Cellular Microbiology (2007) **9**(6), 1385–1396
doi:10.1111/j.1462-5822.2007.00944.x
First published online 19 April 2007

Microreview

Elicitation and suppression of microbe-associated molecular pattern-triggered immunity in plant–microbe interactions

Ping He, Libo Shan and Jen Sheen*
Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, MA 02114, USA.

Summary

Recent studies have uncovered fascinating molecular mechanisms underlying plant–microbe interactions that coevolved dynamically. As in animals, the primary plant innate immunity is immediately triggered by the detection of common pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs). Different MAMPs are often perceived by distinct cell-surface pattern-recognition receptors (PRRs) and activate convergent intracellular signalling pathways in plant cells for broad-spectrum immunity. Successful pathogens, however, have evolved multiple virulence factors to suppress MAMP-triggered immunity. Specifically, diverse pathogenic bacteria have employed the type III secretion system to deliver a repertoire of virulence effector proteins to interfere with host immunity and promote pathogenesis. Plants challenged by pathogens have evolved the secondary plant innate immunity. In particular, some plants possess the specific intracellular disease resistance (R) proteins to effectively counteract virulence effectors of pathogens for effector-triggered immunity. This potent but cultivar-specific effector-triggered immunity occurs rapidly with localized programmed cell death/hypersensitive response to limit pathogen proliferation and disease development. Remarkably, bacteria have further acquired virulence effectors to block effector-triggered immunity. This review covers the latest findings in the dynamics of MAMP-triggered immunity and its interception by virulence factors of pathogenic bacteria.

Received 2 February, 2007; revised 20 March, 2007; accepted 26 March, 2007. *For correspondence. E-mail sheen@molbio.mgh.harvard.edu; Tel. (+1) 617 726 5916; Fax (+1) 617 643 3050.

Introduction

Plants face constant threat from a wide range of microorganisms in their natural habitat. Without the adaptive immune system, plants rely on innate immunity to defend against most potential pathogens. The first line of plant innate immune response is triggered upon the detection of many common pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) that are not found in host cells (Boller, 1995; Nürnberger *et al*., 2004; Ausubel, 2005; Zipfel and Felix, 2005). The term MAMPs is used here since both non-pathogenic and pathogenic microbes produce effective MAMPs to activate immune responses (Ausubel, 2005; He *et al*., 2006; Jones and Dangl, 2006). Moreover, some pathogens actively suppress MAMP-triggered immunity (Kim *et al*., 2005; Li *et al*., 2005; He *et al*., 2006). The perception of different MAMPs by specific pattern-recognition receptors (PRRs) induces the defence responses that contribute plant immunity to both pathogenic and non-pathogenic microbes. In mammals, MAMPs are recognized by a limited number of PRRs, including Toll-like receptors (TLRs) and CATERPILLER/NOD-like receptors (NLRs), which detect distinct microbial components and activate immune responses (Ting and Davis, 2005; Akira *et al*., 2006; Fritz *et al*., 2006; Meylan *et al*., 2006). In contrast, there are hundreds of single-transmembrane receptor-like kinase (RLK) and receptor-like protein (RLP) genes in plant genomes. Some of them may play roles in the perception of different MAMPs in plants (Gomez-Gomez and Boller, 2000; Shiu and Bleecker, 2003; Shiu *et al*., 2004; Kaku *et al*., 2006; Zipfel *et al*., 2006).

The early immune responses downstream of MAMP perception share some common features in plants and animals, including the activation of conserved mitogen-activated protein kinase (MAPK) signalling cascades and the production of antimicrobial compounds (Nürnberger *et al*., 2004; Ausubel, 2005). In plants, different MAMPs likely activate convergent defence responses including changes in cytoplasmic Ca^{2+} levels, activation of MAPK cascades, induction of defence-related genes, the

production of phytoalexin, reactive oxygen species (ROS) and nitric oxide (NO), deposition of callose to reinforce the cell wall and stomatal closure to prevent bacterial entry (Ligterink *et al*., 1997; Felix *et al*., 1999; Lee *et al*., 2001; Asai *et al*., 2002; Fellbrich *et al*., 2002; Navarro *et al*., 2004; Zeidler *et al*., 2004; Ramonell *et al*., 2005; Daxberger *et al*., 2006; Kaku *et al*., 2006; Melotto *et al*., 2006; Qutob *et al*., 2006).

Notably, successful pathogens have acquired multiple virulence factors to suppress host immunity for their survival. Many Gram-negative pathogenic bacteria inject a set of effector proteins into host cells through the type III secretion system (T3SS) to interfere with host innate immunity (Büttner and Bonas, 2003; Alfano and Collmer, 2004; Beth Mudgett, 2005; Nomura *et al*., 2005; Abramovitch *et al*., 2006; Galan and Wolf-Watz, 2006; Grant *et al*., 2006). However, some of these specific virulence effector proteins are counteracted by the disease resistance (R) proteins in plants. Hundreds of plant genes encode putative R proteins that share conserved nucleotide-binding and leucine-rich repeat domains (NB-LRR) for pathogen sensing and defence signalling (Meyers *et al*., 2003). Although absent from flies and worms, NB-LRR proteins have recently been found in mammalian systems for intracellular MAMP sensing (Ting and Davis, 2005; Akira *et al*., 2006; Fritz *et al*., 2006; Meylan *et al*., 2006). Through direct or indirect interactions between specific effectors and R proteins, a second line of potent plant immunity, the so-called gene-for-gene resistance, is triggered. The gene-for-gene resistance, also termed effector-triggered immunity, occurs between a specific plant cultivar possessing an *R* gene and a pathogen carrying an avirulence (*avr*) gene (Chisholm *et al*., 2006). The effector-triggered immunity is probably one of the most powerful forms of plant defence responses, and has been widely used by farmers and extensively studied by geneticists for decades. Several excellent recent reviews provide detailed information on the current understanding of effector-triggered immunity (Abramovitch *et al*., 2006; Chisholm *et al*., 2006; DeYoung and Innes, 2006; Jones and Dangl, 2006). This review focuses on the most recent breakthroughs of the molecular mechanisms underlying MAMP-triggered immunity to detect and fight off potential infections, and how successful bacterial pathogens subsequently switch it off to promote pathogenicity.

