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REVIEW

Regulation of the Type III Secretion System in Phytopathogenic Bacteria

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The type III secretion system (TTSS) is a specialized protein secretion machinery used by numerous gram-negative bacterial pathogens of animals and plants to deliver effector proteins directly into the host cells. In plant-pathogenic bacteria, genes encoding the TTSS were discovered as hypersensitive response and pathogenicity (*hrp*) genes, because mutation of these genes typically disrupts the bacterial ability to cause diseases on host plants and to elicit hypersensitive response on nonhost plants. The *hrp* genes and the type III effector genes (collectively called TTSS genes hereafter) are repressed in nutrient-rich media but induced when bacteria are infiltrated into plants or incubated in nutrient-deficient inducing media. Multiple regulatory components have been identified in the plant-pathogenic bacteria regulating TTSS genes under various conditions. In *Ralstonia solanacearum*, several signal transduction components essential for the induction of TTSS genes in plants are dispensable for the induction in inducing medium. In addition to the inducing signals, recent studies indicated the presence of negative signals in the plant regulating the *Pseudomonas syringae* TTSS genes. Thus, the levels of TTSS gene expression in plants likely are determined by the interactions of multiple signal transduction pathways. Studies of the *hrp* regulons indicated that TTSS genes are coordinately regulated with a number of non-TTSS genes.

Numerous gram-negative bacterial pathogens of plants and animals rely on the type III secretion system (TTSS) to invade their hosts (Galan and Collmer 1999). The TTSS is a syringe needle-like structure consisting of inner and outer membrane rings and a protruding filament called hrp pilus in the plant pathogens (Kubori et al. 1998; Marlovits et al. 2004; Roine et al. 1997; Van Gijsegem et al. 2000; Weber et al. 2005). The hrp pilus functions as a conduit to guide the translocation of the type III effectors to the interior of the host cells (Jin and He 2001; Li et al. 2002). The TTSS is encoded by a cluster of approximately 20 hypersensitive response and pathogenicity (*hrp*) genes that are organized into several operons on either the chromosome or plasmid of plant-pathogenic bacteria (Arnold et al. 2003). The *hrp* gene clusters often are flanked by several type III effectors and other types of virulence-related genes; together, these genes form the pathogenicity island that is defined from the rest of the genome by tRNA or mobile elements (Alfano et al. 2000; Arnold et al. 2003). Additional type III effector genes are scattered in the rest of the bacterial genomes either in clusters or singly (Arnold et al. 2003). The expression of TTSS genes is coordinately regulated by various host and environmental factors (Arlat et al. 1992; Rahme et al. 1992; Schulte and Bonas 1992; Wei et al. 1992; Xiao et al. 1992). According to the *hrp* operon structures and the regulatory systems controlling TTSS gene expression, the *hrp* genes of the plant bacterial pathogens are divided into two main groups. The *hrp* genes of *Erwinia* spp., *Pantoea stewartii*, and *Pseudomonas syringae* belong to group I, and those of *Xanthomonas* spp. and *Ralstonia solanacearum* are in group II (Alfano and Collmer 1997). Multiple signal transduction components have been identified for both groups of the *hrp* genes (Fig. 1A and B). This review will highlight *hrp* gene regulation in these phytopathogenic bacteria.

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The regulatory system for group I *hrp* genes.

Group I *hrp* genes in *Erwinia* spp., *Pantoea stewartii*, and *P. syringae* are regulated by HrpL, a member of the ECF family of alternative sigma factors (Frederick et al. 2001; Wei and Beer 1995; Xiao et al. 1994). The HrpL proteins of these bacteria are highly conserved in amino acid sequences and all recognize a consensus sequence (GGAACC-N15/16-CCACNNA) named the "hrp box" in the promoters of the *hrp* operons and type III effectors (Fouts et al. 2002; Frederick et al. 2001; Wei and Beer 1995). The hrp box motif contains two conserved modules. Recent studies in *Pantoea agglomerans* pv. *gypsophilae* indicated that the hrp box consensus can be divided into crucial and noncrucial nucleotides. The five nucleotides (GGAAC) in the upstream module and the three nucleotides (ACNNA) in the downstream module are crucial, whereas other consensus and adjacent nonconsensus nucleotides exert a significant effect on the promoter's strength (Nissan et al. 2005). The hrp box consensus sequence has served as an important genomic marker for the identification of candidate type III effector genes of the group I bacteria via computational analysis (Fouts et al. 2002; Zwiesler-Vollick et al. 2002). In addition, several functional genomic approaches have been developed to identify the type III effector genes in *Pseudomonas syringae* strains based on their dependence on *hrpL* for induction (Chang et al. 2005; Fouts et al. 2002; Lan et al. 2006; Losada et al. 2004). It was shown recently that HrpL directly binds the hrp box motif in gel shift assay but, surprisingly, mutations of the consensus nucleotides in the downstream module that eliminate the promoter activity do not affect the HrpL-binding (Nissan et al. 2005).

