Site-Specific Transposition of Insertion Sequence IS630

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IS630 is a 1.15-kilobase sequence in *Shigella sonnei* that, unlike many mobile elements, seems not to mediate cointegration between different replicons. To assess its transposition, we constructed composite elements containing inverted copies of IS630 flanking a drug resistance gene. We found that these composite elements transposed to plasmid CoIE1 in *Escherichia coli*. DNA sequencing showed that transposition was, in all cases, to the dinucleotide sequence 5'-TA-3'. There were two preferred insertion sites which corresponded to the TA sequences in the inverted repeats of a 13-base-pair stem region of the [rho]-dependent transcription terminator. IS630 is flanked by TA, and nucleotide substitution by in vitro mutagenesis at these ends did not affect transposition activity of a composite element or its ability to insert preferentially into TA within the 13-base-pair inverted repeat sequences or to duplicate the target sequence.

An insertion sequence (IS) is a discrete DNA segment which can transpose to various sites on the bacterial plasmids and chromosomes, usually showing little specificity for the target sites of insertion (for reviews, see references 10, 12, and 22). Some IS elements mediate the fusion of the replicons containing them with the target replicons to give a cointegrate, in which the two replicons are linked by direct repeats of the IS element (6, 13, 14, 21, 40, 42, 47). Several other IS elements seem not to mediate such cointegration, although composite transposons, which contain IS elements in pairs, can transpose (2, 3, 17).

IS elements are often present in the bacterial chromosomes as repetitive sequences. Recently, Matsutani et al. isolated several kinds of repetitive sequences from the *Shigella sonnei* chromosome (33). Some of them are variants of the well-known IS elements IS1, IS2, and IS3, whereas others are unrelated but resemble canonical IS elements, containing short terminal inverted repeat sequences (<50 base pairs [bp]) and an open reading frame(s) that might encode a transposase protein(s). Representative of this class is IS630, a 1.15-kilobase element with terminal inverted repeats of 32 bp and a large open reading frame encoding a 343-amino-acid protein. IS630 does not mediate cointegration between two replicons in *Escherichia coli* (33), and hence, additional tests were needed to see if it was in fact mobile. We report here that a segment consisting of inverted copies of IS630 flanking a drug resistance gene can transpose, and we describe unusual features of these mobile IS630-based composite transposons.

MATERIALS AND METHODS

Bacterial strains, plasmids, and a phage. Bacterial strains used were the *E. coli* K-12 derivatives W3110 (38), JM109 (49), BW313 (26, 27), MV1184 (48), JE5519 (41), and JE5519 harboring plasmid CoIE1 (30).

Plasmid pUC18 (49) was used for cloning the fragments containing transposable DNA segments, as described below. Plasmid pSAM31 carried IS630 (33). Plasmids pINJ1622 and pINJ1624 (1.-N. Jin, Ph.D. thesis, Tokyo University, Tokyo, Japan, 1986) carried the cat gene (encoding chloramphenicol acetyltransferase), which could be isolated as an *EcoRI*-BamHI fragment. Plasmid pH1 is a temperature-sensitive replication mutant of the tetracycline resistance plasmid pSC101 (18). pH7 plasmids used are described below.

A phage M13mp18 derivative, φT31, contained IS630 which is identical to that contained in plasmid pSAM31.

Media. Culture media used were L broth, L-rich broth, φ medium (50) and 2× YT broth (35). L agar plates and L soft agar contained 1.5 and 0.6% Bacto-Agar (Difco Laboratories) in L broth, respectively. Antibiotics were added to L agar plates, if required, as follows: 50 μg of ampicillin per ml, 5 or 8 μg of tetracycline per ml, and 20 μg of chloramphenicol per ml (Sigma Chemical Co.). Dilution buffer (0.1% tryptone [Difco], 0.3% NaCl) was used for diluting the cell culture.

Enzymes. Restriction endonucleases used were BbII, SmaI, BamHI, EcoRI, PstI, HincII, MluI, DraI, PvuII, SstI, AccI, BsiRI, NspI(524)V, Ncol, ApaLI (all from Takara Shuzo Co.), SspI, and HgiAI (both from New England BioLabs, Inc.). Phage T4 DNA ligase, Klenow fragment of *E. coli* DNA polymerase I, and RNase A (Takara) 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 crude lysis method (29) was employed to isolate a small amount of plasmid DNA. The alkaline lysis method (32) was used to prepare pUC plasmid derivatives. DNAs of CoIE1 derivatives (CoIE1::Tn4731 or CoIE1::Tn4733) were amplified by adding chloramphenicol by the method of Maniatis et al. (32) and extracted from cells by the alkaline lysis method or the method of Ohnsubo et al. (39).

