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REVIEW ARTICLE

Host Immunity-Suppressive Molecular Weapons of Phytopathogenic Bacteria

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The co-evolution of phytopathogenic bacteria and their hosts has determined the outcome of interactions between these organisms. The sophisticated plant immune system ensures the resistance of most plants to most microbial attacks and thus the ability of phytopathogens to cause disease is an exception rather than the rule in nature. In susceptible plants, however, bacterial virulence factors manipulate a number of host cellular pathways, thereby facilitating successful colonization. These virulence factors include effector proteins that are delivered into host cells *via* the type III secretion system (TTSS) and suppress host defenses. The type III effectors (TTEs) perturb various host cellular processes including the hypersensitive response, MAPK signaling, cellular trafficking, transcription, hormone signaling, host protein modification, and stomatal reopening. This review summarizes the observations of recent studies focusing on the interactions between the two model organisms *Arabidopsis thaliana* and *Pseudomonas syringae* that have shed light on the TTSS and the virulent activities of TTSS-translocated effector

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EFFECTOR PROTEINS AND PLANT DEFENSE RESPONSES

Plants have developed sophisticated systems to defend themselves from microbial attack (Dangl and Jones, 2001; Jones and Dangl, 2006). Since plants do not have specialized immune cells, all plant cells appear to have the innate ability to recognize pathogens and turn on an appropriate defense response (Ausubel, 2005). Hallmarks of the response to microbial attack by *Arabidopsis* include synthesis of reactive oxygen species and antimicrobial secondary metabolites, fortification of host cell walls, and activation of a large number of defense-related genes (Hammond-Kosack and Jones, 1997; Yang et al., 1997). In addition, some resistant plants attacked by *Pseudomonas syringae* generate the hypersensitive response (HR), which halts the growth of *P. syringae* and prevents the development of symptoms (Hammond-Kosack and Jones, 1997; Heath, 2000). In susceptible plants, however, *P. syringae* multiplies to high population levels.

The ability of plants to combat pathogens is often conferred by R-proteins. Directly or indirectly, R-proteins recognize effector molecules encoded by *avr* genes. When a pathogen has an *avr* gene, and a plant host has the corresponding disease resistance (*R*) gene, the host can react to the pathogen by activating a battery of defense responses. This type of resistance is called 'cultivar level resistance' and the interaction is one characterized by incompatibility (Keen et al., 1990). Cultivar level resistance is often associated with the hypersensitive response (HR), which is characterized by localized cell death. However, when the pathogen does not have the appropriate *avr* gene, it can successfully colonize the host and cause disease (Holt et al., 2000). While different R-proteins detect different pathogens, they share several structural features. The well-characterized R-proteins have been shown to contain a central NBS (nucleotide-binding site) and C-terminal LRRs (leu-rich repeats). The NBS-LRR R-proteins can be divided into two subfamilies based on whether they have at their N-terminus a coiled-coil (CC) motif (CC-NBS-LRR) or a motif related to the *Drosophila* Toll and mammalian interleukin (IL-1) receptors (TIR-NBS-LRR) (Dangl and Jones, 2001). The different R proteins initiate very similar downstream plant defense responses after effector recognition. Consequently, it is postulated that different pathogens are countered by a common signaling pathway that mediates gene-for-gene resistance.

BACTERIAL PATHOGEN WEAPONS THAT SUPPRESS HOST DEFENSES

There are many strains of *P. syringae* that collectively infect hundreds of plant species and cause various disease symptoms ranging from leaf spots to stem cankers (Cuppels, 1986; Whalen et al., 1991). One important function of the bacterial virulence factors that allow pathogenic bacteria to propagate themselves in plants is the suppression of host defense responses, including the basal defenses, the gene-for-gene resistance, and nonhost resistance. *P. syringae* suppresses host defenses by delivering effector proteins from its cytosol into the host cell *via* the TTSS (type III secretion system). Moreover, *P. syringae* strains are known to produce a variety of phytotoxins that also impair host defense. An example of such a phytotoxin is coronatine, which is necessary for the full virulence of individual *P. syringae* strains in their host plants (Brooks et al., 2004; He et al., 2004). How coronatine and the TTSS-delivered effector proteins subvert host immunity will be described below.

