilities of the extracellular domains of the RLK PRRs, and the facts that XA21 is present in microsomal fractions (39) and that Pi-d2 localizes to the cell membrane (37), suggest a simple model in which XA21 and other RLKs recognize Avr proteins in the extracellular space, directly or indirectly, as the tomato Cf RLPs do (40).

Recent discoveries, including the results described here, call for a blurring of the distinctions between PRR proteins that recognize PAMPs and those that recognize Avr proteins and other conceptual dichotomies that have been established in plant pathology. Rather, a continuum of classes seems to exist, with plants making use of a diversity of strategies for innate immunity (41). Although the AvrXa21 molecule(s) itself has not yet been identified, we show here that AvrXa21 activity (*i*) depends on a bacterial type one secretion system (TOSS) not on a TTSS, (*ii*)

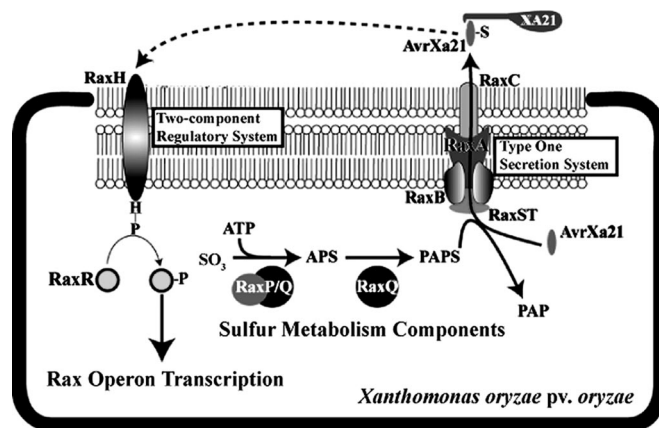


Fig. 1. Working model for the synthesis, regulation, and function of AvrXa21. Functions assigned to each of the *rax* gene products based on sequence homology and/or functional studies are as follows (43–45): RaxH, histidine kinase; RaxR, response regulator; RaxP, ATP sulfurylase; RaxQ, adenosine-5'-phosphosulfate kinase; RaxST, sulfotransferase; RaxA, membrane fusion protein, spanning the inner membrane and the periplasmic space; RaxB, ATP-binding cassette transporter; and RaxC, outer-membrane protein. See text for elaboration.

is regulated by a two-component regulatory system that responds to *Xoo* cell population density, and (*iii*) may be conserved in most *Xanthomonas* spp. Furthermore, we show that a high-pressure liquid chromatography (HPLC)-purified fraction carrying AvrXa21 activity can induce *raxST* gene expression at low cell density. These data suggest that AvrXa21 represents a previously uncharacterized class of signaling molecules, which is used by *Xoo* for quorum sensing (QS) and which does not fall into any of the previously described classes of PAMPs or Avr factors.

PRR XA21 Represents a Large Class of Kinases Predicted to be Involved in Innate Immunity

In our recent survey of kinases in yeast, fly, worm, human, *Arabidopsis*, and rice, a correlation was found between a function in innate immunity and the absence of a conserved arginine (R) adjacent to a conserved aspartate (D) in the activation loop in domain VII of interleukin 1 receptor-associated kinase (IRAK) family kinases (42). Of the 38 characterized IRAK family kinases in plants, all 6 RLKs associated with pathogen recognition fall into this “non-RD” class, as do 6 of the 9 kinases associated with early PRR signal transduction in animal innate immunity, IRAK and receptor-interacting protein kinases. Although there are only 7 non-RD IRAK-family kinases in humans, there are 47 in *Arabidopsis* and 371 in rice (42). Some of these may, like XA21, represent RLK PRRs that confer dominant and strong resistance to important diseases. Characterization of the molecule that XA21 recognizes could have significant impact toward understanding the mode of action of this large but poorly understood class of non-RD associated receptors.