Construction of plasmids pTT4 and pTT8. pTT4 carrying Tn4730 was constructed as follows. pSAM31 (Fig. 1, top) was digested with EcoRI and BamHI, and the fragment (1,192 bp in length) containing IS630 was purified by 0.7% agarose gel electrophoresis. The fragment was treated with ligase and then digested with BamHI to yield a dimer form of the fragment (Fig. 1). The DNA sample was then mixed with the fragment (831 bp in length) containing the cat gene which was purified after digestion of pH1624 with BamHI, and the mixture was treated with ligase. The product was digested by EcoRI to yield a composite DNA fragment containing Tn4730 (Fig. 1, bottom). This composite DNA fragment was then ligated with pUC18 which had been digested by EcoRI, and the ligated sample was introduced into JM109.
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TABLE 1. Primers used for DNA sequencing

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5' → 3')</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>ColN1</td>
<td>AGCAGTTTACTGTCGCA</td>
<td>265-248</td>
</tr>
<tr>
<td>ColP1</td>
<td>GGCAGATTTTTTATCA</td>
<td>6,521-6,538</td>
</tr>
<tr>
<td>Col38A</td>
<td>TGAAGTGAGAAGAAACGA</td>
<td>5,661-5,679</td>
</tr>
<tr>
<td>Col38B</td>
<td>ATTTGACTTTTGGATGAT</td>
<td>5,925-5,907</td>
</tr>
<tr>
<td>Col33A</td>
<td>TCTAAGCCCGAGATCC</td>
<td>586-569</td>
</tr>
<tr>
<td>Col33B</td>
<td>TAGCATCTTACTGACAC</td>
<td>281-298</td>
</tr>
<tr>
<td>Col8A</td>
<td>ACAGGCGGAAGACGCC</td>
<td>2,521-2,537</td>
</tr>
<tr>
<td>Col8B</td>
<td>AAGATGTCTATAAAA</td>
<td>2,880-2,864</td>
</tr>
<tr>
<td>Col45A</td>
<td>ATGAAAAGGAGTCTCGGTG</td>
<td>5,826-5,843</td>
</tr>
<tr>
<td>Col45B</td>
<td>TTAAGAGATATTACGGT</td>
<td>6,164-6,147</td>
</tr>
</tbody>
</table>

fragment containing cat and portions of IS630 containing the IRR regions were replaced with the MluI fragment in plasmid pTT4 (see Fig. 2), yielding mutants m1, m2, and m3 of pTT4 (see Fig. 7).

Transposition. pTT4 (or pTT8) was introduced into recA mutant JE5519 already harboring ColE1 by transformation, and JE5519 harboring both ColE1 and pTT4 (or pTT8) was selected on an L-agar plate containing 5 μg of tetracycline per ml at 30°C. A single colony of JE5519 harboring the two plasmids was inoculated into 5 ml of L broth and grown at 30°C overnight. The culture was diluted 10-fold, and 0.1 ml of the diluted culture was inoculated into each of 50 or 60 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 L-agar plates containing tetracycline (8 μg/ml), and the plates were incubated at 42°C for 1 to 2 days to select colonies of cells harboring ColE1::Tn4731 from pTT4 (or ColE1::Tn4733 from pTT8). Plasmids containing 20 μg of chloramphenicol per ml were used to select colonies of cells harboring ColE1::Tn4730 from pTT4.

Only one colony was picked up from each plate, and cells were grown in L broth at 37°C overnight. Plasmids were extracted and analyzed for their sizes by 0.7% agarose gel electrophoresis. About one-third of plasmids were the same size as pTT4. These were revertants of pTT4 in temperature sensitivity for its replication and thus were able to replicate at 42°C. The others were larger than parental plasmids. We analyzed only the latter plasmids for their structures by cleavage with restriction endonucleases NcoI (see Results).