MAMP perception through cell-surface LRR-RLK receptors

Plants respond to a wide array of MAMPs from both non-pathogenic and pathogenic microbes, including bacterial flagellin, lipopolysaccharide (LPS), elongation factor EF-Tu, and harpin (HrpZ), oomycete-derived Pep13, NEP1-like proteins (NLPs) and β-glucan, as well as fungal chitin and ergosterol (Boller, 1995; Ligterink *et al*., 1997; Zhang *et al*., 1998; Cardinale *et al*., 2000; Desikan *et al*., 2001; Lee *et al*., 2001; Asai *et al*., 2002; Fellbrich *et al*., 2002; Wan *et al*., 2004; Daxberger *et al*., 2006; Qutob *et al*., 2006; Zipfel *et al*., 2006). The perception of several MAMPs is mediated by cell-surface receptors. The best-studied plant PRR is *Arabidopsis* flagellin receptor FLS2, a RLK protein with extracellular LRR (Gomez-Gomez and Boller, 2000). The 22 amino acid peptide (flg22) corresponding to the highly conserved amino terminus of flagellin is sufficient to trigger immune responses in *Arabidopsis,* tomato, tobacco and barley but not rice (Felix *et al*., 1999; Peck *et al*., 2001; Taguchi, 2003; Chinchilla *et al*., 2006; Hann and Rathjen, 2007; Shen *et al*., 2007). Affinity cross-linking and immunoprecipitation demonstrate direct interaction of flg22 with FLS2 (Chinchilla *et al*., 2006), but do not exclude the requirement of additional plant factors in receptor complex signalling. The established method will facilitate future studies of the receptor complexes for the initiation of MAMP-induced signalling.

Recently, one of the most abundant and conserved bacterial proteins, elongation factor EF-Tu from *E. coli* and many other bacteria, has been found to function as an MAMP in *Arabidopsis*. An N-acetylated peptide comprising the first 18 amino acids, termed elf18, is fully active (Kunze *et al*., 2004). Treating *Arabidopsis* seedlings with elf18 or flg22 induces a common set of responses including whole genome transcriptome profiles (Zipfel *et al*., 2006). Affinity cross-linking labelled a putative receptor protein, which was later identified as EF-Tu receptor (EFR) by a reverse genetic screen of mutants in LRR-RLKs related to FLS2 (Zipfel *et al*., 2006). The *efr* mutant is more susceptible to *Agrobacterium tumefaciens* infection. Intriguingly, EF-Tu has been found on the surface of *Mycoplasma pneumoniae* and *Lactobacillus johnsonii,* and may play a role in adhesion to mammalian host cells and activation of inflammatory responses (Dallo *et al*., 2002; Granato *et al*., 2004).

Many genes encoding LRR-RLKs, such as *FLS2* and *EFR*, are MAMP-inducible (Navarro *et al*., 2004; Zipfel *et al*., 2004; Qutob *et al*., 2006; Thilmony *et al*., 2006; Truman *et al*., 2006). However, the level of receptor protein is tightly controlled upon ligand binding. New evidence suggests that the receptors undergo endocytosis after MAMP signal perception (Robatzek *et al*., 2006). In the presence of flg22, membrane localized FLS2 quickly accumulates in intracellular vesicles that are likely followed by degradation. Mutational analysis indicates that T867 located in the intracellular juxta membrane region of FLS2 is critical for both FLS2 internalization and response. The cytoplasmic region of FLS2 possesses a PEST-like motif, implicated in ubiquitin-triggered receptor

endocytosis. A proteasome inhibitor MG132 blocks FLS2 internalization and degradation (Robatzek *et al*., 2006). It will be important to further define the cellular and molecular steps that connect FLS2 phosphorylation, ubiquitination and endocytosis with downstream signalling.

