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Horizontal Transfer of the High-Pathogenicity Island of *Yersinia pseudotuberculosis*†

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The horizontal transfer of genetic elements plays a major role in bacterial evolution. The high-pathogenicity island (HPI), which codes for an iron uptake system, is present and highly conserved in various *Enterobacteriaceae*, suggesting its recent acquisition by lateral gene transfer. The aim of this work was to determine whether the HPI has kept its ability to be transmitted horizontally. We demonstrate here that the HPI is indeed transferable from a donor to a recipient *Yersinia pseudotuberculosis* strain. This transfer was observable only when the donor and recipient bacteria were cocultured at low temperatures in a liquid medium. When optimized conditions were used (bacteria actively growing in an iron-deprived medium at 4°C), the frequency of HPI transfer reached $\sim 10^{-8}$. The island was transferable to various serotype I strains of *Y. pseudotuberculosis* and to *Yersinia pestis*, but not to *Y. pseudotuberculosis* strains of serotypes II and IV or to *Yersinia enterocolitica*. Upon transfer, the HPI was inserted almost systematically into the *asn3* tRNA locus. Acquisition of the HPI resulted in the loss of the resident island, suggesting an incompatibility between two copies of the HPI within the same strain. Transfer of the island did not require a functional HPI-borne insertion-excision machinery and was RecA dependent in the recipient but not the donor strain, suggesting that integration of the island into the recipient chromosome occurs via a mechanism of homologous recombination. This lateral transfer also involved the HPI-adjacent sequences, leading to the mobilization of a chromosomal region at least 46 kb in size.

Genomic islands are large pieces of chromosomal DNA which display characteristics of horizontally acquired elements (17). These elements frequently harbor phage- and/or plasmid-derived sequences, but little is known about their mechanisms of lateral transfer. To date, only two genomic islands have been shown to be transmissible. The 105-kb *clc* ecological island of *Pseudomonas* spp. is transferable at low frequencies ($\approx 10^{-8}$) to various γ- and β-*Proteobacteria* (34, 41). The genetic bases for this transfer have not yet been elucidated. The 500-kb symbiosis island of *Mesorhizobium loti* is transferable at a frequency of 10^{-7} to three *Mesorhizobium* species (42). The transmissibility of this island has been associated with the presence of the *trb* operon and the *traG* gene, which display homology to genes required for the conjugative transfer of some plasmids (43).

The hypothesis that genomic islands could be transmitted by phages has been put forward because, as with bacteriophages, these islands are most often inserted into tRNA genes, they excise from the host chromosome by site-specific recombination between flanking direct repeats homologous to phage attachment sites, and their excision is mediated by a phagelike integrase and sometimes a recombination directionality factor (22, 25, 36). Pathogenicity islands (PAIs) are a subset of genomic islands characterized by their capacity to encode virulence functions (17). Two PAIs have been shown to be transferable by helper phages: SaPI1 of *Staphylococcus aureus*, which uses the helper phage 80α (24, 35) for its transfer, and VPI of *Vibrio cholerae*, which is transduced by CP-T1 (30).

The high-pathogenicity island (HPI) is a 36- to 43-kb PAI which encodes an iron uptake system. This island was first identified in highly pathogenic strains of *Yersinia* (*Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica* 1B) (1, 8, 18) and has been identified subsequently in various other *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp., *Salmonella enterica*, and *Serratia liquefaciens* (2, 20, 21, 28, 29, 38). The presence of highly conserved HPI-borne genes among different species (98 to 100% nucleotide identity between *Yersinia* and *E. coli* genes [13, 23, 33, 39]) argues for a recent acquisition of the island by these bacteria. However, the mechanism mediating the lateral transfer of the HPI (HPI transfer) remains unknown. In one particular *E. coli* strain (ECOR31), an HPI harboring an additional 35-kb fragment which encodes genes involved in conjugative DNA transfer has been identified (37). This element, whose organization resembles that of an integrative and conjugative element (9), has been proposed to be the progenitor of the transferable HPI, although the role of the additional fragment in HPI transfer awaits further demonstration. The fact that this additional 35-kb region is absent from all other HPI-positive strains and that the putative integrative and conjugative element is inserted into a locus different from that of the HPIs on the chromosomes of other *E. coli* strains suggests that this element may represent a particular type of HPI rather than the progenitor of all HPIs.

