JOURNAL OF BACTERIOLOGY, Nov. 1990, p. 6348–6354
0021-9193/90/116348-07$02.00/0

Vol. 172, No. 11

Intramolecular Transposition by a Synthetic IS*50* (Tn*5*) Derivative

TIHAMER TOMCSANYI,[1]† CLAIRE M. BERG,[2] SUHAS H. PHADNIS,[1] AND DOUGLAS E. BERG[1]*

Departments of Molecular Microbiology and Genetics, Box 8230, Washington University Medical School, St. Louis, Missouri 63110-1093,[1] and Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269-2131[2]

Received 17 July 1990/Accepted 27 August 1990

We report the formation of deletions and inversions by intramolecular transposition of Tn*5*-derived mobile elements. The synthetic transposons used contained the IS*50* O and I end segments and the transposase gene, a contraselectable gene encoding sucrose sensitivity (*sacB*), antibiotic resistance genes, and a plasmid replication origin. Both deletions and inversions were associated with loss of a 300-bp segment that is designated the vector because it is outside of the transposon. Deletions were severalfold more frequent than inversions, perhaps reflecting constraints on DNA twisting or abortive transposition. Restriction and DNA sequence analyses showed that both types of rearrangements extended from one transposon end to many different sites in target DNA. In the case of inversions, transposition generated 9-bp direct repeats of target sequences.

Transposable elements are a diverse group of specialized DNA segments that move to new sites in a genome without need for extensive sequence homology. They cause insertion mutations and rearrangements, alter the expression of nearby genes, are found in both procaryotes and eucaryotes, and are valuable as tools for molecular genetics (7).

Tn*5* is one of the most intensively studied and widely used of the bacterial transposons. It is a composite element, consisting of terminal inverted repeats of the insertion sequence IS*50* and genes for resistance to several antibiotics (6). Transposition is mediated by the *cis*-acting transposase protein (encoded in IS*50*), along with host factors. Segments of about 19 bp at each end of IS*50* and of Tn*5* are essential for transposition (15, 27). Tn*5* can insert into dozens of sites in any typical gene, a few of which may be used preferentially (hot spots) (8, 18). This pattern of relatively low target specificity contributes to the value of Tn*5* as a research tool (2, 3). Early studies showed that the movement of Tn*5* and IS*50* between DNA molecules generates simple insertions (4, 5), not the cointegrates that are the hallmark of replicative transposition (28), and led to a model in which transposition occurs by a conservative cut-and-paste mechanism (4–6, 22). Several other elements are now also known to undergo conservative transposition (9, 16, 21), and the present experiments lend support to the nonreplicative transposition model for Tn*5*.

Most previous studies of Tn*5* transposition have involved movement between different molecules (intermolecular transposition), but insertion into sites within the same molecule (intramolecular transposition) should also occur. Deletion and inversion products are expected in the case of transposition to a site within the element itself: the resultant circular molecules should consist solely of transposon sequences (Fig. 1). A linear fragment, designated the vector because it is outside the transposon, should also be formed and then lost (4–6). Rearranged DNA molecules that were probably products of intramolecular conservative Tn*5* (IS*50*) transposition were obtained from complex λ-IS*50*-pBR322-Tn*5* chimeras that contained three copies of IS*50*, by selecting for loss of the λ phage component (14).

We began the experiments reported here for two reasons: (i) to determine whether Tn*5* would mediate intramolecular transposition, and if so, whether the products obtained supported the conservative model; and (ii) to assess whether Tn*5*-derived elements would generate nested deletions suitable for functional and DNA sequence analyses. We show here that rearrangements mediated by a synthetic IS*50* (Tn*5*) element can be obtained easily by using a contraselectable gene (*sacB*, sucrose sensitivity) and that both deletions and inversions arise, but at unequal frequencies.

MATERIALS AND METHODS

Bacteria were grown in LN broth (1% Humko-Sheffield NZamine, 0.5% Difco yeast extract, 1% NaCl) at 37°C. When indicated, the media also contained 12.5 μg of tetracycline per ml, 50 μg of ampicillin per ml, 60 μg of kanamycin per ml, and/or 5% sucrose. Solid medium contained 1.5% Difco Bacto agar. The strains of *Escherichia coli* K-12 used in this study were MC1061 (10) and DB4351, a *dam*::Tn*9* derivative of MC1061. The plasmids used (Fig. 2A) are derivatives of pBR322 and were constructed by standard recombinant DNA methods (20, 25). Sequences critical to the present study are shown in Fig. 2B.

To enrich for intramolecular transposition in the plasmids depicted in Fig. 2, overnight cultures of plasmid-carrying cells from young single colonies were plated on LN-tetracycline agar containing sucrose (35). Colony hybridization (25) to identify plasmids likely to have lost the vector site was carried out by using the vector-specific oligonucleotide designated "19 bp primer" in reference 23. Plasmid DNAs were extracted from representative sucrose-resistant colonies, screened for size and diagnostic restriction sites by agarose gel electrophoresis, and, when appropriate, sequenced by the double-stranded dideoxy method (26, 37) with Sequenase (United States Biochemical) and oligonucleotides specific for sequences near the O and I ends in these plasmids (Fig. 2B).