MAMP perception through cell-surface receptors without a kinase domain

MAMP receptors for certain fungal proteins and chitin fragments have been identified as cell-surface receptors without kinase domain in plants (Ron and Avni, 2004; Kaku *et al*., 2006). The 22-kDa fungal protein ethylene-inducing xylanase (EIX) activates defence responses independent of its enzymatic activity in many plant species (Furman-Matarasso *et al*., 1999). Two tomato genes, *LeEix1* and *LeEix2*, have been identified by map-based cloning. They encode proteins with EIX binding activities when ectopically expressed in plant and mammalian cells (Ron and Avni, 2004). The EIX receptors contain a leucine zipper, an extracellular LRR domain, a transmembrane domain and a C-terminal domain with a mammalian endocytosis signal. The structure of EIX receptors is similar to a family of RLPs, such as tomato Cf2 and Cf9, the plasma membrane R proteins that respond to specific extracellular fungal avirulence (Avr) proteins for effector-triggered immunity. FLS2 and EFR also resemble a rice R protein XA21 as an LRR-RLK recognizing a specific molecule secreted from a pathogenic bacterium *Xanthomonas oryzae* pv *oryzae* (*Xoo*) (Lee *et al*., 2006).Thus, the signalling events acting downstream of MAMP and extracellular effector perception by cell-surface PRRs may share certain common features. Transcriptome and cDNA-AFLP analyses of early response genes identified some shared target genes activated by flg22 in *Arabidopsis* and Avr9-Cf9 signalling in tobacco (Navarro *et al*., 2004). It remains a future research challenge to precisely determine the shared or distinct signalling pathways triggered by MAMPs, extracellular and intracellular effectors.

The rice transmembrane protein with two extracellular LysM motifs binds to fungal cell wall elicitor, chitin oligosaccharide (Kaku *et al*., 2006). The LysM motif is found among both prokaryotes and eukaryotes, and was proposed to function as a peptidoglycan- and chitin-binding site. Interestingly, two RLKs with LysM motif have been recently indicated as the receptors of Nod-factor, a lipochitin-oligosaccharide produced by rhizobial bacteria to establish symbiosis with legume plants (Madsen *et al*., 2003; Radutoiu *et al*., 2003). Thus, the LysM motifs of these receptors could recognize both MAMP signal for defence and rhizobial signal for symbiosis, two distinct outcomes in plant–microbe interactions.

MAMP perception through intracellular receptors

Although the mammalian cell-surface receptor TLR5 has been identified for bacterial flagellin (Akira *et al*., 2006), latest findings provide evidence that flagellin can also be delivered into the cytosol of mammalian macrophages during infection to trigger innate immune responses (Franchi *et al*., 2006; Miao *et al*., 2006; Molofsky *et al*., 2006; Ren *et al*., 2006; Subramanian and Qadri, 2006). Cytosolic flagellin of *Salmonella* is sensed by the procaspase-1-activating protein Ipaf, a member of the NOD-like receptors (NLRs) closely related to plant intracellular NB-LRRs for effector-triggered immunity (Franchi *et al*., 2006; Miao *et al*., 2006). Many mammalian NLRs are intracellular receptors for MAMPs, bacterial toxins and danger signals (Fritz *et al*., 2006; Meylan *et al*., 2006). Most strikingly, cytosolic flagellin-mediated immune responses require bacterial T3SS, but not the evolutionarily related flagellar secretion system (Miao *et al*., 2006). Introduction of purified flagellin into the cytoplasm activates Ipaf-dependent immune responses. From an evolutionary point of view, T3SS, a potent bacterial weapon, seems to mistakenly secrete some MAMPs that are recognized by hosts to trigger immune responses. Cytosolic flagellin of *Legionella pneumophila* is perceived by Naip5, a member of NLRs, and activates caspase-1-mediated macrophage death (Molofsky *et al*., 2006; Ren *et al*., 2006). It seems that the pore-forming activity of *L. pneumophila* type IV secretion system is required for cytosolic flagellin immune responses in macrophage (Molofsky *et al*., 2006). Currently, no evidence has emerged yet to support the existence of cytosolic MAMP receptors in plants. We have observed that non-host bacteria induce stronger defence responses than their T3SS mutants (P. He, L. Shan and J. Sheen, unpubl. data). It is possible that either type III effectors in these bacteria or MAMPs including structural components of the T3SS travel through T3SS to trigger defence responses in host cells that warrants further characterization.

Specificity of MAMP perception

In general, MAMPs are evolutionarily conserved molecules that are present in many microbes and are often essential for their fitness, survival or virulence. However, a recent report has revealed surprisingly narrow recognition specificity of bacterial flagellin proteins at the subspecies level (Sun *et al*., 2006). The significant variation of defence-eliciting ability occurs among flagellin purified from different strains of *Xanthomonas campestris* pv *campestris* (*Xcc*). A single amino acid polymorphism in *Xcc* flagellin is critical for the FLS2-mediated defence-eliciting ability. However, the mutation of FLS2 does not significantly affect the growth of virulent *Xcc* strains and

symptom development in *Arabidopsis* using different infection assays (Sun *et al*., 2006). In contrast, FLS2 is important in the defence against the virulent *Pseudomonas syringae tomato* DC3000 in *Arabidopsis* (Zipfel *et al*., 2004). The finding suggests that the contribution of flagellin to elicit plant immunity may vary in different bacterial species, and raises the question that some MAMPs may exist as variable but not conserved products. MAMP specificity is not solely determinated by sequence variation. For example, the flagellin from *P. s. glycinea*, but not from *P. s. tabaci*, triggers defence responses in tobacco although the flagellins from these two bacterial strains share identical sequence. A post-translational modification, glycosylation, of flagellin is responsible for the difference (Takeuchi *et al*., 2003; Taguchi *et al*., 2006). Apparently, some successful pathogens can acquire active mechanisms to modify MAMPs in order to avoid host recognition. However, most microbes are likely recognized by plant innate immune systems through the perception of multiple and distinct MAMPs.

