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REVIEW

Evolution of pathogenicity islands of *Salmonella enterica*

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Abstract

Virulence genes located on pathogenicity islands play a crucial role in the pathogenesis of *Salmonella enterica* infections. *Salmonella* pathogenicity islands (SPI) contribute to host cell invasion and intracellular pathogenesis. At present, 12 SPI have been described. Although size, structure and function of these SPI, as well as the distribution in *Salmonella* subspecies and serovars can be markedly different, several common motifs are present among SPI. In this review, the characteristics of SPI are described with focus on the evolution of these genetic elements.

Keywords: *Salmonella* pathogenicity island; Evolution; Invasion; Intracellular pathogenesis; Host adaptation

Content

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Introduction

Salmonella spp. is one of the most frequent bacterial food-borne pathogens of humans. *Salmonella* infections range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhoea and vomiting, to typhoid fever, a life-threatening systemic infection. In addition to the different disease outcome, there is also a remarkable range of host specificities. While certain serovars of *Salmonella enterica* cause disease in humans and a variety of animals, other serovars are highly restricted to a specific host. Current taxonomy distinguishes the species *S. enterica* with a large number of clinically important serovars and *S. bongori*, a phylogenetically older species that is rarely associated with human disease.

Salmonella infections show a complex pathogenesis. The pathogen is usually taken up by contaminated food. A variety of fimbrial adhesins are involved in initiation of contact to host cells. The first hallmark of bacteria-host cell interaction is the invasion of non-phagocytic cells, such as epithelial cells of the intestinal mucosa. *S. enterica* is also a facultative intracellular pathogen that survives phagocytosis and is able to proliferate in infected host cells within a specific compartment, the *Salmonella*-containing vacuole.

Pathogenicity islands (PAI) are genetic elements on the chromosomes of a large number of pathogens (for a recent overview, see Schmidt and Hensel, 2004) and are considered to be 'quantum leaps' in bacterial evolution (Groisman and Ochman, 1996). The acquisition of PAI by horizontal gene transfer enables bacteria to rapidly gain complex virulence functions from other species. Although PAI are diverse in structure and function, several common characteristics were observed. PAI represent distinct, often large regions on the chromosome that contain virulence genes. The acquisition of PAI by horizontal gene transfer is frequently reflected in the base composition of a PAI that is different from that of the core genome, and the association with insertion sites such as tRNA genes. A subset of PAI is genetically instable, and in these cases sequences associated with DNA mobility (integrases, transposases, direct repeats, bacteriophage genes) can be detected within the PAI.

Many of the virulence phenotypes of *S. enterica* are encoded by genes on PAI, which are referred to as '*Salmonella* Pathogenicity Islands' or 'SPI'. This includes the most prominent virulence phenotypes, i.e. host cell invasion and intracellular pathogenesis. At present, 12 different SPI have been described. Some of these SPI are conserved throughout the genus *Salmonella*, while others are specific for certain serovars. There are also chromosomal regions that are referred to as SPI with several characteristics of PAI but without a clear function in pathogenesis of salmonellosis. In the following sections, the structure, role in pathogenesis and evolutionary aspects of known SPI are described. The features of the various SPI are summarized in Table 1.

SPI-1

SPI-1 forms an insertion of 40 kb between genes that are consecutive in *E. coli* K-12 (Mills et al., 1995). The base composition of SPI-1 is significantly lower than the average G+C content of the *Salmonella* genome of 52%, but SPI-1 is not associated with a tRNA gene. SPI-1 encodes a type III secretion system (T3SS) that mediates the contact-dependent translocation of a complex set of effector proteins into eukaryotic host cells (reviewed by Galan, 2001). One subset of SPI-1 effector proteins mediates the invasion of non-phagocytic cells by *Salmonella*, a process that involves the modification of the actin cytoskeleton. A second subset is associated with the enteropathogenesis and a role of these proteins in inflammation of the intestinal epithelium and diarrhoeal symptoms was observed in animal models (reviewed by Wallis and Galyov, 2000).

