

Homologs of the *Shigella* IpaB and IpaC Invasins Are Required for *Salmonella typhimurium* Entry into Cultured Epithelial Cells

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Entry into host cells is an essential feature in the pathogenicity of *Salmonella* spp. The *inv* locus of *Salmonella typhimurium* encodes several proteins which are components of a type III protein secretion system required for these organisms to gain access to host cells. We report here the identification of several proteins whose secretion into the culture supernatant of *S. typhimurium* is dependent on the function of the *inv*-encoded translocation apparatus. Nucleotide sequence analysis of the genes encoding two of these secreted proteins, SipB and SipC, indicated that they are homologous to the *Shigella* sp. invasins IpaB and IpaC, respectively. An additional gene was identified, *sicA*, which encodes a protein homologous to IpgC, a *Shigella* protein that serves as a molecular chaperone for the invasins IpaB and IpaC. Nonpolar mutations in *sicA*, *sipB*, and *sipC* rendered *S. typhimurium* unable to enter cultured epithelial cells, indicating that these genes are required for bacterial internalization.

Many bacterial pathogens have evolved various mechanisms to enter, survive, and replicate within nonphagocytic cells (33). Reaching the intracellular compartment constitutes an essential feature of their pathogenic life cycle since this environment may protect them from the host's immune system, provide them with nutrients to ensure their growth, or simply allow them to gain access to deeper tissues. The entry mechanisms involve a rather sophisticated manipulation of the host cell machinery (7, 12). Since different microorganisms have evolved various strategies to gain access to host cells, it is not surprising that the determinants that mediate entry vary among different bacterial species. For example, *Yersinia* spp. encode several entry pathways mediated by the outer membrane proteins invasin (24), YadA (6, 47), and Ail (31). Invasin, the best characterized of these determinants, mediates entry by binding with high affinity to multiple β 1 integrin receptors on the host cell surface (41). The molecular genetic bases of entry of other microorganisms such as shigellae and salmonellae are much more complex. In *Shigella* spp., more than 30 proteins encoded in a large virulence-associated plasmid are necessary for bacterial internalization into host cells (37). Likewise, a large number of genetic loci are required for *Salmonella* entry into cultured mammalian cells (15). Although there are significant differences between the ways in which shigellae and salmonellae interact with their hosts, it is now clear that both microorganisms share a dedicated protein secretion system required to present or deliver determinants that are essential for triggering bacterial uptake (28). This system is also present in other bacterial pathogens such as *Yersinia* spp. and a number of plant pathogens, including *Pseudomonas* spp., *Aeromonas* spp., and *Xanthomonas* spp., and is also a part of the flagellar assembly apparatus (42). Such widespread distribution has led to its consideration as an independent protein secretion system termed type III (36).

A number of targets of this type of secretion system have been identified in various microorganisms. These include the invasion plasmid antigens of *Shigella* spp. (Ipa proteins) (37),

the *Yersinia* outer proteins or Yops (39), and the Harpins of several plant pathogens (22, 46). These proteins lack typical signal sequences and have no obvious sequence similarities. Thus, although the export systems are functionally homologous, they have been adapted in each microbial pathogen for the export of the specific effector molecules.

We have recently shown that contact of *Salmonella typhimurium* with cultured mammalian cells leads to the transient assembly of appendage-like structures (invasomes) on the surface of these organisms (17). This process seems to be required for entry since mutations that prevent the normal assembly of the invasomes rendered the salmonellae unable to gain access to cultured cells. Formation of these surface structures is dependent on the type III protein secretion system encoded in the *Salmonella* invasion loci *inv* and *spa* (11, 14, 16, 19, 25). Therefore, identification of the targets of this secretion system in *Salmonella* spp. may help to characterize this invasion organelle and to identify potential effector molecules that trigger bacterial uptake. To date, only one such target, InvJ, has been identified (8). InvJ shares homology with EaeB, a protein from enteropathogenic *Escherichia coli* which is involved in triggering the signaling events leading to the characteristic cytoskeletal rearrangements induced by these organisms (10). Interestingly, EaeB is also a target of a type III secretion system encoded in these strains of *E. coli*.