MAMP perception can also be variable in different plants. For example, EF-Tu as an MAMP is recognized by *Arabidopsis* but not plants outside the *Brassicaceae* (Zipfel *et al*., 2006). Pep-13, a surface-exposed peptide of a cell wall transglutaminase in *Phytophthora*, acts as an MAMP in parsley and potato but not in *Arabidopsis* (Nürnberger *et al*., 1994; Brunner *et al*., 2002). The perception of flg22 in *Arabidopsis* by FLS2 displays characteristics distinct from that in tomato and rice, suggesting the specificity in ligand–receptor interactions (Felix *et al*., 1999; Chinchilla *et al*., 2006). There are also a large family of NLP proteins produced by bacteria, fungi and oomycetes that play dual roles as MAMPs to elicit innate immune responses as well as phytotoxins to induce cell death in dicotyledonous plants but not monocots (Qutob *et al*., 2006). Continued evolution of MAMPs and their PRRs may be more dynamic than previously expected.

Convergent intracellular signalling triggered by MAMPs

The recent genome-wide transcriptome analyses have provided solid evidence for remarkable convergent signalling downstream of MAMP receptors. A set of largely overlapping genes are activated or repressed by different MAMPs, including bacterial flg22 and EF-Tu, and oomycete NLP (Zipfel *et al*., 2004; Qutob *et al*., 2006; Zipfel *et al*., 2006). The convergent MAMP signalling is also evident from the whole plant-bacteria infection experiments, in which the bacterial mutants with deficiency in flagellin production do not significantly alter the MAMP-triggered regulation of transcript profile at the genome level (Thilmony *et al*., 2006). These results collectively suggest that the responses to multiple MAMPs converge at a very early step very likely immediately after signal perception (He *et al*., 2006). The convergence of MAMP signalling is probably an evolutionary advantage for plants to secure MAMP-triggered immunity. The modification of individual MAMPs by microbes will not significantly dilute the overall MAMP-triggered immunity. Consequently, it has been difficult to study the functions of individual MAMPs using whole microbial organisms that carry multiple MAMPs. Many unknown MAMPs remain to be discovered.

MAPK signalling cascades in MAMP-triggered immunity

Activation of MAPK signalling cascades is a convergent and immediate-early response in MAMP-triggered immunity (Tena *et al*., 2001; Zhang and Klessig, 2001; Pedley and Martin, 2005). A MAPK cascade generally involves a MAPKKK(MTK)-MAPKK(MKK)-MAPK(MPK) module that transduces extracellular signals through the receptors into a wide range of intracellular responses in eukaryotic cells. *Arabidopsis* genome encodes at least 20 MPKs (MAPK Group, 2002). *Arabidopsis* MPK3, MPK4 and MPK6, and their orthologues in other plant species are rapidly activated by multiple MAMPs including bacterial elicitors flg22, HrpZ, EF-Tu, oomycete elicitors Pep 13, NPP1 and β-glucan and fungal elicitor chitin and ergosterol (Ligterink *et al*., 1997; Zhang *et al*., 1998; Cardinale *et al*., 2000; Desikan *et al*., 2001; Lee *et al*., 2001; Asai *et al*., 2002; Fellbrich *et al*., 2002; Droillard *et al*., 2004; Wan *et al*., 2004; Daxberger *et al*., 2006; Zipfel *et al*., 2006). A functional genomic approach has led to the identification of *Arabidopsis* MAPK cascades comprising MEKK1, MKK4/MKK5 and MPK3/MPK6 that provide redundant functions downstream of the flagellin receptor FLS2 and upstream of WRKY and other transcription factors (Asai *et al*., 2002). Activation of the MAPK cascades triggers massive transcript changes and confers resistance to multiple pathogens, suggesting the importance of MAPK cascade signalling in plant innate immunity. More comprehensive functional genomic screens of *Arabidopsis* MAPK cascades with 68 MTKs, 10 MKKs and 20 MPKs have revealed surprisingly large number of MTKs that may share similar functions with MEKK1 (G. Tena and J. Sheen, unpubl. data). Due to functional redundancy, thorough genetic evidence for the critical roles of specific MAPK cascade components in MAMP-induced signalling requires quantitative analysis of single and higher order mutants.

Recently, several groups have reported the isolation and characterization of *mekk1* knockout plants (Ichimura *et al*., 2006; Nakagami *et al*., 2006; Suarez-Rodriguez *et al*., 2007). The *mekk1*-deficient plants display constitutive cell death during the emergence of first pair of true

leaves accompanied by the production of H_2O_2, deposition of callose and activation of pathogenesis-related (PR) genes. The lethality is partially eliminated by high temperature or the removal of high level of salicylic acid (SA). Surprisingly, the lethal defect of *mekk1* knockout plants is rescued by the MEKK1 protein without the kinase activity (Suarez-Rodriguez *et al*., 2007). The results suggest that MEKK1 has both protein kinase (PK)-dependent and PK-independent functions. It is possible that MEKK1 acts as a scaffold protein to hold other proteins together to prevent cell death and SA/H_2O_2 accumulation. In addition, the regulatory domain, not the kinase domain, of MEKK1 interacts directly with MPK4 in the yeast two-hybrid assay (Ichimura *et al*., 1998). As a kinase, MEKK1 can activate MPK4 in response to signals, such as flg22 or H_2O_2 (Teige *et al*., 2004; Nakagami *et al*., 2006). It is important to determine which MKKs are involved in this MAPK cascade. The results indicate that MEKK1 has multiple functions as the positive and negative regulators in plant development and innate immunity. In the absence of external signals, MPK4 kinase activity is required to prevent SA accumulation (Petersen *et al*., 2000). The functional significance of MPK4 activated by external signals through MEKK1 remains to be elucidated. The analysis of one *mkk1* mutant has also suggested very complex roles of MKK1 as both a positive regulator in MPK3/4/6 activation by flg22 and a negative regulator in flg22-activation of gene expression (Meszaros *et al*., 2006). It remains unclear how MKK1 controls MPK3/6 that do not serve as its substrate (Asai *et al*., 2002; Hua *et al*., 2006). In the future, it is necessary to employ integrative approaches and pathway-specific assays to study the complexity of the MAPK cascade signalling networks in plant innate immunity.

