Recent Advances in PAMP-Triggered Immunity against Bacteria: Pattern Recognition Receptors Watch over and Raise the Alarm[1]

Valerie Nicaise[2], Milena Roux[2], and Cyril Zipfel*

Sainsbury Laboratory, Norwich NR4 7UH, United Kingdom

In an environment rich in potentially harmful microbes, plant survival depends on efficient microbe perception and fast defense responses. Contrary to the mammalian immune system composed of cells specialized for defense (e.g. lymphocytes), plant immunity relies on the ability of each cell to recognize pathogens. A first level of microbe recognition is performed by membrane proteins termed pattern recognition receptors (PRRs), which perceive molecular signatures characteristic of a whole class of microbes, termed pathogen-associated (or microbe-associated) molecular patterns (PAMPs; Medzhitov and Janeway, 1997). Perception of PAMPs by PRRs is common to all multicellular organisms and leads to an array of defense responses and redeployment of cellular energy in a fast, efficient, and multiresponse manner, which prevents further pathogen ingress. PAMP recognition leads to a chain of signaling events, broadly referred to as general defense responses in plants. PAMP perception also results in plant systemic acquired resistance (Mishina and Zeier, 2007b).

Faced with PAMP-triggered immunity (PTI), successful pathogens evolved secreted effectors targeting key PTI actors to interfere with plant defense. In turn, some plant cultivars have evolved resistance (R) proteins to directly or indirectly detect these effectors (previously termed avirulence or Avr proteins) according to the gene-to-gene theory and leading to effector-triggered immunity (ETI), which is often accompanied by the hypersensitive response, a form of programmed cell death. This model illustrates the dynamic coevolution between plants and pathogens (Chisholm et al., 2006; Jones and Dangl, 2006).

Gaining knowledge related to recognition and signaling in PTI constitutes a challenge in plant pathology research, as many of the underlying molecular mechanisms remain largely unknown. In this review, we summarize our knowledge of PTI with a special focus on recognition of bacteria.

PAMP RECOGNITION BY PRRS: THE PLANT SENTINELS ARE AT THE PLASMA MEMBRANE

PAMPs are molecular components highly conserved within a class of microbes, where they carry out an essential function for fitness or survival (Medzhitov and Janeway, 1997). Plants recognize a wide range of bacterial PAMPs, most of which are derived from structural components of the bacterial cell. Although the number of identified bacterial PAMPs recognized by plants is increasing constantly, very few plant PRRs have been discovered.

Flagellin and FLS2

The protein flagellin, the building block of the motility organ flagellum, is recognized by most plants, indicating that detection of flagellin is evolutionarily ancient (Boller and Felix, 2009). Synthetic peptides corresponding to a highly conserved part of the flagellin N terminus act as potent elicitors at subnanomolar concentrations (Felix et al., 1999), although a certain degree of recognition specificity exists. The peptide flg22 (corresponding to 22 amino acids localized in the conserved region) elicits responses in most plant species and is as active as the full-length flagellin. Interestingly, the flg22 region is required for bacterial virulence and motility, consistent with the fact that PAMP mutation has a fitness cost for microbes (Naito et al., 2008). Rice (*Oryza sativa*) was reported to be insensitive to flg22 (Felix et al., 1999). However, recent results showed that flg22 is recognized by rice but that this response is weaker than with full-length flagellin (Takai et al., 2008). A shorter version of the flg22 peptide, called flg15, acts as an antagonist in Arabidopsis (*Arabidopsis thaliana*), while it is fully active in tomato (*Solanum lycopersicum*; Meindl et al., 2000; Bauer et al., 2001; Robatzek et al., 2007). The recent finding that flg22, as well as flagellin, induces the hypersensitive response (Naito et al., 2008) revoked the apparent dogma that PAMPs generally do not induce this response. In addition, flagellins derived from nonadapted bacteria but having identical protein sequences differentially induce strong defense responses in nonhost plants, suggesting that other

[1] This work was supported by the Gatsby Charitable Foundation, the Biotechnology and Biological Sciences Research Council, European Research Area Network Plant Genomics, the Royal Society, and the Two Blades Foundation.

[2] These authors contributed equally to the article.

* Corresponding author; e-mail cyril.zipfel@tsl.ac.uk.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Cyril Zipfel (cyril.zipfel@tsl.ac.uk).

www.plantphysiol.org/cgi/doi/10.1104/pp.109.139709

domains and/or posttranslational modifications of flagellin are also recognized (Taguchi et al., 2003, 2006; Takeuchi et al., 2003, 2007). In rare examples, some virulent phytopathogenic bacteria are able to mask recognition of a PAMP (e.g. flagellin) by mutating residues within the recognized epitope (Felix et al., 1999; Pfund et al., 2004; Sun et al., 2006). This reflects a virulence strategy evolved by successful pathogens complementary to effector secretion. In other words, while flagellins from nearly all bacteria are recognized by plants, only a few from plant pathogenic bacteria are not.

The Leu-rich repeat receptor kinase (LRR-RK) FLS2 is the PRR for flagellin. It belongs to subfamily XII of LRR-RK and consists of an extracellular domain with 28 LRR motifs, a transmembrane domain, and a cytoplasmic Ser/Thr kinase domain (Gomez-Gomez and Boller, 2000). First identified in Arabidopsis, FLS2 orthologs have since been cloned in tomato, *Nicotiana benthamiana*, and rice (Hann and Rathjen, 2007; Robatzek et al., 2007; Takai et al., 2008). Although FLS2 directly binds flg22 and is responsible for recognition specificity, the flg22 binding site is still unknown (Chinchilla et al., 2006; Robatzek et al., 2007). Nevertheless, the AtFLS2 LRR domains 9 to 15 contribute significantly to flg22 binding (Dunning et al., 2007).

In Arabidopsis, pretreatment with flg22 restricts the growth of the pathogenic bacterium *Pseudomonas syringae* pv *tomato* DC3000 (*Pto* DC3000), and *fls2* mutants are more susceptible to this bacterium (Zipfel et al., 2004). In addition, lack of flagellin recognition allows more growth of the nonadapted bacteria *P. syringae* pv *phaseolicola* and *P. syringae* pv *tabaci* (Li et al., 2005; de Torres et al., 2006). These data demonstrate the importance of flagellin perception in innate immunity.

EF-Tu and EFR

The elongation factor Tu (EF-Tu) acts as a very potent bacterial PAMP in Arabidopsis and other members of the Brassicaceae family (Kunze et al., 2004). EF-Tu is one of the most abundant and conserved bacterial proteins. Similar to flg22 and flagellin, a synthetic peptide corresponding to the N-acetylated first 18 amino acids of EF-Tu, elf18, is sufficient for recognition, while the shorter peptide elf12 is an agonist in Arabidopsis (Kunze et al., 2004). Although EF-Tu is mostly intracellular, its release from lysis of dying bacteria during plant colonization should be sufficient to trigger its subnanomolar recognition. Moreover, EF-Tu is clearly present in the secretome of several bacteria and serves as an adhesion factor at the bacterial surface, in addition to its primary role in translation (Zipfel et al., 2006, and refs. therein).

The LRR-RK EFR is the PRR for EF-Tu and, like FLS2, belongs to subfamily XII (Zipfel et al., 2006). Similar to FLS2, the exact elf18 binding site is still unknown. EFR structure is highly similar to FLS2, with a 21-LRR extracellular domain, a transmembrane domain, and a cytoplasmic Ser/Thr kinase domain. As for FLS2, EFR autophosphorylation has been reported (Xiang et al., 2008), suggesting that both FLS2 and EFR carry active kinase domains. EF-Tu responsiveness was found only in Brassicaceae species (Kunze et al., 2004), suggesting that EFR is an innovation of this family. Nevertheless, genes with high similarities with *EFR* exist in Arabidopsis and other plants; their function as PRRs needs to be determined.

Arabidopsis plants lacking *EFR* are more amenable to transformation by *Agrobacterium tumefaciens*, revealing that plant transformation is normally restricted by plant defenses (Zipfel et al., 2006). In addition, *efr* mutant plants are more susceptible to colonization by weakly virulent mutant strains of *Pto* DC3000 (C. Zipfel, unpublished data). Interestingly, transient heterologous expression of *AtEFR* in *N. benthamiana*, a plant that normally lacks elf18 responsiveness, restores elf18 binding and responses (Zipfel et al., 2006), demonstrating that downstream signaling components are conserved between Brassicaceae and Solanaceae.

