BACTERIAL PATHOGENICITY

Distribution and structural variation of the *she* pathogenicity island in enteric bacterial pathogens

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Shigella flexneri serotype 2a carries a chromosomal pathogenicity island (PAI), termed the *she* PAI, that has been implicated in the pathogenesis of diarrhoeal disease [1, 2]. The complete nucleotide sequence and genetic organisation of the *she* PAI of *S. flexneri* 2a strain YSH6000T was determined recently [3]. In the current study the distribution and structure of the *she* PAI was investigated by PCR and Southern analysis in 65 isolates of enteric pathogens including *Shigella* spp., enterohaemorrhagic *Escherichia coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), *Yersinia enterocolitica* and *Salmonella enterica* serovar Typhimurium. The study showed that the *she* PAI has undergone a variety of structural changes, defined by the presence or absence of specific marker genes in the PAI. The *she* PAI or structural variants of this element were found in all species of *Shigella* as well as in EIEC, EHEC and EPEC. No evidence of the PAI was found in *Y. enterocolitica* or *Sal. Typhimurium*. The structural form of the *she* PAI that exists in strain YSH6000T was present in all strains of *S. flexneri* serotype 2a and in some strains of *S. flexneri* serotypes 2b and 3c. Variants of the PAI that were missing one or more marker regions were found in all species of *Shigella* and in pathogenic strains of *E. coli*. In all strains, the PAIs have inserted into either *pheV* or a *phe* tRNA gene in another location on the chromosome. It was concluded that the *she* PAI is one of several closely related genetic elements that have disseminated throughout *Shigella* and pathogenic strains of *E. coli* and diverged into distinct structural forms.

Introduction

The *Shigella* spp. are gram-negative bacteria that cause bacillary dysentery, a diarrhoeal disease responsible for the deaths of more than 1.1 million people every year [4]. Infections are transmitted *via* the faecal-oral route usually as a result of direct person-to-person contact or through the consumption of contaminated food or water [5]. Infected individuals develop a watery diarrhoea that often progresses to the bloody, mucoid diarrhoea typical of bacillary dysentery [6]. Although shigellosis is caused by all four species of *Shigella*, *S. flexneri* serotype 2a appears to be the most prevalent endemic strain in developing countries [4].

In *S. flexneri* 2a, some of the genes implicated in the pathogenesis of diarrhoea are carried on the *she*

pathogenicity island (PAI) [1, 2]. These include *set1A* and *set1B* (Fig. 1) that encode the two subunits of ShET1 enterotoxin and *sigA* that encodes a cytopathic, autotransported protease that contributes to fluid accumulation in a rabbit ileal loop model of infection [2]. A third putative virulence gene, *pic*, encoding a mucinase with haemagglutinin activity [7], is also carried on the PAI [1].

The complete nucleotide sequence and genetic organisation of the *she* PAI was determined recently and revealed that it has many of the features that are characteristic of PAIs from various gram-negative pathogens [3]. Such features include the carriage of IS elements, plasmid-related sequences and phage-related sequences including a phage-like integrase gene termed *int* situated near the *pheV*-proximal boundary of the PAI (Fig. 1). The *she* PAI appears to have been acquired by lateral gene transfer and, like several other PAIs, has a chromosomal insertion site in a tRNA gene, *pheV*. The *she* PAI is an unstable genetic element that spontaneously excises from the chromosome at

Received 23 Nov. 2000; revised version received 9 April 2001; accepted 9 April 2001.
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Fig. 1. Genetic organisation of the *she* PAI. Arrows show the position and direction of transcription for each open-reading frame. ORFs are labelled by name or number, or both. Genes similar to the putative prophage 933L from the LEE PAI of EHEC strain EDL933 are indicated by the black bar. Bulleted lines indicate regions A–H amplified in linkage analyses.

frequencies of 10^{-5} – 10^{-6} [1]. The excision of the PAI reconstitutes the interrupted *pheV* gene but results in a single base pair deletion 5 bp from the 3' terminus of the gene [3]. By analogy to prophages and conjugative transposons, excision of the PAI may be dependent on the phage-related integrase gene, *int*. This excision event may represent the first step in the lateral transfer of the PAI. However, transmission of the PAI from one host to another has not yet been demonstrated experimentally.