The *Y. pseudotuberculosis* HPI can spontaneously excise from the bacterial chromosome by site-specific recombination

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TABLE 1. Strains and plasmids used in this study[a]

Strain or plasmid	Serotype	Characteristic(s)	Source or reference
Strains			
Y. pseudotuberculosis			
IP32637	I	Wild type (HPI$^+$)	IP collection
IP32637/do	I	*irp2*::*kan* Rif^r used as donor strain	This study
IP32637/do Δ*hef*	I	*hef*::*kan* Rif^r	22
IP32637/do(pBAD33-*hef*)	I	IP32637/do harboring pBAD33-*hef*	22
IP32637/do Δ*int*	I	*int*::*kan* Rif^r	22
IP32637/do Δ*recA*	I	*recA*::*spe*	This study
IP32637/do Δor1188	I	or1188::*tmp*	This study
IP32637/re	I	ΔHPI Nal^r used as recipient strain	22
IP32637/re HPI$^+$	I	HPI$^+$ Nal^r	This study
IP32637/re Δ*recA*	I	*recA*::*spe*	This study
IP32533	I	Wild type (HPI$^+$)	IP collection
IP32533/re	I	ΔHPI Nal^r	22
IP32781	I	Wild type (HPI$^+$)	IP collection
IP32781/re	I	ΔHPI Nal^r	22
IP32953	I	Wild type (HPI$^+$)	IP collection
IP32953/re	I	Nal^r	This study
IP33306	II	Wild type (HPI$^-$)	IP collection
IP33306/re	II	Nal^r	This study
IP31830	IV	Wild type (HPI$^-$)	IP collection
IP31830/re	IV	Nal^r	This study
Y. enterocolitica			
IP845	O:20	Wild type (HPI$^+$)	IP collection
IP845/re	O:20	Nal^r	This study
IP11666	O:16	Wild type (HPI$^-$)	IP collection
IP11666/re	O:16	Nal^r	This study
Y. pestis			
6/69	NA	Wild type (HPI$^+$)	IP collection
6/69/re	NA	Nal^r	This study
Plasmids			
pUC4K		High copy number, Km^r	Amersham
pSW25		*oriT ccdB* Spe^r	11
pBAD33-*hef*		*hef* cloned under the pArab promoter, low copy number, Cm^r	22
pKOBEG*sacB*		Ts, low copy number, *sacB* Cm^r	12
pGP704N		Suicide vector, Amp^r	27
pNot::Ptac-*dfrB1*		Tmp^r	5
pGP704N-*dfr*		*dfr* amplified from pNot::Ptac-*dfrB1*, Tmp^r Amp^r	This study

[a] IP, Institut Pasteur; NA, not applicable.

between two 17-bp flanking repeats homologous to the *att* site of bacteriophage P4 (8). The excision process requires the combined actions of Int, an HPI-borne integrase homologous to that of phage P4, and Hef (for HPI excision factor), a recombination directionality factor which is assumed to drive the function of Int towards an excisionase activity (22). A circular HPI molecule is generated upon excision (22). Insertion of the island into the bacterial chromosome is mediated by Int (31). Notably, the *Y. pseudotuberculosis* HPI is mobile within the genome of its host strain; it is able to excise from the bacterial chromosome and to reinsert into another site on the chromosome (8). The fact that the HPI has kept its mobility and is highly conserved in various bacterial genera suggests that the island has been acquired recently and may have retained its ability to be transmitted horizontally to new bacterial hosts. The aim of this work was to experimentally determine whether the HPI has the intrinsic capacity (i.e., without the artificial addition of a helper phage or a conjugative plasmid) to be transferred to a new host.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. The wild-type *Yersinia* isolates were taken from the collection of the Yersinia Research Unit (Institut Pasteur). Spontaneous rifampin-resistant (Rif^r) and nalidixic acid-resistant (Nal^r) derivatives were obtained on Luria-Bertani (LB) agar containing Rif (100 μg/ml) and Nal (50 μg/ml), respectively.

Media. The media used in this study were LB broth or agar, Mueller-Hinton (MH) broth or agar, and M63 liquid medium supplemented with 1% glucose and 20% Casamino Acids. When necessary, kanamycin (Kan) (30 μg/ml or 100 μg/ml), spectinomycin (50 μg/ml), Rif (100 μg/ml), Nal (25 μg/ml), trimethoprim (Tmp) (20 μg/ml), or the iron chelator α,α′-dipyridyl (0.2 mM) was added to the medium. Selection for Tmp resistance was done in MH broth. Overexpression of *hef* was induced by adding 0.2% L-arabinose to the medium.

