

## The Future Challenge

Our path seems clear. We have come to understand many things about the unique biology of bacterial biofilms. Biofilms represent microbial societies with their own defense and communication systems. We have an arsenal of microscopic, physical chemical, and molecular techniques available to examine biofilms. There are many basic questions regarding the biology of biofilms that can now be answered. Our modern view of biofilm infections leads to the realization that their effective control will require a concerted effort to develop therapeutic agents that target the biofilm phenotype and community signaling-based agents that prevent the formation, or promote the detachment, of biofilms. The techniques are now available to undertake such efforts.

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### REVIEW

# Type III Secretion Machines: Bacterial Devices for Protein Delivery into Host Cells

Jorge E. Galán<sup>1\*</sup> and Alan Collmer<sup>2</sup>

Several Gram-negative pathogenic bacteria have evolved a complex protein secretion system termed type III to deliver bacterial effector proteins into host cells that then modulate host cellular functions. These bacterial devices are present in both plant and animal pathogenic bacteria and are evolutionarily related to the flagellar apparatus. Although type III secretion systems are substantially conserved, the effector molecules they deliver are unique for each bacterial species. Understanding the biology of these devices may allow the development of novel prevention and therapeutic approaches for several infectious diseases.

A number of bacterial pathogens have evolved the capacity to engage their hosts in complex intimate interactions aimed not necessarily at causing disease but rather at securing the microbe's ability to multiply and move on to a new host. The relationship between bacterial pathogens and their hosts is most often a peaceful one, because it has been shaped by a coevolutionary process aimed at securing the survival

of both the pathogen and the host. This is particularly the case for microbial pathogens that, through the process of host adaptation, have lost the ability to explore other niches. Sometimes, however, these pathogens cause harm to the host. In some instances, disease symptoms may simply be unpleasant manifestations of a self-limiting process that leads to the transmission of the bacteria from one host to

the next. However, in other cases, fatal disease may occur when these bacterial pathogens encounter a host that has been weakened by circumstances that alter the delicate balance of the microbe-host interaction.

Recent advances in the fields of immunology and of molecular, cell, and structural biology are allowing the detailed investigation of the interactions between these highly adapted pathogens and their hosts. This close examination is not only helping in the understanding of microbial pathogenesis but is also providing

<sup>1</sup>Section of Microbial Pathogenesis, Boyer Center for Molecular Medicine, Yale School of Medicine, New Haven, CT 06536, USA. <sup>2</sup>Department of Plant Pathology, Cornell University, Ithaca, NY 14853–4203, USA.

\*To whom correspondence should be addressed. e-mail: jorge.galan@yale.edu

insight into basic cellular processes. A recent development in this field is the identification of a highly specialized protein secretion system in several Gram-negative pathogenic bacteria (Table 1). This system, which is termed type III, has evolved to deliver proteins from the bacterial cytoplasm into the host cell cytosol. These bacterial proteins can then stimulate or interfere with host cellular processes, thereby dictating the terms of the bacterial–host cell interaction. Type III secretion systems are present in both animal and plant pathogenic bacteria, which indicates that they are capable of operating not only across bacterial genera but also across host kingdoms. This article will highlight the general features of type III secretion systems as well as the cellular responses that result from their activities. More detailed reviews on this subject can be found elsewhere (1).

**From Flagellar Assembly to Bacterial Pathogenesis**

The architecture of the cell envelope of Gram-negative bacteria dictates that proteins destined to be delivered to the outside must traverse several barriers: the inner membrane, the periplasmic space, the peptidoglycan layer, and finally the outer membrane. Consequently, these bacteria have evolved a variety of mechanisms to transfer proteins from the cytoplasm to the extracellular environment (2). The discovery of sequence homologies between proteins implicated in the secretion of virulence factors in several different bacterial pathogens and proteins implicated in the export of flagellar com-

ponents prompted the proposal that a common protein secretion pathway existed, termed type III (3). Since then, the commonality of these secretion mechanisms has been corroborated experimentally (4), and the complexity of these systems has become more apparent. Made up of more than 20 proteins, type III secretion systems are the most complex of all known protein secretion systems in bacteria. The observation that these virulence-associated systems were always linked to phenotypes related to interactions between bacterial pathogens and their animal or plant hosts intrigued researchers in this field from the outset. It is now known that such associations are due to the central function of these systems, which is the delivery of bacterial effector proteins into the cytosol of the host cells (5). Type III secretion systems have three distinguishing features: (i) the absence in the secreted proteins of a cleavable signal peptide that is characteristic of proteins secreted via the *sec*-mediated general secretory pathway, (ii) the requirement for customized accessory proteins (chaperones) for many of the secreted proteins, and (iii) a widespread requirement for host cell contact for full activation of the secretory pathway.