In this paper, we report the identification of several additional targets of the type III system encoded in the *inv* locus. Two of these proteins have extensive sequence similarity to the *Shigella* invasins IpaB and IpaC.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains used in this study have been described previously (25). Strain SB181, a derivative of the wild-type *Salmonella typhimurium* SL1344 which expresses active alkaline phosphatase, was used for certain experiments in which monitoring the presence of this enzyme was required. Strains were grown in L broth or on L agar, and when required, the following antibiotics were added at the concentrations indicated: kanamycin, 50 μ g/ml; ampicillin, 100 μ g/ml; chloramphenicol, 30 μ g/ml; and streptomycin, 100 μ g/ml. Growth conditions to stimulate the ability of *Salmonella typhimurium* to enter cultured epithelial cells have been described elsewhere (8).

Invasion assay. Entry of *Salmonella typhimurium* strains into cultured Henle-407 cells was assayed in 24-well tissue culture plates as described previously (14).

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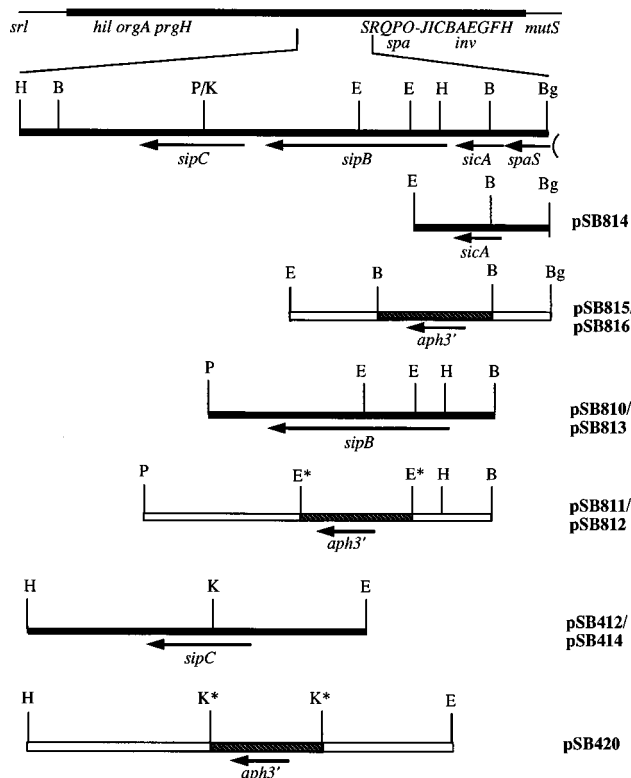


FIG. 1. Partial restriction endonuclease maps of the inserts of relevant plasmids utilized in this study. The position of the relevant region in relation to other invasion loci in the 59-min region of the *Salmonella* chromosome is indicated. The location and direction of transcription of the different genes are shown by arrows. Abbreviations: H, *Hind*III; B, *Bam*HI; P, *Pst*I; E, *Eco*RI; Bg, *Bgl*II.

Recombinant DNA, genetic techniques, and nucleotide sequencing. All recombinant DNA procedures were carried out by standard protocols (27). P22HT \int transduction and bacterial conjugation were carried out as described elsewhere (25). Nucleotide sequence determination was carried out by the dideoxy chain termination procedure with Sequenase as described in the manufacturer's instructions (U.S. Biochemical Corp., Cleveland, Ohio).

