

How *Salmonella* became a pathogen

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At least 60 genes are required for virulence in the Gram-negative enteric bacterium *Salmonella enterica* (Table 1). The requirement for so many virulence determinants is thought to reflect the complex life cycle of this pathogen in infected animals¹. *Salmonella* are typically acquired through the consumption of contaminated water or food and must endure the acid pH of the stomach before they adhere to and enter the cells lining the intestinal epithelium (Fig. 1). Those invasive microorganisms destined to cause systemic disease must also survive in blood and replicate in the macrophages of the liver and spleen (Fig. 1).

For many enteric bacteria, a single DNA segment can convert the microorganism into a pathogen. For example, the determinants responsible for invasion and intercellular spreading by *Shigella flexneri* are encoded within its large virulence plasmid², and transfer of this plasmid into a laboratory strain of *Escherichia coli* will render it invasive³. Similarly, a 35-kb region [termed the locus of enterocyte effacement (LEE)] in enteropathogenic *E. coli* mediates the production of attachment and effacing lesions on intestinal epithelial cells⁴. And this phenotype can be reproduced by a laboratory strain of *E. coli* upon introduction of a plasmid carrying LEE (Ref. 5).

These experiments suggest that nonpathogenic *E. coli*, which are normal constituents of the human intestinal flora, harbor many of the genes necessary for interaction with animal cells and are, thus, predisposed to become pathogens upon acquisition of a particular virulence gene cluster. For example, P fimbriae mediate adhesion to cells of the urinary tract in uropathogenic strains of *E. coli* and are encoded by genes that are normally present in commensal strains of *E. coli*, where they increase persistence in the colon⁶. Moreover, the biosynthetic genes *argC* and *guaA* are present in benign forms of *E. coli*, yet they are both required for the survival and replication of uropathogenic strains of *E. coli* during extraintestinal infections⁷.

In further support of this notion, many of the genes implicated in *Salmonella* virulence are also present in nonpathogenic strains of *E. coli* (Table 1). These genes encode enzymes responsible for the biosynthesis of nutrients that are scarce within host tissues, transcriptional and post-transcriptional regulatory factors, pro-

In many pathogens, virulence can be conferred by a single region of the genome. In contrast, the facultative intracellular lifestyle of *Salmonella* demands a large number of genes distributed around the chromosome. The evolution of *Salmonella* has been marked by the acquisition of several 'pathogenicity islands', each contributing to the unique virulence properties of this microorganism.

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teins necessary for the repair of damaged DNA, and products necessary for defense against host microbicidal mechanisms. The presence of these genes in nonpathogenic species suggests that they promote survival within nutritionally deprived and/or potentially lethal environments that microorganisms encounter inside and outside animal hosts.

Apart from the requirement for loci that are also present in nonpathogenic species, *Salmonella* virulence demands several genes that are absent from benign microorganisms (Table 1). Although some of these genes reside on a plasmid common to many *Salmonella* serovars, the vast majority are encoded within pathogenicity

islands – large clusters of virulence genes not found in related nonpathogenic species⁸. *Salmonella*-specific virulence genes reside in several regions of the chromosome (Fig. 2) and often encode determinants responsible for establishing specific interactions with the host. Therefore, these *Salmonella*-specific genes help define the molecular basis for pathogenicity in this intracellular pathogen.

The multiple virulence features of the SPI-1 island

The best-characterized pathogenicity island of *Salmonella* is SPI-1, a 40-kb region harboring some 25 genes and mapping to the 63' region of the *S. enterica* sv. Typhimurium chromosome⁹. Genes within the SPI-1 island were originally identified because their inactivation prevented *Salmonella* from invading epithelial cells *in vitro*¹⁰. To date, 13 of these genes have been directly implicated in the invasion of nonphagocytic cells, as assayed using strains harboring non-polar mutations in SPI-1 genes. Consistent with the role of SPI-1 in the early steps of infection (i.e. the invasion of the intestinal epithelia), strains harboring mutations in the SPI-1-encoded *invA* and *orgA* genes are attenuated if inoculated orally but virulent if introduced into mice by the intraperitoneal route^{10,11}.

The SPI-1 island encodes two distinct regulatory proteins, InvF and HilA, as well as the components of a type III secretion system, termed Inv/Spa, and several proteins secreted by the Inv/Spa system⁹. The Inv/Spa secretion system is necessary for the transient formation of cell-surface appendages whose appearance is induced upon bacterial contact with host cells (Fig. 1). Proteins secreted by the Inv/Spa system stimulate a

