INTERNATL MICROBIOL (1998) 1:197–204

RESEARCH ARTICLES

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Molecular mechanisms of *Salmonella* invasion: the type III secretion system of the pathogenicity island 1

Received 11 February 1998
Accepted 15 May 1998

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Summary *Salmonella* spp. are facultative intracellular pathogens which are able to enter into non-phagocytic cells as an essential step in their pathogenic life cycle. The majority of the molecular determinants involved in this entry process are encoded in a pathogenicity island located at the centisome 63 of the bacterial chromosome, and belong to a specialized protein secretion system termed “type III” or “contact-dependent”. This secretion system is used by *Salmonella* spp. and several other bacterial pathogens to translocate bacterial effector proteins into the eukaryotic cell. Thus, a bidirectional biochemical cross-talk with the host cell is initiated, which leads to several responses such as membrane ruffling, bacterial internalization and the activation of various transcription factors.

Key words *Salmonella* · Virulence · Protein secretion · Bacterial invasion · Intracellular

Introduction

A great number of animal species, including humans, can be infected by different *Salmonella* serotypes. Some of them are highly host-adapted for example, *Salmonella typhi*, *Salmonella gallinarum* and *Salmonella typhi-suis*, which specifically infect humans, poultry and pigs, respectively. In contrast, other serotypes, like *Salmonella enteritidis*, can infect a broad range of hosts. The type of disease caused by *Salmonella* depends on the serotype, the infected species and the immunological status of the host. The clinical manifestations of salmonellosis range from a mild gastroenteritis to a severe systemic infection [25].

Salmonella is transmitted to animals and humans by contaminated water or food [40]. There is little information available about the interaction of *Salmonella* spp. and host cells in vivo. In pigs, *Salmonella typhimurium* was found within absorptive mucosal cells and mucosa-associated macrophages of the small intestine and colon. The role of M-cells in the entry process of *Salmonella* spp. into the intestinal tract is still controversial. There is evidence that at least some *Salmonella* serotypes use M-cells as an entry port to invade deeper tissues of the intestine [14, 50]. After adhesion and invasion of epithelial cells, bacteria can proliferate intracellularly, residing in endocytic vacuoles.

During adherence, bacteria trigger a complex set of signaling events in the host cell which cause cytoskeletal rearrangements, and concomitant changes in the morphology of the host plasma membrane, ultimately leading to the entry of *Salmonella* into the eukaryotic cell. Bacterial invasion correlates with the appearance of transient *Salmonella*-surface appendage-like structures termed “invasomes” [36], modifications of the eukaryotic cell-surface similar to pseudopodia termed “membrane ruffling” [25], massive uptake of extracellular fluid in the form of macropinosomes [34], formation of filamentous endocytic vacuoles that contain lysosomal membrane glycoproteins [33], and the intracellularly replicating bacteria. *Salmonella* invasion-defective mutants are unable to induce these responses in host cells [29, 33]. The biochemical bases of these morphological changes are the accumulation of a number of cytoskeletal-associated proteins such as actin, α-actinin, talin and erzin [24, 26], a marked increase in the concentration of intracellular free calcium [35, 69, 73], and the induction of nuclear responses [12]. Interestingly, these processes resemble events triggered by growth factors such as EGF [30] or by oncogene activation [9, 52].

Salmonella species adhere to and invade a number of different mammalian cell lines, such as Henle cells, Caco-2 and HEp-2 cells. Most of the information about the pathogenic mechanisms of these bacteria was obtained by using such in vitro systems. The insights into the molecular and genetic bases of *Salmonella* virulence provided by these studies are believed to be highly relevant to the understanding of the natural infection process [25, 28, 50]. The ability of *S. typhimurium* to invade

non-phagocytic cells is an important pathogenic property since it provides bacteria with shelter against host defense mechanisms. Several laboratories have identified genetic loci involved in the entry process of *Salmonella* spp. into host cells [31, 38, 43, 45, 61, 76]. Among these loci there are, for instance, determinants encoding surface structures such as flagella [49, 62], lipopolysaccharide (LPS) or type-1 pili [23], which facilitate the contact of bacteria with the eukaryotic cell. In this review, we focus on a large, discrete region of the *S. typhimurium* chromosome localized at centisome 63, which plays a major role in bacterial invasion and in the above mentioned invasion-associated host-cell signal transduction processes.

