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Review

The invasion-associated type III secretion system of *Salmonella typhimurium*: common and unique features

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Abstract. Several bacterial pathogens make use of a specialized protein secretion system to inject effector proteins into host cells. This system, commonly referred to as type III secretion, is always associated with phenotypes related to intimate interactions between the pathogen and its respective host cells. The enteric pathogen *Salmonella typhimurium* utilizes a type III secretion system to invade nonphagocytic intestinal epithelial cells. Whereas the invasion-associated type III system of *S. typhimurium* has evolved to perform a specific function, many of the components of this system are conserved among the type III systems of other bacterial pathogens. This review will discuss the common and unique features of the *S. typhimurium* system in relation to the type III systems of other human pathogens. Topics discussed include the phenotypes associated with various type III systems, the genetic loci encoding these systems, the components of the type III secretion apparatus, the effector proteins and the mechanisms by which they enter host cells as well as the mechanisms used to regulate the expression of type III systems.

Key words. Type III secretion; *S. typhimurium*; bacterial invasion; bacterial pathogenicity.

Introduction

The ability to invade host epithelial cells is an essential step in the pathogenic life cycle of the enteric pathogen *Salmonella enterica*. The invasion process is mediated by a contact-dependent secretion system that enables this pathogen to deliver effector proteins to the cytosol of host cells. This system, commonly referred to as type III secretion, has been found to be present in several animal pathogens and, remarkably, is also present in a number of plant pathogenic bacteria. The fact that infection of both animal and plant cells can be facilitated by the same virulence mechanism demonstrates the versatility of this unique secretion system. Although many of the phenotypes associated with the *Salmonella* system are unique to this pathogen, various features appear to be broadly conserved among all type III systems. This review will focus on the invasion-associated type III secretion system of *S. enterica* subspecies *typhimurium* (*S. typhimurium*) and will compare this system with those of other human pathogens, including members of the *Shigella*, *Yersinia* and enteropathogenic *Escherichia coli* (EPEC) spp., as well as *Pseudomonas aeruginosa*. More comprehensive discussions of this topic can be found elsewhere [1, 2].

General features of type III secretion systems and their associated phenotypes

The cell envelope of Gram-negative bacteria acts as an effective barrier, separating the cytoplasm from the external environment. Proteins destined for delivery to the external environment must pass through the inner

membrane, the periplasmic space and finally the outer membrane. To accomplish this feat, bacteria have evolved a number of different secretion systems [1]. These include the type I, type II and type IV secretion systems. Type I systems, exemplified by the *E. coli* α-hemolysin secretion system, consist of three main proteins: an inner membrane-localized adenosine triphosphate (ATP)-binding protein, an outer membrane-localized channel-forming protein and a membrane fusion protein which serves to connect the other two components. Secretion of type I substrate proteins through the membrane, which occurs in a single step, is promoted by the presence of a carboxyl-terminal signal sequence which is not cleaved upon secretion [3]. Proteins secreted by type II systems, such as the *Klebsiella oxytoca* pullulanase secretion system, contain cleavable amino-terminal signal sequences which allow these proteins to be recognized and exported through the inner membrane by the *sec* machinery. Upon export through the inner membrane, the substrates traverse the outer membrane via a pore formed by other components of the secretion system [4]. Type IV systems constitute a family of secretion systems which are capable of transporting both protein and DNA through the bacterial envelope and into host cells. The *vir* system of *Agrobacterium tumefaciens* [5] and the pertussis toxin exporter system of *Bordetella pertussis* are [6] examples of type IV secretion systems. The secretion of type IV substrates occurs in a single step and requires at least 11 different type IV-specific proteins.

The discovery of a protein secretion system in *Yersinia enterocolitica* dedicated to the secretion of virulence factors [7] and the subsequent discovery of sequence homologies between proteins involved in the secretion of virulence factors by different bacterial pathogens led to the proposal of a novel secretion system, termed type III secretion [8–10]. Features common to all type III secretion systems include (i) the absence of a cleavable amino-terminal signal sequence in the secreted protein, (ii) the requirement of specific chaperones for the secretion of many of the effector proteins, (iii) the requirement of an inducing signal (typically contact with the host cell) for full activation of the system and (iv) the ability to deliver the secreted proteins to the cytosol of host cells.

Several components of type III secretion systems have been shown to share sequence similarities with proteins involved in the export and assembly of flagella subunits in both Gram-negative and Gram-positive bacteria [1, 11]. Sequence comparisons of the most conserved components of type III secretion and flagellar assembly systems show a clustering of family members into discrete groups [2]. Components of the type III systems of plant pathogenic bacteria appear to be the most closely related to components of the flagellar system, suggesting that type III secretion first evolved in plant pathogens as an evolutionary adaptation of the flagellar export apparatus. In addition, one component of all type III systems is a member of the secretin family of proteins. This family includes proteins involved in type II secretion [4] as well as proteins involved in the assembly of type IV pili [12]. It therefore appears that the type III secretion systems of modern bacterial pathogens may have evolved by combining proteins from various other secretion systems.

It was noticed early on that type III secretion systems were always associated with phenotypes related to intimate interactions between the bacterial pathogen and their respective host cell (table 1). It is now known that this association is due to the primary purpose of these systems, which is to deliver bacterial effector proteins to the cytosol of host cells. Upon reaching the host cell cytosol, the effector proteins subvert signal transduction pathways in a manner that is beneficial to the invading bacteria. In the case of *S. typhimurium*, the subversion of host cell signaling pathways is accomplished by two separate type III systems. Translocation of effector proteins into host epithelial cells by the invasion-associated type III system results in profuse cytoskeletal rearrangements, membrane ruffling and the production of

Table 1. Phenotypes and diseases associated with different type III secretion systems.

Type III system	Associated phenotypes	Associated diseases
S. typhimurium-SPI-1	invasion of nonphagocytic cells and the induction of apoptosis in macrophages	food poisoning and typhoid fever
S. typhimurium-SPI-2	survival inside macrophages and systemic infection	food poisoning and typhoid fever
Shigella spp.	invasion of nonphagocytic cells and induction of apoptosis in macrophages	dysentery
Yersinia spp.	antiphagocytosis, induction of apoptosis in macrophages and inhibition of cytokine production	gastroenteritis and plague
P. aeruginosa	antiphagocytosis and cytotoxicity	opportunistic infections
EPEC	attachment to and effacement of intestinal epithelial cells	diarrhea in young children

proinflammatory cytokines [13, 14]. The cytoskeletal rearrangements and membrane ruffling result in macropinocytosis, leading to the entry of the invading bacteria, whereas the production of cytokines results in a localized inflammatory response at the site of infection. This inflammatory response is likely exacerbated by the ability of the invasion-associated type III system to induce apoptosis in macrophages [15].