Consistent with the role as the transcriptional activator of TTSS genes, *hrpL* itself also is induced under the *hrp*-inducing

conditions (Chatterjee et al. 2002; Merighi et al. 2003; Xiao et al. 1994). The *hrpL* induction requires *hrpS* in *Erwinia* spp. and *Pantoea stewartii* and *hrpS* and *hrpR* in *P. syringae* (Hutcheson et al. 2001; Merighi et al. 2003; Nizan-Koren et al. 2003; Wei et al. 2000b). The HrpS proteins in *Erwinia* spp., *Pantoea stewartii*, and *P. syringae* share significant amino acid identify. The *hrpR* gene is present in *P. syringae* but not in *Erwinia* spp. and *Pantoea stewartii*. The *hrpR* and *hrpS* genes are organized in an operon controlled by the promoter upstream of *hrpR* (Grimm et al. 1995; Hutcheson et al. 2001; Xiao et al. 1994). The HrpR and HrpS proteins belong to the NtrC family of two-component regulator proteins but lack the N-terminal receiver domain, suggesting that the transcription regulator activity of HrpR and HrpS is not regulated directly by phosphorylation (Xiao et al. 1994). The two proteins are highly homologous in sequence and form a heterodimer. The dimerization is believed to be crucial for the transcriptional activation of *hrpL* (Hutcheson et al. 2001). *hrpS* alone is capable of activating the *hrpL* gene in *P. syringae* but only at a very low level, and the maximal induction of *hrpL* requires both *hrpR* and *hrpS* (Bretz et al. 2002; Grimm et al. 1995; Hutcheson et al. 2001). Both HrpR and HrpS contain an enhancer-binding domain and a motif that interacts with the σ^{54}-RNA polymerase holoenzyme. Consistently, transcription of *hrpL* is under the control of a σ^{54}-dependent promoter in an alternate σ factor RpoN-dependent manner (Chatterjee et al. 2002; Hendrickson et al. 2000). It was hypothesized that, under inducing conditions, HrpR and HrpS form a heterodimer on the *hrpL* promoter to stimulate the transcription of *hrpL* by interacting with the RpoN-RNA polymerase holoenzyme (Hutcheson et al. 2001). Additional regulators of *hrpL* were reported in *Erwinia carotovora* subsp. *carotovora* that include the RsmA/rsmB RNA-mediated post-transcriptional system and genes affecting the RsmA/rsmB production (Chatterjee et