Table 1. *Salmonella* genes necessary for virulence in mice^{a,b}

Gene	Function/ virulence phenotype	Map position ^c	Presence in <i>Escherichia coli</i> K-12	Refs
<i>crp</i>	Transcriptional control	75.0	Yes	44
<i>cya</i>	Transcriptional control	85.7	Yes	44
<i>flgM</i>	Transcriptional control	26.4	Yes	45
<i>furA</i>	Transcriptional control	16.9	Yes	46
<i>ompRenvZ</i>	Transcriptional control	76.0	Yes	47
<i>phoPQ</i>	Transcriptional control	27.4	Yes	32,48
<i>mviA</i>	Transcriptional control	38.9	Yes	49
<i>rpoS</i>	Transcriptional control	63.6	Yes	50
<i>rpoE</i>	Transcriptional control	57.7	Yes	^d
<i>slyA</i>	Transcriptional control	30.8	Yes	51,52
<i>hns</i>	Chromosome structure	38.4	Yes	53
<i>pheSThimA</i>	tRNA/transcriptional control	30.3	Yes	35
<i>recA</i>	DNA repair	62.2	Yes	54
<i>recBC</i>	DNA repair	65.2	Yes	54
<i>hemA</i>	Nutrient biosynthesis	38.8	Yes	55
<i>aroA</i>	Nutrient biosynthesis	19.9	Yes	56
<i>purA</i>	Nutrient biosynthesis	95.1	Yes	57
<i>purB</i>	Nutrient biosynthesis	27.5	Yes	57
<i>purC</i>	Nutrient biosynthesis	54.0	Yes	57
<i>purF</i>	Nutrient biosynthesis	51.7	Yes	57
<i>purG</i>	Nutrient biosynthesis	56.3	Yes	57
<i>purJHD</i>	Nutrient biosynthesis	90.8	Yes	57
<i>guaAB</i>	Nutrient biosynthesis	54.4	Yes	57
<i>thyA</i>	Nutrient biosynthesis	65.3	Yes	58
<i>carAB</i>	Nutrient biosynthesis	1.6	Yes	35
<i>eutE</i>	Nutrient biosynthesis	53.3	Yes	59
<i>eutG</i>	Nutrient biosynthesis	53.3	Yes	59
<i>tonB</i>	Nutrient uptake	38.2	Yes	60
<i>sapABCDF</i>	Peptide resistance	37.2	Yes	61
<i>sapG</i>	Peptide resistance	75.0	Yes	61
<i>sapE</i>	Peptide resistance	3.4	Yes	61
<i>sapI</i>	Peptide resistance	38.7	Yes	61
<i>sapJ</i>	Peptide resistance	87.7	Yes	61
<i>sapM</i>	Peptide resistance	6.6	Yes	61
<i>metL</i>	Nitric oxide resistance	89.4	Yes	62
<i>sodC</i>	Superoxide dismutase	NM	Yes	^e
<i>zwf</i>	Glucose-6-P dehydrogenase	NM	Yes	^f
<i>poxA</i>	Pyruvate oxidase	94.6	Yes	^g
<i>htrA</i>	Periplasmic protease	5.1	Yes	63
<i>atp</i>	ATPase	84.6	Yes	46
<i>galE</i>	LPS biosynthesis	17.8	Yes	64
<i>pml</i>	LPS biosynthesis	30.0	Yes	65
<i>rfb</i>	LPS biosynthesis	45.2	Yes	66
<i>rfc</i>	LPS biosynthesis	35.7	No	65
<i>ompD</i>	Growth within the host	33.7	NT	47
<i>pagC</i>	Survival in the macrophage	25.0	No	32
<i>msgA</i>	Survival in the macrophage	25.0	No	28
<i>tolC</i>	Host recognition/invasion	60-73	Yes	67
<i>cdt</i>	Host recognition/invasion	75.0	NT	44
<i>invA</i>	Host recognition/invasion	63.1	No	10
<i>orgA</i>	Host recognition/invasion	63.9	No	11
<i>prgH</i>	Host recognition/invasion	62.9	No	68
<i>lpfC</i>	Host recognition/invasion	80.0	No	69
<i>sifA</i>	Filamentous structure formation	27.3	No	27
<i>spiA</i>	Intramacrophage survival	30.7	No	19
<i>spiR</i>	Systemic disease	30.7	No	19,20
<i>spvABCD</i>	Growth within the host	Plasmid	No	70
<i>spvR</i>	Transcriptional control	Plasmid	No	70

^aDetermined in mice *in vivo* as a direct comparison between wild-type and mutant strains for the gene of interest. These results were obtained from several *Salmonella enterica* serovars, including Typhimurium, Enteritidis, Choleraesuis and Dublin, and do not include genes whose roles have only been assayed in tissue culture systems.

^bAbbreviations: LPS, lipopolysaccharide; NM, not mapped; NT, not tested.

^cMap positions, in minutes, were taken from the linkage map of *S. enterica* sv. Typhimurium strain LT2 (edn VIII) (Ref. 71) or primary sources.

^dT.L. Testerman and F.C. Fang (1997) Am. Soc. Microbiol. Meeting, Miami, FL, USA, Abstr. B-280.

^eM.A. de Groot *et al.* (1997) Am. Soc. Microbiol. Meeting, Miami, FL, USA, Abstr. B-279.

^fB.E. Lundberg, M.C. Dinauer and F.C. Fang (1997) Am. Soc. Microbiol. Meeting, Miami, FL, USA, Abstr. B-278.

^gK. Kaniga, P. Sundaram and R. Curtiss, III (1997) Am. Soc. Microbiol. Meeting, Miami, FL, USA, Abstr. B-289.

signal transduction cascade that elicits the internalization of bacteria into the host cell⁹. Strains defective in *inv* or *spa* genes are not invasive, and nonpathogenic *E. coli* can be passively internalized into host cells by co-infection with wild-type *Salmonella*¹².

