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Getting across—bacterial type III effector proteins on their way to the plant cell

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Pathogenicity of most Gram-negative bacterial plant pathogens depends on *hrp* (hypersensitive response and pathogenicity) genes, which control the ability to cause disease and to elicit specific defense responses in resistant plants. *hrp* genes encode a specialized type III secretion (TTS) system that mediates the vectorial delivery of bacterial effector proteins across both bacterial membranes as well as across the eukaryotic plasma membrane into the host cell cytosol. One well-studied effector protein is AvrBs3 from *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial spot in pepper and tomato. AvrBs3 induces hypertrophy symptoms in susceptible plants and triggers a resistance gene-specific cell death reaction in resistant plants. Intriguingly, AvrBs3 has characteristic features of eukaryotic transcription factors, suggesting that it modulates the host's transcriptome. Here, we discuss the TTS system of *X.campestris* pv. *vesicatoria* in the light of current knowledge on type III-dependent protein secretion in plant pathogenic bacteria.

Keywords: AvrBs3/*hrp* genes/pathogenicity island/PIP box/secretion

Introduction

Plants provide an attractive nutrient reservoir and ecological niche for bacterial pathogens. In most higher plants, bacterial colonization leads to a variety of severe diseases. However, disease is the exception rather than the rule since most plants possess a battery of defense mechanisms that repel invading microbes. Therefore, Gram-negative plant pathogenic bacteria have evolved sophisticated strategies to colonize their host plants. They enter the plant through natural openings such as stomata, or wounds, and multiply in the intercellular spaces of the tissue at the expense of the host.

Over the past two decades, genetic and molecular studies unraveled important mechanisms underlying bacterial pathogenicity. Essential for the molecular cross-talk between pathogens and their host plants is a specialized protein delivery system, the type III secretion (TTS) system. TTS systems are conserved in plant and animal pathogenic bacteria and mediate the vectorial delivery of bacterial effector proteins into the host cell (Hueck, 1998; Cornelis and Van Gijsegem, 2000). In plant pathogens, TTS systems are encoded by *hrp* (hypersensitive response

and pathogenicity) genes, essential determinants of bacterial pathogenicity that control the ability to multiply in susceptible hosts and to cause disease (Alfano and Collmer, 1997). In addition, *hrp* genes are required to induce the hypersensitive response (HR), a rapid localized programmed death of plant cells at the infection site, in resistant host and in non-host plants (Klement, 1982). The HR is part of the plant's innate immune response that halts bacterial ingress. Induction of the HR is due to the specific recognition of a bacterial effector protein [designated avirulence (Avr) protein] by a corresponding plant resistance (R) protein (Flor, 1971; Table I).

Among the model organisms for the molecular and genetic characterization of host–plant interactions and the functional analysis of TTS systems are *Erwinia amylovora*, *Ralstonia solanacearum*, pathovars (pv.) of *Pseudomonas syringae* and species (spp.) of *Xanthomonas*, all infecting important crop plants. The pathovar designation refers to differences in the host range of the bacteria. For some of these bacteria, the genome sequence has become available recently, initiating a new era in molecular plant pathology (Da Silva *et al.*, 2002; Salanoubat *et al.*, 2002; www.tigr.org). Our laboratory studies *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial spot in pepper and tomato plants, which is the focus of this review.

The *hrp* pathogenicity island—genetic requisite for effector protein traffic

Gram-negative bacteria utilize different protein secretion systems to transport proteins across the inner and outer membrane. Among the six main groups of secretion systems, TTS systems exhibit the most complex architecture (Thanassi and Hultgren, 2000). Around 20 proteins are involved in the formation of a membrane-spanning secretion apparatus, which is associated with an extracellular filamentous structure (Hueck, 1998; see below).

Type III-mediated protein secretion into the extracellular medium was discovered initially in the animal pathogen *Yersinia enterocolitica* (Heesemann *et al.*, 1984). However, the first genes encoding components of the TTS system were identified by the analysis of non-pathogenic mutants of the plant pathogens *P.syringae* pv. *syringae* and *P.syringae* pv. *phaseolicola* (Niepold *et al.*, 1985; Lindgren *et al.*, 1986). Except for *Agrobacterium* spp., *hrp* genes are present in all Gram-negative biotrophic plant pathogens and are generally organized in large clusters comprising >20 genes (Boucher *et al.*, 1987; Steinberger and Beer, 1988; Barny *et al.*, 1990; Arlat *et al.*, 1991; Bonas *et al.*, 1991). Based on similarities in *hrp* gene organization and regulation, plant pathogenic bacteria have been classified into two groups, group I (*E.amylovora* and *P.syringae*) and group II (*R.solanacearum* and species

of *Xanthomonas*) (Alfano and Collmer, 1996). At least nine *hrp* genes (termed *hrc* for *hrp* conserved) are conserved in both groups and encode components of the TTS system, which are also present in animal pathogenic bacteria (Bogdanove *et al.*, 1996; He, 1998; Hueck, 1998). Hrc proteins presumably constitute the core components of the secretion apparatus in the inner and outer membrane. With the exception of HrcC—the best studied Hrc protein, which belongs to the secretin family of outer membrane proteins—Hrc proteins share sequence similarities with flagellar assembly components. The flagellar assembly apparatus serves as a protein export system and probably represents an evolutionary ancestor of the TTS system (Hueck, 1998; Macnab, 1999; Aizawa, 2001; Young and Young, 2002).

In contrast to conserved *hrp* genes, the precise role of non-conserved *hrp* genes remains to be investigated. Genetic studies of *X.campestris* pv. *vesicatoria* revealed that type III secretion requires at least six non-conserved *hrp* genes, some of which encode type III-secreted

proteins, e.g. HrpB2 (Rossier *et al.*, 2000; Table II). The *hrp* region also contains so-called *hrp*-associated (*hpa*) genes (Figure 1), which are not essential for bacterial pathogenicity but contribute to the interaction with the host plant (Huguët *et al.*, 1998; Noël *et al.*, 2002; O.Rossier, D.Büttner and U.Bonas, unpublished data).

How did *hrp* gene clusters evolve? Genes involved in bacterial virulence often are located in regions that show characteristics of pathogenicity islands. These DNA regions usually are flanked by direct repeats, insertion sequence (IS) elements, tRNA genes and/or genes for integrases and transposases. Pathogenicity islands often differ in G + C content from the genomic DNA, indicating horizontal gene transfer (Hacker and Kaper, 2000). In *X.campestris* pv. *vesicatoria*, mobility of the *hrp* region has indeed been observed (Basim *et al.*, 1999). Furthermore, sequence analyses of DNA regions flanking the *hrp* gene cluster revealed the presence of an IS-like element and putative effector genes with lower G + C content than the genomic DNA (Noël *et al.*, 2002; Figure 1).

Typical features of pathogenicity islands are also present in DNA sequences flanking the *hrp* gene cluster of *P.syringae*. Here, the region adjacent to *hrpK* has a lower G + C content and contains sequences homologous to IS elements, transposases and tRNA genes. Interestingly, the genes located in this region, termed exchangeable effector locus (EEL), vary in pathovars of *P.syringae* (Alfano *et al.*, 2000; one example is given in Figure 1).

Table I. *R* gene-specified pathogen recognition according to gene-for-gene interactions^a

Pathogen genotype	Plant reaction	
	Host plant genotype ^b <i>R1/R1</i> or <i>R1/r1</i>	<i>r1/r1</i>
<i>avr1</i>	HR ^d	Disease
– ^c	Disease	Disease

^aGene-for-gene hypothesis (Flor, 1971).

^b*R1*, resistance locus allowing recognition of a corresponding avirulence (*avr*) gene (designated *avr1*). Most resistance (*R*) genes are single dominant genes. *r1* refers to the absence of a functional *R1* allele.

^cThe avirulence gene is absent or mutated, resulting in loss of recognition by plants carrying the corresponding *R* gene.

^dHR, hypersensitive reaction.

