INFECTION AND IMMUNITY, Apr. 1998, p. 1806–1811 Vol. 66, No. 4
0019-9567/98/$04.00+0
Copyright © 1998, American Society for Microbiology

Trafficking of Porin-Deficient *Salmonella typhimurium* Mutants inside HeLa Cells: *ompR* and *envZ* Mutants Are Defective for the Formation of *Salmonella*-Induced Filaments

SCOTT D. MILLS,† SHARON R. RUSCHKOWSKI, MURRY A. STEIN,‡ AND B. BRETT FINLAY*

Biotechnology Laboratory, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

Received 17 July 1997/Returned for modification 1 September 1997/Accepted 14 January 1998

Outer membrane porin genes of *Salmonella typhimurium*, including *ompC*, *ompF*, and *tppB*, are regulated by the products of *ompB*, a two-component regulatory locus encoding OmpR and EnvZ. *S. typhimurium ompR* mutants are attenuated in mice, but to date no one has studied the intracellular trafficking of *S. typhimurium* porin-deficient mutants. In this study, isogenic transposon mutants of *S. typhimurium* with insertions in *ompR*, *envZ*, *ompF*, *ompC*, *ompD*, *osmZ*, and *tppB* were compared with wild-type SL1344 for trafficking in the human epithelial cell line HeLa. We found that *ompR* and *envZ* mutants were reduced or completely inhibited for the formation of *Salmonella*-induced filaments (Sifs). This result was confirmed with an *ompB* deletion mutant. Sifs are tubular structures containing lysosomal glycoprotein which are induced specifically by intracellular *Salmonella*. Genetic analysis showed that the *ompR* mutation could be complemented in *trans* by cloned *ompR* to restore its ability to induce Sifs. In contrast, mutations in the known *ompR*-regulated genes *ompF*, *ompC*, and *tppB* (as well as the *ompR*-independent porin gene, *ompD*) had no effect on Sif formation relative to that of wild-type SL1344, thus indicating that OmpR does not exert its role on these genes to induce Sif formation. The *omp* mutants studied were able to invade and replicate in HeLa cells at levels comparable to those in wild-type SL1344. We conclude that OmpR and EnvZ appear to regulate Sif formation triggered by intracellular *S. typhimurium*.

Salmonella spp. are facultative intracellular pathogens which cause a variety of diseases in humans, ranging from acute gastroenteritis (*Salmonella typhimurium*) to enteric fever (*Salmonella typhi*) (20). *S. typhimurium* causes self-limiting illnesses in humans, such as gastroenteritis (food poisoning), but in mice it causes fatal enteric fever resembling human typhoid fever. Therefore, mice provide a useful animal model in which to study enteric fever. After ingestion, *Salmonella* organisms colonize the lower intestine and invade Peyer's patches to gain access to the lamina propria; from there, they can be disseminated systemically. The first cellular barriers that *Salmonella* faces in the body are epithelial cells and M cells of Peyer's patches (19). The mechanism by which *S. typhimurium* invades epithelial cells has been well studied and shown to involve many genes encoding a type III secretion system and specific secreted proteins (7, 8, 16, 18). At the site of bacterial contact with the host cell surface, *S. typhimurium* induces massive host membrane ruffling (6), capping of specific plasma membrane proteins (12), and macropinocytosis (11). After invasion, the host plasma membrane normalizes and the internalized bacteria reside within a host membrane-derived vacuole, where they are able to survive and replicate. The vacuolar membrane enclosing *S. typhimurium* acquires and maintains the host lysosomal marker, lysosomal glycoprotein (lgp), within 30 min after invasion (13). Similar mechanisms for invasion and intracellular trafficking have been reported for *S. typhi* (9, 25).

After cell invasion, there is a lag period in *S. typhimurium* growth, during which time the bacteria presumably acclimate to their new intracellular surroundings (for example, altered pH and osmolarity). Within 4 to 6 h after invasion, *S. typhimurium* organisms begin to replicate, and by 10 to 16 h postinvasion the bacteria fill the cell, resulting in lysis. Intracellular replication is an essential *S. typhimurium* virulence trait, since prototrophic nonreplicating mutants are attenuated in mice (22). Coincident with the onset of *S. typhimurium* replication within cultured epithelial cells is the formation of lgp-containing tubular structures that appear to connect multiple *Salmonella*-containing vacuoles within the cell (10). These tubular structures are induced specifically by *Salmonella* spp., and thus far no other known invasive bacterial pathogen, including *Yersinia*, *Shigella*, enteropathogenic *Escherichia coli*, and *Listeria*, has been shown to induce these structures (10, 32a). It is thought that these tubular structures, termed Sifs (*Salmonella*-induced filaments), are somehow involved in *Salmonella*'s ability to acquire nutrients and replicate intracellularly, since all intracellular nonreplicating mutants of *S. typhimurium* tested thus far are unable to induce Sifs (22).

