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Preferential Transposition of an IS*630*-Associated Composite Transposon to TA in the 5′-CTAG-3′ Sequence

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A composite transposon, Tn*4731*, associated with IS*630* has been shown to transpose preferentially to 5′-TA-3′ sequences that are located at two sites in a rho-dependent transcription terminator in plasmid ColE1 in *Escherichia coli* (T. Tenzen, S. Matsutani, and E. Ohtsubo, J. Bacteriol. 172:3830–3836, 1990). Here we demonstrated that Tn*4731* preferentially transposes to TA sequences at four sites in plasmid pUC118 and its derivatives: the TA sequence (hot spot I) in the intergenic region of phage M13 within the pUC sequence, the TA sequence (hot spot II) in the *Xba*I site in multiple cloning sites of the *lacZ* coding region, the TA sequence (hot spot III) in a spacer region flanked by inverted repeat sequences of a transcription terminator located downstream of the *bla* gene, and the TA sequence (hot spot IV) in the middle of *bla*. Transposition of Tn*4731* to hot spot III was found not to require the inverted repeats in the terminator. Transposition of Tn*4731* to hot spot II, which is located immediately downstream of the *lacZ* promoter, was not affected by mutations introduced into the promoter. There appear to be no particular sequences important for transposition of Tn*4731* around each of the hot spots, except a palindromic sequence, 5′-CTAG-3′, that contains the target sequence. Mutations introduced within the CTAG sequence at a hot spot inhibited Tn*4731* from transposing to it, indicating that the CTAG sequence is responsible for the preferential transposition of Tn*4731*.

An insertion sequence (IS) is a discrete DNA segment which can transpose from one site to another in bacterial plasmids, chromosomes, and phages (for a recent review, see reference 5). A DNA segment which is flanked by the same IS elements can also transpose together with the IS elements as a unit called a composite transposon (Tn). Transposition of IS and Tn elements occurs at various sites with or without specificity (2, 8, 11, 15, 22, 29).

IS*630* is a 1,153-bp element present in the *Shigella sonnei* chromosome in multiple copies (19, 29). A composite transposon, Tn*4731*, which has two inverted IS*630* insertion sequences that flank the sequence of the tetracycline resistance plasmid pHS1, has been shown to transpose to the dinucleotide 5′-TA-3′ in the core of at least 4-bp palindromic sequences, such as CTAG, TTAA, and ATAT, in ColE1 in *Escherichia coli* (29). There are two hot spots for transposition of Tn*4731* within each of the inverted repeat sequences of 13 bp in a rho-dependent transcription terminator located downstream of the *cea* gene in ColE1 (29).

To clarify the determinants for the site-specific transposition of Tn*4731*, we have studied transposition of Tn*4731* to pUC118 and its derivatives. We report here that Tn*4731* can transpose also to TA sequences at four sites in pUC118 and its derivatives. We also show that the preferential transposition is determined by the CTAG sequence containing the target site, but not by the sequences surrounding the CTAG sequence. The two transpositional hot spots previously identified in ColE1 by Tenzen et al. (29) are actually TA sequences in CTAG. Since the CTAG sequence was within each of the 13-bp inverted repeat sequences, we could not determine that it was the sequence responsible for the preferential transposition of Tn*4731* in the previous study.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and a phage. Bacterial strains used were *E. coli* K-12 derivatives JE5519 (24, 25), MV1184 (30), and BW313 (13, 14).

Plasmid pTT4 was described previously (29). pUC118 and pUC119 (30) were purchased from Takara Shuzo. Helper phage M13K07 (Takara Shuzo) was used for site-directed mutagenesis as described by Kunkel et al. (14) to construct pUC118 derivatives.

