Palindromic Unit-Independent Transposition of IS1397 in *Yersinia pestis*†

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Palindromic units (PUs) are intergenic repeated sequences scattered over the chromosomes of *Escherichia coli* and several other enterobacteria. In the latter, IS1397, an *E. coli* insertion sequence specific to PUs, transposes into PUs with sequences close to the *E. coli* consensus. Reasons for this insertion specificity can relate to either a direct recognition of the target (by its sequence or its structure) by the transposase or an interaction between a specific host protein and the PU target DNA sequence. In this study, we show that for *Yersinia pestis*, a species deprived of PUs, IS1397 can transpose onto its chromosome, with transpositional hot spots. Our results are in favor of a direct recognition of target DNA by IS1397 transposase.

Despite their small size, microorganisms contain a wide variety of repeated sequences, sometimes accounting for 10% or more of the total genome. Bacterial interspersed mosaic elements (BIMEs) are a family of small extragenic sequences scattered over the *Escherichia coli* chromosome (2). They could play a role in the functional organization of the bacterial nucleoid. Their mosaic structure associates palindromic units (PUs) (or repetitive extragenic palindromic sequences) (10, 28) with extra-PU motifs. PUs are imperfect palindromic sequences of about 40 bp, transcribed but not translated (4), which can confer a stem-loop secondary structure to mRNA. A complete description of BIMEs can be found on the World Wide Web (http://www.pasteur.fr/recherche/unites/pmtg/repet/index.html).

Insertions sequences (ISs) are small (800 to 2,500 bp) DNA segments capable of inserting into target DNA molecules with a more or less pronounced target specificity. More than 500 ISs have been identified so far for more than 40 bacterial species and classified into 17 families on the basis of open reading frame (ORF) organization, length of the target duplications, similarity of their terminal inverted repeats (IRs), and signature motifs among the transposases (see reference 16 for a review). They play important roles in DNA translocations and other rearrangements in bacteria (9, 13).

Insertion sequence IS1397 has been found in several *E. coli* natural isolates, always inserted into the central part (loop) of a PU. This 1,432-bp insertion sequence belongs to the IS3 family. It has 25-bp-long terminal inverted repeats (IRL and IRR) and encodes two proteins, OrfA and OrfB, which are in phase 0 and −1, respectively. A −1 translational frameshifting leads to a fusion protein, OrfAB, which is the transposase.

Members of our group have shown that IS1397 is able to transpose into PUs with sequences close or identical to the *E. coli* consensus, even in other enterobacteria (*Salmonella Enterica* serovar Typhimurium, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*) (6, 31).

The nature of PU recognition by IS1397 is still unclear. The transposase could directly bind PU DNA sequence. Alternatively, since PUs were shown to interact specifically with integration host factor (5, 20), DNA gyrase (8, 32), and DNA polymerase (11), they could be the central part of complex nucleoproteic structures which could be recognized by IS1397 transposase. *Yersinia pestis* is an enterobacterium phylogenetically close to *E. coli* but in which no sequences homologous to *E. coli* PU were found. This offered the opportunity to analyze whether a PU-specific host factor is required for IS1397 transposition. Furthermore, if transposition could take place in this host, the analysis of target sites could reveal possible structural or functional PU homologs.

MATERIALS AND METHODS

Media and bacterial strains. Luria-Bertani (LB) medium was used for bacteria growth. Kanamycin (KAN) and chloramphenicol (CHL) were used at concentrations of 25 and 50 μg/ml, respectively. Isopropyl-β-D-thiogalactopyranoside (IPTG) was used at a final concentration of 10^{-3} M, except for the transposition with pBLOCKS, where it was used at a final concentration of 10^{-6} M. LBS medium is LB medium without NaCl and containing 10% sucrose. Hemin was added at a concentration of 25 μg/ml in *Y. pestis* culture plates. *E. coli* strains were grown at 37°C, and *Y. pestis* was grown at 28°C.

Strains used were the following: *Escherichia coli* BW19610 [DE3(*lac*)X74 Δ*uidA::pir*-116 *recA*1 Δ*phoA*532 Δ(*phnC?D-P*)33-30] (18) and *Yersinia pestis* 6/69c, a derivative of *Yersinia pestis* 6/69 (12), which was cured of the pYV virulence plasmid.