Sif formation in epithelial cells requires the *Salmonella*-specific gene *sifA* (33). *sifA* encodes a single protein and is found specifically on the *Salmonella* chromosome located within the *pot* operon at approximately 59 min. It contains inverted repeats on either end to suggest horizontal transfer via transposition and shows no clear homology to genes thus far identified. *sifA* mutants display several features that distinguish them from the parental strain. They are unable to induce Sifs in epithelial cell lines, they replicate at a faster rate than the wild-type parent in these cells, and they are attenuated in mice (33). *sifA* is the only *Salmonella*-specific gene shown thus far to influence intracellular trafficking in HeLa cells.

Biosyntheses of some *S. typhimurium* (and *E. coli*) outer membrane porin proteins such as OmpF, OmpC, and TppB

* Corresponding author. Mailing address: Biotechnology Laboratory, Room 237, Wesbrook Building, 6174 University Blvd., Vancouver, British Columbia V6T 1Z3, Canada. Phone: (604) 822-2493. Fax: (604) 822-9830. E-mail: bfinlay@unixg.ubc.ca.

† Present address: Astra Research Center Boston, Inc., Cambridge, MA 02139-4239.

‡ Present address: Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405-0068.

TABLE 1. Strains of *S. typhimurium* and plasmids used in this study

Strain or plasmid	Description	Source or reference
Strains		
SL1344	Wild type	17
CJD359	SL1344 *ompR*::Tn*10*	5
SR-11	Wild type	8
SWL350	SR-11 *ompR*276::Mu dJ-*lacZ*	23
ARD3	SL1344 *ompR*276::Mu dJ-*lacZ*-P22 from SWL350	A. Richter-Dalfours (unpublished)
CJD372	SL1344 *ompC*396::Tn*10*, *ompF*1006::Mu d1-8	3
BRD455	SL1344 *ompD*159::Tn*10*	3
BRD454	SL1344 *ompC*396::Tn*10*	3
J1-3	SL1344 *sifA*::Tn*10* dCm	33
CJD408	SL1344 *tppB*83::Mu dJ	3
CH1082	LT2 *supD zeb*609::Tn*10*, *ompF*1004::Mu dJ	G. Dougan and C. F. Higgins
SDM1082	SL1344 *ompF*1004::Mu dJ − P22 from CH1082	This study
CJD409	SL1344 *ompC*396::Tn*10*, *ompF*1006::Mu d1-8, *tppB*83::Mu dJ	3
CH1118	LT2 *envZ*1005::Mu dJ	14
TT15265	LT2 *envZ*1005::Mu dP	1
SDM1118	SL1344 *envZ*1005::Mu dJ − P22 from CH1118	This study
SDM15265	SL1344 *envZ*1005::Mu dP − P22 from TT15265	This study
Plasmids		
pWSK29	Cloning vector	35
pSWLOMP	pWSK29 containing *ompR-envZ* operon from SR-11	23

are regulated by the *ompB* locus (26, 29). The *ompB* locus encodes OmpR-EnvZ, a two-component regulatory system in which EnvZ, a transmembrane sensory protein with histidine kinase activity, controls the activity of OmpR, a transcriptional regulator, in response to changes in external environmental factors such as osmolarity, temperature, and pH. These environmental changes that regulate the activity of OmpR-EnvZ are likely encountered by *Salmonella* during its adaptation to the intracellular environment after invasion, and thus *S. typhimurium omp* mutants may be affected in their intracellular interactions in epithelial cells. It has been previously reported that *ompR* mutants of *S. typhimurium* are avirulent in a mouse model (3, 5). In addition, OmpR-EnvZ has been shown to play an important role in the virulence of *Shigella flexneri* (2). Given these in vivo data, we looked at several isogenic *S. typhimurium* strains with mutations in various porin biosynthesis genes (*ompC*, *ompF*, *ompD*, and *tppB*) and regulatory genes (*osmZ*, *ompR*, and *envZ*) influencing porin gene expression to determine their influence on intracellular trafficking of *S. typhimurium* in HeLa cells, as determined by Sif formation.

Isogenic transposon mutants affected in outer membrane protein biosynthesis in *S. typhimurium* SL1344, which is virulent in mice, were either obtained from the various sources listed in Table 1 or constructed by P22 transduction into wild-type strain SL1344 with P22 HT105/1 (30) as previously described (34). Outer membranes were prepared from these strains as described previously (31) and analyzed by sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis to verify the mutations as described previously (references 5 and 21 and data not shown). Lack of OmpC in mutants containing insertions in *ompR*, *envZ*, or *ompC* was further supported by Western blotting with monoclonal mouse anti-OmpC antibody CM 95.3 (reference 32 and data not shown). The *tppB* mutants were confirmed by their resistance to the antibiotic peptide alafosfalin relative to the situation for wild-type SL1344, as in the procedure of Gibson et al. (15).

