Plant pathogenic bacterial type III effectors subdue host responses
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Like animals, plants sense bacterial pathogens through surface-localized pattern recognition receptors (PRRs) and intracellular nucleotide-binding leucine-rich repeat proteins (NB-LRR) and trigger defense responses. Many plant-pathogenic bacteria secrete a large repertoire of effector proteins into host cells to modulate host responses, enabling successful infection and multiplication in plants. A number of these effector proteins target plant innate immunity signaling pathways, while others induce specific host genes to enhance plant susceptibility. Substantial progress has been made in the past two years concerning biochemical functions of effectors and their host targets. These advances provide new insights into regulatory mechanisms of plant immunity and host–pathogen co-evolution.

Introduction
Similar to animals, plants possess highly effective innate immunity that uses surface-localized pattern recognition receptors (PRRs) to detect pathogen-associated molecular patterns (PAMPs) [1], which are also referred to as microbe-associated molecular patterns (MAMPs) [2]. PAMP-triggered immunity (PTI) is considered an ancient form of plant immunity that acts as a first line of inducible defense against diverse pathogenic microbes [3,4]. Plant-pathogenic bacteria including *Pseudomonas, Xanthomonas,Ralstonia, and Erwinia* live in the plant intercellular spaces and use conserved type III secretion system to deliver effector proteins into host cells to promote parasitism [5]. It is now clear that PTI is repeatedly targeted and consequently inhibited by plant bacterial effectors [6–8]. To counteract, plants have evolved nucleotide-binding leucine-rich repeat (NB-LRR) proteins to detect the activity of effector proteins and elicit strong immune responses called effector-triggered immunity (ETI) [3,4]. This review focuses on recent advances on biochemical functions of plant-pathogenic bacterial type III effectors and mechanisms by which they enhance host susceptibility and trigger ETI.

Plant PTI signaling pathway
PAMPs/MAMPs derived from plant bacteria include lipopolysaccharides, flagellin, elongation factor Tu (EF-Tu), cold-shock protein [1], and peptidoglycans [9]. FLS2 and EFR, two closely related leucine-rich repeat (LRR) receptor kinases are the only known plant receptors for PAMPs/MAMPs derived from plant-pathogenic bacteria. FLS2 perceives flagella peptide flg22, whereas EFR detects EF-Tu peptide elf18 [10,11]. Arabidopsis contains more than 200 LRR receptor-like kinases (RLKs), many of which are induced by PAMPs/MAMPs at the transcriptional level, suggesting that more PRRs are yet to be found.

Following flg22 stimulation, FLS2 rapidly associates with BAK1, another receptor-like kinase previously known to dimerize with the brassinosteroid receptor BR1 [12,13]. Arabidopsis plants lacking BAK1 and *Nicotiana* plants silenced for the BAK1 ortholog SERK3 are largely insensitive to flg22 [12,13]. Furthermore, *Nicotiana* plants silenced for SERK3 are more susceptible to *Pseudomonas syringae*, indicating an important role of BAK1 in disease resistance [13].

Little is known about the signaling mechanism downstream of FLS2–BAK1 association. Like tyrosine receptor kinases, FLS2 rapidly internalizes following the binding of flg22, leading to the accumulation of FLS2 in the cytosol [14]. This may allow FLS2 to activate cytoplasmic signaling components. It is also known that several MAP kinases including MPK3, MPK4, and MPK6 are activated in Arabidopsis within minutes after stimulation by PAMPs/MAMPs [1]. Although the exact constituents remain to be defined, it is generally accepted that MAP kinase cascades are involved in both positive and negative regulation of the PTI pathway.

In addition to MAP kinases, PAMP/MAMP perception rapidly stimulates several other molecular events including production of reactive oxygen species (ROS), nitric oxide, and ion channel opening [1]. These events are thought to generate secondary signals and activate defenses. However, the relationship between these events and MAP kinase activation and the specific role of the secondary signals in PTI responses are yet to be adequately addressed. A role of ROS production in
PAMP/MAMP-induced cell wall defense was shown recently. Flg22 induces ROS through the NADPH oxidase AtRbohD. By using the bacterial effector HopA11 that directly inhibits MAP kinases, Zhang et al. [15] showed that AtRbohD acts downstream of MAP kinases to positively regulate callose deposition.