Once inside the host, *Salmonella* resides within a membrane-bound compartment (spacious vacuole) where it is able to survive and replicate. Survival within these intracellular compartments is dependent on the function of the second type III system [16, 17]. Genes in this second system are preferentially expressed in the intracellular environment of macrophages [18], and strains carrying mutations in this system exhibit severely attenuated virulence in both mice and macrophage virulence assays [19]. It is interesting to note that whereas the invasion-associated type III system confers upon *S. typhimurium* the ability to kill resident macrophages, the second type III system affords this pathogen the capacity to survive the bactericidal capabilities of infected macrophages.

Like *Salmonella*, *Shigella* spp. use their type III secretion systems to subvert host cell signal transduction pathways in order to effect cytoskeletal changes which facilitate entry of the invading bacteria [20]. However, once inside the cell, *Shigella* escapes the confines of the vacuole and resides in the nutrient-rich cytoplasm, where it replicates and eventually spreads to adjacent cells [20]. The ability of *Shigella* to escape the vacuole also appears to be type III-dependent since this step is closely correlated with the production of proteins secreted by the *Shigella* type III system [21]. Infection with *Shigella* also results in a localized inflammatory response; however, in the case of this pathogen, inflammation is predominantly due to the type III-associated killing of resident macrophages [20].

Other bacterial pathogens make use of type III secretion to evade the host immune system while remaining in the extracellular environment. The capacity of all three pathogenic *Yersinia* spp. to resist phagocytosis is associated with the presence of a functional type III system [22]. Translocation of *Yersinia* type III effector proteins (Yops) into macrophages results in alterations in actin cytoskeleton dynamics which help to prevent bacterial uptake, and ultimately lead to cell death [22]. This phenotype is similar to that attributed to the closely related type III system of *P. aeruginosa*. Although much of what is known about the *P. aeruginosa* system is based on genetic comparisons [23], studies have shown that the type III system of this opportunistic pathogen delivers proteins into epithelial cells which, like the Yops, interfere with normal host signaling pathways, resulting in cytoskeletal changes that ultimately lead to the death of the host cell [24–26].

The type III secretion system of EPEC spp. is also responsible for inducing cytoskeletal rearrangements in host cells that lead to the attachment of the bacteria to the cell surface. Upon contact with epithelial cells, pathogenic *E. coli* induce a specific cytopathic effect termed the attaching and effacing (A/E) lesion [27]. Host cells are induced to form pedestals of densely packed cytoskeletal proteins which protrude from the apical surface and intimately cup individual bacteria. These pedestals are not formed when cells are infected with strains that carry mutations in genes essential for type III secretion [27], clearly implicating this system in the formation of these structures. The type III secretion system of EPEC spp. has apparently also endowed this pathogen with the ability to resist phagocytosis by macrophages [28].

Genetic loci encoding type III secretion systems

The genes that encode the *S. typhimurium* invasion-associated type III secretion system are located in a contiguous 40-Kb region of the chromosome at centisome 63. This region is an example of a pathogenicity island (SPI-1) which is widely distributed among all *Salmonella* serotypes but is absent from the corresponding region of the chromosome in a closely related nonpathogenic *E. coli* strain [29]. The GC content of this region is significantly lower than that of the rest of the chromosome, suggesting that this region was acquired by horizontal transmission from another organism. This theory is strengthened by the findings that this region is often flanked by IS-3-like elements [30] and that natural deletions frequently occur in environmental isolates of *Salmonella* [31]. It is now believed that all type III secretion systems of modern bacteria emerged from a common source, perhaps as an adaptation of a preexisting secretion system. In other pathogens, the genes encoding the type III system also bear evidence of having been acquired through horizontal transmission from another organism. The *Shigella* and *Yersinia* systems are encoded on virulence plasmids [20, 22], whereas pathogenic *E. coli* spp. carry this locus in a chromosomally located pathogenicity island which has a significantly different GC content from the rest of the chromosome [32].

The *Salmonella* invasion-associated type III system bears a remarkable degree of similarity to the type III system of *Shigella*, suggesting that these organisms may have acquired their type III systems from a common source and at similar times in their evolutionary history. Several blocks of genes in the *Salmonella* system including the *inv*, *spa*, *sip* and *prg* operons have direct homologs in the *Shigella* system [11, 33]. These homologous genes encode structural components as

well as a number of the secreted targets. In many cases, these homologies extend to functional complementation [11, 34]. The second *Salmonella* type III secretion system, which is encoded in another pathogenicity island (SPI-2) located at centisome 30, appears to be more similar to the system in EPEC spp. than to the *Salmonella* SPI-1 and *Shigella* systems [35], suggesting that the SPI-2 type III system was acquired at a different time and from a different source than the SPI-1 system.

The *Yersinia* system, which represents the most well characterized example of type III secretion systems, is encoded on a 70-Kb virulence plasmid which is present in all three pathogenic *Yersinia* spp. [22, 36]. Whereas the regions of this plasmid encoding the structural elements of the type III systems are almost identical in the different *Yersinia* spp., subtle differences in the proteins secreted by the different species indicate a minor degree of evolutionary divergence [22]. These subtle differences are in contrast to the large degree of variation seen between the *Yersinia* type III systems and those found in *Salmonella* and *Shigella* spp. A number of proteins are present in the *Yersinia* system for which no direct homologs have been found in the systems of these other two pathogens [22]. This is not surprising, given the differences in the phenotypes associated with these different systems. The recently discovered type III system of *P. aeruginosa* bears a striking resemblance to the *Yersinia* system. Although still not completely characterized, the *P. aeruginosa* type III locus appears to be organized in a manner similar to that of *Yersinia* and to contain homologs to proteins which are not found in the type III systems of other pathogens [23]. It seems that the type III systems found in *P. aeruginosa* and pathogenic *Yersinia* spp. represent a different subset of these systems from those found in *Salmonella* and *Shigella* spp.

The genes encoding the type III system of EPEC spp. are located on a 35-Kb pathogenicity island called the locus of enterocyte effacement (LEE). The site of the EPEC chromosome where the LEE is located [the *selC* transfer RNA (tRNA) locus] is identical to the site at which another virulence element in uropathogenic *E. coli* is located, suggesting that this region of the *E. coli* chromosome is a hotspot for insertion events [32]. The LEE appears to be conserved as an intact unit in all intestinal pathogens which are known to produce the A/E phenotype, including pathogenic *Citrobacter* and *Hafnia* spp. [32]. While there is currently no evidence that these pathogens possess functional type III systems, future studies will likely prove this to be the case. As mentioned previously, within the family of type III systems, the LEE shares the highest level of sequence similarity and genetic organization with the SPI-2 system of *S. typhimurium*. It is surprising that two systems which are associated with such different phenotypes and which function in such different environments appear to be so closely related.

