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# Global virulence regulation networks in phytopathogenic bacteria

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**Phytopathogens coordinate multifaceted life histories and deploy stratified virulence determinants via complex, global regulation networks. We dissect the global regulation of four distantly related model phytopathogens to evaluate large-scale events and mechanisms that determine successful pathogenesis. Overarching themes include dependence on centralized cell-to-cell communication systems, pervasive two-component signal-transduction systems, post-transcriptional regulation systems, AraC-like regulators and sigma factors. Although these common regulatory systems control virulence, each functions in different capacities, and to differing ends, in the diverse species. Hence, the virulence regulation network of each species determines its survival and success in various life histories and niches.**

## Global virulence regulation networks dictate life histories and infection strategies

Phytopathogens survive in diverse environments, not only as pathogens but also as benign epiphytes on plant surfaces or saprophytes in soil and water. Consequently, expression of virulence factors and behaviors associated with virulence must be coordinated for energy conservation, appropriate disease development, evasion of host defense and eventual dispersal. The survival of a phytopathogen, therefore, relies on a controlled global virulence regulation network. Here, we discuss such networks in four distantly related and well-studied phytopathogens (Figure 1). *Ralstonia solanacearum* (hereafter *R.s.*) is a hemibiotrophic agent (with initial biotrophy followed by necrotrophy during infection) of bacterial wilt diseases across a wide range of hosts (for review, see Ref. [1]). *R.s.* also exists as a saprophyte in soil until it invades the roots of susceptible plants. *R.s.* colonizes xylem tissue and migrates to aerial parts of the plant where it accumulates and disables the vascular system of the plant (Figure 1a). *Pectobacterium carotovorum* subsp. *carotovorum* (hereafter *P.c.c.*) (previously *Erwinia carotovora subsp. carotovora*) is a necrotrophic soft-rot pathogen with a large host-range (for review, see Ref. [2]). *P.c.c.* can live as an epiphyte or as a saprophyte in soil and ground water until it encounters a susceptible host. It secretes pectolytic enzymes to macerate the host tissue (Figure 1b). *Xanthomonas campestris* pv. *campestris* (hereafter *X.c.c.*) is a hemibiotrophic, narrow-host-range agent of black rot (for review, see Ref. [3]) (Figure 1c). *Pseudomonas syringae* (hereafter *P.s.*) is the hemibiotrophic agent of bacterial speck, spot and rot diseases typically associated with a narrow host-range (for review, see Ref. [4]) (Figure 1d). Both *P.s.* and *X.c.c.* exist as epiphytes on plant surfaces until opportunity enables them to enter the intercellular apoplastic space inside leaf tissue. These disease stages and life-history transitions require information sensed from the environment, the host and the pathogen population. These inputs are received and integrated by central regulators to produce survival and virulence outputs through a global virulence regulation network.

Here, we focus on the global regulatory networks of these pathogens. We discuss the mechanisms that control expression of vital type III secretion systems (T3SS; Box 1), toxins, extracellular enzymes and various behaviors. We begin by presenting cell-to-cell communication systems and their influence on prominent regulators in each species. Then, we compare two-component regulators that report information about the environment to central regulator(s) or influence distinct aspects of virulence independently. We finish with a discussion of common transcription regulators that control T3SS expression, but that also have global impacts. By comparing global virulence-regulation strategies of these four pathogen groups, we aim to identify common mechanisms that are essential to plant infection and areas of divergence that enable each pathogen to take advantage of its biological niche.

## Cell-density-dependent regulation of virulence factors is crucial for all four phytopathogens

Cell-to-cell communication systems enable temporally coordinated gene expression within bacterial populations. The infection strategies of phytopathogens, which often require swift global changes in gene expression and physiology in response to environmental cues, are particularly reliant on cell-to-cell communication to coordinate crucial steps in pathogenesis. Some of the communication systems of phytopathogens are paradigmatic quorum-sensing systems, analogous in mechanism to the conserved LuxR/I system of *Photobacterium fischeri* (Box 2), whereas others represent mechanistically distinct systems particular to related pathogen groups. Here, we compare the main features and functions of cell-to-cell communication systems with respect to central regulators in each of our model species.

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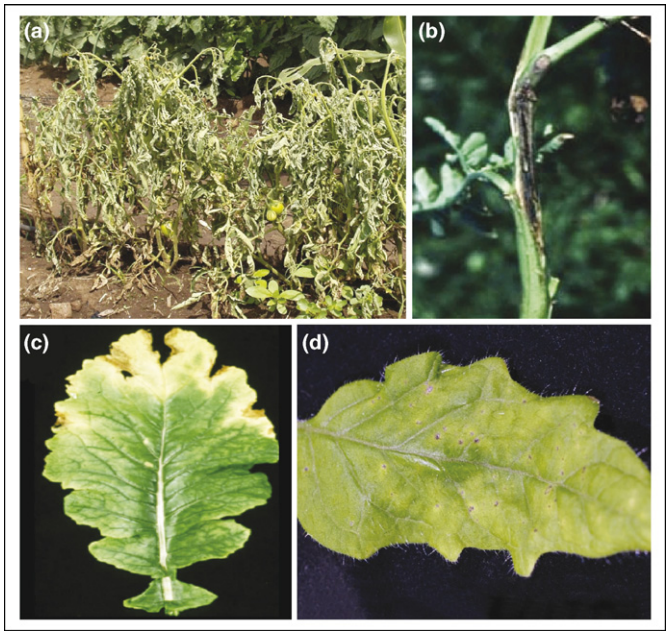


**Figure 1**. Disease symptoms of selected phytopathogens. (**a**) Bacterial wilt of *Ralstonia solanacearum* on tomato (courtesy of Caitilyn Allen, University of Wisconsin-Madison). (**b**) Stem rot by *Pectobacterium carotovorum* subsp. *carotovorum* on potato (courtesy of Amy Charkowski, University of Wisconsin-Madison). (**c**) Black rot by *Xanthomonas campestris* on cabbage (courtesy of Max Dow, University of Ireland). (**d**) *Pseudomonas syringae* bacterial spot on tomato (courtesy of Marc Nishimura, University of North Carolina-Chapel Hill).

### *Cell-to-cell communication in R.s. is responsible for the transition from early- to late-stage infection*

*R. s.* uses a genus-specific communication system that senses the diffusible 3-OH-palmitic acid methyl ester (3-OH-PAME) signal. The 3-OH-PAME system enables *R.s.* to make the transition from early- to late-stage pathogenesis by controlling the activity of the global virulence regulator, PhcA (Figure 2a). The 3-OH-PAME system is encoded by the *phc* (phenotypic conversion) operon [5], which encodes the 3-OH-PAME synthase, PhcB, the membrane-bound sensor, PhcS, and a downstream response regulator, PhcR [6]. At low cell-density, or in unconfined conditions, PhcA is inhibited by the 3-OH-PAME system. When the bacterial population density reaches $\sim 10^7$ cells $mL^{-1}$ and/or the concentration of 3-OH-PAME reaches 5 nM, 3-OH-PAME binds to PhcS [7] and PhcA is released from repression by PhcS. PhcA then either directly or indirectly activates virulence factors required for late-stage infection, including exopolysaccharides (EPS) [8] and cellulase [5], and represses virulence factors required for early-stage infection including the T3SS [9], swimming motility and siderophore expression [10].

