JOURNAL OF BACTERIOLOGY, Mar. 1994, p. 1332–1338
0021-9193/94/$04.00+0

Vol. 176, No. 5

Inversions and Deletions Generated by a Mini-γδ (Tn*1000*) Transposon

GAN WANG,[1] XIAOXIN XU,[1] JIAN-MIN CHEN,[1] DOUGLAS E. BERG,[2] AND CLAIRE M. BERG[1]*

Department of Molecular and Cell Biology, The University of Connecticut, Storrs, Connecticut 06269-2131,[1] and Departments of Molecular Microbiology and Genetics, Washington University Medical School, St. Louis, Missouri 63130[2]

Received 29 September 1993/Accepted 13 December 1993

Intramolecular transposition by an engineered derivative of the transposon γδ (Tn*1000*) is described. This 1-kb element contains inverted repeats of the 40 bp of the δ end of γδ, bracketing a *kan* gene, but it contains no resolution site. Transposition was analyzed in two plasmids; one contained two contraselectable (conditional lethal) genes (*thyA* and *sacB*) adjacent to the mini-γδ element in a 13.0-kb pBR322/pUC-based two-component plasmid (a heterodimer), and the other contained a different contraselectable gene (*strA* [*rpsL*]) in a 13.2-kb three-component plasmid (a heterotrimer). Selection for loss of function of a single contraselectable gene yielded inversions and deletions. Each inversion plasmid was 1 kb larger than the parent plasmid: it had a second copy of mini-γδ inserted in the contraselected gene, with that copy plus the intervening segment inverted, and the 5-bp target site duplicated. Each deletion plasmid was smaller than the parent plasmid and had a deletion that extended from one transposon end into or through the contraselected gene for distances of up to 9.4 kb. The frequencies of deletions versus inversions ending in a single target gene were similar, although overall, deletions outnumbered inversions because deletions, but not inversions, into sites beyond the contraselected gene inactivate it. This work also demonstrates that *thyA* (which encodes thymidylate synthetase) is a useful contraselectable marker.

Transposon γδ, a member of the Tn*3* family (21), has been used extensively for molecular genetic analysis of cloned DNAs (2, 13). Recently, small, engineered derivatives of γδ have been constructed to facilitate molecular analysis of cloned fragments (4, 6, 28) and marker exchange (4, 6). γδ generally moves by a replicative mechanism that is believed to involve transposase-catalyzed single-stranded breaks at each transposon end, a five-base staggered cut in the target DNA, ligation of the free transposon ends to target DNA, repair of the five-base gaps, and replication of the element from these newly generated forks. The transposon is duplicated, and no DNA is lost in the transposition process.

When γδ moves from one molecule to another (intermolecular transposition), the product is a cointegrate in which donor and target molecules are joined by direct repeats of the transposon, with the five-base target site duplicated at the new transposon-target junctures. If the resolvase gene is functional and the resolution site is present in the transposon, these cointegrates are broken down into a target molecule containing a copy of the element (bracketed by the 5-bp target site duplication) plus an unchanged donor molecule, even in *recA* cells. However, when γδ moves to a site in the same molecule (intramolecular transposition), the product is either a deletion or an inversion, depending on how DNA between the donor and target sites was twisted (21) (Fig. 1). As described below, an inversion plasmid has the predicted duplication of γδ and of the 5-bp target sequence. On the other hand, only one of the two putative reciprocal deletion plasmids is recoverable in these experiments, because the other lacks a replication origin. Deletions generated by γδ and Tn*1* (Tn*3* family) and inversions generated by Tn*1* transposition have been described previously (7, 10), but they were not analyzed at the molecular level, leaving key elements of the transposition model unconfirmed.

For members of the Tn*3* family, the presence of one or more transposon ends reduces the frequency of subsequent transposition into that plasmid (21). This transposition immunity does not, however, appear to operate during intramolecular transposition in plasmids that contain a single pair of transposon ends (7, 21; this work), although transposition immunity is found when three ends are present (7).

