Cell Host & Microbe
Review

Intercepting Host MAPK Signaling Cascades by Bacterial Type III Effectors

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DOI 10.1016/j.chom.2007.04.008

The evolutionarily conserved MAP kinase (MAPK) cascades play essential roles in plant and animal innate immunity. A recent explosion of research has uncovered a myriad of virulence strategies used by pathogenic bacteria to intercept MAPK signaling through diverse type III effectors injected into host cells. Here, we review the latest literature and discuss the various mechanisms that pathogenic bacteria use to manipulate host MAPK signaling cascades.

Introduction

Plants and animals are constantly exposed to a wide range of microbes and have evolved sophisticated immune systems to defend against potential pathogens. Innate immunity, the first line of host defense, is triggered instantaneously upon the detection of conserved pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs). The recognition of different MAMPs by specific pattern-recognition receptors (PRRs) activates defense responses that are important for host immunity to both pathogenic and nonpathogenic microbes (Akira et al., 2006; Ausubel, 2005; Chisholm et al., 2006; He et al., 2007; Jones and Dangl, 2006; Meylan et al., 2006). The early immune responses activated by different MAMPs, such as bacterial flagellin, lipopolysaccharide, and peptidoglycan, include the activation of mitogen-activated protein kinase (MAPK) signaling cascades and transcriptional reprogramming in plants and animals. (Akira et al., 2006; Ausubel, 2005; Dong et al., 2002; He et al., 2007; Nürnberger et al., 2004). A MAPK signaling cascade generally involves three functionally tiered protein kinases, a MAPK kinase kinase (MAPKKK/MEKK/MTK), a MAPK kinase (MAPKK/MEK/MKK), and a MAP kinase (MAPK/MPK), that transduce and amplify extracellular and intracellular stimuli into a wide range of overlapping or specific intracellular responses in eukaryotic cells. MAPKKKs are the conserved entry point of MAPK cascades and are regulated by upstream signals through diverse receptors, adaptors, and signaling molecules. In mammals, Toll-like receptors (TLRs) play prominent roles in the activation of MAPK signaling in response to MAMPs (Akira et al., 2006; Ausubel, 2005; Dong et al., 2002). In plants, leucine-rich repeat receptor-like kinases (LRR-RLK) act upstream of MAPK cascade in innate immune responses (Gomez-Gomez and Boller, 2000; Zipfel et al., 2006). Transcription factors are important substrates of MAPKs, through which MAPK signaling cascades regulate gene expression in response to stimuli. Several informative reviews have provided comprehensive insights into MAPK cascades that regulate diverse cellular activities and control plant and animal development, growth, survival, cell death, and immunity (Chang and Karin, 2001; Dong et al., 2002; Nakagami et al., 2005; Pedley and Martin, 2005; Tena et al., 2001; Zhang and Klessig, 2001).

Many Gram-negative bacteria inject a repertoire of virulence effector proteins into host cells through type III secretion system (T3SS) to manipulate host immunity and promote pathogenicity (Alfano and Collmer, 2004; Galan and Wolf-Watz, 2006; Grant et al., 2006; He et al., 2004; Mudgett, 2005; Navarro et al., 2005). Although the structural components of T3SS are relatively conserved, the sequences and functions of individual effectors secreted from T3SS can be highly divergent in different species of plant and animal pathogenic bacteria. Some species, such as *Yersinia* of animal pathogens, secrete just a few effectors, whereas *Pseudomonas* species of plant pathogens deliver more than 30 effectors (Lindeberg et al., 2006; Navarro et al., 2005). Different effectors suppress host immunity through overlapping or distinct molecular actions (Abramovitch et al., 2006; Alfano and Collmer, 2004; Galan and Wolf-Watz, 2006; Grant et al., 2006; Mudgett, 2005; Navarro et al., 2005; Nomura et al., 2005; Stebbins and Galan, 2001). Recent findings have uncovered novel strategies and biochemical activities used by various type III effectors to target the evolutionarily conserved MAPK signaling cascades. This review aims to provide a timely overview of this specific aspect by following the sequence of host MAPK cascade modules from the latest discoveries on the inhibition of downstream MAPKs to the suppression of their upstream regulators, namely MAPKKs and MAPKKKs.