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Architecture of the TTSS Apparatus and Regulation of gene Expression

The TTSS is an essential virulence system used by many Gram-negative bacterial pathogens to deliver effector proteins into host cells and cause disease in susceptible plants and animals. The TTSS is also required to trigger the HR in resistant plants (Lindgren et al., 1986; Galan and Collmer, 1999; Collmer et al., 2000; Collmer et al., 2002; Jin et al., 2003). Plant pathogenic bacteria express *hrp* genes, which are essential for the secretion of type III effectors. The *hrp* genes were discovered in *P. syringae* pv. *phaseolicola* in a study searching for mutations that prevented the bacterium from eliciting the HR in a nonhost plant (tobacco) and causing disease in the host plant (bean) (Lindgren et al., 1986). *hrp* gene expression is only induced in plant tissues or in *hrp*-inducing medium that presumably mimics the conditions in the apoplast (Rahme et al., 1992; Xiao et al., 1992). The *hrp* locus of *P. syringae* consists of 27 open reading frames (ORFs) that are organized into six operons, and their expression is coordinately regulated. After the *hrp* genes are expressed, the secretion apparatus, which has the shape of a needle, is constructed. This complex is morphologically similar to the flagellar basal body. This observation, along with the sequence similarities between *hrp* genes and flagella assembly genes, indicates an evolutionary relationship between TTSS and flagella (He, 1997). The needle complex is composed of two parts, namely, an envelope-embedded multi-ring base and a short protruding surface appendage called the needle that is about 8 nm in diameter and ~ 100 nm in length (Kubori et al., 1998; Kubori et al., 2000). It has been proposed that the TTSS needle provides a continuous

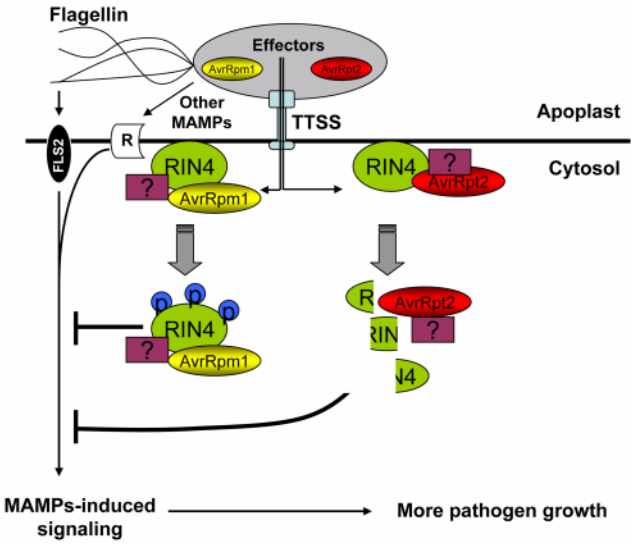

Figure 2. RIN4 is a target of at least three different effector proteins, namely, AvrRpm1, AvrB, and AvrRpt2.
AvrRpm1 and AvrB induce the phosphorylation of RIN4, which is also cleaved by AvrRpt2. Manipulation of RIN4 in the absence of the corresponding R-protein increases virulence.