The 3-OH-PAME system also up-regulates an acyl homoserine lactone (AHL)-dependent quorum-sensing system via PhcA activity. The quorum-sensing system is encoded by *solR* and *solI*, which are *luxR* and *luxI* homologs, respectively [11] (Box 2). SolR activates expression of at least one gene, *aidA*. The function of the novel AidA protein is unknown, and other SolR-regulated genes have yet to be identified. *solR* and *solI* are members of the 3-OH-PAME communication-system regulon, which controls the phenotypic switch from a saprophyte and early-stage colonizer to a full-blown pathogen. Because co-regulated genes usually function in a similar manner, the SolR–SolI system might be expected to have a role in virulence. Surprisingly, *solI* and *solR* mutants have been shown to retain full virulence when inoculated into cut petioles [11]; however, reduction in the virulence of these mutants might be evident if they were tested using different infection conditions such as soil soaking, which more closely mimic a natural infection.

### *Cell-to-cell communication in P.c.c. regulates global virulence expression*

Just as in *R.s.*, the cell-to-cell communication systems of *P.c.c.* are central to virulence regulation. However, the

**Box 1. The evolution of virulence regulation and the incorporation of T3SS**

Many Gram-negative bacterial pathogens of both animals and plants depend on T3SS for virulence [53]. The T3SS translocates effector proteins into host cells, where they interact and interfere with components of host-defense responses (for reviews, see Refs [54,55]). Many proteins of the T3SS apparatus retain structural similarity to bacterial flagellum proteins (for review, see Ref. [53]). The genes encoding the T3SS lie in clusters of structural and regulatory genes called *hrp* clusters and they tend to be flanked by genes encoding secreted effectors and their chaperones [56]. The structural genes of the T3SS are highly conserved in diverse species but the regulators linked within the complexes are variable (for review, see Ref. [48]). Phylogenetic comparisons indicate that T3SS have been acquired by horizontal transmission in diverse plant and animal pathogens at independent stages in their evolutionary histories [57,58]. Two distinct clades of T3SS emerge in our model phytopathogens; Group I in *Pectobacterium* and *Pseudomonas* species and Group II T3SS in *Ralstonia* and *Xanthomonas* species [48]. The shared importance of *hrpL* and *hrpS* in virulence regulatory pathways of *P.s.* and *P.c.c.* is probably due to the linkage of these loci to the T3SS on similar pathogenicity islands that were incorporated into the ancestors of these bacteria independently. In both cases, other loci that are essential in the regulation of virulence (*hrpX* and *hrpY* in *P.c.c.*, and *hrpR* in *P.s.*) are also linked to the T3SS on the pathogenicity island. Likewise, genes regulating virulence (*prhI*, *prhJ*, *prhR* and *hrpG*) of *R.s.* are also linked to the T3SS [59]. However, in *X.c.*, the regulatory genes *hrpG* and *hrpX* do not seem to be linked to the T3SS itself, but might have moved or even been incorporated later because they are found in close proximity to transposases in some strains. In each species, T3SS-encoded regulators seem to have become independently incorporated into the global virulence regulation system.

**Box 2. The paradigmatic LuxR/I quorum-sensing system**

The conserved LuxR/I quorum-sensing system was first identified in the bioluminescent *Photobacterium fischeri* (previously *Vibrio fischeri*), and the related LuxS system was characterized in *Vibrio harveyi*. In the LuxR/I system, the signal molecule is a diffusible acylated homoserine lactone (AHL) that is produced by the AHL synthase, LuxI. When it reaches critical concentration, AHL diffuses into the cell where it binds to and activates LuxR, a transcription regulator (for review, see Ref. [60]). Density-dependent communication systems centered on AHL are common among Gram-negative bacteria, although variable acyl side chains determine specificity. In the LuxS system, the signal molecule AI-2 is produced by LuxS and was identified as furanosyl borate diester in *V. harveyi*. The AI-2 signal is sensed by a signal-transduction system that, in turn, regulates gene expression. AI-2 signaling has been found in a range of bacteria, both Gram-negative and Gram-positive species, leading to the suggestion that it might be a 'universal signal'. However, AI-2 has a variable structure and metabolic roles making its definition as a signaling molecule, particularly a 'universal' one, controversial (for review, see Ref. [61]).



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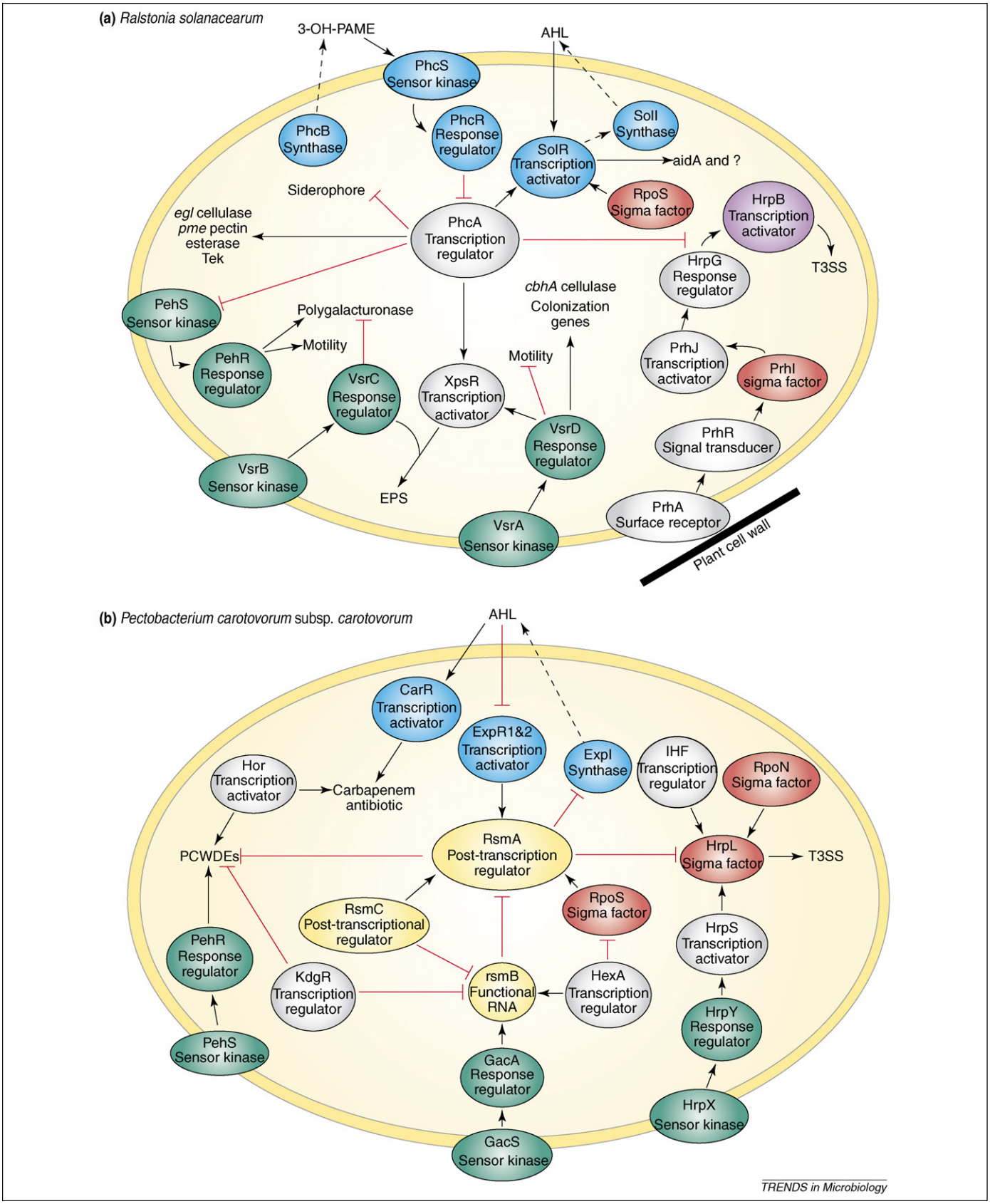