Cloning of the *Salmonella typhimurium* chromosomal DNA region downstream from the *inv* locus. *Salmonella typhimurium* SB219 is a derivative of the wild-type strain SL1344 with a locked-in mini-*TnphoA* transposon insertion in *invJ*. This element carries an R6K origin of replication which is functional in strains expressing the *pir* gene. Total cell DNA from strain SB219 was digested with *Sac*I, religated, and introduced into the *E. coli* strain CC118 λ *pir* by transformation. One of such transformants carried a plasmid, pSB411, which contained DNA expanding beyond the *inv* and *spa* loci of *Salmonella typhimurium*. DNA fragments were then subcloned into the plasmid pBluescript-SKII and used for nucleotide sequence determination, mutant construction, and complementation analysis.

Construction of nonpolar mutations. Strains carrying nonpolar mutations in *sicA*, *sipB*, and *sipC* were constructed as follows (Fig. 1). Mutations in *sicA* were constructed by inserting into the unique *Bam*HI site of pSB814 a cassette containing a modified aminoglycoside 3'-phosphotransferase (*aphT*) gene from which the transcription terminator had been removed (16), yielding plasmid pSB815. An *Eco*RI-*Xba*I fragment from pSB815, carrying the mutated *sicA* gene and flanking sequences, was cloned into the *Eco*RI and *Xba*I sites of the R6K-derived replicon pGP704 (32). The resulting plasmid, pSB816, was then mobilized into *Salmonella typhimurium* by conjugation, and transconjugants were selected for streptomycin and kanamycin resistance. One of the transconjugants, strain SB221, showed a Southern hybridization pattern consistent with the presence of the *aphT* cassette in the proper location (data not shown) and therefore was used in the various functional assays. Mutations in *sipB* were constructed by use of a similar strategy, i.e., by replacing in plasmid pSB810 an internal *Eco*RI fragment of *sipB* with an *aphT* cassette, yielding plasmid pSB811. A *Kpn*I-*Xba*I fragment from this plasmid, carrying the mutated *sipB* gene and flanking sequences, was cloned into the *Kpn*I and *Xba*I sites of pGP704, yielding plasmid pSB812, which was used to construct the *sipB* *Salmonella typhimurium* mutant SB169 as described for *sicA*. The correct position of the *aphT* cassette in the mutant strain was verified by Southern hybridization (data not shown). To con-

struct the *sipC* mutant strain, an *aphT* cassette was inserted in the unique *Kas*I site of plasmid pSB412, and the mutated allele was introduced into the wild-type strain with the plasmid pGP704 by use of a strategy similar to that described for the *sipB* mutation. The proper integration of the mutated *sipC* allele in the resulting strain SB220 was verified by Southern hybridization analysis.

Analysis of *Salmonella typhimurium* culture supernatant proteins. Wild-type and mutant strains of *Salmonella typhimurium* were grown in 250 ml of L broth containing 0.3 M NaCl to an optical density at 600 nm of 0.450. Bacterial cells were removed from cultures by centrifugation at $7,000 \times g$ for 20 min and subsequent filtration through a 0.22- μ m-pore-size filter. Proteins from the cell-free culture supernatants were then precipitated by adding 10% (vol/vol) trichloroacetic acid and recovered by centrifugation at $7,000 \times g$ for 20 min. Pellets were resuspended in 4 ml of phosphate-buffered saline (PBS), and proteins were precipitated again by adding 20 ml of cold acetone. After centrifugation at $7,000 \times g$ for 20 min, the pellets were washed once with cold acetone, dried, and resuspended in 250 μ l of PBS. Polyacrylamide gel electrophoresis, Coomassie blue staining, and Western blot (immunoblot) analysis of proteins were carried out by standard protocols (27). N-terminal sequence determination of electroeluted proteins was carried out at the Center for Analysis and Synthesis of Macromolecules, State University of New York at Stony Brook.

Computer analysis of nucleotide and protein sequences. Nucleotide sequence analysis was performed with the Genetics Computer Group package from the University of Wisconsin (version 8) and the MacProt software package (9). Comparison of translated nucleotide sequences with sequences in the available databases was carried out with the program BLAST from the National Center for Biotechnology Information at the National Library of Medicine (2).