* Corresponding author.
† Present address: Agricultural Biotechnology Center, H-2101 Gödöllö, PF. 170, Hungary.

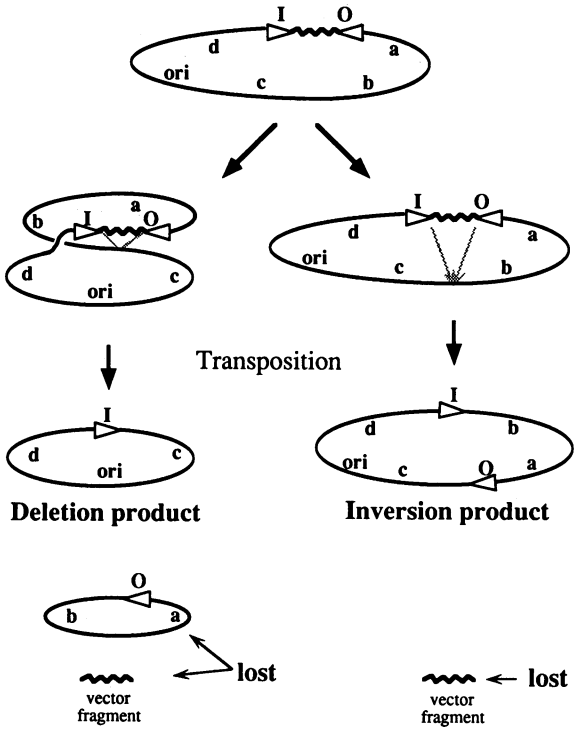


FIG. 1. Expected products of intramolecular transposition mediated by a pair of IS*50* I and O ends. Open triangles indicate IS*50* I and O end segments that face each other across the vector segment (jagged line).

RESULTS

The first plasmids used to test for intramolecular transposition (plasmids I and II; Fig. 2A) contained a synthetic IS*50* (Tn*5*) transposon in which the 19-bp O and I end segments face each other across a 300-bp nonessential segment designated the vector (jagged line in Fig. 1). Interior to the transposon ends (within the transposon) are the origin of replication and genes encoding sucrose sensitivity (*sacB*), tetracycline resistance (*tet*), and transposase (*tnp*). Sucrose-resistant (Suc^r) mutants of strain MC1061 carrying these plasmids were found at frequencies of about 10^{-8} (Table 1). Plasmid DNAs from six independent Suc^r mutants of plasmid I were screened on agarose gels. One mutant plasmid was 1.3 kb larger than its Suc^s parent, had an extra *Eco*RI site, and probably resulted from transposition of a resident IS sequence; the other five plasmids were unchanged in size and probably contained *sacB* point mutations.

Because Tn*5* and IS*50* have a higher transposition frequency in Dam$^-$ cells than in Dam$^+$ cells (11, 17, 33, 36), the selection for sucrose resistance was repeated in Dam$^-$ strain DB4351. Suc^r mutants were about 200-fold more frequent in derivatives of strain DB4351 carrying plasmids I and II than in the isogenic Dam$^+$ strains (Table 1). About one-third of the Suc^r plasmids were at least 300 bp smaller than their Suc^s parents. This suggested that Tn*5*-mediated rearrangement events contribute significantly to the overall mutation frequency in Dam$^-$ cells.

To provide a larger target for transposition and also to efficiently identify many deletions before DNA analysis, we cloned a 1.5-kb *kan* gene adjacent to *sacB* to make plasmids I-a and II-a (Fig. 2A). Suc^r mutants of strain DB4351 (Dam$^-$) carrying these plasmids were obtained at frequencies of 3 × 10^{-6}. More than 1/10 of them were Kan^s (Table 2), and each of the 24 Suc^r Kan^s isolates screened by gel electrophoresis contained plasmids at least 2 kb smaller than its parent plasmid. Among 91 Suc^r plasmids tested that had remained Kan^r, 13 were more than 300 bp smaller than their parents and had lost diagnostic *Bam*HI (plasmid I-a) or *Pvu*II (plasmid II-a) fragments (sites boxed in Fig. 2). This outcome suggested that they contained deletions beginning at the I end (plasmid I-a) and the O end (plasmid II-a). Three other derivatives of plasmid I-a were just 300 bp smaller than their parents, had retained the *Bam*HI site between the O end and *sacB* but on a different-sized fragment, and thus were likely to contain inversions. Two other mutant classes were not due to Tn*5* transposition: eight plasmids were 1 to 2 kb larger than their parents and were attributable to insertions of IS elements resident in the bacterial chromosome, and 67 plasmids were unchanged in size and probably contained *sacB* point mutations (Table 2).