**Figure 2**. The global virulence regulation of *Ralstonia solanacearum* (*R.s.*) and *Pectobacterium carotovorum* subsp. *carotovorum* (*P.c.c.*). Color code: blue, associated with cell-to-cell communication; green, TCST systems; red, sigma factors; yellow, part of the Rsm system; purple, AraC-like transcription regulators; grey, other members that do not fit into major systems discussed in detail here. Cell-to-cell communication systems are located at the top of each figure, TCST systems are located on the bottom, extracellular enzymes and EPS regulation are found on the left, T3SS regulation is found on the right, and global regulators are situated in the center. Arrows represent positive regulation, barred arrows represent negative regulation, broken arrows represent synthesis. (**a**) PhcA is a global virulence regulator in *R.s.*; it controls motility, polygalacturonase, EPS and the T3SS. PhcA is controlled by a species-specific cell-to-cell communication system and, in turn, PhcA regulates the secondary quorum-



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communication systems are more complex in their regulatory mechanisms (and, sadly, their nomenclature). The *P.c.c.* cell-to-cell communication systems are responsible for regulating the T3SS, plant cell-wall-degrading enzymes (PCWDE) and antibiotic production. *P.c.c.* employs quorum-sensing systems that include up to three transcription activators that are responsive to two acylated AHL molecules that, in turn, are encoded by one synthase (Figure 2b). The LuxI synthase homolog, ExpI (also referred to as CarI, AhlI and HslI), was first described as the exoenzyme-production inducer [12]. ExpI can synthesize 3-oxo-C6-HSL or 3-oxo-C8-HSL. Strains that produce both variations of AHL have been designated class I strains, whereas strains that produce only 3-oxo-C6-HSL have been designated class II [2]. Once AHL signaling molecules accumulate, they can interact with CarR, ExpR1 or ExpR2. The most straightforward of these is the CarR regulator. CarR binds 3-oxo-C6-HSL and subsequently binds the *carA* promoter, which controls the *car* operon that encodes the carbapenem antibiotic [13] (Figure 2b). The *car* operon is also controlled by the transcription regulator Hor, but by unknown means. The other two LuxR homologs, ExpR1 (also known as ExpR and EccR) and ExpR2 (also known as VirR), directly inhibit virulence in the absence of threshold levels of AHL by up-regulating *rsmA* [14]. RsmA is a member of the post-transcriptional Rsm system (Box 3) and destabilizes mRNA transcripts that encode PCWDEs, including cellulase, pectate lyase and protease. The Rsm system also includes *rsmB,* which binds to and down regulates RsmA, enabling translation of RsmA-targeted mRNAs. Threshold levels of 3-oxo-C8-HSL bind to ExpR1, whereas 3-oxo-C6-HSL binds to ExpR2. Binding of their cognate AHL inactivates ExpR1 and ExpR2, inhibiting *rsmA* expression and freeing the mRNA transcripts that encode PCWDEs [14,15]. Mutant *P.c.c.* lacking *expI* cannot produce AHLs and PCWDEs, and are consequently avirulent [12]. Thus, *P.c.c.* quorum sensing feeds into the central coordination of virulence for successful infection via synergistic negative regulation by two LuxR homologs ExpR1 and ExpR2.

### *The cell-to-cell communication in X.c.c. is crucial to global virulence regulation and basic cellular functions*

*X.c.c.* possesses a previously unknown cell-to-cell communication system that might be shared among bacterial species [16]. This system relies on a diffusible signal factor [DSF (*cis*-11-methyl-2-dodecenoic acid)] that has been linked to the regulation of motility, toxin and oxidative-stress resistance, aerobic respiration [17], biofilm dispersal, and extracellular enzyme and EPS production [18] (Figure 3a). DSF is synthesized by products of the *rpf* virulence-regulation cluster genes *rpfB* and *rpfF* [16]. DSF accumulates in the early stationary phase and is sensed by a unique two-component signal-transduction (TCST) system consisting of RpfC and RpfG. This leads to degradation of cyclic di-GMP [19]. Cyclic di-GMP was first described as an activator of bacterial cellulose synthase [20] but was subsequently ascribed global regulatory functions (for review, see Ref. [21]). Decreased levels of cyclic di-GMP in *X.c.c.* lead to dispersal of biofilms, increased production of EPS and extracellular enzymes, and alterations in T3SS expression [22]. Cyclic di-GMP is also involved in the regulation of iron up-take, metabolic activities, LPS production, multi-drug resistance and detoxification, but the regulatory network(s) responsible for cyclic di-GMP–dependent activation is poorly understood. During the writing of this review, microarray data have been used to identify three hierarchical transcription regulators (Clp, Zur and FhrR) downstream of cyclic di-GMP activation that are involved in EPS, T3SS and extracellular enzyme production among other cellular functions [23]. This novel and pleiotropic regulation system links virulence to basic, essential cellular functions.

### *P.s. cell-to-cell communication is needed for both early and late stages of disease*

Unlike the three systems already described, the cell-to-cell communication system of *P.s.* does not seem to be the first

**Box 3. Post-transcriptional Rsm-mediated regulation is important in both *P.c.c.* and *P.s.***

The Rsm system has an important role in the virulence regulation of *P.c.c.* but it is broadly distributed throughout the eubacteria, and thus might be a common central player in virulence regulation [62].