Entering the plant—green light for *hrp* gene expression

Type III secretion is a regulated process. Genes encoding components of the secretion apparatus are not constitutively expressed but activated *in planta* and in minimal media mimicking the environmental conditions present in the plant apoplast (Lindgren, 1997). Proteins involved in

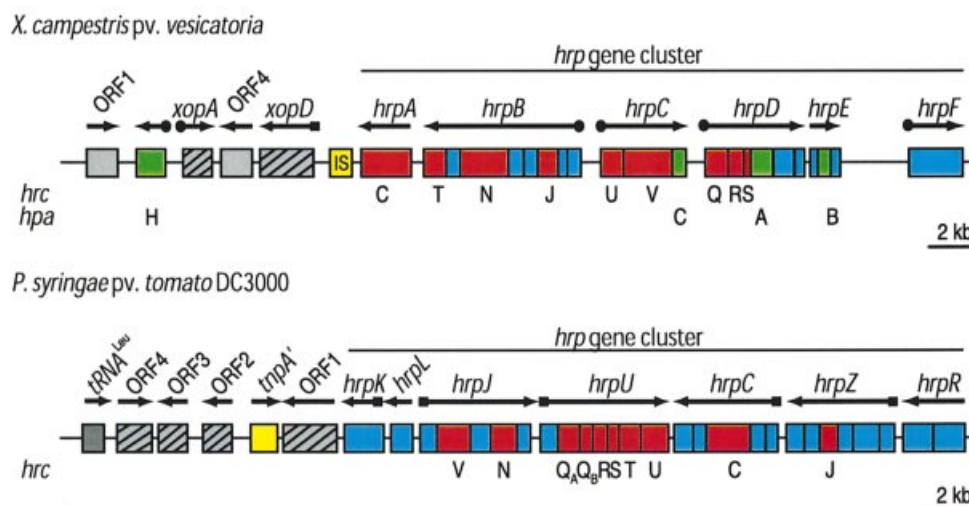


Fig. 1. Schematic overview of the *hrp* gene clusters and the left flanking regions from *X.campestris* pv. *vesicatoria* (group II) and *P.syringae* pv. *tomato* DC3000 (group I). The regions contain *hrp*, *hrc* and *hpa* genes (represented in blue, red and green, respectively). Arrows indicate the direction of transcription. Black dots and squares refer to the presence of PIP and *hrp* boxes, respectively. Hatched regions correspond to sequences with low G + C content; yellow regions refer to mobile genetic elements.

Table II. *Xanthomonas campestris* pv. *vesicatoria* type III-secreted proteins

Protein ^a	Characteristics/homology ^b	Expression ^c	References
Components of the TTS apparatus			
HrpB2 [‡]	Extracellular component of the TTS system	Induced; PIP box	Wengelnik and Bonas (1996); Rossier <i>et al.</i> (2000)
HrpE1 [‡]	Major Hrp pilus subunit	Basal expression, induction	Wengelnik and Bonas (1996); Rossier (1999); T.Ojanen-Reuhs and U.Bonas (unpublished data)
HrpF	Translocon protein	Induced; PIP box	Wengelnik and Bonas (1996); Rossier <i>et al.</i> (2000); Büttner <i>et al.</i> (2002)
Xops ^d			
√XopA	Hpa1 (<i>X.oryzae</i> pv. <i>oryzae</i>)	Induced; PIP box	Noël <i>et al.</i> (2002)
XopB	AvrPphD (<i>P.syringae</i> pv. <i>phaseolicola</i>)	Induced	Noël (2001); Noël <i>et al.</i> (2001)
XopC		Induced	Noël (2001); Noël <i>et al.</i> (2001)
XopD	PsvA (<i>P.syringae</i> pv. <i>eriobotryae</i>)	Induced; <i>hrp</i> box	Noël <i>et al.</i> (2002)
XopJ	AvrRxv/YopJ family; putative cysteine protease	Induced	Noël (2001); Noël <i>et al.</i> (2001)
√HpaA	NLS	Induced; PIP box	Wengelnik and Bonas (1996); Huguier <i>et al.</i> (1998)
AvrBs1*	AvrA (<i>P.syringae</i> pv. <i>glycinea</i>)	Constitutive	Ronald and Staskawicz, (1988); Escolar <i>et al.</i> (2001)
√AvrBs2*	Agrocinopine synthase (<i>A.tumefaciens</i>); phosphodiesterase (<i>E.coli</i>)	ND	Kearney and Staskawicz (1990); Swords <i>et al.</i> (1996); Mudgett <i>et al.</i> (2000)
√AvrBs3*	NLS; AAD; AvrBs3 family	Constitutive ^e	Van den Ackerveken <i>et al.</i> (1996); Rossier <i>et al.</i> (1999); Szurek <i>et al.</i> (2001); Marois <i>et al.</i> (2002)
AvrBs4*	NLS; AAD; AvrBs3 family	Constitutive ^e	Bonas <i>et al.</i> (1993); Ballvora <i>et al.</i> (2001)
AvrBsT*	AvrRxv/YopJ family; putative cysteine protease	Constitutive	Escolar <i>et al.</i> (2001)
AvrRxv	AvrRxv/YopJ family; putative cysteine protease	Constitutive; PIP box	Ciesiolka <i>et al.</i> (1999); Rossier <i>et al.</i> (1999)
AvrXv3*	AAD	Induced; PIP box	Astua-Monge <i>et al.</i> (2000a)
AvrXv4	AvrRxv/YopJ family; putative cysteine protease	ND; PIP box	Astua-Monge <i>et al.</i> (2000b)

[‡], essential for type III secretion *in vitro*; √, virulence activity demonstrated; *, indicates ability of Avr proteins to induce the HR upon transient expression in resistant plants.

^bAAD, acidic activation domain; NLS, nuclear localization signal; Yop, *Yersinia* outer protein.

^cExpression *in planta* or under *hrp* gene-inducing conditions. ND, not determined; PIP, plant-inducible promoter.

^dXops, *Xanthomonas* outer proteins, include type III-secreted proteins with unknown destination as well as avirulence (Avr) proteins; Hpa, *hrp* associated.

^eRecent *in vitro* expression experiments indicate that *hrpG** leads to a 2- to 3-fold increase in expression (U.Bonas *et al.*, unpublished data).

√, virulence activity demonstrated.

*, indicates ability of Avr proteins to induce the HR upon transient expression in resistant plants.

hrp gene regulation vary in the different groups of plant pathogens. In *X.campestris* pv. *vesicatoria*, *hrp* gene expression is controlled by HrpX, an AraC-type transcriptional activator (Wengelnik and Bonas, 1996). In minimal medium or *in planta*, the expression of *hrpX* is activated by HrpG, a transcriptional activator of the OmpR family of two-component regulators (Wengelnik *et al.*, 1996; Figure 2A). Recent transcriptome analysis revealed that HrpG, in most cases via HrpX, controls a genome-wide regulon including *hrp* genes and genes encoding *Xanthomonas* outer proteins (Xops; Wengelnik and Bonas, 1996; Astua-Monge *et al.*, 2000a; Noël *et al.*, 2001, 2002).

Interestingly, one of the *xop* genes, *xopD*, contains an *hrp* box-like motif in the promoter region (Figure 1; Table II). The *hrp* box is a conserved consensus sequence which was identified in promoters of *hrp* and effector genes in *P.syringae*. It presumably provides the binding site for HrpL, a member of the extracytoplasmic function family of sigma factors (Innes *et al.*, 1993; Xiao and Hutcheson, 1994; Xiao *et al.*, 1994; Fouts *et al.*, 2002). In *X.campestris* pv. *vesicatoria*, however, expression of *xopD* is controlled by HrpG and HrpX (Noël *et al.*, 2002). *xopD* encodes a putative type III effector protein with homology to the virulence factor PsvA from *P.syringae* pv. *eriobotryae* (Noël *et al.*, 2002; Table II). The presence

of an *hrp* box in the *xopD* promoter and the low G + C content of *xopD* support the hypothesis that genes involved in bacterial virulence might have been acquired during evolution by horizontal gene transfer.

Many *hrpX*-regulated genes of *X.campestris* pv. *vesicatoria* contain a PIP (plant-inducible promoter, consensus TTCGC-N₁₅-TTCGC) box in their promoter regions. This sequence motif might be involved in the HrpX-mediated gene regulation (Fenselau and Bonas, 1995; Wengelnik and Bonas, 1996; Noël *et al.*, 2002). However, there are also *hrpX*-independent promoters that contain a PIP box, e.g. *avrRxv* (Table II), indicating that the PIP box is not sufficient to confer inducibility by HrpX. In addition, the promoters of several *xop* genes that are controlled by HrpG and HrpX do not contain PIP boxes (Table II). Thus, it remains speculative whether the PIP box serves as a control element. So far, direct binding of HrpX to PIP box-containing promoter sequences could not be demonstrated (L.Escolar and U.Bonas, unpublished data).