Inhibiting MAPKs by Bacterial Effectors of Animal Pathogens

Shigella species are intracellular bacterial pathogens that cause bacillary dysentery in humans by infecting colonic epithelial cells. In intestinal epithelial cells, recognition of bacterial MAMPs by mammalian cell-surface or intracellular PRRs activates proinflammatory signaling pathways, including MAPKs and transcription factor NF-κB, which leads to the expression of cytokines and chemokines (Akira et al., 2006; Meylan et al., 2006). There are at least

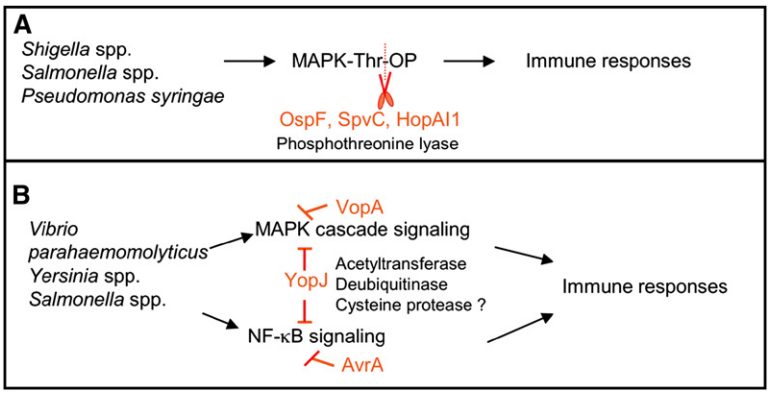

Figure 1. Homologous Effectors with Similar or Distinct Functions in Manipulating Host Innate Immunity

(A) Homologous OspF, SpvC, and HopAI1 dephosphorylate MAPKs with similar phosphothreonine lyase activity. Phosphothreonine lyase activity of SpvC is deduced from sequence homology and its MAPK dephosphorylation activity.

(B) Homologous YopJ, VopA, and AvrA have distinct host targets in suppressing immune responses.

three major families of MAPKs in mammals: the extracellular signal-regulated protein kinases (ERK), the p38 kinases, and the c-Jun NH_2-terminal kinases (JNK) (Chang and Karin, 2001; Dong et al., 2002). All MAPKs contain a conserved Thr-X-Tyr motif within their activation loop, and the phosphorylation of both Thr and Tyr is essential for their activation. Several independent studies now provide compelling evidence that a *Shigella* type III effector, OspF, directly targets and inhibits MAPK activities to promote infection (Arbibe et al., 2007; Kramer et al., 2007; Li et al., 2007).

Using *Shigella flexneri* infection assays in HeLa and Caco-2 cells, Arbibe et al. (2007) show that OspF inactivates MAPKs, ERK1/2, and p38, thereby preventing the phosphorylation of a critical Ser in histone H3 (H3pS10) that is required for the transcription of a subset of NF-κB-regulated genes. By analyzing a series of *Shigella* deletion mutants, OspF was found to be essential for the inactivation of ERKs and p38. OspF accumulates in the nucleus of HeLa cells within 15 min of *Shigella* infection, which is consistent with the nuclear accumulation of ERKs in a dephosphorylated form after *Shigella* infection. However, since there is no obvious nuclear localization signal in OspF, the mechanism by which OspF enters the nucleus and whether the nuclear retention of OspF is required for its activity remains to be determined. Through dephosphorylation of MAPKs, OspF downregulates the expression of a subset of MAPK-dependent genes. Although OspF does not directly block the activation of the IκB kinase-NF-κB pathway, it selectively represses the expression of NF-κB-dependent genes encoding chemokines, e.g., *IL8*. OspF inhibits MAPK-mediated H3pS10, thus blocking the recruitment of transcription machinery components including transcription factor NF-κB and RNA polymerase II at the selected *IL8* promoter. The selective repression of NF-κB targets by OspF suggests that *Shigella* may have evolved the ability to precisely modulate the immune responses rather than nonspecifically shutting them down. Perhaps some aspects of the host immune responses are beneficial for the dissemination of the pathogen, and it might be productive for *Shigella* to incorporate selectivity in repressing NF-κB target genes. It will be interesting to decipher the logic of this selective repression and the precise mechanism by which OspF achieves its narrow specificity.

Contrary to the proposed mode of OspF action as a dual-specificity phosphatase (Arbibe et al., 2007), using tandem mass spectrometry analysis with recombinant protein, Li et al. (2007) make the surprising discovery that OspF functions as a phosphothreonine lyase in the suppression of MAPK activity. OspF cleaves the C-OP bond in the phosphothreonine residue in the activation loop to irreversibly inactivate MAPKs. Different from a protein phosphatase, phosphothreonine lyase produces a modified Thr residue without a free hydroxyl group. This chemical modification prevents the rephosphorylation of the Thr residue by kinases. Significantly, several members of the OspF family, including SpvC from an animal pathogen *Salmonella* (Li et al., 2007) and HopAI1 from a plant pathogen *Pseudomonas syringae* (Li et al., 2007; Zhang et al., 2007), also dephosphorylate MAPKs, probably through the same phosphothreonine lyase activity (Figure 1A). Phosphothreonine lyase activity has not been reported previously. A recurrent characteristic of bacterial type III effector function is to mimic the function of host proteins (Alto et al., 2006; Galan and Wolf-Watz, 2006; Stebbins and Galan, 2001). It is possible that phosphothreonine lyase activity may also be a commonly used eukaryotic mechanism to irreversibly dephosphorylate kinases or other phosphorylated substrates.