Plasmids. pBLOCK (Fig. 1) has already been described (31). This derivative of pACYC184 carries the P15A origin of replication placed between *lacI*q and the CHL resistance gene. The P*tac* promoter is found twice, upstream of *orfA* and upstream of *orfAB*, allowing overproduction of the two proteins upon IPTG induction. OrfA and OrfAB are both toxic to the cell (6). The borders of the IROK transposable module (delineated with arrows on Fig. 1) are IS1397 inverted repeats flanking the KAN resistance gene and the R6K origin of replication (26, 27), which is functional in strains expressing Pir protein, such as
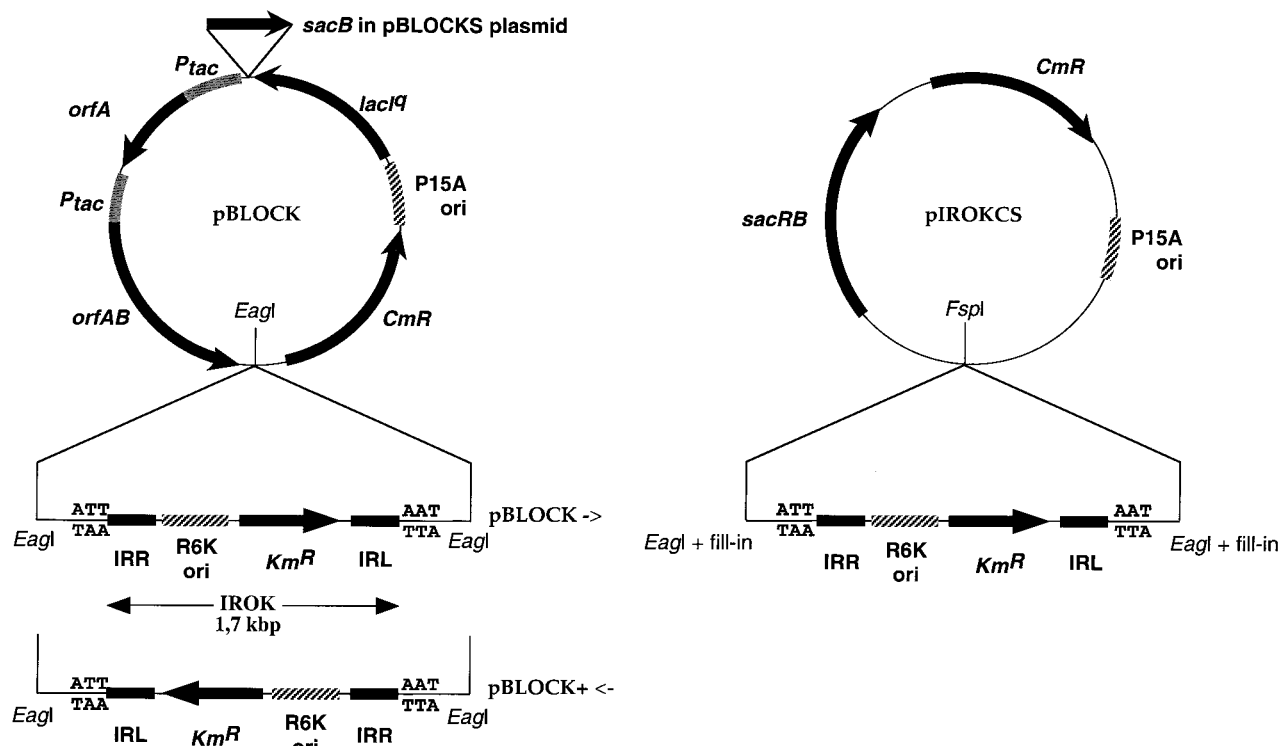

FIG. 1. Plasmids pBLOCK, pBLOCK+, pBLOCKS, and pIROKCS. pBLOCK (31) contains the transposase coding gene *orfAB* and a transposable module, IROK, which is delineated by the two IS*1397* inverted repeats (IRL and IRR). This module is flanked by two *Eag*I sites, and it is in the opposite orientation in pBLOCK+. pBLOCKS contains the *sacB* gene cloned between *orfA* and *lacI^q*. pIROKCS contains IROK, but no transposase gene.

BW19610. In pBLOCK+ (Fig. 1), the *Eag*I DNA fragment containing IROK is in the opposite orientation from that in pBLOCK.

pBLOCKS (Fig. 1) is a derivative of pBLOCK in which the *sacB* gene was cloned at the *Nhe*I site. The *sacB* gene was amplified by PCR with oligonucleotides SacB1 (5′CCCCCCGCTAGCGGCCCGTAGTCTGCAAATCC3′) and SacB2 (5′CCCCCCGCTAGCGCTGTTCACCATGAACAGATCG3′), using pKO3 (15) as a template.

pIROKCS (Fig. 1) is a derivative of the pSU2718 cloning vector (17), in which *sacRB* genes have been cloned at the *Hin*dIII site and the IROK transposable module has been cloned at the *Sma*I site.

DNA technologies. Restriction enzymes and DNA-modifying enzymes were purchased from New England Biolabs or Boehringer Mannheim and used as recommended. Plasmid DNA manipulations were carried out by using standard procedures (24). Extraction of total cellular DNA was performed with the DNA Easy Tissue kit (Qiagen).

DNA sequencing. DNA sequencing was performed by MWG-Biotech AG and ESGS (Cybergene). The two oligonucleotides used to sequence the junctions of IS*1397* chromosomal insertions were purchased from Genset. Their sequences are the following: seqIRL, 5′CGGTTGTGGACAACAAGCCAGGG3′ (complementary to a region of R6K origin of replication); and Kmseqout, 5′CAC-GAGGCAGACCTCAGCGC3′ (corresponding to a region located between the end of the KAN resistance gene and IRR of the module as found on pBLOCK).

Southern blot hybridization. Total DNA was extracted from overnight cultures. One to five micrograms of DNA were loaded on a 1% agarose Tris-acetate gel. DNA transfer onto Hybond N^+ membrane (Amersham) was performed as previously described (6). Hybridization was carried out using the DIG Nucleic Acid Labeling and Detection system (Boehringer Mannheim) at 56°C. The probe used was digoxigenin-dUTP-labeled with the Promega Nick-Translation kit.