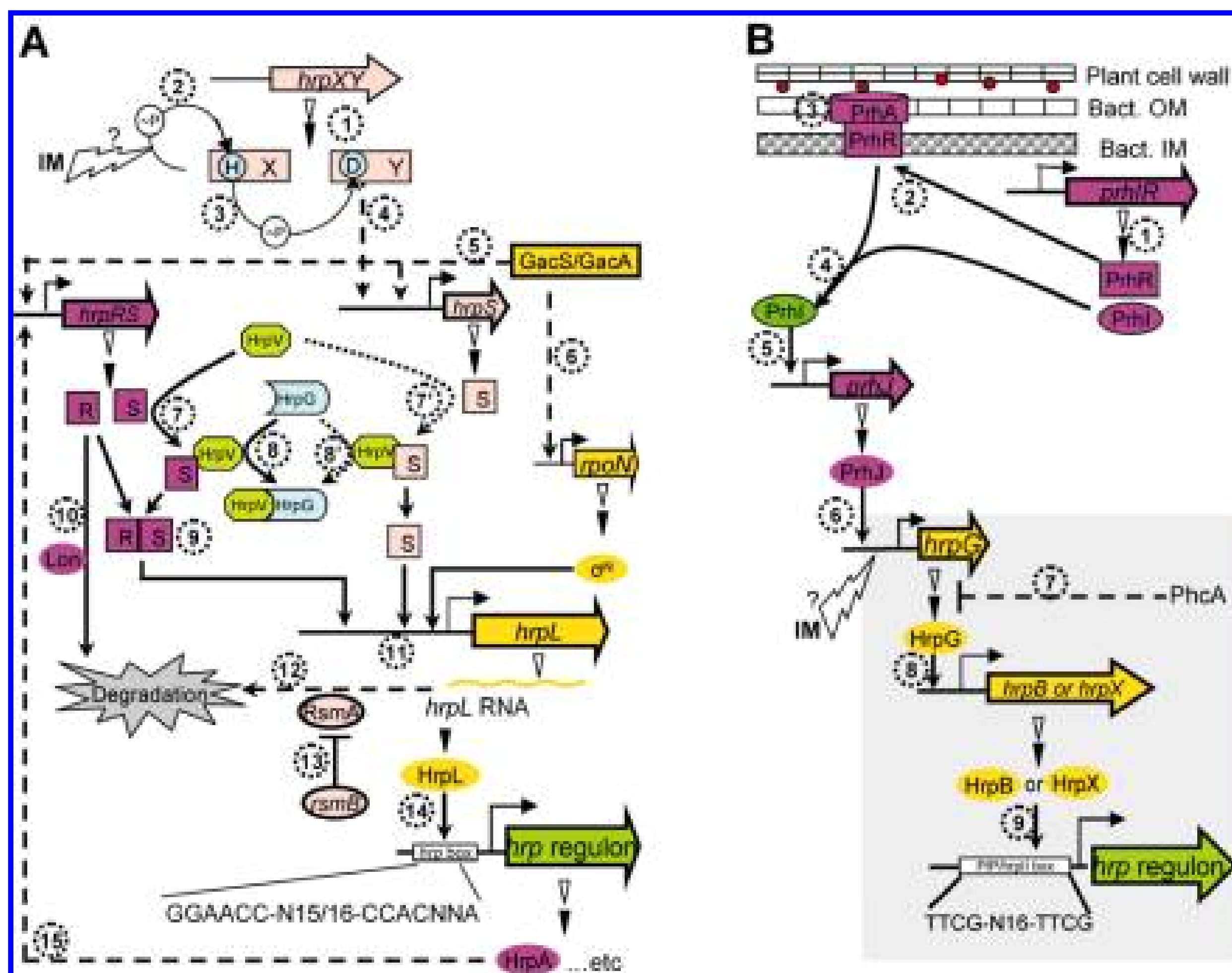

Fig. 1. Models of type III secretion system (TTSS) gene regulation in phytopathogenic bacteria. The numbers indicate the major signaling events. **A,** Group I bacteria. 1, The *hrpXY* operon in *Erwinia* spp. and *Pantoea stewartii* is transcribed and translated into HrpX and HrpY proteins. 2, Signal in inducing medium (IM) triggers autophosphorylation of HrpX. 3, HrpX phosphorylates HrpY. 4, HrpY activates the transcription of *hrpS* through an unknown mechanism. 5, GacS/GacA activates the transcription of *hrpS* in *Erwinia* spp. and *Pantoea stewartii* and *hrpRS* in *Pseudomonas syringae*. 6, GacS/GacA activates the transcription of *rpoN* in *Erwinia* spp., *Pantoea stewartii*, and *P. syringae*. 7 and 7', HrpS is repressed by HrpV via protein–protein interaction. 8 and 8', HrpG interacts with HrpV and derepresses HrpS. 9, HrpS and HrpR proteins form heterodimer in *P. syringae*. 10, HrpR protein is degraded by Lon protease in *P. syringae*. 11, HrpR and HrpS in *P. syringae and* HrpS in *Erwinia* spp. and *Pantoea stewartii* associate with RpoN in the *hrpL* promoter to activate *hrpL* expression. 12, *hrpL* RNA is degraded by RsmA-dependent mechanism. 13, *rsmB* RNA inhibits the function of RsmA. 14, HrpL recognizes the hrp box promoter and activates the transcription of hrp regulons. 15, HrpA acts upstream of *hrpRS* transcription to stimulate TTSS gene expression. **B,** Group II bacteria. 1, PrhI and PrhR proteins are encoded by the *prhIR* operon. 2, PrhR protein is located in the bacterial inner membrane. 3, PrhA protein located in the outer membrane perceives the plant cell wall signal and transfers the signal to PrhR. 4, PrhR activates PrhI. 5, PrhI activates the transcription of *prhJ*. 6, PrhJ activates the transcription *hrpG*. 7, PhcA represses *hrpG* at the post-transcriptional level. 8, HrpG activates the transcription of *hrpX* in *Xanthomonas* spp. and *hrpB* in *Ralstonia solanacearum*. 9, HrpX and HrpB activate the PIP/hrpII box promoters. Components of group II bacteria in the shaded area are required for *hrp* gene activation in both inducing medium and plants. Components in the unshaded area are *R. solanacearum* genes specifically required for *hrp* gene activation in plants. IM indicates unknown signal in inducing medium.