FIG. 2. Structures of plasmid pHS1 and its derivatives pTT4 and pTT8. pHS1 contains the tetracycline resistance gene (tet). Another transposable element, IS1020 preexists in pHS1 and in its derivatives pTT4 and pTT8. pTT4 and pTT8 carry the fragments containing Tn4730 and Tn4732, respectively (see Fig. 1). IS630 is shown by an open arrow whose head and tail represent IRR and IRL, respectively. IRL is the end region located upstream of an open reading frame, possibly coding for transposase. cat is shown by a solid arrow whose orientation indicates the direction of its coding frame. Note that pTT4 carries transposon Tn4731, whereas pTT8 carries another transposon, Tn4733. Cleavage sites of restriction endonucleases EcoRI (E) and MluI (M) are shown. ori, Origins of replication of plasmids.

by transformation as described by Yoshioka et al. (50). Cells harboring plasmid pTT2 that carries the composite DNA fragment were selected on L-agar plates containing ampicillin. The composite DNA fragment in pTT2 was recloned into the EcoRI site of plasmid pHS1, and cells of W3110 harboring plasmid pTT4 (see Fig. 2) were selected at 30°C on L-agar plates containing 5 μg of tetracycline per ml.

pTT8 carrying Tn4732 was made similarly. The fragment (1,180 bp in length) containing IS630 was obtained by digesting pSAM31 with EcoRI and HincII (Fig. 1). The fragment was self-ligated, digested by EcoRI to yield a dimer, and then ligated with the 873-bp EcoRI fragment containing cat from plnJ1622 (Fig. 1). This DNA was cut with HincII to yield the composite DNA fragment containing Tn4732 (Fig. 1, bottom). The fragment was cloned into the HincII site of pUC18, yielding plasmid pTT6, and then recloned into the EcoRI site of pHS1, which had been made blunt by Klenow fragment of DNA polymerase I to yield plasmid pTT8 (see Fig. 2).

Construction of mutant pTT4. A phage M13mp18 derivative, 6TT31, which carried IS630 was grown in E. coli BW313 (dut ung). Single-stranded phage DNA extracted from phage particles and synthetic oligonucleotides (21mers) with altered sequences in the inverted repeat at the right end (IRR) of IS630 were mixed and treated with T4 DNA polymerase, four deoxynucleoside triphosphates, and E. coli DNA ligase (Takara). The sample DNA was transfected in a strain MV1184 to recover mutated phage DNA by the method of Kunkel et al. (27). The mutated regions were confirmed by DNA sequencing. The mutated DNA fragments containing IS630 were prepared and were inserted into pUC18 together with the cat fragment used to construct plasmid pTT2, as described in the previous section. Mutant pTT2 were digested with MluI, and the MluI

FIG. 1. Scheme of construction of the DNA fragment containing Tn4730 or Tn4732. Each step used in constructing the fragment is explained in the text. IS630 (→) and cat (→) are indicated. Location and orientation of the ampicillin resistance gene bla are shown by a thin arrow. The sites of restriction endonucleases used are abbreviated as follows: B, BamHI; E, EcoRI; and H, HincII. kb, Kilobases.
Note that pHS1 carries another IS, IS02 (42), which mediates cointegration between pTT4 (or pTT8) and ColE1. The IS02-mediated cointegrates have two copies of IS02, and these were distinguished from ColE1::Tn4731 and ColE1::Tn4733 with NcoI (see Fig. 3).

Nucleotide sequencing. The dye-chain-termination method of DNA sequencing (35, 45) was employed, with ColE1 derivatives carrying Tn4731 or Tn4733 as templates and synthetic oligonucleotides which could hybridize near the target site on ColE1 as primers. The oligonucleotides used are listed in Table 1. These oligonucleotides were synthesized by using DNA synthesizer 380B (Applied Biosystems). For the reaction, a 2'-deoxy-7-deazaguanosine triphosphate sequencing kit (Takara) was used. The elongating DNA chains were labeled with [γ-32P]dCTP (15 TBq/mmol) (Amersham Corp.) and separated by 6 or 8% polyacrylamide gels containing 8 M urea.

RESULTS

Transposition ability of the DNA segments flanked by inverted repeats of IS630. To test whether IS630 could mediate transposition, we constructed a DNA fragment containing the cat gene that was flanked by inverted copies of IS630, and we inserted it into the EcoRI site of the tetracycline resistance plasmid pH51 to generate plasmid pTT4 (Fig. 2). We designated this segment Tn4730. Similarly, the pTT4 plasmid contains another transposon-like segment (here called Tn4731) in which the inverted copies of IS630 flank the pH51 sequence rather than cat (Fig. 2).

