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IS*1397* Is Active for Transposition into the Chromosome of *Escherichia coli* K-12 and Inserts Specifically into Palindromic Units of Bacterial Interspersed Mosaic Elements

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We demonstrate that IS*1397*, a putative mobile genetic element discovered in natural isolates of *Escherichia coli*, is active for transposition into the chromosome of *E. coli* K-12 and inserts specifically into palindromic units, also called repetitive extragenic palindromes, the basic element of bacterial interspersed mosaic elements (BIMEs), which are found in intergenic regions of enterobacteria closely related to *E. coli* and *Salmonella*. We could not detect transposition onto a plasmid carrying BIMEs. This unprecedented specificity of insertion into a well-characterized chromosomal intergenic repeated element and its evolutionary implications are discussed.

Bacterial interspersed mosaic elements (BIMEs) are repeated structures found on the chromosomes of *Escherichia coli* and other enterobacteria (3, 13). They are positioned in intergenic regions, between convergent operons, or between genes belonging to the same operon and are composed of several motifs assembled with a precise organization, which is summarized in Fig. 1. The basic motif is a palindromic unit (PU), also known as a repetitive extragenic palindromic (REP) sequence (11, 17, 26), which is an imperfect palindromic sequence (Fig. 1) that can confer a stem-loop secondary structure to mRNA. Two PU types, Z and Y, which differ at positions 7 and 32 of their sequence (respectively T/A and G/C), have been defined. The Z family can be divided in two classes (Z^1 and Z^2) according to their size (top of Fig. 1). PUs can be found singly or in clusters, in which they alternate in orientation and type. In this case, they are separated by other conserved motifs (extra PU motifs) to form BIMEs (bottom of Fig. 1). Two BIME families have been described previously (13). In BIME-1, one Z^1 and one Y PU are placed head to head, are separated by an L motif, and are flanked by an A motif on the Z^1 side and a B motif on the Y side. The BIME-2 family structure involves a basic element consisting of one Z^2 and one Y placed head to head, separated by an l, r, or s motif. This basic element can be repeated (up to six times), and each repeat is separated from the following one by an S motif. The chromosome of *E. coli* K-12 contains 61 BIME-1, 71 BIME-2, and 49 occurrences of other associations between PUs and extra PU motifs which are different from the ones mentioned (hereafter referred to as atypical BIMEs).

IS*1397* is a putative insertion sequence related to members of the IS*3* family. This sequence was discovered during the study of intergenic regions in several isolates of *E. coli* (2). These regions had been chosen because they contained typical BIMEs. In the three cases analyzed, IS*1397* was found to be inserted in a PU. In a second step, cloning and analysis of chromosomal DNA fragments from EPEC25 and ECOR49, two strains hosting numerous copies of the IS, confirmed this observation and supported the hypothesis of a target site specificity for IS*1397* insertion into PUs. In this study, we investigated IS*1397* in K-12, the laboratory strain of *E. coli* which normally does not contain IS*1397*. We developed a genetic tool which allowed us to select for transposition events from a donor plasmid carrying a genetically tagged version of IS*1397*. Our results show that IS*1397* is a fully active insertion sequence for transposition into the chromosome of *E. coli* K-12 and complete the demonstration of the high specificity of insertion into extragenic PUs. This case represents the most striking example of sequence specificity for IS insertion.

MATERIALS AND METHODS

Media and standard procedures. Luria-Bertani (LB) medium was used for growth of all *E. coli* strains. Kanamycin and ampicillin were used at 25 and 50 μg/ml, respectively. Restriction enzymes and DNA-modifying enzymes were purchased from New England Biolabs or Boehringer Mannheim and used as recommended. Oligonucleotides were purchased from Genset. Plasmid DNA manipulations were carried out by standard procedures (24). PCR was performed with the Amersham PCR kit as recommended and with an MJ Research Inc. PTC-100 apparatus.

Plasmids. pNABI (Fig. 2) is a composite P15A-based plasmid carrying a modified version of IS*1397* in which a kanamycin resistance cassette has been inserted between *orfB* and the right-end inverted repeat (IRR). This IS is flanked by the same sequences (an interrupted PU sequence with a 4-bp duplication) that were found in the *mtlA* to *mtlD* region of EPEC25, a natural enteropathogenic isolate of *E. coli* in which IS*1397* had been described originally. This module was inserted between the *Xba*I (bp 1424) and *Tth*111-1 (bp 3698) sites of plasmid pACYC184 (8). The second important component of pNABI is an isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *orfAB* cassette composed of a *Ptac* promoter, a ribosome binding site, an open reading frame encoding the expected OrfAB fusion protein (22), and *lacI*^{q}. This module was inserted between the *Nco*I (bp 3944) and *Xmn*I (bp 635) sites of plasmid pACYC184 (8). The structure of pNABI is as follows: bp 1 to 795, P15A origin of replication from pACYC184 (bp 635 to 1429) (8); bp 796 to 798, linker (CTT); bp 799 to 837, EPEC25 *mtlA* to *mtlD* intergenic region, interrupted PU (2); bp 838 to 887, IS*1397* IRL (bp 1 to 51) (2); bp 888 to 1409, IS*1397 orfA* coding sequence (bp 52 to 573) (2); bp 1410 to 1513, IS*1397 orfA-orfB* intergenic region (bp 574 to 677) (2); bp 1514 to 2236, IS*1397 orfB* coding sequence (bp 678 to 1400) (2); bp 2237 to 2261, pUC18 multiple cloning site (MCS) (bp 424 to 452) (33); bp 2262 to 2282, pUC19 MCS (bp 399 to 419) (33); bp 2283 to 3549, pUC4K kanamycin resistance cassette (*Bam*HI fragment, bp 408 to 1674) (28); bp 3550 to 3554, linker TCTAG; bp 3555 to 3586, IS*1397* IRR (bp 1401 to 1432) (2); bp 3587 to 3606, EPEC25 *mtlA* to *mtlD* intergenic region, interrupted PU (2); bp 3607 to 3631, pUC18 MCS (bp 430 to 454) (33); bp 3632 to 3879, end of the chloramphenicol resistance gene