Media. Culture media used were L broth, L-rich broth, φ medium (31), and 2× YT broth (21). L-agar plates contained 1.5% agar (Eiken) in L broth. Antibiotics were added to L broth or L-agar plates, if required, as follows: 150 μg of ampicillin (Wako) per ml, 5 to 8 μg of tetracycline (Sigma Chemical Co.) per ml, and 70 μg of kanamycin (Sigma) per ml. Dilution buffer (0.1% tryptone [Difco], 0.3% NaCl) was used for diluting the cell cultures.

Enzymes. Restriction endonucleases used were *Eco*RI, *Xba*I (Takara Shuzo), and *Mae*I (Boehringer Mannheim). Phage T4 DNA ligase (Takara Shuzo) and RNase A (Sigma) were also used. The reaction conditions for these enzymes were as recommended by the suppliers.

DNA preparation. An *E. coli* strain harboring a plasmid was grown in L broth or L-rich broth. The alkaline lysis method (18) or the method of Ohtsubo et al. (23) was used to prepare plasmid DNA for DNA sequencing. The crude lysis method (17) was used to isolate a small amount of plasmid DNA.

Transposition assay. *recA* strain JE5519 harboring pTT4 and each of the recipient plasmids (pUC118, pUC119, pTT70, pTT71, pTT75, pTT80, pTT81, and pTT82) was prepared by transformation with L-agar plates containing tetracycline and ampicillin, as described previously (29). JE5519 harboring the two plasmids was inoculated in 5 ml of L broth and grown at 30°C overnight. The culture was diluted 10^6-fold, and 0.1 ml of the diluted culture was

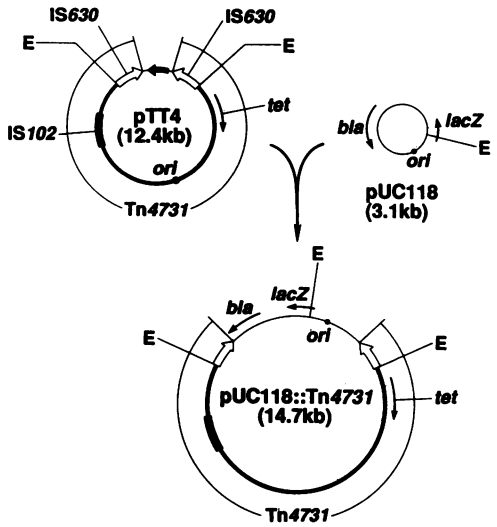


FIG. 1. Structures of pTT4, pUC118, and a pUC118 derivative having received Tn*4731*. Open arrows represent IS*630*. Thick lines represent the sequence of pHS1 from which pTT4 was made (29). Note that Tn*4731* consists of the pHS1 sequence and two IS*630* sequences. Thin lines on circles represent the pUC118 sequence. pTT4 carries an insertion sequence IS*102* (solid box). The pUC118::Tn*4731* drawn here is just one of the pUC118 derivatives with Tn*4731*. A similar construction can be drawn for pTT4 and each of pUC derivatives, pUC119, pTT70, pTT71, pTT75, pTT80, pTT81, and pTT82. A thick solid arrow indicates the chloramphenicol acetyltransferase gene (*cat*). Thin arrows along circles indicate locations and orientations of the tetracycline resistance gene (*tet*), the β-lactamase gene (*bla*), and the β-galactosidase gene (*lacZ*). *ori*, origin of replication; E, *Eco*RI; kb, kilobase.

inoculated into each of 25 culture tubes containing 5 ml of L broth. After incubation at 30°C for 30 h, 0.2 ml of the culture from each tube was spread onto a plate containing tetracycline (8 μg/ml), and the plates were incubated at 42°C for 2 days for selection of colonies of cells harboring the recipient plasmid having Tn*4731*.