Selection of transposition events for *Y. pestis* using pBLOCK. We slightly modified the strategy used for the selection and the study of transposition events for *K. oxytoca* and *Salmonella* serovar Typhimurium (31) in order to minimize liquid culture steps of *Y. pestis*. Independent clones of *Y. pestis* 6/69c electroporated with pBLOCK or pBLOCK+ were grown overnight on LB plates containing hemin, KAN, and IPTG to induce transposition. They were then streaked on LB plates containing hemin, CHL, and IPTG to check the loss of the plasmid.

Cm^s clones were grown overnight in LB liquid medium containing KAN and IPTG prior to DNA extraction. Total cellular DNAs were digested with *Mlu*I (which has a single site in pBLOCK, in the *lacI* gene), and 1 to 5 μg was analyzed by Southern blot with IROK (Fig. 1) as a probe. Fragments of DNA digested with *Mlu*I or *Bst*EII were circularized with T4 DNA ligase and used to transform BW19610, a strain which allows autonomous replication of circles containing R6K origin of replication present in IROK. Recombinant clones were selected on LB plates containing KAN. After DNA sequencing, chromosomal regions flanking the module were identified using FASTA software (21) from Infobiogen (http://www.infobiogen.fr/) and BLAST (1) from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) or the Sanger Centre (http://www.sanger.ac.uk/Projects/Y_pestis/). Chromosomal locations given thereafter between brackets refer to strain CO-92 biovar Orientalis, which was sequenced there.

Selection of transposition events in *E. coli* using pBLOCKS. Transposition has been tested as previously described for *Salmonella* serovar Typhimurium and *K. oxytoca* (31), with a slight modification: 1 μl of overnight cultures of independent pBLOCKS-transformed clones was plated on LBS containing KAN or LBS containing KAN and IPTG (10^{-6} M) (which is not toxic at this concentration). Sucrose-resistant clones were then tested for CHL and IPTG sensitivity. Clones presenting the correct phenotype (i.e., sucrose, KAN, and IPTG resistant and CHL sensitive) were analyzed as before.

Transposition assays using pIROKCS. Independent clones of *E. coli* and *Y. pestis* transformed with pIROKCS were grown overnight in liquid LB medium containing KAN and CHL. Ten microliters for *E. coli* and 500 μl for *Y. pestis* of each culture were plated on LBS containing KAN, incubated overnight, and then tested for CHL sensitivity on LBS containing CHL. All clones tested were still CHL resistant, indicating that they had kept the plasmid and that transposition could not be detected in this case.

RESULTS AND DISCUSSION

IS*1397* is an IS*3* family member discovered in natural isolates of *E. coli*. It is always inserted into small extragenic

repeated sequences scattered on the *E. coli* chromosome, the PUs (3). PUs are the basic motif of BIMEs, which associate PUs with extra-PU motifs according to precise rules. They have been described for several enterobacteria, where slight sequence variations allowed us to define species-specific consensuses (6, 31). We previously tested IS*1397* transposition with PU-containing species (*E. coli* [6], *Salmonella* serovar Typhimurium, *K. pneumoniae*, and *K. oxytoca* [31]) and showed that it is able to transpose from a donor plasmid into their chromosomes, almost exclusively into PUs. Interestingly, transposition for *K. pneumoniae* took place preferentially into PUs which were close to the *E. coli* consensus rather than into characteristic *K. pneumoniae* PUs (which are more GC rich). The more the PU consensus differs from the *E. coli* PU consensus, the more we observed non-PU insertions (such as in *K. oxytoca*). *Y. pestis* is phylogenetically close to *E. coli*, but a systematic computer analysis of intergenic regions in this species did not reveal the presence of any repeated sequence similar to PUs or BIMEs. We investigated the ability of IS*1397* to transpose onto the chromosome of this PU-free species and analyzed the nature of transposition targets.

We studied the transposition of IS*1397* in *Y. pestis* using pBLOCK or pBLOCK+, two plasmids previously used in *Salmonella* serovar Typhimurium and *K. oxytoca* (31). Selection of transposition events relied on the Km^r IPTG^R Cm^s phenotype, which should result from transposition of the module into the chromosome followed by the loss of the plasmid. The presence of both *orfA* and *orfAB* (both lethal when overexpressed) minimized the recovery of IPTG-resistant clones due to mutations in these genes. A deletion encompassing both these genes and the CHL resistance gene would not allow the maintenance of an autonomously replicating plasmid, since the p15A origin of replication is located between the CHL resistance gene and *orfA* (Fig. 1).

Forty-eight and forty-four independent *Y. pestis* clones containing pBLOCK and pBLOCK+, respectively, were streaked on IPTG and KAN plates and subsequently replica plated on CHL and IPTG plates to check for the loss of the plasmid. A total of 57 independent Km^r IPTG^r Cm^s clones were obtained and examined for the presence of IROK on the chromosome by Southern blot hybridization. They displayed one or several bands ranging from 2.5 to 15 kb. Chromosomal DNA fragments encompassing IROK contain a KAN resistance gene and an R6K origin of replication, which is active in *E. coli* BW19610. After recircularization, 25 plasmids were recovered and used to sequence the new flanking regions of the transposable module. Several classes of transposition events were observed.