PIP box-like motifs have also been identified in *Xanthomonas axonopodis* pv. *citri* and *X.campestris* pv. *campestris* in the promoters of *hrp* genes as well as genes encoding putative proteins with type II signal peptides and sequence homologies to cell wall-degrading enzymes, proteases and an iron receptor (Da Silva *et al.*, 2002). Furthermore, PIP box-like promoter sequences have been

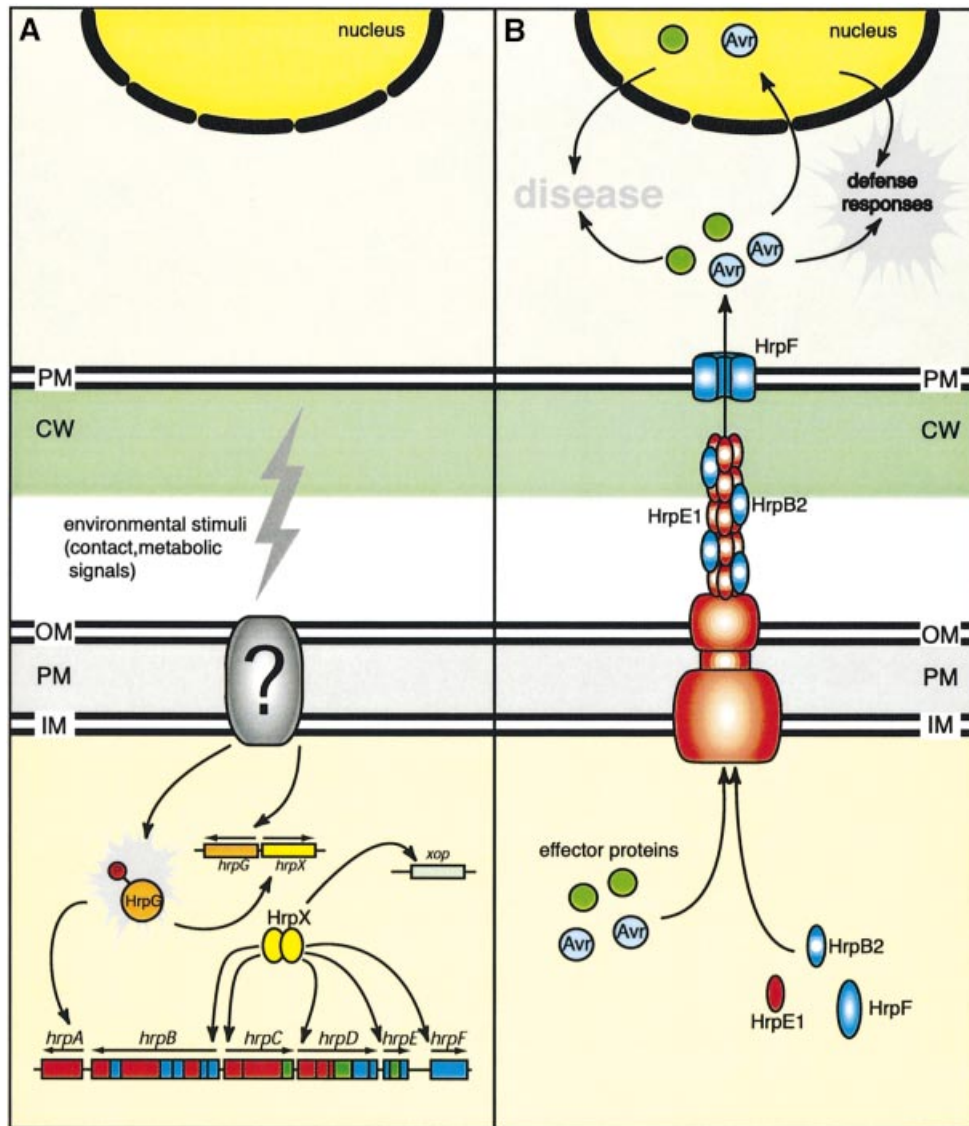


Fig. 2. Model for *hrp* gene regulation and type III secretion in *X.campestris* pv. *vesicatoria*. (A) A so far uncharacterized signal transduction system in the bacterial envelope (indicated by a question mark) senses environmental stimuli and transduces the signal to HrpG. HrpG activates the expression of *hrpA* and, via HrpX, the expression of *hrpB–hrpF* as well as of a number of *xop* genes. (B) Expression of *hrp* genes is essential for the formation of the TTS apparatus, which spans both bacterial membranes and mediates secretion of Hrp and effector proteins. The TTS apparatus is associated with the Hrp pilus, which presumably spans the cell wall (200 nm thick; not drawn to scale). The major subunit of the Hrp pilus is HrpE1. Translocation of effector proteins across the plant plasma membrane requires HrpF, the putative pore-forming component of the type III translocon. Effector proteins are targeted to different locations in the plant cell and presumably modulate cellular processes leading to disease symptom formation in susceptible plants. In resistant plants, effector proteins (designated Avr proteins) can be recognized and trigger the activation of specific defense responses. CW, cell wall; IM, inner membrane; OM, outer membrane; PM, plasma membrane.

identified in *R.solanacearum*, upstream of *hrp* transcription units, the *popA* gene and several *avr* gene homologs (Fenselau and Bonas, 1995; Wengelnik and Bonas, 1996; Salanoubat *et al.*, 2002). In *R.solanacearum*, *hrp* genes are controlled by HrpG and HrpB, which are homologous to HrpG and HrpX, respectively, from *X.campestris* pv. *vesicatoria* (Genin *et al.*, 1992; Brito *et al.*, 1999). In *R.solanacearum*, the outer membrane protein PrhA (plant regulator of *hrp* genes) presumably is on top of the regulatory cascade leading to *hrp* gene expression. PrhA is homologous to TonB-dependent siderophore receptors and acts as a sensor for a non-diffusible molecule present in the plant cell wall (Marenda *et al.*, 1998; Brito *et al.*, 1999,

2002; Aldon *et al.*, 2000). In contrast to *R.solanacearum*, the receptor(s) in *X.campestris* pv. *vesicatoria* that transmits external stimuli into the bacterial cell is still unknown (Figure 2).

Hrp pilus—tunnel to the host cell

TTS systems have been visualized in the animal pathogens *Salmonella typhimurium*, *Shigella flexneri* and *Escherichia coli*, and show striking morphological similarities to flagellar basal bodies: a membrane-embedded complex is associated with an extracellular hollow struc-

ture, the needle (Kubori *et al.*, 1998; Tamano *et al.*, 2000; Blocker *et al.*, 2001; Sekiya *et al.*, 2001).

Type III-dependent surface appendages have also been identified in plant pathogenic bacteria, i.e. *P.syringae* pv. *tomato*, *E.amylovora*, *R.solanacearum* and *X.campestris* pv. *vesicatoria*. These so-called Hrp pili have a similar diameter (6–8 nm), but are considerably longer than the needles of animal pathogens (Roine *et al.*, 1997; Van Gijsegem *et al.*, 2000; Hu *et al.*, 2001; Jin *et al.*, 2001; T.Ojanen-Reuhs and U.Bonas, unpublished data). Since Hrp pili can extend to a length of several micrometers, they have been proposed to cross the plant cell wall (Romantschuk *et al.*, 2001; Figure 2). In *R.solanacearum* and *P.syringae* pv. *tomato*, the pilin, which is the major subunit of the Hrp pilus, is required for type III secretion *in vitro* (Van Gijsegem *et al.*, 2000; Wei *et al.*, 2000). Recent immunocytochemical analyses in *E.amylovora* and *P.syringae* pv. *tomato* elegantly demonstrated that Hrp pili serve as conduits for secreted proteins (Brown *et al.*, 2001; Jin and He, 2001; Jin *et al.*, 2001; Li *et al.*, 2002). So far, there are no indications that Hrp pili also mediate bacterial contact with the host cell. In *R.solanacearum*, mutation of *hrpY*, the gene encoding the major pilus subunit, does not affect attachment of the bacteria to cultured plant cells (Van Gijsegem *et al.*, 2000).