TABLE 1. Frequencies of sucrose-resistant colonies[a]

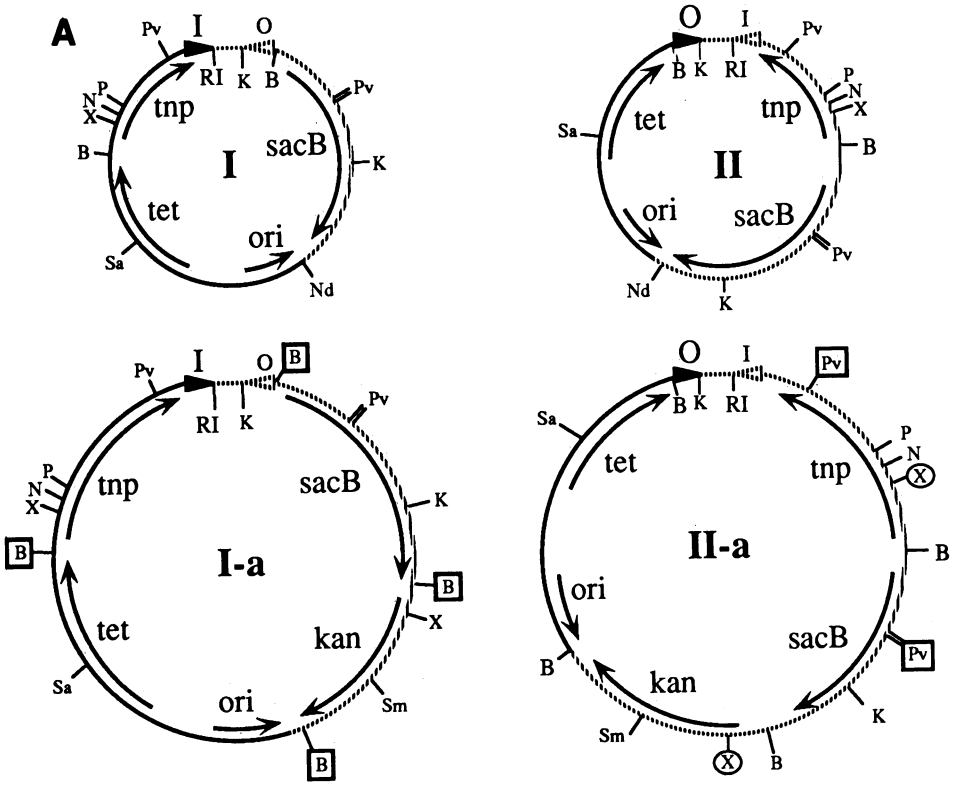
Plasmid	Suc^r frequency (10^6) with host strain:	
	dam$^+$	*dam*
I	0.018	4.7
I-a	0.015	2.6
II	0.022	4.0
II-a	0.042	3.0

[a] Sucrose-resistant mutants were selected on LN-tetracycline agar containing 5% sucrose. Each value reported represents the median frequency obtained with five independent clones (Materials and Methods). Host strains: *dam*$^+$, MC1061; *dam*, isogenic *dam*::Tn*9* strain DB4351.

The DNA sequences adjacent to the transposon ends in representative smaller plasmids were determined by using primer oligonucleotides specific to these ends (Fig. 2B). Plasmids from each of nine Suc^r Kan^s mutants tested contained a simple deletion extending from one transposon end into sites in *kan* or, in one case, into the adjacent pBR322 segment (nos. 6 through 10 and 14 through 17; Table 3, Fig. 3 and 4). Similarly, each of eight Suc^r mutants tested that had lost more than 300 bp and the diagnostic *Bam*HI or *Pvu*II site but remained Kan^r and contained a deletion from the transposon into sites in *sacB* (sites 1 through 5 and 11 through 13). Three other aspects of the data merit attention. (i) The complete sequence of one transposon end was present at the junction with target DNAs in each of the 17 mutants examined (I end in plasmid I-a derivatives, O end in plasmid II-a derivatives). (ii) The base pair of the vector that was adjacent to the I end in plasmid I-a, and the O end in plasmid II-a had been replaced in at least 11 of 17 cases (in the other 6 cases the possibility that this adjacent base pair was from the vector rather than the target could not be ruled out). (iii) Each deletion endpoint was at a different site in the target DNA.

Restriction mapping and sequencing of three of the plasmids that had lost just 300 bp confirmed that they contained inversions of the type diagrammed in Fig. 1. In each case, both the I and O ends were present, the 300-bp vector fragment had been lost, each transposon end was fused to a site in *sacB* in the orientation opposite that in the parental plasmid, and the 9-bp sequence next to the I end was duplicated next to the O end (Fig. 3, Table 3).

Because no inversions were initially obtained from plasmid II-a, additional Suc^r colonies that remained Kan^r were selected and screened by colony hybridization with an oligonucleotide probe specific for vector sequences. Plasmid



B I end region (plasmids I-a and II-a)

5'-GGATCTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGCATTAAAGCTCGAATTC

Primer #1 ——I End—— EcoRI

O end region (plasmid I-a)

5'-CGGCAGGTATATGTCATGGGTTAAAAAGGATCCCCTACTTGTGTATAAGAGTCAGGGTACC

Primer #2 BamHI ——O End—— KpnI

O end region (plasmid II-a)