Recent studies have shown that a number of effector proteins secreted by the invasion-associated type III system of *Salmonella* are encoded by genes located outside of SPI-1. The genes encoding SopD and SopE are located at the centisome 61 region [37, 38], whereas the *sopB* gene is located in a different pathogenicity island (SPI-5) at centisome 20 of the *S. typhimurium* chromosome [39]. The *sopE* gene, which is present in only a subset of *S. enterica* serovars, has been shown to be encoded within the genome of a temperate bacteriophage [38]. Similarly, the genes encoding the *P. aeruginosa* type III effectors ExoS and ExoU [40, 41] are not present in all clinical isolates of this pathogen, and the *exoU* gene bears evidence of having been acquired independently from the secretion system [41]. It is not yet known if genes encoding type III effector proteins of other human pathogens are also encoded in mobile genetic elements, but it would be to the evolutionary advantage of these pathogens to maintain a horizontal mobility of effector protein genes. This would allow the bacteria to maximize the chances of assembling the most appropriate set of effector genes for a given host, while maintaining the genes required for the secretion and translocation of these proteins.

Structural components of type III secretion systems

Proteins which form the secretion apparatus are among the most highly conserved components of type III systems (table 2). This is in keeping with the fact that these proteins must all serve the same function, which is to transport the secreted substrates from the cytoplasm to the external environment. The structural components of type III systems consist of several proteins which can be divided into a number of different functional categories, including (i) a putative ATPase, (ii) a group of at least five proteins which are predicted to be located in the inner membrane and which are similar to components of the flagellar export apparatus and (iii) a group of predicted outer membrane proteins, including proteins with sequence similarity to the family of channel-forming secretin proteins, as well as a number of less-conserved lipoproteins. It has recently been shown that, in the case of *Salmonella*, a number of these predicted outer membrane proteins form a supramolecular structure called the needle complex [42]. In addition, there appear to be a number of less well conserved cytoplasmically located accessory proteins which are thought to assist in the assembly of the secretion apparatus.

Table 2. Type III homologs involved in forming the secretion apparatus.

Salmonella (SPI-1)	*Salmonella* (SPI-2)	*Shigella*	*Yersinia*	*P. aeruginosa*	EPEC	Flagellar assembly	Proposed location	Function
InvC	SsaN	Spa47	YscN	PcrN	EscN	FliI	inner membrane	ATPase
InvA	SsaV	MxiA	LcrD	PcrD	EscV	FlhA	inner membrane	export apparatus
SpaP	SsaR	Spa24	YscR	–	EscR	FliP	inner membrane	export apparatus
SpaQ	SsaS	Spa9	YscS	–	EscS	FliQ	inner membrane	export apparatus
SpaR	SsaT	Spa29	YscT	–	EscT	FliR	inner membrane	export apparatus
SpaS	SsaU	Spa40	YscU	–	EscU	FlhB	inner membrane	export apparatus
InvG	SpiA	MxiD	YscC	PscC	EscC	–	outer membrane	needle complex
PrgH	–	MxiG	–	–	–	–	inner membrane	needle complex
PrgK	SsaJ	MxiJ	YscJ	PscJ	EscJ	FliF	inner/outer membrane	needle complex
InvH	–	MxiM	VirG	–	–	–	outer membrane	secretin pilot
IacP	–	OrfX	–	–	–	–	cytoplasm	acyl-transferase
IagB	–	IpgF	–	–	Rorf3	FlgJ	cytoplasm	muramidase

Energizer of the secretion process

An essential component of all type III systems is a protein that exhibits sequence similarity to the β subunit of F_0F_1 ATPases. The *S. typhimurium* homolog, InvC, has been shown to possess ATPase activity [43]. Like the other members of this family, InvC contains putative A and B Walker boxes (sequence motifs commonly found in ATP-binding proteins). A site-directed mutation which changes a conserved lysine residue in Walker box A (Lys-165) to a glutamate results in a loss of function in this protein [43]. In addition, a deletion of a putative Walker box in the *Yersinia enterocolitica* homolog YscN also leads to a loss of function of this protein [44]. Hydrolysis of ATP by SecA is required for the translocation of bacterial proteins through the inner membrane by the *sec* pathway [45]. Likewise, the homologous flagellar assembly protein FliI possesses ATPase activity which is required for the export and assembly of flagellar subunits [46]. The function of the InvC family of proteins may therefore be to couple ATP hydrolysis to the transport of invasion-related proteins across the bacterial membrane and into the host cell.

Inner membrane-localized secretion components

Several proteins have been identified in all type III systems that, based on their secondary structure, are predicted to be located in the inner membrane. In *Salmonella*, these include InvA [47], SpaP, SpaQ, SpaR and SpaS [11]. Homologs of these proteins are found not only in type III secretion systems but also in the flagellar assembly systems of various bacteria [48–50], and in all cases, expression of these homologs is required for secretion. The InvA homologs are predicted to be integral membrane proteins with eight amino-terminal transmembrane domains and a hydrophilic carboxyl-terminal region [8]. The carboxyl terminus is thought to reside in the cytoplasm, where it may interact with other components of the system. The functional conservation between InvA and the *Shigella* homolog MxiA was demonstrated by the ability of the *Shigella* protein to restore invasiveness to a *Salmonella* strain carrying a loss-of-function mutation in *invA* [51]. Whereas the *Yersinia* homolog, LcrD, could not complement the invasion defect of this *Salmonella* mutant, a chimeric protein consisting of the amino terminus of LcrD and the carboxyl terminus of InvA could complement the defect [51]. This finding indicates that the carboxyl-terminal regions of these proteins may contain determinants of species specificity.

The SpaP, SpaQ, SpaR and SpaS proteins contain potential membrane-spanning hydrophobic domains [11]. Data derived from the study of homologous proteins in other systems have helped to rationalize the placement of these proteins in the inner membrane. Protein fusion experiments have indicated that the *Caulobacter crescentus* proteins FliQ and FliR, which are homologous to SpaQ and SpaR and which are required for both the assembly of flagella and cell division, are likely to be membrane-associated [49]. Epitope-tagging of the homologous FliP and FliR proteins of the *S. typhimurium* flagellar assembly system indicate that these proteins are actually embedded in the inner membrane exposed face of the hook-basal body (HBB) structure [52]. In addition, studies in *Yersinia pestis* indicate that the SpaP homolog YscR is an integral membrane protein with at least four membrane-spanning domains [53]. Homologs of these proteins have been found in the type III secretion systems of both plant and animal pathogens, and in many cases the sequences of these proteins are very similar [1]. The similarities between the *Salmonella* and *Shigella* homologs extend to functional complementarity, as

demonstrated by the finding that the invasion defect of a *S. typhimurium spaP* mutant could be complemented by the homologous *Shigella* protein Spa24 [11].