AvrXa21 and Xa21

In rice, the LRR-RK Xa21 confers resistance to *Xanthomonas oryzae* pv *oryzae* strains carrying the Avr gene *AvrXa21* (Song et al., 1995). Strikingly, Xa21 also belongs to subfamily XII of the LRR-RKs and is highly similar to EFR. Similar to FLS2 and EFR, Xa21 possesses a non-RD kinase, whose presence has been proposed to be correlated with a role in innate immunity across kingdoms (Dardick and Ronald, 2006). Although the identity of AvrXa21 was until now unknown, recent work identified AvrXa21 as a type I secreted sulfated peptide (da Silva et al., 2004; Lee et al., 2006, 2008; P. Ronald, personal communication). AvrXa21 is conserved among all *Xanthomonas* strains sequenced (P. Ronald, personal communication), suggesting that AvrXa21/Xa21 constitutes a PAMP/PRR perception system.

Orphan PAMPs

The bacterial cell wall is an important source of PAMPs. Peptidoglycans (PGNs) are polymers of alternating GlcNAc and *N*-acetyl-muramic acid residues in β-1-4 linkage that are cross-linked by short peptides. They constitute the major structural components of the gram-positive bacterial cell wall, while their presence is restricted to the periplasmic space in gram-negative bacteria. PGNs from both gram-positive and gram-negative bacteria are recognized by Arabidopsis (Gust et al., 2007; Erbs et al., 2008). However, while perception of gram-positive PGNs mostly depends on their sugar backbones (Gust et al., 2007), muropeptides derived from gram-negative PGNs are more potent elicitors than intact PGNs (Erbs et al., 2008).

Lipopolysaccharide (LPS) is the principal component of the outer membrane of gram-negative bacteria and

acts as a PAMP in dicots and monocots (Newman et al., 2007). It contains a long-chain polysaccharide, called O-antigen, which is highly variable with respect to composition, length, and the branching of its carbohydrate subunits. In contrast, the oligosaccharide core and the lipid A, which form the sheet of the membrane, are highly conserved in different bacteria. The lipid A part of LPS is as effective as intact LPS in inducing a defense response in Arabidopsis (Zeidler et al., 2004). Interestingly, the phosphorylation and acylation of the lipid A moiety seem to influence LPS elicitor activity (Silipo et al., 2008). In addition, synthetic oligorhamnans, which are common components of the otherwise highly variable O-chain in LPS, can trigger defense responses in Arabidopsis (Bedini et al., 2005). Intriguingly, in addition to activating defenses, LPS and other exopolysaccharides can suppress defense responses, for example by chelating calcium ions (Newman et al., 2007; Tellstrom et al., 2007; Aslam et al., 2008). Recently, rhamnolipids derived from *Pseudomonas aeruginosa* were identified as PAMPs recognized by grapevine (*Vitis vinifera*; Varnier et al., 2009). Cyclic lipopeptides derived from multiple strains of *Bacillus subtilis* have also been demonstrated to stimulate defense responses in tobacco (*Nicotiana tabacum*; Jourdan et al., 2009).

Similar to flg22 and elf18, the highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins (CSPs) acts as a PAMP in Solanaceae via the recognition of the 22-amino acid core of RNP-1 (CSP22; Felix and Boller, 2003). Other proteinaceous PAMPs perceived by plants include the superoxide dismutase SodM (Watt et al., 2006), harpins (Engelhardt et al., 2009), and Nep1 (for necrosis- and ethylene-inducing peptide 1)-like proteins (Qutob et al., 2006).

The bacterial siderophore pseudobactin is also a potential PAMP perceived by Arabidopsis (Meziane et al., 2005). Microbial nucleic acids are classical PAMPs recognized in mammals (Kawai and Akira, 2009). Very recently, bacterial nonmethylated CpG DNA was also shown to be recognized as a PAMP by Arabidopsis (Yakushiji et al., 2009).

The PRRs for all of these PAMPs are still unknown. The LysM motif can bind PGN (Buist et al., 2008) and is present in several receptor kinases and transmembrane proteins in plants (Zhang et al., 2007b), suggesting that they might function as PRRs for carbohydrate PAMPs. Interestingly, the legume Nod factor receptors involved in the establishment of rhizobial nitrogen-fixing symbiosis carry LysM motifs (Radutoiu et al., 2007). In addition, the rice LysM-containing transmembrane protein CeBiP directly binds the fungal PAMP chitin (Kaku et al., 2006), while the LysM-RK CERK1 is required for chitin responses in Arabidopsis (Miya et al., 2007; Wan et al., 2008). PGN, Nod factors, and chitin all contain GlcNAc moieties. Unexpectedly, CERK1 was recently shown to be also involved in bacterial recognition, as *cerk1* Arabidopsis mutants are more susceptible to *Pto* DC3000 (Gimenez-Ibanez et al., 2009). *cerk1* mutants, however, were not impaired in their responsiveness to flg22, elf18, LPS, or PGN (Gimenez-Ibanez et al., 2009; J. Rathjen and S. Gimenez-Ibanez, personal communication), suggesting that CERK1 is involved in the recognition of yet unknown bacterial PAMP(s). Whether CERK1 is a dual-specificity PRR capable of binding different PAMPs or acts as a downstream signaling adaptor needs to be determined.

IMMEDIATE EVENTS AT THE PLASMA MEMBRANE

BAK1: A Signaling Facilitator?

Formation of receptor complexes linking extracellular perception to intracellular signal transduction is a common theme in plant and animal signaling (Aker and de Vries, 2008). The LRR-RK BRI1 is the receptor for brassinosteroids (BRs), a class of phytohormones that control many aspects of growth and development (Vert et al., 2005). BRI1 forms a complex with the LRR-RK SERK1, SERK3/BAK1, and SERK4/BKK1 to ensure full BR signaling (Aker and de Vries, 2008). Unexpectedly, it has recently been demonstrated that FLS2 and BAK1 interact rapidly (less than 2 min) in a ligand-dependent manner (Chinchilla et al., 2007; Heese et al., 2007). The rapid FLS2-BAK1 association suggests that BAK1 may exist in a preformed complex at the membrane, weakly associated with FLS2. Conformational changes induced by flg22 binding would result in tighter interactions, possibly due to mutual transphosphorylation of the kinase domains. Although BAK1 is not required for flg22 binding, early and late flg22 responses are strongly impaired in *bak1* mutants (Chinchilla et al., 2007; Heese et al., 2007). *bak1* mutants also show reduced early elf18-triggered responses (Chinchilla et al., 2007), although a direct interaction between EFR and BAK1 has not yet been reported. In Arabidopsis and *N. benthamiana*, BAK1 is also required for responses triggered by the orphan PAMPs CSP22, HrpZ, PGN, and LPS (Heese et al., 2007; Shan et al., 2008). Together, these data demonstrate that BAK1 is a positive PTI regulator that acts downstream of several PRRs, independently of BR. The fact that BAK1 is involved in BR and PTI responses, as well as in cell death control (He et al., 2007; Kemmerling et al., 2007), suggests that BAK1 is a general signaling adaptor for RKs. Interestingly, a recent report showed that BRI1-BAK1 interaction leads to the transphosphorylation of their respective kinase domains and the subsequent enhancement of BRI1 signaling output (Wang et al., 2008b), suggesting that BAK1 is a signal "amplifier" rather than an integral component of downstream signaling pathways. It would be interesting to test if this model also applies for FLS2-BAK1 and whether CERK1 plays a similar role as BAK1 in BAK1-independent PTI responses.

Bacterial Virulence Effectors Directly Target PRRs and Their Associated Proteins

To infect plants, pathogens need to defeat PTI. Several recent studies clearly showed that one strategy

to do so is to directly target PRRs and their associated proteins by virulence effectors.