Besides conferring the ability to enter nonphagocytic cells, an additional property has recently been ascribed to the SPI-1 island: the induction of apoptosis in *Salmonella*-infected macrophages^{13,14}. Surprisingly, apoptosis and host cell invasion exhibit several of the same phenotypic and genotypic requirements. First, macrophage cytotoxicity occurs under growth conditions that promote *Salmonella* invasion. Second, the SPI-1-encoded *hila*, *orgA*, *spaN*, *spaO*, *sipB*, *sipC* and *sipD* genes are essential for entry into nonphagocytic cells and for killing of infected macrophages^{13,14}. It is unclear at present whether *Salmonella*-induced cell death requires entry of the microorganism into the macrophage.

Several features of *Salmonella* infections suggest that the ability to survive within, rather than kill, macrophages is integral to the etiology of typhoid fever in mice. *Salmonella* are known to replicate within several types of macrophages¹⁵, and mutants that are unable to survive in macrophages *in vitro* are attenuated when inoculated into mice *in vivo*¹⁶. Furthermore, if macrophage cytotoxicity is essential for systemic infection, strains mutated in SPI-1 genes would be expected to be attenuated in mice when inoculated intraperitoneally. However, as noted above, mutants defective in the *invA* or *orgA* genes are attenuated when introduced into mice by the oral route but are fully virulent if inoculated intraperitoneally^{10,11}.

The SPI-1 island has several characteristics indicating that it was acquired by *Salmonella* through horizontal gene transfer (as opposed to being deleted and lost from the lineage leading to *E. coli*). First, the SPI-1 pathogenicity island has a base composition of only 42% G+C (Ref. 9), which is much lower than that of the *Salmonella* genome (52% G+C). (As G+C content is relatively homogeneous over the entire bacterial chromosome, regions of atypical base composition usually denote acquisition by lateral transfer.) Second, the size, order and orientation of the *inv* and *spa* genes within the SPI-1 island are broadly similar to the invasion genes on the *Shigella* virulence plasmid¹⁷, suggesting that these sequences are transmissible. The *inv* and *spa*

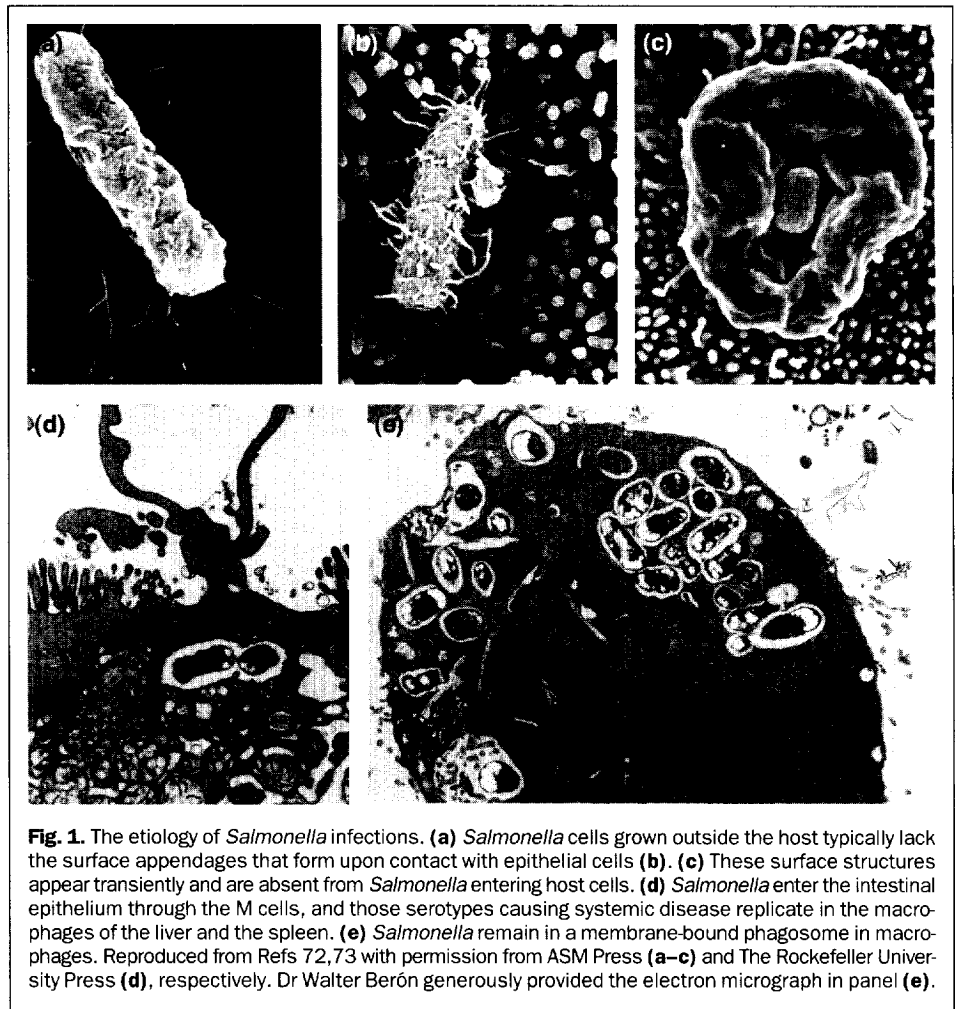


Fig. 1. The etiology of *Salmonella* infections. (a) *Salmonella* cells grown outside the host typically lack the surface appendages that form upon contact with epithelial cells (b). (c) These surface structures appear transiently and are absent from *Salmonella* entering host cells. (d) *Salmonella* enter the intestinal epithelium through the M cells, and those serotypes causing systemic disease replicate in the macrophages of the liver and the spleen. (e) *Salmonella* remain in a membrane-bound phagosome in macrophages. Reproduced from Refs 72,73 with permission from ASM Press (a–c) and The Rockefeller University Press (d), respectively. Dr Walter Berón generously provided the electron micrograph in panel (e).