We show here that (i) γδ-generated inversions and nested deletions can be readily obtained by using any of the three contraselectable genes tested (*strA*$^+$ and *sacB*$^+$, which had previously been used for contraselection in different systems [9, 11]), and *thyA*$^+$, which to our knowledge had not been used previously); (ii) inversions and deletions ending in a target gene are found at similar frequencies; (iii) γδ-generated deletion and inversion products have the predicted structures; (iv) the topography of target sites used in intramolecular transposition is similar to that of sites used in intermolecular transposition; and (v) sets of γδ-generated nested deletions are quite random.

MATERIALS AND METHODS

Chemicals, reagents, and media. Specialized chemicals were from Sigma Chemical Co., St. Louis, Mo. Agar and components of Lennox (L) medium were from Difco Laboratories, Detroit, Mich. Agarose, restriction enzymes, T4 polymerase, DNA size standards, and the double-stranded DNA cycle sequencing kit were from Bethesda Research Laboratories (BRL), Gaithersburg, Md.

L agar (LA) and L broth (LB) plus thymine (10 μg/ml) and Vogel and Bonner medium E plus 0.5% Casamino Acids with the indicated additives were used for growth and selection (4, 6). Ampicillin (100 μg/ml), chloramphenicol (30 μg/ml), kana-

* Corresponding author. Mailing address: U-131, 354 Mansfield Rd., University of Connecticut, Storrs, CT 06269-2131. Phone: (203) 486-2916. Fax: (203) 486-1936. Electronic mail (Bitnet) address: Berg@UCONNVM.uconn.edu.

