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To be or not to be: how *Pseudomonas solanacearum* decides whether or not to express virulence genes

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Abstract

Pseudomonas solanacearum is a soil-borne phytopathogen that causes a lethal wilting disease of many plants, due in part to production of the unusual exopolysaccharide EPS I and numerous extracellular proteins (EXPs). Levels of EPS I and many EXPs are differentially controlled by a complex sensory array whose size, organization, and other properties set it apart from others found in prokaryotes. This network not only controls reversible switching between two morphotypes, each probably specialized for survival in different ecological niches (plant vs. soil), but also fine tunes transcription of virulence genes in response to multiple environmental signals. The interacting and cascading nature of the network is reminiscent of a primitive neural network, apparently designed to guide virulence gene expression during the dynamic interaction of the pathogen with its environment. This minireview focuses on the unique aspects of the network and its regulated targets.

Abbreviations: AHSL – acyl homoserine lactone; EPS – extracellular polysaccharide; EXP – extracellular protein; HR – hypersensitive response; PAME – palmitic acid methyl ester.

Introduction

One of the world's most troublesome prokaryotic phytopathogens, *Pseudomonas solanacearum* causes a lethal wilting disease of hundreds of diverse plants, including important crops such as potato, tomato, and banana (Buddenhagen and Kelman, 1964; Hayward, 1991; 1994). *P. solanacearum* can exist as a soil saprophyte, but also can colonize exudation sites at root tips or secondary root axils (Vasse et al., 1995). It infects plants via root wounds and/or at points of secondary root emergence (Kelman and Sequeira, 1965; Schmit, 1978). It subsequently colonizes the intercellular spaces of the root cortex and vascular parenchyma and disrupts cell walls to facilitate spread through the vascular system into the stem (Wallis and Truter, 1978; Vasse et al., 1995). Populations can rapidly reach $>10^{10}$ cells per stem concomitant with wilting. After killing the plant, *P. solanacearum* returns to a saprophytic life style in the soil to await a new host.

Geographic origins, physiological characteristics, RFLP analysis (Cook et al., 1989; Cook and Sequeira, 1994), and 16s rRNA sequencing (Seal et al., 1993) define two major evolutionary divisions of *P. solanacearum* containing 5 biovars. Within the two divisions, major virulence factors and their regulators are apparently conserved (Kang et al., 1994; Schell, 1987; Schell et al., 1994; Cook and Sequeira, 1989). Other rRNA sequence analysis (Li et al., 1994; Yabuuchi et al., 1992) shows *P. solanacearum* is distinct from the fluorescent *Pseudomonas* sp. and is most closely related to the former *Pseudomonas* sp. in rRNA group II (e.g. *P. pickettii*, *P. gladioli* and *P. cepacia*) that are now designated *Burkholderia* sp. (Yabuuchi et al., 1992). However, recent polyphasic taxonomic studies (Gillis et al., 1995) clearly showed *P. solanacearum*, *P.*

pickettii, and *Alcaligenes eutrophus* do not belong in *Burkholderia*. From these and further analyses Yabuuchi et al. (1995) recently proposed that these three be placed in the new genus *Ralstonia*. The nomenclature *Ralstonia (Pseudomonas) solanacearum* should soon be validated and accepted. However, since this change is not yet official or widely known, here I will use *P. solanacearum* to prevent confusion.

Virulence factors

Like many pathogens, *P. solanacearum* has many specialized genes relating to interactions with its hosts (Boucher et al., 1992; Schell et al., 1994); however, few are well understood. To fully appreciate the intricacies of virulence gene control, one first needs an understanding of the biochemistry and possible physiological functions of *P. solanacearum*'s regulated virulence factors.

Cell-wall-degrading exoenzymes

P. solanacearum produces a large variety and amount of EXPs (Schell et al., 1994; Kang et al., 1994; Arlat et al., 1994; and Figure 1). While many EXPs are exoenzymes that degrade plant cell-wall components, none appear to be absolutely required for disease: When artificially inoculated into the stem, site-directed mutants deficient in endopolygalacturonase A (PglA), exopolygalacturonase B (PglB), pectin methylesterase (Pme), or endoglucanase (Egl) can still cause disease, but take up to 50% longer to wilt and kill (Denny et al., 1990; Schell et al., 1994; Denny et al., unpubl.). Mutants lacking multiple exoenzymes are further reduced in virulence, but still can grow *in planta* and eventually cause some symptoms (Denny et al., 1990; Kang et al., 1994; Y. Kang and C. Allen, pers. comm.). A direct role in nutrition is unlikely since *P. solanacearum* lacks the capacity to use plant cell-wall breakdown products such as cellobiose or galacturonic acid for multiplication (Schell et al., 1988; Yabuuchi et al., 1995; Gillis et al., 1995). Thus, while exoenzymes appear to only increase aggressiveness of the pathogen, they may be much more critical during root invasion and spread into the vascular system. In support of this idea, loss of production of most major EXPs caused by protein export mutations in the *eep* locus greatly reduces *P. solanacearum's* ability to infect via roots and also to colonize and kill plants (Kang et al., 1994), likely because exoenzymes are needed to loosen cell wall structure.