Recent discoveries that PAIs may disseminate to a wide range of bacterial genera [8–10] and may diverge in their genetic organisation [11, 12] prompted an investigation of the distribution and structure of the *she* PAI in a range of gram-negative bacterial enteric pathogens. PCR and Southern analysis were used to identify four distinct marker regions of the *she* PAI, and to investigate whether the *she* PAI – which until now has been identified only in *S. flexneri* 2a – is present in other members of the genus *Shigella* and other diarrhoeal pathogens including *Yersinia enterocolitica*, *Salmonella enterica* serovar Typhimurium and pathogenic strains of *Escherichia coli*.

Materials and methods

Bacterial strains and growth conditions

Bacterial strains used in this study are listed in Table 1 [13]. All strains were maintained at 37°C in Luria-Bertani medium except for *Y. enterocolitica* which was maintained at 30°C.

General molecular techniques

Genomic DNA from bacterial cells that were grown overnight was prepared as described previously [14]. *EcoRI* digestions were performed as described by the suppliers – Roche Molecular Biochemicals (Basel, Switzerland) or NEB (Beverly, MA, USA). DNA

probes for Southern hybridisation consisted of the digoxigenin-labelled PCR products amplified from strain YSH6000T with the DIG DNA labelling and detection kit supplied by Roche Molecular Biochemicals. Hybridisations were performed as described by Southern [15].

PCR assays

The presence of *int*, *sigA*, *pic* and *orfs* 28–31 on the *she* PAI was determined by PCR. PCR primers were designed with reference to the *she* PAI of strain YSH6000T [3]. Sixteen pairs of primers used for amplification of the above sequences and additional regions on the *she* PAI are listed in Table 2.

Results

Distribution and structural variation of the *she* PAI in *Shigella* spp.

Forty-seven strains, representing all four species and several serotypes of *Shigella*, were surveyed by PCR for the presence of four marker regions present within the *she* PAI of *S. flexneri* 2a strain YSH6000T. The four marker regions spanning the PAI included an internal region of the *int* gene which is situated at the left extremity of the PAI, a region spanning the phage-related *orfs* 28–31 at the right extremity of the PAI and intragenic sequences of the *sigA* and *pic* genes located in internal regions of the PAI (Fig. 1). With oligonucleotide primers (Table 2), one or more of the four marker regions were amplified by PCR from 38 (81%) of the 47 *Shigella* strains tested. The *int* marker was present in all strains that carried markers for the PAI (Table 3). Furthermore, in all but one strain, the *she* PAI marker regions appeared to be of identical length to those of strain YSH6000T (data not shown). However, in one serotype 9 strain of *S. dysenteriae*, SBA1395, the *int* marker fragment was 1.8 kb in length rather than 0.4 kb as in YSH6000T (Table 3), sug-