Mutagenesis. All primers used in this study are listed in supplemental Table S1. The Δ*hef* and Δ*int* mutants of IP32637 were constructed in a previous work (22). The chromosomal *irp2*, *recA*, and or1188 genes were inactivated by allelic exchange with nonpolar *kan*, *aadA*, and *dfr* cassettes, respectively, by following the LFH-PCR procedure (12). The three antibiotic resistance cassettes were obtained by PCR amplification using plasmids pUC4K (primer pair *kan*F/*kan*R), pSW25 (primer pair *aadA*F/*aadA*R), and pGP704N-*dfr* (primer pair *dfr*F/*dfr*R) as templates (Tables 1 and S1), respectively. The *dfr* cassette was first amplified from the pNot::Ptac-*dfrB1* plasmid (kindly

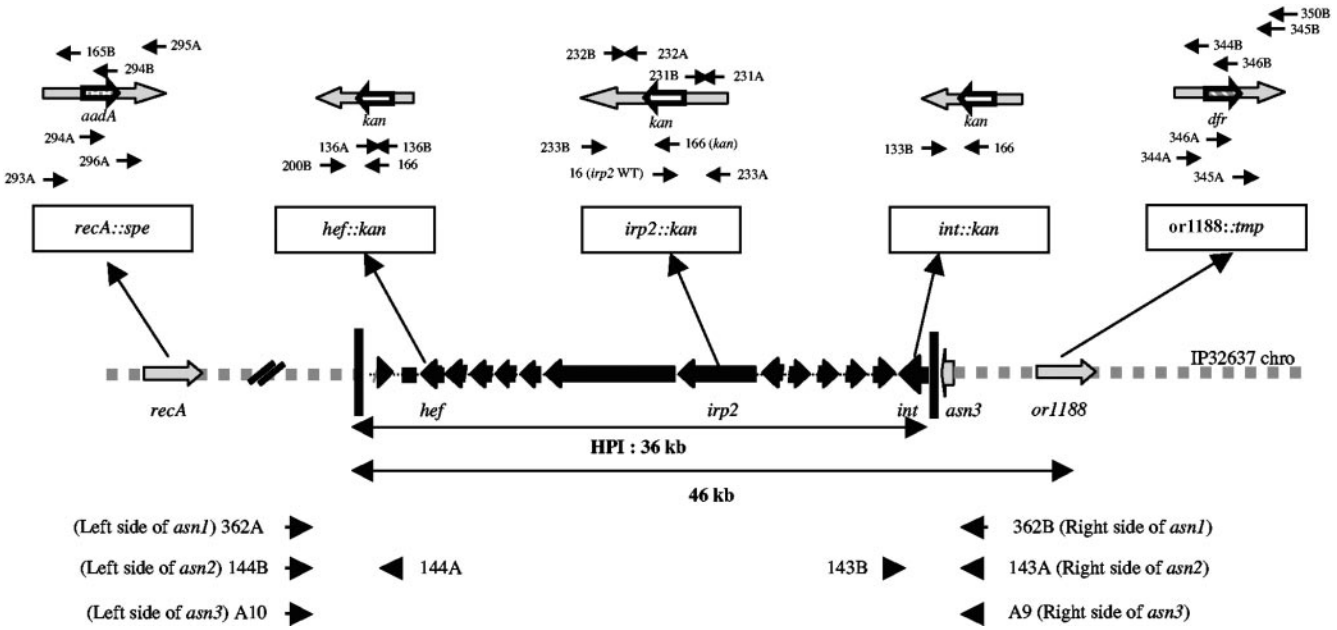

FIG. 1. Genetic map of the HPI and locations of the primers used to generate mutants and to check for the transfer of the island.

provided by D. Mazel, Institut Pasteur) with primer pair *dfr*-RV/*dfr*-No and cloned into the EcoRV and NotI sites of the suicide vector pGP704N (27).

Conditions for HPI transfer on solid medium. Each experiment involved a Rif^r donor strain and a Nal^r recipient strain. After an overnight culture of the donor and the recipient strains at 28°C in LB broth supplemented with the corresponding antibiotics, the bacteria were washed twice in LB broth, and the bacterial concentration was adjusted to 10^{10} bacteria/ml (donor) and 10^9 bacteria/ml (recipient). One hundred microliters of each bacterial suspension was mixed on a 0.45-μm nitrocellulose filter on the surface of an LB plate and incubated for 4 h, 24 h, or 5 days at 28°C, 37°C, or 4°C. The bacterial mixtures were then suspended in 1 ml of LB broth and simultaneously streaked on LB plates containing Rif (number of donors), Nal (number of recipients), and Nal-Kan (number of recipient colonies that had acquired the HPI).