Only one colony was picked up from each plate, and cells were grown in L-rich broth at 37°C overnight. Plasmids were extracted and analyzed for their sizes by 0.7% agarose gel electrophoresis. A few plasmids which had the same size as pTT4 were revertants of pTT4 (about 12.4 kb in size) for temperature sensitivity and could replicate at 42°C. The rest of the plasmids were larger than parental plasmids. Most of them were recipient plasmids carrying Tn*4731* (about 14.7 kb) (Fig. 1); the others were cointegrates (about 16.5 kb), formed between pTT4 carrying IS element IS*102* (26) and the recipient plasmid, in which IS*102* was duplicated at the junctions of the two plasmid sequences. The cointegrates were readily distinguished from the plasmids carrying Tn*4731* by digestion with *Eco*RI, as described previously (29). Only the plasmids with Tn*4731* were further analyzed as described in Results. Frequency of transposition of Tn*4731* was calculated from the number of colonies of cells harboring the plasmids with Tn*4731* by a fluctuation test (16, 24).

Nucleotide sequencing. The dideoxy chain termination method of DNA sequencing (21, 27) was employed. The plasmid DNA containing Tn*4731* was used as template, and synthetic oligonucleotides which could hybridize near the site of transposition were used as primers (Table 1). To sequence the plasmid DNA, a 2′-deoxy-7-deazaguanosine triphosphate sequencing kit (Takara Shuzo) was used, and the elongating DNA chains were labeled with [α-^{32}P]dCTP (15 TBq/mmol) (Amersham). The labeled DNA fragments were electrophoresed in 6% acrylamide gels containing 8 M urea.

TABLE 1. Primers used for sequencing and mutagenesis

Primer	Sequence (5′ to 3′)	Position[a]
Blam1	ATGTAACTCGCCTTGATC	2496–2479
118 prime	GTTTTTTTGTTTGCAAGCAGC	1882–1902
M4	GTTTTCCCAGTCACGAC	835–851
HSIVA	CTCGTCAAAGCAACCAT	187–203
TER1	TTCACCTAGAGCATGTGAAATTAAAAATGAAGT	2025–2057
TER2	GGTCATGAGATTATCACACATGCTATTCACCTA	2000–2032
TER80	ATCTTCACCTAAAGCATGTGAAA	2022–2044
TER81	GATCTTCACCTCGAGCATGTGAA	2023–2045
TER82	AGGATCTTCACTTAAAGCATGTGAAA	2022–2047
PRO1	AGCCGGAAGCATAAAGGTGACCGCCTGGGGTGCC	998–1035

[a] Coordinate numbers to pUC118.

Mutagenesis of pUC118. The method of mutagenesis of pUC118 with strain BW313 was described previously (13, 14). Primers TER1, TER2, and PRO1 (Table 1) were hybridized to single-stranded pUC118 DNA to make pTT70, pTT71, and pTT75, respectively. Primers TER80, TER81, and TER82 were hybridized to single-stranded pTT70 DNA to make pTT80, pTT81, and pTT82, respectively. For the reaction, Muta-Gene (Bio-Rad) was used. Mutations in pUC118 or pTT70 were confirmed by nucleotide sequencing.

RESULTS

Transposition of IS*630*-associated transposon Tn*4731* to pUC plasmids and locations of target sites. We have previously reported that the tetracycline resistance transposon Tn*4731* in plasmid pTT4 (Fig. 1), a temperature-sensitive replication mutant originally derived from plasmid pSC101, transposes to ColE1 to give ColE1::Tn*4731* plasmids, whose replication systems are temperature resistant and thus can

TABLE 2. Transposition of Tn*4731* to hot spots in pUC derivatives

Recipient plasmid[a]	Transposition frequency/ division cycle	No. of plasmids with Tn*4731* transposed to hot spot[b]			
		I	II	III	IV
pUC118	6.6×10^{-8}	2 (1)	3 (2)	5 (3)	8 (5)
pTT70	1.6×10^{-7}	3	2	13 (5)	4
pTT71	1.2×10^{-7}	0	1	13 (6)	5
pUC119	5.4×10^{-8}	0	2 (1)	7 (4)	9 (5)
pTT75	1.0×10^{-7}	1	1	4	8
pTT80	7.7×10^{-8}	2 (1)	1	0	7
pTT81	8.5×10^{-8}	1 (1)	2	0	6
pTT82	5.9×10^{-8}	1	0	0	5
ColE1	1.8×10^{-9}				

[a] Plasmids pTT70, pTT71, and pTT75 were derived from pUC118. Plasmids pTT80, pTT81, and pTT82 were derived from pTT70.