Porin-deficient *S. typhimurium* mutants (Table 1) were then evaluated for their abilities to induce Sifs in HeLa cells (ATCC CCL2) at 6 h postinvasion relative to the abilities of wild-type SL1344 and J1-3 (*sifA*::Tn*10* dCm). J1-3 has previously been shown to be defective for Sif formation due to inactivation of *sifA* (33). HeLa cells were infected and processed for detection of Sifs by epifluorescence microscopy as previously described (25). Sif formation was assessed as the percentage of infected cells that contained Sifs, as stained by anti-lgp antibody. One hundred infected cells were counted per treatment, and each experiment was performed a minimum of three times. The results from these experiments showed that mutations in *ompR* and *envZ* (the *ompB* regulatory locus) render these strains completely defective or highly reduced for the induction of Sif formation (Fig. 1). Further, genetic *trans* complementation of ARD3 (*ompR*::Mu dJ) with a cloned *ompB* locus from *S. typhimurium* (pSWLOMP) (Table 1) demonstrated that the ability of this mutant to induce Sifs could be restored to wild-type levels, while the vector alone (pWSK29) had no effect on the ability of ARD3 to induce Sifs (Fig. 1). The cloned *ompB* locus (pSWLOMP) also restored the ability of ARD3

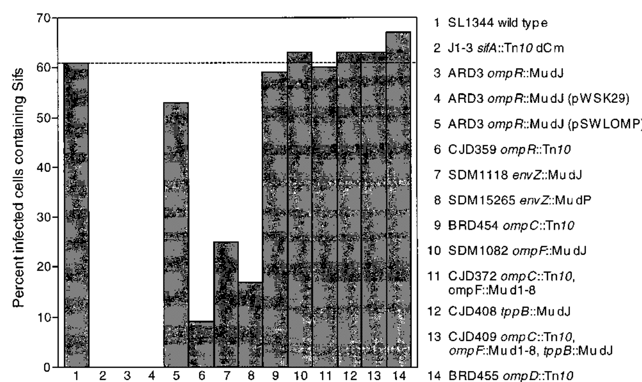

FIG. 1. *omp*B (*ompR* and *envZ*) mutants are defective or highly reduced for Sif formation. Induction of Sif formation in HeLa cells by the *Salmonella* strains listed in Table 1 was determined at 6 h postinvasion. This graph represents results from one of four experiments where cells (100 for each strain) infected by each *S. typhimurium* strain tested were evaluated for Sif formation at 6 h post-invasion. Values are given as the percentage of infected cells containing Sifs. The dashed line provides a reference for Sif induction by wild-type SL1344.