genes have been recovered from representatives of all eight subspecific groups of *Salmonella*, and the phylogenetic tree based on these genes is congruent with that of several housekeeping genes¹⁸, indicating that these genes were acquired before the diversification of all extant serovars of *Salmonella* (Fig. 3). Finally, homologs of the Inv/Spa system occur in a broad range of bacterial pathogens: structurally similar type III secretion systems have been detected in *Salmonella*, *Shigella*, *Yersinia*, *Erwinia*, *Xanthomonas* and *Pseudomonas*. This phylogenetic distribution is attributable to the independent acquisition of these secretion genes by each taxon.

In summary, the SPI-1 island, which was acquired very early in the evolution of *S. enterica* (Fig. 3), encodes

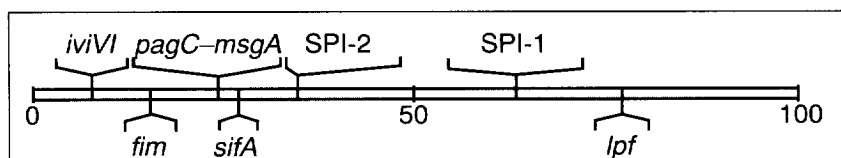


Fig. 2. Distribution of pathogenicity islands and islets in *Salmonella*. The chromosome of *Salmonella enterica* sv. Typhimurium is portrayed linearly, and the relative map positions of horizontally acquired virulence regions are shown. Abbreviation: SPI, *Salmonella* pathogenicity island.

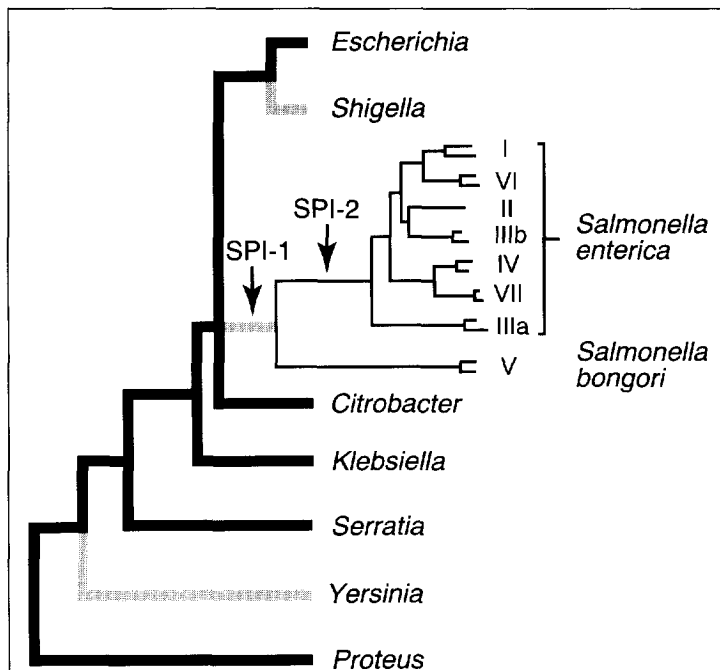


Fig. 3. Phylogenetic relationships among enteric bacteria. The branches shown in gray denote taxa that are typically capable of invading eukaryotic cells. Within *Salmonella enterica*, the acquisition of two pathogenicity islands (SPI-1 and SPI-2) is based on the phylogenetic distribution of island-specific virulence genes among strains representing the eight subspecific groups (I-VII) of this species.

determinants that mediate: (1) the invasion of non-phagocytic host cells, (2) macrophage apoptosis *in vitro* and (3) an as yet unknown function mediated by the SipA and SptP proteins, which are also secreted by the Inv/Spa secretion apparatus but are dispensable for invasion. The role of SPI-1 in invasion is supported by both *in vitro* and *in vivo* analyses; however, its putative role in bacteria-mediated macrophage apoptosis requires further investigation into the pattern of expression of SPI-1-encoded genes in host tissues during infection.

The SPI-2 island confers distinct virulence attributes in *Salmonella*

A second 40-kb pathogenicity island, designated SPI-2, has been mapped to 31' on the *S. enterica* sv. Typhimurium chromosome^{19,20}. SPI-2 contains at least 17 genes that code for a two-component regulatory system and a type III secretion system, designated Spi/Ssa (Refs 19,21), that are distinct in structure and function from the SPI-1-encoded Inv/Spa system and the type III secretion system that mediate the export and assembly of flagellar components in Gram-negative and Gram-positive bacteria.