The Rsm system is made up of the proteins RsmA, RsmC and the regulatory RNA *rsmB* [15]. RsmA is the main regulator and targets mRNA transcripts to regulate virulence in addition to cellular functions. RsmA and the related CsrA from *Escherichia coli* are thought to function similarly: they bind mRNA transcripts using a KH domain (K homology domain originally identified in the heterogeneous nuclear ribonucleoprotein particle hnRNP K binds single-stranded RNA) and can either stabilize or promote the decay of targeted transcripts [63]. CsrA destabilizes messages by binding near the ribosome-binding site, thereby interfering with translation and leading to transcript degradation by RNases [64]. In *P.c.c.,* RsmA is independently down-regulated by the quorum-sensing system and the stationary sigma factor, RpoS [65] (see Figure 2a of main text). RsmA, in turn, targets and destabilizes the transcripts of PCWDEs, which are crucial virulence factors [66], and HrpL, the alternative sigma factor that regulates expression of the T3SS (for review, see Ref. [2]) (see Figure 2a of main text). *rsmB* (also known as *aepH* in *P.c.c.*) and *csrB* in *E. coli* are non-coding, functional RNAs that are antagonists of RsmA and CsrA (for review, see Ref. [67]). *rsmB* is up-regulated by the GacA/S TCST system [68] and HexA, a global regulator that also inhibits RpoS expression [69]. *rsmB* is repressed by the KdgR regulator, which is responsive to breakdown products of plant cell walls such as 2-keto-3-deoxygluconate [70]. The *rsmB* structure contains multiple stem loops with GGA motifs thought to be binding sites of RsmA [71]. CsrA and *csrB* can be co-purified in a stoichiometry of 18:1, indicating that each *csrB* molecule binds 18 CsrA molecules and thus efficiently inhibits its binding to target transcripts [72]. In *P.c.c.*, *rsmB* also efficiently inhibits RsmA. *rsmB* exists in two forms, a 479-nucleotide unprocessed form and a processed 259-nucleotide form that is thought to sequester RsmA via nine CsrA-like binding motifs [72]. Lastly, RsmC, also known as HexY, negatively regulates *rsmB* and, directly or indirectly, activates *rsmA* expression [73]. RsmC has no known homologs outside of *P.c.c.* and has no known DNA-binding motif. The complete regulon of RsmA and the Rsm regulation system is unknown.

sensing system. (**b**) The prominent virulence mechanism in *P.c.c.* is the post-transcriptional Rsm system. The Rsm system is mediated by cell-to-cell communication and the GacA/S TCST system and controls the T3SS and cell-wall-degrading enzymes.



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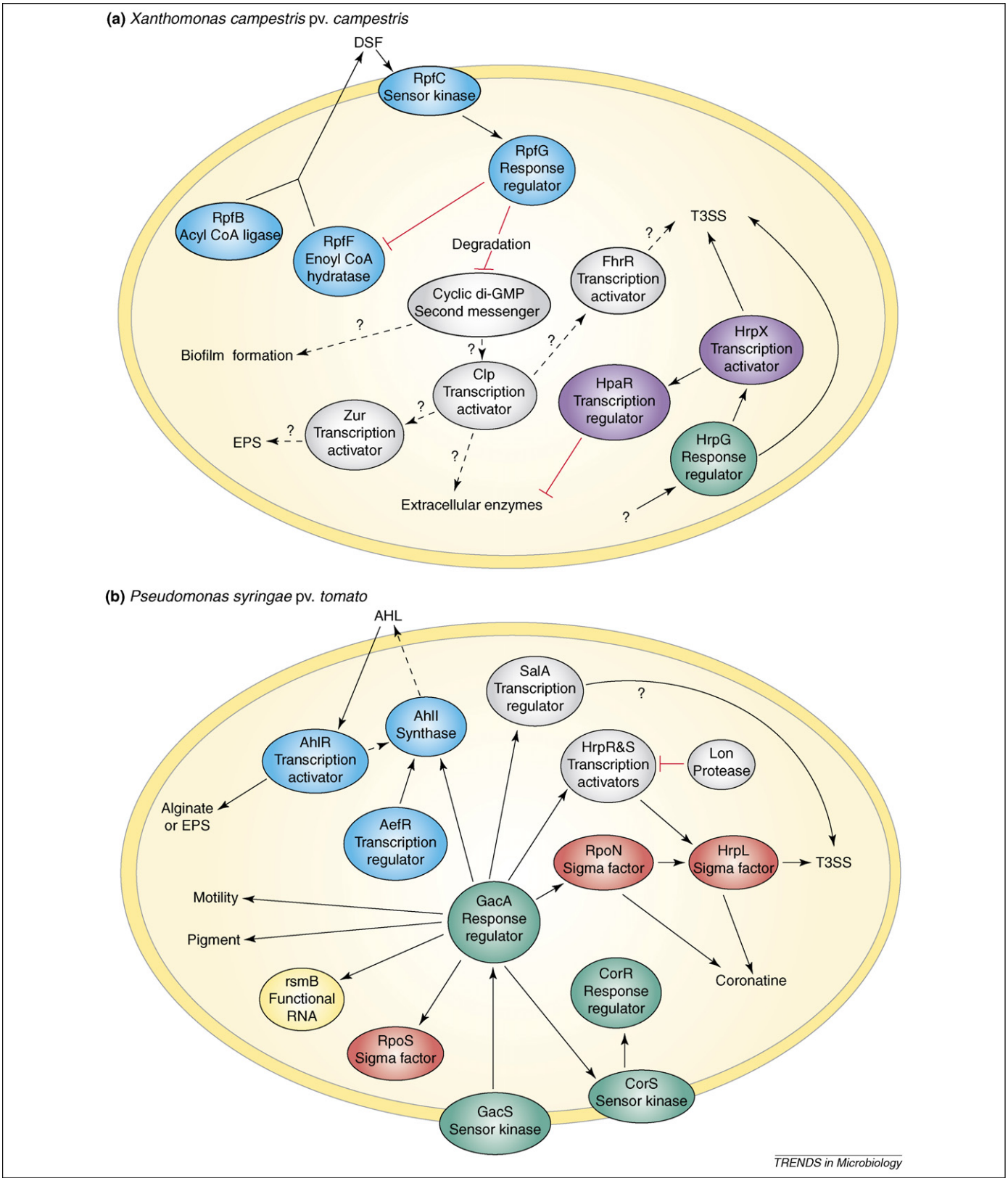


**Figure 3**. The global virulence regulation of *Xanthomonas campestris* pv. *campestris* (*X.c.c.*) and *Pseudomonas syringae* pv. *tomato* (*P.s.t.*). Color code: blue, associated with cell-to-cell communication; green, TCST systems; red, sigma factors; yellow, part of the Rsm system; purple, AraC-like transcription regulators; grey, other members that do not fit into major systems discussed in detail here. Arrows represent positive regulation, barred arrows represent negative regulation, broken arrows represent synthesis, and '?' represent unknown interactions. (**a**) The global regulator in *X.c.c.* is the small molecule cyclic di-GMP. Cyclic di-GMP is degraded by cell-to-cell communication via a unique TCST system. Cyclic di-GMP levels subsequently effect biofilm formation, extracellular enzymes, EPS and the T3SS. (**b**) The global regulation system in *P.s.t.* is the GacA/S TCST system. GacA is known to directly or indirectly regulate quorum sensing, coronatine production, the T3SS, motility and pigment production.



