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Inverted repeats of Tn*5* are transposable elements

(antibiotic resistance/IS*50*/DNA sequence analysis/evolution/transposon)

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ABSTRACT **Experiments presented here show that each of the 1.5-kilobase inverted repeats of the kanamycin-resistance transposon Tn5 is transposable; we designate them IS*50*-L (left) and IS*50*-R (right). By DNA sequence analyses, IS*50* is 1533 base pairs (bp) long and generates 9-bp direct repeats of target sequences. The ends of IS*50* comprise a hyphenated 8-of-9-bp inverted repeat and are not used with equal efficiency; the outside ends are more active than the inside ends, suggesting that a strong transposase recognition site at the outside end extends beyond the 8 bp common to both ends.**

Transposable elements, defined by their ability to move to numerous sites in a genome without need for extensive DNA sequence homology, mutate genes, alter gene expression, and induce genome rearrangements. In bacteria, they speed the proliferation of genes determining pathogenicity and antibiotic resistance and serve as valuable laboratory tools. Their movement is believed to depend on element-specific transposase proteins that bind sequences near the termini of each element (1–3).

Simple insertion sequence (IS) transposable elements contain only genes and sites necessary for their own transposition. In some of the more complex transposons, auxiliary genes encoding functions unrelated to transposition are bracketed by direct or inverted repeats of IS elements (4–7). Other transposons are different in organization: In Tn*1721*, an intact IS element encoding transposase and a nonfunctional derivative of this element bracket the tetracyline-resistance (*tet*^{r}) gene (8). In Tn*3*, a transposition gene segment apparently related to that in Tn*1721* does not transpose autonomously (9), possibly because the internal end of an ancestral IS element was deleted during evolution.

The 5700-base-pair (bp) kanamycin-resistance (*kan*^{r}) transposon Tn5 (Fig. 1) contains 1534-bp inverted repeats [here called "arms," to distinguish them from the short inverted repeats near the ends of each arm (ref. 12; this work)]. The right arm of Tn5 contains a functional transposase gene; the left contains an ochre mutant allele of the transposase gene (13). DNA sequence determination of the arms of Tn5 (12) revealed no inverted repeats longer than 8 bp near their termini. These arms are not homologous to any known IS elements (14) and, since terminal inverted repeats of other transposable elements are longer than 8 bp (1, 3), it was important to determine whether the arms of Tn5 are transposable or whether Tn5, like Tn*3* and Tn*1721*, contains immobile vestiges of once active IS elements.

Our experiments show that (*i*) the exterior 1533 bp of the 1534-bp arms of Tn5 are transposable (IS*50*) elements; (*ii*) the two ends of IS*50* are not equally active in transposition; (*iii*) IS*50*, like Tn5, generates 9-bp duplications of host sequences when it transposes; and (*iv*) the termini of IS*50* comprise an 8-of-9-bp hyphenated inverted repeat.

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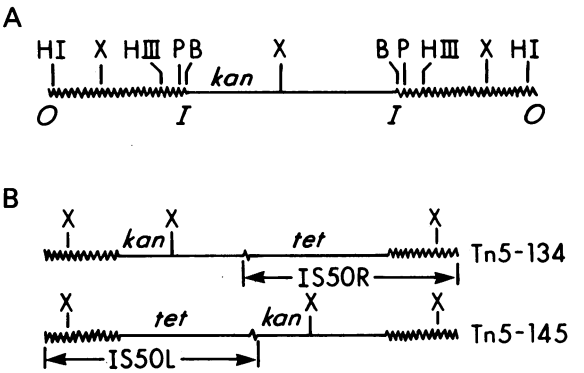

FIG. 1. Map of wild-type Tn*5* and derivatives. (*A*) Wild type (10–12). (*B*) Tn*5*-134 and Tn*5*-145, which contain a 2700-bp *tet*^{r} segment in the *Bgl* II sites of the right and left arms, respectively (10). Restriction endonuclease cleavage sites and their distances in bp from the outside ends of IS*50* are HI, *Hpa* I, 185; X, *Xho* I, 485; HIII, *Hin*dIII, 1195; P, *Pvu* II, 1423; B, *Bgl* II, 1515 (12).