Gram-negative pathogenic bacteria most likely acquired their type III secretion systems through some mechanism of horizontal gene transfer. This is supported by the observation that these systems are often encoded in extra chromosomal elements or within pathogenicity islands, which are segments of chromosomal DNA that are absent from related nonpathogen-

ic bacteria (6). These islands often have a GC content that deviates from that of the chromosome of the host organism, and they are usually bounded by remnants of insertion sequences, bacteriophage genes, or transposable elements. Amino acid sequence comparison of the most conserved components of type III secretion and flagellar export systems shows a clustering of different family members in discrete groups (Fig. 1). The flagellar assembly proteins form a distinct group among this protein family, and components of type III secretion systems of plant and animal pathogenic bacteria also cluster in distinct groups. Intriguingly, type III secretion components of plant pathogenic bacteria are more closely related to components of the flagellar machinery than are their counterparts in animal pathogenic bacteria. Thus, it is possible that type III secretion systems first emerged in plant pathogenic bacteria as an evolutionary adaptation of the flagellar export apparatus in order to secrete proteins other than flagellin, thereby facilitating the ability of these bacteria to form a close association with plant cells.

**Structural Components of the Type III Secretion Apparatus**

All known type III secretion systems of animal and plant pathogenic bacteria share a number of core structural components that are highly conserved (1). These components can be divided into at least two groups. One group consists of predicted outer membrane proteins, including a protein with sequence similarity to the secretin family of protein transporters, as well as several

**Table 1.** Type III protein secretion systems in animal- and plant-associated bacteria.

Bacterial species	Consequence of infection	Phenotypes associated with type III secretion systems
<b>Animal pathogens</b>		
<i>Bordetella bronchiseptica</i>	Closely related to <i>B. pertussis</i> , the cause of whooping cough	Unknown. Regulated by BvgAS, which is a major virulence control system
<i>Chlamydia</i> spp.	Sexually transmitted, respiratory, and ocular diseases	Unknown
Enteropathogenic <i>E. coli</i>	Diarrhea in young children	Attachment to and effacement of intestinal epithelial cells
Enterohemorrhagic <i>E. coli</i>	Hemorrhagic colitis and hemolytic-uremic syndrome	Attachment to and effacement of intestinal epithelial cells
<i>P. aeruginosa</i>	Opportunistic infections	Cytotoxicity and antiphagocytosis
<i>S. enterica</i>	Food poisoning and typhoid fever	These bacteria have two systems, one involved in bacterial entry into nonphagocytic cells and the induction of apoptosis in macrophages and the other required for survival inside macrophages and systemic infection
<i>Shigella</i> spp.	Dysentery	Bacterial entry into nonphagocytic cells and induction of macrophage apoptosis
<i>Yersinia</i> spp.	Plague and gastroenteritis	Antiphagocytosis, inhibition of cytokine production, and induction of apoptosis in macrophages
<b>Plant pathogens</b>		
<i>Erwinia amylovora</i>	Fire blight of apple and pear	Hrp*
<i>Erwinia chrysanthemi</i>	Soft rots and parenchymatal necroses	Infectivity at low levels of inoculum
<i>Erwinia herbicola</i> pv. <i>gypsophila</i>	Gypsophila galls	Hrp*
<i>Erwinia stewartii</i>	Stewart's wilt of corn	Water-soaking symptoms in host
<i>P. syringae</i>	Foliar spots and blights	Hrp*
<i>Rhizobium</i> spp.	N <sub>2</sub> fixation: root nodule symbiosis	Nodulation host range
<i>Ralstonia solanacearum</i>	Wilts of solanaceous plants	Hrp*
<i>Xanthomonas</i> spp.	Foliar spots and blights	Hrp*

\* HR in incompatible plants and pathogenesis in permissive plants.

less conserved lipoproteins. The other group consists of several integral membrane proteins with close similarity to components of the flagellar export apparatus. Recently, a supramolecular structure associated with a type III secretion system of *Salmonella typhimurium* was isolated and visualized under the electron microscope (7). This structure, termed the needle complex, spans both the inner and outer membranes of the bacterial envelope and closely resembles the flagellar basal body, further supporting the evolutionary relationship between flagella and type III secretion systems. The needle complex is a long hollow structure about 120 nm long and is composed of two clearly identifiable domains: a needlelike portion projecting outward from the surface of the bacterial cell and a cylindrical base that anchors the structure to the inner and outer membranes (Fig. 2). Biochemical analysis of the purified needle complexes revealed that they are composed of at least three proteins: InvG (a member of the secretin family) and two lipoproteins, PrgH and PrgK. Despite the architectural similarity between flagella and type III systems, the structural components of the needle complex share limited sequence similarity with components of the flagellar basal body (8). Although information on the supramolecular organization of type III secretion systems from other bacteria is currently not available, the high degree of sequence similarity among several structural components indicates that all these systems are likely to have a similar architecture.