The needle complex

The discovery of the *S. typhimurium* needle complex has helped to shed light on the mechanism by which substrates of type III secretion systems traverse the bacterial envelope [42]. The complex appears to be embedded in both the inner and outer membranes, spanning the periplasmic space, and thus allows for the direct transport of proteins from the bacterial cytosol to the external surface in a single step (fig. 1). The ultrastructure of this complex closely resembles that of the flagellar HBB structure, lending further support to an evolutionary relationship between the flagellar and type III systems. The needle complex consists of two upper (or outer) and two lower (or inner) rings connected by a short rod. The lower rings, which interact with the cytoplasmic membrane, are 40 nm in diameter and 20 nm wide, whereas the upper rings, which appear to interact with both the outer membrane and the peptidoglycan layer, are 20 nm in diameter and 18 nm wide. Extending from the surface of the structure is a stiff, straight tube (the needle), 80 nm long and 13 nm wide, which appears to be hollow. Similar structures have recently been visualized in the membranes of *S. flexneri* [54].

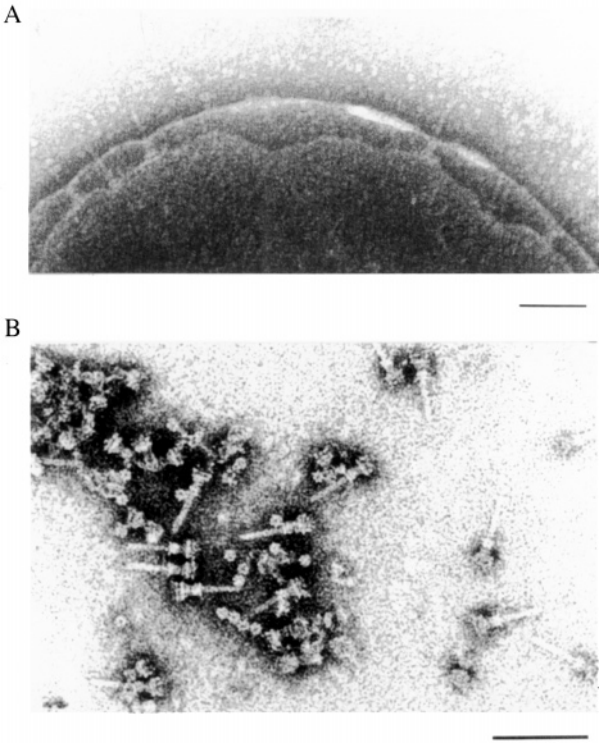

Figure 1. Needle complex of the *S. typhimurium* type III secretion system. (*A*) Electron micrograph of osmotically shocked *S. typhimurium* exhibiting needle complexes in the bacterial cell envelope. (*B*) Electron micrograph of purified needle complexes. Scale bar, 100 nm.

Biochemical analysis of purified *S. typhimurium* needle complexes revealed that they are composed of at least three proteins (InvG, PrgH and PrgK) which are predicted to be located in the bacterial membrane [42]. One of these proteins, InvG, is a member of a family of proteins collectively known as secretins. These proteins, exemplified by the *Klebsiella oxytoca* protein PulD—which is a component of a type II secretion system [4]—have been shown to be associated with systems involved in DNA uptake, type IV pili assembly and filamentous bacteriophage extrusion [55]. Secretins isolated from different bacteria and associated with various systems have been shown to form outer membrane-localized complexes which are often resistant to denaturation with SDS [56, 57]. Examination of these complexes by electron microscopy has shown that they form ring-shaped structures with a central channel and an outer diameter of 15–20 nm, similar to that of the outer rings of the *S. typhimurium* needle complex [12, 58, 59]. Members of the type III family of secretins include MxiD in *Shigella* [60], YscC in *Yersinia* [58] as well as the predicted products of the LEE-encoded *escC* gene [35], the *P. aeruginosa pscC* gene [24] and the *Salmonella* SPI-2-encoded *spiA* gene [16, 17].

The other two identified components of the needle complex are PrgH and PrgK. Both of these proteins are predicted to be lipoproteins based on the presence of amino-terminal bacterial lipoprotein-processing sites [61]. Like all bacterial outer membrane proteins, PrgH and PrgK (as well as InvG) carry amino-terminal hydrophobic domains which are normally recognized and cleaved by signal peptidase upon export to the periplasm by the *sec* pathway. Surprisingly, amino-terminal sequencing of the isolated needle complex components has shown that the signal sequence of PrgH is not cleaved, suggesting that it may remain associated with the inner membrane [42]. While homologs of both PrgH and PrgK have been identified in the *Shigella* system (MxiG and MxiJ) [62, 63], the type III systems of the other human pathogens only contain PrgK homologs [1], suggesting that the complexes formed by these pathogens may differ from the *S. typhimurium* needle complex. In addition, the sequence of a component of the flagellar basal body structure, FliF, is similar to that of PrgK [64], indicating that these two structures are indeed evolutionarily related.

Another protein which has been implicated in playing a role in the assembly of the needle complex is InvH. This protein was first identified as being involved in the

attachment of bacteria to the surface of host cells [30]. It was later shown that strains deficient in expression of InvH were unable to induce fluid accumulation and neutrophil transmigration in a ligated ileal loop model [65]. More recently, two different groups have demonstrated that InvH is required for proper membrane localization of InvG [59, 66]. Mutants unable to express InvH mislocalized InvG to the inner membrane, where it was subject to proteolytic degradation. This proposed role for InvH is analogous to that proposed for the PulS family of proteins [56]. These predicted lipoproteins, which tend to exhibit limited degrees of sequence similarity, are thought to be required for the proper localization of their cognate secretin. While these proteins were originally thought to act as chaperones, it is now believed that they may actually be attached to the secretins as part of a larger multimeric complex. This has recently been shown to be the case with PulS [67]. Lipoproteins in other type III secretion systems which have been shown to play roles similar to InvH include MxiM in *Shigella* [68] and VirG in *Yersinia* [58].

Accessory proteins

In addition to the structural components of the secretion apparatus, two proteins encoded in the SPI-1 region of the *S. typhimurium* chromosome are thought to play accessory roles in the assembly of the apparatus. These proteins, which are located in the bacterial cytoplasm, appear to be involved in the enzymatic modification of either components of the secretion apparatus or the region of the bacterial envelope in which the apparatus is assembled. The *Salmonella* protein IacP belongs to the family of acyl carrier proteins and may therefore be involved in the posttranslational modification of secreted proteins [69]. Modifications of secreted proteins have been observed in other systems, including the type I hemolysin secretion system [70]. However, the target(s) of IacP may be a component of the secretion apparatus itself, since PrgH and PrgK both contain potential lipidation sites [61], and it has been shown that InvH is lipidated in vivo [66]. In *Shigella*, the product of a gene located upstream of *ipaA* shares sequence homology with IacP [71].