MATERIALS AND METHODS

The *Escherichia coli* K-12 strains used are listed in Table 1. Bacterial plasmids pBRG1, pBRG3, and pBRG5, used for sequence analysis, were generated by insertion of IS*50* into pBR322 (10). Bacteriophage λ *red*$^+$ is λ *b*515 *b*519 *xis*am6 *c*I857 *nin*5 Sam7 (15); λ *red*$^-$ is λ Dam15 *b*515 *b*519 *int*am29 *red*Δ15 *imm*21*c*^{ts} Sam 7 (17). λ::Tn5 *red*$^+$ and λ::Tn5 *red*$^-$ were generated by transposition of Tn5 from chromosomal sites to the λ *red*$^+$ and λ *red*$^-$ phage, respectively. Described elsewhere are media and standard techniques for bacterial growth and conjugation (16), phage induction and transducing phage selection for the detection of transposition events (15), plasmid DNA extraction, transformation and agarose gel electrophoretic analyses of plasmid DNAs (10, 16, 17), and DNA sequence analysis (18).

RESULTS

Inverse Transposition. Infection of *E. coli* by a λ::Tn5 phage often generates Kan^r nonlysogenic transductants (19) due to joining of the outside ends of the arms of Tn5 to target DNA sequences (Fig. 2). If the arms of Tn5 are IS elements, their inside ends should also mediate insertion, an event termed inverse transposition (20). We tested for inverse transposition from phage λ::Tn5 to plasmid pBR322 by identifying Kan^s λ Amp^r Tet^r (Amp^r indicating ampicillin resistance) transducing phage (Fig. 2); 42 of 118 λ Amp^r Tet^r phage from Rec$^-$ strain DB1879-6 and 2 of 107 from Rec$^+$ strain DB1820-10 were Kan^s. Agarose gel electrophoresis of DNA extracted from transduc-

Abbreviations: IS, insertion sequence; bp, base pair(s); kb, kilobase(s); *kan*^{r}, *amp*^{r}, and *tet*^{r}, genes conferring resistance to kanamycin, ampicillin, and tetracycline, respectively.

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Table 1. *E. coli* K-12 strains used

Designation	Genotype	Source
DB114	F⁻ Δ*trpE5 hfl-1 supE*	Ref. 15
DB1504	F⁻ Δ*trpE5* Δ*proBlac supE*	Ref. 16
DB1648	F⁻ Δ*trpE5* Δ*proBlac recA1 supE rpsL*	Ref. 15
DB1816-4	F′ *proB⁺lacZ*155::Tn*5*-134/Δ*proBlac* Δ*trpE5 supE rpsL* (λ*red*⁺)	This work
DB1816-5	F′*proB⁺lacZ*155::Tn*5*-145 Δ*proBlac* Δ*trpE5 supE rpsL* (λ*red*⁺)	This work
DB1820-5	F⁻Δ*trpE5* Δ*proBlac supE rpsL* (λ*red*⁺) [pBRG5]	This work
DB1820-10	F⁻Δ*trpE5* Δ*proBlac supE rpsL* (λ::Tn*5 red*⁺) [pBR322]	This work
DB1820-11	F⁻ Δ*trpE5* Δ*proBlac supE rpsL* (λ *red*⁺) [pBR322]	This work
DB1876-4	F′ *proB⁺lacZ*155::Tn*5*-134/Δ*proBlac* Δ*trpE5 recA1 supE rpsL* (λ*red*⁻)	This work
DB1876-5	F′ *proB⁺lacZ*155::Tn*5*-145/Δ*proBlac* Δ*proBlac recA1 supE rpsL* (λ*red*⁻)	This work
DB1879-6	F⁻ Δ*proBlac* Δ*trpE5 recA1 supE rpsL* (λ::Tn*5 red*⁻) [pBR322]	This work
DB1891	F⁻ Δ*trpE5 hfl-1 recA*Δ306	Ref. 17

tants grown at low temperature (32°C) revealed large [>40-kilobase (kb)] plasmids (Fig. 3, lane 2). Thermal inactivation of the prophage repressor led to production of λ Amp^r Tet^r phage.