Pathogenicity island at centisome 63 of the *Salmonella* chromosome

Virulence genes of Gram-negative and Gram-positive bacteria are very often organized in clusters known as "pathogenicity islands", located either on the bacterial chromosome or on large virulence-associated plasmids [39, 42]. A DNA region of about 40 kb located at the centisome 63 of the *Salmonella* chromosome is required for the entry of the pathogen into host cells [30, 40, 65], and it is also involved in macrophage cytotoxicity [13] (Fig. 1). This region is designated as "*Salmonella* pathogenicicity island 1" (SPI-1), and it bears the genetic information for a large number of proteins belonging to a type III secretion system [65]. A second 40-kb pathogenicity island, designated SPI-2, has been also described in *S. typhimurium* [45, 68]. This region, located at the centisome 30.7 of the bacterial chromosome, encodes a two-component regulatory system and a second type III secretion system [40, 76]. Both SPIs seem to be important for different stages of the infectious life cycle of *Salmonella:* in the mouse model of infection SPI-1 it is crucial for the pathogen to initiate the infection and to invade the intestinal tract for further dissemination in the host. In contrast, SPI-2 was shown to be required for the survival of the pathogen within macrophages [46, 68]. Interestingly, there is evidence that mutations in SPI-2 can interfere with the normal function of the SPI-1 secretion apparatus [46]. In addition to these pathogenicity islands, there are smaller regions that also contribute to *Salmonella* virulence. These "pathogenicity islets" include genes such as *sifA*, *pagC-msgA*, *iviVI*, and some fimbrial genes [40].

SPI-1 is the best-characterized pathogenicity island of *Salmonella* so far [17, 31, 40]. Phylogenetic analyses of the SPI-1 genes suggest that *Salmonella* has acquired this region by horizontal gene transfer from another microorganism [29, 35, 38]. In fact, the G + C content of SPI-1 is significantly lower than that of the remaining bacterial genome, and there is an IS3-like element in the vicinity of this invasion region (Fig. 1). Moreover, this region belongs to one of the nine loops of the *Salmonella* chromosome that share no homology with the *Escherichia coli* K-12 chromosome [45, 65], and it is naturally deleted in certain *Salmonella* serotypes such as *Salmonella lichtfield* and *Salmonella seftenberg* [37].

Nucleotide sequence analyses revealed that SPI-1 contains at least 29 genes flanked by the *flhA* and the *mutS* genes [2, 31, 38, 43, 51, 61, 66] (Fig. 1). There are no more invasion functions upstream of *invH* (Fig. 1), as determined by mutational analysis [31]. It is still unknown if additional invasion determinants are encoded downstream of *orgA* (Fig. 1). Non-polar mutations in SPI-1 genes are usually associated with a diminished entry into epithelial cells and a lower invasiveness in the mouse model of infection. However, *invB* [20] and *sipA* [54] (Fig. 1) mutants are not defective for entry, which suggests that these genes are not required for this essential step in *Salmonella* pathogenesis, or have redundant functions. The role of other genes such as *prgK*, *prgI* and *prgJ*, *iagB* and *iacP* in the invasion process, has not been investigated. Interestingly, only mutations in *invH* [2] have an effect on the ability of the bacteria to attach to tissue culture cells.

General features of type III protein secretion systems

A type III or host-cell contact-dependent protein secretion system has been identified in several animal (*Shigella*,

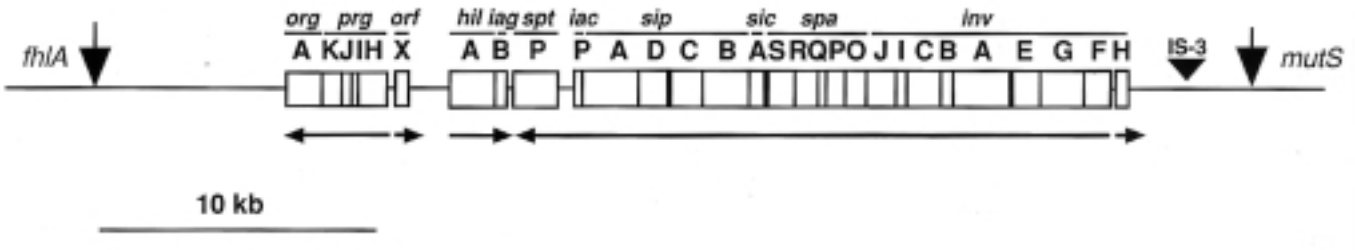

Fig. 1 Gene organization of the pathogenicity island encoded at centisome 63 of the *Salmonella typhimurium* chromosome (SPI-1). Vertical arrows indicate the estimated boundaries of the SPI-1 and the location of the IS3-like sequences. Horizontal arrows indicate the direction of transcription of the genes