al. 2002). RsmA is a small RNA-binding protein that promotes RNA decay (Chatterjee et al. 1995). The *rsmB* RNA, on the other hand, acts as positive regulator, presumably by sequestering RsmA (Liu et al. 1998). The *hrpL* RNA level is barely detectable in the mutant strain of *rsmB* but accumulates to a high level in a mutant lacking RsmA (Chatterjee et al. 2002).

A two-component system consisting of *hrpX* and *hrpY* acts upstream of the *hrpS-hrpL* cascade in *Erwinia* spp. and *Pantoea stewartii* (Merighi et al. 2003; Nizan-Koren et al. 2003; Wei et al. 2000b). *hrpX* encodes the sensor histidine kinase, whereas *hrpY* encodes the corresponding response regulator. The *hrpX* and *hrpY* genes constitute an operon between the *hrpL* and *hrpS* loci in the *hrp* gene cluster (Merighi et al. 2003; Mor et al. 2001; Nizan-Koren et al. 2003; Wei et al. 2000b). Mutagenesis analysis indicated that phosphorylation of HrpY, likely by HrpX, is crucial to its function in activating the *hrpS-hrpL-hrp* cascade (Nizan-Koren et al. 2003). *hrpXY* is required for the *hrp* gene induction in inducing medium. Perhaps HrpX perceives chemical or physiological signals in the inducing medium, which in turn activates HrpY by phosphorylation. How HrpY activates the *hrpS* gene remains to be elucidated. The *hrp* gene cluster of *P. syringae* does not harbor a *hrpXY*-like locus. However, the *P. syringae* genomes contain a large number of two-component system gene loci (Buell et al. 2003; Feil et al. 2005; Joardar et al. 2005); it is unknown if any of these loci encodes the functional cognates of HrpX/HrpY.

Multiple components have been found to regulate the *hrpR* and *hrpS* genes in *P. syringae* at different levels. The *hrpRS* transcription displayed two- to fourfold induction in both inducing medium and plants, and this induction precedes the induction of *hrpL* and other TTSS genes. This induction was detected in several *P. syringae* pv. *phaseolicola* strains as well as the *P. syringae* pv. *tomato* DC3000 strain (Rahme et al. 1992; Thwaites et al. 2004) (L. Lan and X. Tang, *unpublished results*). The GacS/GacA two-component system was found to play a role in regulating the *hrpRS* expression in *P. syringae* (Chatterjee et al. 2003). GacS is the sensory histidine kinase, whereas GacA is the cognate response regulator. The GacS/GacA system is found in numerous bacteria, and this system serves as a master regulator of many bacterial phenotypes, including pathogenicity, production of toxin and antibiotics, quorum sensing, motility, production of exopolysaccharides, biofilm formation, and stress tolerance (Heeb and Haas 2001). In *P. syringae* pv. *tomato* DC3000, the *gacA* mutation attenuates significantly the transcription of *hrpRS*, *rpoN,* and *hrpL* (Chatterjee et al. 2003). Because *hrpL* is regulated by *hrpRS* and *rpoN*, the reduced *hrpL* expression in the *gacA* mutant likely results from the reduced expression of *hrpRS* and *rpoN*. The signal perceived by GacS and the mechanism by which GacA regulates the expression of *hrpRS* and *rpoN* are unknown. In addition to the GacA/GacS system, HrpA, the major component of the type III pilus, also was found to affect the transcription of the *hrpRS* operon (Wei et al. 2000a). In an *hrpA* mutant, transcription of the *hrpRS* operon, *hrpL*, and other TTSS genes is severely reduced, and this can be restored by *hrpRS* overexpression. The mechanism by which HrpA regulates the *hrpRS* locus is unclear.