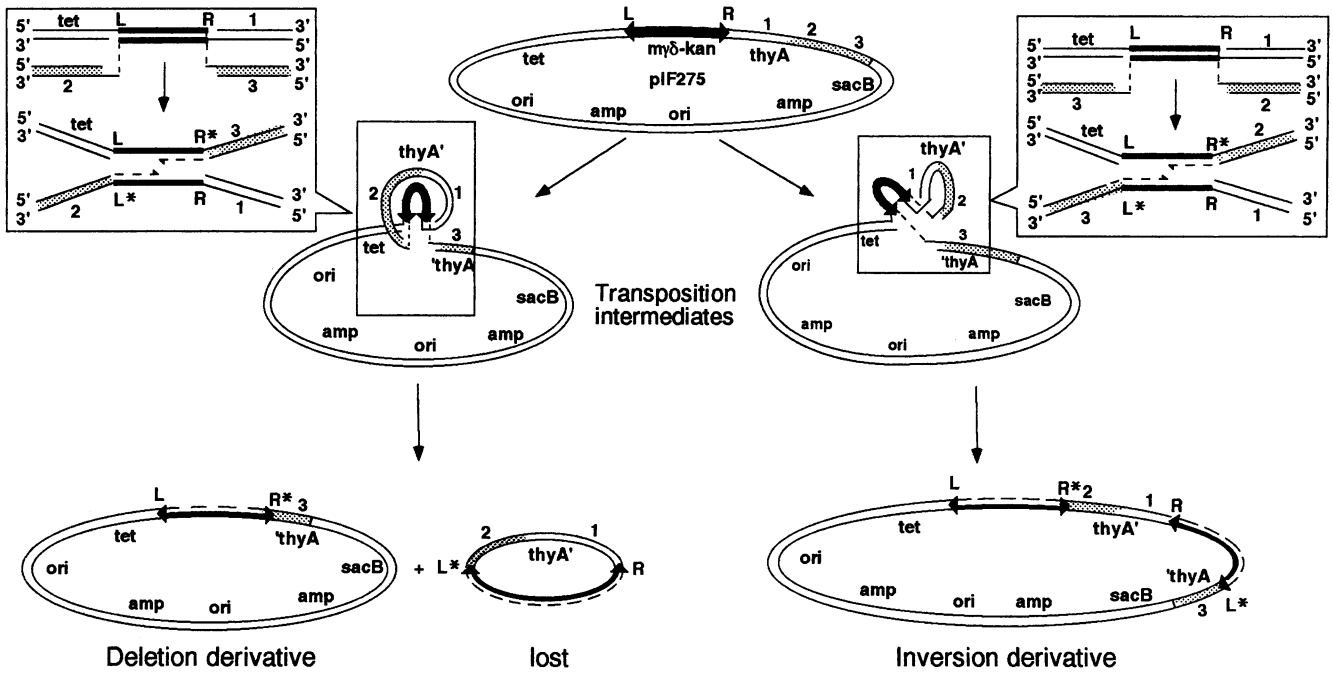


FIG. 1. Expected products of intramolecular γδ transposition into the *thyA* gene of pIF275. Left, deletion formation; right, inversion formation. The 5-bp duplication at the target site is not shown. Symbols: L and R, left and right ends of mini-γδ in pIF275; L* and R*, left and right ends of mini-γδ inserted at new sites; solid bar with arrows, mini-γδ in pIF275; heavy lines, conserved strand of mini-γδ after transposition (one round of replication); dashed lines, newly replicated mγδ strand; thyA', *thyA* gene with clockwise truncation; 'thyA, *thyA* gene with counterclockwise truncation; 1, 2, and 3, sites in the *thyA* gene; sacB, sucrose sensitivity; thyA, TMP sensitivity; ori, pBR322- or pUC-derived replication origin; amp, ampicillin resistance; kan, kanamycin resistance; tet, tetracycline resistance.

mycin (30 μg/ml), and/or tetracycline (25 μg/ml) were used for plasmid maintenance and testing. Streptomycin (100 μg/ml) and sucrose (5%) were used in LA to select against Str^+ and $SacB^+$ cells, respectively. Trimethoprim (TMP; 50 μg/ml) plus thymine (50 μg/ml) in LA or medium E plus Casamino Acids was used to select against Thy^+ cells. Isopropylthiogalactopyranoside (IPTG) was used at 1 mM to induce transposase synthesis.

DNA manipulations and sequencing. Plasmids were constructed using standard recombinant DNA methods (20). Plasmid DNAs were extracted by a miniprep method from purified representative colonies and screened for size and for diagnostic restriction sites by agarose gel electrophoresis. DNA was sequenced by the linear amplification (cycle) sequencing method (8, 15).

The mγδ primer (bases 1946 to 1968 of the Tn*903 kan* gene [17]; 5'-AGTTTCATTTGATGCTCGATGAG-3') was synthesized in the University of Connecticut Biotechnology Center. The M13/pUC 23-base forward sequencing primer and the T7 promoter primer were from BRL.

Strains, plasmids, and general techniques. The *recA rpsL Escherichia coli* K-12 strains used in this study were CBK884 (4); CBK929, a Δ*thyA* derivative of CBK884 (this work); and DH10B (12). The conditions for bacterial growth and transposition were as described previously (4, 6). All incubations were at 37°C.

The plasmids used in this study, the two-component heterodimer pIF275 (Fig. 1 and 2) and the three-component heterotrimer pIF279 (Fig. 3), were constructed as described below. Both plasmids contain 40-bp inverted repeats of the δ end of γδ bracketing the Tn*903 kan* gene. The presence of two (pIF275) or three (pIF279) pBR322/pUC-derived plasmid replication origins had no deleterious effect on the survival of these plasmids.