Although the role of the SPI-2 island has not been fully elucidated, the virulence properties of mutants defective in SPI-2-encoded genes suggest that this pathogenicity island is required for systemic disease. First, mutations in several SPI-2 genes result in strains with a very high median lethal dose (LD₅₀) for mice when inoculated either orally or intraperitoneally^{19,20}, whereas mutants defective in SPI-1 are only attenuated when in-

oculated orally^{10,11}. Second, very low numbers of microorganisms are recovered from the spleen of mice infected with SPI-2 mutants when inoculated together with virulent strains²². Third, despite similarity between the sequences of the SPI-2-encoded SpiA and the SPI-1-encoded InvG proteins, a *spiA* mutant displayed wild-type levels of invasion in nonphagocytic cells but was unable to survive within macrophages¹⁹. Finally, the SPI-2-encoded *ssaH* gene is expressed within macrophages (R. Valdivia and S. Falkow, pers. commun.), which is consistent with SPI-2 being required for survival within host cells. However, not all genes contained in SPI-2 appear to be required for intramacrophage survival, and some SPI-2 mutants are slightly reduced in their ability to invade epithelial cells²¹.

The profile of proteins secreted by strains harboring mutations in SPI-2-encoded genes differs from that of the parent strain¹⁹. Most conspicuously, flagellin is 45 kDa in the wild-type strain and 47 kDa in the *spi* mutants, suggesting that the Spi system exports a protease, or a protein that modifies the activity of a secreted protease. However, flagellin is unlikely to be the relevant substrate of this protease because it is not required for *Salmonella* virulence²³, and *spi* mutants are motile. In addition, the supernatant of certain SPI-2 mutants lacks SipC, an invasion protein encoded within the SPI-1 island²¹. Although this raises the possibility that the type III secretion systems encoded within SPI-1 and SPI-2 interact, the absence of SipC from SPI-2 mutant supernatants may actually result from the unbalanced production of type III secretion proteins encoded by SPI-2 titrating components of the type III secretion system specified within SPI-1.

Analogous to SPI-1, the SPI-2 island has a low base composition (45% G+C) (Ref. 20), which suggests that it was also acquired by horizontal transfer. Furthermore, many pathogenicity islands and prophages reside near tRNA genes^{8,24}, and recent comparisons of the corresponding regions from the *E. coli* K-12 and *S. enterica* sv. Typhimurium chromosomes have revealed that the SPI-2 island inserted just downstream of a tRNA^{Val} gene at 31' (Ref. 25). However, the phylogenetic distribution of SPI-2 differs from that of SPI-1 in two respects: first, SPI-2 sequences are restricted to the genus *Salmonella*^{25,26}, whereas SPI-1-hybridizing sequences have been detected in other bacterial pathogens¹⁷. Second, several genes from within SPI-2 are not present in strains of *Salmonella bongori*, the most divergent subspecific group of *Salmonella*, but they are found in all seven remaining subspecific groups of *S. enterica*^{25,26}. Two of these *S. bongori* strains were originally isolated from nonmammalian hosts (a frog and a bird) and have not been implicated in human disease. Moreover, these *S. bongori* strains do not survive in macrophages *in vitro* (Keiichi Uchiya and Eduardo A. Groisman, unpublished), which is consistent with the macrophage survival defect displayed by *spiA* mutants of *S. enterica* sv. Typhimurium. Thus, it would appear that the SPI-2 pathogenicity island was acquired by the ancestral *S. enterica* after its split from *S. bongori* but before the diversification of groups I, II, IIIa, IIIb, IV, VI and VII (Fig. 3).

Pathogenicity islets of *Salmonella*

Several other *Salmonella*-specific sequences are essential for virulence in this facultative intracellular pathogen. These regions are much smaller than SPI-1 and SPI-2 and may be referred to as 'pathogenicity islets' (Fig. 2).

The *sifA* gene

At 27' on the *S. enterica* sv. Typhimurium chromosome, there is a 1.6-kb segment that harbors a gene, *sifA*, required for the formation of filamentous structures in the lysosomal vacuoles of infected epithelial cells²⁷. The *sifA*-containing segment is situated between the *potB* and *potC* genes, which correspond to the *potABCD* operon of *E. coli*. The housekeeping *potABCD* operon of nonpathogenic bacteria mediates polyamine uptake, whereas the *sifA* gene has no homologs in the sequence databases and is apparently restricted to *Salmonella*. Moreover, *sifA* mutants are attenuated for virulence, whereas a strain harboring a deletion in the *potC* gene could still cause a lethal infection in mice and induce the formation of filamentous structures in host cells²⁷.

Apart from its unusual genomic location, additional features of the *sifA* gene support the view that this locus was integrated into the *Salmonella* chromosome and is not ancestral to the Enterobacteriaceae. First, the base composition of the *sifA* gene is only 41% G+C, whereas the adjoining *pot* genes have G+C contents typical of the *Salmonella* genome. Second, the *sifA*-containing fragment is flanked by 14-bp direct repeats, suggesting that the incorporation of the *sifA* fragment into the *Salmonella* genome occurred by a site-specific recombination mechanism similar to those mediating the integration of phage genomes and the insertion of transposable elements.

pagC and *msgA* genes

Two virulence genes, *pagC* and *msgA*, have been localized to a low G+C region at 25' on the *S. enterica* sv. Typhimurium chromosome²⁸. The *pagC* gene encodes an outer membrane protein that is similar in sequence to several proteins found in enteric bacteria, including Ail from *Yersinia*²⁹. Although the *ail* locus of *Yersinia* has been implicated in the invasion of nonphagocytic cells³⁰, *pagC* mutants are still invasive but cannot replicate in macrophages or cause a lethal infection in mice when administered intraperitoneally^{31,32}, suggesting that the PagC protein plays a role in systemic disease.