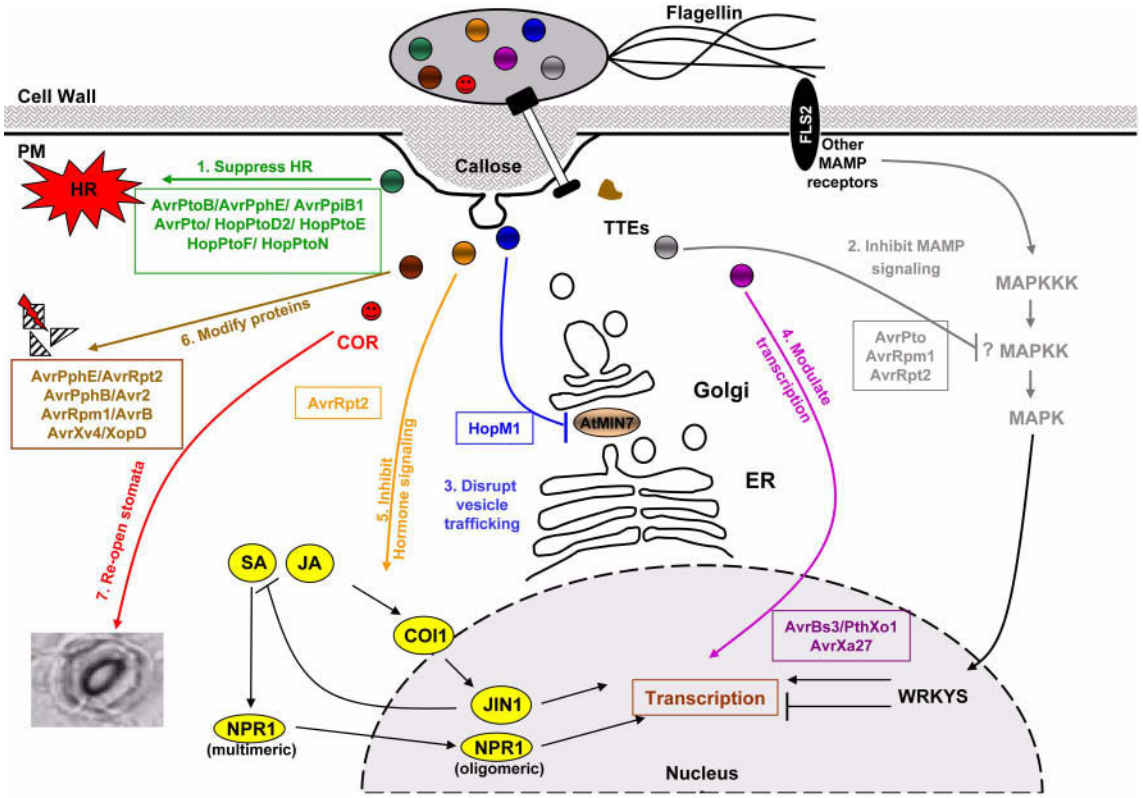

Figure 1. Phytopathogenic bacterial virulence factors perturb a variety of host pathways.
Except for coronatine, which induces stomatal re-opening (depicted in red), the virulence factors all influence processes that take place within the plasma membrane. These cellular processes include 1. the HR; 2. the MAPK signaling cascade; 3. vesicle trafficking; 4. transcription; 5. hormone signaling; 6. host protein modification; 7. stomatal re-opening. Figure is redrawn from da Cunha et. al., 2006.

channel for the secretion of effector proteins from the bacterial cytoplasm into the host cytoplasm. This proposal is supported by the fact that many type III effectors function within the host cell.

Suppression of the HR by TTEs

It has been shown that, to promote virulence in plants, type III effectors (TTEs) perturb various plant cellular and multi-cellular processes (Figure 1). Most of the host processes targeted by bacterial effector proteins are directly or indirectly involved in plant defense responses. Thus, the characterization of the biochemical activity, mode of action, and function of individual effector proteins is currently a principal objective in plant molecular pathology. This section will examine several TTEs whose host cellular targets have been identified recently.

Direct or indirect recognition of TTEs by resistant plants evokes a rapid cell death response at the site of pathogen infection that is called the HR. Thus, the HR sacrifices a small part of the plant to protect the remainder of the plant (Lindgren et al., 1986; Galan and Collmer, 1999; Collmer et al., 2000; Collmer et al., 2002; Jin et al., 2003). One TTE that induces an HR is AvrPtoB (Abramovitch et al., 2003; Pedley and Martin, 2003). Recognition of AvrPtoB by the R-protein Pto activates the HR, leading to disease resistance in tomato plants.Pto recognizes the N-terminal region of AvrPtoB, while the C-terminal region of AvrPtoB suppresses *Rsb*-dependent HR (*Rsb* is another R-gene) but not *Pto*-dependent HR (Abramovitch et al., 2003; Pedley and Martin, 2003). In the *Pto* background, AvrPtoB lacking its C-terminus induces *Rsb*-dependent HR. The crystal structure of the protease-resistant sub-fragment of the C-terminal domain of AvrPtoB displays structural homology to the RING finger and U-box components of E3-ligase (Janjusevic et al., 2006). It has since been shown that the C-terminal E3-ligase activity of AvrPtoB is required for the suppression of the Rsb-dependent immune response in *pto* tomato (Abramovitch et al., 2006). Other TTEs that suppress the HR are AvrPphE, AvrPpiB1, AvrPto, HopPtoD2, HopPtoE, HopPtoF, and HopPtoN (Abramovitch et al., 2003; Bretz et al., 2003; Espinosa et al., 2003; Jamir et al., 2004; Kang et al., 2004; Lopez-Solanilla et al., 2004; Lin and Martin, 2005).