Getting in touch—the type III translocon

Translocation across the eukaryotic plasma membrane probably requires the presence of type III-secreted bacterial proteins that form the type III translocon, a channel-like complex in the host plasma membrane (Büttner and Bonas, 2002). Putative components of the translocon have been described mainly in animal pathogens whereas they have not been identified so far in most plant pathogenic bacteria. To our knowledge, HrpF from *X.campestris* pv. *vesicatoria* is the first known candidate for a type III translocon protein in bacterial plant pathogens. Mutant studies revealed that HrpF, which is secreted by the TTS system, is dispensable for type III secretion *in vitro* but essential for the interaction with the plant (Rossier *et al.*, 2000; Büttner *et al.*, 2002). *hrpF* mutants are not able to grow and cause disease in susceptible plants and to induce the HR in resistant plants. When tested in artificial lipid bilayer systems, HrpF induced pore formation, suggesting that it might be the channel-forming core component of the type III translocon (Büttner *et al.*, 2002; Figure 2). Pore-forming activity has been demonstrated for the putative type III translocon proteins LcrV and PcrV from *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*, respectively, which do not show any sequence similarity to HrpF (Holmström *et al.*, 2001).

In animal pathogenic bacteria, observations of protein–protein interactions between putative translocon proteins suggest that the type III translocon is a heterogeneous protein complex. For instance, LcrV presumably interacts with YopB and YopD to build a functional translocon (Sarker *et al.*, 1998). In *X.campestris* pv. *vesicatoria*, it remains to be investigated whether additional proteins besides HrpF are involved in the formation of the type III translocon. So far, studies to identify HrpF interaction partners failed since HrpF is a ‘sticky’ protein, making it

difficult to show interaction specificity (D.Büttner and U.Bonas, unpublished data).

Carte d'accès—recognition by the TTS system

The mechanisms that control type III secretion *in planta* are still unknown. In *Erwinia* spp., *P.syringae* and *R.solanacearum*, several type III-secreted proteins could be detected in the culture supernatant after incubation of the bacteria in *hrp* gene-inducing medium (e.g. Gaudriault *et al.*, 1997; Mudgett and Staskawicz, 1999; Van Gijsegem *et al.*, 2000).

In *X.campestris* pv. *vesicatoria*, the isolation of a point mutation in *hrpG* (E44K, designated *hrpG**), which leads to constitutive expression of *hrp* genes, was key for the establishment of an *in vitro* secretion assay (Rossier *et al.*, 1999; Wengelnik *et al.*, 1999). However, expression of the *hrp* genes is not sufficient to trigger type III secretion. The identification of secreted proteins requires the incubation of *hrpG** bacteria in acidic minimal medium, which probably mimicks the plant's apoplast. Interestingly, the *X.campestris* pv. *vesicatoria* TTS system also secretes heterologous proteins such as PopA from *R.solanacearum*, AvrB from *P.syringae* and YopE from *Y.pseudotuberculosis*, indicating that the secretion signal is conserved among plant and animal pathogenic bacteria (Rossier *et al.*, 1999).

What is the nature of the secretion signal in proteins traveling the TTS systems? It has been proposed that the signal resides in the N terminus of the secreted proteins. In *Yersinia* spp., the first 11–17 amino acids of *Yersinia* outer proteins (Yops) are sufficient to drive the type III-dependent secretion of a reporter protein (Sory *et al.*, 1995; Schesser *et al.*, 1996; Lloyd *et al.*, 2001b). Similarly, in *X.campestris* pv. *vesicatoria*, the first 28 amino acids of AvrBs2 contain a functional secretion signal (Mudgett *et al.*, 2000). Type III-secreted proteins in both plant and animal pathogens do not share any sequence conservations in their N termini. However, comparative sequence analyses of multiple type III-secreted proteins of *P.syringae* pathovars revealed similarities in their N-terminal amino acid composition, including a high content of serine residues (on average 16–18%; Guttman *et al.*, 2002; Petnicki-Ocwieja *et al.*, 2002). In *X.campestris* pv. *vesicatoria*, the serine content within the first 25 amino acids of known TTS substrates varies between 8% (HrpB2) and 32% (HrpF). This is significantly higher than the serine content in the N termini of non-secreted components of the TTS system (between 0%, as in HrcN, and 12%, as in HrcT).

Since frameshift mutations in the N-terminal coding sequence did not abolish type III secretion of a reporter protein in *Y.enterocolitica*, the secretion signal was also predicted to reside in the 5' region of the mRNA (Anderson and Schneewind, 1997). This hypothesis, which assumes a co-translational secretion, is, however, discussed controversially in the field. For instance, the *Yersinia* YopE and YopH proteins are expressed even in the absence of a functional TTS system. In addition, mutations in YopE resulting in an altered mRNA structure did not abolish its type III secretion (Lloyd *et al.*, 2001b).

The situation is complicated further by the finding that several effector proteins from animal pathogens require specific chaperones for type III secretion and translocation (Bennett and Hughes, 2000; Lloyd *et al.*, 2001a). Recently, TTS chaperones have also been identified in plant pathogenic bacteria. DspB from *E.amylovora* and ShcA from *P.syringae* are essential for the stability and/or secretion of the pathogenicity factor DspA and the effector protein HopPsyA, respectively (Gaudriault *et al.*, 2002; van Dijk *et al.*, 2002).

Quo vadis—type III-secreted proteins

Harpins

The first proteins known to be secreted by the TTS system of bacterial plant pathogens were the harpins; HrpZ from *P.syringae* and PopA from *R.solanacearum* (He *et al.*, 1993; Arlat *et al.*, 1994). Harpins are small, heat-stable, glycine-rich proteins that lack cysteines and elicit a necrosis-like reaction when infiltrated into non-host plants (Wei *et al.*, 1992; He *et al.*, 1993; Arlat *et al.*, 1994; Alfano *et al.*, 1996; Gaudriault *et al.*, 1998). Interestingly, HrpZ from *P.syringae* was found to bind to the plant plasma membrane and to form ion-conducting pores in artificial lipid bilayers (Lee *et al.*, 2001a,b). However, the role of harpins is not well understood. In most cases, a contribution to bacterial virulence could not be demonstrated. Only in *E.amylovora*, a mutation of the harpin gene *hrpN* results in the formation of reduced disease symptoms in susceptible plants (Wei *et al.*, 1992; Barny, 1995).

Effector proteins

The best studied effector proteins are the products of *avr* genes, which were first identified genetically without knowing that they encode TTS substrates. Since the isolation of the first *avr* gene, *avrA* from *P.syringae* pv. *glycinea* (Staskawicz *et al.*, 1984), >40 bacterial *avr* genes have been identified, mainly in species of *Pseudomonas* and *Xanthomonas* (Vivian and Arnold, 2000). As mentioned above, *avr* genes trigger an *R* gene-specific plant defense reaction which often culminates in the HR. The HR phenotype is easy to follow and has been instrumental in the dissection of both bacterial pathogenicity and specific defense reactions in the plant. In the absence of the corresponding *R* gene, no recognition occurs and the infection leads to disease. There is accumulating evidence that Avr proteins probably act as virulence factors, manipulating host cellular processes for the pathogen's benefit and thus contributing to bacterial fitness and/or symptom formation in susceptible plants (White *et al.*, 2000). However, it should be emphasized that mutations in putative effector genes often do not affect bacterial virulence under laboratory conditions, indicating that they play a minor role or have redundant functions.

Until recently, type III-dependent delivery of bacterial effector proteins into the host cell has not been proven. Strong indirect evidence for translocation was provided by the fact that *avr* genes induced an *R* gene-specific HR when expressed inside the plant cell (Bonas and Van den Ackerveken, 1997; Cornelis and Van Gijsegem, 2000). Furthermore, several type III-secreted proteins from plant pathogens contain typical eukaryotic features, indicating an activity inside the host cell (White *et al.*, 2000). For

instance, the putative myristoylation motifs of several Avr proteins in pathovars of *P.syringae* suggest a localization to the plant plasma membrane, which has indeed been shown for AvrB and AvrRpm1 (Nimchuk *et al.*, 2000). In these proteins, the myristoylation motifs are crucial for the avirulence function. Further support for the hypothesis of type III-dependent delivery of bacterial effector proteins into the plant cell was provided by the analysis of the effector protein AvrBs2 from *X.campestris* pv. *vesicatoria*, which was fused translationally to an adenylate cyclase reporter from *Bordetella pertussis* (Casper-Lindley *et al.*, 2002). Recently, the direct detection of a bacterial effector protein in the plant cell has been reported: AvrBs3 from *X.campestris* pv. *vesicatoria* could be visualized in nuclei of infected plant cells, using an AvrBs3-specific antibody (Szurek *et al.*, 2002; see below).