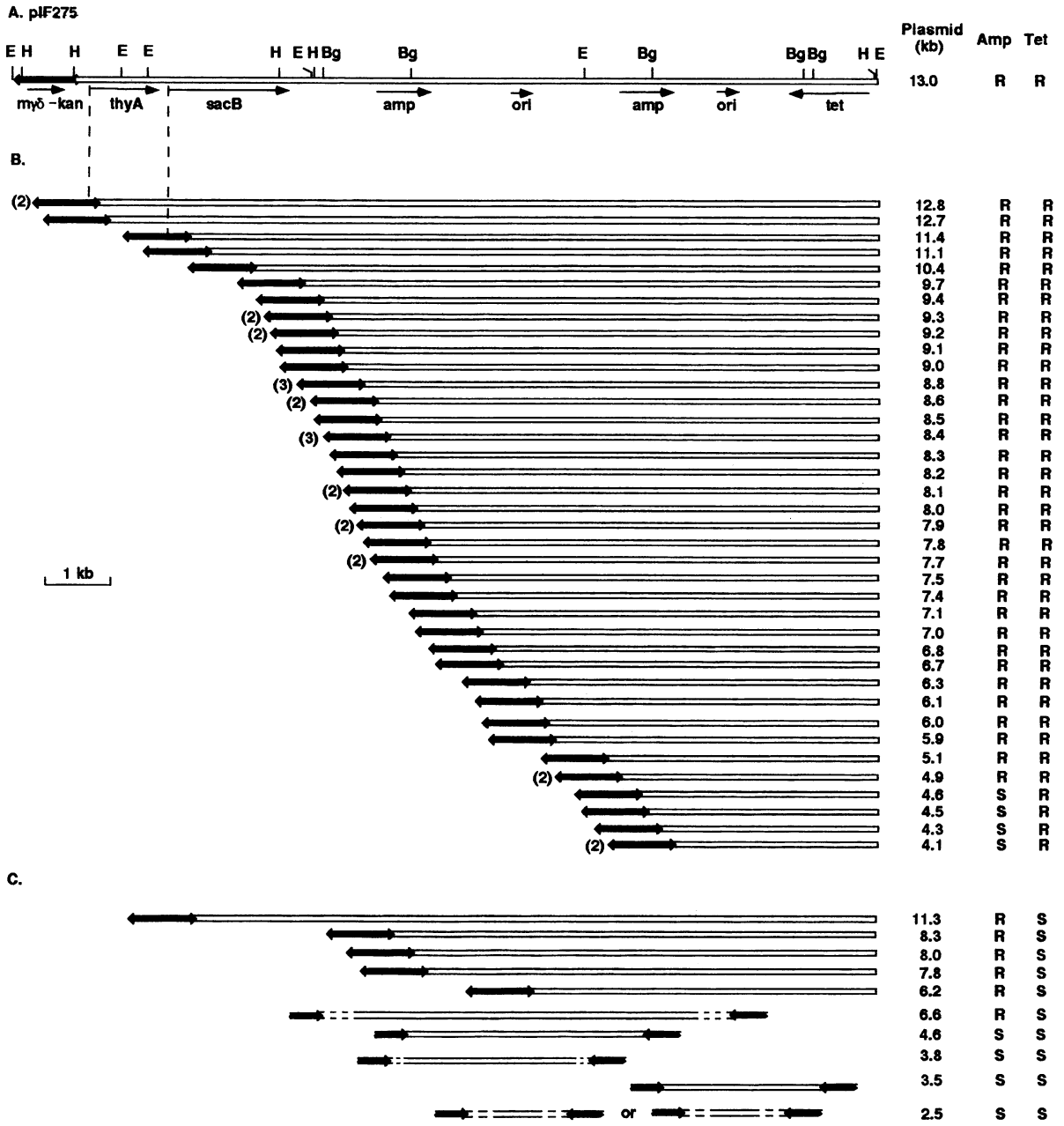
To construct the 13-kb pIF275 plasmid, the *Kpn*I mini-γδ fragment from pLAW168 (29) and the *Xba*I-*Nde*I *thyA-sacB* fragment from pBS-TS (a pBR322-based plasmid containing the *thyA* and *sacB* gene cassette) were cloned into the *Bam*HI site of pSPORT (BRL) (after all fragment ends were made blunt with T4 DNA polymerase or the DNA polymerase I Klenow fragment) and transformed into CBK929, selecting for Amp^r Kan^r Thy^+, to give pIF271. The *Sma*I *amp-ori-tet* fragment from pBR322ΩE (19) was cloned into the filled-in *Sst*I site of pIF271, to give pIF275.

To construct the 13.2-kb pIF279 plasmid, pLAW168 (29) and pNO1523 (9) were each cut once with *Eco*RI and ligated together, selecting for Kan^r Amp^r and screening for Str^s, to give pIF276. pIF276 and pBR322 were digested with *Sty*I and ligated together, selecting for Tet^r Kan^r, to give pIF279.