The ATP-dependent Lon protease is a negative regulator of the HrpR protein (Bretz et al. 2002). Lon was identified as a negative regulator of the *P. syringae* TTSS genes, of which the mutant displayed constitutive *hrp* gene activation in the rich medium (Bretz et al. 2002) (Y. Xiao and X. Tang, *unpublished results*). Mutation of *lon* in *P. syringae* increased the protein stability of HrpR but not HrpS in both the rich medium and the inducing medium (Bretz et al. 2002). In the wild-type *P. syringae* strain, the HrpR protein displayed greater instability in the rich medium than in the inducing medium. This is consistent with the reduced level of the Lon protein in the inducing medium (X. Deng and X. Tang, *unpublished result*). The constitutive activation of TTSS genes in the *lon* mutant in the rich medium likely results from the HrpR protein accumulation. The *lon* mutation does not significantly affect the transcription of the *hrpRS* locus in the rich medium (L. Lan and X. Tang, *unpublished result*). Thus, it is likely that the wild-type *P. syringae* strain and the *lon* mutant have similar levels of HrpS protein, because the Lon protease does not modulate the HrpS protein stability. However, the *lon* mutant displays higher expression of TTSS genes in the repressive medium than does the wild-type strain. This observation further supports the role of HrpR as coactivator of HrpS in inducing the *hrpL* gene. In addition to its role in regulating the HrpR protein, Lon protease was reported to regulate the stability of type III effectors in *P. syringae* (Losada and Hutcheson 2005).

It was reported recently that the HrpS function is regulated by HrpV and HrpG by a mechanism similar to the anti-anti-activator mechanism in *P. aeruginosa* (Wei et al. 2005). In *P. aeruginosa*, expression of TTSS genes is controlled at the transcriptional level by ExsA, an AraC-like transcriptional activator (Hovey and Frank 1995). The anti-activator ExsD interacts with ExsA in repressive conditions and, thus, represses the transcription of TTSS genes (McCaw et al. 2002). When the bacteria are grown in the inducing conditions, a third regulator, ExsC, interacts with ExsD and releases ExsA from inhibition by ExsD that, in turn, activates TTSS gene expression (Dasgupta et al. 2004). In this model, ExsD acts as an anti-activator of ExsA, and ExsC acts as an anti-anti-activator. In *P. syringae*, *hrpG* and *hrpV* genes are located in the *hrcC* operon and are specific to the group I *hrp/hrc* gene cluster. HrpV is a negative regulator of TTSS genes upstream of HrpR/HrpS (Preston et al. 1998). In inducing medium, *hrp* genes are expressed at an elevated level in *hrpV* mutants, whereas overexpression of the *hrpV* gene abolishes TTSS gene expression. HrpG, a chaperone-like protein, can derepress TTSS gene expression in an *hrpV*-overexpression strain without affecting the *hrpV* transcription (Wei et al. 2005). It has been demonstrated further that HrpV interacts with HrpS and HrpG in vivo (Wei et al. 2005). Thus, it is likely that HrpG suppresses HrpV via a protein–protein interaction to free HrpS in the inducing conditions. In this hypothesis, HrpV acts as anti-activator of HrpS, and HrpG is an anti-anti-activator.

The regulatory system for group II *hrp* genes.

Most group II *hrp* operons are activated by an AraC-like activator, which was designated HrpB in *R. solanacearum* (Genin et al. 1992) and HrpX in *Xanthomonas* spp. (Kamdar et al. 1993; Wengelnik and Bonas 1996). The HrpX and HrpB proteins are highly similar at the amino acid level. The promoters of *hrp* operons and type III effector genes regulated by HrpX and HrpB often carry a conserved motif termed plant-inducible promoter (PIP)-box (TTCGC-N15-TTCGC) in *Xanthomonas* spp. (Buttner and Bonas 2002) and hrpII-box (TTCG-N16-TTCG) in *R. solanacearum* (Cunnac et al. 2004a). Individual mutations of the PIP/hrpII-box consensus nucleotides generally reduce the promoter activity; however, alterations of the space between the two conserved modules abolish the promoter induction (Cunnac et al. 2004a; Tsuge et al. 2005). A search of the *Xanthomonas* spp. and *R. solanacearum* genome sequence with this consensus sequence identified a large number of candidate type III effector genes (Cunnac et al. 2004b; da Silva et al. 2002; Thieme et al. 2005). Some of these genes were identified as the *hrpX/hrpB*-induced genes by microarray, promoter trap assay, and cDNA differential display (Mukaihara et al. 2004; Noel et al. 2001; Occhialini et al. 2005). Nonetheless, it should be pointed out that several well-

characterized type III effector genes in *Xanthomonas* spp., including *avrBs1* and members of the *avrBs3* family, are constitutively expressed and do not have a PIP-box in their promoters. Even the promoters of some HrpX/HrpB-regulated effector genes do not have the PIP/hrpII-box and, thus, probably are regulated indirectly by HrpX/HrpB (Buttner and Bonus 2002; Occhialini et al. 2005).