The other *Salmonella* protein which is thought to assist in the formation of the secretion apparatus is IagB [72]. This protein, along with the *Shigella* homolog IpgF [73] and a protein encoded by rorf3 in the LEE of EPEC spp. [35], is homologous to a family of predicted glycosidases [74]. These proteins, which are commonly associated with the assembly of supramolecular membrane structures, are thought to function as peptidoglycan-processing enzymes. The *S. typhimurium* flagellar assembly homolog FlgJ, which is essential for flagella formation, has recently been shown to possess peptidoglycan-hydrolyzing activity [75]. Similarly, the type III homologs are thought to be involved in the localized modification of the peptidoglycan layer in order to facilitate the insertion of the secretion apparatus. Whereas it has been shown that, in the case of *Shigella*, this protein is not required for secretion [73], it is likely that homologous proteins encoded elsewhere in the bacterial chromosome and required for other tasks (such as cell division) are able to partially compensate for the loss of this protein. This hypothesis is strengthened by the finding that the initial secretion of Ipa proteins in *Shigella* occurs predominantly at the septation furrows of dividing cells [76].

Effector proteins and their mechanisms of action

As previously stated, the phenotypes associated with the type III systems of different pathogens vary considerably. This is due in large part to the different types of proteins secreted by these different bacteria. However, despite the fact that each bacteria secretes an array of effectors which provoke a pathological outcome unique to that particular organism, many of these effectors either perform similar functions in the host cells or target similar host cell proteins (table 3).

Formation of surface appendages

The induction of many type III secretion systems has been shown to be accompanied by the formation of appendages on the surface of the induced pathogens. Contact with cultured epithelial cells results in the transient formation of appendages (invasomes) on the surface of *S. typhimurium* [77]. Mutant strains with defective SPI-1 systems lacked invasomes, suggesting that the formation of these structures is dependent on a functional SPI-1 type III system. Another study has demonstrated cell contact-dependent formation of appendages on *S. typhimurium* which is independent of a functional SPI-1 system [78]. It is possible that the structures identified in the second study were not the so-called invasomes since these structures were observed 60 min after infection at a time when the transiently formed invasomes would have already disappeared from the bacterial surface [77]. However, it is possible that the formation of the invasomes is not entirely dependent on a functional SPI-1 type III system. In vitro studies have shown that *Shigella* spp. form sheet-like surface structures which are composed of secreted type III proteins [79]. It is not known whether these structures play a role in the pathogenesis of *Shigella*, since their formation has never been observed when this pathogen makes contact with host cells. The surface

Table 3. Type III effectors and their mechanisms of action.

Protein	Biochemical function	Effect on host cells
Salmonella (SPI-1)		
SipA	binds plastin and actin/promotes actin bundling	promotes localized cytoskeletal rearrangements
SipB	activation of caspase-1	induces apoptosis
SipC	actin nucleation	localizes cytoskeletal rearrangements
SopB	inositol phosphate phosphatase	promotes cytoskeletal rearrangements/promotes Cl^- secretion
SopE	GTPase exchange factor	promotes cytoskeletal rearrangements/activates MAP kinase pathway
SptP	tyrosine phosphatase/GTPase activating protein	rebuilds cytoskeleton
Salmonella (SPI-2)		
SpiC	unknown	inhibits phagosome-lysosome fusion
Shigella		
IpaA	binds vinculin/promotes actin depolymerization	stimulates bacterial entry
IpaB	activation of caspase-1	induces apoptosis
IpaC	unknown	stimulates bacterial entry
IpgD	putative inositol phosphate phosphatase	unknown
Yersinia		
YopE	putative GTPase-activating protein	disrupts cytoskeleton/antiphagocytosis
YopH	tyrosine phosphatase	disrupts cytoskeleton/antiphagocytosis
YopJ/P	inhibition of MAP kinase kinase activity	induces apoptosis
YpkA/YopO	serine/threonine kinase	unknown
P. aeruginosa		
ExoS	ADP-ribosyl transferase/putative GTPase-activating protein	disrupts cytoskeleton/antiphagocytosis
ExoT	ADP-ribosyl transferase	cytotoxicity
ExoU	unknown	cytotoxicity
ExoY	adenylate cyclase	cytotoxicity
PpkA	serine/threonine kinase	unknown
EPEC		
Tir	intimin receptor	formation of actin pedestals

appendages of EPEC spp., which are composed of the secreted protein EspA, form a bridge between the bacterium and the host cell surface, and the assembly of these structures is required for the translocation of EspB into infected epithelial cells [80]. Similar appendages have been shown to be produced by the type III systems of many plant pathogenic bacteria [2], suggesting that these appendages represent a general feature of all type III systems. Although the exact role played by these structures in the infection process is not fully understood, they may represent a mechanism by which effector proteins are delivered to the surface of the host cells.

Changes in host phosphorylation patterns

In eukaryotic cells, the regulated phosphorylation and dephosphorylation of proteins and lipids in response to specific stimuli forms one component of the signal transduction pathways that control various cellular functions. A number of type III effectors of different human pathogens have been shown to influence host cell signaling by specifically altering host protein and lipid phosphorylation patterns. The *Salmonella* effector SopB, which is involved in the induction of fluid secretion and inflammation in infected ileal mucosa [81], has been shown to act as an inositol phosphate (InsP) phosphatase. Purified SopB exhibits phosphatase activity, hydrolyzing several inositol phospholipids and polyphosphates [82]. Inositol phospholipids play key regulatory roles in many cellular processes, including actin cytoskeletal rearrangements, activation of transcription factors and modulation of chloride secretion [83]. Thus, by altering the levels of these phospholipids in host cells, SopB is able to provoke substantial changes in host cell signaling. The *Shigella* type III effector IpgD shows significant sequence similarity to SopB [73], and it is possible that the *Shigella* protein also acts as an InsP phosphatase, although no such activity has yet been observed.

The type III effectors YopH and SptP have been shown to act as protein tyrosine phosphatases (PTPases) [84, 85]. YopH, together with YopE (see below), is responsible for the cytoskeletal disruption and antiphagocytosis associated with the *Yersinia* system. The PTPase activity of YopH, which has been shown to induce the dephosphorylation of $p130^{cas}$ and focal adhesion kinase (FAK), is essential for the pathogenicity of *Y. pseudotuberculosis* [86, 87]. Strains expressing catalytically inactive forms of YopH were found to be avirulent, due most likely to a loss of the ability to resist phagocytosis [22]. The target of the *S. typhimurium* tyrosine phos-

phatase has not been identified, and it is not yet known what role the PTPase activity of SptP plays.

The *Yersinia* effector YpkA/YopO and the *P. aeruginosa* effector PpkA are homologous to eukaryotic serine/threonine protein kinases. Strains carrying loss-of-function mutations in the genes encoding these proteins display diminished virulence compared with wild-type strains [88, 89]. However, no target proteins for these kinases have been identified. Whereas many of the type III secretion-induced changes in host phosphorylation patterns may similarly be attributed to the enzymatic activity of specific effector proteins, in some cases these responses may be induced by a less direct mechanism. Although contact with EPEC spp. results in the tyrosine dephosphorylation of several host cell proteins, no tyrosine phosphatase activity has been detected in EPEC-secreted proteins [28], suggesting that this pathogen induces dephosphorylation by an indirect mechanism.