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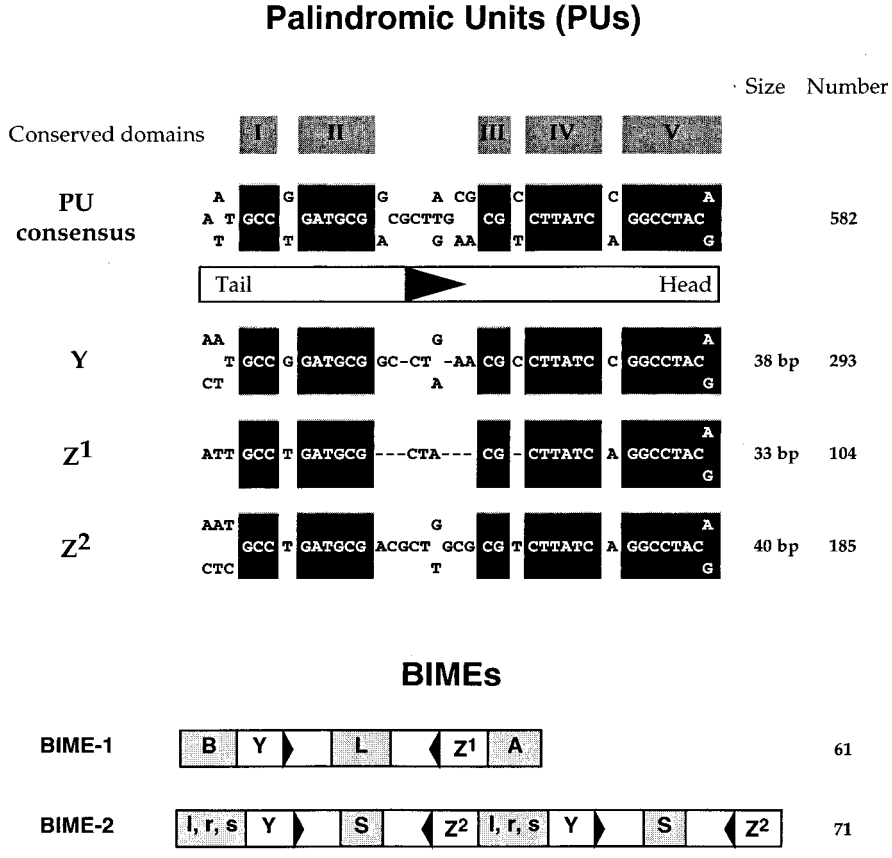

FIG. 1. BIME organization. PUs (11, 17) and BIMEs (3, 13) have been described previously. Important features are summarized here. (Top) Consensus sequences for PUs (upper part) and for the three PU types, Y, Z^1, and Z^2 (lower part). Domains conserved between the three types are boxed in black. Nucleotides G between domains I and II as well as C between domains IV and V are found in Y, while the same positions are occupied respectively by T and A in Z. These sequences are globally palindromic, with asymmetry elements which allow orientation of the structure, which is drawn under the PU consensus, from tail to head. A black triangle indicates PU orientation. The right-hand column shows the number of occurrences on the *E. coli* chromosome (1). (Bottom) BIMEs are composed of PU repeats in which both PU types (Z and Y) and orientation alternate (13). Members of BIME-1 are typically composed of one Y and one Z^1 in head-to-head orientations, placed between A and B and separated by L. Members of BIME-2 are repeats of Y and Z^2 alternating in opposite orientations, separated by S (between heads) and/or by either an s, l, or r motif (between tails).

from pACYC184 (bp 3703 to 3950) (8); bp 3880 to 5048, *lacI*^{q} PCR fragment generated with plasmid CIMER (27) as a template and the two oligonucleotides C_6TCTAGACCATGGTCACTGC$_3$GCT$_3$CCAGTCG$_3$ and C_6TCTAGAGCTA GCACCATCGAATGGTGCA$_4$CCT$_3$CGCGG as primers—this fragment was cut with *Xba*I and introduced into the *Nhe*I site artificially introduced downstream of the *orfAB* gene; and bp 5049 to 6394, assembly of two PCR fragments generated with, as a template, a pUC19 recombinant plasmid in which a *Hin*dIII EPEC25 chromosomal DNA fragment overlapping the *mtlA*-to-*mtlD* intergenic region contains IS*1397* (2). The first fragment, containing *orfA*, was made with the following two primers: G_6ATCCAAGGACCATAGATTATGA$_3$CATTCAT $_3$GAAGTA$_4$CTTGCCGC and G_6ACGCGTGCTAGCTCCTGGCGCCT$_7$CCA GAAGATGCTCCTGCATGGC. The second fragment, containing *orfB*, was made with the following two primers: GAACATCTTCTGGA$_7$GGCGCCTGG AGCAGGTGA$_6$CGA$_3$GTCATCC and CAGT$_3$CAGCTAGCCGGCT$_5$GATAC TC. These fragments were cloned separately and recombined by being cut with *Kas*I before their insertion into pNABI. This created an artificial in-frame fusion between *orfA* and *orfB* in which the wild-type palindromic sequence characteristic of frameshift windows (22),

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                  --- -   ---  >                        <-- --    -- --
CTG GAA AAA AAG CGC CAG GAG CTG GAG AAA AAA CGA AAG TCA TCC AGA GCC TGA GGT
 L   E   K   K   R   Q   E   L   E   K   K   R   K   S   S   R   A   *
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has been replaced by the following, which creates *orfAB*, an in-frame fusion between *orfA* and *orfB* due to the deletion of one nucleotide and the disruption of the palindrome (underlined):

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CTG GAA AAA AAG GCG CCT GGA GCA GGT GAA AAA ACG AAA GTC ATC CAG AGC CTG AGG T
 L   E   K   K   A   P   G   A   G   E   K   T   K   V   I   Q   S   L   R
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The structure of pNABI continues as follows: bp 6395 to 6413, ribosome binding site from pMAL-p2 bp (New England Biolabs) (bp 1513 to 1527); and bp 6414 to 6794, *Ptac* promoter from pDR540 (Pharmacia) (bp 1 to 389) (23).