Arrival—AvrBs3 localizes to the plant cell nucleus

Characteristic eukaryotic protein motifs are also present in members of the AvrBs3 protein family in species of *Xanthomonas* (Gabriel, 1999; Lahaye and Bonas, 2001). AvrBs3-like proteins are highly homologous (90–97% amino acid sequence identity) and all contain C-terminal nuclear localization signals and an acidic activation domain, which are features of eukaryotic transcription factors (Yang and Gabriel, 1995; Van den Ackerveken *et al.*, 1996; Zhu *et al.*, 1998, 1999; Yang *et al.*, 2000; Ballvora *et al.*, 2001; Szurek *et al.*, 2001). Differences between the family members are restricted mainly to the central protein region, which consists of 13.5–25.5 nearly perfect 34-amino-acid repeats (Lahaye and Bonas, 2001).

The AvrBs3 protein family is named after the first isolated member, AvrBs3 from *X.campestris* pv. *vesicatoria* (Bonas *et al.*, 1989). AvrBs3 is one of the few Avr proteins for which a role in symptom formation could be demonstrated. In susceptible host plants, AvrBs3 induces hypertrophy, an enlargement of mesophyll cells (Marois *et al.*, 2002). Since the induction of hypertrophy symptoms depends on functional nuclear localization signals and the acidic activation domain, we speculate that AvrBs3 acts as a transcription factor in the host cell nucleus. The nuclear localization signals probably provide the admission ticket for AvrBs3 to use the host's protein traffic road into the nucleus. Indeed, yeast two-hybrid studies and *in vitro* pull-down assays revealed that AvrBs3 interacts with pepper importin α which, together with importin β , mediates nuclear protein import (Görlich *et al.*, 1995; Szurek *et al.*, 2001; Figure 3). Immunocytological analyses demonstrated that the nuclear localization signals are essential for the targeting of AvrBs3 to nuclei of infected plant cells (Szurek *et al.*, 2002).

The hypothesis that AvrBs3 acts as a transcription factor is supported by transcriptome analyses of infected susceptible pepper plants. cDNA-AFLP (cDNA-amplified fragment length polymorphism) studies unraveled AvrBs3-induced genes, designated *upa* (up-regulated by AvrBs3; Marois *et al.*, 2002). Sequence analyses revealed that several *upa* genes show homologies to auxin-induced

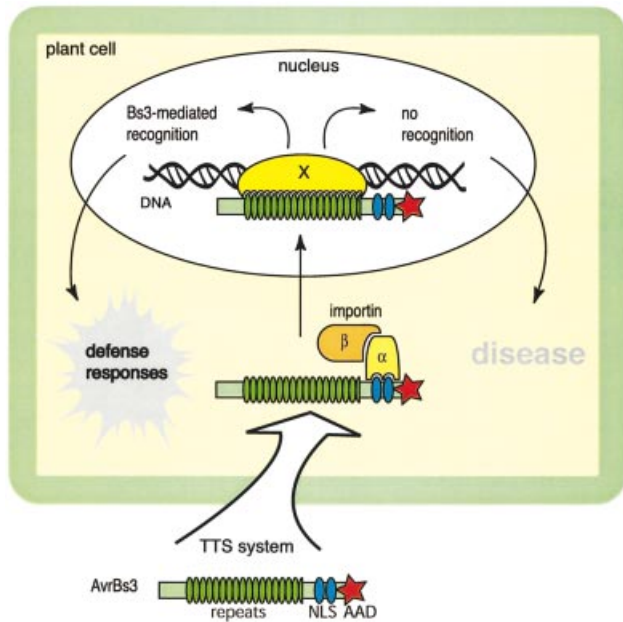


Fig. 3. Proposed model for the molecular mechanisms underlying virulence and avirulence activity of AvrBs3 from *X.campestris* pv. *vesicatoria*. Characteristic features of AvrBs3 are the central 17.5 nearly identical 34 amino acid repeats, two functional C-terminal nuclear localization signals (NLSs) and an acidic activation domain (AAD). Delivery of AvrBs3 into the host cell is mediated by the TTS system. In the plant cell, the NLSs bind to importin α , which together with importin β targets AvrBs3 to the plant cell nucleus. Direct or indirect (via a target protein X) interaction of AvrBs3 with the plant DNA leads to the modulation of the host's transcriptome and presumably results in hypertrophy, a disease symptom in susceptible plants. In resistant plants, specific plant defense responses are induced upon recognition of AvrBs3 by the R protein Bs3 (Bs, bacterial spot).

and expansin-like genes that usually play a role in cell enlargement.

Whether AvrBs3 induces gene expression with the aid of plant transcription factors or directly targets plant promoter sequences is not known (Figure 3). Support for a direct interaction of AvrBs3-like proteins with the host DNA comes from recent studies on AvrXa7, an AvrBs3 homolog from the rice pathogen *Xanthomonas oryzae* pv. *oryzae*, which directly binds to AT-rich DNA sequences (Yang *et al.*, 2000).

Perspectives

In the past decade, tremendous progress has been made in dissecting the plethora of type III-secreted proteins in plant pathogenic bacteria. Genetic and biochemical studies have led to the identification of a variety of effector proteins that travel the TTS system, the bacterial main road into the host cell. The next major challenge is the functional analysis of effector proteins: what are their targets in the plant and how do they interfere with host cellular processes? Expression of individual effector proteins in plant cells followed by transcriptome analysis and biochemical approaches will advance our understanding of the molecular processes in infected plant cells. Interdisciplinary approaches and comparative analyses of different pathogen–host systems should not only provide

a better understanding of the molecular basis of bacterial pathogenicity but also give us some clues about plant defense and last, but not least, solutions for disease management.

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References

- Aizawa,S.I. (2001) Bacterial flagella and type III secretion systems. *FEMS Microbiol. Lett.*, **202**, 157–64.
- Aldon,D., Brito,B., Boucher,C. and Genin,S. (2000) A bacterial sensor of plant cell contact controls the transcriptional induction of *Ralstonia solanacearum* pathogenicity genes. *EMBO J.*, **19**, 2304–2314.
- Alfano,J.R. and Collmer,A. (1996) Bacterial pathogens in plants: life up against the wall. *Plant Cell*, **8**, 1683–1698.
- Alfano,J.R. and Collmer,A. (1997) The type III (Hrp) secretion pathway of plant pathogenic bacteria: trafficking harpins, Avr proteins and death. *J. Bacteriol.*, **179**, 5655–5662.
- Alfano,J.R., Bauer,D.W., Milos,T.M. and Collmer,A. (1996) Analysis of the role of the *Pseudomonas syringae* pv. *syringae* HrpZ harpin in elicitation of the hypersensitive response in tobacco using functionally non-polar *hrpZ* deletion mutations, truncated HrpZ fragments and *hrmA* mutations. *Mol. Microbiol.*, **19**, 715–728.
- Alfano,J.R., Charkowski,A.O., Deng,W.L., Badel,J.L., Petnicki-Ocwieja,T., van Dijk,K. and Collmer,A. (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. USA*, **97**, 4856–4851.
- Anderson,D.M. and Schneewind,O. (1997) A mRNA signal for the type III secretion of Yop proteins by *Yersinia enterocolitica*. *Science*, **278**, 1140–1143.
- Arlat,M., Gough,C.L., Barber,C.E., Boucher,C. and Daniels,M.J. (1991) *Xanthomonas campestris* contains a cluster of *hrp* genes related to the larger *hrp* cluster of *Pseudomonas solanacearum*. *Mol. Plant-Microbe Interact.*, **4**, 593–601.
- Arlat,M., Van Gijsegem,F., Huet,J.C., Pernollet,J.C. and Boucher,C.A. (1994) PopA1, a protein which induces a hypersensitivity-like response on specific *Petunia* genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum*. *EMBO J.*, **13**, 543–553.
- Astua-Monge,G., Minsavage,G.V., Stall,R.E., Davis,M.J., Bonas,U. and Jones,J.B. (2000a) Resistance of tomato and pepper to T3 strains of *Xanthomonas campestris* pv. *vesicatoria* is specified by a plant-inducible avirulence gene. *Mol. Plant-Microbe Interact.*, **13**, 911–921.
- Astua-Monge,G., Minsavage,G.V., Stall,R.E., Vallejos,C.E., Davis,M.J. and Jones,J.B. (2000b) *Xv4-avrXv4*: a new gene-for-gene interaction identified between *Xanthomonas campestris* pv. *vesicatoria* race T3 and the wild tomato relative *Lycopersicon pennellii*. *Mol. Plant-Microbe Interact.*, **13**, 1346–1355.
- Ballvora,A., Pierre,M., van den Ackerveken,G., Schornack,S., Rossier,O., Ganal,M., Lahaye,T. and Bonas,U. (2001) Genetic mapping and functional analysis of the tomato *Bs4* locus governing recognition of the *Xanthomonas campestris* pv. *vesicatoria* AvrBs4 protein. *Mol. Plant-Microbe Interact.*, **14**, 629–638.
- Barny,M.A. (1995) *Erwinia amylovora hrpN* mutants, blocked in harpin synthesis, express a reduced virulence on host plants and elicit variable hypersensitive reactions on tobacco. *Eur. J. Plant Pathol.*, **101**, 333–340.
- Barny,M.A., Guinebretière,M.H., Marçais,B., Coissac,E., Paulin,J.P. and Laurent,J. (1990) Cloning of a large gene cluster involved in *Erwinia amylovora* CFBP1430 virulence. *Mol. Microbiol.*, **4**, 777–786.
- Basim,H., Stall,R., Minsavage,G. and Jones,J. (1999) Chromosomal gene transfer by conjugation in the plant pathogen *Xanthomonas axonopodis* pv. *vesicatoria*. *Phytopathology*, **89**, 1044–1049.
- Bennett,J.C. and Hughes,C. (2000) From flagellum assembly to