Interactions with small GTP-binding proteins

One common theme observed among the effector proteins of different pathogens is the targeting of small GTP-binding proteins (GTPases) in host cells. These eukaryotic proteins alternate between an active GTP-bound form and an inactive GDP-bound form and function as molecular switches. By cycling between the two forms, these proteins are able to regulate signaling pathways that lead to cellular processes such as cytoskeletal rearrangements and cell cycle progression as well as nuclear and mitogenic responses [90]. It has been shown that, in the case of *S. typhimurium*, type III-associated invasion is dependent on the activity of members of the Rho subfamily of GTPases, including Cdc42 and Rac-1 [91]. Studies have shown that a number of *Salmonella* effector proteins exert their influence on host signal transduction pathways by interacting directly with GTPases. By stimulating GDP/GTP exchange in Cdc42 and Rac-1, SopE activates these GTPases, which in turn stimulates cytoskeletal reorganization and JNK kinase activation, ultimately leading to nuclear responses [92]. More recent work has shown that SptP functions as a GTPase-activating protein (GAP) and serves to downregulate the SopE-induced activation of Cdc42 and Rac, thus helping to rebuild the host cytoskeleton [93].

Another type III effector which has been shown to interact with host GTPases is the *P. aeruginosa* protein ExoS. This protein is composed of two separate domains which each appear to interact with GTPases in different ways. The carboxyl-terminal domain of ExoS has been shown to be responsible for the adenosine diphosphate (ADP)-ribosyltransferase activity of this protein. Both in vitro and in vivo studies have shown that ExoS ADP ribosylates a number of targets, including members of the Ras family of GTPases [26]. In addition, the amino-terminal domain of ExoS has recently been shown to be responsible for the induction of changes in host cytoskeletal dynamics by acting on host GTPases [94]. Whereas the exact mechanism by which this domain of ExoS influences the state of host GTPases is not yet known, the similarity of this domain with the domain of SptP containing the GAP activity would suggest that the *P. aeruginosa* protein is also acting as a GAP. The *Yersinia* effector YopE bears sequence homology with the amino-terminal domains of SptP and ExoS [85], and like both these proteins is also associated with actin depolymerization [95], suggesting that this effector also exerts its influence on host cells by activating GTPases.

The ability of *S. flexneri* to invade epithelial cells is also dependent on the activity of members of the GTPase family, including Rho, Rac and Cdc42 [96, 97]. However, no *Shigella* effector protein has yet been shown to interact directly with host cell GTPases. Similarly, no EPEC effector protein has yet been shown to interact directly with host GTPases. However, a recent study has shown that a novel GTPase plays an important role in the formation of EPEC-induced pedestals [98].

Interactions with host cytoskeletal proteins

The ability to modulate the host cytoskeleton is a prominent feature of pathogens which possess type III systems, and it is not surprising that these organisms utilize a number of different mechanisms to achieve this goal. While many of the signaling events elicited by different type III effectors ultimately result in a reorganization of the host cytoskeleton, it is now known that certain effectors are able to cause these changes by interacting directly with components of the cytoskeleton. By binding to both actin and the actin-binding protein plastin, the *S. typhimurium* protein SipA is able to decrease the critical concentration of actin required for polymerization, inhibit the depolymerization of F-actin and increase the actin-bundling activity of T-plastin [99, 100]. These activities of SipA are thought to be responsible for the spatial localization and pronounced outward extension of *Salmonella*-induced membrane ruffles. Another *Salmonella* protein, SipC, has been reported to bind and nucleate actin at the site of bacterial contact [101]. Thus, by working cooperatively, SipA and SipC are able to effect dramatic changes in the organization of the host cytoskeleton independent of other signaling mechanisms.

The *Shigella* proteins IpaA and IpaC bear sequence similarity to the *Salmonella* SipA and SipC proteins, respectively. IpaA is thought to aid in the formation of focal-adhesion-like structures at the point of bacterial-

host contact by binding to vinculin [102]. A recent study has shown that the vinculin-IpaA complex appears to induce the depolymerization of actin filaments [103]. Mutants deficient in the expression of IpaA demonstrate a reduced ability to invade host epithelial cells. IpaC has been reported to induce actin polymerization and filopodia formation in a semipermeabilized cell model [104]. However, it is not yet known if IpaC induces these changes by directly interacting with components of the host cytoskeleton.

Another type III effector which may interact with the host cytoskeleton is the Tir protein of EPEC spp. This protein, which was originally thought to be a component of the host cell membrane, serves as a receptor for intimin, another protein secreted by the EPEC type III system [105]. Upon binding to intimin, Tir triggers host signaling events and actin nucleation [106]. Although strains lacking Tir are unable to localize actin beneath adherent bacteria, it is not known whether this effector directly interacts with actin or any other component of the host cytoskeleton.

Induction of apoptosis in host macrophages

Another phenotype associated with the type III secretion system of *Salmonella* which is common to the systems of other human pathogens is the induction of apoptosis in infected macrophages. This phenomenon is characterized by chromosomal fragmentation, membrane blebbing and the presence of apoptotic bodies in the cell cytosol. Recent studies have implicated SipB as playing a role in *Salmonella*-induced apoptosis of macrophages [107]. The cell death resulting from microinjection of macrophages with purified SipB was shown to be due to the activation of the proapoptotic protease caspase-1 (ICE). This postulated role for SipB is analogous to that proposed for the *Shigella* effector IpaB, which is also thought to initiate apoptosis in infected macrophages by activating caspase-1 [108]. In *Yersinia* spp., the induction of apoptosis is dependent on the function of the type III effector YopJ/YopP. This effector has been shown to be responsible for the inhibition of tumor necrosis factor α (TNF-α) production and the downregulation of mitogen-activated protein (MAP) kinase activity in *Yersinia*-infected macrophages [109]. It has recently been shown that YopJ effects these changes by blocking the activation of host MAP kinase kinases (MKKs) [110]. YopJ shares sequence similarity with other type III effectors, such as AvrA in *S. typhimurium* [111] and AvrRxv in the plant pathogen *Xanthomonas campestris* [112]. This homology constitutes the only example of a family of type III effectors which includes members of both animal and plant pathogens. This is of particular significance since AvrRvx is required for the initiation of the hypersensitive response in infected plants which involves the induction of apoptosis [113]. It is not yet known whether the sequence similarity displayed among these different effectors is indicative of a conserved mechanism by which these proteins coopt host signaling pathways to induce apoptotic events. More recently, the type III system of *P. aeruginosa* has been shown to mediate apoptosis of both macrophages and epithelial cells [114]. This phenotype is independent of the previously identified ExoU-mediated necrosis of epithelial cells.

Table 4. Proteins that control various aspects of type III secretion.