SPI-1 is present in *S. bongori* and all subspecies and serotypes of *S. enterica* analyzed so far (Ochman and Groisman, 1996; Hensel et al., 1997a). Genes associated with DNA mobility are absent from SPI-1 and deletions

Table 1. Characteristics of *Salmonella* pathogenicity islands

Designation (alternative)	Size[a] in kb	Base composition %G+C (range)	Insertion point	Distribution	Variability (stability)	Virulence functions
SPI-1	39.8	47	*flhA-mutS*	*Salmonella* spp.	Conserved	T3SS, iron uptake
SPI-2	39.7	44.6	tRNA *valV*	*S. enterica*	Conserved	T3SS
SPI-3	17.3	47.3 (39.8–49.3)	tRNA *selC*	*Salmonella* spp.	Variable	Mg^{2+} uptake
SPI-4	23.4	44.8	(tRNA like)	*Salmonella* spp.	Conserved	Unknown
SPI-5	7.6	43.6	tRNA *serT*	*Salmonella* spp.	Variable	T3SS effectors
SPI-6 (SCI)	59	51.5	tRNA *aspV*	subsp. I, parts in IIIB, IV, VII	?	Fimbriae
SPI-7 (MPI)	133	49.7 (44–53)	tRNA *pheU*	subsp. I serovars	Instable	Vi antigen, pilus assembly, *sopE*
SPI-8	6.8	38.1	tRNA *pheV*	sv. Typhi	?	Unknown
SPI-9	16.3	56.7	prophage	subsp. I serovars	?	Putative toxin, unknown
SPI-10	32.8	46.6	tRNA *leuX*	subsp. I serovars	?	Sef fimbriae
SGI-1	43	48.4	*thdF–yidY*	subsp. I serovars	Variable	5 antibiotic resistance genes
HPI	?	?	tRNA *asnT* (*ychF*)	subspecies IIIa, IIIb, IV	?	High-affinity iron uptake

[a]Size of the PAI as calculated for sv. Typhi, sv. Typhimurium DT104 for SGI-1.

of portions of SPI-1 were only reported for some environmental isolates (Ginocchio et al., 1997). This led to the hypothesis that SPI-1 is a rather ancient acquisition gained at the separation of the genera *Escherichia* and *Salmonella* from a common ancestor (Bäumler, 1997). A high degree of sequence similarity was observed for genes encoding the T3SS in SPI-1 and the *mxi/spa* invasion gene cluster on the virulence plasmid of *Shigella* spp. (Groisman and Ochman, 1993), suggesting a common ancestry. More recently, a related locus was detected in *Burkholderia pseudomallei*, the causative agent of melioidosis. In contrast to functions in *Salmonella* and *Shigella*, the locus in *B. pseudomallei* is important for intracellular motility (Stevens et al., 2002).

SPI-2

The SPI-2 locus is 40 kb in size and inserted adjacent to the *valV* tRNA gene (Hensel et al., 1997a). Comparison to *E. coli* K-12 revealed a smaller species-specific element that is present at this location in *E. coli*. SPI-2 is composed of at least two distinct elements (Hensel et al., 1999b). A portion of 25 kb is only present in *S. enterica* and essential for systemic pathogenesis and has a G+C content of 43%. This element encodes a second T3SS in *S. enterica* that is activated by intracellular bacteria. Another portion of SPI-2 is 15 kb in size and was detected in *S. bongori* and *S. enterica*. This element has a G+C content of 54%, is dispensable for systemic virulence and encodes the tetrathionate reductase (Ttr) involved in anaerobic respiration (Hensel et al., 1999a).