5'-CCATCGCGTCCGCCATCTCCAGCAGCCGCACGCGGCGCATCTCGGGATCCCCTACTTGTGTATAAGAGTCAGGGTACC

Primer #3 BamHI ——O End—— KpnI

FIG. 2. Structures of plasmids containing engineered Tn*5* elements. (A) Restriction maps of plasmids used in this work: – – –, deletable segment; sacB, sucrose sensitivity; ori, pBR322 replication origin; tet, tetracycline resistance; tnp, transposase; kan; kanamycin resistance. Restriction endonuclease cleavage sites: RI, *Eco*RI; K, *Kpn*I; B, *Bam*HI; Nd, *Nde*I; Sa, *Sal*I; X, *Xho*I; N, *Not*I; P, *Pst*I; Sm, *Sma*I; Pv, *Pvu*II. Restriction sites that were used to help distinguish deletions and inversions are outlined with boxes and circles, respectively (see the text). Plasmids I and II are 6.3 kb; plasmids I-a and II-a are 7.8 kb. The segment containing the IS*50* I and O ends and *tnp* (transposase) gene came from a derivative of pBRG1305 (23) in which *tnp* was at the *Bam*HI site interior to the two ends (K. Dodson, unpublished data). A 300-bp segment between the first positions of the O and I ends (the *Eco*RI-*Cla*I fragment) from pBRG1305 served as the vector as described in the text. *sacB* (the levansucrose gene from *Bacillus subtilis*) causes sucrose sensitivity in *E. coli* and was derived from pLS302 (13, 30) as a *Bam*HI-*Nde*I fragment. The *kan* gene of Tn*903* was obtained as an *Eco*RI fragment from pUC4K (31, 34). The *Eco*RI site in *sacB* was removed by the linearized plasmid method (19) and a bridging oligonucleotide (5′ TATCAAGTTCCTGAGTTCGATTCGTCCAC) designed to retain the amino acid sequence of the *sacB* protein and to avoid rare codons in the gene. The *Bam*HI site in *tet* was eliminated by using bisulfite mutagenesis of *Bam*HI-cleaved plasmid DNA (18). The unmarked segment between *tet* and *ori* contains the 3′ portion of the *amp* gene, distal to the pBR322 *Pst*I site. (B) Sequences in the vicinity of the transposon ends. The 19 bp of the O and I end segments and the sequences used as primers are boxed. Primers: 1, nucleotides 1492 to 1501 of IS*50*; 2, nucleotides 32 to 13 of *sacB*; 3, nucleotides 1384 to 1403 of pBR322.

TABLE 2. Analysis of plasmids from sucrose-resistant colonies[a]

Parental plasmid	No. of colonies tested			No. of Kan^s colonies		No. of Kan^r colonies[b]				
	Kan^r	Kan^s	Total	Loss of >300 bp	Total screened	Loss of: >300 bp	Loss of: 300 bp	Gain of >1 kb	No change	Total screened
I-a[c]	415	85	500	11	11	7	3	2	27	39
II-a[c]	447	53	500	13	13	6	0	6	40	52
II-a[d]						24	3	ND	ND	100

[a] DB4351 (Dam$^-$) was used as the host. Plasmid DNAs were tested for size by agarose gel electrophoresis.
[b] Loss of >300 bp was a deletion; loss of 300 bp was an inversion; gain of >1 kb was an insertion, probably of IS element; no change indicated a point mutation. ND, Not determined.
[c] 20 separate single-colony isolates were grown to the stationary phase in 3 ml of broth. Samples were plated on LN-tetracycline-sucrose agar, and 25 sucrose-resistant isolates from each culture were tested for kanamycin sensitivity. Plasmid DNAs from representative Kan^s and Kan^r isolates were then screened by plasmid size and *Bam*HI and *Pvu*II digestion to distinguish deletions and inversions (Fig. 2A).
[d] Ten separate single-colony isolates were tested as described above, and 10 sucrose-resistant, kanamycin-resistant derivatives from each culture were screened by colony hybridization for loss of the vector segment. Plasmid DNAs from each of the 27 isolates identified as having sustained rearrangement were then examined by plasmid size and *Xho*I digestion to distinguish deletions and inversions (Fig. 2A).

size determination and *Xho*I restriction analysis (*Xho*I sites circled in Fig. 2) of 27 isolates that did not hybridize with the probe indicated that 24 had deletions extending into *sacB* and that 3 isolates had inversions with endpoints in *sacB*. Although the endpoints were not mapped in detail, the diagnostic fragments varied in size, indicating that the insertions in *sacB* were not clustered.

DISCUSSION

The conservative (nonreplicative) model of Tn*5* transposition predicts the formation of adjacent deletions and inversions by transposition to sites within the element itself (Fig. 1). Formally, the deletions arise when one twist or an odd number of twists intervene between the transposable element ends and the target, whereas inversions arise when there are no twists or an even number of twists in this interval. In both situations the vector segment is linearized and lost and viable circular products are formed only when transposition is targeted to sites within the element itself (Fig. 1). In contrast, the replicative transposition model predicts that the vector is not linearized when adjacent deletions and inversions are formed, and that circular products are formed by intramolecular transposition only when sites in the vector are targeted (reviewed in references 2, 3, 21, 28, and 29). The experiments presented here are in accord with the conservative model for intramolecular Tn*5* transposition.