AvrXa21 Activity Requires a TOSS

As previously described, we have cloned the *raxC* gene and seven additional genes in three operons (*raxSTAB*, *raxPQ*, and *raxRH*), that are required for AvrXa21 (*rax*) activity of *Xoo*, Philippine race 6 (strain PXO99) (43–45). Mutations in any of the eight *rax* genes allow this normally avirulent strain to form lesions when inoculated onto XA21-containing rice plants. Fig. 1 shows our working model for the action of the *rax* gene products in producing AvrXa21 activity. Based on sequence analysis and functional studies, the *rax* gene products can be grouped into three functional classes as follows: RaxP, RaxQ, and RaxST are sulfur metabolism enzymes; RaxA, RaxB, and RaxC form a

Table 1. Comparison of lesion lengths on rice leaves indicating that the backbone of the AvrXa21 PAM is conserved in *Xcc*

Supernatant pretreatment	Inoculation	Lesion length, cm
PXO99	<i>raxST</i> ⁻	1.3 ± 0.43
<i>raxST</i> ⁻	<i>raxST</i> ⁻	10.1 ± 5.2
<i>Xcc</i>	<i>raxST</i> ⁻	10.0 ± 4.8
<i>Xcc (raxSTAB)</i>	<i>raxST</i> ⁻	1.8 ± 0.8
<i>Xcc (raxSTA)</i>	<i>raxST</i> ⁻	2.0 ± 1.0
<i>Xcc (raxST)</i>	<i>raxST</i> ⁻	12.7 ± 7.3

Data are average ± SD from 10 scored leaves in three time repeats.

products, or that RaxST activity or stability is altered in the absence of RaxA. There are many examples of posttranslational modification affecting extracellular recognition. Typically, sulfated molecules are directed outside the cell to serve as modulators of cell–cell interactions (49). A notable example pertinent to agriculture is sulfation of the *Sinorhizobium meliloti* Nod factor that is required for specific recognition by its host, alfalfa (50).

Another element of the definition of a PAMP is that it is essential for the pathogen. The fact that the core of AvrXa21 appears to be conserved in *Xcc* suggests that the molecule(s) has a function that makes it selectively advantageous. In addition, although we did not observe differences in virulence in the *raxSTAB* and *raxC* knockout strains under controlled conditions, in a field study, 37 *Xoo* Korean strains lacking AvrXa21 activity appeared to have reduced fitness (51). These results suggest both that the core AvrXa21 molecule is conserved between *Xoo* and *Xcc* and that loss of AvrXa21 results in a fitness cost to *Xoo*. Therefore, AvrXa21, which is detected by the dominant, resistance-conferring XA21 PRR, shares characteristics of PAMPs and Avr proteins.

Cell Density-Dependent Expression of *rax* Genes

What is the function of AvrXa21? Like other living organisms, bacteria have developed multiple systems for responding to environmental variation, such as changes in temperature, osmolarity, pH, nutrient availability, and even population size. Two-component systems, composed of histidine kinases and response regulators, have an important role in sensing the environment (52). Phosphorylation of a response regulator by a histidine kinase regulates gene expression and governs the response to an environmental stimulus (53). Bacteria themselves produce some stimuli. In a process called “quorum sensing” (QS), small molecules serve as signals to recognize cell population size, leading to changes in expression of specific genes when a signal has accumulated to some threshold concentration (54). QS molecules regulate their own expression and are called autoinducers (55). Interestingly, recent findings demonstrate that the QS signal molecules are not only active in communication between bacteria but also can affect host immune responses. A QS molecule from *Pseudomonas aeruginosa* stimulates phagocytic activity in human macrophages through a MAPK pathway (56, 57).

To test whether *rax* gene expression depends on cell population, *rax* gene expression in wild-type *Xoo* was measured by using real-time quantitative PCR with three primer sets specific for each of the following four *rax* genes, one in each operon: *raxST*, in *raxSTAB* (Fig. 3A); *raxP*, in *raxPQ*; *raxR*, in *raxRH*; and *raxC* (data not shown). Remarkably, the expression of all four *rax* genes was observed only in the wild-type strain at high cell densities, whereas levels of a ribosomal RNA were unaffected (Fig. 3A). We also measured *rax* gene expression in a strain constitutively expressing RaxR. Density dependence of *rax* gene