Of the four additional genes that have been localized to the region harboring *pagC*, only *msgA* is required for intramacrophage survival and mouse virulence²⁸. Despite the similarity in their virulence phenotypes, the *pagC* and *msgA* genes differ in their regulation and phylogenetic distribution: expression of *pagC*, but not *msgA*, is dependent on the PhoP regulatory protein²⁸, and *msgA*-hybridizing sequences are detected in enteric species that lack the *pagC* gene^{28,33}.

Fimbrial genes

At least five fimbrial operons, *fim*, *agfA*, *lpf*, *ser* and *pef*, have been identified in *Salmonella*, the majority of

which have not been detected in other enteric species³⁴. Phylogenetic analysis of these sequences indicates that the *fim* operon and *agfA* gene (which specifies a *Salmonella* homolog of the *E. coli* gene encoding Curli) are ancestral to *Salmonella*. In contrast, the *lpf*, *pef* and *ser* operons are each marked by events of acquisition and/or loss by lineages or subspecific groups of *Salmonella*. The specific combination of fimbrial genes and, hence, adhesive properties of the cell appear to be associated with the host range of certain serovars and subspecies³⁴. However, the mechanism by which the presence or absence of a fimbrial operon affects the colonization of a particular host is unknown, and the observed relationship could have arisen by chance.

The sporadic distribution of fimbrial operons within *Salmonella* and the absence of hybridizing sequences from other enteric species suggest that these regions are subject to extensive transfer³⁴. Certain fimbrial operons have been detected on the virulence plasmid present in several *Salmonella* serovars, and genes encoding type 1 fimbriae map to different genomic locations in the *E. coli* and *S. enterica* sv. Typhimurium chromosomes, suggesting that fimbrial adhesins were acquired independently by these enteric species. Moreover, many fimbrial systems are flanked by short repeats, and the movement or rearrangement of these genes is thought to regulate fimbrial phase variation.

iviVI-A and *iviVI-B* genes

Two genes of low G+C content were recently recovered by *in vivo* expression technology (IVET), a procedure that allows the identification of promoter sequences induced during the course of infection³⁵. These genes, termed *iviVI-A* and *iviVI-B*, are organized in an operon that is regulated by the PhoP protein³⁶, and they may mediate the adhesion and/or invasion of host cells because the products of both genes exhibit sequence similarity to proteins that specify these traits in other pathogens. The *iviVI-AB* operon maps to 7' on the *S. enterica* sv. Typhimurium chromosome³⁶, a region that contains another *Salmonella*-specific gene of low G+C content, *sinR* (Ref. 37). However, it is unknown at present whether the *sinR* region is part of the *iviVI* virulence gene cluster.

Regulation of horizontally acquired virulence determinants

The acquisition of virulence cassettes offers a rapid means of evolving new functions. However, for the introgressed sequences to be immediately useful to the recipient microorganism, these regions need to encode regulators that control expression of the virulence genes contained within the acquired regions. It is therefore not surprising that several transcriptional regulators have been identified within the large pathogenicity islands of *Salmonella*: the SPI-1 proteins InvF and HilA control the expression of proteins involved in host cell invasion³⁸, and SPI-2 contains genes encoding a two-component regulatory system^{19,20}.

The incorporation of virulence cassettes by lateral gene transfer, however, poses a problem for the recipient microorganism because the acquired sequences are

only effective if their expression is coordinated with that of the rest of the genome and the life cycle of the microbial host. Thus, genes contained in many pathogenicity islands and islets are often controlled by regulatory systems that were present in the genome of the recipient microorganism before the acquisition of the pathogenicity island. For example, *pagC*, *iviVI-A*, the SPI-1-encoded *hilA* and the plasmid-encoded *spvB* genes are dispersed throughout the *Salmonella* genome and were acquired in independent events of horizontal transfer, but each is regulated by the PhoP/PhoQ two-component system^{32,36,38}.

Apart from the PhoP protein, two additional regulatory proteins, which are also present in nonpathogenic *E. coli*, have been implicated in the transcriptional control of horizontally acquired virulence determinants of *Salmonella*: the RpoS sigma factor regulates expression of the plasmid-linked *spv* genes³⁹, and the *Salmonella* homolog of the *E. coli* *uvrY* gene, which codes for a regulator of the two-component family, controls transcription of SPI-1-encoded invasion genes⁴⁰.

The PhoP/PhoQ system and the RpoS sigma factor respond to environmental and growth phase cues, respectively. PhoP/PhoQ is controlled by extracellular Mg²⁺ (Ref. 41), which varies from millimolar concentrations in extracellular fluids⁴² to micromolar concentrations in *Salmonella*-containing phagosomes⁴³, and RpoS modulates numerous genes when a microorganism enters the stationary phase³⁹. (The signals modulating the UvrY protein are presently unknown.) Thus, it appears that the horizontally acquired virulence genes of *Salmonella* have been appropriated by these global regulators to ensure their proper temporal and spatial expression within the host. In this manner, pathogenicity islands can become established in a recipient microorganism and provide new abilities that expand its ecological niche.