**Effector targets that mediate ETI**

The mechanism by which NB-LRR proteins activate disease resistance is poorly understood. Recent studies indicated that at least some NB-LRR proteins enter nucleus to activate defenses, probably as a consequence of effector recognition [16,17]. For example, the NB-LRR protein MLA10, a barley resistance protein against the powdery mildew fungus, was shown to associate with the transcription repressors HvWRKY1/2 in the nucleus upon activation by its cognate effector [17**]. Significantly, these WRKY proteins negatively regulate PAMP/MAMP-induced genes, suggesting that the ETI and PTI pathways converge at the step of gene regulation.

Plant NB-LRR proteins specifying ETI are similar to animal NOD-like receptors (NLRs) [4]. No NB-LRR protein structure has been solved, but modeling studies based on the conserved NB-ARC domain from Apaf-1 and CED-4 suggested that NB-LRR proteins cycle between the ADP-bound inactive state and ATP-bound active state [21]. It is likely that NB-LRR proteins in the resting state are locked in an inactive conformation through intramolecular autoinhibition, whereas effector proteins promote the exchange of ADP for ATP, thus relieving the autoinhibition and subsequently triggering downstream signaling.

Most NB-LRR proteins detect effector proteins indirectly by associating with host proteins that are targeted by effectors [3,4,6,22,23]. These effector targets play a crucial role in the activation of NB-LRR proteins. For example, the membrane-localized protein RIN4 constitutively associates with the NB-LRR proteins RPM1 and RPS2 to mediate the recognition of effectors AvrRpm1, AvrB, and AvrRpt2 [6]. RPS2 is held in an inactive state by RIN4, and the proteolytic cleavage of the latter by AvrRpt2, a cysteine protease, releases RPS2 for defense activation. AvrRpm1 and AvrB trigger RPM1 resistance probably by inducing the phosphorylation of RIN4 [6,24]. The Arabidopsis protein kinase PBS1 primes the NB-LRR protein RPS5 for the activation by *P. syringae* effector AvrPphB, another cysteine protease [22]. The proteolytic cleavage of PBS1 by AvrPphB probably causes a conformational change in the PBS1–RPS5 complex and relieves the autoinhibition of RPS5. Likewise, the tomato serine/threonine protein kinase Pto constitutively associates with the NB-LRR protein Prf, allowing the latter to recognize *P. syringae* effectors AvrPto and AvrPtoB [25]. The recently solved crystal structure of the AvrPto–Pto complex indicated that AvrPto interacts with two loops of Pto [26**]. In the absence of AvrPto, both loops are required for maintaining Prf in an inactive state because mutations disrupting these loops result in the autoactivation of Prf in plants [26**,27]. These results suggest that AvrPto triggers ETI by releasing the inhibitory effect of Pto on Prf. Like the PBS1–RPS5 and RIN4–RPM1 complexes, the Pto–Prf complex is necessary for the activation of Prf resistance. It is possible that AvrPto also triggers a conformational change in Pto, which in turn disturbs the intramolecular interaction in Prf to initiate resistance. Further structural and biochemical studies on NB-LRR proteins are needed to understand how effectors trigger ETI.

**Bacterial effectors modulate diverse host processes to enhance virulence**

How do effectors promote virulence in the host plant? As has been covered by previous reviews [6–8], plant-pathogenic bacterial effector proteins are known to modulate different host processes, particularly the inhibition of PTI and ETI pathways and alteration of plant hormone signaling. Microarray analysis showed that AvrPtoB and other *P. syringae* effectors modulate the ABA pathway to enhance host susceptibility [28,29]. AvrRpt2 delivered by bacteria or transgene enhances auxin accumulation and sensitivity in Arabidopsis plants, suggesting that AvrRpt2 modulates the auxin pathway to enhance plant susceptibility [30]. The *P. syringae* effector HopI1 modifies host chloroplast, suppresses the production of defense hormone salicylic acid, enhances heat tolerance in the host, and increases susceptibility of host to a strain lacking HopI1, probably through the J domain postulated to interact with HSP70 [31]. The *P. syringae* effector HopAO1 possesses tyrosine phosphatase activity [6] and is shown recently to suppress flg22-induced callose deposition and resistance when expressed as a transgene in the plant [32]. The *P. syringae* effector HopAA1 was shown to localize to mitochondria and inhibit respiration in yeast [33]. Taken together, pathogenic bacteria use effectors to modulate diverse host responses to their advantage. The identification of direct targets for these effectors will greatly enhance our understanding of the biochemical basis of bacterial virulence. Because many effector proteins are specialized to take control of host immunity/susceptibility, the characterization of these effectors and their host targets will help the construction of regulatory pathways for plant immune systems.