Yersinia, enteropathogenic *E. coli*, *Chlamydia*) and plant (*Pseudomonas*, *Erwinia* and *Xanthomonas*) pathogens, which have in common the ability to interact with eukaryotic host cells and secrete virulence factors [48, 80]. Some proteins of these systems are homologous to components of the flagellar assembly apparatus of Gram-positive and Gram-negative bacteria [19] (Table 1). The type III secretion system differs significantly from (a) the type I system, exemplified by the *E. coli* haemolysin export system, or (b) the type II secretion system (General Secretory Pathway), exemplified by the *Klebsiella oxytoca* pullulanase export system. In contrast to the latter systems, the type III secretion system is characterized by the following features: (a) the requirement for a large number of proteins for the exportation process, (b) the absence of a *sec*-dependent typical amino-terminal sequence in the secreted proteins, (c) the export of the target proteins across the periplasmic space without modification, and (d) the requirement for an extracellular signal for the complete activation of the secretory apparatus [18, 31, 60]. Recently, it has been described in *Yersinia* that the signal which leads to the export of the type III secreted proteins appears to be encoded in their messenger RNA, rather than the peptide sequence [6].

By using the type III export system, the microorganism is able to manage the simultaneous and coordinated secretion of a wide range of proteins and, in some cases, their translocation into the eukaryotic host cell [18, 60]. Most of the proteins investigated in these secretion systems belong to one of the following groups: (i) components of the secretion apparatus, including structural components localized in the inner or outer membrane, proteins involved in the energy transduction, chaperones, and proteins with regulatory function; and (ii) secreted proteins involved in the secretion process or with effector functions inside the host cell.

Table 1 Homology[a] of some *Salmonella* proteins encoded in SPI-1

Salmonella spp.	*Shigella* spp.	*Yersinia* spp.	EPEC	*Chlamydia* spp.	*Xanthomonas* spp.	Flagellar assembly	Possible localization	Possible function
InvA	MxiA	LcrD	SepA	Cds2	HrpC2	FlhA	IM	channel forming
SpaP	Spa24	YscR	–	–	ORF2	FlipP	IM	structural
SpaQ	Spa9	YscS	–	–	–	FliQ	IM	structural
SpaR	Spa29	YscT	–	–	HrpB8	FliR	IM	structural
SpaS	Spa40	YscU	–	Cds1	–	FlhB	IM	structural
InvG	MxiD	YscC	SepC	–	HrpA1	–	OM	channel forming
PrgH	MxiG	–	–	–	–	–	OM	structural
PrgK	MxiJ	YscJ	SepD	–	HrpB3	FliF	OM	structural
InvE	MxiC	LcrE (YopN)	–	CopN	–	–	unknown	regulator
InvB	Spa15	–	–	–	–	FliH	unknown	unknown
InvC	Spa47	YscN	SepB	–	HrpB6	FliI	C, IM associate	ATPase
IagB	IpgF	–	–	–	–	–	unknown	unknown
Prgl	MxiH	YscF	–	–	–	–	unknown	unknown
PrgJ	Mxil	–	–	–	–	–	unknown	unknown
OrgA	MxiK	–	–	–	–	–	unknown	regulator
SicA	IpgC	LcrH (SycD)	–	–	–	–	C	chaperone
IacP	OrfX	–	–	–	–	–	unknown	unknown
InvF	MxiE	VirF	–	–	HrpX	–	unknown	regulator
HilA	VirF	–	–	–	–	–	unknown	regulator
SipA	IpaA	YopE	EspA	–	–	–	E	effector
SipB	IpaB	YopH (Yop51)	EspB (EaeB)	–	–	–	E	pore forming toxin
SipC	IpaC	YopQ (YopK)	–	–	–	–	E	effector
SipD	IpaD	YopB	–	–	–	–	E	secretion modulator
InvJ	VirA	YopD	–	–	–	–	E	Sips secretion
SpaO	Spa33	YscQ	–	–	–	FliN/ FliY	E	Sips secretion
SptP	–	YpkA (YopO)	–	–	–	–	E	tyrosine phosphatase
AvrA	–	YopJ	–	–	AvrRxv	–	E	unknown

[a] Searches were conducted at the NCBI server using the Blast program. Sequences were from Genbank release 92.0. (Modified from [32]).
Abbreviations: EPEC, enteropathogenic *E. coli*; IM, inner membrane; OM, outer membrane; C, cytoplasm; E, extracellular.
– Not investigated/Not found.