The low frequency of IS*50*-mediated rearrangements recovered ($<10^{-8}$ in Dam$^+$ cells; $\sim10^{-6}$ in Dam$^-$ cells) does not accurately reflect the rate of transposition, since the

TABLE 3. Products of intramolecular transposition[a]

Type of plasmid	Isolate	Target site duplication[b] sequences and positions	Phenotype
Deletion[c]	1	ATAAAAAAG s443-451	Kan^r
	2	AACATCAAA s467-475	Kan^r
	3	GTCTTTAAA s1499-1507	Kan^r
	4	TACTCACAC s1694-1702	Kan^r
	5	TCCTTGAAC s1851-1859	Kan^r
	6	CTCATACCA k468-476	Kan^s
	7	GAAACTGCA sk747-754	Kan^s
	8	TTTTGAAAA k787-795	Kan^s
	9	CTGAGCGAG k1083-1091	Kan^s
	10	CTTCCCATA k1398-1406	Kan^s
	11	CTGCTGGCA s518-526	Kan^r
	12	GGCGACAAC s1181-1189	Kan^r
	13	ATTCTACGC s1765-1773	Kan^r
	14	AGATGCGTG k676-684	Kan^s
	15	ATGCATCAT k1253-1261	Kan^s
	16	GCTTTCCCC k1641-1649	Kan^s
	17	GTGAAATAC p2306-2314	Kan^s
Inversion	1	CAGTGCGGT s520-512 (I end), ACCGCACTG s512-s20 (O end)	Kan^r
	2	GTTGATGTT s1090-1082 (I end), AACATCAAC s1082-1090 (O end)	Kan^r
	3	AAGTGTGAC s1704-1696 (I end), CTCACACTT s1696-1704 (O end)	Kan^r

[a] The rearrangements were obtained by selecting Suc^r mutants and then screening plasmids for size and diagnostic *Bam*HI and *Pvu*II sites (Fig. 2A legend). Putative deletion plasmids were >300 bp smaller than plasmid I-a and lacked a diagnostic site, whereas inversion plasmids were just 300 bp smaller than plasmid I-a.
[b] The deletion and inversion endpoints were determined by double-stranded dideoxy sequencing with the primers indicated in Fig. 2B. The inversions were from plasmid I-a and were sequenced in both directions as detailed in Fig. 3. The sequence listed indicates the 9-bp duplication adjacent to the transposon end. Numbers refer to the nucleotide sequence coordinates of the 1,960-bp *sacB* gene (s) (30), the 1,270-bp *kan* segment from pUC4K (k) (31, 34), and pBR322 (p) (20).
[c] See Fig. 4.