pUP3 has been described previously (12). It consists of a DNA fragment with *Nco*I ends filled with Klenow polymerase and cloned into the *Sma*I site of pUC18. This fragment contains a 3-PU BIME-2 present in the *E. coli malE-malF* intergenic region. The structure of this region is *malE*→**Y′→s←Z^2 S Y**→*malF*→ (see the legend of Fig. 3 for explanations of the symbols). The BIME is flanked by *Sac*I and *Bcl*I sites on one side and by a *Bam*HI site on the other side, so that successive rounds of ligations between *Sac*I-*Bcl*I and *Sac*I-*Bam*HI fragments created a series of recombinant plasmids with increasing numbers of PUs, up to 99 occurrences (33 tandem repeats of the same BIME-2 repeated in tandem). The largest plasmid, called pUP99, was digested with *Bam*HI and *Eco*RI, and its insert was transferred into pTZ18 (Pharmacia), which contains an f1 origin of replication. Since the new plasmid, called pTZ99, contained sequences located between the *Eco*RI (bp 213) and *Pvu*II (bp 415) sites of pTZ18 that were also present in pNABI, these were removed by ligating the large *Pvu*II-*Rsa*I fragment from pTZ99 to the large *Pvu*II fragment from pTZ18, creating pTZ99Δ.

Bacterial strains. We used strains JM109 [*recA1 endA1 gyrA96 thiA hsdR17* (r_K^- m_K^+) *relA1 supE44* Δ(*lac-proAB*) (F′ *traD proAB lacI*^{q}*ZΔM15*)] (33), TG1 [*supE hsdΔ5 thiA* Δ(*lac-proAB*) (F′ *proAB lacI*^{q}*ZΔM15*)] (Promega), PL0 [F$^-$ Δ(*lac-proAB*) *strA trp ara thiA* Val^r *galE* ϕ80d*lac lacIΔ169*) (25), JC10289 (a gift from A. J. Clark), PL1 [F$^-$ Δ(*lac-pro*AB) *strA trp ara thiA* Val^r *galE* ϕ80d*lac lacIΔ169 recA* [see below—*recA* transduction of strain PL0 by a P1 phage stock made on JC10289]), PL2 (PL0/pNABI), PL3 (PL1/pNABI), and P4 (JM109/pNABI/pTZ99Δ).

Selection of transposition events. Independent clones of PL2 and PL3 strains were grown overnight at 37°C in liquid LB medium containing kanamycin, and a 500-μl volume of each culture was plated on an LB plate containing kanamycin

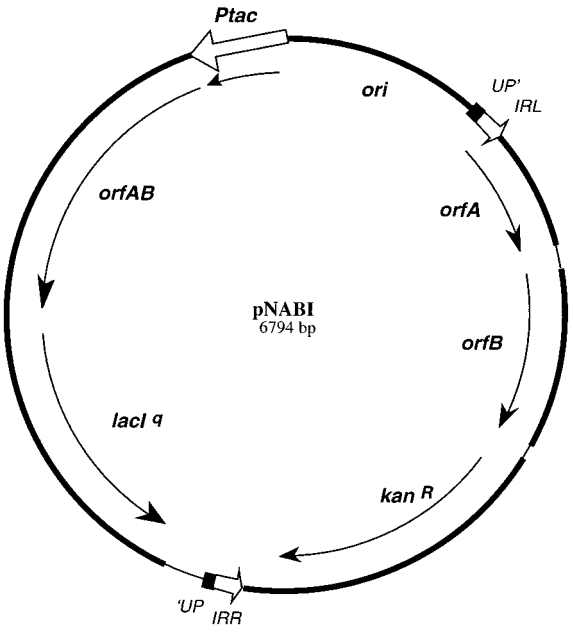

FIG. 2. Plasmid pNABI. pNABI is a composite low-copy-number plasmid composed of two modules. The first (clockwise) is IS*1397*, in which a kanamycin resistance gene has been inserted downstream of *orfAB*. In the second (counterclockwise), the expression of OrfAB is under the control of the *Ptac* promoter and is repressed by the product of *lacI* (IPTG inducible). Details are given in Materials and Methods.

and 10^{-3} M IPTG. After overnight incubation at 37°C, the plates were replicated onto LB plus kanamycin (without IPTG). After a 24-h incubation at 37°C, these plates were replicated on LB plus kanamycin plus 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) plates to check the LacI$^+$ phenotype of IPTG-resistant colonies. Blue colonies (which had possibly lost the plasmid) were restreaked and grown in liquid medium for subsequent plasmid preparation by the minilysate alkaline lysis technique. These preparations were analyzed by agarose gel electrophoresis and used to transform strain PL0 to Km^r.

Chromosomal DNA extraction. The centrifugation pellet from a 1-ml overnight culture was resuspended in 500 μl of TE (10 mM Tris-HCl [pH 8], 1 mM EDTA). Then 14 μl of 10% sodium dodecyl sulfate (SDS) and 13 μl of proteinase K (20 mg/ml in 100 mM Tris-HCl [pH 7.5]–100 mM $CaCl_2$) were added, and incubation at 37°C was continued until lysis. Two phenol and one chloroform-isoamyl alcohol extractions were followed by ethanol precipitation. The DNA pellet was resuspended in 400 μl of TE.

Southern hybridization. Chromosomal DNAs were digested with *Bgl*II (which does not cut inside IS*1397*), and 1 to 5 μg was loaded on a 1% agarose gel. After electrophoresis, DNA was transferred to a Hybond N$^+$ membrane (Amersham) by using a Transvac TE80 vacuum blotter (Hoeffer Scientific Instruments) as follows: 0.25 N HCl for 15 min and 0.4 N NaOH for 1 h. After transfer, the membrane was neutralized for 15 mn in 1 M Tris-HCl (pH 7.5) and incubated for 1 h in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 2 h in hybridization buffer (6× SSC, 0.1% SDS, 0.05% skim milk powder) at 65°C. The heat-denatured probe was added, and hybridization was carried out overnight at 65°C. The probe was an internal *Eco*RI fragment from IS*1397* ^{32}P labelled with the Promega nick-translation kit. The membrane was washed twice at room temperature with 2× SSC–0.1% SDS for 30 min and twice at 68°C in 0.2× SSC–0.1% SDS for 45 min. The membrane was wrapped in Saran Wrap and exposed on an autoradiographic film.