In a third recently published study using yeast as a surrogate host, Kramer et al. (2007) show that OspF targets the yeast cell wall integrity pathway, a highly conserved MAPK signaling pathway. The finding is enabled by combining sophisticated yeast functional genomic screens, statistical data mining, and microarray analysis. Expression of OspF inhibits phosphorylation of all tested yeast MAPKs, including SLT2, HOG1, KSS1, and FUS3. However, when tested in HeLa cells after *Shigella* infection, OspF specifically inhibits phosphorylation of ERK and p38, but not JNK. Similar to the results of Arbibe et al. (2007), Kramer et al. (2007) find that inhibition of MAPK signaling by OspF attenuates the host immune responses to *Shigella* infection, particularly recruitment of polymorphonuclear leukocytes to the infected tissues in mouse and rabbit animal models.

There are some contradictory data in the studies by Li et al. (2007) and by Arbibe et al. (2007). Arbibe et al. (2007) report that OspF specifically dephosphorylates ERK1/2 and p38 in vivo and in vitro, but not JNK, while Li et al. (2007) show that OspF dephosphorylates all three families of MAPKs in vitro. An independent study by Kramer et al. (2007) supports the specificity of OspF inhibition in ERK and p38 activation in HeLa cells infected with *Shigella*. The conflicting observations may be due to the different experimental conditions and OspF overexpression, since a significantly higher amount of OspF is required to dephosphorylate JNK than p38 (Li et al., 2007). Intriguingly, in an earlier report, Zurawski et al. (2006) show that OspF promotes, rather than blocks, phosphorylation of ERK1/2 in the polarized T84 cells 3 hr after *Shigella* infection. Besides different hosts and bacterial strains used, the conflicting results may be mainly caused by the different time points, 30 min versus 3 hr, for detection of phosphorylated MAPKs, which can be activated by multiple signals for distinct functions in different subcellular compartments. Alternatively, OspF may play dual roles in manipulating MAPK activity at different infection stages. To ultimately resolve the detailed molecular actions of OspF, it is important to determine its expression dynamics, subcellular localization, and quantity in physiological conditions during *Shigella* infection in cells or animal models.

Modulating MAPKs by Bacterial Effectors of Plant Pathogens

Most type III effectors of plant and animal pathogens do not share obvious sequence similarity to eukaryotic proteins. However, some effectors of plant pathogens have putative homologs in animal pathogens. It remains mostly unknown whether these homologs from plant and animal pathogens share conserved molecular actions in promoting pathogenicity. Now, Zhang et al. (2007) suggest that HopAI1, a conserved effector from *Pseudomonas syringae* strains and a homolog of OspF, also acts as a phosphothreonine lyase (Figure 1A) that removes the phosphate group from phosphothreonine to inactive MAPKs. Shown by in vitro pull-down and in vivo coimmunoprecipitation assays, HopAI1 directly interacts with two *Arabidopsis* MAPKs, MPK3 and MPK6. In plants, several MAPKs are quickly activated by a conserved 22 amino acid peptide (flg22) from *P. syringae* flagellin (Asai et al., 2002). Overexpression of HopAI1 in transgenic *Arabidopsis* plants or in mesophyll cells suppresses endogenous MPK3 and MPK6 activation by flg22. In overexpression experiments, the ratio of HopAI1 to MAPK protein level may be critical for HopAI1 suppressor activity, since HopAI1 does not inhibit flg22-induced MAPK activation when HopAI1 and MAPK are expressed under the same promoter in the mesophyll cell transient expression assays (He et al., 2006). MPK3 and MPK6 are activated by many biotic and abiotic stresses (Nakagami et al., 2005). It is likely that HopAI1 can also block the activation of MPK3 and MPK6 by other signals in addition to flg22. OspF specifically inactivates ERK and p38, but not JNK (Arbibe et al., 2007; Kramer et al., 2007). It will be interesting to learn whether HopAI1 also specifically dephosphorylates MPK3 and MPK6, but not other MAPKs in *Arabidopsis*.