Nucleotide sequence accession number. The nucleotide sequence described in this paper has been deposited in GenBank under the accession number U25631.

RESULTS

Identification of proteins whose export into the culture supernatant of *Salmonella typhimurium* is dependent on the type III secretion system encoded in the *inv* locus. Although the secretory system encoded in the *inv* locus is clearly stimulated upon contact with epithelial cells (17, 48), a target of this system, InvJ, was detected by Western blot analysis in the culture supernatant of *Salmonella typhimurium* grown in vitro under conditions that stimulate invasion competency (8). Therefore, we reasoned that we should be able to identify additional proteins whose export is dependent on this secretion system by comparing the protein profiles of culture supernatants from wild-type *Salmonella typhimurium* with those of different *inv* mutants. As shown in Fig. 2A, this proved to be the case. Several proteins with molecular sizes ranging from 85 to 26 kDa were detected in the culture supernatant of wild-type *Salmonella typhimurium* but were absent from that of the *invG* isogenic mutant strain SB161. The presence of these proteins in the culture supernatant of wild-type salmonellae was not the result of bacterial lysis or nonspecific leakage of proteins from internal compartments since neither the periplasmic protein alkaline phosphatase nor the cytoplasmic protein 6-phosphogluconate dehydrogenase was identified in these preparations by Western blot analysis with antibodies specific for these proteins (data not shown; antibodies to alkaline phosphatase and 6-phosphogluconate dehydrogenase were generously provided by Donald Oliver). Introduction of the complementing plasmid pSB405 encoding *invG* restored the ability of the mutant strain to secrete these proteins to the culture supernatant (Fig. 2). Similar results were obtained with strains carrying mutations in other components of the type III secretion system (data not shown). These data indicate that the secretion of these proteins requires the function of the type III secretion system encoded in the *inv* and *spa* loci. These proteins are therefore candidates for effector molecules that are required for bacterial entry.

Western blot analysis of the culture supernatant proteins with a monoclonal antibody specific to InvJ recognized one of the secreted proteins with a molecular size of 37 kDa (Fig. 2B). These findings and the observation that the 37-kDa protein was absent from culture supernatants of an *invJ* mutant of

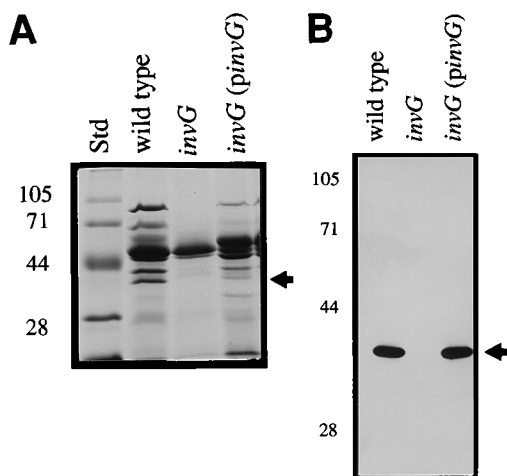


FIG. 2. InvG-dependent secretion of proteins to the culture supernatant of wild-type *Salmonella typhimurium*. Culture supernatant proteins from wild-type *Salmonella typhimurium* SL1344 and the *invG* isogenic mutant SB161 with and without its complementing plasmid pSB405 were obtained as indicated in Materials and Methods, separated on an SDS-10% polyacrylamide gel, and stained with Coomassie blue (A) or transferred to nitrocellulose membranes and probed with an anti-InvJ monoclonal antibody (B). The numbers on the left of the gels indicate the positions of the molecular size standards (in kilodaltons), and the arrows on the right indicate the position of InvJ. The amino-terminal sequence of the prominent ~50-kDa protein observed in the culture supernatants of wild-type, *invG*, and complemented *invG* strains was determined and corresponds to flagellin. Std, molecular size standards.

Salmonella typhimurium (data not shown) allowed us to identify this polypeptide as the *invJ* gene product.