Type III effectors as suppressors of MAMP-triggered immunity

It has long been observed that the T3SS-deficient bacteria can trigger broader plant defence responses than bacteria with functional T3SS (Jakobek *et al*., 1993; Espinosa and Alfano, 2004; Beth Mudgett, 2005; Nomura *et al*., 2005; Abramovitch *et al*., 2006; Grant *et al*., 2006). This observation led to the hypothesis that virulent bacteria secrete type III effectors to suppress plant defence.

Recently, many type III effectors of plant pathogenic bacteria have been reported to suppress effector-triggered immunity or MAMP-triggered immunity (Espinosa and Alfano, 2004; Beth Mudgett, 2005; Abramovitch *et al*., 2006; Grant *et al*., 2006). *Arabidopsis* transgenic plants expressing a *P. s. tomato* effector, AvrPto, inhibit the callose deposition-mediated cell wall defence triggered by a T3SS-deficient mutant and support the growth of this bacterium (Hauck *et al*., 2003). Two *P. syringae* type III effectors, AvrRpm1 and AvrRpt2, suppress flg22-mediated callose deposition and *GST6* expression (Kim *et al*., 2005). Nine effectors from *P. s. tomato* have been found to inhibit flg22-triggered expression of *NHO1*, which encodes a glycerol kinase and is important for *Arabidopsis* non-host immunity to certain bacteria. The deletion of flagellin from a non-host bacterium enhances its growth in *Arabidopsis*, supporting an essential role of flagellin in plant non-host immunity (Li *et al*., 2005). However, this result is surprising because multiple MAMPs are expected to provide redundant functions in triggering defence signalling, and the bacteria with or without flagellin induce the same *Arabidopsis* transcriptome changes (Thilmony *et al*., 2006).

Specific effectors as suppressors of early MAMP-induced signalling

It is possible that a large number of type III effectors have the ability to suppress MAMP-triggered immunity. Currently, it remains a great challenge to elucidate the overlapping or specific mechanisms of individual effectors as suppressors of MAMP-triggered immunity. Commonly used assays rely on relatively late defence outcomes, including cell wall modification, disease symptom development and bacterial growth. New assays with higher temporal and spatial resolution are required to differentiate the molecular actions of many effectors that seemingly share similar suppressor activities.

Based on an effector screen using a MAMP-specific and early responsive marker gene in *Arabidopsis* leaf cells, AvrPto and AvrPtoB from *P. s. tomato* have been identified as unique and potent suppressors of MAMP-triggered early defence gene transcription and MAPK signalling (He *et al*., 2006). AvrPto and AvrPtoB intercept the convergent signalling mediated by multiple MAMPs upstream of MAPK cascades immediately after signal perception. Although AvrPto and AvrPtoB do not share significant sequence homology, they both interact with a tomato R protein Pto kinase in yeast and activate the effector-triggered immunity in tomato (Kim *et al*., 2002). Thus, AvrPto and AvrPtoB may target the same host protein(s) to impede the MAMP-triggered immunity in *Arabidopsis*. Nine other type III effectors tested by the same assay do not exhibit the same suppressor function as AvrPto and AvrPtoB although they have all been shown to suppress other types of defence responses. It is possible that these effectors can also suppress MAMP-induced signalling at different and perhaps later steps. Most effectors, such as HopPtoD2, HopAI1 and AvrBsT, do not suppress flg22-mediated MAPK activation as AvrPto and AvrPtoB do. This conclusion is based on the cotransfection of effector and MAPK genes under the same expression condition. In this assay, the expression

level of MAPKs is relatively high. Thus, only the most potent and early suppressors could stand out. The results may be different if the expression level of effectors is much higher than that of MAPKs. As effectors are hypothesized to function at minute amount during natural infection, it is critical to control the relative expression level of effectors and their host targets in the study of effector functions. Confirmation with bacterial strains carrying specific effector gene mutation will provide further support for the biological relevance of effector functions, if functional redundancy of effectors can be overcome (He *et al*., 2006).