System	Protein
Gene expression	
Salmonella (SPI-1)	HilA, InvF
Salmonella (SPI-2)	SpiR
Shigella	VirF, VirB
Yersinia	VirF, LcrQ
P. aeruginosa	ExsA
EPEC	PerA, Ler
Secretion	
Salmonella (SPI-1)	SipB, SipD, InvJ, SpaO
Shigella	IpaB, IpaD
Yersinia	YopN, LcrG
EPEC	Pas
All systems	chaperones
Translocation	
Salmonella (SPI-1)	SipB, SipC
Shigella	IpaB, IpaC
Yersinia	YopB, YopD, LcrV
P. aeruginosa	PopB, PopD, PcrV
EPEC	EspB, EspD

Proteins that mediate translocation

A number of proteins secreted by type III systems have been shown to be responsible for mediating the translocation of effector proteins into the cytosol of host cells (table 4). These include SipB and SipC in *Salmonella* [115], IpaB and IpaC in *Shigella* [20], YopB and YopD in *Yersinia* [22] and PopB and PopD in *P. aeruginosa* [23]. Although the translocation process is not well understood, studies in *Yersinia* have helped to gain some insight into this aspect of type III secretion. The *Yersinia* proteins YopB and YopD have been shown to be required for the translocation of the effector Yop proteins [22]. These two proteins interact prior to secretion and are thought to form an extracellular complex, although this has not yet been demonstrated. The sequence of YopB is similar to that of SipB, IpaB and PopB in *Salmonella*, *Shigella* and *P. aeruginosa*, respectively, as well as the sequences of the LEE-encoded EspB and EspD proteins [35]. All of these proteins contain a hydrophobic domain which shares homology

with the RTX family of pore-forming toxins such as HlyA of *E. coli*. This homology suggests that these type III substrates may serve to form pores in the membranes of host cells, allowing the entry of effector proteins into the cell cytosol. This hypothesis is supported by the findings that *Yersinia* possesses a YopB-dependent hemolytic activity and that purified YopB possesses the ability to disrupt lipid bilayers [116]. YopD also has a potential membrane-spanning domain, and it may be that both YopB and YopD interact in the host cell membrane to form a translocation pore. However, studies have shown that YopD is actually translocated into the host cell cytosol [117], suggesting that it may also act as an effector. It has been shown that expression of the *P. aeruginosa* proteins PopB and PopD in a *Y. pseudotuberculosis yopB/yopD* mutant restored the contact-dependent cytotoxicity of this strain [118]. This finding demonstrates that not only are the type III systems of these two pathogens closely related but the mechanism of effector translocation is functionally conserved at the molecular level.

The *Shigella* secreted proteins IpaB and IpaC are absolutely required for the efficient entry of these pathogens into cultured epithelial cells [119]. These two proteins are similar to the *Salmonella* SipB and SipC proteins, which are also required for entry [33]. Studies on the *Shigella* homologs have shown that IpaB and IpaC form a complex in the extracellular medium. Latex beads coated with this Ipa complex were efficiently internalized into HeLa cells, and this internalization was accompanied by membrane ruffling and actin polymerization similar to that associated with bacterial invasion [120]. Although the Ipa proteins appear to be the major proteins secreted by the *Shigella* type III system, the role of IpaB and IpaC may be, like YopB and YopD, to form a pore in the host cell membrane in order to promote the entry of IpaA or other as yet unidentified *Shigella* effectors into the host cell. It is believed that the *Salmonella* homologs SipB and SipC, which have also been shown to form a complex [101] [S. Tucker and J. E. Galán, unpublished results], are involved in the delivery of the effector proteins into the cytosol of host cells. However, these proteins also play roles as effectors (see above), and both SipB and SipC have been shown to be translocated into host cells [121].

The EspB and EspD proteins of EPEC spp. have been implicated in playing a role in the translocation of effector proteins into host cells. EspB has been shown to be translocated into the host cell cytosol [122], whereas EspD appears to localize to the membrane of host cells [123].

Recent studies have shown that the *Yersinia* secreted protein LcrV may also play a role in the translocation process. This protein was previously shown to be involved in the expression and secretion of Yop proteins [124]; however, bacteria treated with antibodies directed against LcrV were unable to translocate effector Yops into host cells, suggesting that this protein is also involved in the translocation process [125]. The only known type III homolog of LcrV is the *P. aeruginosa* protein PcrV [23]. Expression of the *Pseudomonas* protein in a *Y. pseudotuberculosis lcrV* mutant restored the wild-type phenotype [125], reaffirming the close evolutionary relationship between the type III systems of these two pathogens.

Secretion signals and substrate recognition

Type III systems make use of several strategies to target substrates to the membrane-bound secretion apparatus. These include signal sequences encoded within the messenger RNA (mRNA) transcripts of the secreted proteins and intracellularly located chaperone proteins. In addition, a number of proteins, which are themselves secreted, have been shown to control the secretion of effector proteins.

mRNA secretion signals

Experiments with the secreted *Yersinia* proteins YopE and YopN, and more recently YopQ, have demonstrated that translation and secretion of these proteins may be coupled, and that the signals controlling both processes reside in the mRNA [126, 127]. Frameshift mutations in these putative mRNA signal sequences, which resulted in completely different polypeptide sequences, did not prevent secretion through the type III apparatus. The 5′ mRNA regions of the *yop* transcripts are predicted to form stem-loop structures which bury the AUG translational start codon, suggesting that the translation of these transcripts is arrested until the 5′ mRNA is able to interact with a component of the secretion machinery. Studies on YopQ have shown that this protein cannot be secreted posttranslationally, suggesting that the translation and secretion of this protein are coupled [127]. While it is not yet known whether the expression of proteins secreted by the type III systems of other human pathogens are similarly regulated at the translational level, the synthesis and secretion of proteins by the type III system of the plant pathogen *P. syringae* may also be regulated by signals which reside in the 5′ mRNA [2], suggesting that this type of regulation may be a common feature of all type III systems.