Transposase was provided in *trans* from a pBR322-compatible plasmid, pXRD4043, that contains the γδ *tnpA* gene under *tac* promoter control (24). However, some constitutive transposase synthesis was found in the strains used in this study. Consequently, transposition that occurred early during colony growth often resulted in the recovery of identical sibling deletion and inversion products (listed in Tables 1 and 2 but not in Fig. 2 and 3).

Intramolecular transposition does not involve a resolution step (Fig. 1), so neither resolvase nor a resolution (*res*) site was needed.

To select colonies in which transposition might have occurred, young (16-h) single colonies grown on LA plus ampicillin, kanamycin, and chloramphenicol were picked into 1 ml of LB plus kanamycin, chloramphenicol, and IPTG and grown



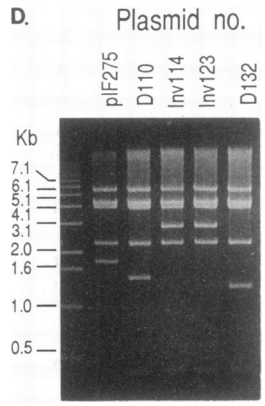


FIG. 2. Positions of deletion endpoints in pIF275 derivatives. pIF275 is depicted opened at the *Eco*RI site just to the left of mini-γδ. Colonies containing deletion plasmids were selected on kanamycin-TMP or on kanamycin-sucrose medium and then screened for TMP, Suc, and Amp phenotypes. Deletion endpoints were mapped first by plasmid size and then by digestion with *Bgl*I (Bg), *Eco*RI (E), and/or *Hin*dIII (H). Numbers to the left of the deletions indicate the number of independent insertions mapped in the same segment. Plasmids from the same single colony that could not be distinguished by phenotype or size are listed in Tables 1 and 2 but not in the figure because most were probably siblings that arose early during growth of the culture (data not shown). The vertical dashed lines indicate the beginning of the *thyA* and *sacB* genes (only deletions that extend beyond them will inactivate the gene). (A) pIF275. (B) Sizes and deletion endpoints of 3 TMP^r Suc^s (see panel D for linear depictions of D110 [top line] and D132 [second line]) and 48 TMP^r Suc^r independent deletion survivors from 21 different colonies. All plasmids retained the *Eco*RI site between *tet* and mini-γδ (which starts 14 bp outside the γδ end),