Identification of *Salmonella typhimurium* sipB, sipC, and sicA which encode proteins homologous to the *Shigella* sp. invasins IpaB and IpaC and their molecular chaperone IpgC. As part of our ongoing effort to characterize the 59-min region of the *Salmonella typhimurium* chromosome, which is required for bacterial entry into cultured epithelial cells, we identified three open reading frames (ORFs) located immediately downstream of the previously characterized *spa* locus (19). The first ORF encodes a protein of 165 amino acids with a predicted molec-

ular size of 19,208 Da and an isoelectric point of 4.61. Comparison of the predicted protein sequence with those in the available databases showed similarity to the *Shigella* sp. IpgC (4) and the *Yersinia* sp. LcrH proteins (5, 35) (Fig. 3). IpgC is required for *Shigella* entry into host cells by serving as a chaperone that stabilizes the *Shigella* invasins IpaB and IpaC, thereby impeding their premature association in the cytoplasm which leads to their degradation (30). LcrH has also been shown to act as a chaperone for the *Yersinia* sp. virulence proteins YopB and YopD, which are also targets of a type III secretion system (45). Since the *Salmonella* homologous gene product is likely to have a similar function, we have termed this ORF SicA (*Salmonella* invasion chaperone A). The second ORF, located immediately downstream of *sicA* in an arrangement that suggests translational coupling, is capable of encoding a 593-amino-acid polypeptide with a predicted molecular size of 62,411 Da. Secondary structure analysis of the predicted polypeptide with the algorithm of Klein et al. (26) predicts the presence of two transmembrane regions in a central domain of the protein (residues 313 to 360 and 403 to 439). No indication of the presence of a typical signal sequence was found by use of the algorithm of von Heijne (44). Sequence homology analysis revealed that this ORF, which we have termed SipB (*Salmonella* invasion protein B), encodes a protein with significant similarity to the *Shigella* invasin IpaB (4) (Fig. 4). The overall identity between these two proteins is 28%, which increases to 58% when conservative substitutions are allowed. Interestingly, the similarity is significantly higher (65% identity and 81% similarity) in the central domain (SipB residues 300 through 450) which contains the potential membrane-spanning regions. This region also shares significant similarity (28% identity and 58% similarity) with YopB (21), a protein secreted by *Yersinia* spp. which is also a target of a type III secretion system. The third ORF encodes a 409-amino-acid polypeptide with a predicted molecular size of 43,997 Da which is homologous to the *Shigella* invasin IpaC (32% identity and 48% similarity) (4) (Fig. 5). We have named this ORF SipC (*Salmonella* invasion protein C). Secondary structure analysis of SipC with the algorithms of von Heijne (44) and Klein et al. (26) showed no indication of the presence of a typical signal sequence or potential transmembrane domains. The organiza-