Other EXPs

The *tek* gene encodes the 28-kDa EXP, a basic exoprotein produced in great abundance by many *P. solanacearum* strains (Figure 1 and Schell, 1987). However, inactivation of *tek* and resultant loss of the 28-kDa EXP does not affect virulence, EPS I production, or any other obvious characteristic (Denny et al., 1996). DNA sequence and other analyses show production of the 28-kDa EXP follows an unusual pathway: *Tek* actually encodes a 59-kDa lipid-modified precursor protein that, after export, is cleaved to release its C-terminus which becomes the 28-kDa EXP (Denny et al., 1996). While the function of the 28-kDa EXP remains obscure (no amino acid sequence homologs are known), the facts that it is produced at such high levels, is co-regulated with EPS I and other important virulence factors (below), and appears to be physically associated with EPS I (Schell, unpubl.), implies an involvement in disease. Similarly, *P. solanacearum* produces many other major and minor EXPs of unknown function that are co-regulated with known virulence factors (Figure 1 and below). Similar to protein export mutants, some regulatory mutants also missing sets of EXPs are dramatically reduced in virulence further suggesting that, as individuals or as a group, some EXPs are critical for disease.

The importance of EXPs in disease is further emphasized by studies of the *hrp* locus, a 23-kb gene cluster comprised of at least 6 environmentally-regulated transcription units that encode functions essential for pathogenicity on host plants, elicitation of a hypersensitive defense response (HR) in non-hosts, and *in planta* multiplication (Boucher et al., 1992; Genin et al., 1992). The predicted amino acid sequences of *hrp* genes and other experiments suggest that *hrp* encodes an apparatus of at least 15 polypeptides that exports pathogenesis-related EXPs across the cell envelope. Very similar and likely isofunctional genes are found in most plant pathogens and to some extent in animal pathogens (Van Gijsegem et al., 1995). One Hrp-exported protein of *P. solanacearum* is PopA1, a 33-kDa EXP that is processed extracellularly to yield the 23-kDa derivative PopA3 (Arlat et al., 1994). Purified PopA1 or PopA3 induces an HR, but *popA* mutants lacking both remain pathogenic and HR-inducing. It is likely that the Hrp system exports PopA1 and other similar (perhaps redundant) proteins which as a group are essential for pathogenicity, but as individuals are not. Analyses of different types of *P. solanacearum*, *Erwinia*, and *Xanthomonas* mutants