- virulence: the extended family of type III export chaperones. *Trends Microbiol.*, **8**, 202–204.
- Blocker, A., Jouihri, N., Larquet, E., Gounon, P., Ebel, F., Parsot, C., Sansonetti, P. and Allaoui, A. (2001) Structure and composition of the *Shigella flexneri* 'needle complex', a part of its type III secretin. *Mol. Microbiol.*, **39**, 652–663.
- Bogdanove, A. et al. (1996) Unified nomenclature for broadly conserved *hrp* genes of phytopathogenic bacteria. *Mol. Microbiol.*, **20**, 681–683.
- Bonas, U. and Van den Ackerveken, G. (1997) Recognition of bacterial avirulence proteins occurs inside the plant cell: a general phenomenon in resistance to bacterial diseases. *Plant J.*, **12**, 1–7.
- Bonas, U., Stall, R.E. and Staskawicz, B. (1989) Genetic and structural characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. *Mol. Gen. Genet.*, **218**, 127–136.
- Bonas, U., Schulte, R., Fenselau, S., Minsavage, G.V., Staskawicz, B.J. and Stall, R.E. (1991) Isolation of a gene-cluster from *Xanthomonas campestris* pv. *vesicatoria* that determines pathogenicity and the hypersensitive response on pepper and tomato. *Mol. Plant-Microbe Interact.*, **4**, 81–88.
- Bonas, U., Conrads-Strauch, J. and Balbo, I. (1993) Resistance in tomato to *Xanthomonas campestris* pv. *vesicatoria* is determined by alleles of the pepper-specific avirulence gene *avrBs3*. *Mol. Gen. Genet.*, **238**, 261–269.
- Boucher, C.A., Van Gijsegem, F., Barberis, P.A., Arlat, M. and Zischek, C. (1987) *Pseudomonas solanacearum* genes controlling both pathogenicity on tomato and hypersensitivity on tobacco are clustered. *J. Bacteriol.*, **169**, 5626–5632.
- Brito, B., Marena, M., Barberis, P., Boucher, C. and Genin, S. (1999) *prhJ* and *hrpG*, two new components of the plant signal-dependent regulatory cascade controlled by PrhA in *Ralstonia solanacearum*. *Mol. Microbiol.*, **31**, 237–251.
- Brito, B., Aldon, D., Barberis, P., Boucher, C. and Genin, S. (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.
- Brown, I.R., Mansfield, J.W., Taira, S., Roine, E. and Romantschuk, M. (2001) Immunocytochemical localization of HrpA and HrpZ supports a role for the Hrp pilus in the transfer of effector proteins from *Pseudomonas syringae* pv. *tomato* across the host plant cell wall. *Mol. Plant-Microbe Interact.*, **14**, 394–404.
- Büttner, D. and Bonas, U. (2002) Port of entry—the type III secretion translocon. *Trends Microbiol.*, **10**, 186–192.
- Büttner, D., Nennstiel, D., Klüßener, B. and Bonas, U. (2002) Functional analysis of HrpF, a putative type III translocon protein from *Xanthomonas campestris* pv. *vesicatoria*. *J. Bacteriol.*, **184**, 2389–2398.
- Casper-Lindley, C., Dahlbeck, D., Clark, E.T. and Staskawicz, B. (2002) Direct biochemical evidence for type III secretion-dependent translocation of the AvrBs2 effector protein into plant cells. *Proc. Natl Acad. Sci. USA*, **99**, 8336–8341.
- Ciesiolka, L.D. et al. (1999) Regulation of expression of avirulence gene *avrRxv* and identification of a family of host interaction factors by sequence analysis of *avrBsT*. *Mol. Plant-Microbe Interact.*, **12**, 35–44.
- Cornelis, G.R. and Van Gijsegem, F. (2000) Assembly and function of type III secretory systems. *Annu. Rev. Microbiol.*, **54**, 735–774.
- Da Silva, A.C. et al. (2002) Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature*, **417**, 459–463.
- Escolar, L., Van den Ackerveken, G., Pieplow, S., Rossier, O. and Bonas, U. (2001) Type III secretion and *in planta* recognition of the *Xanthomonas* avirulence proteins AvrBs1 and AvrBsT. *Mol. Plant Pathol.*, **2**, 287–296.
- Fenselau, S. and Bonas, U. (1995) Sequence and expression analysis of the *hrpB* pathogenicity operon of *Xanthomonas campestris* pv. *vesicatoria* which encodes eight proteins with similarity to components of the Hrp, Ysc, Spa and Fli secretion systems. *Mol. Plant-Microbe Interact.*, **8**, 845–854.
- Flor, H.H. (1971) Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.*, **9**, 275–296.
- Fouts, D.E. et al. (2002) Genomewide identification of *Pseudomonas syringae* pv. *tomato* DC3000 promoters controlled by the HrpL alternative sigma factor. *Proc. Natl Acad. Sci. USA*, **99**, 2275–2280.
- Gabriel, D.W. (1999) The *Xanthomonas* *avr/pth* gene family. In Stacey, G. and Keen, N.T. (eds), *Plant-Microbe Interactions*. Vol. 4. APS Press, St Paul, MN, pp. 39–55.
- Gaudriault, S., Malandrin, L., Paulin, J.P. and Barny, M.A. (1997) DspA, an essential pathogenicity factor of *Erwinia amylovora* showing homology with AvrE of *Pseudomonas syringae*, is secreted via the Hrp secretion pathway in a DspB-dependent way. *Mol. Microbiol.*, **26**, 1057–1069.
- Gaudriault, S., Brisset, M.N. and Barny, M.A. (1998) HrpW of *Erwinia amylovora*, a new Hrp-secreted protein. *FEBS Lett.*, **428**, 224–228.
- Gaudriault, S., Paulin, J.P. and Barny, M.A. (2002) The DspB/F protein of *Erwinia amylovora* is a type III secretion chaperone ensuring efficient secretion of the DspA/E essential pathogenicity factor. *Mol. Plant Pathol.*, in press.
- Genin, S., Gough, C.L., Zischek, C. and Boucher, C.A. (1992) Evidence that the *hrpB* gene encodes a positive regulator of pathogenicity genes from *Pseudomonas solanacearum*. *Mol. Microbiol.*, **6**, 3065–3076.
- Görlich, D., Vogel, F., Mills, A.D., Hartmann, E. and Laskey, R.A. (1995) Distinct functions for the two importin subunits in nuclear protein import. *Nature*, **377**, 246–248.
- Guttman, D.S., Vinatzer, B.A., Sarkar, S.F., Ranall, M.V., Kettler, G. and Greenberg, J.T. (2002) A functional screen for the type III (Hrp) secretome of the plant pathogen *Pseudomonas syringae*. *Science*, **295**, 1722–1726.
- Hacker, J. and Kaper, J.B. (2000) Pathogenicity islands and the evolution of microbes. *Annu. Rev. Microbiol.*, **54**, 641–679.
- He, S.Y. (1998) Type III protein secretion systems in plant and animal pathogenic bacteria. *Annu. Rev. Phytopathol.*, **36**, 363–392.
- He, S.Y., Huang, H.C. and Collmer, A. (1993) *Pseudomonas syringae* pv. *syringae* harpin_{ps}: a protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. *Cell*, **73**, 1255–1266.
- Heesemann, J., Algermissen, B. and Laufs, R. (1984) Genetically manipulated virulence of *Yersinia enterocolitica*. *Infect. Immun.*, **46**, 105–110.
- Holmström, A., Olsson, J., Cherepanov, P., Maier, E., Nordfelth, R., Pettersson, J., Benz, R., Wolf-Watz, H. and Forsberg, A. (2001) LcrV is a channel size-determining component of the Yop effector translocon of *Yersinia*. *Mol. Microbiol.*, **39**, 620–632.
- Hu, W., Yuan, J., Jin, Q.L., Hart, P. and He, S.Y. (2001) Immunogold labeling of Hrp pili of *Pseudomonas syringae* pv. *tomato* assembled in minimal medium and *in planta*. *Mol. Plant-Microbe Interact.*, **14**, 234–241.
- Hueck, C.J. (1998) Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev.*, **62**, 379–433.
- Huguet, E., Hahn, K., Wengelnik, K. and Bonas, U. (1998) *hpaA* mutants of *Xanthomonas campestris* pv. *vesicatoria* are affected in pathogenicity but retain the ability to induce host-specific hypersensitive reaction. *Mol. Microbiol.*, **29**, 1379–1390.
- Innes, R.W., Bent, A.F., Kunkel, B.N., Bisgrove, S.R. and Staskawicz, B.J. (1993) Molecular analysis of avirulence gene *avrRpt2* and identification of a putative regulatory sequence common to all known *Pseudomonas syringae* avirulence genes. *J. Bacteriol.*, **175**, 4859–4869.
- Jin, Q. and He, S.Y. (2001) Role of the Hrp pilus in type III protein secretion in *Pseudomonas syringae*. *Science*, **294**, 2556–2558.
- Jin, Q., Hu, W., Brown, I., McGhee, G., Hart, P., Jones, A.L. and He, S.Y. (2001) Visualization of secreted Hrp and Avr proteins along the Hrp pilus during type III secretion in *Erwinia amylovora* and *Pseudomonas syringae*. *Mol. Microbiol.*, **40**, 1129–1139.
- Kearney, B. and Staskawicz, B.J. (1990) Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. *Nature*, **346**, 385–386.
- Klement, Z. (1982) Hypersensitivity. In Mount, M.S. and Lacy, G.H. (eds), *Phytopathogenic Prokaryotes*. Vol. 2. Academic Press, New York, pp. 149–177.
- Kubori, T., Matsushima, Y., Nakamura, D., Uralil, J., Lara-Tejero, M., Sukhan, A., Galan, J.E. and Aizawa, S.I. (1998) Supramolecular structure of the *Salmonella typhimurium* type III protein secretion system. *Science*, **280**, 602–605.
- Lahaye, T. and Bonas, U. (2001) Molecular secrets of bacterial type III effector proteins. *Trends Plant Sci.*, **6**, 479–485.
- Lee, J., Klessig, D.F. and Nürnberger, T. (2001a) A harpin binding site in tobacco plasma membranes mediates activation of the pathogenesis-related gene *hin1* independent of extracellular calcium but dependent on mitogen-activated protein kinase activity. *Plant Cell*, **13**, 1079–1093.
- Lee, J. et al. (2001b) HrpZ_{Psph} from the plant pathogen *Pseudomonas syringae* pv. *phaseolicola* binds to lipid bilayers and forms an ion-conducting pore *in vitro*. *Proc. Natl Acad. Sci. USA*, **98**, 289–294.
- Li, C.M., Brown, I., Mansfield, J., Stevens, C., Boureau, T., Romantschuk, M. and Taira, S. (2002) The Hrp pilus of *Pseudomonas*