Interestingly, the N-terminal region of AvrPtoB is required and sufficient for its suppressor activity in MAMP-triggered immunity, and the C-terminal region with E3 ligase activity is indispensable for the inhibition of hypersensitive response (HR), a defence-associated cell death (Janjusevic *et al*., 2006; He *et al*., 2006). These studies suggest that a single type III effector could exhibit distinct virulence functions using different domains. In *Nicotiana benthamiana*, AvrPto and AvrPtoB also suppress diverse defence responses triggered by flagellin and the oomycete elicitor INF1 (Hann and Rathjen, 2007). However, AvrPtoB, but not AvrPto, when delivered from a non-host bacterium, suppressed *Arabidopsis* MAMP-triggered immunity (de Torres *et al*., 2006). This is likely caused by the restricted bacteria–host interaction specificity of these two effectors (Kim *et al*., 2002). Importantly, expression of AvrPto (He *et al*., 2006) or AvrPtoB (de Torres *et al*., 2006; Hann and Rathjen, 2007) in *Arabidopsis* or *N. benthamiana* blocks flg22 signalling and supports the growth of non-host bacterial strains. A non-host *P. s. phaseolicola* strain expressing AvrPtoB causes disease in *Arabidopsis* (de Torres *et al*., 2006). The data collectively support the importance of MAMP-induced signalling in plant non-host immunity.

Host targets of type III effectors

Type III effectors suppress MAMP-triggered immunity through modification of host proteins. It has been proposed that AvrRpm1 and AvrRpt2 suppress flg22-mediated responses via the manipulation of RIN4, a repressor of plant basal defence. RIN4 is phosphorylated by AvrRpm1 and cleaved by AvrRpt2 and it also plays a pivotal role in AvrRpm1- and AvrRpt2-triggered immunity (Mackey *et al*., 2002; Axtell and Staskawicz, 2003; Mackey *et al*., 2003). However, whether degradation or phosphorylation of RIN4 is directly connected to AvrRpm1 and AvrRpt2 virulence remains to be resolved. Interestingly, AvrB, another effector interacting with RIN4, suppresses flg22-mediated cell wall defence through RAR1, a protein regulating the stability of some NB-LRR disease resistance proteins (Shang *et al*., 2006). Like *rin4* plants, *rar1* mutants display enhanced cell wall defence in response to flg22, suggesting that both RIN4 and RAR1 negatively regulate MAMP-triggered immunity. It is interesting that AvrB coimmunoprecipitates with RAR1, but does not interact directly with RAR1 in the yeast two-hybrid assay. The result suggests that RAR1 and AvrB are in a same complex through the interaction of other proteins. One candidate to bridge RAR1 and AvrB is RIN4 because it interacts with AvrB, AvrRpm1 and AvrRpt2. However, RIN4 is not detected in the RAR1 complex (Shang *et al*., 2006). Interestingly, RAR1 is also required for AvrPtoB but not AvrPto virulence (Hann and Rathjen, 2007). It would be interesting to see whether there is differential suppression of MAPK and early gene activation by flg22 between AvrPto and AvrPtoB in *rar1* mutant. RAR1 likely collaborates with cytosolic HSP90 to maintain NB-LRR protein levels (Shirasu *et al*., 1999; Mackey *et al*., 2002; Hubert *et al*., 2003). The exact mechanism of RIN4 and RAR1 as negative regulators of MAMP-triggered immunity will be future research interests.

Recent novel findings show that a conserved *P. syringae* type III effector HopM1 promotes pathogenicity by interacting and destabilizing a host protein MIN7 (HopM1 interacting protein) through proteasome. MIN7 encodes an ARF GEF (guanine nucleotide exchange factor), a key component of the vesicle trafficking system in eukaryotic cells. HopM1 may be involved in the inhibition of a host vesicle trafficking pathway whose acceleration is associated with a polarized cell wall-associated defence in plants (Nomura *et al*., 2006). Importantly, the *Arabidopsis min7*-deficient plants only exhibit increased susceptibility to the *P. syringae* ΔCEL mutant, which lacks *hopM1* gene, but not to the virulent *Pst* DC3000 or the T3SS mutant. The use of specific bacterial mutants can greatly facilitate the dissection of individual effector functions in plants.

It appears to be common that a single type III effector targets multiple host components to enhance bacterial pathogenicity. For example, both AvrRpt2 and AvrRpm1 still contribute to *Pseudomonas* virulence in the *rin4*-deficient plants (Belkhadir *et al*., 2004; Lim and Kunkel, 2004). HopM1 expressing plants are more susceptible to the infection of *P. syringae* ΔCEL mutant than *min7* knockout plants, suggesting that other host factors could be targeted by HopM1 (Nomura *et al*., 2006). Alternatively, HopM1 could more efficiently target MIN7 when it is overexpressed in plants than it is delivered naturally by bacteria. Nevertheless, plants may use multiple host components to subduce the virulence of bacterial effectors.

Reprogramming of host transcriptome by type III effectors

The global gene expression or proteomic changes of *Arabidopsis* plants in response to different pathogenic and

non-pathogenic bacteria have provided valuable information on the function of type III effectors (Jones *et al*., 2006; Thilmony *et al*., 2006; Truman *et al*., 2006; de Torres-Zabala *et al*., 2007). Not surprisingly, non-pathogenic bacteria including T3SS mutants and human pathogens induce a large set of genes predominately through MAMP signals. Many of these genes are suppressed by type III effectors secreted by virulent bacteria. Among these MAMP-inducible and type III effector-repressible genes, the genes encoding receptor-like kinases are over-represented. The results suggest that diminution of MAMP perception is a powerful means for the successful virulent pathogens.