The model bacterium *Pto* DC3000 secretes more than 30 effectors (Jones and Dangl, 2006). Among them, AvrPto is a small triple helix protein that could act as a kinase inhibitor (Xing et al., 2007), and AvrPtoB contains an E3 ligase domain (Janjusevic et al., 2006). In resistant tomato plants, AvrPto and AvrPtoB are directly recognized by the cytoplasmic protein kinase Pto in plants carrying the nucleotide-binding site-LRR gene *Prf*, leading to ETI responses (Mucyn et al., 2006). Interestingly, ETI triggered by Pto and Prf in resistant tomato plants results from the inhibition of the AvrPtoB E3 ligase activity by Pto (Ntoukakis et al., 2009). In susceptible tomato and Arabidopsis plants, *AvrPto* contributes to virulence and inhibits all responses induced by several PAMPs (He et al., 2006; Hann and Rathjen, 2007; Xiao et al., 2007), suggesting that AvrPto might target very early PTI events. Indeed, based on homology between Pto, FLS2, and EFR kinase domains, Zhou and colleagues have shown that AvrPto interacts in vivo with FLS2 and EFR and inhibits their autophosphorylation in a dose-dependent manner (Xiang et al., 2008). In parallel, FLS2 and CERK1 have recently been identified as targets of AvrPtoB, leading to their degradation (Gohre et al., 2008; Shan et al., 2008; Gimenez-Ibanez et al., 2009). In addition, AvrPto and AvrPtoB are also able to target BAK1, thereby preventing the formation of PRR/BAK1 complexes (Shan et al., 2008). Together, these discoveries illustrate an effective strategy employed by pathogens to suppress PTI by directly targeting PRRs.

PRR Endocytosis

Analysis of FLS2-GFP fate using confocal microscopy revealed that FLS2-GFP is rapidly internalized into intracellular vesicles after flg22 treatment (Robatzek et al., 2006). This finding parallels the fact that other plant RKs are endocytosed (Shah et al., 2002; Russinova et al., 2004; Gifford et al., 2005; Geldner et al., 2007). FLS2 endocytosis depends on receptor activation, its PEST motif present in the cytoplasmic domain, the proteasome, cytoskeleton functions, and BAK1 (Robatzek et al., 2006; Chinchilla et al., 2007). Whether FLS2 internalization regulates its recycling, degradation, or signaling is still unclear.

Negative Regulation by Phosphatases and E3 Ubiquitin Ligases

Phosphorylation/dephosphorylation events are efficient regulatory mechanisms for signaling pathways involving kinases. The kinase-associated protein phosphatase (KAPP) is a member of the protein phosphatase 2C (PP2C) family. KAPP binds the kinase domain of FLS2 in yeast two-hybrid experiments (Gomez-Gomez et al., 2001), and transgenic Arabidopsis plants overexpressing KAPP are affected in flg22 binding and induced responses. Therefore, KAPP is a negative regulator of FLS2 (Gomez-Gomez et al., 2001). The fact that KAPP interacts with many plant RKs through their phosphorylated kinase domains (Chevalier et al., 2009) suggests that it is a general regulator of RKs. Another PP2C, the rice XB15 (for Xa21-binding protein 15), interacts both in vitro and in vivo with the kinase domain of Xa21 (Park et al., 2008). The dephosphorylation of Xa21 by XB15 inactivates the receptor and compromises Xa21-mediated bacterial resistance.

In mammals, E3 ligases are known to act as mediators of immune responses, via the degradation of negative regulators of PRR pathways as well as the activation of mitogen-activated protein kinase (MAPKs) and transcription factors (Liu et al., 2005; Moynagh, 2009). Recently, a triplet of plant U-box E3 ligases (PUBs), PUB22, PUB23, and PUB24, was shown to act as a negative regulator of PTI in response to various PAMPs in Arabidopsis (Trujillo et al., 2008), probably through the degradation of positive regulators or conceivably by interaction with PRRs. Conversely, a reduced level of the E3 ubiquitin ligase XB3, a substrate of the Xa21 kinase, correlates with a reduction of Xa21 accumulation and compromises Xa21-mediated resistance (Wang et al., 2006).

Heterotrimeric G Proteins

In mammals, the heterotrimeric G protein complexes (composed of three subunits, α, β, and γ) are associated to the plasma membrane and interact with specific receptors to initiate intracellular signaling cascades (Luttrell, 2006). Heterotrimeric G proteins are involved in many diverse physiological processes in plants (Temple and Jones, 2007; Chen, 2008; Gao et al., 2008b; Oki et al., 2009). The gene *AGB1*, encoding the β-subunit in Arabidopsis, is highly induced after flg22 and elf18 treatment (Zipfel et al., 2006). Based on this observation, Ishikawa (2009) investigated whether AGB1 is involved in FLS2- and EFR-mediated responses and showed that *agb1* mutants are impaired in the oxidative burst and seedling growth inhibition triggered by flg22 and elf18. Similarly, *XLG2* (for extra-large protein G 2) gene expression is induced after bacterial infection, and *xlg2* mutant plants are more susceptible to *P. syringae* (Zhu et al., 2009). How heterotrimeric G proteins regulate PTI responses is still unclear and requires further investigation.

DOWNSTREAM SIGNALING: PLANTS COUNTERATTACK

Ion Fluxes

The first easily detectable physiological response to PAMPs in plant cell cultures is the alkalinization of the growth medium. Occurring 0.5 to 2 min after elicitation, this event relies on drastic changes in ion fluxes across the plasma membrane (Nurnberger et al., 2004; Garcia-Brugger et al., 2006). Fluxes of H^+, K^+, Cl^-, and Ca^{2+} have been observed after PAMP treatment (Jabs

et al., 1997; Pugin et al., 1997; Garcia-Brugger et al., 2006).

Elevation of cytoplasmic calcium is a critical step in plant innate immunity and is mediated by an increase in Ca^{2+} influx (Ma and Berkowitz, 2007). The cyclic nucleotide-gated channel 2 mediates this influx after LPS perception (Ali et al., 2007). Changes in $[Ca^{2+}]_{cyt}$ are perceived by calcium-binding proteins such as calmodulin, calcium-dependent protein kinases, and calcineurin B-like proteins (Reddy and Reddy, 2004). Some of these have a demonstrated role in plant defense, particularly in the control of reactive oxygen species (ROS) and salicylic acid (SA) production (Chiasson et al., 2005; Takabatake et al., 2007; Xing et al., 2007; Galon et al., 2008; Du et al., 2009; Wang et al., 2009). Interestingly, the Arabidopsis S-locus RK CBRLK1 interacts with calmodulin and acts as a negative regulator of plant defense against bacteria (Kim et al., 2009a, 2009b). The importance of Ca^{2+} in defense signal transduction is further supported by the demonstration that Ca^{2+} chelation by bacterial exopolysaccharides is a virulence strategy used by pathogens to overcome PTI (Aslam et al., 2008).

Oxidative Burst

PAMPs induce rapid and transient production of ROS in an oxidative burst within a few minutes after treatment. ROS are highly toxic intermediates corresponding to reduced oxygen forms, such as the superoxide anion and hydrogen peroxide. ROS produced during pathogen challenge are largely derived from the activity of membrane-localized NADPH oxidases (respiratory burst oxidase homologs [Rboh]; Torres et al., 2006), with *AtRbohD* being the most important for PAMP-triggered oxidative burst (Meszaros et al., 2006; Nuhse et al., 2007; Zhang et al., 2007a). The relative position of oxidative burst in the sequence of signaling events during PTI is still unclear. In Arabidopsis, RbohD-dependent ROS production seems to be downstream or independent of MAPK activation (Zhang et al., 2007a).

From MAPKs to Defense Gene Expression

Protein phosphorylation occurs in diverse cellular processes as a means of controlling protein activity. Signaling via the MAPK network relies on directional and sequential phosphorylation events between three elements, MAPK kinase kinases, MAPK kinases, and MAPKs. MAPKs are involved in various processes in eukaryote cells, including plant defense (Colcombet and Hirt, 2008).

In Arabidopsis, a complete MAPK cascade including MEKK1-MKK4/5-MPK3/6 was initially proposed to be involved in PTI downstream of FLS2 (Asai et al., 2002). More recent work showed that MEKK1 does not regulate flg22-activated MPK3/6 but rather activates MPK4, known as a negative regulator of defense (Ichimura et al., 2006; Nakagami et al., 2006; Su et al., 2007; Suarez-Rodriguez et al., 2007; Gao et al., 2008a). At the MAPK kinase level, flg22-induced activation of MPK3/4/6 is dependent on MKK1, while MPK3 and MPK6 are also activated by MKK4 (Meszaros et al., 2006). Furthermore, MKK1 and MKK2 seem to act redundantly to control MPK4 (Gao et al., 2008a; Qiu et al., 2008). Thus, FLS2 activates two simultaneous MAPK cascades: one consists of an unknown MEKK-MKK4/5-MPK3/6 and acts positively on PTI, while the other, consisting of MEKK1-MKK1/2-MPK4, acts negatively on PTI.