DNA cloning of IS*1397* insertion sites. *Bgl*II-digested chromosomal DNAs were ligated to pUC18 vector cut with *Bam*HI and treated with phosphatase (pUC18 *Bam*HI/BAP; Pharmacia Biotech), and *E. coli* TOP10 cells were transformed as recommended by the supplier (Invitrogen). For fragments which were too long to be cloned in pUC18, chromosomal DNAs were codigested with *Apa*LI, *Bgl*II, *Nco*I, and *Nde*I (none of which have restriction sites in the IS), and a 3′-A residue was added by using *Taq* polymerase activity (Invitrogen). DNAs were then ligated to the pGEM-T vector, and *E. coli* JM109 cells were transformed as recommended by the supplier (Promega). In both cases, ampicillin- and kanamycin-resistant clones were selected on LB-kanamycin-ampicillin plates.

DNA sequencing. DNA sequencing was performed with Qiagen-purified plasmid DNAs (Qiagen spin minipreps or Qiagen midi preps as recommended) and with a Thermo Sequenase Cy5.5 dye terminator cycle-sequencing kit (Amersham Pharmacia Biotech). Sequencing reactions were run on an Amersham Pharmacia Biotech 4X4 automatic sequencer. The following primers were used for sequencing: lig-PCR-A (GCCGTAGAAATGATGCCTGC), complementary to codons 20 to 26 of *orfA*) (2) and Km seq out (CACGAGGCAGACCTCAGCGC), corresponding to a region located between the end of the Km^r gene and the IRR of IS*139* (2), as found on plasmid pNABI (Fig. 2 and see below). Chromosomal regions flanking the IS were identified on the Colibri Web Server (8a).

RESULTS

Transposition events. To study IS*1397* transposition, pNABI was constructed. It is composed of two modules. The first contains IS*1397* flanked by an interrupted PU and containing a kanamycin resistance gene inserted between *orfB* and IRR. The second contains an *orfAB* in-frame fusion under the control of the IPTG-inducible *Ptac* promoter. *lacI* was included in the construct to achieve repression of the toxic OrfAB protein in all *E. coli* strains. pNABI was used to transform PL0, a *lacZY*$^+$ *lacI* strain. In the presence of IPTG, OrfAB is expressed, and this induction was found to be lethal to the cells. IPTG-resistant colonies could be isolated after overnight culture on LB-kanamycin-IPTG plates at 37°C (see Materials and Methods). These were found to arise from distinct events: (i) a mutation within *orfAB* resulting in a nontoxic protein (in this case, LacI is still expressed and the presence of this marker is revealed by a white phenotype after restreaking on LB–kanamycin–X-Gal plates without IPTG; (ii) a deletion encompassing *orfAB* and *lacI*; or (iii) transposition of the IS*1397*-Km^r module into the chromosome, with a loss of the pNABI donor plasmid. Due to the loss of *lacI*, these last two events would give blue colonies on kanamycin–X-Gal plates. To discriminate between them, the presence or absence of a plasmid carrying the kanamycin resistance gene was checked by analyzing minilysates by agarose gel electrophoresis or by analyzing for the ability to transform a kanamycin-sensitive strain into a resistant one.

Approximately 5×10^8 CFU from each of 26 separate liquid cultures of independent PL2 (*rec*$^+$) or PL3 (*rec*) clones were plated on IPTG plates. Resistant colonies were obtained for 24 PL2 and 22 PL3 plates. Of these, 19 PL2 and 20 PL3 plates contained blue colonies when streaked on LB–X-Gal plates. Two LacI$^-$ colonies from each plate were checked for the absence of plasmid, either by analyzing plasmid minipreparations by agarose gel electrophoresis or by using the same minipreparations to transform PL0 to kanamycin resistance. When used in parallel, both tests always led to the same conclusion: approximately half of the clones tested had lost the plasmid, so that 11 PL2 and 7 PL3 clones could be examined for the presence of an IS*1397*-Km^r insertion in the chromosome. *Bgl*II digests of chromosomal DNA were analyzed by Southern hybridization with an internal IS*1397* DNA fragment as a probe (results not shown). Under these conditions, all candidates showed a single band (ranging from approximately 7 to 15 kb) hybridizing to the probe. In all cases, the two colonies from the same plate proved to be identical, i.e., containing a plasmid or, when plasmid free, displaying a single IS-containing chromosomal *Bgl*II DNA fragment of identical size. We cloned these *Bgl*II fragments directly in pUC18. When this was not successful, we used the TA cloning procedure on DNA fragments obtained after chromosomal DNA was cut with a cocktail of enzymes which did not cut within IS*1397*-Km^r. This allowed us to analyze the new junctions between the IS and the chromosome. The results are presented in Fig. 3.

As we expected from our previous results, all insertions were found to have occurred within a PU, with two possible exceptions (see Discussion) in the *recA* context (Fig. 3, column b). In all instances, insertion took place in the loop, with a 3- to 4-bp