[b] Transposition of Tn*4731* to each hot spot was determined by nucleotide sequencing and by cleavage first with *Eco*RI and then with *Mae*I and also with *Xba*I when Tn*4731* transposed to hot spot II (Fig. 2). The numbers of plasmids examined by sequencing are in parentheses. pUC119 has a sequence containing the multiple cloning sites in the orientation opposite to the corresponding sequence in pUC118; thus, pUC119::Tn*4731* plasmids show restriction patterns different from those of pUC118::Tn*4731* derivatives, although the pUC119 derivatives with Tn*4731* at each hot spot give unique restriction patterns, as observed in the pUC118 derivatives (Fig. 2).

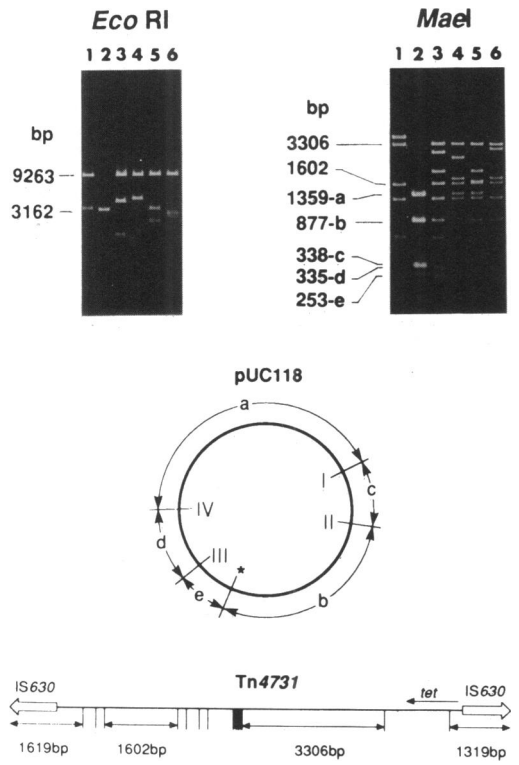


FIG. 2. Determination of Tn*4731* transposition to each hot spot in pUC118 through analysis of restriction digests of pUC118::Tn*4731* plasmids by gel electrophoresis. Ethidium bromide-stained agarose gels (0.7 and 1.4%) showing fragments in the *Eco*RI and *Mae*I digests, respectively, are shown at the top. DNA samples in lanes 1 to 6 are pTT4, pUC118, pUC118 with Tn*4731* at hot spot I, pUC118 with Tn*4731* at hot spot II, pUC118 with Tn*4731* at hot spot III, and pUC118 with Tn*4731* at hot spot IV, respectively. In *Eco*RI digests, the gel band patterns of three fragments generated from each of the pUC118 derivatives with Tn*4731* at a hot spot are the same, regardless of the orientation of Tn*4731* inserted in pUC118. In *Mae*I digests, the gel band patterns of several fragments generated from each of the pUC118 derivatives with Tn*4731* at a hot spot are the same when Tn*4731* is present in one orientation in pUC118. The second and third largest *Mae*I-digested fragments, which do not correspond to those of parental plasmids, contain terminal regions of Tn*4731* (1,319 and 1,619 bp) (see *Mae*I cleavage map of Tn*4731* at bottom), each of which is joined with one of the *Mae*I fragments a to e of pUC118 (see *Mae*I cleavage map of pUC118 in middle). Identification of the pair of fragments, which are characteristic for each sample, can determine transposition of Tn*4731* to each hot spot as well as the orientation of the sequence in pUC118. Note that all pUC118 derivatives carrying Tn*4731* except two give one of the four patterns shown in lane 3 to 6, indicating that Tn*4731* transposed almost exclusively in one orientation, as depicted in Fig. 1. (Two exceptions contained Tn*4731* at hot spot III in an orientation opposite to that shown in Fig. 1.) The *Mae*I-digested DNA samples in lanes 3 to 6 do not contain two fragments (a and c, b and c, d and e, and a and d, respectively), although bands of small DNA fragments were not separated better in the 1.4% agarose gel shown here (but could be separated in a 4% polyacrylamide gel [data not shown]). Identification of each pair of the fragments can also determine transposition of Tn*4731* to each hot spot in pUC118.