To obtain physical evidence that these λ–pBR322 cointegrates had been generated by inverse transposition, 20 independent isolates were digested with *Bgl* II, which cleaves the arms of Tn5 (near their inside ends) but not pBR322. A constant pattern of four fragments was obtained: three from cleavage in λ sequences and a fourth that was 4.4 kb long and contained pBR322 and the inside ends of the two IS*50* elements (Fig. 3, lane 4). Digestion with *Hin*dIII, which cleaves pBR322, generated pairs of fusion fragments unique for each isolate, indicating inverse transposition to numerous sites in pBR322 (Fig. 3, lanes 6–11).

Transposition of IS*50*. Analysis of the Kan^r Amp^r Tet^r λ–pBR322 cointegrate plasmids described below indicated that they contained three copies of IS*50* and that many may have been formed by IS*50* transposition to pBR322 followed by homologous recombination between this IS*50* element and IS*50* in the λ::Tn5 phage (Fig. 4). (*i*) Agarose gel electrophoresis showed that these >40-kb cointegrates were stable in *recA*⁻ strain DB1648 and did not segregate smaller plasmid derivatives; in *recA*⁺ strain DB114, a 5.8-kb (pBR322::IS*50*) recombinant plasmid species accumulated (≈10% of total plasmid DNA after 30 generations). (*ii*) *Xho* I digestion of 19 cointegrate DNAs extracted from *recA*⁻ strain DB1648 generated a set of five fragments reflecting cleavage at single *Xho* I sites in λ, in the central region of Tn5, and in the three copies of IS*50*. (*iii*) *Hin*dIII digestion of pBR322::IS*50* plasmids generated by recombination from the cointegrate DNAs (Fig. 4) resulted in a pair of fragments whose sizes reflect the sites and orientations of IS*50* insertion in pBR322 (Fig. 4 and Fig. 5, lanes 2, 4, 6, 8, and 10). These pBR322::IS*50* *Hin*dIII fragments were also found among the seven fragments generated by digestion of each parental cointegrate extracted from *recA*⁻ cells (Fig. 5, lanes 1, 3, 5, 7, and 9).

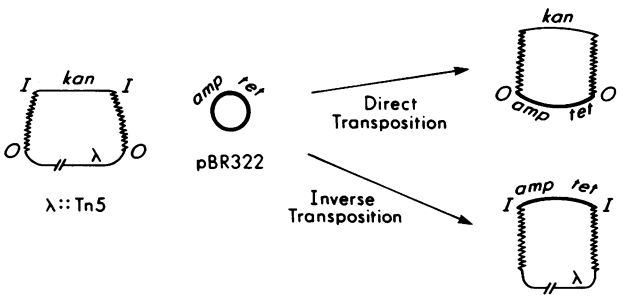

FIG. 2. Direct and inverse transposition using a λ::Tn*5* donor and pBR322 as the target. Jagged lines indicate the arms of Tn*5*.

Demonstration that both Arms of Tn5 Can Transpose. The relative transposabilities of each of the arms of Tn5 was assessed by using Tn5 derivatives in which IS*50* components were marked with a *tet*^{r} determinant (Fig. 1*B*). Lytic phage growth was induced in lysogens carrying the marked Tn5 elements as insertions in *lac*, and Tet^r transducing phage resulting from transposition of IS*50* (*tet*^{r}) or the entire Tn5 (*kan*^{r} *tet*^{r}) element were selected. About one-tenth and one-third of λ Tet^r phage formed in strains carrying Tn5 elements with marked left (IS*50*-L) and right (IS*50*-R) arms, respectively, were Kan^s, indicating transposition of the marked IS element only; the remainder were Tet^r Kan^r due to transposition of the entire element (Table 2). *Xho* I restriction endonuclease digestion of representative λ Tet^r Kan^s and λ Tet^r Kan^r DNAs confirmed that they had resulted from transposition of marked IS*50* (*tet*^{r}) and Tn5 (*kan*^{r}, *tet*^{r}) elements, respectively.