Table 1. Bacterial strains

Strain no	Genus and species	Source*
SBA1273 (YSH6000T)	<i>S. flexneri</i> 2a	see ref. [13]
SBA1317	<i>S. flexneri</i> 2a	1
SBA1318	<i>S. flexneri</i> 2a	1
SBA1319	<i>S. flexneri</i> 2a	1
SBA1320	<i>S. flexneri</i> 2a	1
SBA1322	<i>S. flexneri</i> 2a	1
SBA1388	<i>S. flexneri</i> 2a	2
SBA1402	<i>S. flexneri</i> 2a	1
SBA1404	<i>S. flexneri</i> 2a	1
SBA1299	<i>S. flexneri</i> 1a	2
SBA1173	<i>S. flexneri</i> 1b	1
SBA1300	<i>S. flexneri</i> 2b	2
SBA1316	<i>S. flexneri</i> 2b	1
SBA1390	<i>S. flexneri</i> 2b	2
SBA1401	<i>S. flexneri</i> 2b	1
SBA1407	<i>S. flexneri</i> 3a	1
SBA1389	<i>S. flexneri</i> 3a	2
SBA1297	<i>S. flexneri</i> 3b	2
SBA1298	<i>S. flexneri</i> 3c	2
SBA1403	<i>S. flexneri</i> 4	1
SBA1391	<i>S. flexneri</i> 4a	2
SBA1408	<i>S. flexneri</i> 4a	1
SBA1387	<i>S. flexneri</i> 5	2
SBA1405	<i>S. flexneri</i> 6	1
SBA1406	<i>S. flexneri</i> 6	1
SBA1381	<i>S. boydii</i> 1	2
SBA1382	<i>S. boydii</i> 2	2
SBA1384	<i>S. boydii</i> 4	2
SBA1308	<i>S. boydii</i> 4	2
SBA1383	<i>S. boydii</i> 3	2
SBA1385	<i>S. boydii</i> 7	2
SBA1386	<i>S. boydii</i> 8	2
SBA1393	<i>S. dysenteriae</i> 1	2
SBA1395	<i>S. dysenteriae</i> 9	2
SBA1306	<i>S. dysenteriae</i> 3	2
SBA1394	<i>S. dysenteriae</i> 3	2
SBA1397	<i>S. dysenteriae</i> 4	2
SBA1398	<i>S. dysenteriae</i> 5	2
SBA1396	<i>S. dysenteriae</i> 6	2
SBA1375	<i>S. sonnei</i>	2
SBA1376	<i>S. sonnei</i>	2
SBA1377	<i>S. sonnei</i>	2
SBA1378	<i>S. sonnei</i>	2
SBA1379	<i>S. sonnei</i>	2
SBA1400	<i>S. sonnei</i>	1
SBA1399	<i>S. sonnei</i>	1
SBA1301	<i>S. sonnei</i>	2
AL21	<i>Sal. Typhimurium</i>	3
AL22	<i>Sal. Typhimurium</i>	3
AL23	<i>Sal. Typhimurium</i>	3
AL27	<i>Y. enterocolitica</i>	3
AL28	<i>Y. enterocolitica</i>	3
AL29	<i>Y. enterocolitica</i>	3
AL24	EHEC O157:H	3
AL25	EHEC O157:H	3
AL26	EHEC O157:H	3
AL30	EPEC O111:H	3
AL31	EPEC O119:H	3
AL32	EPEC O127:H6	3
SBA885	EIEC 28ac	3
SBA886	EIEC 167	3
SBA887	EIEC 112ac	3
SBA889	EIEC O28	2
SBA890	EIEC O29	2

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gesting that an insertion into the *int* gene had occurred in this strain.

Many *Shigella* strains carried an incomplete complement of the *she* PAI markers (Table 3). However, in 9 (19%) of the 47 *Shigella* strains, belonging to *S. flexneri* and *S. dysenteriae*, none of the marker regions was detected by PCR, indicating that none of these strains carried the PAI. To exclude the possibility that this was due to target sequence variations preventing the PCR detection of the markers, the same strains were analysed by Southern hybridisation. As *int* is common to all *Shigella* strains carrying markers of the PAI, strains were probed with a fragment of the *int* gene. Southern analysis with the *int* probe confirmed that although the positive control strain (YSH6000T) carried *int*, none of the nine test strains carried the gene (data not shown), supporting the proposition that the *she* PAI and structural variants of the PAI were absent from these strains.

The full complement of *she* PAI markers was found in all nine *S. flexneri* 2a strains, two of the four serotype 2b strains (SBA1390 and SBA1300) and the single serotype 3c strain of *S. flexneri* (SBA1298) tested (Table 3). To determine whether the four marker regions in the serotype 2b and 3c strains constituted an intact, ordered set of genes representing the *she* PAI, the study tested whether they were physically linked to each other as they are in the control strain YSH6000T (Fig. 1). One serotype 2b strain, SBA1300, the serotype 3c strain, SBA1298, and the serotype 2a strain YSH6000T were analysed by PCR with primers (Table 2) that amplify a set of regions designated A–H, extending across the 46-kb *she* PAI (Fig. 1). In both test strains, PCR products corresponding to regions A–H in strain YSH6000T were amplified with the appropriate primers and, with the exception of region A in strain SBA1298, all were of the same length (Table 4). These results demonstrated the presence of elements in strains SBA1300 and SBA1298 that appear to have similar structures to the *she* PAI.

In a single *S. flexneri* 2b strain, SBA1316, all the *she* PAI marker regions except for *orfs* 28–31 were present (Table 3). However, in most *Shigella* strains two or more of the *she* PAI markers, including *orfs* 28–31, were not amplified (Table 3). The *int* marker occurred more frequently than *sigA*, while *sigA* occurred more frequently than *pic*, suggesting that the right end of the *she* PAI may be unstable and undergo deletions of varying lengths to yield a variety of structural forms of the PAI. Alternatively, such structural variations may have occurred by the sequential acquisition of sequences in various strains.