By comparing the *Pst* DC3000 wild type and the T3SS mutant bacterial strains, it is striking that type III effectors from virulent bacteria activate a significant number of host genes (Thilmony *et al*., 2006; Truman *et al*., 2006). Among 880 type III effector-regulated genes, 282 were repressed and 598 were induced by effector activities (Truman *et al*., 2006). It is known that effectors of *Xanthomonas* could activate the transcription of plant genes, some of which may encode factors to increase plant susceptibility (Yang *et al*., 2006). One *Pst* effector-regulated gene, *RAP2.6* (*At1g43160*) encoding an ERF family transcription factor, has been shown to be activated by a few type III effectors, and its activation is associated with the virulence of different *P. syringae* strains (He *et al*., 2004). Many *Pst* DC3000 effector-activated genes can potentially attenuate the essential kinase signalling pathways in MAMP-triggered immunity and/or associate with plant hormone biosynthesis and signalling pathways to promote pathogenicity (Truman *et al*., 2006; de Torres-Zabala *et al*., 2007). The activation of these genes may negatively regulate plant immune responses through novel mechanisms.

Surprisingly, many SA-mediated defence genes are dramatically activated by type III effectors from *Pst* DC3000. These genes have been shown extensively to function as positive regulators in SA-mediated defence to *Pst* DC3000 (Glazebrook, 2005). They are either not or weakly activated by MAMP signals in T3SS-deficient bacteria, but are highly induced by *Pst* DC3000 type III effectors (Thilmony *et al*., 2006). Our studies indicate that these genes are specifically activated at an early time point in effector-triggered immunity in a gene-for-gene-dependent manner (P. He, L. Shan and J. Sheen, unpubl. data). It is possible that some 'virulence' type III effectors could also be recognized by host proteins to activate part of the secondary immune responses, such as early defence gene transcription without inducing cell death (HR). In effector-triggered immunity, emerging evidence suggests that defence gene transcription and cell death could represent two separable immune responses (Yu *et al*., 1998; P. He, L. Shan and J. Sheen, unpubl. data). Our understanding of the roles of bacterial effectors in plant defence is limited and deserves further exploration.

Coronatine as suppressor of MAMP-triggered immunity

Phytotoxin coronatine contributes to virulence in several pathovars of *P. syringae* by promoting bacterial multiplication and lesion formation in many plant species. Coronatine consists of two components, coronafacic acid and coronamic acid, which are structurally similar to jasmonic acid (JA) and aminocyclopropane carboxylic acid, the immediate precursor of ethylene, respectively (Bender *et al*., 1999). Coronatine is known to function as a molecular mimic of JA, a plant hormone that could activate the defence to necrotrophic pathogens and suppress SA-mediated defence to biotrophic pathogens (Zhao *et al*., 2003; Glazebrook, 2005; Nomura *et al*., 2005). The coronatine insensitive mutants, *coi1* and *jai1* of *Arabidopsis* and tomato, display enhanced resistance to *P. syringae* infection through de-repression of SA signalling (Kloek *et al*., 2001; Zhao *et al*., 2003). Evidence also suggests the synergistic effect of T3SS and coronatine in targeting JA signalling pathway to promote *P. syringae* pathogenesis (Zhao *et al*., 2003; He *et al*., 2004).

It was puzzling that the growth of coronatine-deficient bacteria was greatly reduced compared with the wild-type strain only when inoculated onto the leaf surface, but not when infiltrated directly into the apoplast (Mittal and Davis, 1995). This mystery is solved recently in the discovery of the role of coronatine in disabling stomatal-mediated defence. Pathogenic bacteria enter the internal plant tissues through natural openings or wounds in the plant surface. Stomata, the microscopic pores in plant epidermis, have been thought to function as passive ports of bacterial entry during infection. An interesting study reveals that stomatal closure is an active innate immune response targeted and inhibited by coronatine (Melotto *et al*., 2006). As many defence responses, stomatal closure could be activated by non-pathogenic bacteria and different MAMPs, such as flg22 and LPS, and suppressed by pathogenic *Pst* DC3000. However, unlike many other responses, flg22-mediated stomatal closure is dependent on intact SA signalling. Coronatine, but not type III effector, is the surprising virulence factor responsible for suppressing stomatal defence. The purified coronatine could interfere with flg22- or LPS-induced stomatal closure. Although coronatine is thought to function as a molecular mimic of JA, unlike coronatine, JA actually causes stomatal closure (Suhita *et al*., 2004).

Concluding remarks

Although plants lack the elaborate adaptive immune system found in mammals, they have expanded the