	Strain	Origin (a)	PU consensus and insertion site (b)	BIME structure (c)	BIME type (d)	Coordinates in K-12 (kb)	ref.
	Y consensus		AA -------> ---> G <--- <-------- A T GCCGGATG C GGCG T AA CGCC T TATCCGGC CTAC CT A G				
1	ECOR49	Seq. IS	GTT GCCGGATG C GGC TAAG CACC T TATCCGGC CCAGG	←*malM* Y→ S ←Z^2 s Y'] → [`Y→ ←*lamB*	2	4246,9	(2)
2	ECOR49	Seq. IS	AAT GCCGGATG C GGCG TGAA CGGC C TGTCCGGC CTACA	*malE*→ Y'] → [`Y→ s ←Z^2 S Y→ *malF*→	2	4244,0	(2)
3	EPEC25	Seq. IS	TTT GCCGGATG C GGCG TAAA CGCC T TATCCGGC CTACG	←*mtlD* Y'] → [`Y→ S ←Z^2 I Y→R←Z^1 ←*mtlA*	2	3770,0	(2)
4	EPEC25	Cloning	ATT GCCGGATG C GGTG TATAA CGCC T TATCCGGC CTACA	*acs*→ Y'] → [`Y→ *b4068*→	AB	4285,0	(2)
5	EPEC25	Cloning	AAT GCCGGATG C GGCG TGAA CGCC T TATCCGGC CTACA	*mpl* → ←Z^2 I Y'] → [`Y→ ←*b4234*	2	4454,8	(2)
6	EPEC25	Cloning	ATT GCCGGATG C GGCG TGAA CGCC T TATCCGGC CTACA	*b2712*→ Y'] → `Y→ S ←Z^2 I Y→ S ←Y ←*b2711*	2	2835,5	(2)
7	EPEC25	Cloning	GTT GCCGGATG T GGCG TAAA CGCC T TATCCGGC CTACA	*nhoA*→ Y'] → [`Y→ S ←Z^1 ←*b1464*	1	1533,9	(2)
8	EPEC25	Cloning	AAT GCCGGATG C GGCG TGAA CGCC T TATCCGGC CTACG	*walX*→ Y→ S ←Z^2 s Y'] → [`Y→ S ←Z^2 B *wcaK*→	2	2116,5	(2)
9	EPEC25	Cloning	CAT GCCGGATG C GGCG TAA CGCC T TATCCGGC CTACA	*livJ*→ Y'] ← [`Y→ L ←Z^1 *livK*→	1	3596,1	(2)
10	EPEC25	Lig-PCR	TGG CGTC T TATCCGG CTACA	*cca*→ Y'] → [`Y→ S ←Z^2 s Y→B ←*bacA*	2	3200,9	(2)
11	EPEC25	Lig-PCR	AA CGCC T TATCCGGC CTACA	*fdnI*→ B Y'] → [`Y→ L ←Z^1 A B Y→ L ←Z^1 A ←*b1477*	1	1550,1	(2)
12	K-12 (*rec*+)	*rhsB* sequence	TAAA CGCC T TATCCGGC CTAAA	*b3481*→ *rhsB* [`→ [`Y→ L ←Z^1 *b3483*→	1	3621,0	(2)
13	K-12 (*rec*+)	Transposition	GAT GCCGGATG C GGCG TAAA CGCC C TATCCGGC CTACA	*b3815*→ Y'] → [`Y→ L ←Z^2 ←*uvrD*	2	3997,8	this work
14	K-12 (*rec*+)	Transposition	AAT GCCGGATG GC AGAGTT GCCA C CCGGC TGATT	*ung*→ Y'] ← [`Y→ ←*b2581*	AB	2715,5	this work
15	K-12 (*rec*+)	Transposition	ACT GCCGGATG C GGCA TAAA CGCT T TATCCGGC CTACC	*fhi*A→ Y→ S ←Z^2 I Y'] ← [`Y→ S ←Z^2 ←*yafM*	2	248,2	this work
16	K-12 (*rec*+)	Transposition	ACT GCCGGATG C GGCG TAAA CGCC T TATCCGGC CTACG	*b3875*→ Y'] → [`Y→ S ←Z^1 ←*b3874*	AB	4061,9	this work
	Z^2 consensus		AAT -------> ----> G <---- <-------- A GCCTGATG C GACGC T GC GCGTC T TATCAGGC CTAC GTC T G				
17	ECOR49	Seq. IS	AAC GCCTGATG C GACGC TGAC GCGTC T TATCAGGC CTACA	*araA*→ $Z^{2\prime}$] ← [`Z^2→ S ←Y *araD*→	2	66,9	(2)
18	EPEC25	Cloning	AAT GCCTGATG C GACGC TTGC ACGTC T TATCAGGC CTACA	*b3150*→ $Z^{2\prime}$] → [`Z^2→ S ←Y ←*b3151*	2	3294,7	(2)
19	K-12 (*rec*+)	Transposition	GAT GCCTGATG C GACGC TACC GCGTC T TATCAGGC CTACA	*b0801*→ ←Y S $Z^{2\prime}$] ← [`Z^2→ s ←Y ←*b0802*	2	836,9	this work
20	K-12 (*rec*+)	Transposition	AAT GCCTGATG C GACGC TGCC GCGTC T TATCAGGC CTACG	*b2989*→ ←Y I $Z^{2\prime}$] ← [`Z^2→ ←*hybG*	2	3137,7	this work
21	K-12 (*rec*+)	Transposition	AGT GCCTGATG C GACGC TGAC GCGTC T TATCAGGC CTACA	←*wcaK* Z^2→ S ←Y s $Z^{2\prime}$] ← [`Z^2→ S ←Y ←*walX*	2	2116,5	this work
22	K-12 (*rec*+)	Transposition	AGT GCCTGATG C GACGC TGAC GCGTC T TATCAGGC CTACA	←*wcaK* Z^2→ S ←Y s $Z^{2\prime}$] → [`Z^2→ S ←Y ←*walX*	2	2116,6	this work
23	K-12 (*rec*+)	Transposition	ATT GCCTGATG C GATGC TAAT GCGCC G CATCAGGC CTACG	←*appA* $Z^{2\prime}$] → [`Z^2→ ←*appB*	AB	1039,7	this work
24	K-12 (*rec*+)	Transposition	CAC GCCGGATG C GACGC AAAT GCGTC T TATCCGGC CTACA	*glnA*→ $Z^{2\prime}$] → [`Z^2→ S ←Y *glnL*→	2	4054,1	this work
25	K-12 (*recA*)	Transposition	CAC GCCGGATG C GACGC AAAT GCGTC T TATCCGGC CTACA	*glnA*→ $Z^{2\prime}$] → [`Z^2→ S ←Y *glnL*→	2	4054,1	this work
26	K-12 (*recA*)	Transposition	ATT GCCTGATG C GACGC TTAC CCGTC T TATCAGGC CTACA	*b0483*→ ←Y I $Z^{2\prime}$] ← [`Z^2→ S ←Y I Z^2→ S ←Y ←*b0484*	2	508,0	this work
27	K-12 (*recA*)	Transposition	ATT GCCGGATG C GACGC TGAC GCGTC T TATCCGGC CTACA	*tktB*→ Z'] $Z^{2\prime}$] ← [`$Z^2$→ [`Z→ ←*b2466*	AB	2579,7	this work
28	K-12 (*recA*)	Transposition	ATT GCCGGATG C GACGC TGAC GCGTC T TATCCGGC CTACA	*tktB*→ Z'] $Z^{2\prime}$] → [`$Z^2$→ [`Z→ ←*b2466*	AB	2579,7	this work
29	K-12 (*recA*)	Transposition	ATT GCCGGATG C GACGC TGAC GCGTC T TATCCGGC CTACA	*tktB*→ Z'] $Z^{2\prime}$] ← [`$Z^2$→ [`Z→ ←*b2466*	AB	2579,7	this work
	Z^1 consensus		-------> -> <- <-------- A ATT GCCTGATG C GC TAC GC T TATCAGGC CTAC G				
30	EPEC25	Lig-PCR	ACT GCCTGATG C GC TACCGA	*yacK*→ A $Z^{1\prime}$] → `Z^1→ L ←Y B ←*gcd*	1	138,7	(2)
31	K-12 (*recA*)	Transposition	CCT GCCGGAT ATTCA CAG CCGCGCC AACAATC CTACC	-*b3712*→	-	3892,1	this work
32	K-12 (*recA*)	Transposition	CAA GCCGGAT C GTCCGGGAACT CCA TCATCATGGC CCGCA	-*b3656*→	-	3830,3	this work