The actual mechanisms of type III secretion are poorly understood. Similar to what has been proposed for the export of flagellin through the basal body, it is possible that the type III secretion needle complex serves as a hollow conduit through which the type III secreted proteins traverse the different barriers of the bacterial envelope. It is likely that adenosine triphosphate

(ATP) hydrolysis provides the energy for the secretion process. This is supported by the observation that a conserved component of the secretion systems shares sequence similarity with the  $\alpha$  and  $\beta$  subunits of the F1 component of the bacterial F0F1 proton-translocating ATPase and that in at least two systems this protein has been shown to hydrolyze ATP in vitro (9).

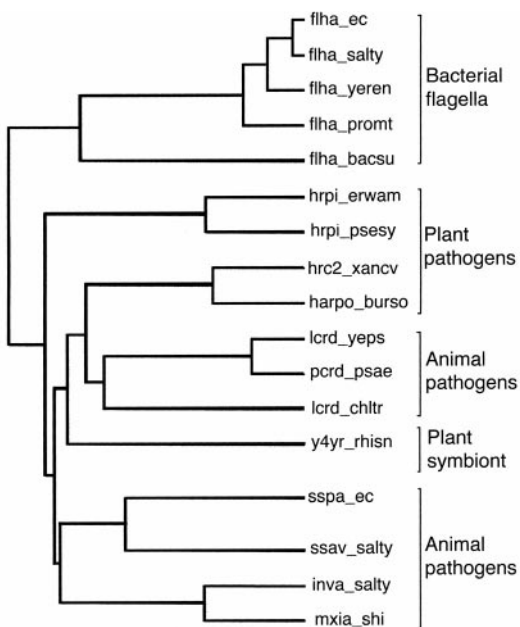
In addition to the needle complex, some type III secretion systems display other supramolecular structures that are likely to function in the delivery of effector proteins into the host cell (10–12). These are structures composed of proteins secreted via the type III secretion machinery that assemble on the surface of the bacteria upon contact with host cells. The proteins and associated surface structures differ widely among different pathogens. For example, *S. typhimurium* and enteropathogenic *Escherichia coli* produce filamentous appendages about 50 nm in diameter that bridge the bacteria and the host cells and appear to be shed when cellular responses are stimulated (10, 11). In contrast, *Pseudomonas syringae* produces a much thinner (6 to 8 nm in diameter) pilus that may penetrate the ~200-nm-thick plant cell wall (12). Although these structures appear to be required for bacteria to deliver effector proteins to the host cell through the type III systems, it is not known whether they function as conduits or attachment factors that facilitate contact between the bacteria and the host cell.

### Substrate Recognition and Delivery into the Host Cell

Substrate proteins for the type III pathway carry multiple signals that route them to the secretion pathway and eventually to subcellular targets within the host. Experiments carried out with YopE and YopN, two type III secreted proteins

of the enteropathogen *Yersinia*, revealed that the first ~15 amino acids of these proteins were sufficient to direct the secretion of a heterologous protein (13). Closer scrutiny demonstrated that the secretion signal must reside in the structure of the mRNA because mutations that shifted the reading frame of the putative secretion signal, yielding completely different polypeptide sequences, were still able to direct secretion through the type III machinery (14). The 5' mRNA regions of some of the *yop* messages are predicted to have stem-loop structures that bury AUG translational start signals, which suggests that the translation of these proteins may be arrested until the 5' mRNA interacts with a component of the secretion apparatus. Such a coupling of translation and secretion has been demonstrated for YopQ (14). The secretion signal of two proteins from the plant pathogen *P. syringae*, AvrB and AvrPto, also appears to reside in their 5' mRNA, which suggests that this type of substrate recognition may be a general feature of type III secretion systems (15). However, some type III secreted proteins can be secreted posttranslationally through an alternative secretion signal (14). This alternative mechanism involves the function of specific chaperones that bind the cognate secreted proteins on discrete domains located within the first 100 amino acids of many proteins that travel the type III pathway (13, 16). Unlike other well-characterized chaperones such as GroEL or Hsp70, type III secretion-associated chaperones have a rather narrow binding specificity and appear to lack nucleotide-binding or nucleotide-hydrolyzing activities. Although they exhibit little amino acid sequence similarity, type III secretion chaperones share a number of properties such as relatively small size (15 to 18 kD), a low isoelectric point, and a predominantly  $\alpha$ -helical secondary structure. Proteins carrying chaperone-binding domains are not secreted in culture or translocated into the host in the absence of their cytosolic chaperone. Removal of this domain alleviates the requirement for a chaperone for secretion, although it also prevents translocation of the protein into host cells. Various chaperones may have differing or overlapping roles as secretion/translocation pilots or as partitioning factors that prevent the premature association of secreted proteins in the bacterial cytoplasm, a process that would target them for premature degradation. The activity as secretion pilots is evident in the residual ability of some bacterial effector proteins to be secreted (albeit less efficiently) in a chaperone-dependent manner despite deletion of the 5'-terminal mRNA secretion domain (14). The function as partitioning factors is most evident with proteins that strongly associate after secretion, such as the IpaB and IpaC proteins of *Shigella*, SipB and SipC of *Salmonella*, and YopB and YopD of *Yersinia* (17). The use of different or multiple targeting mechanisms may determine the timing of secretion of some effectors and contribute to the robust se-