No direct homologues with known functions in virulence have been reported for SPI-2. However, in *Yersinia pestis* a chromosomal locus was identified with high levels of sequence similarity to SPI-2 genes (Parkhill et al., 2001b). Interestingly, the *Y. pestis ysa* locus lacks the homologues of *sse* genes, that encode translocon proteins SseBCD as well as translocated effectors SseFG. In light of these observations, it is questionable whether a functional T3SS is encoded by the *ysa* locus. Comparison between T3SS of various pathogens placed the SPI-2-encoded system in a group with the T3SS of EPEC, but only a distant relation to the SPI-1-encoded T3SS was observed (Hensel et al., 1997b; Foultier et al., 2002). This observation suggests that the SPI-1- and SPI-2-encoded T3SS result from independent events of horizontal gene transfer, but not from duplication of a gene cluster. The *ttr* gene cluster was detected in genome sequences of several species (*Haemophilus somnus*, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Vibrio parahaemolyticus*, etc.).

SPI-3

A further *Salmonella*-specific locus was identified by analysis of the tRNA *selC* locus, that is also an insertion point for PAI in *E. coli* and *Shigella* spp. (McDaniel et al., 1995; Blanc-Potard and Groisman, 1997). SPI-3 is a *Salmonella*-specific insertion of 17 kb with a G+C content of 47.5% (Blanc-Potard et al., 1999). The major virulence function encoded by SPI-3 is the MgtCB high-affinity Mg^{2+} uptake system that is required for adaptation to the nutritional limitations of the intra-phagosomal habitat (Blanc-Potard and Groisman, 1997). Further putative virulence determinants are MisL (similar to AIDA-I autotransporter) and MarT (similar to *Vibrio cholerae* ToxR).

SPI-3 is conserved between *S. enterica* sv. Typhi and Typhimurium. Analyses of the distribution of SPI-3 in various subspecies (Blanc-Potard et al., 1999) and serotypes (Amavisit et al., 2003) revealed extensive variations in the structure of SPI-3, ranging from deletions to insertions of additional gene clusters. These variations were mainly located in the portion of SPI-3 adjacent to *selC*, while a portion containing *mgtCB* and various other genes appeared to be conserved in various isolates (Amavisit et al., 2003). It was also detected in the genome sequence of *S. bongori*. Genes in the central region of SPI-3 are flanked by remnants of IS elements, indicating an insertion within this SPI (Blanc-Potard et al., 1999). The variability of SPI-3 in *Salmonella* and the observation of insertions of different PAI at this locus in various pathogens indicate that *selC* is a hot spot for the integration of foreign DNA elements.

SPI-4

SPI-4 is an insertion of 27 kb and is located adjacent to a tRNA-like *ssb* gene (Wong et al., 1998). The role of SPI-4 in *Salmonella* virulence has not yet been analyzed in detail, but several putative virulence factors are present, e.g. a putative type I secretion system and ORFs with weak similarity to RTX toxins.

SPI-4 appears to be conserved between various serovars of *S. enterica* (Wong et al., 1998; Amavisit et al., 2003). However, comparison of genome sequences revealed differences in the organization of SPI-4 in sv. Typhi and Typhimurium (Parkhill et al., 2001a).

SPI-5

SPI-5 is a small locus of 7.6 kb that is inserted adjacent to tRNA *serT*. SPI-5 encodes effector proteins for both, the T3SS encoded by SPI-1 and SPI-2. SopB is translocated by the SPI-1-encoded T3SS and expression of *sopB* is under control of HilA, the central transcriptional regulator of SPI-1. SopB is an inositol phosphatase involved in triggering fluid secretion resulting in diarrhoeal symptoms. In contrast, PipB is a translocated effector of the SPI-2-encoded T3SS under control of the SsrAB two-component system.