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step in the regulatory hierarchy of virulence that controls a regulatory molecule that subsequently integrates many virulence responses. Instead, it is the virulence integrating GacA/S TCST system (described later) that controls the AhlI/R (LuxI/R-homologous) quorum-sensing system in combination with the TetR-family transcription activator AefR [24] (Figure 3b). The AhlI/R system is essential for the regulation of intercellular host-tissue maceration [25] and epiphytic fitness [26] because it controls EPS production and swarming motility in diverse environments [25]. By regulating swarming motility, quorum sensing becomes a means to disperse epiphytic aggregates on the leaf surface. Quorum-sensing mutants are also unable to macerate tissue at later stages of infection. Therefore, the AhlI/R system is important for regulating virulence at various stages of infection. The complete regulon controlled by AhlI/R, and the mechanisms by which it is regulated, are unknown.

## TCST systems provide environmental signal input to global virulence regulation

TCST systems are commonly used by bacteria to sense and adapt to extracellular environmental signals. The two common, and well studied, components are a membrane-bound sensor kinase and a cognate, cytoplasmic response regulator (for review, see Ref. [27]). Subsequent to signal perception, the cytoplasmic response regulator is either activated or inactivated by phosphorylation. The response regulator can carry out a variety of tasks, including transcriptional regulation and protein–protein interactions. In the phytopathogens discussed here, TCST systems are typically used to directly regulate single or a few related virulence determinants, whereas only a few TCST systems have global effects on virulence.

### *TCST systems in R.s., P.c.c. and X.c.c. complement global virulence regulation*

In *R. s.*, it seems that global virulence regulation is coordinated by quorum sensing (see earlier), and TCST systems have ancillary roles in virulence regulation. For example, the sensor kinase and response regulator pairs VsrA/D and VsrB/C, respectively, both function in combination with PhcA (discussed earlier) to regulate the key virulence factor EPS (for review see Ref. [1]). Together, activated VsrD and PhcA activate XpsR, an atypical response regulator [8,28] (Figure 2a). Activated XpsR, together with activated VsrC, initiates transcription of the 16-kb *eps* operon, which is responsible for production of exopolysaccharide 1 (EPS I) [1,29]. In addition, *R.s.* uses the NtrB/C-like TCST system PehS/R. PehS/R functions in opposition with VsrC to activate the transcription of *pglA,* one of three polygalacturonases used to degrade the plant cell wall. In addition, PehR activates swimming and twitching motility [30] (Figure 2a).

Similarly, *P.c.c.* deploys TCST systems that are activated independently from the global regulator RsmA and that have ancillary roles in virulence regulation. For example, the PehS/R TCST system in *P.c.c.* also regulates PCWDEs (Figure 2b), as does the system with the same name in *R.s.* However, the PehS/R system in *P.c.c.* is not a member of the NtrB/C family like the *R.s.* PehS/R. Instead, it is a member of the EnvZ/OmpR family of the TCST systems [31]. Notably, this system is homologous to the PhoP/Q system that regulates virulence throughout the Enterobacteria, indicating that the theme of ancillary roles for TCST systems in virulence regulation extends beyond phytopathogens [31].

The OmpR-family response regulator of *X.c.c.*, HrpG, regulates the T3SS [32] (Figure 3a). Although a cognate sensor kinase for this response regulator has not been identified, this finding indicates that a TCST might be involved in T3SS regulation, in addition to the cyclic di-GMP interactions, which are regulated by quorum sensing (discussed earlier). The lack of an identified cognate sensor kinase indicates the possibility of cross-talk between multiple systems and/or redundancy in the function of a sensor kinase. Future studies on HrpG activation will be useful in understanding this seemingly incomplete TCST system, and will define the cues for T3SS system expression.

These are exemplary cases of TCST systems that contribute supplemental regulatory function to global virulence regulation networks. However, a recent study using reporter gene studies has shown that *R.s.* VsrA is a direct regulator of motility, T3SS and quorum sensing, which indicates more than a supplemental role in virulence regulation (J. Yao and C. Allen, unpublished) (Figure 2a). Although the mechanisms of this regulation are unknown, further studies and fully identified regulons of TCST systems might shift the view of their roles in global virulence regulation.

### *The GacA/S and RpfC/G TCST systems are exceptional in their global impact on virulence*

*The GacA/S system is crucial to virulence regulation in both P.c.c. and P.s.* In contrast to all other TCST systems discussed so far, the GacA/S TCST system has a key global role in virulence regulation. The Gac system is found in a variety of Gram-negative bacteria and its name was coined from *Pseudomonas fluorescens* as the global regulator of antibiotic and cyanide production [33], although the homolog LemA (lesion manifestation) was identified first in *P.s.* [34]. The Gac system in *P.c.c.* and *P.s.* interacts with the Rsm global virulence regulation system (Box 3). Activated GacA increases the expression of the functional RNA *rsmB,* which down-regulates RsmA, thereby enabling translation of RsmA targeted mRNAs.

*The Gac system is the central virulence regulator in P.s.* The GacA/S TCST system is directly or indirectly involved in the regulation of every known virulence factor in *P.s.* including coronatine phytotoxin, EPS, and the T3SS and its effectors [35]. The GacA/S system achieves this breadth by positively regulating the quorum-sensing system, which is crucial to motility and EPS regulation, and the sigma factors RpoS, RpoN and HrpL [35] (Figure 3b). The Gac system is activated in the host apoplast by a combination of signals, including low pH, low osmolarity, sucrose or fructose sugars and a lack of complex carbon and nitrogen sources [35,36]. However, the exact signal(s) GacS receives and the mechanism of GacA–GacS interaction remain unknown. In addition, the molecular means by which GacA/S regulates downstream regulators is largely unknown. Answers to these questions should reveal how a pathogen such as



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*P.s.* relies so heavily on a TCST system that functions in one of two states, inactivated or activated, while coordinating quantitative, analog outputs such as the expression of virulence factors.

### *The RpfC/G TCST system in X.c.c. links cell-to-cell signaling to diverse behavioral changes*

Unlike in *P.s.* and *P.c.c.,* a well-characterized TCST system constitutes the novel cell-to-cell communication system of *X.c.c*. The unique RpfC/RpfG TCST system (described earlier) is not only central to cell-to-cell communication but also to the regulation of diverse behaviors mediated via the degradation of cyclic di-GMP (Figure 3a). RpfC is a hybrid membrane-bound, bi-functional sensor kinase composed of three distinct functional domains that autophosphorylate and phosphor-relay to both activate RpfG and repress DSF biosynthesis. RpfG is a novel cyclic di-GMP phosphodiesterase that degrades the bacterial second-messenger cyclic di-GMP [37,38]. As mentioned, cyclic di-GMP has global regulatory functions, but the proteins with which it interacts downstream of RpfG are only beginning to be identified. Thus, the RpfC/G TCST system seems to connect large-scale virulence regulation with cell-to-cell communication. However, the many gaps that exist in the network could, when discovered, change our perception of the importance of RpfC and RpfG in global virulence regulation.