During PTI, MAPK cascade activation leads to the activation of WRKY-type transcription factors, key regulators of plant defenses (Eulgem and Somssich, 2007; for review, see Pandey and Somssich, 2009). For example, the positive regulators WRKY22 and WRKY29 act downstream of the MPK3/6 cascade (Asai et al., 2002), while MPK4 directly regulates gene expression by interaction with WRKY25 and WRKY33 and the MPK4-interacting protein MKS1 (Andreasson et al., 2005; Zheng et al., 2007; Qiu et al., 2008). Interestingly, MPK4 exists constitutively in nuclear complex with MKS1 and WRKY33. Pathogen challenge leads to MPK4 activation and MKS1 phosphorylation, leading to the release of MKS1 and WRKY33 and the activation of gene expression (Qiu et al., 2008).

Although PAMPs trigger the simultaneous activation of positive (MPK3/6) and negative (MPK4) regulators of defense gene expression, these antagonistic pathways are regulated by the same PP2C phosphatase, AP2C1 (Schweighofer et al., 2007). It may seem counterintuitive, but in practice this may provide a sensitive mechanism for the control of defense responses by maintaining a careful balance of positive and negative regulators during signaling. Interestingly, MPK3 and MPK6 are directly targeted by the bacterial virulence effector HopAI1 via its phosphothreonine lyase activity (Zhang et al., 2007a).

MAPKs are involved in many different aspects of plant physiology, including stomata patterning (Wang et al., 2007b), ovule and anther development (Bush and Krysan, 2007; Wang et al., 2008a), and leaf senescence (Zhou et al., 2009). This poses the question of signal specificity, which may occur through the action of as yet unidentified parallel signaling pathways or through time- and location-specific expression (Colcombet and Hirt, 2008). The latter example was recently nicely illustrated during embryonic patterning, where an upstream regulator of the MAPK3 YODA, the receptor-like cytoplasmic kinase SSP, is specifically transcribed in the mature pollen but is only translated in the ovule, where SSP protein transiently accumulates (Bayer et al., 2009).

Callose Deposition

The accumulation of callose, a plant β-1,3-glucan polymer synthesized between the cell wall and the plasma membrane, is a classical marker of PTI re-

sponses after treatment with PAMPs or noninfectious pathogens (Bestwick et al., 1995; Brown et al., 1998; Gomez-Gomez et al., 1999). The callose synthase GSL5/PMR4 is responsible for callose synthesis in response to PAMPs and fungal pathogens in Arabidopsis (Jacobs et al., 2003; Nishimura et al., 2003; Kim et al., 2005). *pmr4* mutant plants allow 20-fold more growth than wild-type plants of the type 3 secretion system (TTSS)-deficient strain *Pto* DC3000 *hrcC*$^-$ (Kim et al., 2005), while the double mutant *pmr4 pad4* (which also reduces SA levels) allows some growth of the nonadapted bacterium *P. syringae* pv *phaseolicola* in comparison with the respective single mutants (Ham et al., 2007). This indicates that PMR4-dependent callose deposition contributes to antibacterial immunity. Although the order of events in PTI is still not clear, callose deposition may be downstream of ROS production, as *AtrbohD* mutants exhibit fewer callose deposits after flg22 treatment (Zhang et al., 2007a). Interestingly, callose deposition was recently shown to depend on PAMP-induced glucosinolates (Clay et al., 2009), components that are linked to antimicrobial immunity (Brader et al., 2001; Mishina and Zeier, 2007a; Bednarek et al., 2009; Clay et al., 2009).

Hormone Action

SA, jasmonic acid (JA), and ethylene (ET) function as classical defense hormones (Bari and Jones, 2009). Bacterial PAMPs, such as flg22, induce the production of SA (Mishina and Zeier, 2007a; Tsuda et al., 2008) that is required for both local and systemic acquired resistances (Durrant and Dong, 2004), consistent with the induction of systemic acquired resistance by flg22 and LPS (Mishina and Zeier, 2007b). Moreover, bacterial PAMPs induce the production of ET (Felix et al., 1999). Interestingly, the Arabidopsis enzyme 1-aminocyclopropane-1-carboxylate synthase 6, involved in ET biosynthesis, is a substrate of PAMP-activated MPK6 (Liu and Zhang, 2004; Joo et al., 2008). Despite the flg22-triggered production of SA and ET, local resistance induced by flg22 does not strictly depend on SA, ET, or JA pathways (Zipfel et al., 2004; Ferrari et al., 2007; Tsuda et al., 2008). However, disruption of key SA signaling components affects the expression of a subset of PAMP-regulated genes (Tsuda et al., 2008).

Flg22 up-regulates the expression of the Arabidopsis microRNA *miRNA393*, which reduces auxin receptor levels by targeting TIR1-like proteins (Navarro et al., 2006), and SA antagonizes auxin signaling by stabilizing auxin response repressors (Wang et al., 2007a). This suggests that PTI and auxin signaling pathways are antagonistic. Consistently, the phytopathogenic bacteria *Xanthomonas campestris* pv *campestris* and *Pto* DC3000 increase plant auxin levels (O'Donnell et al., 2003), potentially by up-regulating the expression of auxin biosynthetic genes (Schmelz et al., 2003).

Recent excellent reviews summarize the role of phytohormones in plant disease resistance in more detail (Spoel and Dong, 2008; Bari and Jones, 2009).

Stomatal Closure

Gaseous exchange and water transpiration influenced by environmental conditions are controlled by pores present in the epidermis of aerial plant organs, called stomata. During plant-pathogen interactions, stomata constitute one entry point for bacteria, which need to reach apoplastic spaces to multiply and cause disease. PAMP treatments induce stomatal closure (Lee et al., 1999; Melotto et al., 2006) in a manner dependent on abscisic acid, SA, K^+ fluxes, and heterotrimeric G proteins (Melotto et al., 2006; Zhang et al., 2008). Consistent with a role of stomatal closure to limit bacterial infection, the phytotoxin coronatine, a JA-Ile mimic secreted by the pathogenic bacterium *Pto* DC3000, reverts PAMP-induced stomatal closure (Melotto et al., 2006). Intriguingly, the Gly-rich binding protein 7, which plays a role in stomatal closure in response to abiotic stress (Kim et al., 2008), has been identified as a positive regulator of PTI against bacteria and is a target of the *Pto* DC3000 effector HopU1 (Fu et al., 2007).

Gene Silencing

Flg22 treatment leads to the rapid down-regulation of several primary auxin response genes (Navarro et al., 2004, 2006; Zipfel et al., 2004). This initial observation was later linked to the flg22-induced accumulation of the conserved microRNA *miRNA393* that targets the auxin receptor (TIR1) and its close paralogs (Navarro et al., 2006). Constitutive overexpression of *miRNA393* drastically restricts *Pto* DC3000 growth. Therefore, antibacterial immunity involves a rapid down-regulation of auxin responses mediated by RNA silencing. Consistently, *Pto* DC3000 effectors target the silencing machinery to achieve full virulence, with AvrPto interfering with the processing of *miRNA393*, while AvrPtoB leads to the degradation of *miRNA393* precursors (Navarro et al., 2008). In addition to *miRNA393*, *miRNA167* and *miRNA160*, which target auxin response factors/receptors to negatively regulate auxin signaling, are also induced after infection with *Pto* DC3000 *TTSS*$^-$ (Fahlgren et al., 2006). Hence, microRNA-deficient Arabidopsis mutants support the growth of *Pto* DC3000 *TTSS*$^-$ and the nonadapted bacterium *P. syringae* pv *phaseolicola* (Navarro et al., 2008), and Arabidopsis plants lacking *Argonaute4*, which is involved in RNA-directed DNA methylation, are more susceptible to *Pto* DC3000 and to the nonadapted strain *P. syringae* pv *tabaci* (Agorio and Vera, 2007). Thus, gene silencing appears as an inherent component of antibacterial immunity.

PERSPECTIVES

Perception of microbes by PRRs represents the first line of plant defense, relies on fast, efficient, and carefully coordinated reactions, and plays a major

role in disease resistance. This is now clearly illustrated by the recent finding that bacterial virulence effectors directly target PRRs and downstream components to cause disease. However, only a few plant PRRs have been identified so far, and our knowledge of the molecular mechanisms underlying PTI is still limited. Therefore, we need to identify more bacterial PAMPs and their corresponding PRRs, and not only from the classical models *Pto* DC3000 and Arabidopsis. Crystallographic studies of PAMP/PRR complexes are required to define PAMP-binding sites and to understand receptor activation. From the numerous signaling outputs occurring after PAMP perception, the identity of the molecular players and the exact sequence of signaling events need to be deciphered. Finally, we still do not know what actually restricts bacterial growth in planta.