Components of the SPI-1 secretion apparatus

The genes located in SPI-1 code for components of a specialized protein secretion system (Fig. 2), which is activated upon *Salmonella* contact with the host cell. Several of these proteins are highly conserved in other bacterial pathogens, such as *Shigella*, *Yersinia* and *Pseudomonas* spp. (Table 1). Protein sequence and biochemical analyses of *Salmonella* components have revealed their possible function and localization [17, 31, 32].

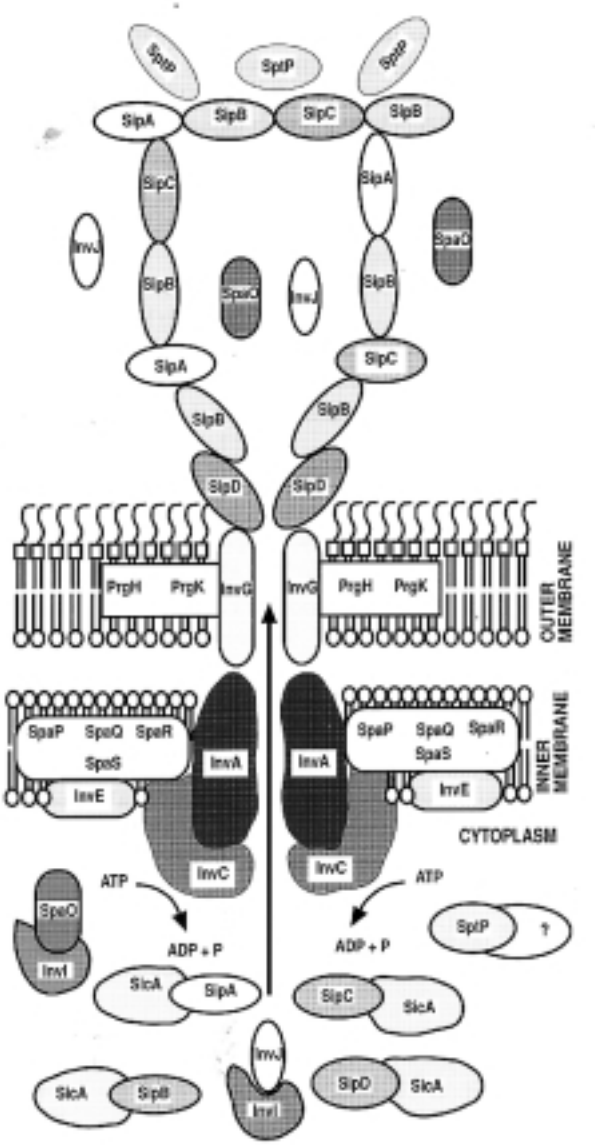

Fig. 2 Fictitious protein model of the SPI-1 type III secretion apparatus spanning the inner and outer bacterial membrane (from Dr. J. E. Galán's laboratory). Inner membrane proteins: InvA, SpaP, SpaQ, SpaR, SpaS. Putative associated-inner membrane proteins: InvA, InvE. Outer membrane proteins: InvG, PrgH, PrgK. Chaperone: SicA. Putative chaperone: InvI. Secreted proteins involved in secretion: InvJ, SpaO. Secreted proteins with a putative effector function (target proteins): SipA, SipB, SipC, SipD, SptP. Some of the target proteins could form part of a supramolecular structure

Inner membrane proteins Based on their secondary structure, InvA, SpaP, SpaQ, SpaR and SpaS [17, 45] are probably inner membrane proteins. InvA is a polytopic membrane protein with seven hydrophobic transmembrane domains and a hydrophilic carboxyterminal domain probably located in the cytoplasm [36]. SpaP, SpaQ and SpaR also contain hydrophobic regions that likely span the inner membrane [38], and some homologues of these proteins, identified in other pathogens, are known to be membrane proteins. Thus, Spa proteins are probably structural components which assist in the translocation process, since non-polar mutations in the *spa* genes abolish *Salmonella* entry into tissue culture cells, and prevent the secretion [16] of type III target proteins.