## T3SS gene expression is regulated by AraC-type regulators or alternative sigma factors

### *AraC-type regulators regulate T3SS expression in R.s. and X.c.c.*

AraC-type regulators commonly have important virulence regulation roles in association with T3SSs in animal and plant bacterial pathogens. Particularly in enterobacterial pathogens such as *Salmonella, Shigella, Yersinia* and *Escherichia coli*, AraC-like regulators are key activators of T3SS expression. *Pseudomonas aeruginosa* also relies on an AraC-like transcription activator, ExsA, to regulate the T3SS [39]. Interestingly, neither *P.s.* nor *P.c.c.* use AraC-like proteins to regulate their T3SS, whereas *R.s.* and *X.c.c.* do. In *R.s.,* the response regulator HrpG responds to contact with the plant cell wall via PrhA and activates the expression of the AraC-like HrpB [9,40] (Figure 2a). HrpB activates the *hrp* regulon (T3SS) promoters by binding a specific plant inducible promoter box sequence [41,42]. HrpB in *R.s.* also regulates chemotaxis and siderophore expression, and coordinates expression of virulence genes, but by unknown means [40,43]. In *X. campestris*, the response regulator HrpG also activates the expression of the AraC-like regulator HrpX [32,41] (Figure 3a). HrpX and HrpB are related, and mutation of HrpX can be complemented by HrpB. Although the importance of AraC-like regulators is clear in T3SS regulation, the means by which they influence global virulence regulation has not been fully established.

### *Alternative sigma factors regulate T3SS and more*

A major theme across T3SS regulation in phytopathogens is the use of alternative sigma factors. Sigma factors are essential transcription initiation factors that direct RNA polymerase to bind specific promoter regions. In *P.s.* and *P.c.c.*, the alternative sigma factor responsible for activating the expression of the whole T3SS and the effectors is HrpL [44]. HrpL is a member of the extracytoplasmic function (ECF) sigma factor family, a family that is essential for controlling transcription during stress responses and morphological changes [45]. In both *P.c.c.* and *P.s.*, another sigma factor, RpoN/sigma-54, activates the expression of HrpL (Figures 2b and 3b). In addition, specific NtrC-like transcription activators regulate HrpL expression in both cases: HrpR and HrpS in *P.s.* [44] and HrpS in *Erwinia* and *Pectobacterium* species [46,47] (for review, see Ref. [48]). In *P.s.*, HrpR/S activation of the T3SS might be reinforced by the transcription activator SalA [35] but repressed by Lon protease via protein degradation by HrpR [49].The alternative sigma factor PrhI in *R.s.* is regulated by the signal transducer PrhR/A, which is activated by contact with the plant cell wall. PrhI then activates expression of the transcription activator PrhJ, which is then responsible for activating the expression of HrpG [50] (Figure 2a). Whereas HrpL and PrhI have been studied in relation to T3SS regulation, whole genome expression profiling studies have begun to expand our knowledge of their regulons beyond T3SS gene expression. Most notably, the regulon of HrpL in *P.s.* pv. *tomato* has recently been expanded to include protein synthesis, metabolic genes [51] and might include the coronatine phytotoxin regulator, CorR [48].

## Concluding remarks

Phytopathogens coordinate transitions in life histories and infection strategies by collecting information from the host plant, the environment and their own population density. These inputs result in a collection of virulence outputs in each species that is determined by integrated, global regulation networks. The current evidence indicates that virulence regulation networks in the phytopathogens we profile here center on global regulators and cell-density-dependent regulation. Complementary TCST systems provide environmental signal inputs to virulence regulation networks. AraC-type regulators and alternative sigma

**Box 4. Outstanding questions**

- What are the host or environmental molecules that activate virulence of phytopathogens? How are they perceived at the bacterial surface?
- The activators and targets of the central regulation system of *X.c.c.* remain poorly defined. Although a quorum-sensing system involved in virulence has been identified, there is potential for unidentified TCST systems that are involved in the perception of environmental signals. Likewise, the proteins responsible for coordination of cyclic di-GMP production with virulence factors, such as expression of extracellular cell-wall-degrading enzymes, remain to be well characterized.
- Does post-transcriptional regulation by systems analogous to the Rsm system of *P.c.c.* exist in other phytopathogens? For example, a *rsmB* homolog exists in *P.s.* but its role in virulence regulation is poorly understood.
- The role of the SolR/I quorum-sensing system of *R.s.* in virulence regulation is poorly defined. How might the system be contributing to disease development or survival of *R.s.*?
- Microarray studies and functional screens have begun to identify full regulons for key transcription activators in the virulence pathways of phytopathogens. The functional importance of many of the identified genes in pathogen life histories remains to be studied.



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factors, which are historically important for T3SS regulation, have global regulatory impacts that are supplemental to global regulators. Although many questions still remain regarding each species (Box 4), the use of DNA microarrays and genome sequences is advancing knowledge quickly. Despite common mechanisms and themes among the phytopathogens discussed here, phylogenetic comparisons of their DNA sequences reveals that each species is more closely related to non-plant pathogen species than they are to each other [52]. Identifying how common mechanisms and horizontally acquired virulence determinants have become incorporated into global regulation networks might be the next step in understanding the success of a phytopathogen in infection processes and their own biological niches.