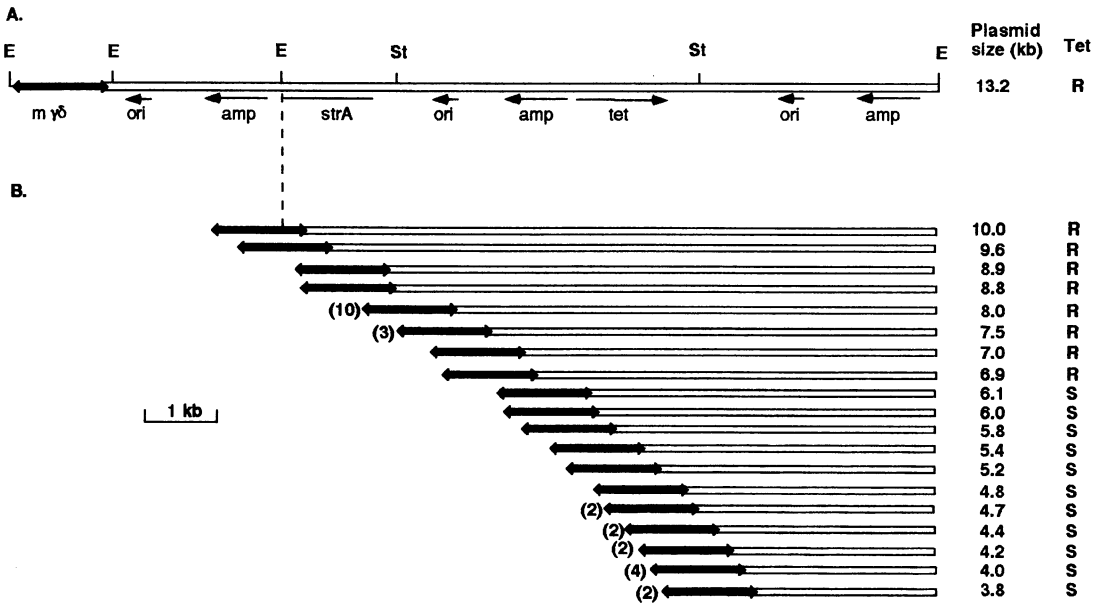


FIG. 3. Positions of deletion endpoints in pIF279. pIF279 is depicted opened at the *Eco*RI site counterclockwise of mini-γδ. Str^r survivors were selected from 20 different parental colonies. Two survivors were tested from each parental colony. Apparent siblings from the same parental colony were eliminated, leaving 37 independent deletion clones. Deletion endpoints were mapped, first by plasmid size and then by digestion with *Eco*RI and *Hin*dIII. Numbers to the left of the deletions indicate the number of independent insertions mapped in the same segment. Plasmids from the same single colony that could not be distinguished by phenotype or size were not listed because most were probably siblings that arose early during growth of the culture (data not shown). The vertical dashed line indicates the beginning of the *strA* gene. (A) pIF279; (B) sizes and deletion endpoints of 19 independent Str^r Tet^r and 18 independent Str^r Tet^s deletion survivors from 20 different colonies.

at 37°C for 4 h. Cells containing pIF275 were plated on LA plus kanamycin and TMP and/or sucrose or on medium E plus Casamino Acids, kanamycin, thymine, and TMP and/or sucrose. Cells containing pIF279 were plated on LA plus kanamycin and streptomycin. Single colonies that arose on the selection plates were tested on appropriate media.

RESULTS

Isolation and characterization of Suc^r and TMP^r mutants of CBK929(pIF275, pXRD4043). Transposition products could be selected because inactivation of *sacB* results in sucrose resistance (11) and inactivation of *thyA* results in TMP resistance in the presence of thymine (22).

Transposition was induced in the 13-kb plasmid, pIF275 (Fig. 1 and 2A), and cells were plated on selective media. Kan^r Suc^r and Kan^r TMP^r mutants were found at frequencies of 10^{-5} to 10^{-4}. Of 500 Kan^r TMP^r colonies, 91.6% were also Suc^r, while of 483 Kan^r Suc^r colonies, 85.3% were also TMP^r. These data indicate that most colonies selected on the basis of resistance to either contraselectable agent contain plasmids with deletions that extend through *thyA* and into or beyond *sacB* (at least 1.5 kb). Among the 870 colonies that were both TMP^r and Suc^r, 34 (3.9%) were also Amp^s, indicating that they had deletions extending into or beyond the rightmost *amp* gene (at least 8.1 kb) (Table 1).

Plasmids from 51 independent Kan^r TMP^r Suc^r colonies fell into at least 38 size classes, ranging from 4.1 to 11.4 kb, with the largest deletion extending almost as far as the rightmost replication origin (Fig. 2B). One Kan^r TMP^r Suc^r plasmid was 14.4 kb, larger than pIF275. It probably had an insertion sequence (IS) element insertion into *thyA* or *sacB* and a point mutation in the other gene (Table 2).

Plasmids from 12 independent Kan^r TMP^r Suc^s colonies were analyzed. Five were 12.7 to 12.8 kb and had deletions into but not beyond *thyA*; six (all from one culture) were 14 kb; and one was 14.4 kb and contained an IS element insertion (Table 2; Fig. 2B and D). The 14-kb plasmids were analyzed further; they contained a second (inverted copy of γδ in *thyA* and an inversion of the intervening sequence. The insertion sites in three of these inversion plasmids were in the 0.5-kb *Hin*dIII-*Eco*RI fragment (Fig. 2A and D), and the sites in the other three were in the adjacent 0.4-kb *Eco*RI fragment (not shown; see Fig. 2A for map). One inversion plasmid is described in more detail below.