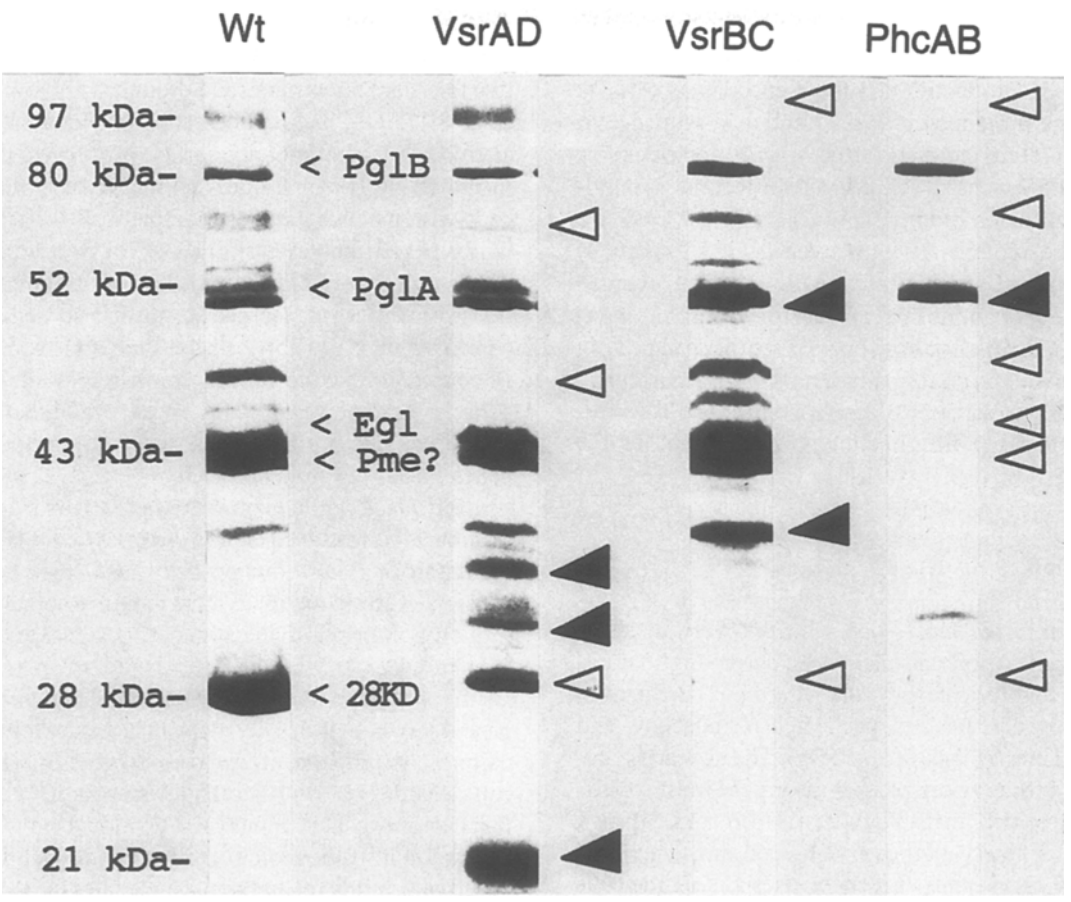

Figure 1. Extracellular proteins of *P. solanacearum* strains. A Coomassie Blue stained SDS-polyacrylamide gel of culture supernatants from: Wt, wild type; VsrAD, *vsrA* or *vsrD* mutant; VsrBC, *vsrB* or *vsrC* mutant; PhcAB, *phcA* or *phcB* mutant. Pgl, polygalacturonase; Egl, endoglucanase; Pme, pectin methylesterase; 28KD, 28-kDa EXP (C-terminus of Tek). Filled arrowheads mark EXPs whose production is increased by mutation; open arrowheads indicate EXPs whose production is decreased by mutation.

deficient in multiple EXPs (Kang et al., 1994 and references therein) lead to a similar conclusion.

Exopolysaccharide

The major known virulence factor of *P. solanacearum* is EPS I which it releases extracellularly in large quantities. EPS I is an acidic polymer ($>10^6$ Da) comprised of a trimeric repeating unit of N-acetyl-galactosamine, 2-N-acetyl-2-deoxy-L-galacturonic acid, and 2-N-acetyl-4-N-(3-hydroxybutanoyl)-2,4,6-trideoxy-D-glucose (Orgambide et al., 1991; Schell et al., 1993a). Many studies suggest that EPS I production is necessary, but alone is insufficient, for wilting and killing (Denny et al., 1990; Kao et al., 1992; Kang et al., 1994; and reviewed by Denny, 1995). While the major effect of EPS I appears to be plugging of the xylem resulting in wilting (Denny et al., 1990), the unusual sugars and high nitrogen content of EPS I suggest additional functions are likely. While the advantages conferred on *P. solanacearum* by high-level EPS I production are not clear, apparently EPS I is not required for significant root invasion or multiplication *in planta*.

The 16-kb *eps* gene cluster contains at least 12 genes which are probably transcribed as an operon from a single environmentally-regulated promoter (Huang and Schell, 1995). Most or all *eps* genes are required for EPS I production and wilting (Denny and Baek, 1991; Schell et al., 1993a). Analysis of the first 7 genes (Huang and Schell, 1995) by DNA sequencing, maxicells, and *phoA* fusion methods suggested: 1) *epsA* encodes a 38-kDa outer membrane lipoprotein similar to CtrA of *Neisseria meningitidis* and other proteins involved in extracellular export of capsular

polysaccharides of animal pathogens (Frosch et al., 1991); *epsP* encodes a phosphatase of ill-defined function in EPS production; 2) *EpsB* encodes an 80-kDa membrane protein similar in amino acid sequence to ExoP, a protein required for polymerization or export of an EPS essential for *Rhizobium*-legume symbiosis (Glucksmann et al., 1994); 3) EpsE and EpsF are membrane proteins also involved in EPS export; 4) EpsC and EpsD are likely cytosolic enzymes involved in synthesis of amino sugar precursors of EPS I. EpsA, EpsP, and EpsB also show marked sequence similarity to the first 3 products of *ams*, a 16-kb gene cluster encoding biosynthesis of amylovoran, an EPS virulence factor of *Erwinia amylovora* (Bugert and Geider, 1995).