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**References**

1 Schell, M.A. (2000) Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. *Annu. Rev. Phytopathol.* 38, 263–292
2 Barnard, A.M. and Salmond, G.P. (2007) Quorum sensing in *Erwinia* species. *Anal. Bioanal. Chem.* 387, 415–423
3 Crossman, L. and Dow, J.M. (2004) Biofilm formation and dispersal in *Xanthomonas campestris*. *Microbes Infect.* 6, 623–629
4 Nomura, K. *et al.* (2005) Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions. *Curr. Opin. Plant Biol.* 8, 361–368
5 Brumbley, S.M. *et al.* (1993) Phenotype conversion in *Pseudomonas solanacearum* due to spontaneous inactivation of PhcA, a putative LysR transcriptional regulator. *J. Bacteriol.* 175, 5477–5487
6 Clough, S.J. *et al.* (1997) A two-component system in *Ralstonia (Pseudomonas) solanacearum* modulates production of PhcA-regulated virulence factors in response to 3-hydroxypalmitic acid methyl ester. *J. Bacteriol.* 179, 3639–3648
7 Flavier, A.B. *et al.* (1997) Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol. Microbiol.* 26, 251–259
8 Huang, J. *et al.* (1998) Joint transcriptional control of *xpsR*, the unusual signal integrator of the *Ralstonia solanacearum* virulence gene regulatory network, by a response regulator and a LysR-type transcriptional activator. *J. Bacteriol.* 180, 2736–2743
9 Genin, S. *et al.* (2005) Control of the *Ralstonia solanacearum* Type III secretion system (Hrp) genes by the global virulence regulator PhcA. *FEBS Lett.* 579, 2077–2081
10 Bhatt, G. and Denny, T.P. (2004) *Ralstonia solanacearum* iron scavenging by the siderophore staphyloferrin B is controlled by PhcA, the global virulence regulator. *J. Bacteriol.* 186, 7896–7904
11 Flavier, A.B. *et al.* (1997) Hierarchical autoinduction in *Ralstonia solanacearum*: control of acyl-homoserine lactone production by a novel autoregulatory system responsive to 3-hydroxypalmitic acid methyl ester. *J. Bacteriol.* 179, 7089–7097
12 Pirhonen, M. *et al.* (1993) A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. *EMBO J.* 12, 2467–2476
13 Welch, M. *et al.* (2000) N-acyl homoserine lactone binding to the CarR receptor determines quorum-sensing specificity in *Erwinia*. *EMBO J.* 19, 631–641
14 Sjoblom, S. *et al.* (2006) Cooperation of two distinct ExpR regulators controls quorum sensing specificity and virulence in the plant pathogen *Erwinia carotovora*. *Mol. Microbiol.* 60, 1474–1489
15 Cui, Y. *et al.* (2005) ExpR, a LuxR homolog of *Erwinia carotovora* subsp. *carotovora*, activates transcription of *rsmA*, which specifies a global regulatory RNA-binding protein. *J. Bacteriol.* 187, 4792–4803
16 Wang, L.H. *et al.* (2004) A bacterial cell–cell communication signal with cross-kingdom structural analogues. *Mol. Microbiol.* 51, 903–912
17 He, Y.W. *et al.* (2006) Genome scale analysis of diffusible signal factor regulon in *Xanthomonas campestris* pv. *campestris*: identification of novel cell–cell communication-dependent genes and functions. *Mol. Microbiol.* 59, 610–622
18 Dow, J.M. *et al.* (2003) Biofilm dispersal in *Xanthomonas campestris* is controlled by cell–cell signaling and is required for full virulence to plants. *Proc. Natl. Acad. Sci. U. S. A.* 100, 10995–11000
19 Slater, H. *et al.* (2000) A two-component system involving an HD-GYP domain protein links cell–cell signalling to pathogenicity gene expression in *Xanthomonas campestris*. *Mol. Microbiol.* 38, 986–1003
20 Ross, P. *et al.* (1987) Regulation of cellulose synthesis in *Acetobacter xylinum* by cyclic diguanylic acid. *Nature* 325, 279–281
21 Romling, U. and Amikam, D. (2006) Cyclic di-GMP as a second messenger. *Curr. Opin. Microbiol.* 9, 218–228
22 Fouhy, Y. *et al.* (2006) Cell–cell signaling, cyclic di-GMP turnover and regulation of virulence in *Xanthomonas campestris*. *Res. Microbiol.* 157, 899–904
23 He, Y.W. *et al.* (2007) *Xanthomonas campestris* cell–cell communication involves a putative nucleotide receptor protein Clp and a hierarchical signalling network. *Mol. Microbiol.* 64, 281–292
24 Quinones, B. *et al.* (2004) Regulation of AHL production and its contribution to epiphytic fitness in *Pseudomonas syringae*. *Mol. Plant Microbe Interact.* 17, 521–531
25 Quinones, B. *et al.* (2005) Quorum sensing regulates exopolysaccharide production, motility, and virulence in *Pseudomonas syringae*. *Mol. Plant Microbe Interact.* 18, 682–693
26 Monier, J.M. and Lindow, S.E. (2003) Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. *Proc. Natl. Acad. Sci. U. S. A.* 100, 15977–15982
27 Beier, D. and Gross, R. (2006) Regulation of bacterial virulence by two-component systems. *Curr. Opin. Microbiol.* 9, 143–152
28 Huang, J. *et al.* (1995) A complex network regulates expression of *eps* and other virulence genes of *Pseudomonas solanacearum*. *J. Bacteriol.* 177, 1259–1267
29 Garg, R.P. *et al.* (2000) Multicomponent transcriptional regulation at the complex promoter of the exopolysaccharide I biosynthetic operon of *Ralstonia solanacearum*. *J. Bacteriol.* 182, 6659–6666
30 Allen, C. *et al.* (1997) A regulatory locus, *pehSR*, controls polygalacturonase production and other virulence functions in *Ralstonia solanacearum*. *Mol. Plant Microbe Interact.* 10, 1054–1064
31 Flego, D. *et al.* (2000) A two-component regulatory system, *pehR-pehS*, controls endopolygalacturonase production and virulence in the plant pathogen *Erwinia carotovora* subsp. *carotovora*. *Mol. Plant Microbe Interact.* 13, 447–455
32 Wengelnik, K. *et al.* (1996) HrpG, a key *hrp* regulatory protein of *Xanthomonas campestris* pv. *vesicatoria* is homologous to two-component response regulators. *Mol. Plant Microbe Interact.* 9, 704–712
33 Laville, J. *et al.* (1992) Global control in *Pseudomonas fluorescens* mediating antibiotic synthesis and suppression of black root rot of tobacco. *Proc. Natl. Acad. Sci. U. S. A.* 89, 1562–1566
34 Hrabak, E.M. and Willis, D.K. (1992) The *lemA* gene required for pathogenicity of *Pseudomonas syringae* pv. *syringae* on bean is a member of a family of two-component regulators. *J. Bacteriol.* 174, 3011–3020
35 Chatterjee, A. *et al.* (2003) GacA, the response regulator of a two-component system, acts as a master regulator in *Pseudomonas syringae* pv. *tomato* DC3000 by controlling regulatory RNA, transcriptional activators, and alternate sigma factors. *Mol. Plant Microbe Interact.* 16, 1106–1117
36 Rahme, L.G. *et al.* (1992) Plant and environmental sensory signals control the expression of *hrp* genes in *Pseudomonas syringae* pv. *phaseolicola*. *J. Bacteriol.* 174, 3499–3507
37 Dow, J.M. *et al.* (2006) The HD-GYP domain, cyclic di-GMP signaling, and bacterial virulence to plants. *Mol. Plant Microbe Interact.* 19, 1378–1384
38 Ryan, R.P. *et al.* (2006) Cell-cell signaling in *Xanthomonas campestris* involves an HD-GYP domain protein that functions in