A subset of these strains, representing each *Shigella* spp., was analysed to determine whether *int* and *sigA* were linked, as they are in strain YSH6000T. *S. flexneri* 6 (SBA1405), *S. boydii* 1 (SBA1381), *S. dysenteriae* 3

Table 2. Primers used in this study

Target DNA	Primer name	Primer sequence (5'–3')	PCR product amplified from strain YSH6000T (kb)	5' position of primers
<i>int</i>	BAP1329	AAAGGATCCCGGACCTGCTATCCCTG	0.4	1697
	BAP1330	ATCTTGGATCCCGGTGTGAG		2097
<i>int</i> + left junction	BAP1330	ATCTTGGATCCCGGTGTGAG	1.2	2097
	BAP1258	GCAATAGCGGCAAAGTGATGATAG		903
left junction + <i>pheV</i>	BAP1258	GCAATAGCGGCAAAGTGATGATAG	0.13	903
	BAP 1486	TTCAATCCCCTGCTCTACCG		1035
<i>int</i> + <i>phe</i>	BAP1330	ATCTTGGATCCCGGTGTGAG	1.2	2097
	BAP1434	TCAGTCGGTAGAGCAGGGGATTG		1011
<i>int</i> + upstream of <i>pheR</i>	BAP1330	ATCTTGGATCCCGGTGTGAG	1.2	2097
	BAP1459	GCTTCGCCCCTGCCGCC		–
<i>sigA</i>	BAP1147	ATCCTCGGTATTATTTATCCTCC	3.0	9077
	BAP1153	GCGGGCTGTGACTTTCC		12598
<i>pic</i>	BAP1403	AGCCCTCAAGAAGACTGCC	2.3	17639
	BAP1404	ATTCTTCTGGCTGGCATTCC		20279
ϕ region (<i>orfs</i> 28–31)	BAP1054	GCAGGAGCCCCGTCGCAG	1.8	45805
	BAP1435	TGTTTCGCTTCGCTGATTTC		47123
Region A	BAP1631	TGGTTCCCGTTACCTGGCGTCTCCG	7.8	1371
	BAP1632	GCACCCGGTCTGAACCTCTCTCTG		9213
Region B	BAP1476	CAGGATCGCGGAATGAGCGTGACC	2.4	12482
	BAP1488	TGGCTCTTCGCGXAACCTGGG		14866
Region C	BAP1477	CCCTTTACCTGACTATGGAGCCCC	4.3	19647
	BAP1487	TGCCAACCGACATCACCACG		15333
Region D	BAP1484	GACATTCCACCAGTTCGTAGCACC	6.2	19509
	BAP1485	GCCAGAGCCACAGGATTTCG		25800
Region E	BAP836	CCAGGCATACCCGACCAG	5.8	25859
	BAP1073	ATCGTCGAAACGCCGAGCAGG		31829
Region F	BAP1016	ATGCCGGTGAAGGTGGTGCCTC	5.8	31080
	BAP1209	TGCTCCAGGCGGTTTGTG		36915
Region G	BAP1212	CTGGGAACCTGGATACGCTGG	4.8	35325
	BAP1017	CGCAGGAGGCACAGATACGAAG		40174
Region H	BAP1313	GCAACGGCGAATGGTCTG	6.7	40309
	BAP1435	TGTTTCGCTTCGCTGATTTC		47014