Conditions for HPI transfer in liquid medium. The donor and recipient strains were grown as described above. In one set of experiments, 2×10^9 donors were mixed with 2×10^8 recipients in 1 ml of LB broth and incubated for 90 min with agitation at 37°C, 28°C, or 4°C. In a second set of experiments, 25 ml of LB broth was inoculated in 150-ml flasks with either 5×10^7 or 25×10^7 donors and 5×10^7 recipients. Cultures were incubated with rotatory shaking (80 rpm) or without shaking at 37°C or 28°C for 24 h or at 37°C, 28°C, 12°C, or 4°C for 4 days. Serial dilutions of the cultures were streaked on LB plates containing Rif, Nal, and Nal-Kan.

Verification of the transformants and determination of the frequency of HPI transfer. To ensure that the Nal^r Kan^r clones were not spontaneous Nal^r colonies of the donor strain, each clone was spotted on Rif-negative plates. The Nal^r Kan^r Rif^s transformants were further checked by PCR for the presence of the inserted genes with primer pair 233B/166 (*irp2*::*kan*), 133B/166 (*int*::*kan*), or 200B/166 (*hef*::*kan*) (Table S1). The frequency of HPI transfer (Tf_{HPI}) was calculated as the number of Nal^r Kan^r Rif^s transformants per Rif^r donor cells.

Analysis of the HPI insertion site into the chromosome of the recipient strain. Primer pairs 362A/362B, 143A/144B, and A9/A10, located on each side of *asn1*, *asn2*, and *asn3*, respectively (Table S1 and Fig. 1), were used to search for loci without HPI insertion. When no amplification product was obtained, the presence of the HPI in this site was confirmed using two primer pairs: for the right boundary, a combination of primers flanking the right side of the *asn* locus and the right-hand border of the HPI (143B), and for the left boundary, primers flanking the left side of the *asn* locus and the left-hand border of the HPI (144A) (Fig. 1).

RESULTS AND DISCUSSION

The capacity of horizontal transfer of the HPI was studied in *Y. pseudotuberculosis* strain IP32637 because this strain was previously shown to harbor an island which has functional excision-integration machinery (22) and which could be mobile within the host chromosome (8). A spontaneous Rif^r derivative of strain IP32637 in which the HPI was tagged by insertion of a Kan resistance cassette into the *irp2* gene (Fig. 1) was used as the HPI donor strain (IP32637/do). A spontaneous Nal^r derivative of an IP32637 strain precisely deleted of its HPI (22) was used as a recipient (IP32637/re) (Table 1).

Search for HPI transfer on mating filters. The donor and recipient bacteria were mixed on nitrocellulose filters and incubated for various periods of time (4 h, 24 h, or 5 days) at different temperatures (37°C, 28°C, or 4°C). These three temperatures were chosen because 37°C is close to the temperature found in vivo, 28°C is the optimal in vitro growth temperature for *Yersinia* (4, 7), and 4°C is a temperature which still supports *Yersinia* growth because of the psychrophilic properties of these bacteria (4). As shown in supplemental Table S2, none of these conditions yielded Nal^r Kan^r transformants, indicating that transfer of the HPI to the recipient bacteria was not detectable under these conditions.

Search for HPI transfer in a liquid medium. Different amounts of IP32637/do were mixed with IP32637/re, and the cocultures were incubated in 25 ml of LB broth for various periods of time (90 min, 24 h, and 4 days) at three different temperatures (37°C, 28°C, and 4°C), with or without shaking. Whatever the duration of contact between the donor and recipient bacteria or the number of donor cells, no Nal^r Kan^r colonies were observed after coculture at 37°C or 28°C (Table S2). The search for HPI transfer at 4°C was not performed when the bacteria were grown for 24 h because the starting

inocula were too low to reach a sufficient bacterial concentration at the end of the experiment. In contrast, after 4 days at 4°C, the number of bacteria increased to reach approximately 10^9 CFU/ml, and Nal^r Kan^r colonies were detected (Table S2). No such colonies were observed when the donor and recipient bacteria were grown separately under the same conditions (data not shown). The susceptibility to Rif of each Nal^r Kan^r transformant was tested to further ensure that they were not spontaneous Nal^r mutants of IP32637/do. Most Nal^r Kan^r colonies (≈80%) were Rif^s. The presence of the *kan* cassette as well as the entire length of the HPI was checked by PCR of the IP32637/re transformants using primer pairs 233B/166 to amplify the *irp2*::*kan* locus, 143B/A9 for the right-hand extremity of the island, and A10/144A for its left-hand extremity (Fig. 1). All Nal^r Kan^r Rif^s transformants gave a positive PCR signal for the three sets of primers (data not shown), indicating that the recipient bacteria had acquired the HPI. This is the first experimental demonstration of the horizontal transfer of the HPI to a new bacterial host.