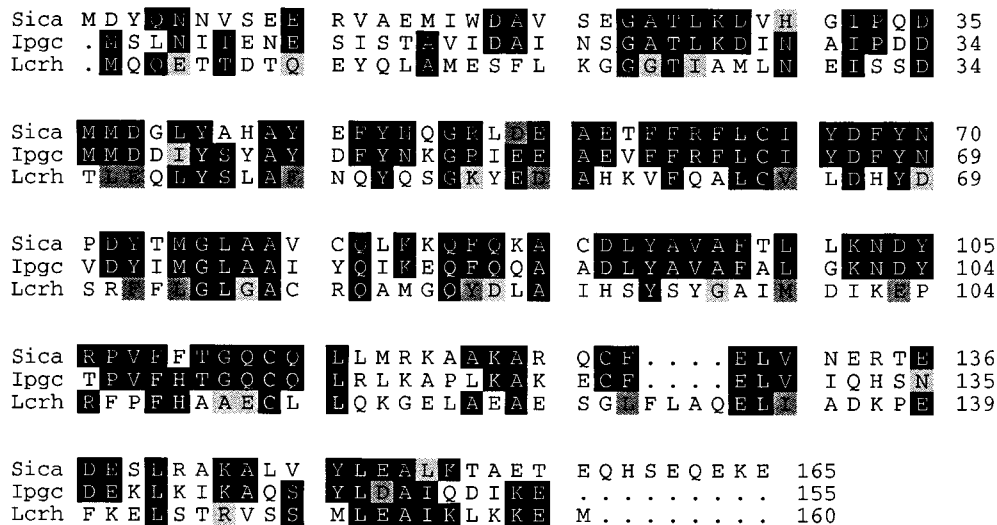


FIG. 3. Sequence alignment of *Salmonella typhimurium* SicA, *Shigella flexneri* IpgC, and *Yersinia pseudotuberculosis* LcrH. Black boxes indicate complete identity, and shaded boxes indicate conservative amino acid substitutions.



FIG. 4. Sequence alignment of *Salmonella typhimurium* SipB and *Shigella flexneri* IpaB. Lines indicate complete identity; colons and periods indicate conservative amino acid substitutions.

tion of the *Salmonella sicA*, *sipB*, and *sipC* genes and that of the *Shigella* homologs are remarkably conserved. Their topological relationship to the genes encoding the secretion apparatus, however, is inverted. While in *Salmonella* spp. the *sicA sipB sipC* operon is immediately downstream of the *spa* locus (19) (equivalent to the *Shigella spa* locus [38, 43]) and transcribed in the same orientation, the equivalent operon in *Shigella* spp. is upstream of the *mxi* locus (equivalent to the *Salmonella inv* locus) and transcribed in the opposite direction (1, 3).

***Salmonella typhimurium sicA*, *sipB*, and *sipC* are required for bacterial entry into cultured mammalian cells.** To investigate the possible involvement of *sicA*, *sipB*, and *sipC* in the invasive

phenotype, *Salmonella typhimurium* strains carrying nonpolar mutations in each ORF were constructed as indicated in Materials and Methods, and the resulting mutant strains were tested for their ability to enter cultured Henle-407 cells. As shown in Table 1, strains SB221, SB169, and SB220 carrying mutations in *sicA*, *sipB*, and *sipC*, respectively, were severely deficient in their ability to gain access to cultured host cells. Introduction of the respective complementing plasmids restored the invasion phenotype, indicating that these gene products are essential for *Salmonella typhimurium* entry into host cells.

SipB and SipC are secreted into the culture supernatant via the type III secretion system encoded in the *inv* locus. SipB and SipC are homologous to IpaB and IpaC, which are exported via a type III secretion system. Therefore, we examined the possibility that these proteins were among those whose secretion to the culture supernatant of invasion-competent *Salmonella typhimurium* is dependent on the protein secretion apparatus encoded in the *inv* locus (see above and Fig. 2). Culture supernatants from the *Salmonella typhimurium sipB* strain SB169 lacked a polypeptide with an apparent molecular size of 68 kDa which was present in wild-type *Salmonella typhimurium* as well as in strain SB169 carrying the complementing plasmid pSB813 (Fig. 6). These results indicate that this polypeptide



FIG. 5. Sequence alignment of *Salmonella typhimurium* SipC and *Shigella flexneri* IpaC. Lines indicate complete identity; colons and periods indicate conservative amino acid substitutions.

TABLE 1. Entry of wild-type, *sicA*, *sipB*, and *sipC* *Salmonella typhimurium* strains into cultured Henle-407 cells

Strain	Relevant phenotype	% Internalization ^a
SL1344	Wild type	35 ± 3
SB221	SicA ⁻	0.03 ± 0.002
SB221(pSB814)	SicA ⁻ (SicA ⁺)	23 ± 3
SB169	SipB ⁻	0.01 ± 0.001
SB169(pSB813)	SipB ⁻ (SipB ⁺)	13 ± 2
SB220	SipC ⁻	0.03 ± 0.004
SB220(pSB414)	SipC ⁻ (SipC ⁺)	38 ± 5

^a Values are means ± standard deviations of triplicate samples and represent the percentages of the initial inoculum that survived gentamicin treatment for 2 h as described in Materials and Methods. Similar results were observed in several repetitions of this experiment.