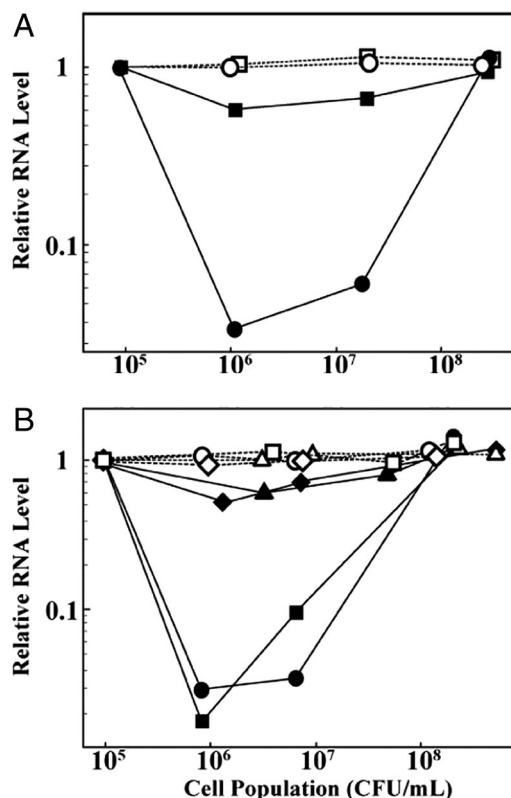


Fig. 3. Cell density and AvrXa21-dependent expression of the *raxST* gene (filled symbols) and rRNA (open symbols). *Xoo* bacteria were cultured for 72 h and then diluted with fresh media to $\approx 10^5$ cfu/ml. The diluted cultures were returned to the incubator, and, as they grew, aliquots were removed for RNA isolation at different cell population densities. Equal amounts (1 μ g) of bacterial RNA extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) were used for real-time quantitative PCR. Three primer sets for each *rax* gene were designed to amplify ≈ 300 -bp segments corresponding to three different parts of each gene. Primer sequences are provided in Table 3, which is published as supporting information on the PNAS web site. Expression levels are reported on a log scale and normalized to the copy number in the samples immediately after dilution. Data are from one of set of primers and two independent experiments. Similar trends were seen with all of the primer pairs. Expression is high at 10^5 cfu/ml because the bacteria in this sample behave as if they are still at high density. (A) Wild-type PXO99 (circles) and *raxR*⁻ (squares) strains. (B) Diluted PXO99 *Xoo* cultures were recultured without treatment (circles), with AvrXa21-active HPLC fractions (no. 2, triangles; no. 17, diamonds) or an inactive HPLC fraction (no. 24, squares).

expression in the *raxR* constitutive expressing strain is mostly lost (Fig. 3A). This result indicates that RaxR is involved in regulation of the density-dependent expression of the *rax* genes, including itself.

Furthermore, we tested *raxST* gene expression in the wild-type strain with and without treatment with HPLC-fractionated supernatant to address the question of whether a semipurified form of AvrXa21 could itself induce *rax* gene expression (Fig. 3B). Reverse-phase HPLC-C18 fractions purified from 10 ml of wild-type *Xoo* were tested for activity in the leaf-dip bioassay described above. Two bioactive fractions (nos. 2 and 17) and one randomly chosen inactive fraction (no. 24) were added separately to 10 ml of the diluted *Xoo* cells (10^5 cfu/ml) for reculture. Fig. 3B shows that treatment with both active fractions strikingly increases the *raxST* gene expression at low cell population density, whereas treatment with inactive fraction had less effect on *raxST* gene expression, similar to untreated cells. This auto-induction is consistent with a role of the product of the *rax* gene pathway, namely AvrXa21, in QS (55).

The characteristics of the *Xoo* *rax* genes suggest a QS system with features similar to those typically found in Gram-positive bacteria. For example, oligopeptides are the predominant signaling molecules for genetic competence in *Bacillus subtilis* (58), virulence in *Staphylococcus aureus* (59), and the production of antimicrobial peptides, inducing bacteriocins and lantibiotics, in lactic acid bacteria (60, 61). As QS molecules, these peptides also activate their own expression and have been given the name autoinducible peptides (AIPs). AIPs are known to be secreted by ABC transporters that are similar to RaxB (44) and are sensed by two-component systems. Significantly, secretion of the putative AvrXa21 peptide by a TOSS would be a previously undescribed occurrence of an AIP in Gram-negative bacteria.