ACKNOWLEDGMENTS

Pamela Ronald and John Rathjen are acknowledged for sharing unpublished results.

Received April 7, 2009; accepted June 23, 2009; published June 26, 2009.

LITERATURE CITED

Agorio A, Vera P (2007) ARGONAUTE4 is required for resistance to *Pseudomonas syringae* in *Arabidopsis*. Plant Cell **19:** 3778–3790

Aker J, de Vries SC (2008) Plasma membrane receptor complexes. Plant Physiol **147:** 1560–1564

Ali R, Ma W, Lemtiri-Chlieh F, Tsaltas D, Leng Q, von Bodman S, Berkowitz GA (2007) Death don't have no mercy and neither does calcium: *Arabidopsis* CYCLIC NUCLEOTIDE GATED CHANNEL2 and innate immunity. Plant Cell **19:** 1081–1095

Andreasson E, Jenkins T, Brodersen P, Thorgrimsen S, Petersen NH, Zhu S, Qiu JL, Micheelsen P, Rocher A, Petersen M, et al (2005) The MAP kinase substrate MKS1 is a regulator of plant defense responses. EMBO J **24:** 2579–2589

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in Arabidopsis innate immunity. Nature **415:** 977–983

Aslam SN, Newman MA, Erbs G, Morrissey KL, Chinchilla D, Boller T, Jensen TT, De Castro C, Ierano T, Molinaro A, et al (2008) Bacterial polysaccharides suppress induced innate immunity by calcium chelation. Curr Biol **18:** 1078–1083

Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant Mol Biol **69:** 473–488

Bauer Z, Gomez-Gomez L, Boller T, Felix G (2001) Sensitivity of different ecotypes and mutants of Arabidopsis thaliana toward the bacterial elicitor flagellin correlates with the presence of receptor-binding sites. J Biol Chem **276:** 45669–45676

Bayer M, Nawy T, Giglione C, Galli M, Meinnel T, Lukowitz W (2009) Paternal control of embryonic patterning in Arabidopsis thaliana. Science **323:** 1485–1488

Bedini E, De Castro C, Erbs G, Mangoni L, Dow JM, Newman MA, Parrilli M, Unverzagt C (2005) Structure-dependent modulation of a pathogen response in plants by synthetic O-antigen polysaccharides. J Am Chem Soc **127:** 2414–2416

Bednarek P, Pislewska-Bednarek M, Svatos A, Schneider B, Doubsky J, Mansurova M, Humphry M, Consonni C, Panstruga R, Sanchez-Vallet A, et al (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Science **323:** 101–106

Bestwick CS, Bennett MH, Mansfield JW (1995) Hrp mutant of *Pseudomonas syringae* pv. *phaseolicola* induces cell wall alterations but not membrane damage leading to the hypersensitive reaction in lettuce. Plant Physiol **108:** 503–516

Boller T, Felix G (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol **60:** 379–406

Brader G, Tas E, Palva ET (2001) Jasmonate-dependent induction of indole glucosinolates in Arabidopsis by culture filtrates of the nonspecific pathogen Erwinia carotovora. Plant Physiol **126:** 849–860

Brown I, Trethowan J, Kerry M, Mansfield J, Bolwell GP (1998) Localization of components of the oxidative cross-linking of glycoproteins and of callose synthesis in papillae formed during the interaction between non-pathogenic strains of *Xanthomonas campestris* and French bean mesophyll cells. Plant J **15:** 333–343

Buist G, Steen A, Kok J, Kuipers OP (2008) LysM, a widely distributed protein motif for binding to (peptido)glycans. Mol Microbiol **68:** 838–847

Bush SM, Krysan PJ (2007) Mutational evidence that the Arabidopsis MAP kinase MPK6 is involved in anther, inflorescence, and embryo development. J Exp Bot **58:** 2181–2191

Chen JG (2008) Heterotrimeric G-proteins in plant development. Front Biosci **13:** 3321–3333

Chevalier D, Morris ER, Walker JC (2009) 14-3-3 and FHA domains mediate phosphoprotein interactions. Annu Rev Plant Biol **60:** 67–91

Chiasson D, Ekengren SK, Martin GB, Dobney SL, Snedden WA (2005) Calmodulin-like proteins from Arabidopsis and tomato are involved in host defense against *Pseudomonas syringae* pv. *tomato*. Plant Mol Biol **58:** 887–897

Chinchilla D, Bauer Z, Regenass M, Boller T, Felix G (2006) The *Arabidopsis* receptor kinase FLS2 binds flg22 and determines the specificity of flagellin perception. Plant Cell **18:** 465–476

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nurnberger T, Jones JD, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature **448:** 497–500

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell **124:** 803–814

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM (2009) Glucosinolate metabolites required for an Arabidopsis innate immune response. Science **323:** 95–101

Colcombet J, Hirt H (2008) Arabidopsis MAPKs: a complex signalling network involved in multiple biological processes. Biochem J **413:** 217–226

Dardick C, Ronald P (2006) Plant and animal pathogen recognition receptors signal through non-RD kinases. PLoS Pathog **2:** e2

da Silva FG, Shen Y, Dardick C, Burdman S, Yadav RC, de Leon AL, Ronald PC (2004) Bacterial genes involved in type I secretion and sulfation are required to elicit the rice Xa21-mediated innate immune response. Mol Plant Microbe Interact **17:** 593–601

de Torres M, Mansfield JW, Grabov N, Brown IR, Ammouneh H, Tsiamis G, Forsyth A, Robatzek S, Grant M, Boch J (2006) *Pseudomonas syringae* effector AvrPtoB suppresses basal defence in Arabidopsis. Plant J **47:** 368–382

Du L, Ali GS, Simons KA, Hou J, Yang T, Reddy AS, Poovaiah BW (2009) $Ca^{(2+)}$/calmodulin regulates salicylic-acid-mediated plant immunity. Nature **457:** 1154–1158

Dunning FM, Sun W, Jansen KL, Helft L, Bent AF (2007) Identification and mutational analysis of *Arabidopsis* FLS2 leucine-rich repeat domain residues that contribute to flagellin perception. Plant Cell **19:** 3297–3313

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol **42:** 185–209

Engelhardt S, Lee J, Gabler Y, Kemmerling B, Haapalainen ML, Li CM, Wei Z, Keller H, Joosten M, Taira S, et al (2009) Separable roles of the *Pseudomonas syringae* pv. *phaseolicola* accessory protein HrpZ1 in ion-conducting pore formation and activation of plant immunity. Plant J **57:** 706–717

Erbs G, Silipo A, Aslam S, De Castro C, Liparoti V, Flagiello A, Pucci P, Lanzetta R, Parrilli M, Molinaro A, et al (2008) Peptidoglycan and muropeptides from pathogens *Agrobacterium* and *Xanthomonas* elicit plant innate immunity: structure and activity. Chem Biol **15:** 438–448

Eulgem T, Somssich IE (2007) Networks of WRKY transcription factors in defense signaling. Curr Opin Plant Biol **10:** 366–371

Fahlgren N, Montgomery TA, Howell MD, Allen E, Dvorak SK, Alexander AL, Carrington JC (2006) Regulation of AUXIN RESPONSE FACTOR3 by TAS3 ta-siRNA affects developmental timing and patterning in Arabidopsis. Curr Biol **16:** 939–944

Felix G, Boller T (2003) Molecular sensing of bacteria in plants: the highly

conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. J Biol Chem **278:** 6201–6208

Felix G, Duran JD, Volko S, Boller T (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J **18:** 265–276

Ferrari S, Galletti R, Denoux C, De Lorenzo G, Ausubel FM, Dewdney J (2007) Resistance to *Botrytis cinerea* induced in Arabidopsis by elicitors is independent of salicylic acid, ethylene, or jasmonate signaling but requires PHYTOALEXIN DEFICIENT3. Plant Physiol **144:** 367–379

Fu ZQ, Guo M, Jeong BR, Tian F, Elthon TE, Cerny RL, Staiger D, Alfano JR (2007) A type III effector ADP-ribosylates RNA-binding proteins and quells plant immunity. Nature **447:** 284–288

Galon Y, Nave R, Boyce JM, Nachmias D, Knight MR, Fromm H (2008) Calmodulin-binding transcription activator (CAMTA) 3 mediates biotic defense responses in Arabidopsis. FEBS Lett **582:** 943–948