Transposition of pBR322::IS*50*. To test whether all Tn5 sequences essential for transposition are in IS*50*-R [which encodes

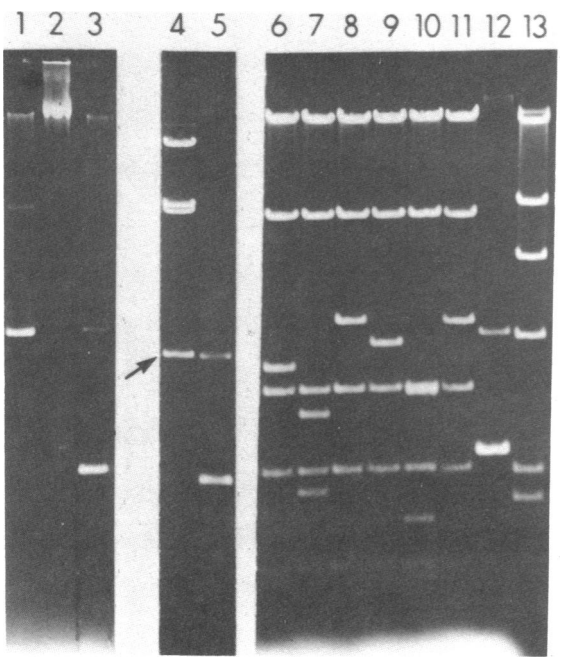

FIG. 3. Agarose gel electrophoresis of λ–pBR322 cointegrates generated by inverse transposition of λ::Tn*5 red*⁻ to pBR322 in strain DB1879-6. Lanes: 1, dimeric pBR322; 2, λ–pBR322 cointegrate; 3, monomeric pBR322; 4, *Bgl* II-digested Kan^s Amp^r Tet^r cointegrate (arrow designates pBR322-containing band); 5 and 12, mixture of uncleaved and *Eco*RI-cleaved pBR322; 6–11, *Hin*dIII-digested Kan^s Tet^r cointegrate DNAs (6 and 9 are insertions into the *amp*^{r} gene); 13, *Hin*dIII-digested λ DNA marker.

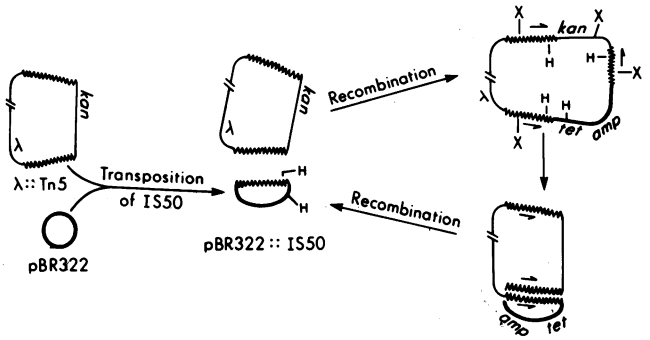

FIG. 4. Formation and breakdown of Kan^r Amp^r Tet^r λ::Tn*5*–pBR322 cointegrates containing three copies of IS*50*. Jagged and thick lines, IS*50* and pBR322 sequences, respectively; H and X, *Hin*dIII and *Xho* I cleavage sites, respectively. Sites in λ DNA are omitted.

transposase (13, 15)], we tested whether a pBR322::IS*50*-R plasmid could transpose to phage λ. Phage lysates prepared by induction of λ in strain DB1820-5 transduced strain DB1891 to Amp^r Tet^r at a frequency of $\approx 10^{-8}$. Each of 40 independent transductants tested carried large λ–pBR322 cointegrate plasmids. Digestion of 15 independent cointegrates with *Xho* I gave a constant 5.8-kb (pBR322::IS*50*) fragment resulting from *Xho*