(SBA1306) and *S. sonnei* (SBA1375) were analysed by PCR for the presence of the *she* PAI region A which links *int* and *sigA* (Fig 1). The *int* gene was found to be linked to *sigA* in *S. sonnei* strain SBA1375, although the length of the *int*–*sigA* intergenic region was greater than that of *S. flexneri* strains (Table 4). However, the linkage of *int* and *sigA* could not be confirmed in *S. flexneri* 6 strain SBA1405, *S. boydii* 1 strain SBA1381 or *S. dysenteriae* 3 strain SBA1306, by the same approach. This finding suggested that either major rearrangements of the genes had occurred or, as a consequence of large insertions into the *int*–*sigA* intergenic region, the *int*–*sigA* fragment was too long to be amplified by PCR. To investigate the latter possibility, chromosomal DNA from each strain was digested with *EcoRI* and analysed by Southern blotting with both *int* and *sigA* probes. Both the *int* and *sigA* genes were found to reside on *EcoRI* fragments of 15 kb in strain SBA1306 and 13 kb in strain SBA1405, suggesting that the two genes were linked in these strains (data not shown). However, the two genes were on *EcoRI* fragments of different lengths in the *S. boydii* 1 strain SBA1381. Therefore, it was not possible to confirm the linkage of *int* to *sigA* in SBA1381. However, in 75% of the non-*S. flexneri* 2a strains tested, *int* was linked to *sigA* as it is in *S. flexneri* 2a strain YSH6000T.

she PAI-related elements in pathogenic strains of *E. coli*

The dissemination of the *she* PAI and its structural variants in *Shigella*, and the recent demonstration that PAIs may disseminate to various gram-negative bacterial genera [8–10], prompted the survey of several enteric pathogens – including *Sal. Typhimurium*, *Y. enterocolitica*, enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli* (EPEC) – for carriage of the four *she* PAI markers. The presence of *orfs* 28–31 was not tested in EHEC and EPEC strains because the locus of enterocyte effacement (LEE) PAIs of EHEC and EPEC strains carry highly related sequences [16].

Three of the five EIEC strains tested carried both *int* and *sigA*, while one strain carried only the *int* marker and one strain did not carry any of the *she* PAI markers (Table 3). A sample EIEC strain, SBA885, was tested by PCR for the linkage of *int* and *sigA* as described above. As in most strains of *Shigella*, *int* and *sigA* were linked in strain SBA885, although the intergenic region between the two genes was slightly shorter than that in *S. flexneri* (Table 4). Two of the three EHEC strains and two of the three EPEC strains carried *int* but no other *she* PAI markers (Table 3). Similarly, none of the three *Y. enteroco-*

Table 3. Distribution of *she* PAI markers in gram-negative enteric pathogens and linkage of *int* to *phe* tRNA genes

Species (number of strains tested)	<i>she</i> PAI markers				Linkage of <i>int</i> to	
	<i>int</i>	<i>sigA</i>	<i>pic</i>	<i>orfs</i> 28–31	<i>pheV</i> ^{†*}	<i>phe</i>
<i>S. flexneri</i> 2a (9)	+	+	+	+	+	+
<i>S. flexneri</i> 1a (1)	–	–	–	–	–	...
<i>S. flexneri</i> 1b (1)	–	–	–	–	–	...
<i>S. flexneri</i> 2b (2)	+	+	+	+	+	...
<i>S. flexneri</i> 2b (1)	+	+	+	–	+	...
<i>S. flexneri</i> 2b (1)	+	+	–	–	+	...
<i>S. flexneri</i> 3a (1)	+	+	–	–	+	...
<i>S. flexneri</i> 3a (1)	–	–	–	–	–	...
<i>S. flexneri</i> 3b (1)	–	–	–	–	–	...
<i>S. flexneri</i> 3c (1)	+	+	+	+	+	...
<i>S. flexneri</i> 4a (3)	–	–	–	–	–	...
<i>S. flexneri</i> 5 (1)	–	–	–	–	–	...
<i>S. flexneri</i> 6 (2)	+	+	–	–	–	+
<i>S. boydii</i> 1 (1)	+	+	–	–	–	+
<i>S. boydii</i> 2 (1)	+	+	–	–	–	+
<i>S. boydii</i> 4 (2)	+	+	–	–	–	+
<i>S. boydii</i> 3 (1)	+	–	–	–	–	+
<i>S. boydii</i> 7 (1)	+	–	–	–	–	+
<i>S. boydii</i> 8 (1)	+	+	–	–	–	+
<i>S. dysenteriae</i> 1 (1)	–	–	–	–	...	...
<i>S. dysenteriae</i> 9 (1)	+ [†]	+	–	–	–	+ [†]
<i>S. dysenteriae</i> 3 (2)	+	+	–	–	–	+
<i>S. dysenteriae</i> 4 (1)	+	+	–	–	–	+
<i>S. dysenteriae</i> 5 (1)	+	+	–	–	–	+
<i>S. dysenteriae</i> 6 (1)	+	+	–	–	–	+
<i>S. sonnei</i> (8)	+	+	–	–	+	...
<i>Salmonella</i> (3)	–	–	...	...	...	...
EHEC	+	–	–	...	+	+
EHEC	+	–	–	...	...	+
EHEC	–	–	–	...	...	...
<i>Y. enterocolitica</i> (3)	–	–	...	...	...	...
EPEC	–	–	–	...	...	...
EPEC	+	–	–	...	+	+
EPEC	+	–	–	...	...	+
EIEC (3)	+	+	–	–	–	+
EIEC (1)	–	...	...	–	...	...
EIEC (1)	+	–	–	–	–	+