Acknowledgements

We thank Jorge Galán, Bradley Jones and Walter Berón for the electron micrographs presented in Fig. 1, and Raphael Valdivia and Stanley Falkow for communicating unpublished data. Work in our laboratories is supported by grants GM55535 (to H.O.) and GM54900 (to E.A.G.) from the NIH, and grant NRI9602159 (to E.A.G.) from the USDA. H.O. and E.A.G. are recipients of Research Career Development Awards from the NIH.

References

1 Jones, B.D. and Falkow, S. (1996) *Annu. Rev. Immunol.* 14, 533–561
 2 Parsot, C. (1994) *Curr. Top. Microbiol. Immunol.* 192, 217–241
 3 Sansonetti, P.J., Kopecko, D.J. and Formal, S.B. (1982) *Infect. Immun.* 35, 852–860
 4 McDaniel, T.K. *et al.* (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 1664–1668
 5 McDaniel, T.K. and Kaper, J.B. (1997) *Mol. Microbiol.* 23, 399–407
 6 Wold, A.E. *et al.* (1992) *J. Infect. Dis.* 165, 46–52
 7 Russo, T.A. *et al.* (1996) *Mol. Microbiol.* 22, 217–229
 8 Groisman, E.A. and Ochman, H. (1996) *Cell* 87, 791–794
 9 Galán, J.E. (1996) *Mol. Microbiol.* 20, 263–272
 10 Galán, J. and Curtiss, R. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 6383–6387
 11 Jones, B.D. and Falkow, S. (1994) *Infect. Immun.* 62, 3745–3752

12 Francis, C.L. *et al.* (1993) *Nature* 364, 639–642
 13 Chen, L.M., Kaniga, K. and Galán, J.E. (1996) *Mol. Microbiol.* 21, 1101–1115
 14 Monack, D.M. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 9833–9838
 15 Buchmeier, N.A. and Heffron, F. (1989) *Infect. Immun.* 57, 1–7
 16 Fields, P.I. *et al.* (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 5189–5193
 17 Groisman, E.A. and Ochman, H. (1993) *EMBO J.* 12, 3779–3787
 18 Li, J. *et al.* (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 7252–7256
 19 Ochman, H. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 7800–7804
 20 Shea, J.E. *et al.* (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 2593–2597
 21 Hensel, M. *et al.* (1997) *Mol. Microbiol.* 24, 155–167
 22 Hensel, M. *et al.* (1995) *Science* 269, 400–403
 23 Lockman, H.A. and Curtiss, R. (1990) *Infect. Immun.* 58, 137–143
 24 Cheetham, B.F. and Katz, M.E. (1995) *Mol. Microbiol.* 18, 201–208
 25 Hensel, M. *et al.* (1997) *J. Bacteriol.* 179, 1105–1111
 26 Ochman, H. and Groisman, E.A. (1996) *Infect. Immun.* 64, 5410–5412
 27 Stein, M.A. *et al.* (1996) *Mol. Microbiol.* 20, 151–164
 28 Gunn, J.S. *et al.* (1995) *J. Bacteriol.* 177, 5040–5047
 29 Pulkkinen, W.S. and Miller, S.I. (1991) *J. Bacteriol.* 173, 86–93
 30 Miller, V.L. and Falkow, S. (1988) *Infect. Immun.* 56, 1242–1248
 31 Miller, V.L. *et al.* (1992) *Infect. Immun.* 60, 3763–3770
 32 Miller, S.I., Kukral, A.M. and Mekalanos, J.J. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 5054–5058
 33 Hohmann, E.L. *et al.* (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 2904–2908
 34 Bäumlér, A.J. *et al.* (1997) *J. Bacteriol.* 179, 317–322
 35 Mahan, M.J., Slauch, J.M. and Mekalanos, J.J. (1993) *Science* 259, 686–688
 36 Heithoff, D.M. *et al.* (1997) *Proc. Natl. Acad. Sci. U. S. A.* 94, 934–939
 37 Groisman, E.A. *et al.* (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 1033–1037
 38 Bajaj, V. *et al.* (1996) *Mol. Microbiol.* 22, 703–714
 39 Guiney, D.G. *et al.* (1995) *Trends Microbiol.* 3, 275–279
 40 Johnston, C. *et al.* (1996) *Mol. Microbiol.* 22, 715–727
 41 García Vescovi, E., Soncini, F.C. and Groisman, E.A. (1996) *Cell* 84, 165–174
 42 Reinhart, R.A. (1988) *Arch. Intern. Med.* 148, 2415–2420
 43 Garcia-del Portillo, F. *et al.* (1992) *Mol. Microbiol.* 6, 3289–3297
 44 Kelly, S.M., Bosecker, B.A. and Curtiss, R., III (1992) *Infect. Immun.* 60, 4881–4890
 45 Schmitt, C.K. *et al.* (1994) *J. Bacteriol.* 176, 368–377
 46 Garcia-del Portillo, F., Foster, J.W. and Finlay, B.B. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 61, 4489–4492
 47 Dorman, C.J. *et al.* (1989) *Infect. Immun.* 57, 2136–2140
 48 Fields, P.I., Groisman, E.A. and Heffron, F. (1989) *Science* 243, 1059–1062
 49 Swords, W.E. and Benjamin, W.H.J. (1994) *Ann. New York Acad. Sci.* 730, 295–296
 50 Fang, F.C. *et al.* (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 11978–11982
 51 Libby, S.J. *et al.* (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 489–493
 52 Oscarsson, J. *et al.* (1996) *Mol. Microbiol.* 20, 191–199
 53 Harrison, J.A. *et al.* (1994) *Mol. Microbiol.* 13, 133–140
 54 Buchmeier, N.A. *et al.* (1993) *Mol. Microbiol.* 7, 933–936
 55 Benjamin, W.H., Jr, Hall, P. and Briles, D.E. (1991) *Microb. Pathog.* 11, 289–295
 56 Hoiseth, S.K. and Stocker, B.A.D. (1981) *Nature* 291, 238–239
 57 McFarland, W.C. and Stocker, B.A.D. (1987) *Microb. Pathog.* 3, 129–141