**PTI pathway components as effector targets**

Consistent with previous findings that PTI is actively inhibited by *P. syringae* effectors, several recent reports demonstrated that regulatory proteins in the PTI pathways are directly targeted by these effectors (Figure 1). For example, earlier studies indicated that AvrPto...
inhibits PTI upstream of the MAP kinase cascade through an unknown mechanism [34]. A surprising finding from the structural and biochemical analyses of the Pto–AvrPto complex is that AvrPto is a kinase inhibitor [26]. The possibility that the kinase inhibition activity is intrinsic to the virulence function of AvrPto was tested recently [35]. AvrPto directly interacts with several receptor kinases, including FLS2 and EFR in Arabidopsis and LeFLS2 in tomato plants to block PAMP/MAMP-induced defenses and enhances bacterial virulence. In Arabidopsis plants, AvrPto allows *P. syringae* to overcome basal resistance mediated by FLS2. Thus, AvrPto is an effector directly targeting PRRs.

Another example of plant pathogenic bacterial effector targeting known PTI pathway components is HopAI1. HopAI1 belongs to a novel family of bacterial effectors highly conserved in animal pathogens such as *Salmonella typhimurium*, *Shigella flexneri*, *Chromobacterium violaceum*, and plant-pathogenic bacterium *P. syringae*. When expressed as a transgene, HopAI1 directly inactivates Arabidopsis MAP kinases MPK3 and MPK6 to block PAMP/MAMP-triggered responses [15]. Similarly, the *Shigella* effector OspF and *Salmonella* effector SpvC were also shown to target MAP kinases ERK1, ERK2, and P38 in animal and yeast cells [36,37,38]. Mass spectrometry analyses showed that these proteins use a novel phosphothreonine lyase activity to irreversibly dephosphorylate phosphothreonine of MAP kinases [38]. Structural studies on SpvC revealed that the recognition of MAP kinases by SpvC is primarily mediated by the dual-phosphorylated residues in the TXY motif, which is shared in all MAPKs. The binding of the phosphorylated substrate triggers conformational changes that sequester the phosphothreonine of MAP kinases in a completely solvent-free environment [39,40]. This not only facilitates dephosphorylation through elimination reaction, but also excludes the possibility that SpvC functions as a conventional phosphatase.

The search for effector targets has identified novel components involved in PTI regulation. The *P. syringae* effector AvrB activates jasmonate signaling pathway in Arabidopsis [41]. Overexpression of AvrB in plants inhibits fgl22-induced callose deposition and enhances host...
susceptibility to the bacterium [42]. Genetic screen for mutants insensitive to AvrB identified RAR1 as a key component required for AvrB to inhibit flg22-induced callose deposition and to enhance host susceptibility. AvrB can interact with RAR1 both in vivo and in vitro ([42]; JMZ, unpublished results), indicating that RAR1 is a direct target of AvrB. Plants lacking RAR1 show enhanced callose deposition in response to flg22, indicating that RAR1 is a negative regulator of PTI. Interestingly, RAR1 is a co-chaperone of the HSP90 complex previously known to function in ETI pathway. The findings suggest a molecular link between the virulence target of an effector protein and the ETI pathway. It remains to be elucidated how the interaction of AvrB with RAR1 leads to the inhibition of PTI and enhanced susceptibility in plants.

HopU1, along with several other \textit{P. syringae} effectors, shares significant homology with mono-ADP-ribosyltransferases (ADP-RT) from bacteria, such as ExoS and ExoT from \textit{P. aeruginosa} [43**]. Recombinant HopU1 possesses ADP-RT activity against an artificial substrate and ADP-ribosylates several proteins in Arabidopsis and tobacco crude protein extracts. Tandem mass spectrometry identified these proteins as chloroplast RNA-binding proteins (CP-RBPs) and glycine-rich RNA-binding proteins (GR-RBPs). At least one of these proteins, GRP7, plays an important role in PTI because \textit{grp7} knockout plants are compromised in flg22-induced callose deposition and display enhanced susceptibility to \textit{P. syringae} bacteria. Putative ribosylation sites R47 and R49 of GRP7 are required for ribosylation by HopU1 \textit{in vitro}. HopU1 expressed directly in plants appears to ribosylate GRP7 \textit{in vivo}. This important work uncovers a novel component in the PTI pathway and raises several questions for future research: How is GRP7 regulated in the PTI pathway? Does GRP7 bind specific RNA? If yes, what are the functions of the RNA in immune responses?