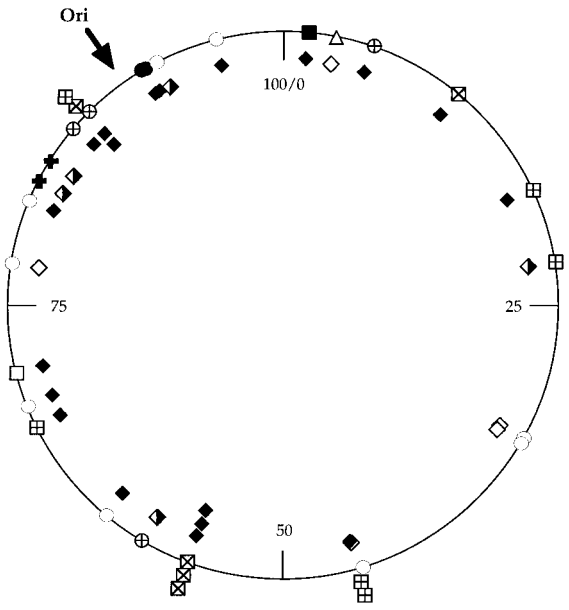

FIG. 4. Chromosomal locations of the various IS*1397* insertions in *E. coli*. Circular map of *E. coli* and positions of the various occurrences of IS*1397* insertions. The origin of replication is indicated by an arrow, and coordinates are indicated in minutes. Symbols for PUs are as follows: ○, Y (EPEC25); ●, Y (ECOR49); ⊕, Y (K-12 *rec*$^+$); ✚, non-PU; △, Z^1 (EPEC25); □, Z^2 (EPEC25); ■, Z^2 (ECOR49); ⊞, Z^2 (K-12 *rec*$^+$); ⊠, Z^2 (K-12 *rec*). Inside the circle, symbols for BIMEs are as follows: ◇, BIME-1; ◆, BIME-2; ◈, Atypical BIME.

FIG. 3. Insertion sites of IS*1397*. Natural or laboratory examples have been separated into four boxes according to the PU type: Y (insertions 1 to 15), Z^2 (insertions 16 to 28), Z^1 (insertion 29), and non-PU (insertions 30 and 31). The consensus sequences for Y, Z^2, and Z^1 are indicated on top of each box. (a) DNA sequencing of cloned PCR fragments from intergenic regions containing IS*1397* ("Seq. IS"), of ligation-mediated PCR cloned fragments ("Lig-PCR"), and of clones from a representative library of EPEC25 chromosomal DNA ("cloning") has been described previously (2). All other examples ("Transposition") are new examples from the selection of transposition events in *E. coli* K-12. (b) The various insertion sites are indicated. Duplicated nucleotides on each side of the IS are doubly underlined. Pairing nucleotides from the stem of the palindrome are singly underlined and also labelled with arrows above consensus sequences for Y, Z^2, and Z^1. (c) Flanking genes are indicated, as well as the structure of the BIME in which IS*1397* had transposed. Small arrows indicate, the orientation of genes or PUs. The various BIME accessory motifs (A, B, S, s, L, l, and r) (13) are included. The large arrow symbolizes IS*1397* and its orientation within the interrupted PU, which is underlined. (d) The BIME type (BIME-1 or BIME-2) is shown. AB, atypical BIMEs which are either isolated PUs or combinations of Z and Y with accessory motifs that are different from BIME-1 or BIME-2.

duplication. This confirmed the tight IS*1397* specificity of insertion into PUs. Insertion localizations seem to follow the PU distribution on the *E. coli* chromosomal map (1) (Fig. 4). However, some regions were overrepresented. (i) In *walX-wcaK*, three independent insertions were characterized. One (insertion 8) occurred in EPEC25 in the central Y PU, while two (insertions 21 and 22), selected in *E. coli* K-12, had occurred in the central Z^2 PU with either IS*1397* orientation. (ii) Two insertions were found between *glnA* and *glnL*, one (insertion 24) in *E. coli* K-12 *rec*$^+$ and the other (insertion 25) in *recA*, both in the same Z^2 PU in the same IS orientation. (iii) Three occurrences (insertions 27 to 29) were identified in *E. coli* K-12 *recA* at the same position within the Z^2 PU, between *b2466* and *tktB*, with the two possible IS*1397* orientations.