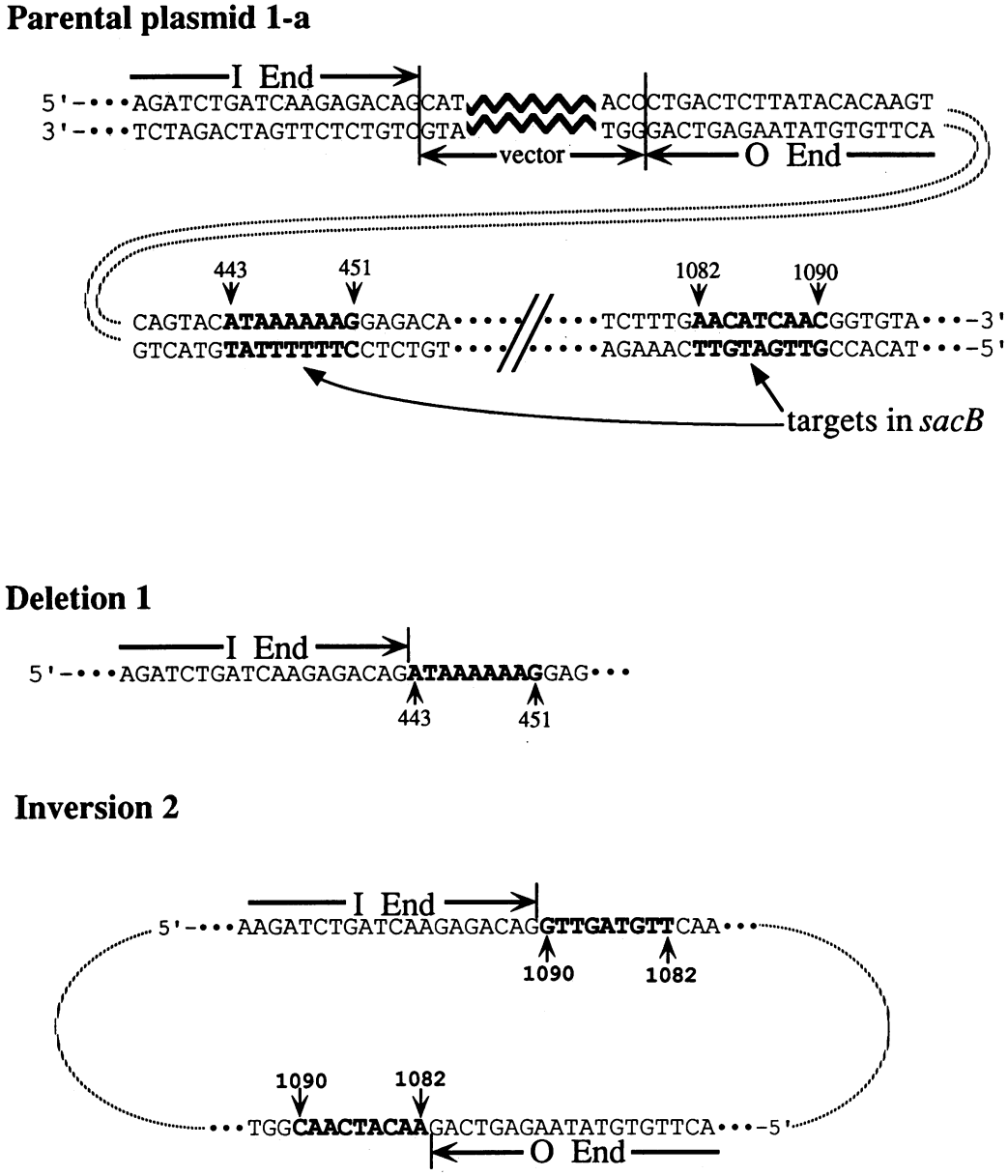


FIG. 3. Strategy for analysis of deletion and inversion mutants. Sequences were determined by using the primers shown in Fig. 2B. Isolate numbers refer to those summarized in Table 3 and Fig. 4.

plasmids are present in multiple copies and several generations of growth and segregation are needed for expression of sucrose resistance.

The alignment that results in deletions should actually generate two complementary circular deletion molecules, each of which lacks vector sequences (Fig. 1). Only one deletion product was found, however, because the other lacked a replication origin and thus was lost during bacterial growth. We found that deletions began precisely at one transposon end and extended to many different sites in target DNAs. The alignment that results in inversions, in contrast, generates one circular DNA molecule consisting of the entire transposon without vector sequences and with the transposon ends moved to new sites. We found that the inversion breakpoints also occurred precisely at the element ends and were associated with 9-bp duplications of target sequences (Fig. 3).

Given that Suc^r Kan^r mutant plasmids (Fig. 2) can result from transposition in either the deletion- or the inversion-generating alignments, what determines which transposition product is formed? The simplest model predicts random alignment of DNA sequences, with both products arising at equal frequencies. However, deletions were more frequent than inversions (37 deletions versus 6 inversions; Table 2). It is possible that topological constraints (32) specifically favor the formation of just one type of product. However, it is also possible that some deletions arise by abortive transposition (Fig. 5). This would be an in vivo equivalent of the exonuclease III-SI in vitro method for making nested deletions (25). It would entail the following: (i) binding of transposition proteins to just one, instead of both, transposon ends; (ii) cleavage at that transposon-vector junction; (iii) incomplete exonucleolytic erosion of the adjacent unprotected DNA; and (iv) spontaneous ligation of the ends to regenerate viable

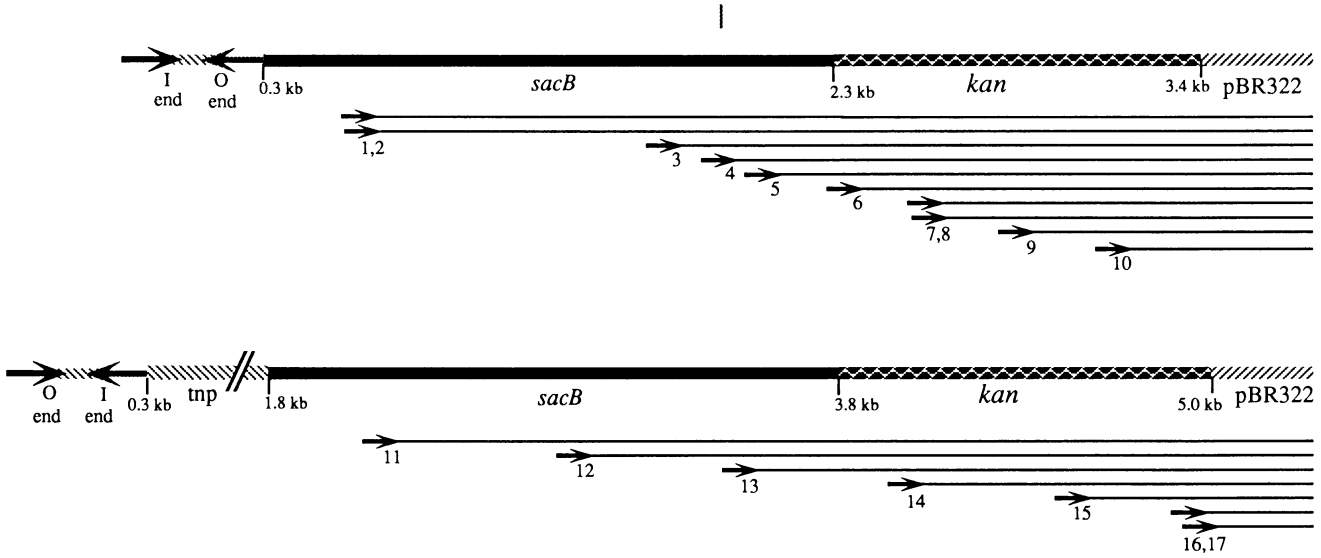


FIG. 4. Positions of deletion endpoints. The relevant segments of plasmids I-a and II-a are shown. Thin horizontal lines and arrows indicate segments left after deletion. The precise endpoint sequences of these deletion mutants are presented in Table 3.

plasmids. Such abortive transposition might explain deletions in vector segments adjacent to a synthetic Tn*5* element obtained in selections different from those used here (R. Jilk and W. S. Reznikoff, personal communication) and the very rare deletions found adjacent to a single IS*10* element (16). However, inversions cannot arise by abortive transposition, since they contain the entire transposon, albeit rearranged.