This transfer was observed whether the starting inoculum of donor bacteria was 5×10^7 or 25×10^7 and whether the cultures were shaken or unshaken (Table S2). Transfer of the HPI at 12°C was also detected in both shaken and unshaken cultures (Table S2). Yersiniae are known to undergo temperature-dependent changes between 28°C and 37°C. However, the properties that are associated with a temperature shift to 4°C are not well characterized. The absence of transfer at temperatures ≥28°C may result from (i) the specific induction at low temperatures of genes that encode proteins involved in the transfer process, (ii) a modification of the bacterial membrane composition which would increase DNA uptake, or (iii) a better resistance of DNA molecules to degradation processes at low temperatures.

Estimation of Tf_{HPI}. To estimate Tf_{HPI}, 5×10^7 CFU of IP32637/do and IP32637/re was incubated for 4 days at 4°C in LB broth with shaking. Tf_{HPI} was calculated as the number of Nal^r Kan^r Rif^s transformants observed per Rif^r donor cell. The experiment was repeated 10 times. Tf_{HPI} varied from 0.6×10^{-9} to 2×10^{-9}, with an average of 1.4×10^{-9} ($\pm 0.7 \times 10^{-9}$). Transfer of the HPI to the recipient bacteria is thus a reproducible but infrequent event.

Conditions influencing the efficacy of HPI transfer. The factors regulating horizontal gene transfer in bacteria are mostly unknown to date. In order to identify conditions that might increase Tf_{HPI}, the donor and recipient bacteria were cocultured for 4 days at 4°C with shaking, and various growth parameters were tested.

Since genetic transfer may be influenced by the degree of bacterial multiplication, various starting inocula (5×10^6, 5×10^7, 5×10^8, and 5×10^9 CFU) of donor and recipient bacteria were mixed in equal amounts in 25 ml of LB broth. At the end of the experiments, all cultures had reached approximately the same final optical density at 600 nm, corresponding to $\sim 10^9$ CFU/ml. Tf_{HPI} was higher (20- to 40-fold) at the lowest initial bacterial concentrations (Table 2), suggesting that actively growing bacteria may be better suited for lateral DNA exchange.

Inducers of the SOS response have been shown to enhance lateral gene transfers in some instances (3). To determine whether this could be true for the HPI, mitomycin C (50 ng/ml) was added at the beginning of the coculture. The presence of this chemical reduced the number of viable cells at the end of the incubation period but did not increase Tf_{HPI} significantly (Table 2).

TABLE 2. HPI transfer frequencies under various coculture conditions[a]

Parameter tested	Amt or type	Tf_{HPI} (10^{-9})
Inoculum[b]	5×10^6	1.7
	5×10^7	1.0
	5×10^8	0.05
	5×10^9	0.04
Medium[c]	LB broth	1.4
	LB broth + mitomycin C	3.0
	MH broth	2.0
	M63	3.1
	LB broth + α,α′-dipyridyl	11.1
	M63 + α,α′-dipyridyl	27.0
Temp[d]	4°C	8.8
	12°C	0.06

[a] IP32637/do and IP32637/re were mixed in equal amounts in 25 ml of liquid medium and incubated for 4 days at 4°C (except when specified) with shaking.
[b] Equal inocula of donor and recipient bacteria were grown in LB broth.
[c] Donor and recipient bacteria (5×10^7) were used.
[d] Donor and recipient bacteria (5×10^6) were cultured in LB broth plus α,α′-dipyridyl.

The impact of the liquid medium composition on Tf_{HPI} was investigated using a rich (MH) and a minimal (M63) medium. No major modification of Tf_{HPI} was observed with these two media (Table 2).