Outer membrane proteins Three proteins may be localized in *Salmonella* outer membrane: InvG [53], PrgH and PrgK [71]. InvG is a member of the PulD family of proteins and is the only component identified so far that shares homology with components of type II protein secretion systems. It has been shown that it plays an essential role in bacterial uptake [53]. Like other members of the PulD family (e.g. pIV protein of filamentous phages), InvG may be a multimeric protein that forms an outer membrane channel, which may serve as a pore for the passage of secreted proteins [57, 74]. PrgH and PrgK are lipoproteins widely conserved in all types of secretion systems [71]. PrgK shares sequence homology with the *Shigella* MxiJ [3] and *Yersinia* YscJ [64]. PrgH is homologous to the *Shigella* MxiG protein [5] and it is involved in PhoQ/PhoP regulation [11].

Proteins involved in energy transduction InvC shares homology with the β-subunit of F_0F_1 ATPases [20]. This homology suggests that InvC may play a role in energizing the secretion system. In biochemical studies, it was demonstrated that InvC has an ATPase activity. This activity was abolished by the change of a single residue in the predicted nucleotide-binding region of the protein [20]. Although sublocalization studies show that InvC could be associated to the inner membrane through InvA, this result needs to be further confirmed.

Proteins involved in the protection of exported proteins (chaperones) Some cytoplasmic proteins assist the secretion process by maintaining the target proteins in a conformation that is competent for their export and/or by preventing their degradation [82]. Usually, these chaperones are encoded next to the genes coding for target proteins. Chaperones are characterized by their high charge, their small size and the potential to form α-helices. They have been described for Yop proteins of *Yersinia* and Ipa proteins of *Shigella* [63, 81]. At least two proteins could serve as chaperone in the SPI-1 type III secretion system. InvI is a 17 kDa protein which is encoded immediately upstream of *invJ* and *spaO* and exhibits some structural features observed in chaperons as described above. The other protein, SicA [55], shares sequence homology to the *Shigella* and *Yersinia* chaperones IpgC and LcrH [11]. A non-polar mutation in *sicA* prevented the secretion of SipA, SipB and SipC, but not of InvJ, suggesting that the function

of SicA is restricted to a selected number of secreted proteins, such as Sips proteins. On the other hand, a mutation in *invI* prevented the secretion of InvJ and SpaO even after activation of the secretion process by contact with Henle cells [16]. Sublocalization studies using sucrose gradients demonstrate that InvI is associated to the inner membrane (M. Suárez and J. E. Galán, unpublished results). It is conceivable that InvI interacts with other components of the *inv/spa* locus to complete its function.

Proteins involved in regulation At least two regulatory proteins associated with the invasion phenotype, InvF and HilA, have been identified at centisome 63 of the *Salmonella* chromosome. InvF [53] is a member of the AraC family of transcriptional activators, but its regulatory target has not been identified yet. In contrast, HilA [66] is a member of the OmpR/ToxR family of transcriptional activators and some of the regulatory targets of this protein are *orgA*, *sipC* and *invF* [8]. OrgA is encoded by an oxygen-regulated gene [51], which might be involved in the ability of *Salmonella* to survive in a low oxygen environment, like that of the intracellular compartment of the host cell. In addition, OrgA has a strong homology with MxiK [3], a protein of *Shigella* involved in the secretion of the Ipa proteins.

InvE has been shown to play a role in the entry and secretion process of *Salmonella* [35]. Although *invE* mutants grown under in vitro conditions are defective in protein secretion (M. Suárez and J. E. Galán, unpublished results), they are able to secrete InvJ upon contact with cultured epithelial cells. These observations suggest an important role of InvE in regulating the secretion process. Moreover, InvE shares homology to YopN of *Yersinia* [27], a protein that is known to be involved in the regulation of Yop secretion.

Other proteins IacP is a protein of the acylcarrier protein (ACPs) family and it is involved in the post-transcriptional modification of exported proteins necessary for the biosynthesis of essential lipids. This protein might take part in the fatty acid acylation of SipB, but this possibility has not been investigated yet [55]. InvH is involved in the ability of *Salmonella* to attach to cultured epithelial cells [2], and it could play a role in the initial interaction of the bacteria with the host cell.