be selected at the restrictive temperature of 42°C in the presence of tetracycline (29). Using the method previously employed, we examined transposition of Tn*4731* to pUC118 (Fig. 1) and found that Tn*4731* transposed to pUC118 at a frequency much higher than to ColE1 (Table 2).

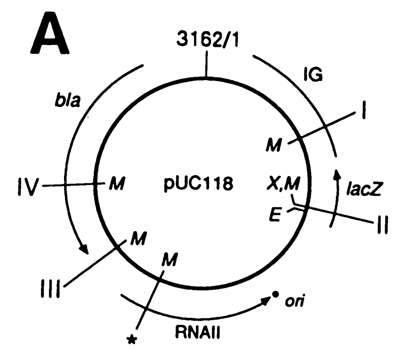
To map the target sites for transposition, each of pUC118 derivatives carrying Tn*4731* was digested with *Eco*RI, which cuts pUC118 at one site and cuts two sites within the Tn*4731* sequence (Fig. 1), and electrophoresed in an agarose gel. Band patterns of the *Eco*RI fragments generated from all pUC118::Tn*4731* plasmids were of four kinds (Fig. 2), although each of the plasmids examined was isolated from an independent clone of cells. This result suggests that there exist hot spots (maybe four) for transposition of Tn*4731* in the pUC sequence.

We then determined the exact positions of several target sites by sequencing junctions of Tn*4731* with the pUC118 sequence and found that Tn*4731* transposed to 5′-TA-3′ sequences at four sites (here called hot spots I, II, III, and IV) to give rise to duplication of the TA sequence (Fig. 3 and Table 2). Hot spot I was located at the TA sequence within the intergenic region of phage M13 in pUC118; hot spot II was located at the TA sequence within the recognition sequence of restriction endonuclease *Xba*I in the multiple cloning sites; hot spot III was located at the TA sequence within a 6-bp spacer region flanked by 10-bp inverted repeat sequences, 60 bp downstream of the *bla* gene; and hot spot IV was located at the TA sequence within the *bla* gene.

None of the pUC118 derivatives carrying Tn*4731*, whose map position was assigned near the *Xba*I site by the *Eco*RI-cleavage analysis, could be cleaved with *Xba*I. This indicates that Tn*4731* in these plasmids transposed to hot spot II (Fig. 3 and Table 2). Note that the TA sequence in hot spot II as well as each in hot spots I, III, and IV is the core of a palindromic sequence 5′-CTAG-3′ (Fig. 3B), which is the recognition site of restriction endonuclease *Mae*I. Cleavage analysis with *Mae*I showed that band patterns of the *Mae*I-digested fragments generated from all the pUC118::Tn*4731* plasmids were essentially of four kinds (Fig. 2); a characteristic pair of fragments containing junctions of Tn*4731* with pUC118 was generated, but a particular pair of the *Mae*I fragments of pUC118 which adjoined each *Mae*I site used as a transpositional target was not generated (see legend to Fig. 2). This indicates that Tn*4731* has been transposed to TA in the four CTAG sequences almost exclusively in one orientation in pUC118 (see legend to Fig. 2).