Class I: circles. We obtained five clones (I.a to I.e) consisting of circles derived from pBLOCK (Fig. 2). Four of them were a truncated version of the transposable module (IROK). IRL was fused to the DNA region located after the KAN resistance gene. Thus, IRR was deleted and no extra bases were found at the junction. These circles probably correspond to an intramolecular transposition event within pBLOCK. Such a phenomenon has already been observed in the case of IS*911* (22, 30) and was interpreted as an alternative pathway to the formation of intermediate transposition circles. These circles could not be detected on Southern blots when the total DNA content (i.e., chromosomal and plasmidic) of the candi-
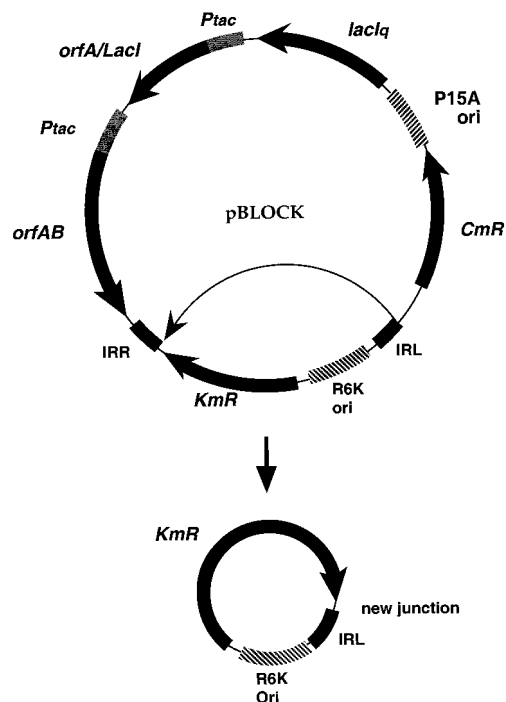

FIG. 2. Structure of the circles observed in clones I.a to I.e. See the legend for pBLOCK in Fig. 1. The thin arrow inside the pBLOCK plasmid indicates the target region in IROK for IRL attack.

dates was analyzed (data not shown), indicating that they are in very low copy numbers and/or very transient. This is consistent with the fact that they do not carry a fully functional origin of replication and must be lost at a high frequency. Covalently closed circles have a high transformation potential in an *E. coli* strain such as BW19610 which can support their replication. If the ligation efficiency of linear *Mlu*I or *Bst*EII chromosomal fragments was low, the recovery of circular molecules at the expense of ligated chromosomal fragments was expected. It is interesting that we never observed such circular forms in other enterobacteria (6, 31), and it may be due to the tendency of *Y. pestis* to keep replicating such suicidal plasmids (E. Carniel, unpublished observation). In clone I.a, which will be discussed below, IRR was present and IRL was modified from $^{5'}$TG-CAGT*T*CA$^{3'}$ to $^{5'}$TGCAGT*G*CA$^{3'}$ (base change is in italic; the same type of change as observed in clone III.d). IRL and IRR were joined by an AAG-triplet which is originally flanking neither IRR nor IRL in pBLOCK (Fig. 1).

Class II: typical chromosomal transposition events. A classical transposition event was observed in 11 cases with a 3-bp duplication at the sites of insertion (Fig. 3, class II).

For seven clones (class IIA, Fig. 3), the transposable module had moved into five distinct regions. It inserted into IS*1541* (19) in clones IIA.a and IIA.b. The insertion site (upstream of IRR) and the duplication (TAT) are identical, but the module orientations and the chromosomal locations (coordinates 4,064,971 and 3,417,535, respectively) are different. An insertion into a palindromic region which precedes another copy of IS*1541* was observed in clone IIA.g. In clones IIA.c to IIA.e, independent transposition events occurred at the same site (position 2,398,820) and with the same module orientation. In

Class[a]	Clones	Y. pestis flanking sequence[b]	Transposable module[c]	Y. pestis flanking sequence	Coordinates[e]	Insertion site features
IIA	IIA.a	Ggcatagccggaggtttttcat**at**	TGAACTGAA ← TGCAGTTCA	**tat**gcgcctataaggctct	4,064,971	into IS*1541*
	IIA.b	ggcatagccggaggtttttcat**at**	TGAACTGCA → TTCAGTTCA	**tat**gcgcctataaggctct	3,417,535	into IS*1541*
	IIA.c, d, e	cagtttgccggatacagccgat**ag**	TGAACTGAA ← TGCAGTTCA	**tag**ccgtcatatcaagctg	2,398,820	none
	IIA.f	agaatacgccggattacccaa**ctg**	TGAACTGCA → TTCAGTTCA	**ctg**tggctatcggtggcat	4,193,142	*thiE-F* intergenic region
	IIA.g	aacaaaatgccggtctggggt**aac**	TGAACTGAA ← TGCAGTTCA	**aac**cctaccggcatttta	1,726,432	next to IS*1541*

Class[a]	Clones	Y. pestis flanking sequence[b]	Transposable module[c]	Y. pestis flanking sequence	Coordinates[e]	Insertion site features
IIB	IIB.a, b, c	Agttttggggttcagtgca**cgg**	TGAACTGCA → TTCAGTTCA	**cgg**ccgcgccccttaggaa	4,137,899	truncated YpIS IRR
	IIB.d	agttttggggttcagtgcacgg**gcc**	TGAACTGCA → TTCAGTTCA	**gcc**gcgccccttaggaa	4,137,899	truncated YpIS IRR