Horizontal transfer of a genetic element may be increased when the bacteria are in an environment where expression of the genes carried by the mobile element is required. For example, transfer of the tetracycline resistance-encoding conjugative transposons CTnDOT and CTnERL in *Bacteroides* spp. and Tn*916* in *Enterococcus faecalis* is increased in the presence of tetracycline (6, 10, 26). Similarly, the presence of 3-chlorobenzoate in the culture medium enhances the transfer of the *clc* genomic island of *Pseudomonas* spp., which carries genes involved in 3-chlorobenzoate metabolism (34, 40). Since the HPI encodes the yersiniabactin system, which provides a growth advantage under iron-limiting conditions (15, 16, 32), we tested the impact of iron deprivation on Tf_{HPI}. Coculture of the donor and recipient bacteria in LB broth supplemented with the iron chelator α,α′-dipyridyl led to an eightfold increase in Tf_{HPI} (Table 2). Addition of the iron chelator to M63 medium resulted in a severe reduction in bacterial growth, but also in a more pronounced (20-fold) increase in Tf_{HPI} (Table 2).

All together, these results indicate that HPI transfer is enhanced when the bacteria are cocultured at low temperatures in an environment where iron and nutrient availability is limiting. These conditions may be close to those encountered by *Y. pseudotuberculosis* in its natural ecological niche. Indeed, enteropathogenic *Yersinia* strains spend a significant part of their life cycle in the environment, and they have a predilection for temperate and cold climates. The incidences of *Y. pseudotuberculosis* infections are highest in cold areas such as Russia, Scandinavia, and Japan (14, 19, 44). In its natural ecological

niche, *Y. pseudotuberculosis* may be in close contact with various environmental microorganisms, and this niche may thus represent a favorable environment for lateral gene transfer.

Based on the above results, the conditions adopted for further experiments were the coculture of 5×10^6 donor and recipient bacteria for 4 days with shaking in LB broth with α,α'-dipyridyl. We did not use the iron-depleted M63 medium because the number of bacteria at the end of the experiment was too low to yield sufficient amounts of transformants for analysis. Under the conditions defined above, Tf_{HPI} was tested for bacteria cocultured at 37°C, 28°C, 12°C, and 4°C. Even under these optimized conditions, no HPI transfer could be detected at 37°C and 28°C. HPI transfer was detected at both 12°C and 4°C but was much more efficient (140-fold) at 4°C (Table 2). This temperature was subsequently used throughout the study.

HPI transfer to other pathogenic *Yersinia* strains. Two derivatives of *Yersinia* strain IP32637 were initially used as donor and recipient bacteria to investigate HPI transfer, because this strain was previously found to have the most efficient HPI excision machinery (22). Strains belonging to the species *Y. pseudotuberculosis* are classically divided into five major serotypes (I to V). Serotype I strains harbor a complete HPI, and those of serotype III carry a truncated island, while those of other serotypes do not harbor an HPI (2). To determine whether the HPI could be transferred to other *Y. pseudotuberculosis* strains, spontaneous Nal^r mutants of two HPI-deleted strains of serotype I (IP32533 and IP32781) and of one strain each of serotypes II (IP33306) and IV (IP31830) were used as recipients (Table 1). HPI transfer from IP32637/do to the two strains of serotype I occurred at frequencies of 15×10^{-9} (IP32533/re) and 45×10^{-9} (IP32781/re), i.e., in the same range as that of IP32637/re (21×10^{-9}). The HPI is thus transferable to various serotype I strains. In contrast, HPI transfer to serotype II and IV strains could not be detected, suggesting that the absence of the HPI in these strains (which naturally do not carry the island) may reflect their inability to acquire the HPI rather than a loss of this element during evolution.

To investigate the transferability of the HPI to other pathogenic *Yersinia* species, spontaneous Nal^r mutants of two *Y. enterocolitica* strains, IP845 (bioserotype 1B/O:20, HPI^+) and IP11666 (bioserotype 1A/O:16, naturally HPI^-), were used as recipients (Table 1). No HPI transfer from IP32637/do to these two recipients was detected. In contrast, the IP32637 HPI was transferred to *Y. pestis* 6/69/re, but Tf_{HPI} could not be accurately determined because of the poor growth of *Y. pestis* under the conditions used.

The HPI is thus transmissible to serotype I strains of *Y. pseudotuberculosis* and to *Y. pestis*. The absence of transfer to *Y. enterocolitica* may indicate that HPI transfer is more efficient when the bacteria are genetically closely related or that *Y. enterocolitica* does not possess the factors necessary to acquire the island.