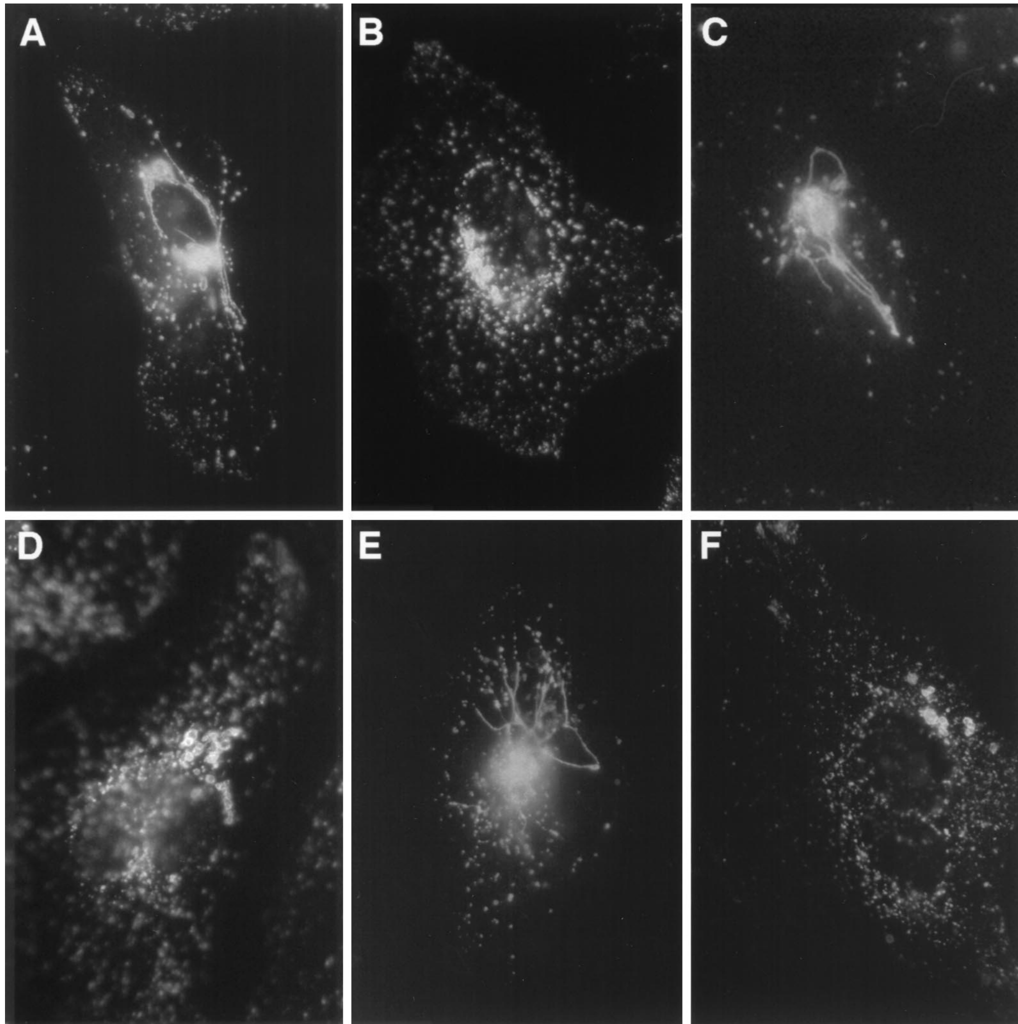

FIG. 2. Micrograph illustrating immunofluorescence labeling of Sifs in HeLa cells infected with wild-type SL1344 and porin-deficient mutants at 6 h postinvasion. Shown is a typical Sif (stained with anti-lgp monoclonal antibody) (A) induced by wild-type SL1344. Induction of Sif formation was defective for *ompR* mutants ARD3 (*ompR*::Mu dJ) (B) and CJD359 (*ompR*::Tn*10*) (D), a situation similar to what was previously shown for J1-3 (*sifA*::Tn*10* dCm) (F) (33). Sif formation was restored to ARD3 (*ompR*::Mu dJ) (C) complemented in *trans* with the cloned *ompB* locus in pSWLOMP. Triple porin-deficient mutant BRD409 (*ompC*::Tn*10*, *ompF*::Mu d1-8, *tppB*::Mu dJ) induced Sifs similarly to wild-type SL1344 (E).

(*ompR*::Mu dJ) to produce OmpC, as shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (data not shown). In contrast, mutations in the genes known to be regulated by the *ompB* locus (*ompC*, *ompF*, and *tppB*) had no effect on the induction of Sifs (Fig. 1). BRD455 (*ompD*::Tn*10*) also induced Sif formation comparable to that of wild-type SL1344, as was expected, since *ompD* is expressed independent of the *ompB* locus. These results indicate that *ompR* and *envZ* are required to induce wild-type Sif formation, while the porin-deficient mutants *ompC*, *ompF*, *ompD*, and *tppB* (and multiple mutants thereof) play no apparent role in Sif formation.

A *S. typhimurium ompB* deletion mutant was constructed in strain SL1344 to confirm the results obtained with the *ompR* and *envZ* transposon insertion mutants. *S. typhimurium* SL1344 *ompB* was PCR amplified with oligonucleotides derived from the *S. typhimurium ompR* and *envZ* genes (accession no. X12374). Oligonucleotide 327U19 (5′-CTGCGGGCGCT ACTGGAAC-3′), corresponding to positions 327 to 345 in the *ompR* sequence, and oligonucleotide 2308L19, corresponding to positions 2308 to 2326 in the *envZ* sequence (5′-GACGCG AGCCACAGGAACC-3′), were used to amplify *ompB* from heat-disrupted *S. typhimurium* as described previously (33). The PCR product was cloned into pCR2.1-TOPO using the TOPO TA cloning kit (Invitrogen). An internal portion of the cloned *ompB* locus was deleted from bases 710 to 1649 by digestion with *Pme*I and *Sma*I and subsequent religation, introducing a stop codon at the 10th and 20th codons after the *Pme*I-*Sma*I junction. The Δ*ompB* was cloned, by using the pCR2.1-TOPO polylinker-encoded *Sac*I and *Xba*I sites, into the positive allelic exchange vector pCVD442 linearized by digestion with *Xba*I and *Sac*I. Allelic exchange and selection were performed as described previously (33). The *ompB* lesion was confirmed by PCR, and this *ompB* deletion strain (MS123) was characterized for Sif formation in HeLa cells at hours 6 and 8 postinvasion. No Sif formation was observed for the *ompB* deletion mutant in two separate experiments when the latter was compared with wild-type SL1344. These results were comparable to the results obtained with the SL1344 *ompR*::Mu dJ and *ompR*::Tn*10* strains.