Expression of HopAI1 in *Arabidopsis* plants blocks all tested flg22-induced responses, including activation of defense genes, production of reactive oxygen species (ROS), and deposition of callose. These responses may function downstream or in parallel to MAPK activation in flg22-induced signaling. In contrast, OspF only suppresses a small subset of MAPK- and NF-κB-activated genes by comparing the transcriptome changes in mammalian cells infected with wild-type or *ospF* mutant *Shigella* strains (Arbibe et al., 2007). The identification of direct MAPK target genes in *Arabidopsis* will reveal the specificity of HopAI1 in the inactivation of MAPKs. Since the effect of HopAI1 is very broad, it may also act on additional substrates besides MAPKs. Unlike in mammals, no histone phosphorylation role for *Arabidopsis* MAPKs inside the nucleus has been reported yet. Understanding MAPK functions in plant innate immunity may help uncover virulence mechanisms of bacterial type III effectors. Although HopAI1 does not seem to function as a virulence effector in *P. s. tomato* DC3000, a model virulent pathogen in *Arabidopsis* and tomato (Lindeberg et al., 2006), it contributes to pathogenicity of *P. s. tomato* 0288-9 in tomato (Zhang et al., 2007). This may be a result of specific adaptation during host and microbe coevolution and adds another layer of complexity in deciphering the precise functions of type III effectors.

HopPtoD2, another effector from *P. s. tomato*, has in vitro protein tyrosine phosphatase activity and a conserved catalytic Cys residue is required for its activity (Bretz et al., 2003; Espinosa et al., 2003). Transient overexpression of HopPtoD2 in tobacco suppresses the cell death induced by an active tobacco MKK, NtMEK2^{DD}. This cell death suppression function of HopPtoD2 requires the conserved catalytic Cys residue (Espinosa et al., 2003). It is likely that HopPtoD2 promotes pathogenicity through inactivation of MAPK signaling. However, since HopPtoD2 is unable to suppress flg22-induced MAPK activation in *Arabidopsis* mesophyll cells (He et al., 2006), it remains unknown whether the effects of HopPtoD2 on MAPK are a direct consequence of dephosphorylating MAPKs through its tyrosine phosphatase activity. Intriguingly, like HopPtoD2, many type III effectors of plant bacterial pathogens could suppress immunity-associated cell death triggered by pathogen effectors or other signals (Abramovitch et al., 2006; Alfano and Collmer, 2004; Grant et al., 2006; Mudgett, 2005). It will be interesting to test the ability of more type III effectors in modulating MAPK signaling, which has been shown to regulate immunity-associated cell death in plants (Pedley and Martin, 2005; Zhang and Klessig, 2001).

Targeting and Interfering with MAPKKs

YopJ is a conserved type III effector protein of *Yersinia* species that disrupts host immune responses by inhibiting both the MAPK and NF-κB signaling pathways. YopJ binds

directly to multiple MAPKKs and the upstream kinase in the NF-κB pathway, IκB kinase β (IKKβ), to prevent their activation (Orth et al., 1999). Based on the sequence and structure prediction, it is suggested that YopJ functions as a cysteine protease (Figure 1B), although the recombinant YopJ protein does not possess in vitro protease activity (Orth et al., 2000). It appears that YopJ may not cleave MAPKKs directly, since the presence of YopJ does not noticeably change the migration pattern of MAPKKs on SDS-PAGE. Using the highly sensitive mass spectrometry and liquid chromatography analyses, Mukherjee et al. (2006) show that YopJ acetylates the recombinant human MAPKK6 on Ser and Thr residues in the activation loop. YopJ is an acetyltransferase that transfers acetyl from acetyl-CoA to MAPKK in vitro. The acetylation of MAPKKs on the conserved Ser and Thr residues by YopJ may inhibit these residues from being further phosphorylated by upstream MAPKKKs.

In a parallel study, Mittal et al. (2006) have observed that a specific peptide antibody against the activation loop of MEK2, a MAPKK upstream of ERK1/2, cannot detect the MEK2 protein in cells expressing YopJ. Since YopJ does not affect the stability of MEK2, it is hypothesized that YopJ may covalently modify MEK2, thereby masking the epitope for its antibody recognition. Mass spectrometry analyses reveal that YopJ causes acetylation of two Ser residues in the activation loop of MEK2. YopJ also acetylates a conserved Thr residue in the activation loop of both IKKα and IKKβ (Mittal et al., 2006). In addition, YopJ specifically acetylates one Ser residue in the kinase domain and one Thr residue near the carboxyl terminus of IKKα. These two residues are not conserved in IKKβ, and the significance of this modification is not known. Future studies may eventually uncover how YopJ determines its substrate specificity. The finding that YopJ can function as an acetyltransferase may have broader impact because YopJ homologs with similar catalytic residues have also been found in *Salmonella* (AvrA), *Vibrio* (VopA) (Figure 1B), and plant bacterial pathogen *Xanthomonas* (AvrBsT) (Orth et al., 2000; Trosky et al., 2004). AvrBsT does not seem to inhibit the flg22-induced MAPK activation when expressed in *Arabidopsis* mesophyll cells (He et al., 2006). Interestingly, instead of inhibiting immunity, AvrBsT triggers potent immune responses, including programmed cell death. The conserved catalytic residues are required for AvrBsT-induced cell death (Orth et al., 2000). It remains to be determined whether AvrBsT also functions as an acetyltransferase to trigger plant immunity. By blocking the NF-κB pathway, YopJ inhibits the synthesis of antiapoptotic factors and promotes the induction of apoptosis, thereby contributing to *Yersinia* pathogenicity (Orth et al., 1999).