Type III chaperones

Whereas it appears that the mRNA signal may be a common mechanism used by *Yersinia* spp. to target Yop proteins to the secretion apparatus, it is unlikely

that either *Salmonella* or *Shigella* spp. make use of this mechanism to secrete many of their type III proteins: these pathogens are still able to invade cultured epithelial cells in the presence of chloramphenicol, indicating that de novo protein synthesis is not required [77, 128]. The type III systems of these pathogens must therefore employ a strategy to maintain an intracellular stockpile of secreted proteins which, upon contact with host cells, is released into the extracellular environment. One such strategy involves the function of chaperone proteins. These chaperones, which specifically interact with one or a small subset of the secreted substrates, are small (15–20 kDa), acidic proteins and often contain a carboxyl-terminal domain which is predicted to form an α helix [129]. The genes encoding the chaperones are commonly located adjacent to the genes encoding their target protein. The *S. typhimurium* chaperone SicA (SipE in *S. typhi*), which is encoded directly upstream of SipB, is required for the secretion of SipB and SipC [33, 130]. Mutations in *sicA* prevent the secretion of these proteins and also result in an inability of the bacteria to invade cultured epithelial cells [33]. Studies of the homologous *Shigella* chaperone IpgC indicate that this protein serves as a partitioning factor for IpaB and IpaC [131]. The IpaB and IpaC proteins form a complex in the extracellular environment; however, premature interactions between these two proteins within the bacterial cytoplasm result in the degradation of both proteins. By independently binding to IpaB and IpaC, IpgC prevents these proteins from interacting until they are secreted, and thus protects them from degradation [131]. Similarly, *Yersinia* mutants which do not express SycD/LcrH are unable to secrete YopB and YopD and are unable to translocate effector proteins into host cells. Other examples of type III chaperones include SicP in *Salmonella* [132], SycE and SycH in *Yersinia* [133] and SpcU in *P. aeruginosa* [134].

In addition to their roles as stabilizing and partitioning factors, type III chaperones also appear to function as secretion pilots which aid in the delivery of their cognate protein to the secretion machinery once the appropriate signal is received. This activity is demonstrated by the residual ability of certain effector proteins in *Yersinia* to be secreted in a chaperone-dependent manner despite the deletion of the 5′ mRNA secretion signal [135]. Binding of the chaperones to their secreted target proteins occurs at discrete domains located within the first 100 amino acids, and deletion of these domains alleviates the requirement of a chaperone for secretion, although it also prevents translocation of the protein into host cells. Since these chaperones have never been shown to interact directly with components of the secretion machinery, it is not known how they are able to fulfill their postulated roles as secretion pilots. Nevertheless, the chaperone-dependent pathway appears to be an important mode by which type III proteins are guided to the membrane-localized secretion apparatus.

Secreted proteins that control secretion

The secretion of effector proteins by type III systems has been shown to be controlled, in part, by proteins which themselves are secreted by these systems (table 4). Two secreted *Salmonella* proteins which are required for secretion through the type III system are InvJ and SpaO. Mutant strains which do not express these proteins are unable to secrete any of the other targets of the secretion apparatus [136]. These proteins are encoded within the highly conserved *inv/spa* locus, but they share little sequence homology with components of other type III systems, and may therefore be involved in functions unique to the *Salmonella* system. Other secreted *Salmonella* proteins which play a role in secretion are SipB and SipD. These proteins are homologous to the *Shigella* proteins IpaB and IpaD. Additionally, SipB also shares sequence homology with the *Yersinia* protein YopB. Loss-of-function mutations in *sipD* and *sipB* led to increased secretion of a subset of the secreted proteins [69]. These findings are similar to the results of studies on IpaD and IpaB. It is not known how these proteins modulate the secretion process, but studies in *Shigella* indicate that IpaB and IpaD may interact to form a complex in the bacterial outer membrane and that this complex serves to block secretion until the proper signal is received [119]. Whereas this model may explain the situation in *Shigella*, it does not explain why, in *Salmonella*, the secretion of InvJ is unaffected in a strain carrying a loss-of-function mutation in *sipD* [115].

In the case of *Yersinia*, the secreted proteins YopN and LcrG appear to participate in the regulation of the secretion process. A loss-of-function mutation in either of the genes encoding these proteins results in uncontrolled secretion, similar to that shown for SipD/IpaD mutants [137, 138]. It is thought that, by interacting with proteoglycans located on the surface of host cells, LcrG is able to sense the presence of the host cell and can then transmit a signal to the secretion apparatus. YopN is similar to the *Salmonella* SPI-1-encoded protein InvE. Whereas the *Salmonella* homolog has been shown to be an indispensable component of the SPI-1 system [139], the fact that InvE is located in the bacterial cytosol and YopN has been localized to the outer membrane argues that these proteins must function differently.

Transcriptional regulation of type III secretion systems

The assembly of the type III secretion apparatus along with the coordinated synthesis and secretion of substrate proteins represents a complex biological process which is undoubtedly energetically taxing to the bacteria. It is therefore not surprising that pathogens have evolved intricate mechanisms with which to regulate the expression of type III systems. Whereas a number of studies have helped to shed light on the regulation of the *S. typhimurium* system, this remains one of the least-understood aspects of type III secretion in *Salmonella*. Regulation of the *S. typhimurium* system is accomplished by a number of specific transcription factors as well as by components of global regulatory networks that respond to environmental cues such as temperature, osmolarity, pH and oxygen tension (table 4). Type III specific regulatory proteins include HilA and InvF. HilA, which shares sequence similarity with the DNA binding domain of the OmpR/ToxR transcriptional regulatory family of proteins [140], activates expression of genes encoding both structural components and secreted substrates of the type III secretion apparatus [141]. HilA also activates expression of InvF, a member of the AraC family of regulatory proteins [142]. InvF has been shown to positively regulate the expression of genes encoding secreted proteins which are located both inside and outside of the SPI-1 region [141, 143]. Expression of *S. typhimurium* type III-associated genes is also indirectly influenced by a number of global regulatory systems, including the PhoP/PhoQ and RcsB/RcsC two-component regulatory systems [61, 144], the flagellum-associated σ factor FliA [145], the SirA response regulatory system [146], DNA adenine methylase [147] and DNA topoisomerase I [148]. These multiple regulatory mechanisms afford *Salmonella* the ability to tightly control expression of the SPI-1 system in response to changes in the environment.

Like the *Salmonella* system, the type III systems of other bacterial pathogens are also subject to complex regulatory mechanisms. Whereas these mechanisms include certain common features, such as the presence of AraC-like transcriptional activators (VirF in *Shigella* and *Yersinia* [149, 150], PerA in EPEC [151]) and the ability to respond to changes in growth temperature, each pathogen appears to regulate its system in a unique manner. The transcriptional regulation of these other systems has been discussed elsewhere [1, 22] and will not be addressed in this review.

Summary

The invasion-associated type III secretion system of *S. typhimurium* confers upon this pathogen the ability to engage in complex biochemical interactions with host cells that ultimately lead to the internalization of the invading bacteria. Although the phenotypes associated with the *Salmonella* system vary considerably from those associated with the systems of other pathogens, it is becoming clear that the essential components required for the secretion and translocation of effector proteins are broadly conserved. In the past 8 years since their existence was first proposed, much has been learned about type III secretion systems. However, many important questions still remain unanswered. How is the mRNA secretion signal recognized by the secretion apparatus? How do the chaperones target the secreted proteins to the apparatus? How are the effectors translocated into the cytosol of host cells? How does contact with host cells activate these systems? Future studies of the type III secretion systems of *S. typhimurium* and other pathogens should provide the answers to many of these questions. Research in this field will also likely provide new knowledge for the development of novel vaccines and therapeutic strategies as well as insights into many basic aspects of cell biology.

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