Transposition into a plasmid-borne target. pTZ99Δ and pNABI carry compatible replication origins and specify different antibiotic resistances, and so both plasmids can be maintained in the same cell. We analyzed whether the recombinant IS*1397*-Km^r borne by pNABI could integrate into PTZ99Δ. For this, minilysates were prepared from cultures of strain P4 (which contains both pNABI and pTZ99Δ) grown overnight in LB containing kanamycin and ampicillin. These preparations were used to transform PL0. All the Amp^r Km^r colonies were found to contain both intact plasmids when checked by agarose gel electrophoresis. To avoid the problem of double transformants, we infected strain P4 with M13mp18 phage, and supernatants of overnight cultures in LB plus ampicillin and kanamycin were first filtered to eliminate parental cells and used to transform JM109 or TG1 for Amp^r or for Amp^r Km^r. This protocol takes advantage of the presence of an active M13 origin of replication on pTZ99Δ, which ensures an effective encapsidation by this bacteriophage. Despite an extensive search for colonies resistant to both antibiotics, we never observed recombinant plasmids harboring IS*1397*-Km^r integrated into pTZ99Δ. Hence, considering the titer of pseudo-viral particles able to confer Amp^r (around 10^9/ml), we can estimate

that the frequency of transposition into pTZ99Δ is less than 10^{-8}. The method we used involved M13 encapsidation of an expected recombinant plasmid carrying IS*1397*-Km^r. One could argue that integration of IS*1397*-Km^r into pTZ99Δ would create a plasmid of excessive length or incompatible for encapsidation. This point was checked by using pTZ99, the progenitor of pTZ99Δ, which has two regions of homology to pNABI. In this case, M13 culture supernatants were able to transduce Amp^r and Km^r in a single step. The plasmid content of the resistant colonies was analyzed and revealed either a large plasmid resulting from the recombination between pTZ99 and pNABI or two plasmids resulting from an intramolecular recombination within the latter (not shown). This rules out the possibility that a large plasmid was encapsidated in M13 or that IS*1397* has a deleterious effect on encapsidation. It thus appears that transposition of IS*1397* into a plasmid is truly a rare event ($<10^{-8}$) which could not be detected by our method.

DISCUSSION

We discovered IS*1397* when we studied the polymorphism of intergenic regions containing BIMEs among natural or laboratory strains of *E. coli* (2). Three independent experiments indicated that IS*1397* was systematically associated with PUs. This paper extends these findings to the case of *E. coli* K-12, a strain which was shown to be free of IS*1397*, with the exception of the *rhsB* locus, which contains a truncated form of the IS (2). Our results demonstrate that IS*1397* is a fully active insertion sequence, able to transpose from a plasmid to the chromosome of *E. coli* K-12.

The genetic procedure used to select for transposition events relies on the toxicity of OrfAB. Such a phenomenon has been described for IS*1* (19) and is well documented for Tn*5* (30, 34, 35). The reasons for the toxicity of the IS*1397* transposase are currently being investigated.

The emergence of IPTG-resistant clones is the result of several events. Beside any alteration rendering OrfAB nontoxic for the cell, which still leaves an intact *lacI* on the plasmid and is easily detected, we observed *lacI* clones still harboring a plasmid. We hypothesized that in this case a deletion encompassing *lacI* and *orfAB* on pNABI had occurred. This was checked in 2 cases of 19 obtained by sequencing the regions of interest. The remaining cases, i.e., IPTG- and kanamycin-resistant clones having lost the plasmid, which represent roughly half of the *lacI* clones, were all caused by transposition events. This class is thus the result of two unrelated events: transposition and loss of the plasmid. The selection procedure we used did not allow us to dissociate them, so that the number of clones having undergone transposition is probably larger than what could be selected. We measured the frequency of spontaneous plasmid loss in the absence of selection pressure (kanamycin resistance). We could estimate this rate to be around 5×10^{-4} per generation. If transposition occurs first, the plasmid can be lost, since kanamycin resistance has moved into the chromosome. We observed a highly heterogeneous proportion (from 0 to nearly 100%) of *lacI* colonies in the different independent PL2 or PL3 clones. This could be explained by a different timing in transposition during preculture, with IPTG allowing us to select subsequently for plasmid loss. Not only is IPTG a selective agent for transposition events, but also it induces the expression of OrfAB, the putative transposase from IS*1397*. Overexpression of this protein could induce transposition. However, it does not seem that IS*1397* moves to many chromosomal sites within the same cell, since we never observed on Southern blots any clone harboring more than one IS chromosomal location. We therefore believe that the result of our selection is the emergence of clones having first undergone transposition of the IS, which can take place anytime during the preculture, followed by the loss of the plasmid, which is no longer required to sustain kanamycin resistance, and then counterselection in the presence of IPTG because of the production of a toxic protein.

Transposition into the chromosome could be readily detected. The sequences flanking IS*1397* are shown in Fig. 3. All but two cases (insertions 31 and 32) are characteristic PUs (column b) with a 4-bp duplication at the junction. This signature is a hallmark of transposition and proves that in *E. coli* K-12 transposition did not occur through recombination between a resident PU and the interrupted PU sequence (originating from *mtlA* to *mtlD*) flanking IS*1397*-Km^r. It will be interesting to examine whether the presence of such a truncated PU is necessary for transposition and target specificity. Insertion took place very precisely in the central part of PUs, particularly in Z^2, where in almost all cases the four nucleotides of the loop were duplicated (the only exceptions are insertion 20, where only three residues were duplicated, and insertion 18, where the last nucleotides of the stem do not match, creating a 6-nucleotide central part). Insertion seems less precise for Y. The duplication always overlapped the central part of the palindrome but often included one residue belonging to the "stem." There might be a slight preference for some central sequences. Half of the 32 examples are distributed among three sequences: TGAC was found six times and accounts for half of the Z^2 examples. This sequence was found only in Z^2 even though it is equally close to the Y consensus sequences, TGAA and TAAA, which were found four and six times, respectively, and account altogether for 62.5% of the Y examples. However, when we compared the distribution of PU or BIME types in *E. coli* to the distribution of the PUs or BIMEs found as targets for transposition, we did not observe a statistical difference, indicating that transposition occurs randomly among PUs and BIMEs (data not shown).