Analysis of the HPI integration site into the chromosome of the recipient strains. The HPI can be inserted into any of the three *Y. pseudotuberculosis asn* loci (8). In strain IP32637, the island is preferentially inserted into *asn3*. To determine the site of HPI insertion into the chromosome of the recipient strain, 60 Nal^r Kan^r Rif^s IP32637/re transformants were selected from three independent experiments and analyzed by PCR for the location of the HPI (see Materials and Methods). In all transformants, the HPI was found inserted into *asn3* (data not shown), i.e., into the usual site of HPI insertion in strain IP32637.

The site of HPI integration was subsequently analyzed in the IP32781/re and IP32533/re transformants, whose natural sites of HPI insertion are the *asn3* and *asn2* loci, respectively (22). In both transformants, the HPI was found inserted into the *asn3* locus (data not shown), suggesting a tropism of the HPI of strain IP32637 for *asn3*.

Impact of the presence of a resident HPI on the acquisition of a foreign island. The presence of an HPI in the recipient strain may prevent the acquisition of a foreign island for at least three reasons: (i) the presence of a resident island renders the strain immune to the acquisition of a new HPI, as seen for some phages, (ii) the occupancy of the HPI insertion site does not allow the insertion of a second island, and (iii) the presence of two islands in the same strains results in an incompatibility of the two islands, as has been observed with some plasmids. HPI transfer from IP32637/do to IP32637/re HPI^+ did occur, at a frequency of 16×10^{-9}, which is similar to that observed with the ΔHPI recipient. Therefore, the presence of a resident HPI does not render the strain immune to the acquisition of a foreign HPI and has no effect on its transfer frequency. In all IP32637/re HPI^+ transformants, the acquired HPI was found inserted into the *asn3* locus (data not shown), i.e., in the same site as the resident HPI. This insertion is expected to have led either to the replacement of the resident island by the entering HPI or to a tandem duplication of the HPI at the *asn3* locus, which is theoretically possible since an intact *asn3* locus is restored upon HPI insertion. A search by PCR for an intact *irp2* gene (carried by the resident HPI) and for the *irp2*::*kan* locus (present on the acquired island) revealed that in all clones tested, only the *irp2*::*kan* locus was present, indicating a replacement of the resident HPI by the incoming island.

This replacement may be the result of an allelic exchange with the incoming HPI during the integration process or of an elimination of the resident HPI due to an incompatibility between the two HPIs. To differentiate between these two hypotheses, IP32637/do was cocultured with IP32953/re, a strain in which the HPI is inserted into *asn2* (22). In the first hypothesis, two HPIs should be present in the recipient bacteria: the incoming HPI inserted into *asn3* and the resident island into *asn2*. In the second hypothesis, the insertion of the foreign island into *asn3* should chase the resident island from the *asn2* locus. Twenty transformants obtained after coculture of IP32637/do with IP32953/re were analyzed by PCR for the presence of the original HPI. In one clone, both an *irp2*::*kan* and a wild-type *irp2* gene were detected, while in the 19 other transformants, the wild-type *irp2* gene was absent (supplemental Table S3). These results indicate that in most clones, acquisition of the foreign HPI led to the deletion of the resident island, suggesting an incompatibility between the two HPIs. Analysis of the site of insertion of the foreign HPI in these 19 clones revealed that in one case, the HPI had inserted into *asn2* and had undergone rearrangements, since its left border was not detectable (Table S3). In the 18 remaining clones, the foreign HPI had inserted into *asn3*. In most of these clones (11/18), the original HPI had precisely excised from the *asn2*

TABLE 3. HPI transfer frequencies in various IP32637 donor and recipient mutants[a]

Donor strain	Recipient strain	Tf_{HPI} ($\times 10^{-9}$) ($\pm$SD)
IP32637/do	IP32637/re	13 ($\pm$2.8)
IP32637/do(pBAD33-*hef*)	IP32637/re	24 ($\pm$8.5)
IP32637/do Δ*int*	IP32637/re	8.2 ($\pm$3.9)
IP32637/do Δ*hef*	IP32637/re	15.3 ($\pm$13.7)
IP32637/do Δ*recA*	IP32637/re	3 ($\pm$1.4)
IP32637/do	IP32637/reΔ*recA*	0
IP32637/do Δor1188	IP32637/re	15.6 ($\pm$0.3)

[a] All experiments were performed twice, and the numbers represent averages from the two experiments.

locus, as evidenced by the restoration of an *asn2* region without HPI insertion (Table S3). In the seven other clones, deletion of the HPI probably encompassed adjacent regions since no PCR product for the *asn2* locus and for the regions overlapping the borders of the HPI was obtained (Table S3). Therefore, the presence of a resident HPI does not prevent the acquisition of a foreign island, but two HPIs cannot usually coexist within the same strain, even at two different loci.