Sequence requirement for the preferential transposition of Tn*4731*. We have previously observed that Tn*4731* transposes preferentially to the TA sequence in each of the inverted repeat sequences of 13 bp in a rho-dependent transcription terminator in ColE1 (29). As described in the previous section, hot spot III in pUC118 was located in a 6-bp spacer sequence flanked by 10-bp inverted repeat sequences (Fig. 3B), which are entirely contained in the transcription terminator for *bla* (28). To test the possibility that the inverted repeats flanking a hot spot are responsible for preferential transposition of Tn*4731*, therefore, we introduced substitution mutations within each one of the inverted repeat sequences flanking hot spot III and examined transposition of Tn*4731* to the resulting mutant plasmids pTT70 and pTT71 (Fig. 4A). Tn*4731* was found to transpose to hot spot III in these plasmids at even a slightly higher frequency than to the same site in pUC118 (Table 2). This result shows that the inverted repeats are not required for transposition of Tn*4731* but rather inhibit it, suggesting that the transcription terminator in pUC118 is not responsible for the preferential transposition of Tn*4731*.

As for hot spot II, we examined transposition of Tn*4731* to plasmid pUC119, which has the same sequence of 58 bp containing multiple cloning sites including the hot spot as that in pUC118 in the inverted orientation (30). Tn*4731* was



B

Hot spot I

```
         .260        .          .            .        .300        .        .
5'-ACGCGCAGCGTGACCGCTACACTTGCCAGCGCCC TA GCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTC-3'
3'-TGCGCGTCGCACTGGCGATGTGAACGGTCGCGGG AT CGCGGGCGAGGAAAGCGAAAGAAGGGAAGGAAAG-5'
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Hot spot II

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    .        .        .890                       .910         .        .
5'-CCAGTGCCAAGCTTGCATGCCTGCAGGTCGACTC TA GAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT-3'
3'-GGTCACGGTTCGAACGTACGGACGTCCAGCTGAG AT CTCCTAGGGGCCCATGGCTCGAGCTTAAGCATTA-5'
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Hot spot III

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      .2000        .        .                     .        .2050        .
5'-TTTGGTCATGAGATTATCAAAAAGGATCTTCACC TA GATCCTTTTAAATTAAAAATGAAGTTTTAAATCA-3'
3'-AAACCAGTACTCTAATAGTTTTTCCTAGAAGTGG AT CTAGGAAAATTTAATTTTTACTTCAAAATTTAGT-5'
```

Hot spot IV

```
         .2340        .        .           .        .        .2390        .
5'-CCTCCATCCAGTCTATTAATTGTTGCCGGGAAGC TA GAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAA-3'
3'-GGAGGTAGGTCAGATAATTAACAACGGCCCTTCG AT CTCATTCATCAAGCGGTCAATTATCAAACGCGTT-5'
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A potential hot spot

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          .1750        .        .           .        .        .1800        .
5'-AGTTCTTGAAGTGGTGGCCTAACTACGGCTACAC TA GAAGGACAGTATTTGGTATCTGCGCTCTGCTGAA-3'
3'-TCAAGAACTTCACCACCGGATTGATGCCGATGTG AT CTTCCTGTCATAAACCATAGACGCGAGACGACTT-5'
```

FIG. 3. Map locations of hot spots I, II, III, and IV and nucleotide sequences around the hot spots and a potential site for transposition of Tn*4731* in pUC118. (A) Locations of hot spots. pUC118 contains multiple cloning sites within the *lacZ* coding frame. RNA primer for replication (RNA II) and the coding regions of *lacZ* and *bla* genes are shown by arrows. An intergenic region (IG), derived from M13 phage, is shown by a line. A dot represents the replication origin where DNA synthesis starts. An asterisk shows the potential integration site of Tn*4731*, where CTAG exists within the RNA II primer region. Abbreviations for cleavage sites of restriction endonucleases: E (*Eco*RI), X (*Xba*I), and M (*Mae*I). (B) Nucleotide sequences including hot spots. The dinucleotide TA boxed in each sequence of hot spots (I, II, III, and IV) is duplicated upon transposition of Tn*4731*. Thick solid lines near the target sites indicate palindromic sequences with the dyad axis in the middle of TA. A pair of inverted arrows in hot spot III shows 10-bp inverted repeat sequences of a transcription terminator (28). Positions of nucleotides are shown by the coordinate numbers to pUC118. Hot spots I to IV were sequenced by using primers HSIVA, M4 (purchased from Takara Shuzo), 118 prime, and Blaml (Table 1), respectively.