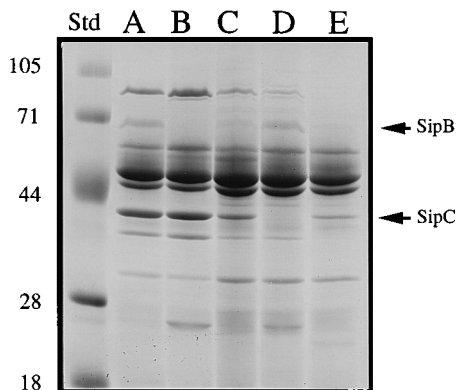


FIG. 6. Protein secretion profile of *Salmonella typhimurium sipB* and *sipC* mutants. Culture supernatant proteins from wild-type *Salmonella typhimurium* SL1344 and the isogenic *sipB* and *sipC* mutants SB169 and SB220 with and without the complementing plasmids were prepared as indicated in Materials and Methods, separated on an SDS-10% polyacrylamide gel, and stained with Coomassie blue. Lanes: A, wild type; B, SB169; C, SB169(pSB813); D, SB220; E, SB220(pSB414); Std, molecular size standards. The amino-terminal sequence of the prominent ~50-kDa protein observed in all lanes was determined and corresponds to flagellin. The numbers on the left of the gel indicate the positions of the molecular size standards (in kilodaltons), and the arrows on the right indicate the positions of SipB and SipC.

corresponds to the *sipB* gene product. The slight discrepancy between the size of SipB deduced from its mobility in a sodium dodecyl sulfate (SDS)-polyacrylamide gel (68 kDa) and that derived from its predicted amino acid sequence (62.4 kDa) has also been observed in the *Shigella* homolog IpaB. Attempts to determine the N-terminal sequence of the 68-kDa polypeptide were unsuccessful. The N-terminal sequence of the 41-kDa polypeptide, however, was successfully determined and found to match exactly that of the predicted SipC protein (MLIS NVGINPAAYLN). In addition, the 41-kDa polypeptide was absent from culture supernatants of the *sipC* *Salmonella typhimurium* strain SB220, further supporting the identification of this polypeptide as SipC (Fig. 6). The N-terminal sequence of the secreted SipC also indicates that this protein is secreted without undergoing cleavage of its amino terminus upon translocation, a feature common to other targets of type III secretion systems (36).

DISCUSSION

Salmonella entry into host cells requires the function of a dedicated, *sec*-independent protein secretion system encoded in the *inv* and *spa* loci located at 59 min on the *Salmonella* chromosome (11, 16, 18, 19, 25). This translocation apparatus participates in the host cell contact-dependent assembly of a supramolecular structure presumably required for the presentation and/or delivery of invasion determinants to the target cell (17).

We report here the identification of several targets of the *inv*- and *spa*-encoded secretion system. Several proteins with molecular sizes ranging from 85 to 26 kDa were observed in culture supernatants from wild-type *Salmonella typhimurium* grown to invasion competency but were absent from those of strains carrying mutations in genes encoding components of the type III secretion system such as *invG* (Fig. 2). The presence of these proteins in the culture supernatant of wild-type salmonellae was not the result of bacterial lysis or nonspecific leakage of proteins from internal compartments since neither the periplasmic protein alkaline phosphatase nor the cytoplasmic protein 6-phosphogluconate dehydrogenase was identified

in these preparations. The identity of three of these proteins was established by a variety of biochemical, immunological, and genetic assays. Western blot analysis identified a 37-kDa secreted protein as InvJ, a previously characterized protein encoded in the *inv* locus and required for *Salmonella* entry into cultured cells (8). Nucleotide sequencing in combination with genetic and biochemical analysis of defined mutants identified two other secreted proteins with molecular sizes of 68 and 41 kDa as the products of *sipB* and *sipC*, two newly identified invasion genes. *Salmonella typhimurium* strains carrying non-polar mutations in *sipB* and *sipC* were unable to enter cultured epithelial cells, indicating that they are essential components of the entry apparatus. N-terminal sequence analysis of the secreted SipC protein indicated that, like other identified targets of the type III secretion systems, it is not processed upon secretion (36).