In summary, our data suggest that the AvrXa21 elicitor is a secreted peptide and hint that the biological function of the AvrXa21 elicitor is as a QS signal molecule, the production of which is regulated in a cell-density-dependent manner in the Gram-negative bacteria *Xoo* by a two-component system. Thus, the rice XA21 PRR effectively eavesdrops on the AvrXa21-mediated molecular conversation among *Xoo*, using AvrXa21 as an indicator of the presence of a pathogenic bacterial population of significant size and leading to plant responses that effectively halt infection (Fig. 1). Sequence analysis of the genomes of several phytopathogenic bacteria that are similar to *Xoo* reveals an abundance of the *rax* classes of molecular components. *Xoo*, *Xcc*, *Xanthomonas axonopodis* pv. *citri*, and *Xylella fastidiosa* each possess genes for ≈ 30 two-component systems together with three to five TOSSs that, like RaxB, are predicted to transport peptides. Furthermore, it is apparent that *X. fastidiosa* does not use the TTSS for pathogenesis because genome analysis has revealed the absence of these components in this bacterium. Rather, *X. fastidiosa* contains TOSS genes similar to *raxA*, *raxB*, and *raxC* as well as sequences encoding as many as 10–12 peptides that are candidates for secretion (C. Dardick and P.C.R., unpublished results). These observations highlight that the interactions among phytopathogens, their hosts, and the environment are more sophisticated than are currently recognized.

Our data suggesting that the function of the AvrXa21 pathogen-associated molecule is as a QS signaling molecule may present a critical clue to answering the question, why is AvrXa21 maintained in *Xoo* regardless of its detection by a host protein? Based on others' results that suggest decreased fitness of *rax* mutants, AvrXa21 may be essential for *Xoo* as a signal molecule for cell–cell communication. We hypothesize that *Xoo* may use QS to coordinate infection and production of virulence factors. QS has global effects on bacterial growth, survival, and interactions with eukaryotes because QS-responsive genes compose up to 10% of the *P. aeruginosa* transcriptome (62). In that report, a large portion of QS-promoted genes code for membrane proteins and protein export apparatuses involved in secretion of virulence factors. A relationship between QS and virulence also has been described for the human pathogen *Vibrio cholerae* (63). Interestingly, in another case, a host can produce QS mimic molecules that interfere with the QS-regulated behaviors of the infecting bacteria (64). Given the abundance of other QS peptides and small molecules such as bacteriocins and lantibiotics in the host vicinity, it will not be surprising if these peptides are shown to play a role in the interaction of other bacteria with their hosts, as has been found for a homoserine lactone QS molecule from *P. aeruginosa* (57). In principle, recognition by plants of TOSS elicitors could be exploited to generate new types of specific and environmentally benign pesticides that induce plant defense responses.

Perspective

The data presented here as well as results of others in recent years establish that there is not a clear dichotomy between PAMP recognition and Avr recognition; rather, plant innate immunity functions as a continuum between the two general

types (Table 2, which is published as supporting information on the PNAS web site) (16). PAMPs have been defined as being derived from conserved structures required for pathogen function, whereas Avr factors are thought to be maintained by specific strains as virulence factors. The features of AvrXa21 are a hybrid between these two categories. The AvrXa21 molecule appears to be conserved in *Xcc*, but sulfation seems to provide specificity to the system, just as flagellin recognition by rice is modulated by glycosylation (65, 66). Further evidence that plant-recognized PAMPs are not entirely conserved comes from a recent article showing that sequence variation of flagellin among *Xcc* strains modulates FLS2-dependent pathogen recognition by *Arabidopsis* (16). Also, compromised virulence and survival of *avrXa21*⁻ strains in the field is consistent with a crucial role for AvrXa21 in Xanthomonad QS. On the other hand, AvrXa21 and XA21 fulfill the standard gene-for-gene model of the dominant resistance gene interacting with the bacterial virulence factor as well (35). Thus, AvrXa21 has features that are similar to both PAMPs and other Avrs.