SPI-5 has a mosaic structure. The *sopB* gene is present in *S. bongori* and all subspecies of *S. enterica*. In contrast, a portion of SPI-5 harbouring *pipAB* is absent from *S. bongori* and *S. enterica* subspecies II (Knodler et al., 2002). There is also a difference in base composition in the different portions of SPI-5, supporting the hypothesis of independent acquisition of two elements at tRNA *serT*. As observed for other effector proteins, the presence of genes encoding the effector proteins correlates with the presence of the PAI encoding the cognate T3SS.

SPI-6 or *Salmonella* chromosomal island (SCI)

A locus of 59 kb in the genome sequence of sv. Typhi (Parkhill et al., 2001a) has been termed SPI-6 and subsequently SCI for sv. Typhimurium (Folkesson et al., 2002). SPI-6 is inserted adjacent to the *aspV* tRNA gene and contains the *saf* gene cluster for fimbriae, *pagN* encoding an invasin and several genes of unknown function. Deletion of the entire SCI locus in sv. Typhimurium had no effect on systemic pathogenesis, but a reduced invasion of cultured cells was observed (Folkesson et al., 2002).

The SCI locus was detected in *S. enterica* subspecies I, and the presence of portions of SCI at the tRNA *aspV* locus of isolates of subspecies IIIb, IV and VII was taken as an indication for the mosaic structure of this PAI. There is partial synteny between SPI-6 and PAI OI#7 of enterohemorrhagic *E. coli* that is also associated with the *aspV* tRNA gene. Further homologues of SPI-6 genes were identified in the *P. aeruginosa* and *Y. pestis* genome sequences, but the function of the homologues in these pathogens is also unknown.

SPI-7 or major pathogenicity island (MPI)

SPI-7 is a locus that is specific for sv. Typhi, Dublin and Paratyphi C. The locus was also referred to as MPI of sv. Typhi (Zhang et al., 1997). SPI-7 is 133 kb in size and inserted adjacent to tRNA *pheU* (Hansen-Wester and Hensel, 2002). An important virulence factor encoded by SPI-7 is the Vi antigen, a capsular exopolysaccaride. The *sopE* phage encoding effector protein SopE of the SPI-1-encoded T3SS is present in SPI-7. A further putative virulence factor is a type IVB pilus encoded by the *pil* gene cluster.

The genetic organization of SPI-7 is rather complex and indicates that this locus is composed of different horizontally acquired elements. The presence of *pil*, *tra* and *sam* genes indicates that SPI-7 originated from a conjugative plasmid or conjugative transposon. A recent analysis by Pickard et al. (2003) demonstrated that a portion of SPI-7 is also present in several other bacteria including the plant pathogen *Xanthomonas axonopodis* pathovar citri and *Pseudomonas aeruginosa* SG17 M, in which the locus is referred to as PAGI-3. Furthermore, loss of the Vi capsule phenotype can be observed in isolates of sv. Typhi, suggesting instability of the SPI-7 locus. The extensive synteny between SPI-7 of *S. enterica* and related loci in plant pathogens and *P. aeruginosa* was considered to be an indication for the acquisition of the locus by contact between *Salmonella* and environmental bacteria (Pickard et al., 2003).

The *sopE* phage is also present in a subset of *S. enterica* subspecies I isolates that lack SPI-7, and it was demonstrated that the *sopE* phage can be activated and transfers the *sopE* gene to other isolates (Mirold et al., 1999). The absence of the *sopE* phage in the SPI-7 of sv. Dublin and Paratyphi C indicates that the insertion in SPI-7 of sv. Typhi is rather recent.

SPI-8

SPI-8 is a further locus that was identified in the genome sequence of *S. enterica* sv. Typhi. The locus consists of 6.8 kb and is located adjacent to the *pheV* tRNA gene (Parkhill et al., 2001a). Putative virulence factors are bacteriocin genes, but no functional data have been reported so far.

The presence of a gene encoding an integrase indicates the mobility of this element. SPI-8 appears to be specific for sv. Typhi, however, the distribution has not been investigated in detail.