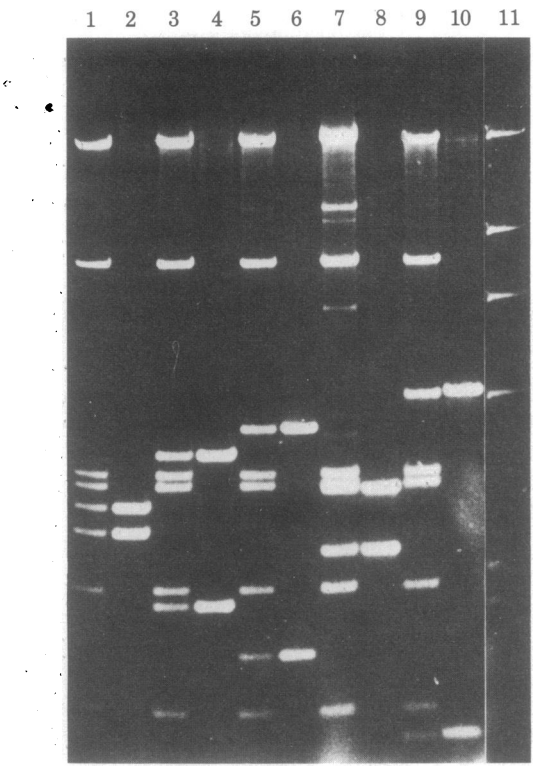

FIG. 5. *Hin*dIII digestion of λ::Tn*5* *red*$^-$–pBR322 cointegrates (lanes 1, 3, 5, 7, and 9) and derived pBR322::IS*50* recombinant plasmids (lanes 2, 4, 6, 8, and 10). The former were extracted as plasmids from *recA*$^-$ strain DB1648; the latter were derived as follows. Phage lysates from cointegrate-carrying strains were used to transduce *recA*$^+$ DB114 to Tet^r at 30°C. Plasmid DNAs extracted from pools of transductants contained 5.8-kb pBR322::IS*50* and large λ–pBR322 cointegrates. These DNA mixtures were used to transform DB1504, and Tet^r transformants were selected at 41°C to kill cells that harbor the λ phage and to select cells that contain the 5.8-kb pBR322::IS*50* plasmid. Eleven of 19 cointegrates (and their descendent pBR322::IS*50* plasmids) analyzed were Amp^s because of IS*50* insertion in the *amp*^{r} gene of pBR322. Amp^s DNAs are shown in lanes 5, 6, 9, and 10. Lane 11: *Hin*dIII-digested λ$^+$DNA.

Table 2. Transposition of IS*50*-R and IS*50*-L marked with *tet*^{r}

Marked arm	Fraction of Kan^s Tet^r/total Tet^r	
	recA$^+$ *red*$^+$	*recA*$^-$ *red*$^-$
IS*50*-R	0.37	0.39
IS*50*-L	0.11	0.12

Six subclones from each of strains DB1816-4, DB1816-5 (*recA*$^+$ *red*$^+$), DB1876-4, and DB1876-5 (*recA*$^-$ *red*$^-$) were prepared, phage development was induced, the lysate was used to lysogenize DB114, and Tet^r transductants were selected. Each fraction is based on tests of 150–200 Tet^r transductants for Kan^r. Kan^s Tet^r transductants carry IS*50* (*tet*^{r}) while Kan^r Tet^r transductants carry the entire Tn*5* element. The frequency of λ Tet^r phages per plaque-forming unit was $\approx 5 \times 10^{-6}$.

I cleavage in the directly repeated IS*50* elements and two variable-size fragments reflecting the positions of insertion relative to the single *Xho* I site in λ DNA. These cointegrates were stable in *recA*$^-$ DB1891 cells; in *recA*$^+$ DB114 cells, they were unstable and gave rise to 5.8-kb (pBR322::IS*50*) plasmids. This indicated that the inserted pBR322 segment was bracketed by direct repeats of IS*50*.