Gao M, Liu J, Bi D, Zhang Z, Cheng F, Chen S, Zhang Y (2008a) MEKK1, MKK1/MKK2 and MPK4 function together in a mitogen-activated protein kinase cascade to regulate innate immunity in plants. Cell Res **18:** 1190–1198

Gao Y, Wang S, Asami T, Chen JG (2008b) Loss-of-function mutations in the Arabidopsis heterotrimeric G-protein alpha subunit enhance the developmental defects of brassinosteroid signaling and biosynthesis mutants. Plant Cell Physiol **49:** 1013–1024

Garcia-Brugger A, Lamotte O, Vandelle E, Bourque S, Lecourieux D, Poinssot B, Wendehenne D, Pugin A (2006) Early signaling events induced by elicitors of plant defenses. Mol Plant Microbe Interact **19:** 711–724

Geldner N, Hyman DL, Wang X, Schumacher K, Chory J (2007) Endosomal signaling of plant steroid receptor kinase BRI1. Genes Dev **21:** 1598–1602

Gifford ML, Robertson FC, Soares DC, Ingram GC (2005) *Arabidopsis* CRINKLY4 function, internalization, and turnover are dependent on the extracellular crinkly repeat domain. Plant Cell **17:** 1154–1166

Gimenez-Ibanez S, Hann DR, Ntoukakis V, Petutschnig E, Lipka V, Rathjen JP (2009) AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. Curr Biol **19:** 423–429

Gohre V, Spallek T, Haweker H, Mersmann S, Mentzel T, Boller T, de Torres M, Mansfield JW, Robatzek S (2008) Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. Curr Biol **18:** 1824–1832

Gomez-Gomez L, Bauer Z, Boller T (2001) Both the extracellular leucine-rich repeat domain and the kinase activity of FSL2 are required for flagellin binding and signaling in *Arabidopsis*. Plant Cell **13:** 1155–1163

Gomez-Gomez L, Boller T (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell **5:** 1003–1011

Gomez-Gomez L, Felix G, Boller T (1999) A single locus determines sensitivity to bacterial flagellin in Arabidopsis thaliana. Plant J **18:** 277–284

Gust AA, Biswas R, Lenz HD, Rauhut T, Ranf S, Kemmerling B, Gotz F, Glawischnig E, Lee J, Felix G, et al (2007) Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in Arabidopsis. J Biol Chem **282:** 32338–32348

Ham JH, Kim MG, Lee SY, Mackey D (2007) Layered basal defenses underlie non-host resistance of Arabidopsis to *Pseudomonas syringae* pv. *phaseolicola*. Plant J **51:** 604–616

Hann DR, Rathjen JP (2007) Early events in the pathogenicity of *Pseudomonas syringae* on *Nicotiana benthamiana*. Plant J **49:** 607–618

He K, Gou X, Yuan T, Lin H, Asami T, Yoshida S, Russell SD, Li J (2007) BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. Curr Biol **17:** 1109–1115

He P, Shan L, Lin NC, Martin GB, Kemmerling B, Nurnberger T, Sheen J (2006) Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in Arabidopsis innate immunity. Cell **125:** 563–575

Heese A, Hann DR, Gimenez-Ibanez S, Jones AM, He K, Li J, Schroeder JI, Peck SC, Rathjen JP (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. Proc Natl Acad Sci USA **104:** 12217–12222

Ichimura K, Casais C, Peck SC, Shinozaki K, Shirasu K (2006) MEKK1 is required for MPK4 activation and regulates tissue-specific and temperature-dependent cell death in Arabidopsis. J Biol Chem **281:** 36969–36976

Ishikawa A (2009) The Arabidopsis G-protein beta-subunit is required for defense response against *Agrobacterium tumefaciens*. Biosci Biotechnol Biochem **73:** 47–52

Jabs T, Tschope M, Colling C, Hahlbrock K, Scheel D (1997) Elicitor-stimulated ion fluxes and O_2^- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. Proc Natl Acad Sci USA **94:** 4800–4805

Jacobs AK, Lipka V, Burton RA, Panstruga R, Strizhov N, Schulze-Lefert P, Fincher GB (2003) An *Arabidopsis* callose synthase, GSL5, is required for wound and papillary callose formation. Plant Cell **15:** 2503–2513

Janjusevic R, Abramovitch RB, Martin GB, Stebbins CE (2006) A bacterial inhibitor of host programmed cell death defenses is an E3 ubiquitin ligase. Science **311:** 222–226

Jones JD, Dangl JL (2006) The plant immune system. Nature **444:** 323–329

Joo S, Liu Y, Lueth A, Zhang S (2008) MAPK phosphorylation-induced stabilization of ACS6 protein is mediated by the non-catalytic C-terminal domain, which also contains the cis-determinant for rapid degradation by the 26S proteasome pathway. Plant J **54:** 129–140

Jourdan E, Henry G, Duby F, Dommes J, Barthelemy JP, Thonart P, Ongena M (2009) Insights into the defense-related events occurring in plant cells following perception of surfactin-type lipopeptide from *Bacillus subtilis*. Mol Plant Microbe Interact **22:** 456–468

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc Natl Acad Sci USA **103:** 11086–11091

Kawai T, Akira S (2009) The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol **21:** 317–337

Kemmerling B, Schwedt A, Rodriguez P, Mazzotta S, Frank M, Qamar SA, Mengiste T, Betsuyaku S, Parker JE, Mussig C, et al (2007) The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. Curr Biol **17:** 1116–1122

Kim HS, Jung MS, Lee K, Kim KE, Yoo JH, Kim MC, Kim DH, Cho MJ, Chung WS (2009a) An S-locus receptor-like kinase in plasma membrane interacts with calmodulin in Arabidopsis. FEBS Lett **583:** 36–42

Kim HS, Jung MS, Lee SM, Kim KE, Byun H, Choi MS, Park HC, Cho MJ, Chung WS (2009b) An S-locus receptor-like kinase plays a role as a negative regulator in plant defense responses. Biochem Biophys Res Commun **381:** 424–428

Kim JS, Jung HJ, Lee HJ, Kim KA, Goh CH, Woo Y, Oh SH, Han YS, Kang H (2008) Glycine-rich RNA-binding protein 7 affects abiotic stress responses by regulating stomata opening and closing in *Arabidopsis thaliana*. Plant J **55:** 455–466

Kim MG, da Cunha L, McFall AJ, Belkhadir Y, DebRoy S, Dangl JL, Mackey D (2005) Two Pseudomonas syringae type III effectors inhibit RIN4-regulated basal defense in Arabidopsis. Cell **121:** 749–759

Kunze G, Zipfel C, Robatzek S, Niehaus K, Boller T, Felix G (2004) The N terminus of bacterial elongation factor Tu elicits innate immunity in *Arabidopsis* plants. Plant Cell **16:** 3496–3507

Lee S, Choi H, Suh S, Doo IS, Oh KY, Choi EJ, Schroeder Taylor AT, Low PS, Lee Y (1999) Oligogalacturonic acid and chitosan reduce stomatal aperture by inducing the evolution of reactive oxygen species from guard cells of tomato and *Commelina communis*. Plant Physiol **121:** 147–152

Lee SW, Han SW, Bartley LE, Ronald PC (2006) From the Academy: colloquium review. Unique characteristics of *Xanthomonas oryzae* pv. *oryzae* AvrXa21 and implications for plant innate immunity. Proc Natl Acad Sci USA **103:** 18395–18400

Lee SW, Jeong KS, Han SW, Lee SE, Phee BK, Hahn TR, Ronald P (2008) The *Xanthomonas oryzae* pv. *oryzae* PhoPQ two-component system is required for AvrXA21 activity, hrpG expression, and virulence. J Bacteriol **190:** 2183–2197

Li X, Lin H, Zhang W, Zou Y, Zhang J, Tang X, Zhou JM (2005) Flagellin induces innate immunity in nonhost interactions that is suppressed by *Pseudomonas syringae* effectors. Proc Natl Acad Sci USA **102:** 12990–12995

Liu Y, Zhang S (2004) Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in *Arabidopsis*. Plant Cell **16:** 3386–3399

Liu YC, Penninger J, Karin M (2005) Immunity by ubiquitylation: a reversible process of modification. Nat Rev Immunol **5:** 941–952

Luttrell LM (2006) Transmembrane signaling by G protein-coupled receptors. Methods Mol Biol **332:** 3–49

Ma W, Berkowitz GA (2007) The grateful dead: calcium and cell death in plant innate immunity. Cell Microbiol **9:** 2571–2585