SPI-9

This locus of 16,281 bp is located adjacent to a lysogenic bacteriophage in the chromosome of sv. Typhi (Parkhill et al., 2001a). Putative virulence factors encoded by SPI-9 are a type I secretion system and a large RTX-like protein.

This locus is also present on the chromosome of sv. Typhimurium, however, the ORF for the RTX-like toxin appears to be a pseudo-gene. Parts of SPI-9 and the adjacent bacteriophage genome are also present in the incomplete genome sequences of other serovars and *S. bongori*, indicating a conserved distribution of this island.

SPI-10

SPI-10 is a large insertion of 32.8 kb located at tRNA *leuX*. There is also a cryptic bacteriophage present within SPI-10 (Parkhill et al., 2001a). Known virulence factors encoded by SPI-10 are the Sef fimbriae.

The distribution of Sef fimbriae is restricted to a subset of serovars including sv. Typhi and Enteritidis and is considered as one factor that determines host specificity (Townsend et al., 2001). The role of the bacteriophage in distribution of SPI-10 has not been elucidated so far.

Salmonella genomic island 1 (SGI-1)

The emergence of multidrug-resistant strains such as DT104 is currently a major problem associated with *Salmonella* infections. The characterization of resistance factors of such isolates led to the identification of a genomic island in multidrug-resistant stains of *S. enterica* serovars Typhimurium DT 104, Paratyphi B and Agona. This locus termed *Salmonella* genomic island 1 (SGI-1) has a size of 43 kb, is flanked by DR and not associated with a tRNA gene (Boyd et al., 2001). The base composition of SGI-1 is variable, with regions of significantly lower and higher G + C-content than the genome average of sv. Typhimurium. Within SGI-1, genes conferring the penta-resistance phenotype (i.e. resistances to tetracycline, ampicillin, chloramphenicol, streptomycin, and sulfonamides) are clustered in the multi-drug resistance region, which is composed of two integrons. Furthermore, a cryptic retronphage was identified in SGI-1. In contrast to plasmid-borne antibiotic resistance factors, the chromosomal SGI-1 appears to be stable in the absence of selective pressure.

The presence of SGI-1 in *S.* Typhimurium DT104 can be associated with a worldwide epidemic appearance of this multidrug-resistant strain. Genes associated with DNA mobility such as transposase, integrase, and excisionase genes with sequence similarities to transposon genes have been detected in SGI-1. Variants of SGI-1 were identified in other serovars at the same chromosomal locations, indicating horizontal transfer and site-specific recombination. Most recently, a new variant of SGI-1 was identified in sv. Albany (Doublet et al., 2003). In this variant, one integron in SGI-1 containing the streptomycin resistance gene was replaced by an integron with the trimethoprim resistance gene. These observations indicate that chromosomal antibiotic-resistance loci are recent acquisitions and still in the process of genetic exchange leading to adaptation to antibiotic resistance.

High pathogenicity island (HPI)

The HPI is a typical PAI that was initially identified and characterized in detail in highly pathogenic isolates of *Yersinia enterocolitica* and *Y. pseudotuberculosis* (see review by Schubert et al. (2004) in this issue). HPI encodes the biosynthesis pathway for a siderophore and the cognate iron uptake system. HPI was detected in a variety of other Gram-negative species and its presence appears to be correlated with the ability of strains to cause septicaemic infections. Recently, the presence of HPI in subspecies IIIa, IIIb and IV of *S. enterica* was reported (Oelschlaeger et al., 2003). However, HPI is absent from human-adapted subspecies I isolates, and the role of HPI in subspecies IIIa remains to be analyzed.