Plasmid pTT4, which was used as a donor, is p derivative for its replication. Transposition of Tn4730 or Tn4731 can therefore be detected by selecting for ability to grow at 42°C in the presence of chloramphenicol or tetracycline, respectively, when we use plasmid ColE1, which can replicate at 42 as well as 30°C, as the recipient. For the actual experiment, we used tetracycline for selection to detect only transposition of Tn4731. Selection by chloramphenicol was not successful, because chloramphenicol-sensitive cells were grown resupported, perhaps because of the use of the cat gene, which lacks its promoter (Jin, Ph.D. thesis).

We were able to obtain tetracycline-resistant colonies at 42°C at a frequency of 10^-3 to 10^-8 per division cycle. Twenty-five independent colonies containing plasmids larger than the parental plasmids (pTT4 and ColE1) were obtained, and their structures were analyzed by cleavage with NcoI, which cuts both IS630 and the cat gene at one site but does not cut pH51 or ColE1 (Fig. 3). Thirteen plasmids resulted from cleavage at the two IS630 sequences in ColE1::Tn4731 that had a Tn4731 insert into ColE1; they did not contain the cat sequence, and they yielded two common fragments (11.2 and 7.0 kb) (Fig. 3). The other 12 plasmids were cointegrates between pTT4 and ColE1 in which two plasmid DNAs were joined by direct repeats of IS02 preexisting in pH51 (Fig. 3). No cointegrates mediated by IS630 or by Tn4731 with two IS630s were found, as was expected.

The ends of the Tn4731 element correspond to just one end of IS630, IRR (Fig. 2). To test whether the other end (inverted repeat at the left terminus [IRL]) of IS630 could also serve as the end of a transposon, we constructed plasmid pTT8, in which the IS630 elements are reversed relative to those in Tn4731 (Fig. 2). In comparable tests of selection for tetracycline-resistant colonies at 42°C, we found that 5 of 21 large plasmids were the result of insertion
of this new transposon, Tn4733, into ColEl (i.e., ColEl::Tn4733) and that the other 16 plasmids were co-integrated by a co-integration mediated by IS102.

Approximate locations of the target sites of Tn4731 and Tn4733 and their orientations in ColEl. The sites of Tn4731 and Tn4733 in ColEl were mapped by using restriction enzyme NspI(7524) V, which cuts ColEl into two fragments (594 and 6,052 bp in length) (Fig. 3 and 4). Most plasmids that were ColEl::Tn4731 (11 of 13 cases) and ColEl::Tn4733 (3 of 5 cases) did not yield the 594-bp fragment but did yield the 6,052-bp fragment of ColEl. This shows that Tn4731 and Tn4733 transposed preferentially to a 594-bp segment in ColEl. By using other enzymes such as PstI, SspI, etc., which cut ColEl at several sites, the target sites were narrowed down to a 283-bp region between the NspI(7524) V and PstI sites (NP region) (Fig. 4). Similarly, the target sites located outside of the NP region were also narrowed down at the positions shown in Fig. 4.

The orientations of the sequences of Tn4731 and Tn4733 in the ColEl derivatives were determined by digesting them with SmaI, which cuts the pHS1 sequence at two sites and ColEl at one site, or with ApalI and HindIII, which cut ColEl and Tn4731 (or Tn4733), respectively, at one site (Fig. 3). Almost all inserts of Tn4731 and Tn4733 were in the same orientation (Fig. 4).

Nucleotide sequences at the target sites. We determined the sequences of the junctions between ColEl and the transposons. Figure 5 illustrates two cases and shows that the sequence 5'-TA-3' is present at the ColEl-transposon junctions. Figure 6 summarizes the target sequences for the Tn4731 and Tn4733 insertions that we found. Six sites in ColEl (a through f) were found, and in each case, TA was present at each site. Two sites (a and b) were in the same 13-bp palindromic target sequence. Tn4731 (five cases) and Tn4733 (three cases), whose target sites were mapped in the NP region (A1 through A5 and B3 through B5 in Fig. 4), transposed to either site a or site b. On the basis of the cleavage patterns, Tn4731, whose target sites were also mapped in the NP region but were not sequenced (Fig. 4, A7, A8, and A10 through A13), would have inserted at the TA in either site a or site b.

Target site e was partially homologous with the 13-bp inverted repeat sequence (Fig. 6, bottom). There was partial homology between target sites c and f (Fig. 6, bottom).

All insertion sites contained short palindromic sequences of 4 to 8 bp with the dyad axis located between T and A at the target site (Fig. 6, bottom).