Immunofluorescence micrographs demonstrate the intra-

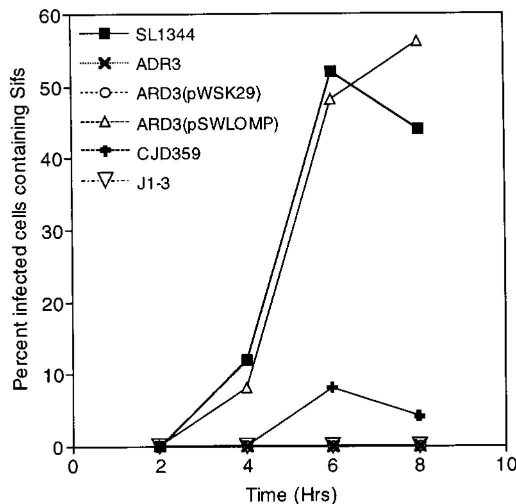

FIG. 3. Kinetics of Sif formation in HeLa cells. SL1344 *ompR* mutants were defective for Sif formation in HeLa cells over a time course of 1 to 8 h postinvasion, compared with wild-type SL1344 and J1-3 (*sifA*::Tn*10* dCm). In these experiments, J1-3 (*sifA*::Tn*10* dCm), ARD3 (*ompR*::Mu dJ), and ARD3 (pWSK29; cloning vector) did not induce any Sifs, while CJD359 (*ompR*::Tn*10*) induced the formation of Sifs at a low frequency. ARD3 complemented in *trans* with the cloned *ompB* locus (pSWLOMP) exhibited Sif formation kinetics similar to those of wild-type SL1344. This graph shows results from one of three experiments where 100 infected cells for each *S. typhimurium* strain were evaluated for Sif formation at 1, 2, 4, 6, and 8 h postinvasion. Results are given as percent infected cells containing Sifs.

cellular Sif phenotype associated with porin-deficient mutants, relative to that of wild-type SL1344 (Fig. 2A) and J1-3 (*sifA*::Tn*10* dCm) (Fig. 2F), in HeLa cells 6 h postinvasion (Fig. 2). *ompR* mutants (ARD3 [Fig. 2B] and CJD359 [Fig. 2D]) were similar to the *sifA* mutant (J1-3 [Fig. 2F]) in that they were defective for Sif formation. Genetic *trans* complementation with the cloned *ompB* locus (pSWLOMP) was able to complement the Sif-negative phenotype of ARD3 (*ompR*::Mu dJ) in *trans* (Fig. 2C). The porin-deficient triple mutant, BRD409 (*ompC*::Tn*10*, *ompF*::Mu d1-8, *tppB*::Mu dJ), was able to induce Sifs at levels comparable to that of wild-type SL1344 (Fig. 1 and 2E).

Time course experiments were performed to ascertain whether the mutants under study were defective and not altered kinetically in their abilities to induce Sif formation. In this study, we compared wild-type SL1344, ARD3 (*ompR*::Mu dJ), CJD359 (*ompR*::Tn*10*), and J1-3 (*sifA*::Tn*10* dCm) for their abilities to induce Sifs at 2, 4, 6, and 8 h postinvasion. The results showed that both of the *ompR* mutants were inhibited or highly reduced for Sif formation over the time course studied, compared with wild-type SL1344 and J1-3 (*sifA*::Tn*10* dCm) (Fig. 3). As shown, at 6 h (Fig. 1), the cloned *ompB* locus (pSWLOMP), but not the vector alone (pWSK29), was able to complement ARD3 (*ompR*::Mu dJ) for Sif formation comparable to that of wild-type SL1344 (Fig. 3). These results show that the *ompR* mutants are defective, and not kinetically altered, for induction of Sif formation.

Invasion and replication by the *ompR* mutants (ARD3 and CJD359) were assessed to determine whether these functions, in addition to Sif formation, were affected relative to their status in wild-type SL1344 and the *sifA* mutant (J1-3). HeLa cell invasion and replication were assessed by means of the gentamicin protection assay as previously described (25). The results from these experiments show that invasion was not significantly affected in the *ompR* mutants (Fig. 4). Replication appeared to be slightly enhanced for the *ompR* mutants, but this was not statistically significant (Fig. 4). These experiments demonstrated that *ompR* mutants (and *envZ* mutants [data not shown]) were not adversely affected for invasion and replication.