The *hrpX* and *hrpB* genes are activated by the HrpG proteins in *Xanthomonas* spp. and *R. solanacearum* (Brito et al. 1999; Wengenilk et al. 1996). The HrpG proteins in *Xanthomonas* spp. and *R. solanacearum* share >40% amino acid identity. HrpG belongs to the OmpR family of the two-component response regulators, with an N-terminal receiver domain and a C-terminal DNA-binding motif (Brito et al. 1999; Wengenilk et al. 1996). Using the yeast two-hybrid analysis, HrpG of *Xanthomonas axonopodis* pv. *citri* was found to interact with a two-component system sensor kinase and a few other bacterial proteins of unknown function (Alegria et al. 2004); it remains to be determined whether this sensor kinase has the ability to phosphorylate HrpG of its receiver domain and whether phosphorylation of HrpG is important to its regulatory activity. Nonetheless, three point mutations in the HrpG protein of *X. campestris* pv. *vesicatoria* render the protein constitutively active even under TTSS repressive conditions (Wengenilk et al. 1999), suggesting that conformational change of the HrpG protein likely plays an important role in TTSS gene regulation.

HrpG in *R. solanacearum* is a converging point of signal transduction pathways mediating TTSS gene induction (Brito et al. 2002). *hrpG* is constitutively expressed in complete and minimal media but induced by a plant-specific signal (Brito et al. 1999). Four signal transduction components acting upstream of *hrpG* to mediate the *hrp* gene induction specifically in plants are discussed in the following section. It has been identified recently that PhcA is a negative regulator of the HrpG protein in complete medium, probably via a post-transcriptional mechanism (Genin et al. 2005). PhcA is a LysR family transcriptional regulator that coordinates the expression of multiple virulence factors such as exopolysaccharides, various plant cell-wall-degrading enzymes, quorum sensing, and bacterial motility (Schell 2000). The *phcA* mutation does not affect the transcription of *hrpG* but elevates the expression of TTSS genes in the complete medium (Genin et al. 2005).

Regulation of TTSS genes by host and environmental signals.

Host sensing is critical for a successful infection by bacterial pathogens. Upon sensing the host, pathogenic bacteria reprogram their transcription, which results in coordinated activation of TTSS genes (Brencic and Winans 2005). Animal bacterial TTSS genes are activated upon contact with host cells (Francis et al. 2002). In *Yersinia pseudotuberculosis*, the contact with Hela cells is believed to trigger the release of LcrQ, a type III secreted protein that negatively regulates TTSS gene expression (Pettersson et al. 1996). This TTSS-dependent feedback regulation also operates the contact-induction of TTSS genes in *Y. enterocolitica* (Stainier et al. 1997).

Several studies elegantly demonstrated that the *R. solanacearum* TTSS genes are induced when the bacterium is in contact with the plant cell (Aldon et al. 2000). Unlike animal bacteria, the contact-dependent induction of type III genes in *Ralstonina* spp. does not require the type III pilus. Instead, it is mediated by PrhA, a membrane protein with significant similarities with siderophore receptors (Marenda et al. 1998). The *prhA* mutation disrupts the contact-dependent type III gene induction but not the induction by the minimal medium (Marenda et al. 1998), suggesting that distinct signals are perceived from host cell and culture medium. The plant signal perceived by PrhA is likely a nondiffusible plant wall component that is resistant to protease and heat treatments (Aldon et al. 2000); however, the chemical nature of the signal remains unknown. Additional components acting downstream of PrhA are PrhR, PrhI, PrhJ, HrpG, and HrpB that function in a sequential order (Brito et al. 1999, 2002). HrpG and HrpB are required for induction of TTSS genes in both minimum medium and plants, whereas PrhR, PrhI, and PrhJ are required specifically for the induction by plant cells. *prhR* and *prhI* are in the same operon and encode a transmembrane protein and a sigma factor of the ECF family, respectively (Brito et al. 2002). PrhJ is a LuxR/UhpA family transcription activator (Brito et al. 1999). PrhR and PrhI act upstream of PrhJ and, together, the three proteins are required for the induction of *hrpG* which, in turn, activates *hrpB* in response to host cell contact. According to Brito and associates (2002), a plant cell-wall-derived signal, likely a macromolecule, is perceived by PrhA. PrhA subsequently transfers the signal to PrhR, which sequentially activates the transcription factor genes *prhI, prhJ, hrpG*, and *hrpB*. The *prhA, prhJ, prhI,* and *prhR* mutants all are affected in pathogenicity in *Arabidopsis* and hypersensitive response (HR) in resistant plants (Brito et al. 1999, 2002; Marenda et al. 1998). In particular, the *prhJ* mutant is completely nonpathogenic in plants. Together, the results demonstrate that host-cell sensing by *R. solanacearum* is fundamental to pathogenesis.