Composite transposons generate a duplication of the TA sequence. There is some ambiguity in the determination of the origin of the TA base pairs, since they could form part of the IRR. To test the idea that the TA sequences at ColEl-transposon junctions represent target duplications made during transposition, we constructed mutant pTT4 with substitution mutations in the TA sequence at both ends of Tn4731 (Fig. 7). One mutant (m1) has GA and the other (m2) has CG instead of TA. In the former mutant, 4 of 28 large plasmids examined were ColEl::Tn4731, while in the latter mutant, 2 of 22 large plasmids were ColEl::Tn4731. DNA sequencing showed (i) that Tn4731 in m1 and m2 transposed preferentially to the TA sequences in sites a and b (Fig. 4 and 6), except one which transposed to TA in site g (Fig. 4 and 6), and (ii) that the target sequence TA was duplicated. This indicates that the TA-flanking insert is duplicated during transposition. There is some homology in the sequence containing site g with the sequences containing sites c and f (Fig. 6, bottom).

We also constructed a second mutant (m3) with an A → C transversion at the first position of the defined Tn4731 sequence (Fig. 7). In this mutant, no ColEl::Tn4731 plas-
FIG. 6. Nucleotide sequences at the target sites of Tn4731 and Tn4733. Numbers are coordinates to the nucleotide sequence of ColEl (Fig. 4). Positions (a to g), to which Tn4731 and Tn4733 had transposed, are boxed. Inverted repeat sequences in A and B are indicated by a pair of arrows. There are palindromic sequences of 4 to 8 bp around each target site (thick lines) with the dyad axis in the middle of the TA sequence. Homologous sequences around target sites are shown at the bottom. Sequences A and B were determined by using synthetic oligonucleotides, named ColN1 and ColP1, as primers; sequences C and D were determined by using Col33A and Col33B; sequence E was determined by using Col8A and Col8B; sequence F was determined by using Col38A and Col38B; and sequence G was determined by using Col45A and Col45B (see Materials and Methods).

mids were present among the 23 large plasmids examined. This shows that the nucleotide adjacent to the TA sequence is essential. This indicates that the A changed in mutant m3 does indeed constitute the first nucleotide of the IS630-based transposable element, since alteration of the first nucleotide of some transposons decreases or abolishes their transposition ability (8, 19, 20, 23, 31, 43, 44, 46).

DISCUSSION

We have shown that DNA segments flanked by inverted repeats of IS630 are transposable. This indicates that IS630 from the S. sonnei chromosome is transposition proficient in E. coli but is unable to mediate cointegration (33). In this sense, IS630 resembles ISSO and IS10, which cannot mediate cointegration but can transpose in pairs, as with the composite transposons Tn5 and Tn10 (for recent reviews, see references 4 and 25).

IS630-associated transposons transpose exclusively to the sequence 5'-TA-3' and generate a duplication of this sequence. There exist two preferred insertion sites within inverted repeats of a 13-bp sequence that correspond to the stem of the [rho]-dependent transcription terminator located

FIG. 7. pTT4 containing wild-type Tn4731 and its derivatives (m1, m2, and m3). The sequence of only one end of Tn4731 is shown; the sequence shown here is actually present at the other end of Tn4731 in the inverted orientation.
downstream of the *cee* gene in CoIE1 (Fig. 4) (9). It is not clear at present, however, whether the terminator function itself is the signal for IS630-mediated transposition.

Some transposons insert preferentially into particular sites (5, 16, 24, 37). Tn7, which is otherwise unrelated to IS630, also inserts preferentially into a site (called *attTn7*) located in the stem of a [rho]-independent transcription terminator downstream of the *glnS* gene in *E. coli* (1, 11, 15, 28). It is assumed that the termination function, however, is not responsible for the existence of the hot spot for Tn7 transposition (15, 34).

The target specificity of IS630 is unusual among bacterial transposons. Only IS30 also generates a 2-bp duplication and inserts preferentially at the 5'-AT-3' sequence (6). Unlike IS630, however, IS30 often transposes to sites other than AT and can also mediate cointegration. In eucaryotes, transposon Tcl of the nematode *Caenorhabditis elegans* transposes specifically to TA sequences that are often within the middle of palindromic sequence (36). Tcl is assumed to generate a duplication of TA upon its transposition (36). The validity of this assumption could be proven by using mutation analysis similar to that employed in this study.

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**LITERATURE CITED**


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