It has been shown previously that YopJ inhibits conjugation of SUMO-1, a ubiquitin-like protein, to the target proteins in mammalian cells (Orth et al., 2000). YopJ has also been found to function as a deubiquitinase in vitro and to inhibit proteasomal degradation of IκBα (Zhou et al., 2005). It seems that the catalytic cysteine residue is required for YopJ inhibition function in MAPKK activation, sumoylation, and ubiquitination. It remains unclear whether YopJ targets MAPKKs for deubiquitination or whether YopJ has different host targets for its acetyltransferase and deubiquitinase activities. Together, these studies suggest that YopJ may be a multifunctional enzyme that is involved in diverse cellular processes (Figure 1B).

Suppressing MAPK Signaling at or Upstream of MAPKKKs

SptP, an effector from *Salmonella*, has been shown to inhibit ERK activation by interfering with Raf-1, a MAPKKK (Lin et al., 2003). In a series of kinase and reporter assays with various constitutively active MAPK cascade components, SptP inhibits active Raf-1-, but not MEK1/2-induced gene induction and ERK activation. SptP contains two functionally independent domains: an amino-terminal domain with GTPase-activating protein (GAP) activity and a carboxy-terminal domain with tyrosine phosphatase activity (Stebbins and Galan, 2001), and both domains are required for its inhibition of ERK activation by active Raf (Lin et al., 2003). However, it is unclear whether SptP directly targets Raf to inactivate its kinase activity or blocks Raf-mediated MEK activation. The physiological role of Raf in mammalian innate immunity remains to be defined.

In plants, different MAMPs are usually perceived by distinct cell-surface PRRs and activate convergent intracellular signaling pathways (Ausubel, 2005; Chisholm et al., 2006; Jones and Dangl, 2006; He et al., 2007). However, in response to pathogenic bacterial infection, MAMP-triggered early defense gene transcription is actively suppressed in a T3SS-dependent manner (He et al., 2006). This observation suggests that type III effectors from pathogenic bacteria could suppress MAMP-triggered immunity. Applying a cell-based genetic screen, AvrPto and AvrPtoB from *P. s. tomato* have been identified as potent suppressors of MAMP-triggered early defense gene transcription and MAPK activation. AvrPto and AvrPtoB suppress the convergent signaling activated by multiple MAMPs from different microorganisms, including harpin and NLP/NPP1 (He et al., 2006; Nürnberger et al., 2004). Epistasis analysis with constitutively active MAPKKs and MAPKKK suggests that AvrPto and AvrPtoB suppress MAMP-triggered signaling upstream of MAPKKK in the MAPK cascade (Figure 2A).

Consistent with the cell-based assays, the inducible expression of AvrPto or AvrPtoB in transgenic *Arabidopsis* plants also blocks flg22 signaling (de Torres et al., 2006; He et al., 2006). In tobacco leaves, transient expression of AvrPto or AvrPtoB inhibits flg22-mediated MAPK activation and ROS production (Hann and Rathjen, 2007). The signaling components downstream of MAMP receptors and upstream of MAPK cascade are largely unknown in plant MAMP-triggered immunity. The identification of host targets of AvrPto and AvrPtoB will help elucidate the conserved mechanism used by multiple MAMPs to transduce signals from receptors to the convergent MAPK cascade (He et al., 2006). Importantly, deletion of AvrPto and AvrPtoB from a pathogenic bacterium significantly reduces its virulence in *Arabidopsis*, suggesting