The fusion of PRR recognition of Avr and PAMP classes proposed in this article has implications for the mechanism of plant innate immunity. Recognition of PAMPs by the animal innate immune system has been proposed to act as guide for the adaptive immune system as to the nature of the infecting pathogen (2). There is not an obvious need for such a function in plants because each receptor can be tied to a specific response through signal transduction. Nonetheless, it is clear that the plant innate immune system recognizes both conserved and less conserved molecules. As recently articulated (67), the fact that plant responses to PAMPs often do not lead to resistance may be attributable to evolutionary selection for plant innate immune perception to require “priming” or “two-hits” so that resources are not unnecessarily wasted in responding to nonpathogenic or small populations of bacteria. In such a model, PAMP perception constitutes an early warning, and, indeed, pretreatment with lipopolysaccharide or flg22 leads to resistance to subsequent treatments with normally pathogenic bacteria (6, 16, 17). However, it may be that the necessity for the factor that primes the plant innate immune system to be a PAMP rather than an Avr molecule is simply a matter of experimental history. Because they are less useful in an agricultural context, plant pathologists have not looked for PRRs and corresponding Avr molecules that do not lead to significant resistance. However, the XA21D receptor, which lacks a transmembrane and kinase domain and confers only partial resistance, may be such a molecule (34). XA21D would likely not have been discovered without experiments showing that its gene family member, Xa21, conferred complete resistance. Conversely, as mentioned above, FLS2-dependent resistance to bacteria that express flagellin also has been observed (17). Physiologically relevant experiments in diverse genetic backgrounds may lead to further evidence that responses mediated by PRRs for both more and less conserved pathogen-associated molecules (i.e., PAMPs and Avrs) are also part of a continuum, with some functioning more often in priming and others more often leading to immediate resistance. Already among described Avr PRRs there is a diversity of strengths, with some leading to superresistance (68). Thus, in possessing receptors for more conserved molecules, it may be that plants are simply taking advantage of the conserved nature of PAMPs as targets for recognition rather than making a functional distinction in terms of responses.

Several examples support the idea that plants gain a selective advantage by making use of a variety of strategies for pathogen recognition beyond the classes of molecules that typically have been discussed (27, 32), both in terms of the molecule detected and the cognate PRRs. AvrXa21 is currently the only known elicitor that is secreted by a TOSS. Until recently, LRRs constituted the only

signal recognition domain described for a cloned PRR RLK; however, a recently cloned RLK conferring rice blast resistance, Pi-d2, instead possesses an extracellular lectin-binding domain (37). Moreover, sequence analyses suggest that RLKs and receptor-like proteins with other, diverse extracellular binding domains also are involved in plant innate immunity (33, 42). Similarly, plants appear to use other, entirely different classes of molecules as PRRs as well. Indeed, the first cloned PAMP high-affinity binding site was the soybean β -glucan elicitor binding protein (69). Localized to the extracellular side of the cell membrane, it is predicted to be tethered to the membrane via interactions with a receptor complex (70). Yet another perception strategy is represented by Xa27, which codes for a defense protein the promoter of which serves as the PRR by directly or indirectly interacting with the cognate, nuclear-localized AvrXa27 type III effector (71).

Our recent results suggest that the AvrXa21 elicitor is a TOSS-dependent secreted peptide that represents a previously unde-

scribed family of signaling molecules for QS via a two-component system. Although QS molecules are known to be widely conserved and essential for bacterial communication, this example illustrates that such a QS molecule triggers plant innate immune responses. Our continuing work to identify the AvrXa21 molecule and understand the physical basis of its recognition may allow for identification of similar pathogen-associated molecules from plants and animal pathogens and their cognate host receptors.