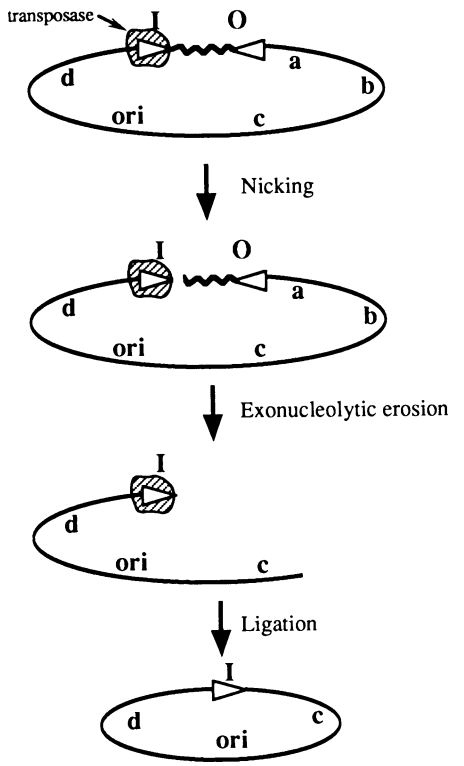


FIG. 5. Abortive transposition model. Cleavage adjacent to one transposon end, exonucleolytic erosion, and then ligation could explain some transposon-associated deletions.

Because inversions account for about 15% of the rearrangements with endpoints in *sacB*, abortive transposition, if it occurs, is not likely to be responsible for all of the deletions obtained.

Regardless of how IS*50*-mediated deletions arise, they extend from a fixed point (the transposon end) to many different sites in target DNAs. Sets of nested deletions are useful for studies of gene function, for mapping, and for DNA sequencing. A Tn*5*-based in vivo deletion strategy should compare favorably with one based on IS*1* (Tn*9*) (1), because Tn*5* insertion is more nearly random than IS*1* insertion (3, 8, 12; this work). The efficient isolation of deletions also depends on a good selection, because deletions are quite rare. In the IS*1*-based system, deletions were selected by loss of plasmid-borne *galK*$^+$ and *galT*$^+$ genes in a *galE* host strain(1). However, *sacB* promises to be more useful: it confers high sensitivity to sucrose, which is inexpensive and nontoxic; it is smaller than *galK* and *galT*; and it can be selected against very strongly in *E. coli* and other gram-negative species, without need for special chromosomal mutations (13, 24). Although several types of mutations were obtained among Suc^r colonies, only deletions were obtained among Suc^r Kan^s colonies. Thus, we anticipate that plasmids that contain synthetic Tn*5* cassettes, *sacB*, a second contraselectable or indicator gene, and a multiple cloning site will be valuable deletion factory vectors for functional and DNA sequence analyses of cloned DNAs.

ACKNOWLEDGMENTS

We are grateful to Lena Henry for skillful technical assistance, to B. R. Krishnan and W. S. Reznikoff for discussions and critical reading of the manuscript, and to W. S. Reznikoff for permission to cite results in advance of publication.

This work was supported by grants to D.E.B. and C.M.B. from the U.S. Department of Energy DEFGO2-89ER60862, by Public Health Service grant GM-37138 from the National Institutes of Health, by National Science Foundation grants DMB8608193 and DMB8802310, and by the Lucille P. Markey Charitable Trust.

LITERATURE CITED

1. **Ahmed, A.** 1987. Use of transposon-promoted deletions in DNA sequence analysis. Methods Enzymol. **155**:177–204.