- syringae* elongates from its tip and acts as a conduit for translocation of the effector protein HrpZ. *EMBO J.*, **21**, 1909–1915.
- Lindgren,P.B. (1997) The role of *hrp* genes during plant–bacterial interactions. *Annu. Rev. Phytopathol.*, **35**, 129–152.
- Lindgren,P.B., Peet,R.C. and Panopoulos,N.J. (1986) Gene-cluster of *Pseudomonas syringae* pv. phaseolicola controls pathogenicity of bean plants and hypersensitivity on nonhost plants. *J. Bacteriol.*, **168**, 512–522.
- Lloyd,S.A., Forsberg,A., Wolf-Watz,H. and Francis,M.S. (2001a) Targeting exported substrates to the *Yersinia* TTSS: different functions for different signals? *Trends Microbiol.*, **9**, 367–371.
- Lloyd,S.A., Norman,M., Rosqvist,R. and Wolf-Watz,H. (2001b) *Yersinia* YopE is targeted for type III secretion by N-terminal, not mRNA, signals. *Mol. Microbiol.*, **39**, 520–532.
- Macnab,R.M. (1999) The bacterial flagellum: reversible rotary propeller and type III export apparatus. *J. Bacteriol.*, **181**, 7149–7153.
- Marenda,M., Brito,B., Callard,D., Genin,S., Barberis,P., Boucher,C. and Arlat,M. (1998) PrhA controls a novel regulatory pathway required for the specific induction of *Ralstonia solanacearum* *hrp* genes in the presence of plant cells. *Mol. Microbiol.*, **27**, 437–453.
- Marois,E., Van den Ackerveken,G. and Bonas,U. (2002) The *Xanthomonas* type III effector protein AvrBs3 modulates plant gene expression and induces cell hypertrophy in the susceptible host. *Mol. Plant-Microbe Interact.*, **15**, 637–646.
- Mudgett,M.B. and Staskawicz,B.J. (1999) Characterization of the *Pseudomonas syringae* pv. *tomato* AvrRpt2 protein: demonstration of secretion and processing during bacterial pathogenesis. *Mol. Microbiol.*, **32**, 927–941.
- Mudgett,M.B., Chesnokova,O., Dahlbeck,D., Clark,E.T., Rossier,O., Bonas,U. and Staskawicz,B.J. (2000) Molecular signals required for type III secretion and translocation of the *Xanthomonas campestris* AvrBs2 protein to pepper plants. *Proc. Natl Acad. Sci. USA*, **97**, 13324–13329.
- Niepold,F., Anderson,D. and Mills,D. (1985) Cloning determinants of pathogenesis from *Pseudomonas syringae* pathovar *syringae*. *Proc. Natl Acad. Sci. USA*, **82**, 406–410.
- Nimchuk,Z., Marois,E., Kjemtrup,S., Leister,R.T., Katagiri,F. and Dangel,J.L. (2000) Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. *Cell*, **101**, 353–363.
- Noël,L. (2001) Utilisation de la technique de cDNA-AFLP pour l'étude d'un transcriptome procaryote: identification et caractérisation du régulon *hrp* chez *Xanthomonas campestris* pv. *vesicatoria*. Université de Paris-Sud, Paris, France.
- Noël,L., Thieme,F., Nennstiel,D. and Bonas,U. (2001) cDNA-AFLP analysis unravels a genome-wide *hrpG*-regulon in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*. *Mol. Microbiol.*, **41**, 1271–1281.
- Noël,L., Thieme,F., Nennstiel,D. and Bonas,U. (2002) Two novel type III system-secreted proteins of *Xanthomonas campestris* pv. *vesicatoria* are encoded within the *hrp* pathogenicity island. *J. Bacteriol.*, **184**, 1340–1348.
- Petnicki-Ocwieja,T. et al. (2002) Genomewide identification of proteins secreted by the Hrp type III protein secretion system of *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl Acad. Sci. USA*, **99**, 7652–7657.
- Roine,E., Wei,W.S., Yuan,J., Nurmiaho-Lassila,E.L., Kalkkinen,N., Romantschuk,M. and He,S.Y. (1997) Hrp pilus: a *hrp*-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl Acad. Sci. USA*, **94**, 3459–3464.
- Romantschuk,M., Roine,E. and Taira,S. (2001) Hrp pilus—reaching through the plant cell wall. *Eur. J. Plant Pathol.*, **107**, 153–160.
- Ronald,P.C. and Staskawicz,B.J. (1988) The avirulence gene *avrBs1* from *Xanthomonas campestris* pv. *vesicatoria* encodes a 50-kDa protein. *Mol. Plant-Microbe Interact.*, **1**, 191–198.
- Rossier,O. (1999) Étude du système de sécrétion de type III chez la bactérie phytopathogène *Xanthomonas campestris* pathovar *vesicatoria*. Université Paris, Paris, France.
- Rossier,O., Wengelnik,K., Hahn,K. and Bonas,U. (1999) The *Xanthomonas* Hrp type III system secretes proteins from plant and mammalian pathogens. *Proc. Natl Acad. Sci. USA*, **96**, 9368–9373.
- Rossier,O., Van den Ackerveken,G. and Bonas,U. (2000) HrpB2 and HrpF from *Xanthomonas* are type III-secreted proteins and essential for pathogenicity and recognition by the host plant. *Mol. Microbiol.*, **38**, 828–838.
- Salanoubat,M. et al. (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature*, **415**, 497–502.
- Sarker,M.R., Neyt,C., Stainier,I. and Cornelis,G.R. (1998) The *Yersinia* Yop virulon: LcrV is required for extrusion of the translocators YopB and YopD. *J. Bacteriol.*, **180**, 1207–1214.
- Schesser,K., Frithz-Lindsten,E. and Wolf-Watz,H. (1996) Delineation and mutational analysis of the *Yersinia pseudotuberculosis* YopE domains which mediate translocation across bacterial and eukaryotic cellular membranes. *J. Bacteriol.*, **178**, 7227–7233.
- Sekiya,K., Ohishi,M., Ogino,T., Tamano,K., Sasakawa,C. and Abe,A. (2001) Supermolecular structure of the enteropathogenic *Escherichia coli* type III secretion system and its direct interaction with the EspA-sheath-like structure. *Proc. Natl Acad. Sci. USA*, **98**, 11638–11643.
- Sory,M.P., Boland,A., Lambermont,I. and Cornelis,G.R. (1995) Identification of the YopE and YopH domains required for secretion and internalization into the cytosol of macrophages, using the *cyaA* gene fusion approach. *Proc. Natl Acad. Sci. USA*, **92**, 11998–12002.
- Staskawicz,B.J., Dahlbeck,D. and Keen,N.T. (1984) Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr. *Proc. Natl Acad. Sci. USA*, **81**, 6024–6028.
- Steinberger,E.M. and Beer,S.V. (1988) Creation and complementation of pathogenicity mutants of *Erwinia amylovora*. *Mol. Plant-Microbe Interact.*, **1**, 135–144.
- Swords,K.M., Dahlbeck,D., Kearney,B., Roy,M. and Staskawicz,B.J. (1996) Spontaneous and induced mutations in a single open reading frame alter both virulence and avirulence in *Xanthomonas campestris* pv. *vesicatoria* *avrBs2*. *J. Bacteriol.*, **178**, 4661–4669.
- Szurek,B., Marois,E., Bonas,U. and Van den Ackerveken,G. (2001) Eukaryotic features of the *Xanthomonas* type III effector AvrBs3: protein domains involved in transcriptional activation and the interaction with nuclear import receptors from pepper. *Plant J.*, **26**, 523–534.
- Szurek,B., Rossier,O., Hause,G. and Bonas,U. (2002) Type III-dependent translocation of the *Xanthomonas* AvrBs3 protein into the plant cell. *Mol. Microbiol.*, in press.
- Tamano,K., Aizawa,S., Katayama,E., Nonaka,T., Imajoh-Ohmi,S., Kuwae,A., Nagai,S. and Sasakawa,C. (2000) Supramolecular structure of the *Shigella* type III secretion machinery: the needle part is changeable in length and essential for delivery of effectors. *EMBO J.*, **19**, 3876–3887.
- Thanassi,D.G. and Hultgren,S.J. (2000) Multiple pathways allow protein secretion across the bacterial outer membrane. *Curr. Opin. Cell Biol.*, **12**, 420–430.
- Van den Ackerveken,G., Marois,E. and Bonas,U. (1996) Recognition of the bacterial avirulence protein AvrBs3 occurs inside the host plant cell. *Cell*, **87**, 1307–1316.
- van Dijk,K., Tam,V.C., Records,A.R., Petnicki-Ocwieja,T. and Alfano,J.R. (2002) The ShcA protein is a molecular chaperone that assists in the secretion of the HopPsyA effector from the type III (Hrp) protein secretion system of *Pseudomonas syringae*. *Mol. Microbiol.*, **44**, 1469–1481.
- Van Gijsegem,F., Vasse,J., Camus,J.C., Marenda,M. and Boucher,C. (2000) *Ralstonia solanacearum* produces Hrp-dependent pili that are required for PopA secretion but not for attachment of bacteria to plant cells. *Mol. Microbiol.*, **36**, 249–260.
- Vivian,A. and Arnold,D.L. (2000) Bacterial effector genes and their role in host pathogen interactions. *J. Plant Pathol.*, **82**, 163–178.
- Wei,W., Plovianich-Jones,A., Deng,W.L., Jin,Q.L., Collmer,A., Huang,H.C. and He,S.Y. (2000) The gene coding for the Hrp pilus structural protein is required for type III secretion of Hrp and Avr proteins in *Pseudomonas syringae* pv. *tomato*. *Proc. Natl Acad. Sci. USA*, **97**, 2247–2252.
- Wei,Z.M., Laby,R.J., Zumoff,C.H., Bauer,D.W., He,S.Y., Collmer,A. and Beer,S.V. (1992) Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. *Science*, **257**, 85–88.
- Wengelnik,K. and Bonas,U. (1996) HrpXv, an AraC-type regulator, activates expression of five of the six loci in the *hrp* cluster of *Xanthomonas campestris* pv. *vesicatoria*. *J. Bacteriol.*, **178**, 3462–3469.
- Wengelnik,K., Van den Ackerveken,G. and Bonas,U. (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.
- Wengelnik,K., Rossier,O. and Bonas,U. (1999) Mutations in the regulatory gene *hrpG* of *Xanthomonas campestris* pv. *vesicatoria* result in constitutive expression of all *hrp* genes. *J. Bacteriol.*, **181**, 6828–6831.