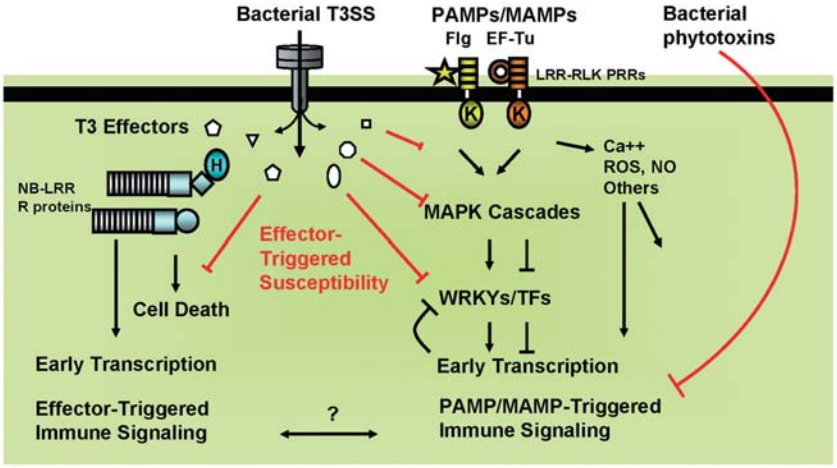

Fig. 1. Interplay between bacterial pathogenesis and plant innate immunity. Plants recognize pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs) through cell-surface pattern recognition receptors (PRRs) and activate the first line of plant innate immune responses. Different PAMPs/MAMPs are usually perceived by specific transmembrane PRRs containing an extracellular leucine-rich repeats and intracellular kinase domain (LRR-RLK), and trigger the convergent immune signalling including MAP kinase (MAPK) cascade activation and early defence gene transcription controlled by WRKY and other transcription factors (TFs). Other common early signalling events include the change of calcium level and the production of ROS (reactive oxygen species) and NO (nitric oxide). Flg (flagellin) and EF-Tu (translation elongation factor) are two well-studied bacterial MAMPs detected by plant cells. Successful bacterial pathogens have evolved multiple virulence factors, such as type III secretion system (T3SS) and phytotoxins, to suppress PAMP/MAMP-triggered immunity and promote pathogenicity. Different type III effectors secreted from T3SS interfere with PAMP/MAMP-triggered immunity through distinct and overlapping molecular actions. To survive, plants have developed the intracellular resistance proteins to counteract specific pathogen-encoded virulence factors and trigger the second line of immunity, effector-triggered immunity. The intracellular resistance proteins are largely encoded by a family of proteins with a nucleotide binding site and leucine-rich repeat domain (NB-LRR) and variable N-terminal region. The interaction between type III effectors and intracellular NB-LRR proteins could be direct or indirect through other host proteins (H). Evidence also suggests that bacterial effectors have the ability to suppress effector-triggered immunity by blocking cell death. A link between early transcription triggered by effectors and PAMPs/MAMPs may exist.

innate immune system through the evolution of a large set of cell-surface PRRs for recognizing generic MAMPs, and intracellular NB-LRR proteins for detecting highly specific pathogen-encoded virulence factors (Fig. 1) (Ausubel, 2005; Chisholm *et al*., 2006; DeYoung and Innes, 2006; Jones and Dangl, 2006). Successful plant bacterial pathogens have evolved numerous type III effectors and other virulence factors to suppress plant MAMP-triggered immunity (Alfano and Collmer, 2004; Beth Mudgett, 2005; Grant *et al*., 2006). To survive, individual plants have further evolved highly specific resistance genes to counteract specific pathogen-encoded virulence factors to trigger potent effector-triggered immunity (DeYoung and Innes, 2006; Jones and Dangl, 2006). The disarmed pathogens continue to evolve new strategies to suppress effector-triggered immunity (Fig. 1) (Espinosa and Alfano, 2004; Abramovitch *et al*., 2006).

Although much effort has been devoted to understand the signalling networks activated downstream of PRRs that respond to highly conserved MAMPs or NB-LRR proteins that respond to specific pathogen-encoded virulence factors, the signalling overlaps and distinctions of these two types of immunity remain unsolved. It has been suggested that MAMP-triggered immunity and effector-triggered immunity are largely overlapping and only differ in quantitative nature and timing (Tao *et al*., 2003; Navarro *et al*., 2004). However, gene expression analysis comparing pathogenic and non-pathogenic bacteria clearly shows that some of the earliest MAMP-induced signalling events are shut down by virulence factors in pathogenic bacteria (He *et al*., 2006). The recent genome-wide transcriptome analyses of *Arabidopsis* plants responding to bacteria with or without T3SS provide better distinction between MAMP-triggered signalling and type III effector-triggered signalling (Jones *et al*., 2006; Thilmony *et al*., 2006; Truman *et al*., 2006). In the future, comparison of early gene-expression profiles induced by purified MAMPs, individual type III effectors, and different bacterial strains will more precisely define genes that are either specific or common to MAMP-triggered and effector-triggered defence pathways or induced by abiotic stresses.

To avoid infection by a wide range of potential pathogens, a robust defence system is essential. The conserved MAMP-induced signalling components, such as MAPK cascades and WRKY transcription factors, are encoded by functionally redundant genes in plants. It has been difficult to genetically dissect the function of MAMP-induced signalling components. Many MAPK cascade genes also play critical and multiple functions in both development and innate immunity, thus creating more complexity and challenges (Asai *et al*., 2002; Wang *et al*., 2007). In addition, existing evidence suggests the involve-

ment of MAPK-independent pathways in MAMP-induced signalling (Asai *et al*., 2002). The reprogramming of massive gene transcription in plant innate immune signalling requires co-ordinated activation or repression of many transcription factors with positive and negative feedback loops (Fig. 1). More efficient methodologies for generating multiple gene silencing and new strategies in integrating genetic, genomic, proteomic, cellular and biochemical approaches will support new discoveries in the signalling pathways downstream of MAMP receptors. Different type III effectors use specific and overlapping mechanisms to suppress multiple plant defence responses at different steps to promote bacterial pathogenicity. Unravelling the detailed molecular mechanisms on how type III effectors target host proteins to interfere with plant immunity will shed light on how the host–microbe interactions shape the evolution of bacterial pathogenicity and disease resistance in plants.

Acknowledgements

Our research has been supported by the NSF (DBI 0077692, MCB 0446109, IOB 0618292) and the NIH (R01 G70567). We thank three anonymous reviewers for valuable comments and suggestions.

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