Regulation of virulence

P. solanacearum must cope with two very different environments: soil and plant. Not surprisingly, it has evolved an intricate network (Figure 2) to control its many genes that are specialized for attacking and killing plants (Huang et al., 1995). The size and complexity of the network place it among the most sophisticated reported in prokaryotes (Hoch and Silhavy, 1995). Its likely purpose is to coordinate expression of *eps* and other virulence genes in simultaneous response to multiple cues encountered during various stages of pathogenesis and during soil survival. The network contains at least 3 different signal transduction arrays, each containing a unique two-component system comprised of a membrane-bound kinase sensor and a response regulator. By analogy to other systems (Parkinson and Kofoid, 1992; Hellingwerf et al., 1995; Hoch and Silhavy, 1995), each sensor detects a different signal causing it to phosphorylate its response regulator which in turn activates or represses promoters of target genes. Additionally, the *P. solanacearum* network has a unique transcriptional regulator, XpsR, which coordinates inputs from 3 signal transduction modules into the *eps* promoter. First, I will discuss individual components and later describe how they interact to form a network.

PhcA /phcBSR, *a global virulence switching system involving an unusual extracellular signal*

PhcA, a member of the large LysR family of transcriptional regulators (Brumbley et al., 1990; 1993; Schell, 1993), appears to function as a global virulence switch for *P. solanacearum*: Cells with active PhcA are highly virulent, producing large amounts of most virulence factors (EPS I and EXPs); cells with inactive PhcA are not virulent and produce very low levels of EPS I, Egl, Pme, and other EXPs (Figure 1). The altered colony morphology, surface properties, 10-fold increased PglA expression, and increased motility of cells with inactive *phcA* also dramatically differ from wild types (Brumbley et al., 1993; Clough et al., 1994). Thus, not only does PhcA positively control transcription of many virulence genes, but it also negatively controls others, possibly because their high expression is counterproductive during some stages of pathogenesis. In some cases, PhcA appears to bind directly to and activate promoters that direct transcription of genes encoding virulence factors (e.g. *egl, tek*, and possibly *pme*), while in other cases it acts in a cascade fashion, indirectly controlling expression via other regulators (e.g. *xpsR* and possibly *pehSR*; see below). Most other LysR-type activators require a coinducer to turn on transcription; the stimulating signal for PhcA is unknown.

Transcription of several PhcA-regulated virulence genes shows cell-density dependence: In log-phase cultures, expression of *eps* or *egl* is 50-fold less at cell densities below 10^7 cells/ml than at 10^9 cells/ml (S. Clough et al., unpubl.). Such quorum sensing regulation in pathogenic bacteria is common, usually involving closely related signal molecules (acyl-homoserine lactones; AHSLs) recognized by LuxR-type transcriptional regulators (Fuqua et al., 1995). While *P. solanacearum* appears to make an AHSL, its relationship to PhcA and cell-density regulation of virulence genes is unclear and not obvious (A. Flavier and T. Denny, unpubl.).

However, Clough et al. (1994; 1995) found that levels of active PhcA appear to be controlled by a new type of endogenous extracellular signal. This process involves the *phcBSR* genes (and likely others) located 11 kb from *phcA*. Inactivation of *phcB* switches off the same multitude of virulence genes as does inactivation of *phcA*. However, *phcB* mutants are fully restored to wild type by exposure to culture supernatants of wild type cells, 1 mM methanol, or 25 uM concentrations of methyl (but not ethyl) esters of C16 or C17 fatty acids (Clough et al., 1994). The active component in wild type culture supernatants was identified as 3-OH palmitic acid methyl ester (3-OH PAME); it fully restores *phcB* mutants at 50 nM (Flavier and Denny, 1994). Production of 3-OH PAME requires *phcB*; response to it requires *phcS*/*phcR* which apparently encode a two-component regulatory system (Clough