The involvement of plant-specific signals in inducing TTSS genes is likely true for other bacteria as well. For example, it was reported that induction of the *P. syringae* pv. *phaseolicola hrpL* gene in planta is much greater compared with the induction by the inducing medium, suggesting the presence of a plant-derived signal (Rahme et al., 1992). In addition, mutants of a conserved hypothetical protein have been isolated recently in two pathovars of *P. syringae* that completely abolish the hrp promoter induction in plant but only marginally affect the hrp promoter induction in the inducing medium. The mutants displayed significantly reduced pathogenicity on their host plants, bean and tomato, and the HR-inducing activity on the nonhost tobacco plants (Y. Xiao and X. Tang, *unpublished results*). Similarly, a heat-stable compound derived from a plant suspension culture was reported to induce TTSS genes of *X. campestris* pv. *vesicatoria* (Schulte and Bonas 1992).

Certain environmental conditions, including temperature, medium composition, and pH, also affect TTSS gene expression (Arlat et al. 1992; Huynh et al. 1989; Rahme et al. 1992; van Dijk et al. 1999; Wei et al. 1992). TTSS genes of plant bacteria are suppressed by rich media but induced by minimal synthetic media. The inhibitory activity of rich media is exerted by high pH and osmolarity and complex carbon and nitrogen nutrient sources. TTSS-inducing media are acidic, low osmotic, nutritionally poor, and contain certain sugars as the carbon source. The optimal expression of TTSS genes can be obtained when the *P. syringae* bacteria are grown at 20 to 30°C (van Dijk et al. 1999). These environmental conditions are thought to simulate the physiological environment encountered by bacteria during infection. The factors influencing TTSS gene expression in the inducing medium differ somewhat between different pathogens, which may reflect the differences between the apoplastic conditions of different host species. For example, the nature of carbon source plays a critical role for TTSS gene activation, and fructose and sucrose are the best inducers of the *P. syringae* TTSS genes among the carbon sources tested (Huynh et al. 1989). Minimal medium supplemented with mannitol induced the *avrB* gene in *P. syringae* pv. *glycinea* almost as well as did the medium supplemented with fructose (Huynh et al. 1989); however, mannitol is unable to induce TTSS genes in *P. syringae* pv. *phaseolicola* strain NPS3121 (Y. Xiao and X. Tang, *unpublished results*). Differ-

ential sugar effects also are established for *R. solanacearum* (Arlat et al. 1992). It is possible that in planta induction of TTSS genes requires both plant-specific signals and suitable physiological and chemical conditions in the plant apoplast.

In a recent study attempting to identify host signals involved in TTSS gene regulation, an *Arabidopsis att1* loss-of-function mutant was isolated that supports significantly higher induction of TTSS genes than did the wild-type plant (Xiao et al. 2004). This observation suggests the existence of plant-associated signals that negatively regulate TTSS genes. *ATT1* encodes a cytochrome *P450* monooxygenase catalyzing fatty acid hydroxylation. In plants, hydroxylated fatty acids form extracellular polyesters that are major constituents of cutin, which envelopes the aerial part of the plant. The cutin content in *att1* mutant is reduced to 30% of the wild-type level, and the composition of cutin monomers also is largely altered in the *att1* mutant compared with the wild-type plant, indicating that ATT1 plays an important role in biosynthesis of cutin monomers. Certain commercially available fatty acids, such as 9,10-epoxy stearic acid, and plant cutin extracts can specifically suppress the induction of TTSS genes (Xiao et al. 2004). Taken together, certain cutin monomer species may act as negative signals to regulate TTSS genes in planta. The negative regulation of TTSS genes by cutin-related signals may be important to epiphytic fitness of *P. syringae* bacteria that repress TTSS genes during epiphytic growth on the leaf surface (Xiao et al. 2004). How the cutin-related negative signal is perceived by *P. syringae* remains to be elucidated.