Suppression of MAPK Signaling Cascades by TTEs

The ability to discriminate between self and non-self is a fundamental feature of living organisms, and it is a prerequisite for the activation of plant defenses specific for microbial infection. Plant cells express receptors that detect extracellular molecules or structures of the microbes, which are collectively called MAMPs (Ausubel, 2005). The most important feature of the molecular signatures in MAMPs is that they are not present in the host and can therefore be perceived as 'non-self by host-encoded receptors (da Cunha et al., 2006). The perception of MAMPs such as flg22, which is a bacterial flagellin peptide, by membrane-localized MAMP receptors activates mitogen-activated protein kinase (MAPK) pathways that then activate WRKY transcription factors (Asai et al., 2002; He et al., 2004; Kim and Zhang, 2004; Nurnberger et al., 2004; Zipfel et al., 2004; He et al., 2006).

The known outputs of MAMP-induced defenses include the induction of defense genes that are exemplified by pathogenesis-related (*PR*) genes, and localized callose deposition in the cell wall (Gomez-Gomez et al., 1999; Zipfel et al., 2004). Several TTEs inhibit MAPK signaling cascades, thereby blocking *PR* gene expression and callose deposition in the cell wall. Supporting this is that transgenic *Arabidopsis* plants expressing the type III effector AvrPto are severely compromised in their ability to deposit callose in the cell wall in response to the TTSS-defective strain *P. syringae* pv.*tomato* (*hrc*) (Hauck et al., 2003). In addition, *P. syringae* pv.*tomato* (*hrc*) grew significantly better in the *AvrPto* transgenic *Arabidopsis* than in wild type *Arabidopsis* plants.

Our recent reports suggest that other effector proteins may also suppress basal defenses such as callose deposition and the expression of host defense genes such as PR-1 (Kim et al., 2005b; Kim and Mackey, 2008). Supporting this is that expression of AvrRpm1 or AvrRpt2 in *Arabidopsis* strongly suppressed its callose deposition and PR-1 accumulation in response to *P. syringae* pv.*tomato* (*hrc*). In addition, when the bacterial strain *P. syringae* pv. *maculicola* expresses AvrRpt2, it can overcome MAMP-induced growth repression (Kim et al., 2005b). The mechanism by which AvrRpt2 and AvrRpm1 suppress MAMP responses appears to involve the host factor RIN4, which negatively regulates MAMP responses (Figure 2). Supporting this is that overexpression of RIN4 also inhibits MAMP-induced defense responses, including callose deposition and defense gene expression, and promotes the growth of *P. syringae* pv. *tomato* (*hrc*). Furthermore, plants lacking RIN4 exhibit increased sensitivity to MAMP stimulation. Thus, RIN4 is a *bona fide* negative regulator of the MAMP responses.

Suppression of Vesicle Trafficking by TTEs

Inter- and intra-cellular trafficking of macromolecules is a crucial process in plants. Recent reports indicate that intracellular vesicle trafficking and polarized secretion pathways play a pivotal role in plant defense responses against bacterial and fungal pathogens (Collins et al., 2003; Wang et al., 2005; Lipka et al., 2007; Kwon et al., 2008). HopM1 is a TTE from *P. syringae* that suppresses cell wall-associated callose deposition in *Arabidopsis*. HopM1 physically interacts with several host proteins and induces 26S proteasome-dependent degradation in *Arabidopsis* (DebRoy et al., 2004). One of the degradation targets is AtMIN7, a guanine nucleotide exchange factor (GEF) protein that positively regulates the ARF family G proteins that are involved in vesicle trafficking. By subverting host proteasome function and inducing AtMIN7 degradation, HopM1 disrupts G protein function and vesicle trafficking. Supporting this is that a mutation in the *Atmin7* gene compromised *Arabidopsis* host immunity and made the mutant more susceptible to a bacterial mutant lacking functional HopM1 (Nomura et al., 2006).

Modulation of Host Transcription by TTEs

The TTE AvrBs3 from *Xanthomonas* and *Ralstonia* is characterized by a central repeat region, nuclear localization signals (NLSs), and an acidic transcriptional activation domain

(AD) (Gurlebeck et al., 2006). An important virulence-promoting function of the AvrBs3 family is the activation of transcription. AvrBs3 localizes to the plant nucleus and induces plant mesophyll cell hypertrophy by binding to the promoter region of upa20 and activating its transcription. Upa20 encodes a transcription factor containing a basic helix-loop-helix domain that is a master regulator of cell size (Kay et al., 2007).

Another example of a TTE that modulates host transcription is PthXo1, a effector of the rice pathogen *X. oryzae* pv. *oryzae* that highly and specifically up-regulates the expression of the *Os8N3* gene in rice (Yang et al., 2006). *Os8N3* and *pthXo1* are both necessary for a compatible interaction. However, the TTE-induced modulation of host transcription does not always benefit pathogens. For example, AvrXa27, an effector from *X. oryzae,* enhances transcription of the *Xa27* R-gene and triggers resistance in some rice varieties (Gu et al., 2005).