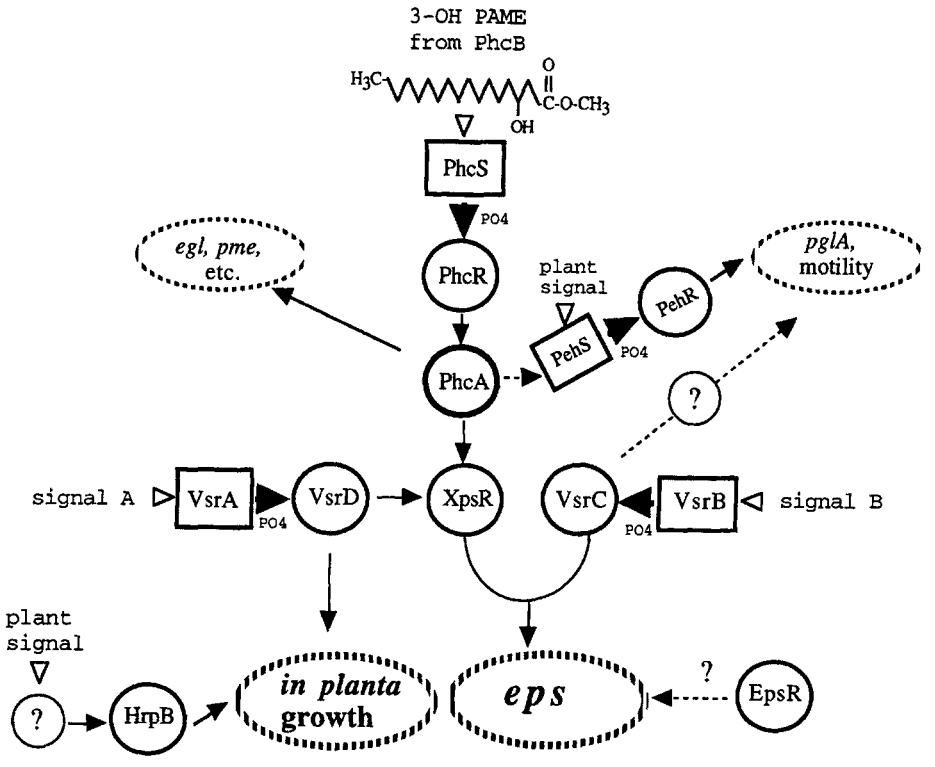

Figure 2. Model showing the organization and operation of virulence gene regulatory network of *P. solanacearum.* Membrane-bound sensory kinase, □; transcriptional regulator, ○; regulated virulence gene target, ⬭; positive transcriptional control, —▶; negative transcriptional control, - - -▶; phosphorylation-mediated signal transfer, ▶.

et al., 1995). How 3-OH PAME and *phcSR* control *phcA* to mediate reversible switching between two very different physiological states of *P. solanacearum* (i.e. virulent and nonvirulent) is not clear. One explanation is that 3-OH PAME affects the ability of the PhcS sensor to phosphorylate the PhcR response regulator leading to increased PhcA synthesis or activity. The role of fatty acid derivatives as extracellular signal molecules may be widespread, since fatty acids appear to trigger complex developmental processes in other bacteria (Downard and Toal, 1995).

VsrA/vsrD, *a two-component system controlling* eps, *EXPs, and* in planta *growth genes*

Inactivation of *vsrA* or *vsrD* gives an identical phenotype: 1) a complete loss of ability to cause disease symptoms likely resulting from a >20-fold decrease in ability to rapidly colonize stems and multiply *in planta*; 2) dramatically altered production of several EXPs (Figure 1); and 3) a 5- to 20-fold growth-medium-dependent reduction in transcription of *eps* (and EPS I synthesis) possibly related to inorganic ion levels (Schell et al., 1993b; Huang et al., 1995). However, the major reduction in ability to multiply in plants is not caused by reduced EPS I production, because some EPS I-deficient mutants multiply normally *in planta* (Denny and Baek, 1991; J. Huang unpubl.). More likely *vsrAD* activates expression of virulence genes that promote *in planta* growth. A role in multiplication *in planta* for the two EXPs whose expression requires *vsrAD* (48-kDa and 66-kDa EXPs; Figure 1) is possible, but speculative. Although reduced *in planta* multiplication is reminiscent of *hrp* mutants (Boucher et al., 1992), *vsrAD* mutants clearly differ from *hrp* mutants because they grow 100-fold better *in planta* and still give a HR (Schell et al., 1993b). Moreover, *vsrD* mutations apparently do not affect expression of several *hrp* transcription units (Schell and C. Boucher, unpubl.). Thus *hrp* and *vsrAD* likely control different genes required for efficient growth in plants.