**Fig. 1.** Phylogram of the LcrD/InvA family of proteins, which constitute a conserved component of type III secretion systems. The unrooted phylogenetic tree was constructed from a distance matrix (by the unweighted pair group method using arithmetic averages) as implemented in the GCG software package (45). Names of sequences correspond to the entries in GenBank.



cretion of others. Thus, proteins to be delivered immediately upon contact may be preformed and therefore delivered through the chaperone-dependent pathway. Effectors delivered later in the interaction may make use of the mRNA signal-dependent pathway, which requires de novo protein synthesis.

Delivery of type III secreted proteins into the host cell requires several accessory proteins that travel the type III pathway to the bacterial cell surface where they function to sense contact with the host, bridge the bacterium with the host cell, and promote translocation across the host cytoplasmic membrane. This process is best understood in the enteropathogen *Yersinia* (13). Host contact activates the pathway, which permits a pool of effector proteins to be injected into the host cell. In these bacteria, this process appears to be polarized, involving the direct transfer of bacterial effectors from the bacterial cytoplasm to the host cell cytoplasm (5, 18). However, polarized transfer may not be a general feature of type III secretion, because it is not observed in *Shigella* spp. (19) or *Salmonella* spp. (20). The sensing of host cells by *Yersinia* is regulated by three extracellular proteins, YopN, LcrG, and TyeA, by unknown mechanisms (21). The actual process of translocation through the eukaryotic host cell membrane is poorly understood; it has been suggested that two type III secreted hydrophobic proteins, YopB and YopD, form a translocation pore in the host cytoplasmic membrane, but their function remains controversial (22).

### Regulation of Type III Secretion

Bacteria use specific and varied strategies to regulate the expression of their type III secretion machineries [for more extensive reviews of this subject, see (1)]. Regulation takes place at both the transcriptional and post-translational levels. Transcriptional regulation is accomplished by one or several specific transcription factors as well as by components of global regulatory networks that control the expression of type III secretion components in response to a variety of environmental cues such as temperature, osmolarity, availability of nutrients, divalent cations (in particular  $Ca^{2+}$ ), pH, and growth phase. In addition, in some bacteria, type III gene expression is also controlled by sensing the secretion process itself. This strategy, first identified in the related flagellar assembly system and more recently described in the enteric pathogen *Yersinia* spp., relies on the secretion of a negative regulator through the type III secretion pathway, thereby coupling the transcription and secretion processes (23).

The posttranslational regulation of the secretion process is less well understood. It appears that, at least in some systems, the physiological signal that stimulates this regulatory pathway involves contact with host factors. For example, contact by the enteric pathogens *Salmonella*,

*Yersinia*, and *Shigella* with host cells results in the stimulation of secretion and translocation of effector proteins through the type III pathway (5, 10, 24). A number of components of type III secretion systems potentially involved in this process have been identified. Loss-of-function mutations in the *Yersinia lcrG* and *lcrE* (*yopN*), the *Salmonella sipD*, or the *Shigella ipaD* genes result in uncontrolled secretion even in the absence of external stimuli (13, 25). This regulation is posttranslational because inhibition of de novo bacterial protein synthesis does not prevent the host cell responses stimulated by type III secretion and translocation (10, 26). Nevertheless, the coupling of secretion to the transcriptional control mechanisms discussed above dictates that the posttranslational stimulation of secretion will eventually result in the activation of transcription of type III secretion genes.

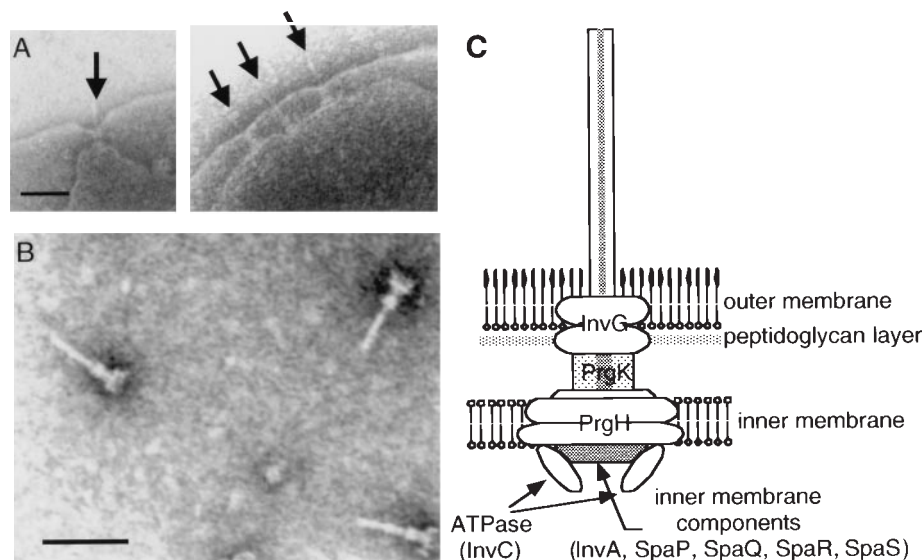
### Effector Proteins and Host Responses