Medzhitov R, Janeway CA Jr (1997) Innate immunity: the virtues of a nonclonal system of recognition. Cell **91:** 295–298

Meindl T, Boller T, Felix G (2000) The bacterial elicitor flagellin activates its receptor in tomato cells according to the address-message concept. Plant Cell **12:** 1783–1794

Melotto M, Underwood W, Koczan J, Nomura K, He SY (2006) Plant stomata function in innate immunity against bacterial invasion. Cell **126:** 969–980

Meszaros T, Helfer A, Hatzimasoura E, Magyar Z, Serazetdinova L, Rios G, Bardoczy V, Teige M, Koncz C, Peck S, et al (2006) The Arabidopsis MAP kinase kinase MKK1 participates in defence responses to the bacterial elicitor flagellin. Plant J **48:** 485–498

Meziane H, Van Der Sluis I, Van Loon LC, Hofte M, Bakker PAHM (2005) Determinants of Pseudomonas putida WCS358 involved in inducing systemic resistance in plants. Mol Plant Pathol **6:** 177–185

Mishina TE, Zeier J (2007a) Bacterial non-host resistance: interactions of Arabidopsis with non-adapted *Pseudomonas syringae* strains. Physiol Plant **131:** 448–461

Mishina TE, Zeier J (2007b) Pathogen-associated molecular pattern recognition rather than development of tissue necrosis contributes to bacterial induction of systemic acquired resistance in Arabidopsis. Plant J **50:** 500–513

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N (2007) CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc Natl Acad Sci USA **104:** 19613–19618

Moynagh PN (2009) The Pellino family: IRAK E3 ligases with emerging roles in innate immune signalling. Trends Immunol **30:** 33–42

Mucyn TS, Clemente A, Andriotis VM, Balmuth AL, Oldroyd GE, Staskawicz BJ, Rathjen JP (2006) The tomato NBARC-LRR protein Prf interacts with Pto kinase in vivo to regulate specific plant immunity. Plant Cell **18:** 2792–2806

Naito K, Taguchi F, Suzuki T, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y (2008) Amino acid sequence of bacterial microbe-associated molecular pattern flg22 is required for virulence. Mol Plant Microbe Interact **21:** 1165–1174

Nakagami H, Soukupova H, Schikora A, Zarsky V, Hirt H (2006) A mitogen-activated protein kinase kinase kinase mediates reactive oxygen species homeostasis in Arabidopsis. J Biol Chem **281:** 38697–38704

Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JD (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science **312:** 436–439

Navarro L, Jay F, Nomura K, He SY, Voinnet O (2008) Suppression of the microRNA pathway by bacterial effector proteins. Science **321:** 964–967

Navarro L, Zipfel C, Rowland O, Keller I, Robatzek S, Boller T, Jones JD (2004) The transcriptional innate immune response to flg22: interplay and overlap with Avr gene-dependent defense responses and bacterial pathogenesis. Plant Physiol **135:** 1113–1128

Newman MA, Dow JM, Molinaro A, Parrilli M (2007) Priming, induction and modulation of plant defence responses by bacterial lipopolysaccharides. J Endotoxin Res **13:** 69–84

Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H, Somerville SC (2003) Loss of a callose synthase results in salicylic acid-dependent disease resistance. Science **301:** 969–972

Ntoukakis V, Mucyn TS, Gimenez-Ibanez S, Chapman H, Gutierrez-Pulgar J, Balmuth AL, Jones A, Rathjen JP (2009) Host inhibition of a bacterial virulence effector triggers immunity to infection. Science **324:** 784–787

Nuhse TS, Bottrill AR, Jones AM, Peck SC (2007) Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant J **51:** 931–940

Nurnberger T, Brunner F, Kemmerling B, Piater L (2004) Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev **198:** 249–266

O'Donnell PJ, Schmelz EA, Moussatche P, Lund ST, Jones JB, Klee HJ (2003) Susceptible to intolerance: a range of hormonal actions in a susceptible Arabidopsis pathogen response. Plant J **33:** 245–257

Oki K, Inaba N, Kitagawa K, Fujioka S, Kitano H, Fujisawa Y, Kato H, Iwasaki Y (2009) Function of the alpha subunit of rice heterotrimeric G protein in brassinosteroid signaling. Plant Cell Physiol **50:** 161–172

Pandey SP, Somssich IE (2009) The role of WRKY transcription factors in plant immunity. Plant Physiol **150:** 1648–1655

Park CJ, Peng Y, Chen X, Dardick C, Ruan D, Bart R, Canlas PE, Ronald PC (2008) Rice XB15, a protein phosphatase 2C, negatively regulates cell death and XA21-mediated innate immunity. PLoS Biol **6:** e231

Pfund C, Tans-Kersten J, Dunning FM, Alonso JM, Ecker JR, Allen C, Bent AF (2004) Flagellin is not a major defense elicitor in *Ralstonia solanacearum* cells or extracts applied to *Arabidopsis thaliana*. Mol Plant Microbe Interact **17:** 696–706

Pugin A, Frachisse JM, Tavernier E, Bligny R, Gout E, Douce R, Guern J (1997) Early events induced by the elicitor cryptogein in tobacco cells: involvement of a plasma membrane NADPH oxidase and activation of glycolysis and the pentose phosphate pathway. Plant Cell **9:** 2077–2091

Qiu JL, Zhou L, Yun BW, Nielsen HB, Fiil BK, Petersen K, Mackinlay J, Loake GJ, Mundy J, Morris PC (2008) Arabidopsis mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1. Plant Physiol **148:** 212–222

Qutob D, Kemmerling B, Brunner F, Kufner I, Engelhardt S, Gust AA, Luberacki B, Seitz HU, Stahl D, Rauhut T, et al (2006) Phytotoxicity and innate immune responses induced by Nep1-like proteins. Plant Cell **18:** 3721–3744

Radutoiu S, Madsen LH, Madsen EB, Jurkiewicz A, Fukai E, Quistgaard EM, Albrektsen AS, James EK, Thirup S, Stougaard J (2007) LysM domains mediate lipochitin-oligosaccharide recognition and Nfr genes extend the symbiotic host range. EMBO J **26:** 3923–3935

Reddy VS, Reddy AS (2004) Proteomics of calcium-signaling components in plants. Phytochemistry **65:** 1745–1776

Robatzek S, Bittel P, Chinchilla D, Kochner P, Felix G, Shiu SH, Boller T (2007) Molecular identification and characterization of the tomato flagellin receptor LeFLS2, an orthologue of Arabidopsis FLS2 exhibiting characteristically different perception specificities. Plant Mol Biol **64:** 539–547

Robatzek S, Chinchilla D, Boller T (2006) Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis. Genes Dev **20:** 537–542

Russinova E, Borst JW, Kwaaitaal M, Cano-Delgado A, Yin Y, Chory J, de Vries SC (2004) Heterodimerization and endocytosis of *Arabidopsis* brassinosteroid receptors BRI1 and AtSERK3 (BAK1). Plant Cell **16:** 3216–3229

Schmelz EA, Engelberth J, Alborn HT, O'Donnell P, Sammons M, Toshima H, Tumlinson JH III (2003) Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. Proc Natl Acad Sci USA **100:** 10552–10557

Schweighofer A, Kazanaviciute V, Scheikl E, Teige M, Doczi R, Hirt H, Schwanninger M, Kant M, Schuurink R, Mauch F, et al (2007) The PP2C-type phosphatase AP2C1, which negatively regulates MPK4 and MPK6, modulates innate immunity, jasmonic acid, and ethylene levels in *Arabidopsis*. Plant Cell **19:** 2213–2224

Shah K, Russinova E, Gadella TW Jr, Willemse J, De Vries SC (2002) The Arabidopsis kinase-associated protein phosphatase controls internalization of the somatic embryogenesis receptor kinase 1. Genes Dev **16:** 1707–1720

Shan L, He P, Li J, Heese A, Peck SC, Nurnberger T, Martin GB, Sheen J (2008) Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. Cell Host Microbe **4:** 17–27

Silipo A, Sturiale L, Garozzo D, Erbs G, Jensen TT, Lanzetta R, Dow JM, Parrilli M, Newman MA, Molinaro A (2008) The acylation and phosphorylation pattern of lipid A from *Xanthomonas campestris* strongly influence its ability to trigger the innate immune response in Arabidopsis. ChemBioChem **9:** 896–904

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, et al (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science **270:** 1804–1806

Spoel SH, Dong X (2008) Making sense of hormone crosstalk during plant immune responses. Cell Host Microbe **3:** 348–351