found to transpose to hot spot II in pUC119 at a frequency similar to that in pUC118 (Table 2). This result indicates that the orientation of the sequence containing hot spot II is not important for transposition of Tn*4731*.

Bernardi and Bernardi (3) have reported that read-through transcription of the target sites enhances transposition of an insertion element. As described above, Tn*4731* transposed to hot spot II, which is within the *lacZ* coding region in pUC118 or pUC119 and located immediately downstream of *lac* promoter. To test, therefore, whether transcription of hot spot II may have caused the preferential transposition of Tn*4731* to the hot spot, we introduced substitution mutations in the −35 region of *lac* promoter in pUC118 to obtain a mutant plasmid, pTT75, with a completely different promoter consensus sequence (Fig. 4B). Tn*4731* was found to transpose to hot spot II in pTT75 as well as to hot spots, I, III, and IV at frequencies as high as those in pUC118 (Table 2). This indicates that transcription of hot spot II from *lac* promoter has little or no effect on transposition of Tn*4731*.

As described in the previous section, the four hot spots in pUC118 are the core of the CTAG sequence (Fig. 3). There seem to be no other common sequences in the region surrounding the palindromic sequences. It is, therefore, likely that the CTAG sequence is responsible for the preferential transposition of Tn*4731*. To prove this, we mutagenized the sequence in hot spot III in pTT70, in which Tn*4731* could transpose most frequently (Table 2). A mutant plasmid, pTT80, has the CTAA sequence instead of CTAG in hot

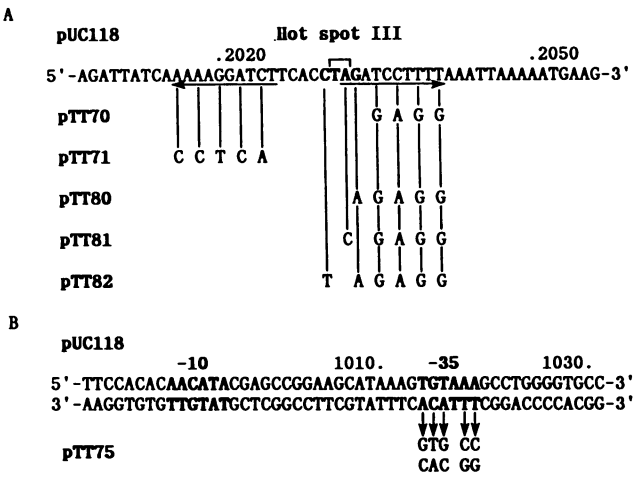

FIG. 4. Substitution mutations introduced into pUC118. (A) Sequence showing the region that contains hot spot III in pUC118. A pair of arrows represents inverted repeat sequences of a transcription terminator. The *bla* gene is located on the right side of this sequence. The TA sequence which is duplicated upon transposition of Tn*4731* in hot spot III is shown by a bracket. Plasmids pTT70 and pTT71 have mutations in either one of the inverted repeat sequences in pUC118. Plasmids pTT80, pTT81, and pTT82 have mutations in the CTAG sequence in pTT70. The nucleotide sequence of only one strand is shown. (B) The sequence of *lacZ* promoter in pUC118 that contains −10 and −35 regions (4). The −35 region of the promoter was mutagenized to abolish the activity of this promoter according to the rule presented by Hawley and McClure (9). Colonies of MV1184 harboring the resulting plasmid pTT75 were in fact white on a plate containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and isopropyl-β-D-thiogalactopyranoside (IPTG), whereas colonies of MV1184 harboring pUC118 were blue.