SPI-1 secreted proteins

At least eight proteins secreted by the type III secretion system have been identified in culture supernatants of invasion-competent *S. typhimurium* [54]. These proteins, with a molecular weight of 26 to 85 kDa, are designated InvJ [15], SpaO [16, 61], SipA, SipB, SipC, SipD [54, 55], SptP [56] and AvrA [43].There are at least two functional categories of secreted proteins: (i) proteins that are unrenounceable for the secretion process, and (ii) proteins with a putative effector function [16]. Effector molecules of type III secretion systems, which are involved in triggering the invasion process, are also known as “target proteins”. Different target proteins have been identified in several bacterial pathogens (Table 1), for example *Yersinia* outer membrane proteins (Yops) [77]; VirA, IpgD and Ipa proteins of *Shigella* [4, 75, 79]; EaeB and Sep proteins of enteropathogenic *E. coli* (EPEC) [20] and the Harpins and Pop proteins of plant pathogenic bacteria [1, 7, 44, 83].

Secreted proteins involved in the secretion process InvJ shows only low similarity with the EaeB protein of enteropathogenic *E. coli*. The latter protein is involved in triggering the host cell responses that lead to the attaching and effacing lesions characteristic of EPEC infection [15]. InvJ and SpaO [61] are absolutely required for protein secretion via the type III pathway, since mutations in their genes prevent the secretion of all other target proteins of the export apparatus [16]. However, a mutation in one of the *sip* genes does not prevent the secretion of InvJ, SpaO or other Sip proteins [54, 55]. Sequence analyses of InvJ and SpaO indicate that they show polymorphisms in several *Salmonella* strains [31], but are very conserved among *Salmonella* serotypes that are strongly host-adapted, such as *S. gallinarum*. The variability of InvJ and SpaO may be the result of their adaptation to host-driven changes in effector target proteins of the secretion apparatus.

SipD shares high homology with the *Shigella* IpaD protein [54]. Mutations in *sipD* and *sip*B lead to increased secretion of SPI-1 target proteins and this effect is similar to that observed in *ipaB* and *ipaD* mutant strains of *Shigella* [60, 66]. This suggests that SipD in *Salmonella* could play a role as modulator of the *Salmonella* secretion process [51]. In *Shigella*, IpaB and IpaD proteins are associated with the membrane, and it has been speculated that they may block the secretion apparatus by forming a plug or lid [63].

Secreted proteins with a putative effector function in the host cell SipA, SipB and SipC [54, 55] are significantly homologous to *Shigella* IpaA, IpaB and IpaC proteins [41], which are involved in the complex interaction of *Shigella* with the host cell [75]. Certain domains of these proteins are either extremely conserved or very divergent [31]. An *ipaB* mutation in *Shigella* could be complemented by expressing SipB in this strain, indicating that both proteins are not only structurally related, but can also perform similar functions in both microorganisms. SipB, IpaB and YopB share a significant homology in a hydrophobic domain which is present in pore-forming toxins belonging to the RTX family. Therefore, it has been suggested that IpaB may induce pore formation [47], which could be significant for triggering signal transduction pathways by inducing ion fluxes in infected cells. But at the moment this function has not fully been demonstrated in this protein.

SptP is a target protein different from the Sip family [56]. The aminoterminal domain of SptP shows a sequence similar to that of the ribosyltransferase exotoxin S from *Pseudomonas aeruginosa* and the cytotoxin YopE from *Yersinia* spp., whereas the carboxyterminal domain is homologous to the catalytic domain of protein tyrosine phosphatases, including YopH from *Yersinia*. In biochemical assays, SptP exhibits tyrosine phosphatase activity [56]. A non-polar mutation in *sptP* did not prevent *Salmonella*

from entering into epithelial cells or macrophages. However, infection studies in the mouse model show that SptP is required for virulence, since *sptP* mutants were defective in the colonization of the spleens of orally infected mice.

Recently, Hardt and Galán described a new effector of the *Salmonella* centisome 63, called AvrA [43]. This protein shares sequence similarity with YopJ of *Yersinia pseudotuberculosis* and the avirulent protein AvrRxv of the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*. Similarity between these two target proteins may reflect the existence of a common virulence mechanism shared by plant and animal pathogenic bacteria. YopJ is necessary for inducing apoptosis in macrophages in vitro [67], and AvrA may have a similar function in *Salmonella*.