λ Amp^r Tet^r transducing phage were also obtained from DB1820-11, a lysogen carrying pBR322 free of IS*50*, although at a frequency of only $\approx 10^{-10}$ (1/100 of that when pBR322::IS*50* was used). Many such λ–pBR322 cointegrates appear to arise by integration of λ into secondary attachment sites (21). As we found that IS*50* stimulated cointegrate formation ≈100-fold and the cointegrates contained IS*50* as direct repeats joining pBR322 to λ sequences, we conclude that IS*50*-R mediates pBR322 transposition.

DNA Sequence Analyses. To identify the exact termini of IS*50*, we determined the sequences of the IS*50*-target junctions in the pBR322::IS*50* plasmids pBRG1, pBRG3, and pBRG5 (Fig. 6). We found that (*i*) in each plasmid analyzed, the inserted IS*50* segment extended from nucleotide 1 at the outside end of IS*50* to nucleotide 1533 of the 1534-bp arm of Tn5; (*ii*) IS*50* insertion generated a 9-bp target sequence duplication; (*iii*) no pBR322 sequences were deleted; and (*iv*) the IS*50* termini consist of 8-of-9-bp hyphenated inverted repeats.

We also analyzed part of the internal DNA sequence of IS*50* in pBRG5 to determine whether pBRG5 contained IS*50*-R or IS*50*-L. The sequence of the *Pvu* II/*Bgl* II fragment of IS*50* was determined from the *Pvu* II site, and guanosine, characteristic of IS*50*-R, not thymidine, characteristic of IS*50*-L (12, 13), was found at position 1442. As only IS*50*-R encodes a functional transposase in these strains (10, 13, 15, 24), it is unlikely that a pBR322::IS*50*-L plasmid would have transposed to λ.

DISCUSSION

Our analyses show that each of the arms of Tn5 are transposable elements and we designate these elements IS*50*-L and IS*50*-R. This conclusion rests on (*i*) Tn5-mediated inverse transposition of λ::Tn5 to pBR322 (Figs. 2 and 3), (*ii*) transposition of IS*50* from Tn5 (Figs. 4 and 5) and the transposability of one of the resulting pBR322::IS*50*-R plasmids in cells free of other Tn5 sequences, (*iii*) the transposition of *tet*^{r}-marked IS*50* elements (Fig. 1) to phage λ (Table 2), and (*iv*) DNA sequence analyses showing that IS*50* is 1 base shorter than the inverted repeat arms of Tn5 and that it, like Tn5, generates 9-bp duplications of target sequences (Fig. 6). These data confirm and extend earlier conclusions (10, 17, 24).

By its 8-bp terminal inverted repeat, IS*50* is distinguished from other IS elements that contain terminal inverted repeats of 15 or more bp (1, 3). The rarity of inverse transposition (Fig. 2) relative to transposition of IS*50* (Fig. 4) indicates that pairs