Type III secretion systems of plant and animal pathogens have evolved to deliver into the host cell an array of effector proteins that have the capacity to stimulate or interfere with host cellular functions (Table 2). This diversity is consistent with the different phenotypes associated with these systems and with their adaptation to carry out specific functions in each bacterial pathogen. However, not all proteins secreted by type III secretion systems are delivered into the host cell or have effector function. For example, several type III secreted proteins are involved in the secretion process itself, its regulation, or the translocation of effector proteins through the host cell membrane. The study of the function of type III secreted effector proteins has been hampered by the functional redundancy of

some of the effectors. Furthermore, the effector proteins most often act in concert with one another, which hampers the use of reductionist approaches for the study of their individual function. Despite these difficulties, the function of some of the effector proteins, and in some instances their biochemical activities, are beginning to be understood.

*Effector proteins in animal pathogens.* Animal pathogenic bacteria often use their type III secretion-associated functions to modulate the actin cytoskeleton of the host cell. For example, some pathogenic bacteria such as *Yersinia* spp. and *P. aeruginosa* deliver effector proteins that interfere with actin cytoskeleton dynamics to prevent phagocytosis. *Yersinia* spp. encode at least two type III secreted proteins, YopE and YopH, that hamper macrophage function (13). YopH appears to exert its effect by dephosphorylating  $p130^{cas}$  and focal adhesion kinase, two components of focal adhesions that become tyrosine phosphorylated upon  $\beta 1$ -integrin stimulation (27). *Pseudomonas aeruginosa* delivers an adenosine diphosphate-ribosylating toxin, ExoS, that targets actin-organizing small guanosine triphosphate (GTP)-binding proteins (28).

Other animal pathogenic bacteria modulate actin cytoskeleton functions to either gain access to nonphagocytic cells (for example, *Salmonella* spp. and *Shigella* spp.) or to attach to epithelial cell surfaces (for example, enteropathogenic *E. coli*). *Salmonella typhimurium* injects into the host cell a set of effector proteins that, through carefully coordinated activities, induce actin cytoskeleton rearrangements, membrane ruffling, and macropinocytosis, ultimately resulting in bacterial uptake (29). The effector



**Fig. 2.** Needle complex of *S. typhimurium* type III secretion system. (A) Electron micrographs of osmotically shocked *S. typhimurium* exhibiting needle complexes on the bacterial envelope (arrows). (B) Electron micrograph of purified needle complexes. (C) Schematic representation of the *S. typhimurium* needle complex and its putative components. The location of the different components is hypothetical. Other proteins not listed in the scheme may also be present. Electron micrographs are reprinted from (7). Scale bars, 100 nm.

proteins include an exchange factor for Rho GTPases (SopE) (30), an inositol phosphate phosphatase (SopB) (31), and an actin-binding protein (SipA) (32). SopE acts as an exchange factor for a subset of Rho GTPases, including CDC42 and Rac (30). SopB modulates the actin cytoskeleton through its inositol phosphate phosphatase activity, which generates a broad range of inositol phospholipids and inositol phosphates with demonstrated signaling capacity (31). By binding to actin, SipA decreases its critical concentration for polymerization and markedly inhibits F-actin depolymerization. These activities result in the spatial localization and more pronounced outward extension of the *Salmonella*-induced membrane ruffles, thereby facilitating bacterial uptake (32). *Shigella* spp. also modulate the host cell cytoskeleton by targeting Rho GTPases using a type III secretion system, although the actual mechanisms by which the bacteria engage these signaling molecules are poorly understood (33). One of its type III secreted proteins, IpaA, is thought to aid the internalization process by binding vinculin and thereby forming a focal adhesionlike structure at the point of bacterial contact with the host cell (33).

Upon contact with the intestinal epitheli-

um, enteropathogenic *E. coli* induces localized actin cytoskeleton rearrangements that result in the formation of characteristic pedestallike structures (34). An essential step in the formation of these structures is the bacterial attachment to the host cell surface, which is mediated by a bacterial outer membrane protein called intimin. The receptor for intimin, called Tir, is also bacterially encoded and is translocated to the host cell membrane via the type III secretion system (35). How the intimin-Tir interaction results in actin cytoskeleton rearrangements leading to attachment is not known, but it is likely to require the function of additional type III secreted proteins encoded by these bacteria.