*Signifies linkage of *int* to a sequence upstream of *pheV*.[†]The amplified fragment was 1.4 kb larger than the test strain *S. flexneri* 2a YSH6000T. ..., not tested.**Table 4.** Amplification with primer pairs

Species	Regions used for linkage studies + expected size of product (kb)							
	A (7.8)	B (2.4)	C (4.3)	D (6.2)	E (5.8)	F (5.8)	G (4.8)	H (6.8)
<i>S. flexneri</i> 2a	7.8	2.4	4.3	6.2	5.8	5.8	4.8	6.8
<i>S. flexneri</i> 2b	7.8	2.4	4.3	6.2	5.8	5.8	4.8	6.8
<i>S. flexneri</i> 3c	7.5	2.4	4.3	6.2	5.8	5.8	4.8	6.8
<i>S. flexneri</i> 6	–	–	–	–	–	–	–	–
<i>S. boydii</i> 1	–	–	–	–	–	–	–	–
<i>S. dysenteriae</i> 3	–	–	–	–	–	–	–	–
<i>S. sonnei</i>	11	–	–	–	–	–	–	–
EIEC	7.5	–	–	–	–	–	–	–

litica strains nor *Sal.* Typhimurium strains carried any of the *she* PAI markers (Table 3).

Chromosomal insertion sites of *she* PAI-related elements

The site of the *she* PAI insertion into the strain YSH6000T chromosome was previously identified as

the 3' terminus of the *pheV* tRNA gene [3]. Therefore, the specificity of the *she* PAI and its structural variants for insertion into *phe* tRNA genes was investigated. *E. coli* K12 carries two identical tRNA genes, *pheV* and *pheR*, at distinct sites on the chromosome [17]. However, the number of identical *phe* tRNA genes in *Shigella* is not known. To investigate whether the *she* PAI and its structural variants insert into the *phe* tRNA

genes of *Shigella* and pathogenic *E. coli*, strains were analysed by PCR with primers BAP1434 and BAP1330 (Table 2), which hybridise to internal sites of the conserved *phe* tRNA genes of *E. coli* K12 and *int*, respectively. In all but one of the *Shigella* and *E. coli* strains that carried *int*, PCR products of 1 kb were obtained, indicating that *int* was linked to a *phe* tRNA gene (Table 3). In a single *S. dysenteriae* serotype 9 strain, SBA1395, which appeared to have a 1.4-kb insertion in the *int* gene, the *int-phe* PCR product was also 1.4 kb larger than in all other strains (Table 3). These results confirmed that all the elements related to the *she* PAI have a strict specificity for insertion into *phe* tRNA genes.

To investigate whether the insertion of the PAIs was restricted to the *pheV* gene, *Shigella* spp. and pathogenic strains of *E. coli* were analysed by PCR with primers BAP1258 and BAP1330. These primers amplify a 1-kb fragment in strain YSH6000T spanning a region upstream of *pheV* and an internal region of the *int* gene, encompassing the boundary between the chromosome and the left end of the PAI. In 27 (57%) of the 47 *Shigella* and *E. coli* strains tested *int* was found to be linked to *pheV* (Table 3). However, in the remaining strains, PCR products demonstrating the linkage of *int* to *pheV* were not amplified, despite the conservation in all test strains of the BAP1486 and BAP1258 priming sites in *pheV* and upstream of *pheV* respectively, as indicated by PCR in all *Shigella* strains (data not shown). These results indicated that the *she* PAI variants are capable of insertion into more than one *phe* tRNA gene in *Shigella* and *E. coli*. With a primer (BAP1459) specific for a DNA sequence upstream of *pheR* in *E. coli* K12 and the *int* primer (BAP1330), *int* was found to be linked to *pheR* in all *int*-carrying strains of EIEC. However, in *Shigella* strains where *int* was not linked to *pheV*, *int* could not be linked to a *pheR* homologue with the same primer pair. Possible explanations of this are that, in these strains, *int* is either linked to a *phe* gene distinct from *pheV* and *pheR* or that the BAP1459 binding site or its position in relation to *pheR* is not conserved in *Shigella*.