DNA sequence and maxicell analysis showed that VsrA is a 53-kDa transmembrane histidine kinase sensor with one 170-residue periplasmic domain (Schell et al., 1993b). The adjacent *vsrD* gene (Huang

et al., 1995) encodes a 24-kDa response regulator for VsrA whose sequence is similar to RO_{III}-type response regulators (Parkinson and Kofoid, 1992), such as BvgA, NarL, and especially GacA, which globally controls production of several antifungal compounds by *P. fluorescens* (Gaffney et al., 1994). Recently, GacA was found to be the partner response regulator of LemA, a sensor controlling multiple genes involved in lesion formation by *P. syringae* (Rich et al., 1994). GacA appears to control important virulence genes of the animal pathogen *P. aeruginosa* (Rahme et al., 1995). Commonalities in global regulation by LemA/GacA, VsrA/VsrD, and other homologs deserve further investigation, largely because they may represent a widespread and important subclass of signal transduction system that responds to similar signals, but is 'connected to' (i.e. controls) different genes depending on the niche inhabited by the host.

The VsrB/VsrC *two-component signal transduction module*

Inactivation of the adjacent *vsrC* or *vsrB* genes causes a 30-fold reduction in *eps* transcription (Huang et al., 1993; 1995). The resultant reduction in EPS I probably causes the attenuated virulence of *vsrBC* mutants because, similar to *eps* mutants, *vsrBC* mutants grow normally *in planta* and cause minor stunting and chlorosis, but do not wilt or kill. *VsrC* encodes a response regulator because its predicted amino acid sequence shows 25% identity to RO_{III} -type response regulators (e.g. NarL, FixJ, and BvgA) and has all the appropriate characteristics of a two-component response regulator (Parkinson and Kofoid, 1992). VsrC probably directly binds to and regulates the *eps* promoter in response to a signal transmitted via VsrB (see below). Inactivation of *vsrBC* also causes a 15-fold increase in expression of PglA endopolygalacturonase. This control, which is independent of *phcA* (Huang et al., 1993) and *pehSR*, may result from direct binding of VsrC to the *pglA* promoter or involve an intermediary.

Analysis of DNA sequence, *phoA* fusions, and maxicells showed that *vsrB* encodes a 67-kDa transmembrane histidine kinase sensor for VsrC with two 8-residue periplasmically exposed segments and a phosphate receiver domain at its C-terminus (Huang et al., 1993). Although this 'extra' phosphate receiver domain is atypical for two-component sensors, some sensors in animal and plant pathogens (VirA, BvgS, ArcA, and LemA) also have one. This domain may modulate the sensor's activity and/or allow cross talk with other two-component systems (Ishige et al., 1994). By analogy to KdpD and ArcB, the two unusually small periplasmic segments of VsrB imply it may control *eps* in response to a physical signal (e.g. membrane potential) rather than a discrete external signal molecule (Huang et al., 1993).

XpsR, *an unusual transcriptional activator that coordinates control at the* eps *promoter*

XpsR, located just downstream of *eps* at the beginning of an operon containing *tek* and region II (Denny and Baek, 1991; above), is the most novel virulence regulator of *P. solanacearum*. *XpsR* encodes a basic, 33-kDa protein with no known amino acid sequence homologs; *xpsR* mutants have an EPS-deficient, reduced-virulence phenotype (Huang et al., 1995). XpsR mediates the indirect control of the *eps* promoter by both the VsrAD and PhcA/PhcSR signal transduction arrays, since the reduced *eps* transcription caused by inactivation of either or both systems can be fully overcome by constitutively expressed *xpsR*. Using primer extension, *lacZ* fusions, and gel-shift assays, Huang et al. (1995) showed that PhcA and VsrAD activate *xpsR* transcription by 10- and 5-fold, respectively, and that this involves direct binding of PhcA to the *xpsR* promoter. Levels of XpsR needed for activation of *eps* are critical, since a 10-fold increase in XpsR caused by PhcA activation does not turn on *eps* transcription; a further 5-fold increase in XpsR affected by VsrAD is additionally required. The biochemical mechanism XpsR uses to activate *eps* transcription may be novel and requires VsrC (see below).

PehS/pehR, *a two-component system controlling polygalacturonase*

Inactivation of *pehSR* decreases virulence and reduces expression of PglA (PehA) endopolygalacturonase by 11-fold, while levels of other polygalacturonases are only reduced by half (Allen et al., 1991). DNA sequence analysis suggests *pehSR* encodes a two-component system (Allen and Gay, 1995). Since polygalacturonase levels increase 10-fold when cells are grown *in planta* (Allen et al., 1991), *pehSR* could mediate a plant-signal-dependent activation of *pglA* expression. Because overexpression of *pehSR* or inactivation of *phcA* both cause increased motility and PglA levels, and because *pehSR* expression increases 10-fold in *phcA* mutants, it is likely that active PhcA

negatively controls *pehSR* to reduce levels of PglA and motility independent of a plant signal.