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- Girardin SE, Sansonetti PJ, Philpott DJ (2002) *Trends Microbiol* 10:193–199.
- Medzhitov R, Janeway CA, Jr (1997) *Cell* 91:295–298.
- Janeway CA, Jr, Medzhitov R (2002) *Annu Rev Immunol* 20:197–216.
- Ramos HC, Rumbo M, Sirard JC (2004) *Trends Microbiol* 12:509–517.
- Leulier F, Parquet C, Pili-Floury S, Ryu JH, Caroff M, Lee WJ, Mengin-Lecreux D, Lemaître B (2003) *Nat Immunol* 4:478–484.
- Erbs G, Newman, M-A (2003) *Mol Plant Pathol* 4:421–425.
- Jurk M, Heil F, Vollmer J, Schetter C, Krieg AM, Wagner H, Lipford G, Bauer S (2002) *Nat Immunol* 3:499.
- Brunner F, Rosahl S, Lee J, Rudd JJ, Geiler C, Kauppinen S, Rasmussen G, Scheel D, Nurnberger T (2002) *EMBO J* 21:6681–6688.
- Werling D, Jungi TW (2003) *Vet Immunol Immunopathol* 91:1–12.
- Barton GM, Medzhitov R (2003) *Science* 300:1524–1525.
- Inohara N, Nunez G (2003) *Nat Rev Immunol* 3:371–382.
- Nurnberger T, Brunner F, Kemmerling B, Piater L (2004) *Immunol Rev* 198:249–266.
- Felix G, Duran JD, Volko S, Boller T (1999) *Plant J* 18:265–279.
- Chinchilla D, Bauer Z, Regenass M, Boller T, Felix G (2006) *Plant Cell* 18:465–476.
- Gomez-Gomez L, Felix G, Boller T (1999) *Plant J* 18:277–284.
- Sun W, Dunning FM, Pfund C, Weingarten R, Bent AF (2006) *Plant Cell* 18:764–779.
- Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JD, Felix G, Boller T (2004) *Nature* 428:764–767.
- Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) *Cell* 125:749–760.
- Kunze G, Zipfel C, Robatzek S, Niehaus K, Boller T, Felix G (2004) *Plant Cell* 16:3496–3507.
- Lindeberg M, Stavrinides J, Chang JH, Alfano JR, Collmer A, Dangi JL, Greenberg JT, Mansfield JW, Guttman DS (2005) *Mol Plant Microbe Interact* 18:275–282.
- van't Slot KAE, Knogge W (2002) *Crit Rev Plant Sci* 21:229–271.
- Flor HH (1971) *Annu Rev Phytopathol* 9:275–296.
- Niño-Liu DO, Ronald PC, Bogdanove AJ (2006) *Mol Plant Pathol* 7:303–324.
- Henderson IR, Navarro-Garcia F, Desvaux M, Fernandez RC, Ala'Aldeen D (2004) *Microbiol Mol Biol Rev* 68:692–744.
- Staskawicz BJ, Mudgett MB, Dangi JL, Galan JE (2001) *Science* 292:2285–2289.
- Mudgett MB (2005) *Annu Rev Plant Biol* 56:509–531.
- Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) *Cell* 124:803–814.
- Thomma BP, Penninckx IA, Broekaert WF, Cammue BP (2001) *Curr Opin Immunol* 13:63–68.
- Mukherjee S, Keitany G, Li Y, Wang Y, Ball HL, Goldsmith EJ, Orth K (2006) *Science* 312:1211–1214.
- Szurek B, Marois E, Bonas U, Van den Ackerveken G (2001) *Plant J* 26:523–534.
- Dodds PN, Lawrence GJ, Catanzariti AM, Teh T, Wang CI, Ayliffe MA, Kobe B, Ellis JG (2006) *Proc Natl Acad Sci USA* 103:8888–8893.
- Dangi JL, Jones JDG (2001) *Nature* 411:826–833.
- Shiu SH, Bleecker AB (2003) *Plant Physiol* 132:530–543.
- Wang GL, Ruan DL, Song WY, Sideris S, Chen L, Pi LY, Zhang S, Zhang Z, Fauquet C, Gaut BS, et al. (1998) *Plant Cell* 10:765–779.
- Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, et al. (1995) *Science* 270:1804–1806.
- Sun X, Cao Y, Yang Z, Xu C, Li X, Wang S, Zhang Q (2004) *Plant J* 37:517–527.