Discussion

This study shows that the entire *she* PAI, as it is organised in *S. flexneri* 2a strain YSH6000T, exists only in a limited number of *Shigella* strains, including serotype 2a strains and some serotype 2b and 3c strains of *S. flexneri*. However, structural variants of the PAI, missing one or more of the marker genes used in this study, were found in most other *Shigella* strains and in some pathogenic strains of *E. coli*. These variations may have occurred through deletion of the *pheV*-distal side of the PAI. If so, the *pheV*-distal side of the PAI may be less stable than the left side, as strains carrying PAI markers always carry *int* and the frequency of

carriage for specific markers decreases with their proximity to the *pheV*-distal end of the PAI. Alternatively, structural variations in the PAI may have occurred through the sequential addition of sequences to the *pheV*-distal end of the PAI. Structural variations also occur in other types of PAI. For example, although most of the high-pathogenicity island (HPI) is conserved in highly virulent *Yersinia* spp., the gene content and structure of the *pheV*-distal end of the HPI in *Y. pestis* and *Y. pseudotuberculosis* differs markedly from that in *Y. enterocolitica* [12]. A second type of PAI, termed the SHI-2 PAI, also exhibits structural variations in *Shigella* spp. [11]. Thus, the divergence of structure appears to be a common theme in the evolution of PAIs in gram-negative bacteria.

she PAI variants, carrying only *int* and *sigA*, were found in both *Shigella* and EIEC, whereas those carrying only the *int* gene were found in *S. boydii*, EHEC, EPEC and EIEC. Whether such elements are simply truncated versions of the *she* PAI or related PAIs that have acquired distinct sequences at the right end of the PAI remains to be determined. Enterogregative strains of *E. coli* were also shown to carry the *pic* gene (data not shown), as described previously [18]. It was found that these strains also carried *int*, but it was not established whether the two genes were linked. In all cases, *int* was linked to *pheV*, *pheR* or an unidentified *phe* tRNA gene. These findings confirmed previous predictions that the *she* PAI and related elements were mobile and capable of insertion into more than one specific type of *phe* tRNA gene [3]. In this sense, the *she* PAI-related elements resemble the HPI in *Y. enterocolitica* which is capable of inserting into any of three *asn* tRNA genes that serve as targets for its insertion into the chromosome [12]. The dissemination of the *she* PAI variants to bacteria outside of the genus *Shigella* resembles the behaviour of the SHI-2 PAI. Like the *she* PAI-related elements, the SHI-2 PAI and its structural variants have been found in *Shigella* spp. and EIEC [11]. In contrast, the HPI or its variants have been found in a wider range of genera, including *Escherichia*, *Citrobacter*, *Klebsiella* and *Yersinia* [8–10].

The roles of the *she* PAI-related elements in pathogenic *E. coli* strains are unknown. However, unless they have acquired additional virulence genes, PAI variants carrying only the *int* gene are unlikely to affect the virulence properties of EHEC, EPEC and EIEC. The presence of *sigA* in three of the five EIEC tested may be particularly significant, given that *Shigella* spp. and EIEC produce similar disease in man and that *sigA* plays a role in intestinal fluid accumulation in *S. flexneri* 2a [2].

In conclusion, like the *Yersinia* HPI and the *Shigella* SHI-2 PAI, the *she* PAI appears to be a member of a family of closely related genetic elements that have evolved into divergent structural forms. *she* PAI-related

elements are found throughout *Shigella* and pathogenic strains of *E. coli*, suggesting that they may be capable of transmission within this group of bacteria.

This work was supported by a project grant from the National Health and Medical Research Council, Canberra, Australia. The technical assistance of Vicki Vallance and Ian McPherson is gratefully acknowledged.

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