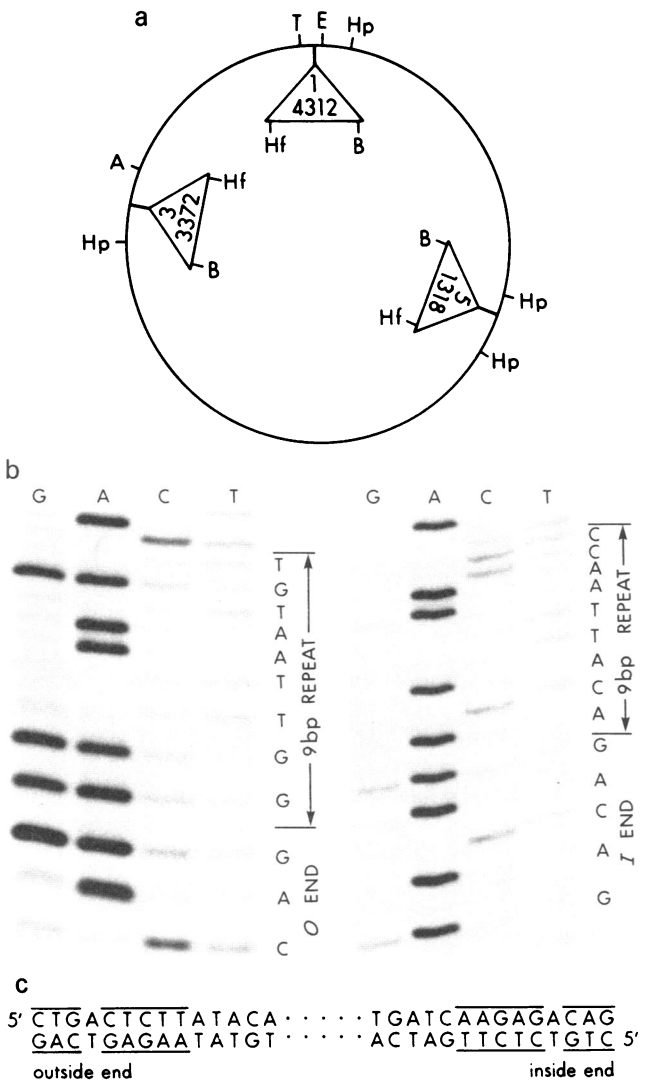

FIG. 6. DNA sequence analysis of IS*50*. (*a*) Map of IS*50* insertions used. Three pBR322::IS*50* plasmids, pBRG1, pBRG3, and pBRG5, were generated by transposition of IS*50* from Tn*5* inserted into the fertility factor pOX38 (22) (referred to as FΔ::Tn*5* in ref. 10) and selected based on the ability of FΔ::Tn*5* to transfer pBR322 carrying IS*50* in conjugation (10). The IS*50* *Hin*fI and *Bgl* II sites [4 and 1515 bp from the outside end, respectively (12)] and the *Alu* I, *Taq* I, and *Hpa* II sites used in the sequence analysis and the *Eco*RI reference site in pBR322 (23) are designated Hf, B, A, T, Hp, and E, respectively. Restriction fragments used for sequence analysis were end labeled with [γ-^{32}P]ATP and polynucleotide kinase (18). The junctions between the outside end of IS*50* and pBR322 were determined after end labeling A in the *Hin*fI site 6 bp from the outside end of IS*50* and cleavage of pBR322 sequences with *Taq* I (pBRG1), *Alu* I (pBRG3), and *Hpa* II (pBRG5). The sequences of the inside junctions were obtained from DNA fragments prepared by end labeling guanosine in the *Bgl* II site 18 bp from the inside end of IS*50* followed by cleavage of pBR322 sequences with *Hpa* II. The 9-bp duplication of pBR322 found at sites of IS*50* insertion were positions 4312–4320 (5′-A-C-A-T-T-A-A-C-C-3′) in pBRG1 (see *b*), 3372–3381 (5′-G-T-G-T-A-G-A-T-A-3′) in pBRG3, and 1318–1326 (5′-G-A-A-T-T-G-G-A-G-3′) in pBRG5. (*b*) Key parts of the DNA sequence of sites of IS*50* insertion in pBRG1. *O*, outside; *I*, inside. (*c*) Sequences at the termini of IS*50* in pBRG1, pBRG3, and pBRG5.

of inside ends act inefficiently. Consequently, we propose that the 8 bp common to the outside and inside ends of IS*50* constitute a minimally efficient transposase recognition site and that a strong recognition site at the outside end extends beyond the common 8-bp segment. However, transposition of IS*50*, which uses one inside plus one outside end detected directly by the *tet*^{r}-marked IS*50* elements (Table 2) or indirectly by the formation of λ::Tn*5*-pBR322 cointegrates (Figs. 4 and 5), is relatively efficient—almost as frequent as transposition of a complete Tn*5* element that used a pair of outside ends. Consequently, we suggest that recognition of the ends of IS*50* by transposase is a two-step process and that the extended DNA sequence of the outside end is needed for efficient binding in only one step.

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