The present study shows that mutations in the *S. typhimurium ompB* locus encoding *ompR* and *envZ* render the resulting mutants defective for inducing the formation of lgp-containing tubules (Sifs) in HeLa cells. In contrast, mutations in the porin genes known to be regulated by the *ompB* locus in *Salmonella* (*ompC*, *ompF*, and *tppB*) had no effect on Sif formation. Disruption of *ompD*, a *Salmonella* outer membrane porin gene whose expression is OmpR independent, also had no effect on Sif formation. The role of OmpR in *Salmonella* pathogenesis, in relation to Sif formation, is interesting because strain SL1344 *ompR* mutants, such as CJD359, have previously been shown to be avirulent in mice (3, 5). This points to a correlation between earlier in vivo studies in mice and the present in vitro study in HeLa cells: virulence in mice and Sif formation both require a functional *ompR* gene. Whether Sif formation has any role in pathogenesis is still unclear. Previous 50% lethal dose experiments comparing wild-type SL1344 and the *sifA* mutant (J1-3) showed that pathogenesis of J1-3 was attenuated, although not to the extent observed for the *ompR* mutant (33).

In addition to these in vivo data, it has been determined that *S. typhimurium ompR* mutants do not induce apoptosis in the mouse macrophage cell line J774A.1 (23), while wild-type *S. typhimurium* does (4, 23, 27). In a study by Lingren et al., the *ompR* mutants were able to replicate to levels similar to those of wild-type *S. typhimurium* in J774A.1 cells, and therefore the noncytotoxic phenotype associated with the *ompR* mutants was not due to nonreplication (23). These results suggested that intracellular fusion of *Salmonella*-containing vacuoles was inhibited in cells infected with *ompR* mutants and that this accounted for their lack of cytotoxicity (23). We also observed an

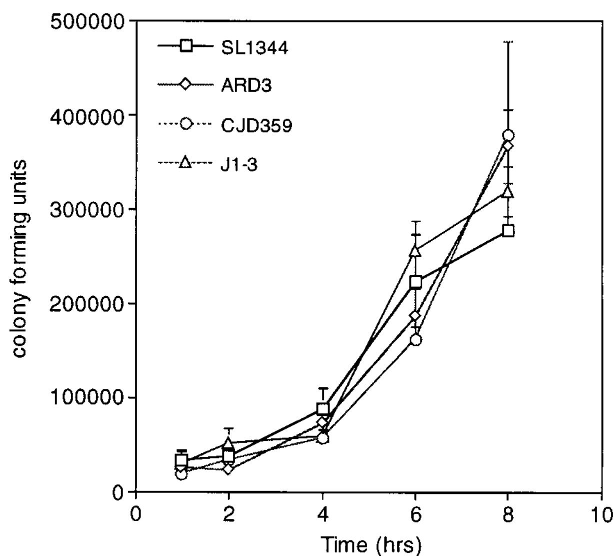

FIG. 4. Invasion and replication in HeLa cells are not affected in *ompR* mutants. This time course replication experiment shows that the *ompR* mutants (ARD3 and CJD359) are able to replicate as well as, if not better than, wild-type SL1344. Values are mean numbers of CFU recovered from three wells of HeLa cells (5×10^4 cells each) infected with *S. typhimurium* at 1, 2, 4, 6, and 8 h postinvasion. This graph depicts results from one of three representative experiments.

apparent lack of fusion of *ompR* or *sifA* mutant-containing vacuoles, which remained as individual vacuoles surrounded by an lgp-containing host membrane (Fig. 2B, D, and F). In contrast, vacuoles containing *S. typhimurium* strains able to induce Sifs generally contained multiple bacteria, presumably as a result of vacuole fusion (Fig. 2A, C, and E). In addition, since Sifs connect *Salmonella*-containing vacuoles throughout the cell (32a), lack of Sif formation may also be viewed as inhibition of fusion. The main question is, then, what selective advantage is conferred by the ability of *Salmonella*-containing vacuoles to fuse in macrophages or by Sif formation in epithelial cells? It is interesting to speculate that fusion provides for a dilution of host defense molecules or for the pooling of nutrients, but at the moment there is no clear answer to this question, especially since the absence of fusion does not seem to inhibit intracellular replication.

At the moment, we can only speculate that *S. typhimurium* uses the *ompB* locus to sense its intracellular surroundings after invasion and then react with appropriate gene expression leading to Sif formation. One of the *ompR* mutants (CJD359) and both *envZ* mutants (SDM1118 and SDM15265) were able to induce Sifs, albeit at low frequencies (8 to 24%) compared with those of wild-type SL1344 (62%). The other *ompR* mutant (ARD3) and the *ompB* deletion mutant (MS123) were completely defective for inducing Sifs (0%). *ompR* mutants were generally more reduced for induction of Sifs than *envZ* mutants. These results indicate that the *ompB* locus plays a regulatory role in the formation of Sifs rather than a direct structural role. Utilization of a two-component regulatory system to regulate intracellular virulence gene expression by *S. typhimurium* is already well documented, with the *phoPQ* system being important for macrophage survival and mouse virulence (24). We have previously found that Sif formation is not affected in HeLa cells infected with a *phoPQ* mutant (unpublished results). The *ompR-envZ* system has also previously been shown to be important for *S. flexneri* virulence gene expression and for regulation of expression of the Vi antigen of *S. typhi* without influencing invasion (2, 28). In conclusion, we have identified a two-component regulatory system (*ompR-envZ*) which affects Sif formation (independent of *ompC*, *ompF*, or *tppB*), and our findings correlate with this system's involvement in virulence in mice.