Class[a]	Clones	Y. pestis flanking sequence[b]	Transposable module[c]	Y. pestis flanking sequence	Coordinates[e]	Insertion site features
III	III.a, b	agttttggggttcagt<u>g</u>ca AAT	TGAACTGCA → TTCAGT<u>g</u>ca	cggccgcgccccttaggaa	4,137,899	truncated YpIS IRR
	III.c	agttttggggttcagt<u>g</u>ca ATT	TGAACTGCA → TTCAGT<u>g</u>ca	cggccgcgccccttaggaa	4,137,899	truncated YpIS IRR
	III.d	agttttggggt<u>t</u>cagt<u>g</u>ca ATT	TGAACTGAA ← T<u>t</u>CAGT<u>g</u>ca	cggccgcgccccttaggaa	4,137,899	truncated YpIS IRR
	III.e	agatctggggttcagt<u>g</u>ca AAT	TGAACTGCA → TTCAGT<u>g</u>ca	cttatggcccccttggcgg	128,549	YpIS IRL
	III.f	agttttggggttcagt<u>g</u>ca ATT	TGAACTGCA → TTCAGT<u>g</u>ca	cttatggcccattggcgg	127,107	YpIS IRR
	III.g	agatctggggttcagt<u>g</u>ca ATT	TGAACTGCA → TTCAGT<u>g</u>ca	ttatgacccctctgaaatg	1,107,464	YpIS IRL
	III.h	actttggggttcagt<u>g</u>ca ATT	TGAACTGCA → TTCAGT<u>g</u>ca	cgcttgtggcccctttggc	2,945,763	solo YpIS IRR
	III.i	ataaatggggttcacttca ATT	TGAACTGCA → TTCAGTtca	gaatggcggtttttttgag	1,811,394	solo YpIS IRL?

Progenitor of I.a		agatctggggttcagt<u>g</u>ca AWT	TGAACTGAA ← TGCAGT<u>g</u>CA	aagcgctggcccccttggaa	4,417,515	hypothetical structure

Transposable module sequence in pBLOCK and pBLOCK+[d]

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ATT   TGAACTGCA  →  TTCAGTTCA   AAT
ATT   TGAACTGAA  ←  TGCAGTTCA   AAT
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FIG. 3. Insertion sites of the transposable module into the chromosome of *Y. pestis*. Superscript italic letters indicate the following. *a*, the three classes have been defined according to the insertion mechanisms and sites (see Results and Discussion). *b*, nucleotides duplicated after transposition are indicated in bold. W stands for A or T. *c*, thick arrows indicate the module orientation on the *Y. pestis* chromosome. Only the nine external base pairs of IRL and IRR are written, and *Y. pestis* IR residues which correspond to changes in the original sequence of IROK are double-underlined. *d*, the sequence of the transposable module in the donor plasmids is written in both orientations. *e*, the coordinates have been determined referring to *Y. pestis* CO-92 biovar Orientalis, sequenced at the Sanger Centre. The progenitor of clone I.a is the hypothetical chromosomal structure resulting from the initial transposition event (see Results and Discussion).

clone IIA.f, the transposable module inserted between *thiE* and *thiF* homologs.

For four clones (class IIB, Fig. 3), transposition has occurred next to a noncharacterized *Y. pestis* resident IS. Screening of the GenBank database with its nucleotide sequence gave the best score with IS*150* (55.5% identity). The distal sequences of its inverted repeats are almost identical to IS*1397*: *Y. pestis* IRL shares 19 nucleotides with IS*1397* IRs, and *Y. pestis* IRR shares 21 nucleotides with IS*1397* IRL and 23 with IS*1397* IRR (Fig. 4). OrfA and OrfB *Y. pestis* IS proteins share 38.9 and 49.5% identity, respectively, with the corresponding proteins of

IS*1397*. The transposable module has inserted three or five nucleotides downstream from the IR in three (IIB.a to IIB.c) or one (IIB.d) clones, respectively. Although this *Y. pestis* IS is present in 10 copies (2 of them are truncated) in the *Y. pestis* genome, the four insertions were all located next to the same copy.

Interestingly, two sites were found either three or four times independently: clones IIA.c to IIA.e at position 2,398,820 and clones IIB.a to IIB.d at position 4,137,899. The exact site of transposition in clone IIB.d is shifted by 2 bp compared with the three others. Hot spots for insertion of IS*1397* have been found in all tested species (6, 31). The finding of a similar phenomenon in *Y. pestis* confirms that some chromosomal regions are more attractive than others for transposition, independently of the presence of PUs.