Another activity associated with type III secretion systems is the induction of apoptosis in infected macrophages. However, different bacteria stimulate apparently similar responses through the activity of different effector proteins. For example, *Shigella* spp. induces apoptosis in macrophages through the activity of the type III secreted protein IpaB, which binds and activates caspase-1, thereby initiating the apoptotic program (36). In *Yersinia* spp., the induction of apoptosis is dependent on the function of the type III secreted protein YopJ (YopP), which functions by an unknown mech-

anism (37). YopJ shares sequence similarity with effector proteins delivered by type III systems in other bacteria, such as the animal pathogen *S. typhimurium* (AvrA) as well as the phytopathogen *Xanthomonas campestris* (AvrRxv) and the legume-associated symbiont *Rhizobium* spp. (the product of the *y410* gene). This is the only example of a type III secreted effector protein family that includes members in both plant and animal pathogens (38). This is of particular significance because the AvrRxv protein has been implicated in the stimulation of the hypersensitive response in plants, which involves the induction of apoptosis. Whether the sequence similarity among these effector proteins points at some basic signaling pathway conserved in both animals and plants is an open question that may be answered when the biochemical function of these proteins is revealed.

*Effector proteins in plant pathogens.* Effector proteins delivered by type III secretion systems in plant pathogenic bacteria have different activities depending on whether the plant is permissive for bacterial virulence or is nonpermissive and renders the pathogen avirulent (Fig. 3). In nonpermissive plants, effectors delivered through the type III secretion system elicit a defense response known as the hypersensitive response (HR), which is characterized

**Table 2.** Examples of effector proteins of known function secreted via type III secretion systems.

Bacterial species	Secreted protein	Biochemical activity	Effect on host cell function
<b>Animal pathogens</b>			
Enteropathogenic <i>E. coli</i>	Tir	Receptor for intimin	Effacement of the microvilli of the intestinal brush border
<i>P. aeruginosa</i> cytotoxicity	ExoS	ADP-ribosyltransferase	Inhibition of phagocytosis
	ExoT	ADP-ribosyltransferase	Cytotoxicity
	ExoY	Adenylate cyclase	Cytotoxicity
<i>S. typhimurium</i>	SopE	Exchange factor for Rho GTPases (such as CDC42 and Rac)	Actin cytoskeleton reorganization; activation of MAP kinase pathways
	SopB (SigA)	Inositol phosphate phosphatase	Actin cytoskeleton reorganization; stimulation of Cl <sup>-</sup> secretion
	SipA	Binds actin, lowering its critical concentration and stabilizing F-actin	Promotes localized actin cytoskeleton reorganization
	SptP	Tyrosine phosphatase	Reorganization of the actin cytoskeleton
<i>Shigella</i> spp.	IpaB	Activation of caspase-1; binds β1-integrins and CD44	Apoptosis
	IpaA	Binds vinculin	Stimulation of bacterial entry
<i>Yersinia</i> spp.	IpgD	Putative inositol phosphate phosphatase	Unknown
	YopE	Unknown	Disruption of the actin cytoskeleton; inhibition of phagocytosis
	YopH	Tyrosine phosphatase	Disruption of focal adhesions; inhibition of phagocytosis
	YpkA YopJ (YopP)	Serine/threonine kinase Unknown	Unknown Apoptosis
<b>Plant pathogens</b>			
<i>P. syringae</i> pv. <i>tomato</i>	AvrPto	Interacts with Pto ( <i>R</i> gene product); activates Pto serine/threonine kinase signaling pathway	HR
<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>	AvrBs2	Interacts with Bs2 <i>R</i> gene product of resistant pepper cultivars; sequence predicts ability to synthesize or hydrolyze phosphodiester linkages, which may be involved in its virulence function	HR
<i>X. campestris</i> pv. <i>vesicatoria</i>	AvrBs3 family	Localized to plant nuclei; probable transcription factors; activity determined by multiple repeats of 34 amino-acid sequence	HR

by a rapid (~24-hour) induction of programmed death in plant cells that are in contact with the pathogen (39). In contrast, in permissive hosts, these pathogens continue to grow and spread within the intercellular spaces of the infected organ for several days before producing visible disease symptoms. In this manner, the Gram-negative pathogens in the genera *Erwinia*, *Pseudomonas*, *Xanthomonas*, and *Ralstonia* cause necrotic spots, blights, wilts, and cankers in most crop plants, and underlying these diverse diseases is the ability of their type III systems to translocate effector proteins across the plant cell wall and into plant cells (39).