Conclusions

The large number of SPI identified in *Salmonella* are indications for the adaptation of a prototrophic, free-living species to a complex pathogenic life-style. *Salmonella* spp. can occur as gastrointestinal commensal, but can cause infections by penetration of the gastrointestinal mucosa and adaptation to extremely different habitats ranging from intracellular locations in various cell types to the persistence in biofilms on gall stones. During speciation of the genus *Salmonella*, different genetic elements were acquired and efficiently combined. For example, about 7.8% of the 4.8 Mb chromosome of *S. enterica* sv. Typhi consist of PAI (Table 1). A similar complexity and high number of PAI is only found in few pathogens, namely certain pathotypes of *E. coli* and in *S. aureus* (Schmidt and Hensel, 2004).

Comparison of structural features and evolutionary characteristics indicated that SPI extend over a range of ancient horizontal acquisitions such as SPI-1 that became stable constituents of the *Salmonella* genome to very variable elements such as SGI that are still in the process of extensive alteration and horizontal distribution.

The majority of SPI, i.e. SPI-2 to SPI-8, SPI-10 and HPI are associated with tRNA loci. This observation demonstrates the role of these loci as insertion points for DNA elements acquired by horizontal gene transfer and may indicate that certain loci functioned as hotspots for repetitive insertion of horizontally acquired DNA elements. Genome-based screening for further *Salmonella*-specific insertions at tRNA genes identified further putative PAI with unknown functions in pathogenesis (Hansen-Wester and Hensel, 2002).

A further common feature of SPI is their genetic stability. Genes associated with DNA mobility are absent in the majority of SPI and this observation correlates with the high stability of SPI. Most SPI have become part of the core set of genes of *S. enterica* and encode species-specific traits. A smaller subset of SPI is limited to certain subspecies or even specific serovars. These SPI harbour genes associated with DNA mobility and are likely to represent more recent acquisitions. It should be mentioned that the stability has not been analyzed systematically for several of the SPI described here, and it is possible that SPI associated with bacteriophages such as SPI-7 or SPI-10 are less stable than SPI-1 to SPI-5.

Significant structural and functional heterogeneity was observed within most of the SPI loci. Examples are the *sit* genes (SPI-1) encoding an iron uptake system and the *ttr* genes (SPI-2) encoding an enzyme system for anaerobic respiration, while other portions of both SPI encode T3SS. The organization of SPI-3 is highly variable in different subspecies of *Salmonella*. These genetic mosaics are indicative for repetitive insertion of foreign DNA elements of various origins into the same insertion site, e.g. at a tRNA gene. Obviously, this situation complicates the definition of functions of SPI genes and the understanding of evolutionary events.

A remarkable observation is the functional interaction of SPI-1 and SPI-2 with further loci encoding effector proteins, including SPI-5. Both SPI-1 and SPI-2 are fixed and stable PAI encoding T3SS and subsets of effector proteins. The majority of the effector proteins are encoded by genes on individual loci outside of SPI-1 and SPI-2. Some of the effector genes are located on mobile DNA elements, as elegantly demonstrated for the *sopE* phage encoding an effector protein for the SPI-1-encoded T3SS (Mirold et al., 1999, 2001). An even more complex set of effector proteins was identified for the SPI-2 system (Miao and Miller, 2000) and some of the loci are also associated with mobile DNA elements (Hansen-Wester et al., 2002).

The acquisition of most SPI in *S. enterica* occurred millions of years ago (Bäumler, 1997), but we also observe that the current acquisition of new SPI leads to the emergence of new antibiotic-resistant epidemic strains. While the roles in pathogenesis of some SPI are well defined, the function in virulence of many genes within SPI is not understood. It is conceivable that several of these SPI contribute to host specificity. This may also apply to the pool of effector genes of the SPI-1- and SPI-2-encoded T3SS, the functions of which are only partially elucidated so far.

Although the contributions of SPI to pathogenesis can be very different, several common motifs were identified between SPI. In conclusion, the acquisition of a large number of PAI had a pivotal role in the evolution of *S. enterica* to a highly successful pathogen. Understanding of the functions of SPI and the interaction with further mobile genetic elements will broaden

our understanding of the molecular mechanisms of virulence of *S. enterica*.

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