2. **Berg, C. M., and D. E. Berg.** 1987. Uses of transposable elements and maps of known insertions, p. 1071–1109. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
3. **Berg, C. M., D. E. Berg, and E. Groisman.** 1989. Transposable elements and the genetic engineering of bacteria, p. 879–925. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
4. **Berg, D. E.** 1977. Insertion and excision of the transposable kanamycin resistance determinant Tn*5*, p. 205–212. *In* A. I. Bukhari, J. A. Shapiro, and S. L. Adhya (ed.), DNA insertion elements, plasmids and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
5. **Berg, D. E.** 1983. Structural requirement for IS*50*-mediated gene transposition. Proc. Natl. Acad. Sci. USA **80:**792–796.
6. **Berg, D. E.** 1989. Transposon Tn*5*, p. 185–210. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
7. **Berg, D. E., and M. M. Howe (ed.).** 1989. Mobile DNA. American Society for Microbiology, Washington, D.C.
8. **Berg, D. E., M. A. Schmandt, and J. B. Lowe.** 1983. Specificity of transposon Tn*5* insertion. Genetics **105:**813–828.
9. **Caparon, M. G., and J. R. Scott.** 1989. Excision and insertion of the conjugative transposon Tn*916* involves a novel recombination mechanism. Cell **59:**1027–1034.
10. **Casadaban, M., and S. N. Cohen.** 1980. Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*. J. Mol. Biol. **138:**179–207.
11. **Dodson, K. W., and D. E. Berg.** 1989. Factors affecting transposition activity of IS*50* and Tn*5* ends. Gene **76:**207–213.
12. **Galas, D. J., and M. Chandler.** 1989. Bacterial insertion sequences, p. 109–162. *In* D. E. Berg and M. M. Howe (ed.). Mobile DNA. American Society for Microbiology, Washington, D.C.
13. **Gay, P., D. Le Coq, M. Steinmetz, T. Berkelman, and C. I. Kado.** 1985. Positive selection procedure for entrapment of insertion sequence elements in gram-negative bacteria. J. Bacteriol. **164:**918–921.
14. **Isberg, R. R., and M. Syvanen.** 1985. Tn*5* transposes independently of cointegrate resolution. Evidence for an alternative model for transposition. J. Mol. Biol. **182:**69–78.
15. **Johnson, R. C., and W. S. Reznikoff.** 1983. DNA sequences at the ends of transposon Tn*5* required for transposition. Nature (London) **204:**280–282.
16. **Kleckner, N.** 1989. Transposon Tn*10*, p. 227–268. *In* D. E. Berg and M. M. Howe (ed.). Mobile DNA. American Society for Microbiology, Washington, D.C.
17. **Krebs, M. P., and W. S. Reznikoff.** 1986. Transcriptional and translational initiation sites of IS*50*. Control of transposase and inhibitor expression. J. Mol. Biol. **192:**781–792.
18. **Lodge, J. K., K. Weston-Hafer, and D. E. Berg.** 1988. Transposon Tn*5* target specificity: preference for G/C pairs. Genetics **120:**645–650.
19. **Mandecki, W.** 1986. Oligonucleotide directed double strand break repair in plasmids of *Escherichia coli*: a method for site directed mutagenesis. Proc. Natl. Acad. Sci. USA **83:**7177–7181.
20. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
21. **Pato, M. L.** 1989. Bacteriophage Mu, p. 23–52. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
22. **Phadnis, S. H., and D. E. Berg.** 1985. *recA*-independent recombination between repeated IS*50* elements is not caused by an IS*50*-encoded function. J. Bacteriol. **161:**928–932.
23. **Phadnis, S. H., and D. E. Berg.** 1987. Identification of base pairs in the IS*50* O end needed for IS*50* and Tn*5* transposition. Proc. Natl. Acad. Sci. USA **84:**9118–9122.
24. **Ried, J. L., and A. Collmer.** 1987. An *nptI-sacB-sacR* cartridge for constructing directed, unmarked mutations in gram-negative bacteria by marker exchange-eviction mutagenesis. Gene **57:** 239–246.
25. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
27. **Sasakawa, C., G. F. Carle, and D. E. Berg.** 1983. Sequences essential for transposition at the termini of IS*50*. Proc. Natl. Acad. Sci. USA **80:**7293–7297.
28. **Shapiro, J. A.** 1979. Molecular model for the transposition and replication of bacteriophage Mu and other transposable elements. Proc. Natl. Acad. Sci. USA **76:**1933–1937.
29. **Sherratt, D.** 1989. Tn*3* and related transposable elements: site-specific recombination and transposition, p. 163–184. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
30. **Steinmetz, M., D. Le Coq, S. Aymerich, G. Gonzy-Treboul, and P. Gay.** 1985. The DNA sequence of the gene for the secreted *Bacillus subtilis* enzyme levansucrase and its genetic control sites. Mol. Gen. Genet. **200:**220–228.
31. **Taylor, L. A., and R. E. Rose.** 1988. A correction in the nucleotide sequence of the Tn*903* kanamycin resistance determinant in pUC4K. Nucleic Acids Res. **16:**358.
32. **Thompson, R. J., J. P. Davies, G. Lin, and G. Mosig.** 1990. Modulation of transcription by altered torsional stress, upstream silencers, and DNA-binding proteins, p. 227–240. *In* K. Drlica and M. Riley (ed.). The bacterial chromosome. American Society for Microbiology, Washington, D.C.
33. **Tomcsanyi, T., and D. E. Berg.** 1989. Effect of adenine (Dam) methylation on transposition activity of O end mutants of IS*50*. J. Mol. Biol. **209:**191–193.
34. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7 derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268.
35. **Weston-Hafer, K., and D. E. Berg.** 1989. Specificity of deletion events in pBR322. Plasmid **21:**251–253.
36. **Yin, J. C. P., M. P. Krebs, and W. S. Reznikoff.** 1988. The effect of *dam* methylation on Tn*5* transposition. J. Mol. Biol. **199:**33–45.
37. **Zagursky, R. J., K. Baumeister, N. Lomax, and M. L. Berman.** 1985. Rapid and easy sequencing of large linear double stranded DNA and supercoiled plasmid DNA. Gene Anal. Tech. **2:**89–94.