**Effectors suppressing ETI**

A series of elegant molecular, genetic, and structural studies on the \textit{P. syringae} effector AvrPtoB revealed remarkable plant–bacterial co-evolution. AvrPtoB is composed of two modules. The N-terminal domain triggers Prf-dependent ETI in tobacco and tomato plants containing Fen or Pto kinases [44**,45,46]. Amino acids 1–307 are sufficient to trigger the Pto/Prf resistance, whereas amino acids 1–387 are required for eliciting the Fen/Prf responses. The C terminus structurally and functionally mimics RING-finger and U-box family E3 ligases [45,46] and directly targets Fen for ubiquitination and degradation, thereby preventing the initiation of ETI in the host plant [44**]. Interestingly, Pto is highly homologous to Fen, but not ubiquitinated by AvrPtoB, and is still capable of recognizing the full-length AvrPtoB to trigger ETI. In Arabidopsis, AvrPtoB inhibits PTI upstream of MAP kinase cascade, and this inhibition does not seem to require the E3 ligase activity [34]. Thus, it appears that the AvrPtoB protein has recorded at least four major events in plant–pathogen co-evolution: the inhibition of PTI by the N terminus of AvrPtoB; the recognition of AvrPtoB N terminus by Fen and triggering of ETI; the emergence of the AvrPtoB C-terminal domain that specifically targets Fen for degradation; and the appearance of Pto in some plants to evade the targeting by AvrPtoB C terminus. HopM1 is an effector conserved in all \textit{P. syringae} strains and is capable of suppressing SA-dependent defenses [47]. HopM1 does not share homology with any proteins of known function. Yeast two-hybrid screen followed by \textit{in vivo} studies showed that HopM1 interacts with at least eight Arabidopsis proteins through its N-terminal domain [48**]. The binding destabilizes at least three of these proteins in a proteasome-dependent manner. One of these proteins, AtMIN7, is a member of the adenosine diphosphate (ADP) ribosylation factor (ARF) guanine nucleotide exchange factor (GEF) family proteins. ARF GEF proteins are crucial for vesicle trafficking. Arabidopsis plant lacking AtMIN7 specifically restores virulence to the \textit{P. syringae} strain lacking HopM1, but does not show enhanced susceptibility to wild-type virulent strain or nonpathogenic strains. It is not clear if AtMIN7 is required for PTI responses. The fact that AtMIN7 contributes to resistance only in the presence of other effectors implies a role of AtMIN7 in ETI that is inhibited by HopM1 (Figure 1).

**Xanthomonas transcription factors regulating host susceptibility genes**

Xanthomonads possess a unique AvrBs3/PthA family of effectors that contains a nuclear-localization sequence (NLS), an acidic transcriptional activation domain, and a leucine-rich repeat domain capable of binding DNA [8]. Mounting evidence indicates that these effectors modulate specific host genes to enhance plant susceptibility. For example, PthXo1 delivered by \textit{X. oryzae pv. oryzae} bacteria strongly induces rice gene \textit{Os8N3} [49]. Rice plants silenced for \textit{Os8N3} display reduced susceptibility to the strain carrying \textit{pthXo1}, indicating that \textit{Os8N3} plays a role in host susceptibility to this bacterium. Likewise, \textit{X. oryzae pv. oryzae} PthXo6 induces rice gene \textit{OsTFX1} to enhance host susceptibility [50]. A recent report provided direct evidence that these effectors target specific genes in the host plant. The \textit{X. campesstri pv. vesicatoria} effector AvrBs3 binds a specific promoter sequence in the pepper gene \textit{upaZ2} to activate the transcription of this gene and enhance hypertrophy, which may prime the host physiology for optimum bacterial colonization or dispersal [51**]. A future challenge will be to understand how the susceptibility genes induced by these effectors assist bacterial colonization.
Evolutionary insights from effector targets

Plant resistance genes might have evolved to guard the intended targets of effectors [4,6]. The comparison of effector targets mediating resistance and susceptibility suggests that at least some resistance genes might have evolved differently. As described above, AvrPto inhibits PTI by interacting with the kinase domain of PRRs but triggers ETI when interacting with the Pto kinase. The Pto family proteins are closely related to receptor kinases in amino acid sequence. Mutagenesis studies indicated that the sequence requirement for FLS2–AvrPto and Pto–AvrPto interactions shares a significant degree of similarity [35**]. It is possible that Pto has evolved to ‘attract’ AvrPto and trigger ETI by at least partially mimicking receptor kinases (Figure 2a). The pepper disease resistance gene Bs3 provides another example of a resistance gene mimicking the virulence target of an effector. AvrBs3 directly binds a specific promoter element that the sequence requirement for FLS2–AvrPto and AvrPto interactions share a significant degree of similarity [35**]. In this case, Bs3 promoter acted as a decoy for AvrBs3, and resistance ensues (Figure 2b). These findings suggest that plant resistance genes can evolve as a decoy for the intended targets of bacterial effectors.