As mentioned, two cases (insertions 30 and 31) are not PUs, since they are both located within a coding sequence and are not palindromic but share the sequence GCCGGAT with the Y subtype. These two cases were observed in a *recA* context. However, from our results it does not seem that RecA is involved in target recognition. We computed the number of occurrences of the sequences GCCGGATG and GCCTGATG, which are part of the stem in the two main PU types in the chromosome of *E. coli*. Only 37% (454 of 1,241) of these sequences are found in PUs, indicating that these sequences are far less attractive targets for transposition when they are found alone than when they belong to a PU. PUs can therefore be considered the true target for IS*1397* transposition.

IS*1397* is to our knowledge the first example of an insertion sequence with such a striking transposition target consensus (for a review, see reference 10). For other IS or transposons, the consensus is very weak or totally undefined. Table 1 summarizes a few examples of recently identified target consensus sequences for different bacterial mobile genetic elements. One can imagine that IS*1397*, being targeted into PUs, will transpose systematically into intergenic regions, which is less detrimental to the host than random jumping, which can inactivate genes. Indeed, we observed that IS*1397*, like many other insertion sequences, had no polar effect on the expression of downstream genes when insertion took place in one orientation. For instance, in strain ECOR49, where IS*1397* insertions have been characterized, IS*1397* is inserted between *malE* and *malF* with the same orientation (Fig. 3, insertion 2). The strain is phenotypically Mal$^-$, but the introduction of a plasmid ex-

TABLE 1. Examples of palindromic target consensus sites for IS insertions

Insertion	Sequence	Reference
IS*231A*	GGGnCCC	15
Tn*10*	GCTnAGC	16
IS50/TN*5*	AGNTY RANCT	14
IS*903*	TTTYAnnnnnnnnnnnTRAAA	18
IS*3*	TAnGAAAnnTTCnTA	20
IS*6*	AnGCAGTnnAAAnTGCnT	20
IS*21*	GGAGSnGGCnYYYRnnGCCnSCTCC	20
IS*30*	TAAAAAWGGCnRYCGCnWTTTTTA	20
IS*1397*	MHTGCCKGATGCGRCG(C)TDRM(G)CGYCTTATCMGGCCTATR (PU)	This work

[a] Symbols: Y, C or T; R, A or G; n, A, G, C, or T; S, G or C; W, A or T; D, A, G, or T; B, G, C, or T; K, G or T; M, A or C. Complementary sequences of the palindromes are underlined.

pressing MalT, the activator of the maltose operons, reversed this phenotype, showing that ECOR49 lacks this protein and that the IS did not inactivate the expression of *malF*. In contrast, IS*1397* is inserted between *araA* and *araD* in the opposite orientation (Fig. 3, insertion 17). The strain is Ara$^-$, but revertants could be obtained and all contained deletions of the IS, showing that the IS had a polar effect in this case. IS*1397* could thus propagate safely for the host, limiting the risk of abortive events to insertions in the wrong orientation into a BIME placed in front of an essential gene. A comparable "safe" strategy has been used by Tn*7*, which chose *att*Tn*7* as a specific site for insertion (9). However, such a strategy limits the possibility of spreading. As discussed below, Tn*7* has developed an alternative strategy to solve this dilemma. IS*1397*, with the choice of PUs, automatically solves this problem in a simpler fashion because these sequences are widespread in the chromosome of *E. coli* (579 occurrences) and also in other enterobacteria. Another advantage of selecting a consensus sequence for integration is that intramolecular transposition (i.e., transposition of an IS within its own sequence) is prevented.

Recognition of target sites by mobile genetic elements results from a variety of different mechanisms which are poorly understood. Homology between the target and the ends of the element has been proposed, for instance in the case of Tn*3* (29). Some transposases have been shown to recognize their target directly (10). In other instances, the recognition occurs through an interaction with a DNA binding protein which is distinct from the transposase. The best-documented case is Tn*7*, which transposes either into a specific site, *att*Tn*7*, or to nonspecific sites (9), with a preference for replicating DNAs in this case (31). Both mechanisms invoke an interaction between a specific protein and the target DNA sequence: transposon-encoded TnsD binds *att*Tn*7* and recruits TnsC, the transposition regulator. DNA-bound TnsD also interacts with the transposase TnsA+B, triggering its endonucleolytic and recombinogenic activities (4, 5). TnsE, another Tn*7*-encoded protein, can substitute for TnsD, leading to transposition into replicating plasmids or episomes. In contrast to transposition into the chromosome, no IS*1397* transposition could be detected when the target was on pTZ99Δ. This multicopy plasmid, which contains 33 tandemly repeated BIMEs totaling 99 PUs, brings a substantial amount of PUs to the cell, probably more than the chromosome, which contains 579 PUs. This observation can be connected to the finding that PUs seem specific to chromosomes, since they were never detected on nonchromosomal genetic elements such as episomes, plasmids, or bacteriophages. An attractive hypothesis is that PUs deal with the organization of the chromosome by scaffolding complex structures which include proteins. Several BIME binding proteins have indeed been described. They all play a role in either DNA replication (e.g., DNA polymerase I [12]) or DNA folding: IHF binds the L motif of BIME-1 (7, 21), and DNA gyrase binds PUs (32). If PU-containing structures involved in nucleoid organization were the actual target for IS*1397* transposition, one could explain why PUs are efficient targets on the chromosome but not on plasmids. This would imply that IS*1397* is driven to its target by an interaction between OrfAB, its transposase, and proteins bound to PUs. Such an example of interaction between a transposase and a DNA binding protein has been described for Tn*5* transposase, which binds topoisomerase I (34, 35). An alternative explanation to IS*1397* transposase specificity would be that chromosomal PUs, bound to proteins, adopt a special conformation which renders them competent for a productive interaction with the transposase. Both hypotheses are currently being investigated.

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