Moreover, IS*1397* seems to target sequences which share similarities with the *E. coli* PU consensus in class IIA clones. Indeed, in five regions (represented by a total of seven independent events [Fig. 3, class II]), transposition events took place 11 to 13 bp from the sequence 5′GCCGG3′ (5′GC-CGGA3′ in 4 examples) (Table 1), which is part of the sequence of an *E. coli* PU stem. This sequence is, however, not found in class IIB clones (next to a *Y. pestis* IS). Similarly, for *E. coli* (6) and for species containing PUs which are different

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IS1397    IRR  TGTTCAGGTTTTGGGGTTCAGTTCA
               |||| | | |||||||||||| ||   19/25
Y. pestis IRL  GATTCAAGATCTGGGGTTCAGTGCA
               |||||| || ||||| |||| ||    19/25
IS1397    IRL  TGTTCAACATTTGGGGTGCAGTTCA


IS1397    IRR  TGTTCAGGTTTTGGGGTTCAGTTCA
               |||||| ||||||||||| |||||   23/25
Y. pestis IRR  TGTTCAAGTTTTGGGGTTCAGTGCA
               |||||||  |||||||| |||| ||  21/25
IS1397    IRL  TGTTCAACATTTGGGGTGCAGTTCA
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FIG. 4. Sequence comparisons between IS*1397* and *Y. pestis* IS IRs. IS*1397* IRL and IRR are compared with homologous sequences of *Y. pestis* IRs. Variable nucleotides between the four sequences are indicated in bold.

TABLE 1. Comparison of non-PU insertion sites of the transposable module in several enterobacteria

Species	No. of clones	Flanking sequence upstream of the insertion site[a]	Duplication	No. of residues identical to 17-bp consensus
E. coli PU consensus		A T AC A T GCC GAT GC G CG T G G T	**NNN**	17
Y. pestis	2	ATA GCCGGAG GT TTTTC A	**TAT**	10
Y. pestis	3	TTT GCCGGAT AC AGCCG A	**TAG**	12
Y. pestis	1	TAC GCCGGAT TA CCCAA	**CTG**	9
Y. pestis	1	AAT GCCGGTC TG GGGT	**AAC**	10
Y. pestis[b]	3	TTT GGGGTTC AG TGCA	**CGG**	6
Y. pestis[b]	1	TTT GGGGTTC AG TGCACG	**GCC**	7
K. pneumoniae	1	TGG GCCGGAT GG GCCGA	**CAG**	10
K. pneumoniae	1	CCT GCCGGAT GA CACGT	**TTA**	13
K. pneumoniae	1	CAG GCCGGAT GG AACGG	**GGT**	12
K. pneumoniae	1	ACG GCCTTCG GC GGCGA	**AGTG**	10
K. pneumoniae	1	ACT TCCACCA GC AGCGG	**AAC**	9
K. oxytoca	1	AGT GCCTGAT GG GCGGC	**ATG**	13
K. oxytoca	1	ACC GCCGGAT CG CGG	**CGT**	9
K. oxytoca	1	GAC GCCGGAT AC CTGC	**GCG**	9
E. coli	1	CTT GCCGGAT AT TCA	**CAG**	9
E. coli	1	CAA GCCGGAT CG TCCGG	**GAAC**	10

[a] Flanking sequences left to insertion sites are aligned under the *E. coli* Y PU consensus 5′ end. Identities with the *E. coli* Y PU consensus are underlined, and identities with the insertion consensus that we have defined (5′ GCCGG3′) are twice underlined. Duplicated nucleotides are indicated in bold.

[b] Insertion in these clones occurred at the same location on the chromosome, but the exact site of transposition in the last clone is shifted by 2 bp compared with the three others.

from those of *E. coli*, such as *K. pneumoniae* and *K. oxytoca*, a few transposition events were found outside PUs but in the vicinity of similar sequences (31). A comparison of all these non-PU insertion sites (Table 1) shows that the 5′GCCGG3′ sequence is present in 11 out of 16 insertion sites, and in these 11 cases, the homology can be further extended (underlined in Table 1). However, this similitude between *Y. pestis* target consensus and *E. coli* PUs is limited to the first part of the PU stem, and no palindromic structure was found in this case. This observation suggests that the transposase binds directly the target sequence and that a secondary DNA structure is not required for IS*1397* transposition.

Class III: nontypical transposition events. Nine transposition events (Fig. 3) occurred next to the same *Y. pestis* IS as in class IIB, but they were different for the following reasons. (i) No target duplication was observed. (ii) Three nucleotides, either ATT or AAT, were found between the end of the target site (*Y. pestis* IR) and IROK. These nucleotides are flanking IS*1397* IRs in pBLOCK or pBLOCK+. (iii) Changes in the end of the IS*1397* IR, which is distal to *Y. pestis* IR, were observed: 5′T*G*CAGT*T*CA3′ (IS*1397* IRL) in clone III.d or 5′TTCAGT*T*CA3′ (IS*1397* IRR) in clones III.a to III.c and III.e to III.h were changed into 5′T*T*CAGT*G*CA3′ (change in italics). In all cases, the original sequence was converted into the sequence found at the target site. In clone III.i, 5′TTCA*G*TTCA3′ (IS*1397* IRR) was unchanged, while the sequence of *Y. pestis* IS at this position is 5′TTCA*C*TTCA3′.

What was observed for class II clones corresponds to the hypothetical classical pathway for IS*1397* transposition, which consists of the excision of the transposable module encompassing 3 bp flanking either IRR or IRL, followed by a joining. The formation of such circles as transposition intermediates is now well documented in a number of cases (14, 29). After removal of the three extra base pairs, the IS is integrated into its target, with a 3-bp duplication.