- Chen X, Shang J, Chen D, Lei C, Zou Y, Zhai W, Liu G, Xu J, Ling Z, Cao G, et al. (2006) *Plant J* 46:794–804.
- Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinhofs A (2002) *Proc Natl Acad Sci USA* 99:9328–9333.
- Xu W-H, Wang Y-S, Liu G-Z, Chen X, Tinjuangjun P, Pi L-Y, Song W-Y (2006) *Plant J* 45:740–751.
- Rooney HC, Van't Klooster JW, van der Hoorn RA, Joosten MH, Jones JD, de Wit PJ (2005) *Science* 308:1783–1786.
- McDowell JM, Woffenden BJ (2003) *Trends Biotechnol* 21:178–183.
- Dardick C, Ronald P (2006) *PLoS Pathog* 2:14–28.
- Shen Y, Sharma P, da Silva FG, Ronald P (2002) *Mol Microbiol* 44:37–48.
- Goes da Silva F, Shen Y, Dardick C, Burdman S, Yadav R, Sharma P, Ronald P (2004) *Mol Plant Microbe Interact* 17:593–601.
- Burdman S, Shen Y, Lee S-W, Xue Q, Ronald P (2004) *Mol Plant Microbe Interact* 17:602–612.
- Kauffman HE, Reddy APK, Hsieh SPY, Merca SD (1973) *Plant Dis Rep* 57:537–541.
- Zhu W, MaGbanua MM, White FF (2000) *J Bacteriol* 182:1844–1853.
- Wang GL, Song WY, Ruan DL, Sideris S, Ronald PC (1996) *Mol Plant Microbe Interact* 9:850–855.
- Bowman KG, Bertozzi CR (1999) *Chem Biol* 6:R9–R22.
- Roche P, Debelle F, Maillet F, Lerouge P, Faucher C, Truchet G, Denarie J, Prome J-C (1991) *Cell* 67:1131–1143.
- Choi SH, Lee SW, Han SS, Lee DK, Noh TH (2003) *Kor J Breed* 35:283–288.
- Lewis HA, Furlong EB, Laubert B, Eroshkina GA, Batiyenko Y, Adams JM, Bergseed MG, Marsh CD, Peat TS, Sanderson WE, et al. (2001) *Structure (London)* 9:527–537.
- Charles TC, Jin S, Nester EW (1992) *Annu Rev Phytopathol* 30:463–484.
- Fuqua WC, Winans SC, Greenberg EP (1994) *J Bacteriol* 176:269–275.
- Waters CM, Bassler BL (2005) *Annu Rev Cell Dev Biol* 21:319–346.
- Smith RS, Fedyk ER, Springer TA, Mukaida N, Iglewski BH, Phipps RP (2001) *J Immunol* 167:366–374.
- Vikstrom E, Magnusson, K-E, Pivoriunas A (2005) *Microbes Infect* 7:1512–1518.
- Tortosa P, Dubnau D (1999) *Curr Opin Microbiol* 2:588–592.
- Novick RP, Muir TW (1999) *Curr Opin Microbiol* 2:40–45.
- Risoen PA, Brurberg MB, Eijsink VG, Nes IF (2000) *Mol Microbiol* 37:619–628.
- Kleerebezem M, Quadri LE, Kuipers OP, de Vos WM (1997) *Mol Microbiol* 24:895–904.
- Wagner VE, Bushnell D, Passador L, Brooks AI, Iglewski BH (2003) *J Bacteriol* 185:2080–2095.
- Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL (2002) *Cell* 110:303–314.
- Teplitski M, Chen H, Rajamani S, Gao M, Merighi M, Sayre RT, Robinson JB, Rolfe BG, Bauer WD (2004) *Plant Physiol* 134:137–146.
- Taguchi F, Shimizu R, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y (2003) *Plant Cell Physiol* 44:342–349.
- Takeuchi K, Taguchi F, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y (2003) *J Bacteriol* 185:6658–6665.
- Ausubel FM (2005) *Nat Immunol* 6:973–979.
- Greenberg JT, Yao N (2004) *Cell Microbiol* 6:201–211.
- Umemoto N, Kakitani M, Iwamatsu A, Yoshikawa M, Yamaoka N, Ishida I (1997) *Proc Natl Acad Sci USA* 94:1029–1034.
- Mithofer A, Fliegmann J, Neuhaus-Url G, Schwarz H, Ebel J (2000) *Biol Chem* 381:705–713.
- Gu K, Yang B, Tian D, Wu L, Wang D, Sreekala C, Yang F, Chu Z, Wang GL, White FF, Yin Z (2005) *Nature* 435:1122–1125.