Induction of the type-III-mediated secretion process

Contact activation Type III secretion systems require certain environmental signals to be fully active. The signals or molecules that stimulate secretion and translocation of proteins are poorly understood. Secretion of Yops is stimulated by growing *Yersinia* in calcium-free medium at 37°C [77], but this stimulation is probably not the activating signal in vivo. Rosqvist et al. [72] have demonstrated that the contact with host cells activates the *Yersinia* secretion apparatus. In *Shigella* and *Salmonella*, contact with eukaryotic cells leads to the secretion of Ipa and Sip proteins, respectively [63, 84]. In *Salmonella*, this contact-dependent secretion does not require de novo protein synthesis since addition of chloramphenicol does not affect the process [84].

Shortly after *S. typhimurium* interacts with cultured epithelial cells, appendage-like structures are detectable on the bacterial surface [36]. These structures, called invasomes, are transient and probably rapidly shed by the bacteria. The invasome assembly does not require de novo protein synthesis and it is dependent on the expression of genes in SPI-1, since mutations in *invG* and *invC* prevent the contact-dependent assembly process. Interestingly, mutations in *invE* result in aberrant appendages exhibiting a longer half-life [36]. These observations suggest that intestinal epithelial cells activate the type III secretion system encoded in SPI-1 and that the entry of *Salmonella* into cultured epithelial cells is the result of a biochemical cross-talk between the bacteria and the host cell [32]. Although the nature of the invasomes has not been defined yet, the fact that their assembly is *inv* gene-dependent suggest that target proteins like InvJ, SpaO or Sip proteins might be components of their structure. In analogy with *Salmonella*, *Shigella* forms organized structures consisting of filamentous sheets when grown under certain inducing conditions [70]. Some *Shigella* target proteins such as Ipas are components of these sheet-like structures [70]. It is conceivable that these sheets are related to the invasomes identified in *Salmonella*. Further investigations will reveal the contribution of secreted proteins to the invasome assembly process at the molecular level.

Secretion regulation In *Salmonella*, the activity of the type III secretion system is tightly regulated at the transcriptional and post-transcriptional levels. Some factors that alter the degree of superhelicity of DNA are known to affect *inv* gene expression, and subsequently the invasion phenotype. These factors are, for instance, the growth phase of the bacteria [58] or environmental conditions such as osmolarity [78] and oxygen tension [23, 58]. The invasion phenotype is also influenced by mechanisms that control intracellular survival, such as PhoP/PhoQ system and RpoS sigma factor [10, 40, 71], flagellar assembly [21] and bacterial motility [59]. A mutation in the *hil* locus (*hilA*) results in hyperinvasiveness for cultured cells and a smooth-swimming phenotype. The regulatory mechanism behind this observation is not known so far [59].

The transcriptional activators InvF and HilA regulate the secretion process through a complex interaction with the regulatory targets [40]. Non-polar mutations in *invF* and *hilA* significantly reduced the expression of *sipC* in *S. typhimurium* and *S. typhi* [22]. The PhoP/PhoQ two-component regulatory system negatively regulates the expression of *prgH* [10, 71] and the *sip* genes [22]. These data suggest that two processes, bacterial entry and intracellular survival, might be co-regulated.

Conclusions

The characterization of the functional organization and interactions of the different components of SPI-1 is leading to a better understanding of the molecular basis of *Salmonella* entry into host cell. The future challenge will be to identify and characterize (i) the actual signaling molecules that mediate the two-way interplay between the bacteria and the host cell, (ii) the mechanisms of interaction of the secreted proteins with their chaperones, (iii) the assembly and organization of supramolecular structures formed by some of the *Salmonella* secreted proteins, (iv) the signal transduction mechanisms triggered upon contact with eukaryotic cells, (v) the translocation process of target proteins into the host cell, and (vi) the regulatory mechanisms involved in the entry process.

Furthermore, the similarity and possible functional interactions between the components of the type III secretion systems encoded by SPI-1 and SPI-2 need to be extensively analyzed. Such studies may contribute in furthering our understanding of how type III secretion systems work, and of their role in the complex regulation of *Salmonella* virulence. Finally, comparison and heterologous complementation of proteins which share structural and functional affinities in different microorganisms should provide new insights into the evolution of bacterial pathogens which could lead to the development of novel therapeutic strategies.

Acknowledgments M.S. and H.R. have been postdoctoral fellows in Dr. Jorge E. Galán's laboratory at the State University of New York, at Stony Brook. M.S. was supported by a postdoctoral

research fellowship from the Universidad Complutense de Madrid, and H.R. was supported by a fellowship from the German Bundesministerium für Forschung und Technologie.

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