Conclusion

The identification of host targets and biochemical functions of bacterial effector proteins is of central importance to our understanding of plant immune system and bacterial pathogenesis. Earlier studies have focused on effector targets required for ETI activation. The role of these targets in bacterial virulence is unknown. The studies on host proteins/genomes targeted by AvrPto and AvrBs3 suggest that some plant proteins/genomes have evolved as a decoy of effector targets to trigger ETI. Many plant-pathogenic bacterial effectors target various host proteins in the PTI and ETI pathways to subdue host responses. The availability of these effectors provides an excellent tool to dissect the poorly understood plant immune pathways.

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References and recommended reading

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

- of special interest
- of outstanding interest


Along with three other reports [36-38], this study showed that the HopA1l/Osfpf/SpvC family effectors from plant and animal bacteria directly target MAP kinases to promote virulence. This work also provides evidence that AtbRahD acts downstream of MAP kinases to generate active oxygen species and regulates cell wall defenses.


The work elegantly demonstrated that MLA acts in the nucleus to trigger ETI by interacting with transcription repressors. The work provides a point of cross-talk between PTI and ETI.


26. Xing W, Zou Y, Liu Q, Liu J, Luo X, Huang Q, She C, Zhu L, Bi R, Hao Q et al: Structural basis for activation of plant immunity by bacterial effector protein AvrPto. Nature 2007, 449:243-247. The authors showed for the first time that the structure of a protein complex composed of plant resistance protein and bacterial effector protein and suggest that AvrPto triggers ETI by masking the inhibitory effects of Pto on Prf. The study also shows a surprising finding that AvrPto is a protein kinase inhibitor, which helped the identification of receptor kinases as virulence targets for AvrPto [35].


The study demonstrated that AvrPto directly targets receptor kinases including FLS2 and EFR to block PTI and enhance virulence. The paper also provides evidence that the Pto kinase, which mediates Ptd-dependent ETI, may be a molecular mimic of receptor kinases.


38. By using mass spectrometry analyses, the authors illustrated that the HopA1l/Osfpf/SpvC family effectors directly inactivate MAP kinases to block immune responses in host cells.


The study takes advantage of yeast genetics to identify MAP kinases as targets of Osfpf.


By using mass spectrometry analyses, the authors illustrated that the HopA1l/Osfpf/SpvC family effectors directly inactivate MAP kinases to block immune responses in host cells.


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43. Fu ZQ, Guo M, Jeong BR, Tian F, Elthon TE, Cerny RL, Staiger D, Alfano JR: A type III effector ADP-ribosylates RNA-binding proteins and quells plant immunity. Nature 2007, 447:284-288. The authors demonstrated that HopU1 is a functional ADP-ribosyl transferase that targets several RNA-binding proteins in plants. One of these targets, GRP7, is a novel component of the PTI pathway and is required for disease resistance.

44. Rosebrock TR, Zeng L, Brady JJ, Abramovitch RB, Xiao F, Martin GB: A bacterial E3 ubiquitin ligase targets a host protein kinase to disrupt plant immunity. Nature 2007, 448:370-374. The authors showed that the tomato protein kinase Fen recognizes the N terminus of the P. syringae effector AvrPtoB to trigger ETI. The C terminus of AvrPtoB directly targets Fen for ubiquitin-dependent degradation, and thereby reverses the outcome of interaction.


49. Yang B, Sugio A, White FF: OsBN3 is a host disease susceptibility gene for bacterial blight of rice. Proc Natl Acad Sci U S A 2006, 103:10503-10508. The paper showed that the X. oryzae pv. oryzae effector PthXo1 activates the transcription of a rice gene, OsBN3, for virulence, providing evidence that the AvrBs3/PthA family effectors function by inducing susceptibility genes in plants.


52. Römer P, Hahn S, Jordan T, Strauss T, Bonas U, Lahaye T: Pathogen recognition mediated by promoter activation of the pepper Bs3 resistance gene. Science 2007, 318:645-648. The authors showed that plant resistance gene Bs3 uses a promoter element to detect the bacterial effector AvrBs3 and trigger ETI, providing evidence that a plant resistance gene may have evolved as a decoy for the intended target of the effector.