Instead, in the class III clones, 3 bp, 5′ATT3′ or 5′AAT3′, which are originally flanking, respectively, the 5′ and 3′ ends of the transposable module on pBLOCK (Fig. 1), were inserted between the *Y. pestis* resident IS and the transposable module. The trinucleotide 5′ATT3′ sequence is found in six clones, either next to IRL (clones III.c and III.f to III.i) or to IRR (clone III.d), keeping the same configuration as on pBLOCK (i.e., 5′ to the transposable module). However, three clones (clones III.a, III.b, and III.e) displayed the nucleotides 5′AAT3′ at the 5′ end of the transposable module (whereas it is found at its 3′ end on pBLOCK).

Moreover, in the class III clones, not only were the three flanking nucleotides kept, but also the end of the distal IR (actually IRR in eight cases and IRL in one case) was substituted for the sequence found in the *Y. pestis* target IR. One possible mechanism for the final structure is depicted in Fig. 5. The first step of transposition would be as previously described, leading to the formation of a circular intermediate. A 3-bp shift of the cutting site would lead to a deletion of the last 3 bp of either IS*1397* IRs (TCA in both cases), and three extra base pairs flanking either IR (AAT or ATT, AWT in the figure) would be kept instead. This could explain the presence of the 3 bp which are flanking the module. The further steps of transposition would be as described previously: the 3-bp duplication of the insertion site (the three last base pairs of *Y. pestis* IR) would reconstitute a complete but modified IS*1397* IR. In clone III.i, IS*1397* IRs were unchanged. However, in this particular example, *Y. pestis* IR ends with CTTCA whereas IS*1397* IRR ends with GTTCA. This means that at the most, the four last nucleotides could have been exchanged with no apparent change in the end product.

Our hypothesis is strengthened by the examination of clone I.a, which harbored a circle where the transposable module has an intact IRR linked to a modified IRL (*Y. pestis* type) by the trinucleotide AAG. It can be assumed that this circle originates
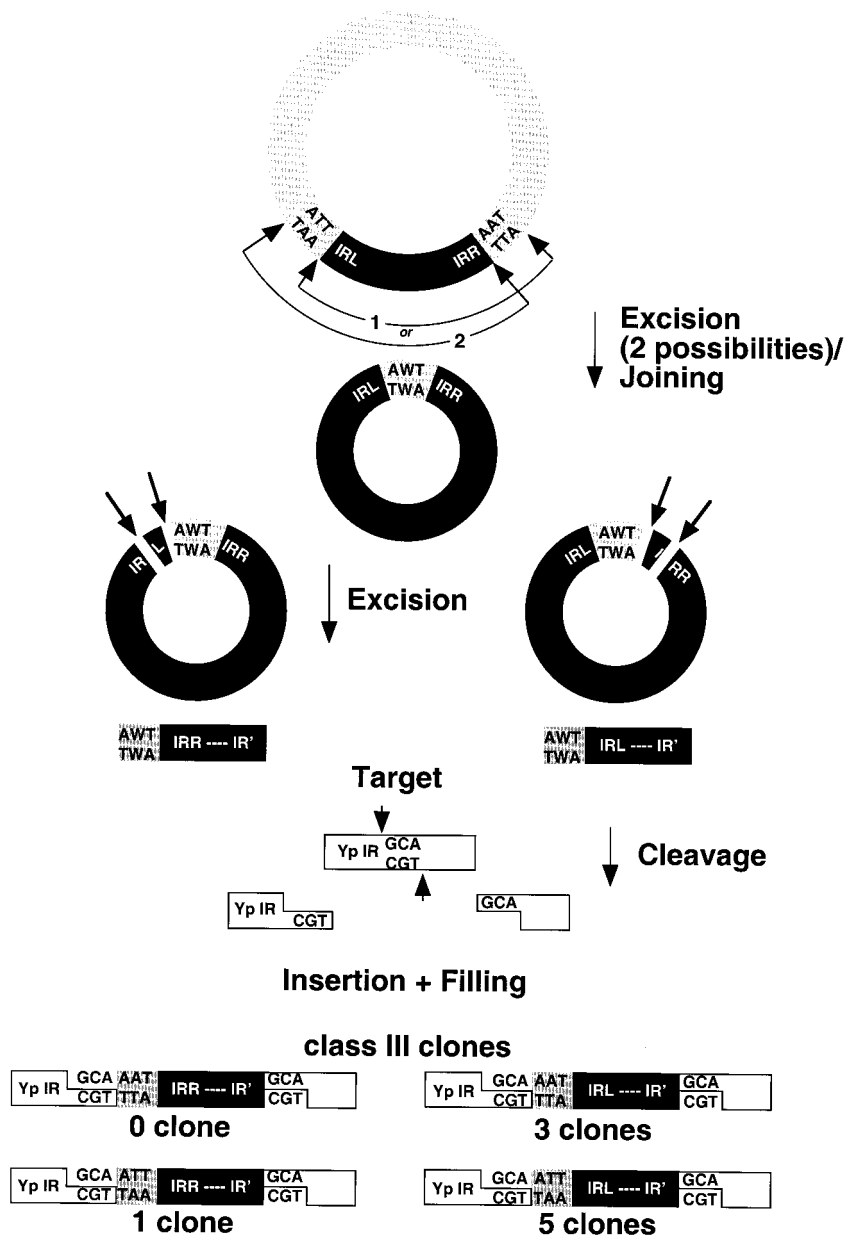
FIG. 5. Hypothetical pathways for the formation of class III (see Fig. 3) clones. Top, the donor plasmid (either pBLOCK or pBLOCK+) is cut 3 bp after IRR (1) or before IRL (2). After recircularization of the module (middle part), the three last base pairs (AWT, which stands for ATT or AAT) of IRL (left) or IRR (right) are removed, instead of the three extra bases, and the module is integrated into the target with a 3-bp duplication (lower part).

from a molecule different from the initial plasmid (pBLOCK), presumably from a chromosomal insertion site with a structure similar to clone III.d (i.e., IROK containing a modified IRL inserted next to a *Y. pestis* IR). It is interesting that one of the *Y. pestis* IRs (at nucleotide 4,417,515) is flanked by AAG, so that the circle observed in clone I.a could come out from the excision of IS*1397* previously integrated at this site.