We thank A. Richter-Dalfours and C. Pfeifer for critical reading of the manuscript. We thank C. Dorman, G. Dougan, K. Sanderson, C. H. Higgins, A. Richter-Dalfours, and S. Lingren for kindly providing strains and plasmids for this study. We also thank Ken Singh (University of Alabama, Montgomery) for providing anti-OmpC antibodies and Heidi Sawatsky of Hoffman-La Roche Limited (Missassauga, Ontario, Canada) for the kind gift of alafosfalin.

This study was supported by the Medical Research Council of Canada. B.B.F. is an MRC scientist and a Howard Hughes Fellow.

REFERENCES

1. **Benson, N. R., and B. S. Goldman.** 1992. Rapid mapping in *Salmonella typhimurium* with Mu*d*-P22 prophages. J. Bacteriol. **174:**1673–1681.
2. **Bernardini, M. L., A. Fontaine, and P. J. Sansonetti.** 1990. The two-component regulatory system OmpR-EnvZ controls the virulence of *Shigella flexneri*. J. Bacteriol. **172:**6274–6281.
3. **Chatfield, S., C. J. Dorman, C. Hayward, and G. Dougan.** 1991. Role of *ompR*-dependent genes in *Salmonella typhimurium* virulence: mutants deficient in both OmpC and OmpF are attenuated in vivo. Infect. Immun. **59:**449–452.
4. **Chen, L. M., K. Kaniga, and J. E. Galán.** 1996. *Salmonella* spp. are cytotoxic for cultured macrophages. Mol. Microbiol. **21:**1101–1115.
5. **Dorman, C. J., S. Chatfield, C. F. Higgins, C. Hayward, and G. Dougan.** 1989. Characterization of porin and *ompR* mutants of a virulent strain of *Salmonella typhimurium*: *ompR* mutants are attenuated in vivo. Infect. Immun. **57:**2136–2140.
6. **Finlay, B. B., S. R. Ruschkowski, and S. Dedhar.** 1991. Cytoskeletal rearrangements accompanying *Salmonella* entry into epithelial cells. J. Cell Sci. **99:**283–296.
7. **Galán, J. E.** 1996. Molecular genetic bases of *Salmonella* entry into host cells. Mol. Microbiol. **20:**263–271.
8. **Galán, J. E., and R. Curtiss III.** 1989. Cloning and molecular characterization of genes whose products allow *Salmonella typhimurium* to penetrate tissue culture cells. Proc. Natl. Acad. Sci. USA **86:**6383–6387.
9. **Galán, J. E., and R. Curtiss III.** 1991. Distribution of the *invA*, *-B*, *-C*, and *-D* genes of *Salmonella typhimurium* among other *Salmonella* serovars: *invA* mutants of *Salmonella typhi* are deficient for entry into mammalian cells. Infect. Immun. **59:**2901–2908.
10. **Garcia del-Portillo, F., M. B. Zwick, K. Y. Leung, and B. B. Finlay.** 1993. *Salmonella* induces the formation of filamentous structures containing lysosomal membrane glycoproteins in epithelial cells. Proc. Natl. Acad. Sci. USA **90:**10544–10548.
11. **Garcia del-Portillo, F., and B. B. Finlay.** 1994. *Salmonella* invasion of nonphagocytic cells induces formation of macropinosomes in the host cell. Infect. Immun. **62:**4641–4645.
12. **Garcia del-Portillo, F., M. G. Pucciarelli, W. A. Jeffries, and B. B. Finlay.** 1994. *Salmonella typhimurium* induces selective aggregation and internalization of host cell surface proteins during invasion of epithelial cells. J. Cell Sci. **107:**2005–2020.
13. **Garcia del-Portillo, F., and B. B. Finlay.** 1995. Targeting of *Salmonella typhimurium* to vesicles containing lysosomal membrane glycoproteins bypasses compartments with mannose 6-phosphate receptors. J. Cell Biol. **129:**81–97.
14. **Gibson, M. M., M. Price, and C. F. Higgins.** 1984. Genetic characterization and molecular cloning of the tripeptide permease (*tpp*) genes of *Salmonella typhimurium*. J. Bacteriol. **160:**122–130.
15. **Gibson, M. M., E. M. Ellis, K. A. Graeme-Cook, and C. F. Higgins.** 1987. OmpR and EnvZ are pleiotrophic regulatory proteins: positive regulation of the tripeptide permease (*tpp*B) of *Salmonella typhimurium*. Mol. Gen. Genet. **207:**120–129.