- 58 Smith, H.W. and Tucker, J.F. (1976) *J. Hyg.* 76, 97–108
- 59 Stojiljkovic, I., Bäumlér, A.J. and Heffron, F. (1995) *J. Bacteriol.* 177, 1357–1366
- 60 Tsois, R.M. *et al.* (1996) *Infect. Immun.* 64, 4549–4556
- 61 Groisman, E.A. *et al.* (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 11939–11943
- 62 De Groote, M.A. *et al.* (1996) *Science* 272, 414–417
- 63 Johnson, K. *et al.* (1991) *Mol. Microbiol.* 5, 401–407
- 64 Germanier, R. and Furer, E. (1971) *Infect. Immun.* 4, 663–673
- 65 Collins, L.V., Attridge, S. and Hackett, J. (1991) *Infect. Immun.* 59, 1079–1085
- 66 Nalue, N.A. and Lindberg, A.A. (1990) *Infect. Immun.* 58, 2493–2501
- 67 Stone, B.J. and Miller, V.L. (1995) *Mol. Microbiol.* 17, 701–712
- 68 Behlau, I. and Miller, S.I. (1993) *J. Bacteriol.* 175, 4475–4484
- 69 Bäumlér, A.J., Tsois, R.M. and Heffron, F. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 279–283
- 70 Gulig, P.A. *et al.* (1993) *Mol. Microbiol.* 7, 825–830
- 71 Sanderson, K.E., Hessel, A. and Rudd, K.E. (1995) *Microbiol. Rev.* 59, 241–303
- 72 Galán, J.E. and Sansonetti, P.J. (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology* (2nd edn) (Neidhardt, F.C. *et al.*, eds), pp. 2757–2773, ASM Press
- 73 Jones, B.D., Ghori, N. and Falkow, S. (1994) *J. Exp. Med.* 180, 15–23
- 74 Blanc-Potard, A-B. and Groisman, E.A. *EMBO J.* (in press)

Note added in proof

A new pathogenicity island, designated SPI-3, has recently been identified at 82' in the *S. enterica* sv. Typhimurium chromosome. SPI-3 is located downstream of *selC*, the site of insertion of the PAI-1 (pathogenicity island 1) and LEE islands in uropathogenic and enteropathogenic strains of *E. coli*, respectively, which suggests a common mechanism for the acquisition of these sequences⁷⁴.

Membrane rearrangements in fusion mediated by viral proteins

Grigory B. Melikyan and Leonid V. Chernomordik

Membrane fusion, a ubiquitous event in cell physiology, is exploited by enveloped viruses to enter their host cells. For many viruses, specialized envelope glycoproteins (fusion proteins) responsible for the fusion of the viral membrane with cellular or endosomal membranes have been identified¹. The relative simplicity of the viral fusion machinery, compared with that of intracellular fusion, should allow us not only to gain an insight into how membranes fuse in disparate cell biological processes but also to design novel antiviral drugs.

Some enveloped viruses (e.g. Sendai and HIV viruses) fuse with the plasma membrane at neutral pH, whereas others (e.g. influenza virus and baculovirus) enter the cell via an endocytotic pathway¹. In the latter category, the low pH within the endosomal compartment triggers the fusion of a viral envelope with an endosomal membrane, allowing the viral nucleocapsid to gain access to the cytoplasm. In this review, we will mainly focus on fusion reactions in which a well-defined trigger (low pH within endosomes) initiates a transformation in the fusion protein from its initially non-fusogenic conformation to a fusogenic form. Despite detailed struc-

Diverse enveloped viruses enter host cells by fusing their envelopes with cell membranes. The mechanisms of merger of lipid bilayers of two membranes mediated by influenza hemagglutinin and other viral fusion proteins apparently involve local lipidic connections that evolve into a bilayer septum in which a pore forms and expands.

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tural and biochemical characterization of some viral fusion proteins, particularly influenza hemagglutinin (HA) (for a review, see Ref. 1; Fig. 1), we have a poor understanding of the molecular mechanisms of the transient, highly localized events of membrane merger. In particular, it is still unclear what comes first in fusion: the merging of membrane lipids or the opening of a proteinaceous fusion pore that connects the aqueous compartments of two membranes. Here, we will discuss the hypothetical mechanisms of viral fusion that have mainly emerged from functional studies

aimed at arresting and characterizing intermediates of fusion preceding pore formation and deducing the structure of fusion pores from their properties and analyzing the driving forces for fusion pore enlargement. We will concentrate on HA-mediated fusion, which is the best-characterized biological fusion reaction.

Fusion – from triggering to pore enlargement

The lipid rearrangements underlying membrane fusion

Although biological membrane fusion is controlled by proteins, lipid bilayers must ultimately rearrange to