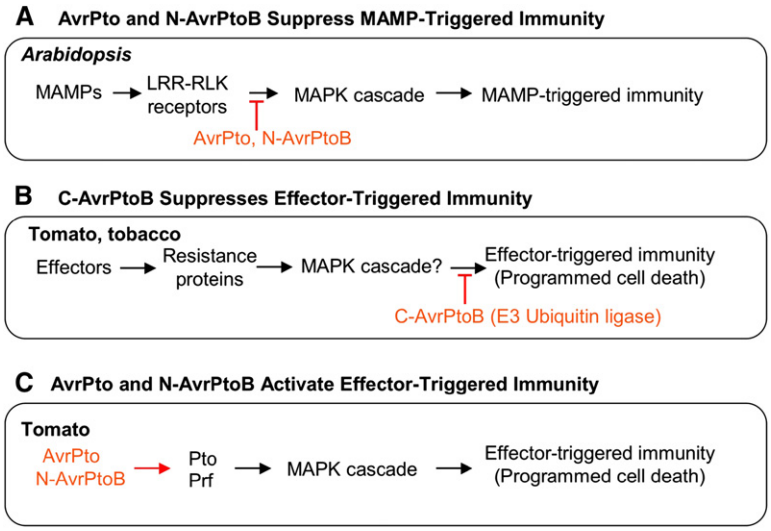

Figure 2. AvrPto and AvrPtoB Do Not Share Overall Sequence Homology but Have Overlapping and Distinct Functions in Plant Innate Immunity

(A) AvrPto and AvrPtoB block MAMP-triggered immunity and MAPK signaling cascade in *Arabidopsis*. The N terminus of AvrPtoB (N-AvrPtoB) is required for the suppression activity.

(B) The C terminus of AvrPtoB (C-AvrPtoB) acts as an E3 ubiquitin ligase to block effector-triggered immunity in tomato and tobacco.

(C) AvrPto and AvrPtoB activate effector-triggered immunity and MAPK signaling cascade through the plant resistance genes *Pto* and *Prf* in tomato. The N terminus of AvrPtoB (N-AvrPtoB) is essential for this activity.

that AvrPto and AvrPtoB contribute to bacterial pathogenicity with in vivo bacterial infection assay (He et al., 2006). In addition, the introduction of AvrPtoB into *P. s. phaseolicola* promotes its virulence in *Arabidopsis* and bean (de Torres et al., 2006).

Future Challenges

MAPK cascades are evolutionarily conserved signaling regulators and are essential components in plant and animal immunity (Ausubel, 2005; Dong et al., 2002; Pedley and Martin, 2005; He et al., 2007; Nürnberger et al., 2004). Many pathogenic bacteria have evolved distinct type III virulence effectors to manipulate host MAPK signaling cascades to promote pathogenicity. These virulence effectors appear to have acquired diverse biochemical activities and target different modules of MAPK cascades in host immunity (Figure 3 and Table 1).

Most type III effectors share no obvious sequence similarity to proteins with known functions in eukaryotes. It has been very challenging to predict the biological and biochemical functions of effectors solely based on amino acid sequences. Structure analysis can provide valuable clues for the function of some effectors (Janjusevic et al., 2006; Stebbins and Galan, 2001). However, understanding the function of most effectors will require versatile and quantitative assays, the determination of their subcellular localization and expression dynamics, and the identification of host targets. The examples presented in this review suggest that pathogens have deployed a set of novel genes to interfere with host cellular functions and processes rather than simply hijacking eukaryotic genes through horizontal gene transfer. Moreover, some effectors are chimeric proteins that possess multiple domains with separate virulence functions. For example, the N-terminal domain of AvrPtoB from *Pseudomonas* shows similar suppressor function with AvrPto in flg22-induced signaling, although AvrPto and AvrPtoB do not share overall sequence homology (Figure 2A) (He et al., 2006). The C-terminal domain of AvrPtoB is structurally similar to the U box and RING finger components of E3 ubiquitin ligases and can block cell death in plants and yeast (Figure 2B) (Janjusevic et al., 2006). In addition to their virulence functions in blocking the two types of innate immune responses, AvrPto and AvrPtoB can also activate potent effector-triggered immune responses in the presence of specific disease resistance genes, *Pto* and *Prf* in tomato and tobacco (Figure 2C) (Pedley and Martin, 2005). Effectors with dual and opposite functions in innate immune responses may be the consequence of unique plant-bacteria coevolution. SptP from *Salmonella* is also a virulence effector with multiple domains and different functions (Lin et al., 2003; Stebbins and Galan, 2001). In addition, some pathogens have developed specific virulence strategies to cope with certain hosts during coevolution. For instance, type III effectors from plant pathogens can modulate plant hormone biosynthesis and signaling pathways or chloroplast structures to evade plant immunity (de Torres-Zabala et al., 2007; Jelenska et al., 2007). Future studies will certainly uncover novel functions of bacterial effectors in their interceptions of host immunity, and perhaps new signaling mechanisms in eukaryotes.