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cyclic di-GMP turnover. *Proc. Natl. Acad. Sci. U. S. A.* 103, 6712–6717
39 Francis, M.S. *et al.* (2002) Regulation of type III secretion systems. *Curr. Opin. Microbiol.* 5, 166–172
40 Valls, M. *et al.* (2006) Integrated regulation of the type III secretion system and other virulence determinants in *Ralstonia solanacearum*. *PLoS Pathog* 2, e82
41 Koebnik, R. *et al.* (2006) Specific binding of the *Xanthomonas campestris* pv. *vesicatoria* AraC-type transcriptional activator HrpX to plant-inducible promoter boxes. *J. Bacteriol.* 188, 7652–7660
42 Mukaihara, T. *et al.* (2004) Genetic screening of Hrp type III-related pathogenicity genes controlled by the HrpB transcriptional activator in *Ralstonia solanacearum*. *Mol. Microbiol.* 54, 863–875
43 Occhialini, A. *et al.* (2005) Genome-wide analysis of gene expression in *Ralstonia solanacearum* reveals that the *hrpB* gene acts as a regulatory switch controlling multiple virulence pathways. *Mol. Plant Microbe Interact.* 18, 938–949
44 Chatterjee, A. *et al.* (2002) Regulation of *Erwinia carotovora hrpL*$_{(Ecc)}$ (sigma-L$_{(Ecc)}$), which encodes an extracytoplasmic function subfamily of sigma factor required for expression of the HRP regulon. *Mol. Plant Microbe Interact.* 15, 971–980
45 Helmann, J.D. (2002) The extracytoplasmic function (ECF) sigma factors. *Adv. Microb. Physiol.* 46, 47–110
46 Hutcheson, S.W. *et al.* (2001) Enhancer-binding proteins HrpR and HrpS interact to regulate *hrp*-encoded type III protein secretion in *Pseudomonas syringae* strains. *J. Bacteriol.* 183, 5589–5598
47 Wei, Z. *et al.* (2000) Regulation of *hrp* genes and type III protein secretion in *Erwinia amylovora* by HrpX/HrpY, a novel two-component system, and HrpS. *Mol. Plant Microbe Interact.* 13, 1251–1262
48 Tang, X. *et al.* (2006) Regulation of the type III secretion system in phytopathogenic bacteria. *Mol. Plant Microbe Interact.* 19, 1159–1166
49 Losada, L.C. and Hutcheson, S.W. (2005) Type III secretion chaperones of *Pseudomonas syringae* protect effectors from Lon-associated degradation. *Mol. Microbiol.* 55, 941–953
50 Brito, B. *et al.* (2002) A signal transfer system through three compartments transduces the plant cell contact-dependent signal controlling *Ralstonia solanacearum hrp* genes. *Mol. Plant Microbe Interact.* 15, 109–119
51 Ferreira, A.O. *et al.* (2006) Whole-genome expression profiling defines the HrpL regulon of *Pseudomonas syringae* pv. *tomato* DC3000, allows *de novo* reconstruction of the Hrp *cis* clement, and identifies novel coregulated genes. *Mol. Plant Microbe Interact.* 19, 1167–1179
52 de Souza, J.T. *et al.* (2003) Conservation of the response regulator gene *gacA* in *Pseudomonas species*. *Environ. Microbiol.* 5, 1328–1340
53 He, S.Y. *et al.* (2004) Type III protein secretion mechanism in mammalian and plant pathogens. *Biochim. Biophys. Acta* 1694, 181–206
54 Chisholm, S.T. *et al.* (2006) Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124, 803–814
55 Jones, J.D. and Dangl, J.L. (2006) The plant immune system. *Nature* 444, 323–329
56 Alfano, J.R. *et al.* (2000) The *Pseudomonas syringae* Hrp pathogenicity island has a tripartite mosaic structure composed of a cluster of type III secretion genes bounded by exchangeable effector and conserved effector loci that contribute to parasitic fitness and pathogenicity in plants. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4856–4861
57 Dale, C. *et al.* (2002) Type III secretion systems and the evolution of mutualistic endosymbiosis. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12397–12402
58 Gophna, U. *et al.* (2003) Bacterial type III secretion systems are ancient and evolved by multiple horizontal-transfer events. *Gene* 312, 151–163
59 Salanoubat, M. *et al.* (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature* 415, 497–502
60 Miller, M.B. and Bassler, B.L. (2001) Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55, 165–199
61 De Keersmaecker, S.C. *et al.* (2006) Let LuxS speak up in AI-2 signaling. *Trends Microbiol.* 14, 114–119
62 White, D. *et al.* (1996) Phylogenetic distribution of the global regulatory gene *csrA* among eubacteria. *Gene* 182, 221–223
63 Whitehead, N.A. *et al.* (2002) The regulation of virulence in phytopathogenic *Erwinia* species: quorum sensing, antibiotics and ecological considerations. *Antonie Van Leeuwenhoek* 81, 223–231
64 Baker, C.S. *et al.* (2002) CsrA regulates glycogen biosynthesis by preventing translation of *glgC* in *Escherichia coli*. *Mol. Microbiol.* 44, 1599–1610
65 Mukherjee, A. *et al.* (1998) RpoS (sigma-S) controls expression of *rsmA*, a global regulator of secondary metabolites, harpin, and extracellular proteins in *Erwinia carotovora*. *J. Bacteriol.* 180, 3629–3634
66 Cui, Y. *et al.* (1995) Identification of a global repressor gene, *rsmA*, of *Erwinia carotovora* subsp. *carotovora* that controls extracellular enzymes, N-(3-oxohexanoyl)-L-homoserine lactone, and pathogenicity in soft-rotting *Erwinia* spp. *J. Bacteriol.* 177, 5108–5115
67 Romeo, T. (1998) Global regulation by the small RNA-binding protein CsrA and the non-coding RNA molecule *csrB*. *Mol. Microbiol.* 29, 1321–1330
68 Cui, Y. *et al.* (2001) Effects of the two-component system comprising GacA and GacS of *Erwinia carotovora* subsp. *carotovora* on the production of global regulatory *rsmB* RNA, extracellular enzymes, and harpin$_{Ecc}$. *Mol. Plant Microbe Interact.* 14, 516–526
69 Mukherjee, A. *et al.* (2000) *hexA* of *Erwinia carotovora* ssp. *carotovora* strain Ecc71 negatively regulates production of RpoS and *rsmB* RNA, a global regulator of extracellular proteins, plant virulence and the quorum-sensing signal, N-(3-oxohexanoyl)-L-homoserine lactone. *Environ. Microbiol.* 2, 203–215
70 Liu, Y. *et al.* (1999) *kdgR*$_{Ecc}$ negatively regulates genes for pectinases, cellulase, protease, Harpin$_{Ecc}$, and a global RNA regulator in *Erwinia carotovora* subsp. *carotovora*. *J. Bacteriol.* 181, 2411–2421
71 Valverde, C. *et al.* (2004) A repeated GGA motif is critical for the activity and stability of the riboregulator RsmY of *Pseudomonas fluorescens*. *J. Biol. Chem.* 279, 25066–25074
72 Liu, Y. *et al.* (1998) Characterization of a novel RNA regulator of *Erwinia carotovora* ssp. *carotovora* that controls production of extracellular enzymes and secondary metabolites. *Mol. Microbiol.* 29, 219–234
73 Cui, Y. *et al.* (1999) *rsmC* of the soft-rotting bacterium *Erwinia carotovora* subsp. *carotovora* negatively controls extracellular enzyme and harpin$_{(Ecc)}$ production and virulence by modulating levels of regulatory RNA (*rsmB*) and RNA-binding protein (RsmA). *J. Bacteriol.* 181, 6042–6052



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