The coregulation of TTSS and other virulence-related genes.

In addition to TTSS genes, in vivo expression technology assays in *P. syringae*, *R. solanacearum,* and *Erwinia* spp. have identified a large number of non-TTSS genes that also are induced in the plant (Boch et al. 2002; Brown and Allen 2004; Yang et al. 2004), indicating coregulation of TTSS genes with other biological processes during the bacterial interaction with plants. The regulatory mechanisms for many of the non-TTSS genes remain unclear. However, a number of the non-TTSS genes are induced by the *hrp* regulators such as *hrpL*, *hrpB*, *hrpG,* and *hrpX*. Co-induction of these genes with TTSS genes likely enables the bacteria to cope with various stress factors encountered during the interaction with the plant and exploit the host nutrients to promote growth.

It has been shown in *P. syringae* pv. *tomato* DC3000 strain that the *iaaL*, *cfa1*, and *cfa6* genes are induced by *hrpL* (Chang et al. 2005; Fouts et al. 2002; Lan et al. 2006). *iaaL* encodes indoleacetate-lysine ligase capable of producing IAA conjugates, an inactive form of the phytohormone auxin. *iaaL* has an "hrp box" motif in its promoter. DC3000 is capable of producing auxin (Glickmann et al. 1998) that may interfere with plant physiology. However, why the bacterium produces IaaL to inactivate auxin is not immediately understood. Genes *cfa1* and *cfa6* are involved in the biosynthesis of the phytotoxin coronatine, an important virulence factor in several *P. syringae* strains (Mittal and Davis 1995). Genes *cfa1* and *cfa6* do not have the hrp box promoter and likely are induced by *hrpL* indirectly via *corR*, the regulator of coronatine biosynthesis genes (Bender et al. 1999), that has a putative *hrp* box in its promoter (Fouts et al. 2002). In addition to these non-TTSS genes, our microarray analysis has identified several other DC3000 genes that apparently do not encode the type III effectors (Lan et al. 2006). Among these genes, PSPTOB0005 encodes a protein of the phosphoesterase family that is secreted from bacterial cells by the twin-arginine translocation pathway (Bronstein et al. 2005). PSPTO0873, PSPTO0874, and PSPTO0875 are likely to be organized in an operon and encode enzymes catalyzing the synthesis of certain secondary metabolites. PSPTO0834, PSPTO0835, and PSPTO0836 probably belong to another transcription unit encoding putative metabolic functions. PSPTO2691 encodes a membrane protein of the TerC family that is implicated in resistance to the toxic tellurium ions. A few of these genes also were shown to be HrpL-induced by Chang and associates (2005) using the HrpL-induced promoter assay. The identification of these genes indicated that the TTSS is coordinately activated with other protective or invasive mechanisms.

The coregulation of TTSS genes with other virulence-related genes also was reported in *R. solanacearum* and *Xanthomonas* spp. In *R. solanacearum*, microarray analysis of *hrpB*-regulated genes has identified TTSS genes as well as genes with metabolic function, signal transduction and regulation, and acquisition and transport of exogenous compounds (Occhialini et al. 2005). Two hemagglutinin-like protein genes and two transposase genes also were shown to be *hrpB*-induced by microarray (Occhialini et al. 2005). A few of the *hrpB* upregulated non-TTSS genes identified by microarray also were identified by the *hrpB*-dependent promoter trap assays (Mukaihara et al. 2004). A significant number of the *hrpB*-activated non-TTSS genes have the hrpII-box in their promoters. Similarly, a number of *hrpG/hrpX*-induced non-TTSS genes were identified by cDNA differential display in *X. campestris* pv. *vesicatoria* (Noel et al. 2001). It was reported recently that a gene encoding a protein secreted by the general secretion pathway in *X. oryzae* pv. *oryzae* has a "PIP box" promoter and is *hrpX*-activated (Furutani et al. 2004).

Perspectives.

The studies of bacterial TTSS gene regulation in the previous years enabled the construction of signal transduction pathways immediately upstream of the *hrp*/effector genes. Although additional regulatory genes have been identified regulating TTSS genes in various plant bacteria, the connection of these regulatory components with the downstream pathways remains obscure for most of these genes. The key puzzles for TTSS gene regulation are the identity and nature of the host and environmental signals regulating TTSS gene expression, the bacterial sensory proteins perceiving the signals, and the links between signal sensors and TTSS gene expression. The possible cross-talk between TTSS gene expression and other biochemical processes in bacteria also remains to be exploited.

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