Evidence from bacterial genome sequences and bioinformatics suggests that some putative effectors from various plant and animal pathogens share certain conserved sequences/motifs or invariant residues. These family members may share similar biochemical activities, molecular functions, and host targets (Alto et al., 2006; Li et al., 2007; Orth et al., 2000; Rohde et al., 2007; Shao et al., 2002; Zhang et al., 2007). For example, OspF and HopAI1 possess the same enzymatic activity to inactivate similar host proteins (Figure 1A; Table 1). However, this may not be generalized for other effector families. YopJ family members include AvrA from *Salmonella*, VopA from *Vibrio*, and AvrBsT from *Xanthomonas* and modulate overlapping or distinct host signaling pathways (Figures 1B and 3). It is well documented that YopJ inhibits both MAPK and NF-κB pathways. AvrA inactivates the NF-κB pathway downstream of IKKβ but not the MAPK pathway

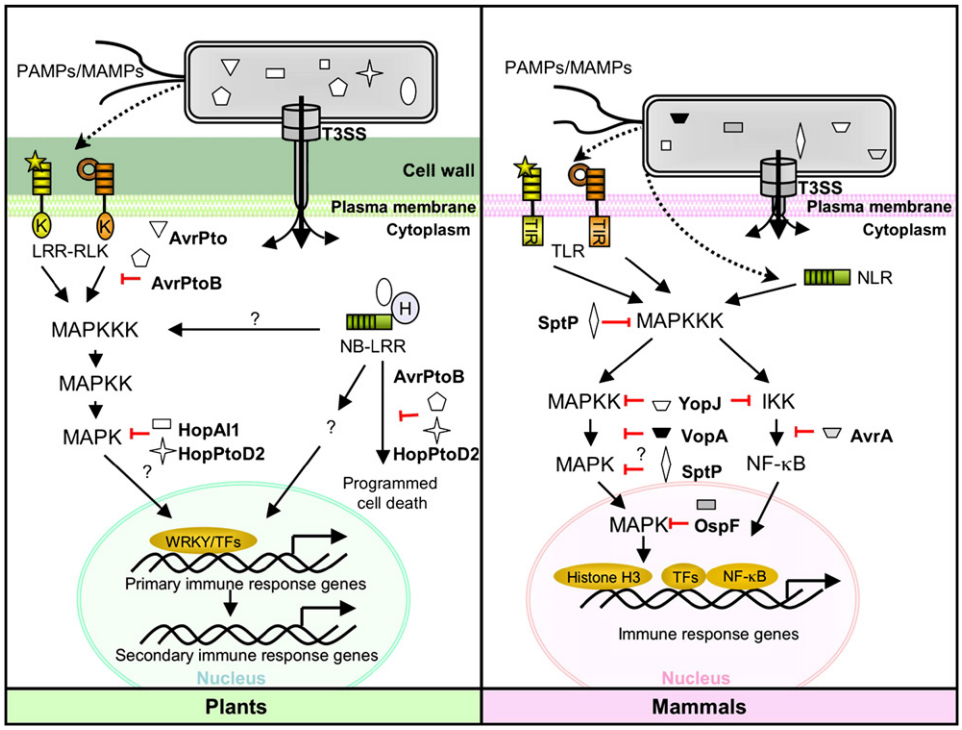

Figure 3. Manipulation of Host MAPK Signaling Cascades by Bacterial Type III Effectors

Innate immunity is triggered by recognition of pathogen- or microbe-associated molecular patterns (PAMPs/MAMPs), such as flagellin, lipopolysaccharide, and peptidoglycan, and activates evolutionary conserved signaling pathways, including MAPK cascades, in plants and mammals. The activation of MAPK cascades induces the expression of downstream transcription factors (TFs), thereby activating immune response genes for host immunity. Pathogenic bacteria inject a set of virulence effectors into host cells through type III secretion system (T3SS) to promote pathogenicity by targeting different MAPK cascade components. In plants, different PAMPs/MAMPs are usually perceived by cell-surface receptors with extracellular leucine-rich repeats and intracellular kinase domain (LRR-RLK). AvrPto and AvrPtoB from *Pseudomonas syringae* suppress MAPK cascade upstream of MAPKKK; HopAI1 from *P. syringae* inactivates MAPKs as a phosphothreonine lyase; HopPtoD2 from *P. syringae* is a protein tyrosine phosphatase that may target MAPKs. Some plants have evolved the specific intracellular resistance proteins to counteract type III effectors and initiate the effector-triggered immunity. The resistance proteins are largely encoded by a family of proteins with a nucleotide-binding site and leucine-rich repeat domain (NB-LRR). The interaction between type III effectors and intracellular NB-LRR proteins could be direct or indirect through other host proteins (H). The recognition of effectors by plant resistance proteins activates immune responses, including defense gene transcription and programmed cell death. MAPK signaling cascade may also be activated in plant effector-triggered immunity. Type III effectors from plant pathogens, such as AvrPtoB and HopPtoD2, could also suppress effector-triggered immunity by blocking cell death. In mammals, PAMPs/MAMPs are recognized by cell-surface Toll-like receptors (TLR) and intracellular CATERPILLER/NOD-like receptors (NLR). SptP from *Salmonella* blocks MAPK cascade by interfering with MAPKKK activation. YopJ, AvrA, and VopA belong to a cysteine protease family. YopJ from *Yersinia* inhibits both MAPK and NF-κB pathways as an acetyltransferase with direct binding to MAPKK and IKK; AvrA from *Salmonella* inhibits the NF-κB pathway downstream of IKK but does not affect the MAPK pathway; VopA from *Vibrio parahaemomolyticus* intercepts MAPK signaling with unknown targets but does not suppress the NF-κB pathway. OspF, a HopAI1 homolog from *Shigella*, inactivates MAPKs as a phosphothreonine lyase. The inactivation of MAPKs by OspF may occur in the nucleus, which in turn prevents the phosphorylation of histone H3 and inhibits the transcription of a subset of NF-κB-regulated genes.