EpsR, *a putative negative regulator of EPS production*

EpsR dramatically represses EPS synthesis and virulence of wild type *P. solanacearum*, but only when put on a multicopy plasmid (Huang and Sequeira, 1990). This repression is apparently caused by a 10-fold, plasmid-directed overexpression of EpsR (Kao et al., 1994). DNA sequence analysis showed *epsR* encodes a 25-kDa protein containing a 65-residue sequence homologous to LuxR/FixJ/MalT-type DNA binding domains. Recently, McWilliams et al. (1995) reported that cloned *epsR* in trans reduced expression of *eps* by 5-fold, implying that high levels of EpsR inhibit transcription of the *eps* biosynthetic genes. However, since inactivation of *epsR* in the genome had no effect on EPS production or any other obvious characteristic (Kao et al., 1994), its role in virulence and/or physiology of *P. solanacearum* remains unclear. While the molecular mechanism of EpsR action remains to be elucidated, it is possible that induction of high levels of EpsR can be used to quickly shut down EPS synthesis.

HrpB, *a transcriptional regulator of* hrp *genes*

Inactivation of *hrpB* gives a classic *hrp*-phenotype (i.e. loss of pathogenicity, HR-induction, and vigorous *in planta* growth), probably because *hrpB* is required for high-level transcription of four *hrp* transcription units *in planta* (Genin et al., 1992). DNA sequence analysis shows *hrpB* encodes a 53-kDa protein whose C-terminus has homology to transcriptional activators in the AraC-XylS family (Genin et al., 1992), including VirF which controls *Yersinia* virulence genes with functional and amino acid sequence homology to *hrp* genes. The 20-fold increase in transcription of *hrp* found in minimal vs. rich medium is probably caused by activation of *hrpB* transcription because: 1) *hrpB* expression is 8-fold higher for cells grown in minimal vs. rich media; and 2) artificial overexpression of *hrpB* in rich medium-grown cells dramatically increases *hrp* transcription (Genin et al., 1992). Environmentally-sensitive regulation of *hrpB* expression (and hence of other *hrp* loci) could be related to *hrpB* autoamplification or may involve additional signal-responsive regulators. HrpB activation of *hrp* genes may also involve another effector, since many AraC-XylS family members require an inducer molecule for transcriptional activation.

Hrp gene expression in other bacteria is influenced by osmolarity, carbon source, and especially *in planta* growth, but no specific plant signal molecules have been identified, although methionine and sucrose have been implicated (see Willis et al., 1994). Control of *hrp* gene expression in *P. syringae* involves two response regulators and an alternate sigma factor (Xiao et al., 1994). The presence or absence of similar *hrp* regulatory elements in *P. solanacearum* is unknown.

How virulence regulators work together to control eps *in simultaneous response to at least 3 signals*

As described above and illustrated in Figure 2, there are at least 12 regulatory genes in *P. solanacearum* involved in control of distinct, but sometimes overlapping, sets of virulence genes. The most novel feature of this virulence control web is the organization of regulators into signal transduction modules that work together to direct synthesis of EPS I in simultaneous response to multiple signals. Interactions between modules and other aspects of this system suggest it may be an example of a phospho-neural network as hypothesized by Hellingwerf et al. (1995).

Primer extension and other analyses (Huang and Schell, 1995) located a promoter just upstream of *epsA* that likely directs transcription of the entire 16-kb *eps* gene cluster as an operon. Deletion analysis showed that a 150-bp region containing this promoter had all sequences necessary for control of *eps* transcription by all network components required for EPS I synthesis. Other experiments (Huang et al., 1995) showed that transcription from the *eps* promoter absolutely requires the VsrBC two-component system and XpsR. Positive input from both the VsrAD and PhcA/PhcSR systems is also required to turn on the *eps* promoter, but indirectly, since they act by controlling the levels of XpsR: While active PhcA increases *xpsR* transcription by 10-fold, the resultant levels of XpsR are not enough to activate *eps*; a further 5-fold increase in XpsR levels caused by signal-activated VsrAD is necessary to actually turn on *eps*. Thus, PhcA activation of *xpsR* transcription is only a prerequisite for subsequent activation and modulation by VsrAD. Active PhcA only allows the possibility of *eps* activation which happens only if VsrAD 'concurs'.