The effector proteins are encoded by *avr* (avirulence) genes, which are so named because they can betray the parasite to the *R* (resistance) gene surveillance system of the plant, an event

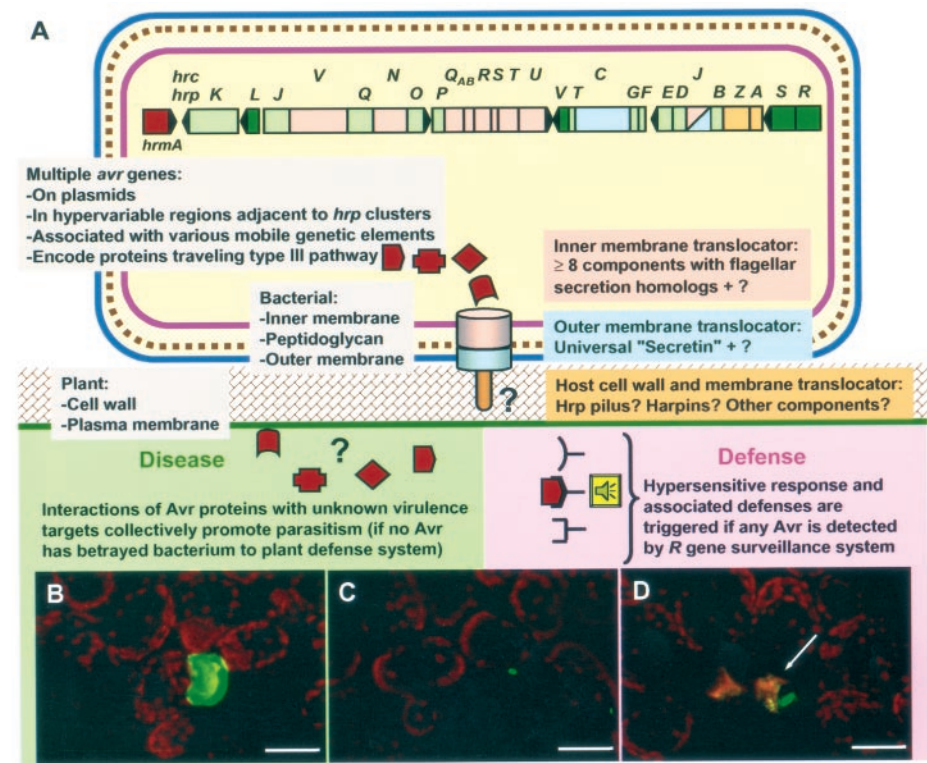
that triggers the HR and renders the parasite avirulent (39). Plants contain many *R* genes, and their products interact with *avr* gene products in a “gene-for-gene” manner, resulting in defensive detection of most potential pathogens. The primary function of Avr proteins is almost certainly to promote parasitism, and mutant phenotypes reveal that approximately one-third of the more than 40 *avr* genes that have been cloned from *P. syringae* and *X. campestris* make a quantitative contribution to virulence in permissive hosts (39). The mechanisms by which these injected proteins promote the growth of bacteria on the surface of plant cells is unknown.

Key properties of three representative Avr proteins are presented in Table 2. Related Avr proteins may function in different genera of plant pathogens. The DspE protein of *Er-*

*winia amylovora* is homologous with *P. syringae* AvrE, and the encoding genes can be functionally exchanged (40). Similarly, an *Erwinia chrysanthemi* type III secretion system expressed in *E. coli* can deliver *P. syringae* Avr signals to plant cells and secrete the proteins in culture (41). The ability of Avr proteins to be delivered by type III systems of bacteria in other genera may be biologically significant, because many *avr* genes are associated with mobile genetic elements (42). Expansions in host range, improvements in parasitic fitness, and evasion of *R* gene surveillance are likely to favor *avr* gene flux among plant-associated bacteria.

To date, there is no direct evidence for the translocation of any Avr protein from the bacterium to the plant cytoplasm. However, there is considerable evidence that Avr proteins function inside plant cells, indirectly supporting the hypothesis that type III secretion systems direct their delivery to that site (43). For example, (i) the amino acid sequence of *R* gene products that are expected to interact with Avr proteins predict an intracellular localization, (ii) several Avr proteins elicit an *R* gene-dependent HR when experimentally produced inside plant cells, and (iii) a physical interaction between at least one Avr protein (AvrPto) and its cognate *R* gene product has been demonstrated. In addition, nuclear targeting signals of an Avr protein (AvrBs3) are required for its ability to elicit the HR (43).

In addition to the effector Avr proteins, type III secretion systems of plant pathogenic bacteria secrete another class of proteins termed harpins (44). These are glycine-rich, cysteine-lacking, heat-stable proteins that can elicit the HR response. Unlike Avr proteins that need to be delivered inside the cell to exert their function, the harpins can elicit the HR when delivered to the surface of plant cells. The function of the harpins, however, remains puzzling. Genetic evidence indicates that harpins may have only a secondary role in bacterial elicitation of the HR, but whether this is to assist the delivery of Avr proteins across the plant cell wall is unknown.