(Collier-Hyams et al., 2002), whereas VopA blocks MAPK signaling with unknown targets but not the NF-κB pathway (Trosky et al., 2004). AvrBsT does not appear to inhibit flg22-mediated MAPK activation in plants (He et al., 2006). Although not yet found in the animal systems, many plants have evolved disease resistance genes to detect virulence effectors and trigger potent and secondary innate immune responses (Figures 2 and 3) (Ausubel, 2005; Chisholm et al., 2006; Jones and Dangl, 2006).

Notably, a large body of research on effector functions is based on the overexpression of individual effectors in host cells. The results from these studies should be interpreted with caution, since the secretion process and biological activities of different effectors are precisely coordinated and temporally regulated. In addition, nonspecific cellular effects may be obtained by overexpression of type III effectors that usually express at a very low level. The understanding of precise functions of individual effectors requires in vivo host-microbe infection assays in the context of the functions of other effectors and the dynamics of host cellular process. Nevertheless, recent studies using plant, mammalian, and yeast model systems have provided tremendous advance and remarkable insights into the plethora of molecular actions of bacterial type III effectors. Similar approaches may be applied to study the functions of effectors delivered into host cells by fungi, Oomycetes, and other microbes. Manipulation of the evolutionary conserved host signaling pathways, such as MAPK cascades, reflects a central mechanism used by bacterial effectors to promote pathogenicity. With the increase of our knowledge of host immune signaling and the development of new technologies, research on

Table 1. Examples of Bacterial Effectors Intercepting Host MAPK Signaling Cascades

MAPK Cascade Target	Effector (Alias)	Organism	Biochemical Function	Reference
MAPK	OspF[a]	*Shigella* spp.	phosphothreonine lyase	Arbibe et al., 2007; Kramer et al. 2007; Li et al., 2007; Zurawski et al., 2006
	HopAI1[a]	*Pseudomonas syringae*	phosphothreonine lyase	Zhang et al., 2007
	HopPtoD2 (HopAO1)	*Pseudomonas syringae*	phosphatase[c]	Espinosa et al., 2003
MAPKK	YopJ[b] (YopP)	*Yersinia* spp.	acetyltransferase	Mittal et al., 2006; Mukherjee et al., 2006
MAPKKK	SptP	*Salmonella* spp.	tyrosine phosphatase, GTPase-activating protein[c]	Lin et al., 2003
Upstream MAPKKK	AvrPto (AvrPto1)	*Pseudomonas syringae*	unknown	He et al., 2006
	AvrPtoB (HopAB2)	*Pseudomonas syringae*	unknown	He et al., 2006
Unknown	VopA[b]	*Vibrio parahaemomolyticus*	unknown	Trosky et al., 2004

[a] OspF, HopAI1, and SpvC (*Salmonella* spp.) belong to a family based on sequence homology.
[b] YopJ, VopA, AvrA (*Salmonella* spp.), and AvrBsT (*Xanthomonas campestris* pv.*vesicatoria*) belong to a family of cysteine proteases with conserved catalytic residues.
[c] The biochemical activities of effectors toward the MAPK cascade components are not known.

bacterial pathogenic mechanisms will continue to generate more exciting breakthroughs.

ACKNOWLEDGMENTS

Our research has been supported by the NSF (DBI 0077692, MCB 0446109, IOB 0618292) and the NIH (R01 GM70567). We are grateful to three anonymous reviewers for valuable suggestions and comments.

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