While the *eps* promoter is regulated by a combination of VsrC and XpsR probably via direct binding, VsrC activity depends on a signal communicated via VsrB and levels of XpsR depend on two other signals communicated through the PhcA/PhcSR and

VsrAD systems. Each of the network's two-component systems is distinct because the amino acid sequences, size and number of periplasmic domains, and other structural features of its sensors differ widely (Huang et al., 1993; Schell et al., 1993b). This makes it likely that each sensor detects a different stimulus before phosphorylating its partner response regulator which then activates (or represses) transcription of its cognate targets. How XpsR and VsrBC interact to activate the *eps* promoter is unknown. XpsR could directly interact with the *eps* promoter to facilitate activation by VsrC or alternatively may enhance or protect VsrC phosphorylation.

Virulence-gene-activating signals perceived *in planta* by VsrA, VsrB, and other sensors of *P. solanacearum* remain to be determined, likely requiring extensive biochemical and *in planta* studies. However, several experiments suggest one of these may be an endogenous extracellular fatty acid derivative, 3-OH PAME. This signal is noteworthy because: 1) via *phcA* (Figure 2) it turns on (or off) so many genes by large amounts (10 to 50 fold); and 2) it may explain why expression of *phcA*-regulated genes is affected by cell density. 3-OH PAME may be a new type of cell density signal that reversibly regulates a transition from saprophytic to pathogenic behavior: When actively growing in the plant or as microcolonies on the roots, sufficient 3-OH PAME may accumulate to switch on virulence gene expression and simultaneously repress unnecessary or deleterious genes. When growing slowly in a more dispersed state (e.g. in soil), 3-OH PAME may not accumulate enough to activate expression of virulence genes, effectively leaving them shut off, and possibly at the same time activating expression of genes for saprophytic survival. However, some data suggests factors besides 3-OH PAME are involved in cell-density regulation of virulence genes (S. Clough et al., unpubl.). While high local concentrations of methanol (possibly from Pme action on cell wall pectins) can substitute for 3-OH PAME, its role as a *bona fide in planta* signal is unclear (Clough et al., 1994). Nonetheless, 3-OH PAME levels appear to control a developmental cycle of *P. solanacearum* characterized by two distinct morphotypes, one specialized for the *in planta* environment and one specialized for soil environment.

While most elements of the *P. solanacearum* regulatory network affect *eps*, some additionally and independently control other virulence genes (Figure 2). For example, *egl* and *pme* expression are affected only by the PhcA/PhcSR system, while *pglA* is affected only by VsrBC and PhcA/PhcSR (probably via PehSR). Only VsrAD and HrpB affect expression of different genes for efficient growth *in planta*. The ability of the network to turn on or off separate sets of virulence genes in response to subsets of signals confers additional utility and efficiency. Inclusion of HrpB and EpsR in the network still needs confirmation, because they have not yet been shown to interact directly with any other regulator or target promoter. Nonetheless, it is likely that there are other virulence genes and regulators of the network.

Conclusion and perspective

A major long range goal of plant pathology is to understand what physiological and genetic factors allow pathogens to invade, colonize, and kill a plant. Many groups are beginning to find that some of the most indispensable systems for successful pathogenesis are those that coordinate production of virulence and pathogenicity factors in response to environmental signals. These systems probably ensure that individual virulence factors are produced in concert at the appropriate time and place during pathogenesis. Further study of these systems should give insight into the types of environmental cues phytopathogens monitor to adjust virulence gene expression, and tell more about where, when, and how specific virulence factors assist bacteria like *P. solanacearum* in colonizing plants. This knowledge may foster development of disease control methods based on interference with a pathogen's ability to monitor and respond to its environment.

Conserved signal transduction systems that control genes in response to single, important environmental parameters (e.g. osmolarity, nitrogen levels, etc.) are universal in prokaryotes and maybe in eukaryotes (Alex and Simon, 1994). Although Hellingwerf et al. (1995) and others (Ishige et al., 1994) hypothesized the existence of primitive sensory arrays in bacteria (comprised of interconnected two-component systems), *bona fide* cross talk between independent signal transducing systems has rarely been documented. However, *P. solanacearum* seems to have evolved just such an integrated, multi-signal sensing/transduction web to control its virulence genes. Although in other plant pathogens (e.g. *Erwinia*) production of EPS, exoenzymes, and other virulence factors is also controlled by multiple systems (including quorum sensors (Fuqua et al., 1994; Von Bodman and Farrand, 1995), sensory kinases (Leigh and Coplin,

1992) and repressors (Cui et al., 1995)), direct interconnections between them (i.e. networking) has not yet been demonstrated. However, the ability to adjust expression of a promoter in simultaneous response to complex sets of environmental signals likely confers a major advantage on pathogens, largely because they must cope with dynamic and diverse situations, including challenges from host defenses. Thus, it is likely that control networks similar to *P. solanacearum's* exist in many other bacteria that need to survive in diverse and changing environments.

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