Su SH, Suarez-Rodriguez MC, Krysan P (2007) Genetic interaction and phenotypic analysis of the Arabidopsis MAP kinase pathway mutations mekk1 and mpk4 suggests signaling pathway complexity. FEBS Lett **581:** 3171–3177

Suarez-Rodriguez MC, Adams-Phillips L, Liu Y, Wang H, Su SH, Jester PJ, Zhang S, Bent AF, Krysan PJ (2007) MEKK1 is required for flg22-

induced MPK4 activation in Arabidopsis plants. Plant Physiol **143:** 661–669

Sun W, Dunning FM, Pfund C, Weingarten R, Bent AF (2006) Within-species flagellin polymorphism in *Xanthomonas campestris* pv *campestris* and its impact on elicitation of *Arabidopsis* FLAGELLIN SENSING2-dependent defenses. Plant Cell **18:** 764–779

Taguchi F, Shimizu R, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y (2003) Post-translational modification of flagellin determines the specificity of HR induction. Plant Cell Physiol **44:** 342–349

Taguchi F, Takeuchi K, Katoh E, Murata K, Suzuki T, Marutani M, Kawasaki T, Eguchi M, Katoh S, Kaku H, et al (2006) Identification of glycosylation genes and glycosylated amino acids of flagellin in *Pseudomonas syringae* pv. *tabaci*. Cell Microbiol **8:** 923–938

Takabatake R, Karita E, Seo S, Mitsuhara I, Kuchitsu K, Ohashi Y (2007) Pathogen-induced calmodulin isoforms in basal resistance against bacterial and fungal pathogens in tobacco. Plant Cell Physiol **48:** 414–423

Takai R, Isogai A, Takayama S, Che FS (2008) Analysis of flagellin perception mediated by flg22 receptor OsFLS2 in rice. Mol Plant Microbe Interact **21:** 1635–1642

Takeuchi K, Ono H, Yoshida M, Ishii T, Katoh E, Taguchi F, Miki R, Murata K, Kaku H, Ichinose Y (2007) Flagellin glycans from two pathovars of *Pseudomonas syringae* contain rhamnose in D and L configurations in different ratios and modified 4-amino-4,6-dideoxyglucose. J Bacteriol **189:** 6945–6956

Takeuchi K, Taguchi F, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y (2003) Flagellin glycosylation island in *Pseudomonas syringae* pv. *glycinea* and its role in host specificity. J Bacteriol **185:** 6658–6665

Tellstrom V, Usadel B, Thimm O, Stitt M, Kuster H, Niehaus K (2007) The lipopolysaccharide of *Sinorhizobium meliloti* suppresses defense-associated gene expression in cell cultures of the host plant *Medicago truncatula*. Plant Physiol **143:** 825–837

Temple BR, Jones AM (2007) The plant heterotrimeric G-protein complex. Annu Rev Plant Biol **58:** 249–266

Torres MA, Jones JD, Dangl JL (2006) Reactive oxygen species signaling in response to pathogens. Plant Physiol **141:** 373–378

Trujillo M, Ichimura K, Casais C, Shirasu K (2008) Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in Arabidopsis. Curr Biol **18:** 1396–1401

Tsuda K, Sato M, Glazebrook J, Cohen JD, Katagiri F (2008) Interplay between MAMP-triggered and SA-mediated defense responses. Plant J **53:** 763–775

Varnier AL, Sanchez L, Vatsa P, Boudesocque L, Garcia-Brugger A, Rabenoelina F, Sorokin A, Renault JH, Kauffmann S, Pugin A, et al (2009) Bacterial rhamnolipids are novel MAMPs conferring resistance to *Botrytis cinerea* in grapevine. Plant Cell Environ **32:** 178–193

Vert G, Nemhauser JL, Geldner N, Hong F, Chory J (2005) Molecular mechanisms of steroid hormone signaling in plants. Annu Rev Cell Dev Biol **21:** 177–201

Wan J, Zhang XC, Neece D, Ramonell KM, Clough S, Kim SY, Stacey MG, Stacey G (2008) A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in *Arabidopsis*. Plant Cell **20:** 471–481

Wang D, Pajerowska-Mukhtar K, Culler AH, Dong X (2007a) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr Biol **17:** 1784–1790

Wang H, Liu Y, Bruffett K, Lee J, Hause G, Walker JC, Zhang S (2008a) Haplo-insufficiency of MPK3 in MPK6 mutant background uncovers a novel function of these two MAPKs in *Arabidopsis* ovule development. Plant Cell **20:** 602–613

Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S (2007b) Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis*. Plant Cell **19:** 63–73

Wang L, Tsuda K, Sato M, Cohen JD, Katagiri F, Glazebrook J (2009) Arabidopsis CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against Pseudomonas syringae. PLoS Pathog **5:** e1000301

Wang X, Kota U, He K, Blackburn K, Li J, Goshe MB, Huber SC, Clouse SD (2008b) Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. Dev Cell **15:** 220–235

Wang YS, Pi LY, Chen X, Chakrabarty PK, Jiang J, De Leon AL, Liu GZ, Li L, Benny U, Oard J, et al (2006) Rice XA21 binding protein 3 is a ubiquitin ligase required for full Xa21-mediated disease resistance. Plant Cell **18:** 3635–3646

Watt SA, Tellstrom V, Patschkowski T, Niehaus K (2006) Identification of the bacterial superoxide dismutase (SodM) as plant-inducible elicitor of an oxidative burst reaction in tobacco cell suspension cultures. J Biotechnol **126:** 78–86

Xiang T, Zong N, Zou Y, Wu Y, Zhang J, Xing W, Li Y, Tang X, Zhu L, Chai J, et al (2008) *Pseudomonas syringae* effector AvrPto blocks innate immunity by targeting receptor kinases. Curr Biol **18:** 74–80

Xiao F, He P, Abramovitch RB, Dawson JE, Nicholson LK, Sheen J, Martin GB (2007) The N-terminal region of *Pseudomonas* type III effector AvrPtoB elicits Pto-dependent immunity and has two distinct virulence determinants. Plant J **52:** 595–614

Xing W, Zou Y, Liu Q, Liu J, Luo X, Huang Q, Chen S, Zhu L, Bi R, Hao Q, et al (2007) The structural basis for activation of plant immunity by bacterial effector protein AvrPto. Nature **449:** 243–247

Yakushiji S, Ishiga Y, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y (2009) Bacterial DNA activates immunity in Arabidopsis thaliana. J Gen Plant Pathol **75:** 227–234

Zeidler D, Zahringer U, Gerber I, Dubery I, Hartung T, Bors W, Hutzler P, Durner J (2004) Innate immunity in *Arabidopsis thaliana*: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. Proc Natl Acad Sci USA **101:** 15811–15816

Zhang J, Shao F, Li Y, Cui H, Chen L, Li H, Zou Y, Long C, Lan L, Chai J, et al (2007a) A *Pseudomonas syringae* effector inactivates MAPKs to suppress PAMP-induced immunity in plants. Cell Host Microbe **1:** 175–185

Zhang W, He SY, Assmann SM (2008) The plant innate immunity response in stomatal guard cells invokes G-protein-dependent ion channel regulation. Plant J **56:** 984–996

Zhang XC, Wu X, Findley S, Wan J, Libault M, Nguyen HT, Cannon SB, Stacey G (2007b) Molecular evolution of lysin motif-type receptor-like kinases in plants. Plant Physiol **144:** 623–636

Zheng Z, Mosher SL, Fan B, Klessig DF, Chen Z (2007) Functional analysis of Arabidopsis WRKY25 transcription factor in plant defense against Pseudomonas syringae. BMC Plant Biol **7:** 2

Zhou C, Cai Z, Guo Y, Gan S (2009) An Arabidopsis mitogen-activated protein kinase cascade, MKK9-MPK6, plays a role in leaf senescence. Plant Physiol **150:** 167–177

Zhu H, Li GJ, Ding L, Cui X, Berg H, Assmann SM, Xia Y (2009) Arabidopsis extra large G-protein 2 (XLG2) interacts with the Gβ subunit of heterotrimeric G protein and functions in disease resistance. Mol Plant **2:** 513–525

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T, Felix G (2006) Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. Cell **125:** 749–760

Zipfel C, Robatzek S, Navarro L, Oakeley EJ, Jones JD, Felix G, Boller T (2004) Bacterial disease resistance in Arabidopsis through flagellin perception. Nature **428:** 764–767