spot III (Fig. 4A). Tn*4731* was found not to transpose to TA in the altered sequence in pTT80 (Table 2). Another mutant plasmid, pTT82, has the TTAA sequence instead of CTAG (Fig. 4A). We have previously observed that the TTAA sequence present in ColE1 has been used as target for transposition of Tn*4731*, though rarely. Tn*4731* did not, however, transpose to TA in the altered sequence (Table 2). These results clearly show that Tn*4731* transposed preferentially to TA in the CTAG sequence. We also constructed plasmid pTT81 having no TA sequence such that the CTAG sequence was altered to CTCG (Fig. 4A). Tn*4731* did not transpose to the altered sequence (Table 2), as expected.

DISCUSSION

We have previously reported that Tn*4731* always transposes to the dinucleotide 5′-TA-3′ in ColE1. We have shown in this article that Tn*4731* can transpose also to the TA sequence in pUC plasmids.

In our previous report, two hot spots for transposition of Tn*4731* are present within each of the 13-bp stem sequences of a rho-dependent transcription terminator in ColE1 (29). We have recently shown that the inverted repeats of the terminator in ColE1 were not required for the preferential transposition of Tn*4731* (our unpublished result). This and our present finding that transposition of Tn*4731* to hot spot III in a transcription terminator in pUC118 does not require inverted repeats indicate that transcription termination is not a signal for the preferential transposition of Tn*4731*. It has been reported that transposon Tn*7* has a transpositional hot spot which is within a transcription terminator downstream of the *glmS* gene in the *E. coli* chromosome (1, 6, 7, 15), but the termination function has also been shown not to be required for transposition of Tn*7* (7, 20).

We have also shown here that the frequency of transposition of Tn*4731* to hot spot II was not affected by mutations introduced into *lac* promoter which is located immediately upstream of the hot spot. This indicates that read-through transcription into hot spot II from the *lac* promoter did not change the frequency of transposition to the hot spot. However, we cannot rule out the possibility that read-through transcription of the target site from other promoters present in pUC plasmids still affects Tn*4731* transposition.

Tn*7* requires a sequence of about 50 bp located about 10 bp away from the hot spot (*att*Tn*7*) for its transposition (7, 20). Some other insertion sites (pseudo-*att*Tn*7*) share homology with the sequence required (12). Unlike for transposition of Tn*7*, however, we cannot find any homologous sequences in the neighborhood of the hot spots which appear to be important for transposition of Tn*4731*. We can find only a palindromic sequence, CTAG, which includes the target sequence TA, in the four hot spots in pUC118.

There are actually five CTAG sequences in pUC118 as well as in pUC119, one of which was not used as target for Tn*4731* transposition. This exception is located within the region that is essential for the initiation of replication of the ColE1-type plasmids, including pUC plasmids (10) (Fig. 3). It is, therefore, quite likely that transposition of Tn*4731* to this potential site could not be detected even if Tn*4731* had transposed to it, since the resulting plasmid would not replicate. In ColE1, Tn*4731* has been shown to transpose to the TA sequence which is the core of at least 4-bp palindromic sequences, such as TTAA and ATAT in addition to CTAG (29). There are four CTAG sequences: three of them were used for transposition of Tn*4731*, and two of the three were hot spots located in a rho-dependent transcription terminator (29). The exception is located at the same essential region for replication of ColE1 as that of pUC plasmids; thus, transposition of Tn*4731* to this site might not be detected as well. There are 30 TTAA sequences and 31 ATAT sequences in ColE1, in which only three TTAA sequences and one ATAT sequence were used as targets for transposition of Tn*4731*. These and our present results indicate that TA in the CTAG sequence becomes a hot spot, although TA in the other 4-bp palindromic sequences is used as target very rarely. Our findings that mutations introduced within the CTAG sequence, including hot spot III in pUC, inhibited the transposition to it support this indication.

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