The analysis of the associations between IS*1397* IRs and the flanking 3 bp showed that six clones displayed the AAT-IR parental structure (found on the donor plasmid) and three clones had the ATT-IR recombinant structure (Fig. 5). This shows that IS*1397* transposase did not strongly discriminate between IRL

and IRR for excision and entrapping of the flanking 3 bp (Fig. 5, top). Contrastingly, eight clones had the structure *Y. pestis* IR-A(A/T)T-IRL-modified IRR and only one (clone III.d) was *Y. pestis* IR-ATT-IRR-modified IRL, indicating that the transposition intermediates were more often opened inside IRR than IRL (Fig. 5, bottom). It should be noted that the terminal region of *Y. pestis* IRs (which are involved in catalysis [7]) is more similar to the IS*1397* IRR than IRL (Fig. 4), which could be related to the prevalence of the first category (*Y. pestis* IR-A(A/T)T-IRL-modified IRR). Clone III.d is actually peculiar, since IS*1397* IRL exhibited a structure (two changes in the last 8 bp) which is not explained by the model above.

In order to know whether class III nontypical transposition events, all occurring next to the *Y. pestis* IS, were due to the activity of *Y. pestis* endogenous transposase(s) rather than to IS*1397* transposase, we tested whether transposition of IROK cloned into a transposase-free plasmid could be selected, using SacB toxicity. We first tested for *E. coli* whether sucrose toxicity was sufficient to counter-select pBLOCKS. Since we did not know if the transposase production under uninduced conditions (i.e., without IPTG) was sufficient to promote transposition, we also induced transposase production under sublethal conditions, i.e., 10^{-6} M IPTG. Twelve independent clones transformed with pBLOCKS were streaked on plates containing Sac and KAN or Sac, KAN, and 10^{-6} M IPTG. In each case, 152 colonies were subsequently checked for plasmid loss on plates containing either CHL or 10^{-3} M IPTG. We obtained 146 independent Km^r IPTG^R Cm^s clones after limited transposase induction (IPTG, 10^{-6} M) and 129 independent Km^r IPTG^R Cm^s clones without transposase induction. Ten of each were examined for the presence of IROK on the chromosome by Southern blot hybridization and displayed one or two bands ranging from 2.5 to 10 kb (data not shown). We sequenced the new flanking regions of the transposable module for six candidates (three induced and three not induced). In all cases, an insertion into the loop of a PU was observed (data not shown), confirming that selection of transposition events can be selected by sucrose toxicity. This result shows that the uninduced level of OrfAB production is sufficient to promote transposition. In a next step, pIROKCS was used to test whether transposition of IROK could occur into *E. coli* and *Y. pestis* chromosomes without IS*1397* transposase. Twelve independent clones containing pBLOCKS were streaked on plates containing Sac and KAN. In the cases of both *E. coli* and *Y. pestis*, all clones obtained had kept the plasmid since they were all CHL resistant. This indicates that IS*1397* transposase is indispensable for IROK transposition in both *E. coli* and *Y. pestis* and that class III transposition events are not due to an endogenous *Y. pestis* transposase activity alone. We can hypothesize that class III transposition events could result from co-action of an endogenous *Y. pestis* transposase and IS*1397* transposase. Further experiments outside the scope of this paper would be necessary to confirm this hypothesis.

Conclusion. Our previous results demonstrated that IS*1397* transposes preferentially into PUs in PU-containing species (6, 31), with a preference for *E. coli* consensus PUs. In the present study, we show that for *Y. pestis*, a species which does not contain PUs, transposition can take place. Circles and nontypical transposition events that we observed suggest that IS*1397* has adopted the two-step transposition mechanism (23) already described for other IS*3* family members (25). We demonstrate here our previous hypothesis: IS*1397* transposition can take place via direct recognition of a consensus (5′GC-CGG^3) target sequence which shares sequence similarities with PUs and which is found 11 to 13 bp upstream from the insertion site. This suggests that transposition of IS*1397* does not necessarily involve PU-associated structures. Indeed the results with *Y. pestis* show that no PU specific host factor is strictly required for IS*1397* transposition. However, IS*1397* transposition depends on factors other than sequence recognition. First, for all species so far examined, hot spots of insertion were observed, which indicates a possible role of local

chromosomal structures. Second, for *E. coli,* PUs are almost exclusive targets for IS*1397* rather than other sites containing the consensus 5′GCCGG3′ sequence, which are, however, abundantly represented throughout the chromosome outside PUs. This indicates that the consensus sequence is sufficient to enable IS*1397* transposition but that the specificity of insertion into PUs could be due to PU structure and/or specifically bound factors.

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