Role of the HPI insertion-excision machinery on the transfer of the island. Excision of the HPI from the host chromosome is mediated by the combined actions of its cognate integrase and the recombination directionality factor Hef and results in the formation of extrachromosomal circular molecules (22). Overexpression of *hef* led to a 100-fold rise in HPI excision (22). Coculture of IP32637/do (pBAD33-*hef*) in which *hef* is overexpressed with IP32637/re did not result in any increase in Tf_{HPI} (Table 3). When *int* and *hef*, which have been shown to be essential for HPI excision (22), were independently mutated in strain IP32637/do, the HPI was still transferable, at a frequency similar to that of the parental strain (Table 3). A functional excision machinery is thus not required for HPI transfer, suggesting that the island is not transferred in an excised form. Furthermore, since the integrase was previously shown to be required for HPI insertion into the bacterial chromosome (31), these results also indicate that integration of the HPI uses a mechanism different from the HPI-borne excision-integration system.

Role of homologous recombination on HPI transfer. As transfer and integration of the HPI into the recipient chromosome are not mediated by the HPI-borne integrase, we wondered whether this mechanism would be RecA dependent. When a *recA* IP32637 donor strain was cocultured with IP32637/re, HPI transfer was observed (Table 3), indicating that a functional *recA* gene is not required in the donor strain. In contrast, no transformants were detected when IP32637/do was cocultured with a *recA* IP32637 recipient (Table 3), indicating that insertion of the HPI into the recipient chromosome is RecA dependent. These results suggest that the HPI integrates into the recipient chromosome via a mechanism of homologous recombination.

Since the HPI is absent in IP32637/re, homologous recombination with the recipient chromosome should involve the regions flanking the HPI. To test this hypothesis, the *dfr* resistance cassette conferring resistance to Tmp was inserted into or1188, a gene adjacent to the HPI in strain IP32637/do (Fig. 1) and coding for a putative regulatory protein for trehalose-maltose transport. When the IP32637 Δor1188 donor bacteria were cocultured with IP32637/re, both the HPI and the or1188 gene were transferred to the recipient bacteria at similar frequencies (Table 3). Analysis of individual colonies revealed that all clones that had acquired or1188 had also acquired the HPI and vice versa. These results demonstrate that the HPI is transferred with the chromosomal flanking regions and, therefore, that this transfer is not limited to the HPI but involves larger chromosomal regions that are at least 46 kb in size.

Conclusion. Several pieces of evidence indicate that PAIs are mobile genetic elements that are horizontally transferable. However, the intrinsic capacity of these islands to be transmitted to new bacterial hosts, without the artificial introduction of helper phages or conjugative functions, remained to be established. This work is the first demonstration that at least one PAI, the *Y. pseudotuberculosis* HPI, can be transferred laterally to new bacterial hosts. This transfer is observed under specific conditions (low temperatures and in iron-deprived liquid medium) which may be close to those encountered by these bacteria in their natural ecological niches. The limitation of iron availability in the environment may thus positively select for lateral transfer of the HPI. Horizontal acquisition of the HPI resulted almost systematically in the loss of the resident island, even when the incoming HPI inserted itself into a different chromosomal site, indicating an incompatibility between different copies of the same PAI. The mechanisms responsible for this incompatibility remain to be characterized. The HPI transfer identified in this study did not require the HPI-borne excision-integration machinery. Insertion of the transferred DNA into the bacterial recipient chromosome was RecA dependent and occurred by homologous recombination between the HPI flanking regions. The existence of such a means of HPI transfer does not preclude the possibility of an additional, HPI-specific mechanism of transfer. In distantly related bacteria with nonconserved genetic organizations and low nucleotide identities, a transfer based on homologous recombination may be of poor efficacy. In contrast, this mechanism may represent an efficient means of propagating the HPI in closely related species. Furthermore, since this horizontal transfer is not limited to the HPI element but involves larger chromosomal regions ($\geq$46 kb), it may participate in the evolution of the *Y. pseudotuberculosis* genome. The mechanism underlying the lateral transfer of large portions of the *Y. pseudotuberculosis* chromosome is not yet known, but future work will be performed to characterize it.

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