**Fig. 3.** The Hrp/Avr system of *P. syringae*. (A) The *hrmA/hrc/hrp* gene cluster depicted is from *P. syringae* pv. *syringae* 61. It encodes a Hrp (type III) secretion system that is thought to inject the HrmA (Avr) protein into plant cells, thus enabling *E. coli* and other nonpathogenic bacteria carrying the cluster to elicit the HR in tobacco leaves (41). At least nine proteins (encoded by *hrc* genes) are universal components of type III secretion systems (1). The remaining *hrp* genes encode regulatory proteins (dark green), known extracellular proteins (orange), and other components presumably specialized for *P. syringae* type III injection into plants (light green). This cluster is part of a larger pathogenicity island that typically contains several of the many *avr* genes that *P. syringae* strains carry. Avr proteins are targeted to various subcellular locations within the host cell and contribute quantitatively to parasitic fitness unless detected by the *R* gene surveillance system. Major unanswered questions include the role of the Hrp pilus and other Hrp proteins in translocating Avr proteins across the plant cell wall, as well as the nature of the virulence targets whose perturbation permits these bacteria to parasitically grow on plant cells. (B through D) Confocal laser scanning micrographs of colonies on the surface of plant cells in tobacco leaves 40 hours after inoculation with *P. syringae* cells constitutively expressing the *Aequorea victoria* green fluorescent protein. (B) *P. syringae* pv. *tabaci* (virulent in tobacco). (C) *P. syringae* pv. *syringae* 61-2089 (unable to grow in plants because of the *hrcC::TnphoA* mutation). (D) *P. syringae* pv. *syringae* 61 (avirulent in tobacco; arrow indicates autofluorescence of an adjacent dead plant cell). All infection sites looked like that shown for the *hrcC* mutant in (C) for at least the first 3 hours after inoculation. Red fluorescence is from chloroplasts lining the cell periphery. Scale bars, 30  $\mu$ m.

**Perspectives and the Future**

The fate of many bacterial pathogens is linked to their ability to gain access and multiply within plant or animal hosts. These bacteria have evolved a vast array of strategies to accomplish this objective. In this article we have provided a brief description of an intricate system that represents a remarkable example of host adaptation by microbial pathogens. Although we are beginning to understand some aspects of the function of this machinery and its secreted proteins, there are many questions to address in the future. How are proteins translocated across the bacterial envelope and across the host cell membrane? How are substrates recognized by the secre-

tion machinery? What is the role of the specific chaperones? What are the cues that stimulate type III secretion? How do the different effector proteins function inside the host cell? The study of these systems will continue to provide insight into the mechanisms of manipulation of host cell functions by bacterial pathogens. The presence of type III secretion systems exclusively in bacteria with pathogenic potential may provide a unique target for the development of therapeutic agents that may spare normal flora. Furthermore, harnessing the type III secretion system for the delivery of heterologous proteins may provide a valuable tool for the development of novel vaccines and therapeutic approaches.

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#### REVIEW

# *Helicobacter pylori* Virulence and Genetic Geography

Antonello Covacci,<sup>1</sup> John L. Telford,<sup>1</sup> Giuseppe Del Giudice,<sup>1</sup> Julie Parsonnet,<sup>2</sup> Rino Rappuoli<sup>1\*</sup>

Isolated for the first time in 1982 from human gastric biopsy, *Helicobacter pylori* is responsible for gastritis, peptic ulcer, and gastric cancer. A pathogenicity island acquired by horizontal transfer, coding for a type IV secretion system, is a major determinant of virulence. The infection is now treated with antibiotics, and vaccines are in preparation. The geographic distribution suggests coevolution of man and *Helicobacter pylori*.

Human, plant, and animal diseases are often caused by infection with unrecognized or uncultivated (or both) etiologic agents. Until 1982, when it was isolated by accidental extended incubation, *Helicobacter pylori* (*Hp*) was part of the unknown microbial world (1). Today it is a well-recognized pathogen that chronically infects up to 50% of the world's

human population. It is a Gram-negative, microaerophilic bacterial rod, associated with gastritis, peptic ulcer, and gastric cancer.

*Hp* lives for decades in the extreme environment of the human stomach. Like other bacteria specialized to live in a single environment, *Hp* has a small genome (1.67 megabases) containing a minimal set of metabolic

genes (2). The mechanisms for environmental adaptation such as the stringent response and the two-component regulatory systems are absent or rare, respectively (3). For example, *Pseudomonas aeruginosa*, an opportunist bacterium able to survive in most environments, contains 90 two-component regulatory systems, whereas *Hp* contains only four (3, 4).

<sup>1</sup>IRIS, Chiron SpA, Via Fiorentina 1, 53100 Siena, Italy.

<sup>2</sup>Department of Medicine/Infectious Diseases Hrp T152, Stanford University, CA 94305, USA.

\*To whom correspondence should be addressed. E-mail: rappuoli@iris02.biocine.it