16. **Groisman, E. A., and H. Ochman.** 1993. Cognate gene clusters govern invasion of epithelial cells by *Salmonella typhimurium* and *Shigella flexneri*. EMBO J. **12:**3779–3787.
17. **Hosieth, S. K., and B. A. D. Stocker.** 1981. Aromatic-dependent *Salmonella typhimurium* are non-virulent and effective as live vaccines. Nature (London) **291:**238–239.
18. **Hueck, C. J., M. J. Hantman, V. Bajaj, C. Johnson, C. A. Lee, and S. I. Miller.** 1995. *Salmonella typhimurium* secreted invasion determinants are homologous to *Shigella* Ipa proteins. Mol. Microbiol. **18:**479–490.
19. **Jones, B. D., N. Ghori, and S. Falkow.** 1994. *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. J. Exp. Med. **180:**15–23.
20. **Jones, B. D., and S. Falkow.** 1996. Salmonellosis: host immune responses and bacterial virulence determinants. Annu. Rev. Immunol. **14:**533–561.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
22. **Leung, K. Y., and B. B. Finlay.** 1991. Intracellular replication is essential for the virulence of *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **88:** 11470–11474.
23. **Lingren, S. W., I. Stojiljkovic, and F. Heffron.** 1996. Macrophage killing is an essential virulence mechanism of *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **93:**4197–4201.
24. **Miller, S. I., A. M. Kukral, and J. J. Mekalanos.** 1989. A two-component regulatory system (*pho*P and *pho*Q) controls *Salmonella typhimurium* virulence. Proc. Natl. Acad. Sci. USA **86:**5054–5058.
25. **Mills, S. D., and B. B. Finlay.** 1994. Comparison of *Salmonella typhi* and *Salmonella typhimurium* invasion, intracellular growth and localization in cultured human epithelial cell lines. Microb. Pathog. **17:**409–423.
26. **Mizuno, T., and S. Mizushima.** 1990. Signal transduction and gene regulation through the phosphorylation of two regulatory components: the molecular basis for the osmotic regulation of the porin genes. Mol. Microbiol. **4:**1077–1082.
27. **Monack, D. M., B. Raupach, A. E. Hromockyj, and S. Falkow.** 1996. *Salmonella typhimurium* invasion induces apoptosis in infected macrophages. Proc. Natl. Acad. Sci. USA **93:**9833–9838.
28. **Pickard, D., J. Li, M. Roberts, D. Maskell, D. Hone, M. Levine, G. Dougan, and S. Chatfield.** 1994. Characterization of defined *ompR* mutants of *Salmonella typhi*: *ompR* is involved in the regulation of Vi polysaccharide expression. Infect. Immun. **62:**3984–3993.
29. **Pratt, L. A., W. Hsing, K. E. Gibson, and T. J. Silhavy.** 1996. From acids to *osm*Z: multiple factors influence synthesis of OmpF and OmpC porins in *Escherichia coli*. Mol. Microbiol. **20:**911–917.
30. **Schmieger, H., and H. Backhaus.** 1976. Altered cotransduction frequencies exhibited by HT-mutants of *Salmonella*-phage P22. Mol. Gen. Genet. **143:** 307–309.
31. **Schnaitman, C. A., and G. A. McDonald.** 1984. Regulation of outer membrane protein synthesis in *Escherichia coli* K-12: deletion of *ompC* affects expression of the OmpF protein. J. Bacteriol. **159:**555–563.

32. **Singh, S. P., S. R. Singh, Y. U. Williams, L. Jones, and T. Abdullah.** 1995. Antigenic determinants of the OmpC porin from *Salmonella typhimurium*. Infect. Immun. **63:**4600–4605.
32a. **Stein, M. A., et al.** Unpublished data.
33. **Stein, M. A., K. Y. Leung, M. Zwyck, F. Garcia del-Portillo, and B. B. Finlay.** 1996. Identification of a *Salmonella* virulence gene required for formation of filamentous structures containing lysosomal membrane glycoproteins within epithelial cells. Mol. Microbiol. **20:**151–164.
34. **Sternberg, N. L., and R. Maurer.** 1991. Bacteriophage-mediated generalized transduction in *Escherichia coli* and *Salmonella typhimurium*. Methods Enzymol. **204:**18–43.
35. **Wang, R. F., and S. R. Kushner.** 1991. Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. Gene **100:**195–199.

Editor: P. J. Sansonetti