- White,F.F., Yang,B. and Johnson,L.B. (2000) Prospects for understanding avirulence gene function. *Curr. Opin. Plant Biol.*, **3**, 291–298.
- Xiao,Y. and Hutcheson,S.W. (1994) A single promoter sequence recognized by a newly identified alternate sigma factor directs expression of pathogenicity and host range determinants in *Pseudomonas syringae* [published erratum appears in *J. Bacteriol.*, **176**, 6158]. *J. Bacteriol.*, **176**, 3089–3091.
- Xiao,Y., Heu,S., Yi,J., Lu,Y. and Hutcheson,S.W. (1994) Identification of a putative alternate sigma factor and characterization of a multicomponent regulatory cascade controlling the expression of *Pseudomonas syringae* pv. *syringae* Pss61 *hrp* and *hrmA* genes. *J. Bacteriol.*, **176**, 1025–1036.
- Yang,B., Zhu,W., Johnson,L.B. and White,F.F. (2000) The virulence factor AvrXa7 of *Xanthomonas oryzae* pv. *oryzae* is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein. *Proc. Natl Acad. Sci. USA*, **97**, 9807–9812.
- Yang,Y. and Gabriel,D.W. (1995) *Xanthomonas* avirulence/pathogenicity gene family encodes functional plant nuclear targeting signals. *Mol. Plant-Microbe Interact.*, **8**, 627–631.
- Young,B.M. and Young,G.M. (2002) YplA is exported by the Ysc, Ysa and flagellar type III secretion systems of *Yersinia enterocolitica*. *J. Bacteriol.*, **184**, 1324–1334.
- Zhu,W.G., Yang,B., Chittoor,J.M., Johnson,L.B. and White,F.F. (1998) AvrXa10 contains an acidic transcriptional activation domain in the functionally conserved C terminus. *Mol. Plant-Microbe Interact.*, **11**, 824–832.
- Zhu,W.G., Yang,B., Wills,N., Johnson,L.B. and White,F.F. (1999) The C terminus of AvrXa10 can be replaced by the transcriptional activation domain of VP16 from the herpes simplex virus. *Plant Cell*, **11**, 1665–1674.

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