Modulation of Hormone Signaling by TTEs

A variety of plant hormones have been suggested to participate in plant defenses. Navaro et al. have shown that MAMP signaling induces the expression of a miRNA that suppresses auxin signaling, and that plants unable to suppress auxin signaling showed increased susceptibility to *P. syringae* (Navarro et al., 2006). In addition, it has been shown that TTEs can stimulate auxin signaling by manipulating the plant. For example, AvrRpt2 prompts *Arabidopsis* to produce more auxin and increase auxin responsiveness (Chen et al., 2007).

Another example of a plant hormone that plays an important role in plant defenses is salicylic acid (SA). SA is an signal molecule that plays a significant part in the plant local defense and systemic acquired resistance (SAR), and SA signaling is mediated by at least two different mechanisms, one that requires the *Non-expresser of PR1* (*NPR1*) gene and one that is independent of *NPR1* (Shah et al., 1997). It has been shown that bacteria use TTEs to manipulate the signaling pathway of ethylene and jasmine acid (JA), which is antagonistic to SA (He et al., 2004; Cohn and Martin, 2005; Thilmony et al., 2006). *P. syringae* also produces the phytotoxin coronatine (COR), which is a functional analog of JA (He et al., 2004) and contributes to *P. syringae* virulence by stimulating JA signaling via JIN1. JIN is a transcription factor that is responsible for JA responsiveness (Lorenzo et al., 2004). Mutation of JIN1 reduces sensitivity to COR, reduces the expression of JA-responsive genes, and increases the expression of SA-responsive genes, which results in increased resistance to *P. syringae* (Laurie-Berry et al., 2006). COR also promotes the virulence of *P. syringae* by inducing closed stomata to re-open (Melotto et al., 2006), as will be described in more detail below.

Modification of Host Proteins by TTEs

Proteolysis of host proteins contributes to the virulence activity of TTEs. AvrPphE and AvrRpt2 from *P. syringae* contain a putative catalytic triad that is characteristic of cysteine proteases along with a conserved N-terminal domain (Axtell et al., 2003; Nimchuk et al., 2007). Host protein proteolysis is known to be required for the virulence activity of AvrPphE (Nimchuk et al., 2007), while AvrRpt2 cleaves not only RIN4 but also numerous other *Arabidopsis* proteins that have RIN4 cleavage sites. It has been speculated that the degradation of one or more of these non-RIN4 targets could also contribute to the suppression of MAMP signaling and/or another virulence function of AvrRpt2 (Chisholm et al., 2005; Kim et al., 2005a). AvrPphB is another TTE from *P. syringae* that also functions as a cysteine protease that cleaves the auto-phosphorylated PBS1 protein; this cleavage is required for RPS5-mediated resistance. (Shao et al., 2003; Ade et al., 2007). Supporting this scenario is that both PBS1, a cytoplasmic serine/threonine protein kinase, and RPS5, a CC-NBS-LRR type R-protein, are required for resistance against a *P. syringae* strain carrying *avrPphB* (Warren et al., 1999). There is also an example of how TTEs modulate host proteins by interfering with host proteolysis: Avr2, a cysteine-rich protein that is secreted into the apoplast of tomato by *Cladosporium fulvum*, binds and inhibits the extracellular cysteine protease Rcr3. This in turn activates the resistance gene *Cf-2* (Rooney et al., 2005). This is another example of how plant R-proteins can indirectly recognize pathogen molecules.

Some TTEs modify host target proteins by altering their phosphorylation status. For example, the *P. syringae* effectors AvrRpm1 and AvrB both induce the multi-phosphorylation of RIN4 and thereby suppress MAMP signaling (Mackey et al., 2002; Kim et al., 2005b; Shang et al., 2006). RPM1 is an R-protein that confers resistance against *P. syringae* bearing AvrRpm1 or AvrB (Dangl et al., 1992), perhaps by detecting the conformational changes in RIN4 that are induced by AvrRpm1- or AvrB-mediated phosphorylation. RIN4 mutation resulted in loss of RPM1-dependent induction of HR cell death and disease resistance, indicating that RIN4 is required for RPM1 function (Mackey et al., 2002). As mentioned above, AvrRpt2 cleaves RIN4, which induces RPS2-dependent defense responses**.** Thus, RIN4 is modified by at least three different TTEs, namely, AvrRpm1, AvrB and AvrRpt2 (Mackey et al., 2002; Axtell and Staskawicz, 2003; Mackey et al., 2003). *P. syringae* also has TTEs that induce the dephosphorylation of host proteins (Bretz et al., 2003; Espinosa et al., 2003; Li et al., 2007).