SipB and SipC were shown to be the homologs of the *Shigella* sp. proteins IpaB and IpaC. The Ipas are encoded in a large virulence-associated plasmid by four contiguous genes, *ipaB*, *ipaC*, *ipaD*, and *ipaA*, arranged in an operon located upstream from the secretion loci *spa* and *mxi*, and transcribed in the opposite orientation (37). These proteins have been postulated to be the effector molecules of different events during the interactions of *Shigella* spp. with phagocytic and nonphagocytic cells such as bacterial entry, vacuolar escape, and macrophage killing (23, 29, 49). The similarity between putative *Shigella* and *Salmonella* effector proteins was unexpected considering the different ways in which these organisms interact with phagocytic and nonphagocytic cells. Subsequent to entering host cells, *Shigella* spp. exit the endocytic vacuole, thus gaining access to the cell cytosol from where they then spread to neighboring cells by manipulating the host cell cytoskeleton (37). In contrast, upon cell entry, *Salmonella* spp. remain in a membrane-bound compartment throughout their intracellular life cycle (40). Furthermore, unlike *Shigella* spp. (34), *Salmonella* spp. can readily enter polarized epithelial cells through the apical side (13). Differences are also apparent in the way these organisms interact with phagocytic cells. Macrophages infected in vitro with *Shigella flexneri* rapidly undergo apoptotic death, while *Salmonella* spp. do not immediately kill macrophages but rather survive and replicate in these cells (20). How could similar proteins mediate such different interactions? At least two explanations are plausible. Despite the homology between these proteins, determinants of specificity may lie in less-conserved regions. For example, it is possible that the *Salmonella* SipB shares with IpaB functional domains related to entry but lacks those implicated in vacuolar exit and apoptosis. Although SipB and IpaB are highly related to each other (65% identity and 81% similarity) in a region extending from residue 300 through 450, the C and N termini of these proteins are significantly less conserved. Those regions may therefore confer SipB and IpaB specificity of function. Trans-complementation and domain-swapping experiments between IpaB and SipB will help to address these issues. Alternatively, the actual effector function may not be dependent on these proteins themselves, but rather, the proteins may be facilitators of the action of yet other effector proteins. For example, the hydrophobic domains of SipB and IpaB, which are also shared by the *Yersinia* protein YopB, are distantly related to those of the RTX family of pore-forming toxins (21). The putative pore-forming function of these proteins may be implicated in the delivery of effector molecules to the host cell as previously postulated by Hakansson et al. (21).

Our nucleotide sequence analysis also revealed an ORF immediately upstream of *sipB* which encodes a protein homologous to IpgC and LcrH from *Shigella* and *Yersinia* spp., re-

spectively. SicA is essential for bacterial entry since a *Salmonella* strain carrying a nonpolar mutation in its coding gene was unable to enter cultured epithelial cells. IpgC has been shown recently to serve as a molecular chaperone of IpaB and IpaC (30). Binding of IpgC to these proteins prevents their association in the cytoplasm, thereby halting their premature degradation. Given the high degree of homology between IpgC and SicA, and between the Ipa proteins and SipB and SipC, it is very likely that the *Salmonella* homolog carries out a similar function.

Finally, it has been shown recently that the Ipa proteins of *Shigella* spp. form a complex in the culture medium (30). If SipB and SipC also form a complex in the extracellular medium, it is tempting to speculate that these proteins may form portions of the appendages observed on the surface of *Salmonella* spp. upon contact with host cells.

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