Some TTEs have cysteine protease activity that they use to remove small ubiquitin-like modifier (SUMO) peptide tags. For example, when AvrXv4 (YopJ family) and XopD (XopD family) are expressed in plants, they reduce the SUMOylated host protein levels (Hotson et al., 2003; Roden et al., 2004). However, the consequences of this deSUMOylation are not clear.

Re-opening of Stomata by Coronatine

Stomata are microscopic pores in the epidermis of aerial plant organs that are composed of a pair of specialized epidermal cells referred to as guard cells. These pores are essential for photosynthesis. At the same time, stomata provide an important niche for bacterial colonization. Moreover, since bacteria, like many fungal pathogens, directly cannot penetrate the leaf epidermis, stomata (along with wounds on the leaf surface) are used by bacteria to enter

the plant (Beattie and Lindow, 1994). Plants regulate the opening and closing of their stomata by changing the turgor pressure within the guard cells. The plant hormone abscisic acid (ABA) plays a central role in the guard cell signaling that leads to stomatal closure (Fan et al., 2004; Israelsson et al., 2006; Young et al., 2006). However, recent evidence suggests that bacterial entry through the stomata is a complex process as it appears that stomatal closure is a part of an active immune response (Melotto et al., 2006). The bacterial surface MAMP molecules are recognized by the FLS2 receptor on the guard cells, which activates SA-dependent stomata closure (Gómez-Gómez and Boller, 2002; Zipfel et al., 2004; Zipfel and Felix, 2005). It has been shown that coronatine inhibits MAMP-induced ABA signaling and reopens stomata, which facilitates bacterial entry into the plant (Melotto et al., 2006). Thus, stomatal closing appears to be part of an active defense response, while coronatine and TTEs have overlapping functions that suppress plant immunity and increase bacterial virulence.

CONCLUDING REMARKS

Since the discovery of *P. syringae* as a pathogen of *Arabidopsis*, these model organisms have been use to study molecular interactions between plants and phytopathgenic bacteria. Considerable progress has been made in our understanding of virulence and avirulence determinants, the mechanism by which the host recognizes avirulence factors, the signaling cascades that activate defense responses, and the mechanisms that pathogens use to suppress host resistance. In particular, significant progress has been made in our understanding of how individual TTEs and coronatine modulate host immunity at the molecular level.

It is of great interest to elucidate further how TTEs block plant defense responses at the molecular level. Recently, several *P. syringae* effectors have been shown to target diverse plant defense components and to suppress different types of plant defense responses. Supporting this is that it has been shown that the transgenic expression of the type III effector proteins AvrRpm1, AvrRpt2, and AvrPto compromises MAMP-induced basal defense responses, including callose deposition, expression of PR proteins, and transcriptional activation of defense genes (Hauck et al., 2003; Kim et al., 2005b; He et al., 2006). In addition, some effector proteins have been shown to suppress the HR (Abramovitch et al., 2003; Bretz et al., 2003; Espinosa et al., 2003; Jamir et al., 2004; Kang et al., 2004; Lopez-Solanilla et al., 2004; Lin and Martin, 2005). For example, AvrPto blocks the HopPsyA-mediated HR in tobacco and *Arabidopsis* ecotype Ws-0 (Jamir et al., 2004). Interestingly, expression of AvrPto does not abolish the HR or the transcriptional activation triggered by AvrRpm1, AvrB, or AvrRpt2 (He et al., 2006). This suggests that different R-proteins use distinct mechanisms to trigger the HR, and that each effector protein utilizes a distinct strategy to compromise the HR. For example, it has been reported that AvrPtoB suppresses the HR by mimicking the host factor E3 ubiquitin ligase (Lin and Martin, 2005; Janjusevic et al., 2006). However, it is not clear how the different effector proteins block the HR and other plant defense responses. Nonetheless, the prominent role TTEs play in enhancing the virulence of bacteria suggests that disabling these proteins may be an effective means of disease control. Such advances may lead to safe and effective methods for enhancing disease resistance in important crops, which is the ultimate goal of studying the molecular interactions between pathogens and their hosts.

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