indicating that the *tet* side of mini-γδ was intact. (C) Sizes and deletion endpoints in plasmids from 10 TMP^r Suc^r Tet^s survivors. The dashes indicate that the deletion endpoints were not mapped precisely. The top five plasmids retained the *Eco*RI site between *tet* and mini-γδ (14 bp from mini-γδ), indicating that the *tet* side of mini-γδ was intact. Therefore, in addition to rightward deletions, they must have undergone mutation in *tet* (point mutations were assumed in deducing the deletion endpoints). The bottom five plasmids had deletions that extended inward from each mini-γδ end. The deletion endpoints were deduced from colony phenotype and the presence or absence of diagnostic *Bgl*I and *Eco*RI fragments. (D) Agarose gel electrophoresis of pIF275 and representative deletion (D110 and D132) and inversion (Inv114 and Inv123) plasmids in which transposition occurred into *thyA*. The plasmids were digested with *Eco*RI and electrophoresed in 0.7% agarose in TAE buffer. Sizes of the DNA standard (BRL) bands are indicated. The 1.7-kb *Eco*RI fragment that contains the mini-γδ element and the *thyA* target sites (the *Eco*RI site at its right boundary is at *thyA* position 447) becomes smaller in these deletion derivatives and larger in these inversion derivatives. The faint 5.4-kb band common to all lanes is the transposase plasmid, pXRD4043.

TABLE 1. Phenotypes of Kan^r TMP^r or Kan^r Suc^r colonies from CBK929(pIF275, pXRD4043)

Colony no.	No. isolated as:									
	TMP^r					Suc^r				
	No. tested	Suc^r		Suc^s		No. tested	TMP^r		TMP^s	
		Amp^r	Amp^s	Amp^r	Amp^s		Amp^r	Amp^s	Amp^r	Amp^s
1	20	0	1	19	0	7	7	0	0	0
2	20	20	0	0	0	20	19	0	1	0
3	20	19	0	1	0	20	17	1	2	0
4	20	13	0	7	0	20	8	0	12	0
5	20	20	0	0	0	20	17	0	3	0
6	20	20	0	0	0	20	18	0	2	0
7	20	19	1	0	0	20	20	0	0	0
8	20	20	0	0	0	20	12	1	7	0
9	20	13	2	5	0	20	13	0	7	0
10	20	20	0	0	0	20	17	1	2	0
11	20	20	0	0	0	20	13	2	5	0
12	20	20	0	0	0	20	17	1	2	0
13	20	20	0	0	0	20	18	2	0	0
14	20	14	6	0	0	20	18	2	0	0
15	20	20	0	0	0	20	14	1	5	0
16	20	20	0	0	0	20	19	0	1	0
17	20	19	0	1	0	20	15	3	2	0
18	20	16	4	0	0	20	16	3	1	0
19	20	17	3	0	0	20	16	0	4	0
20	20	20	0	0	0	20	20	0	0	0
21	100	91	0	9	0	96	81	0	15	0
Total	500	441	17	42	0	483	395	17	71	0
%		88.2	3.4	8.4	0		81.8	3.5	14.7	0

Plasmids from 31 Kan^r TMP^s Suc^r colonies were analyzed; all were 13.0 kb or larger. Four plasmids were 14 kb and had inversions into *sacB* (which inverted *thyA* but left it functional) (Table 2). Twenty-two plasmids were 14.4 kb and had a 1.4-kb IS element inserted into *sacB*. Thirteen of these plasmids were tested, and all of the inserts lacked *Eco*RI and *Bam*HI restriction sites, suggesting they might be IS*4* (14). The remaining four plasmids were 13 kb, like pIF275, and probably had point mutations in *sacB*.

In another experiment, 3,900 colonies from plates containing LA plus kanamycin, TMP, and sucrose were screened for Tet^s; 25 (0.65%) were Tet^s. All 10 plasmids tested contained complex deletions or deletions plus mutations: five retained the *Eco*RI site that starts 14 bases to the left of mini-γδ (the site at which pIF275 is opened in Fig. 2A), while five had lost this site, indicating that they had deletions extending both rightward (through *thyA* and *sacB*) and leftward (into or through *tet*) (Fig. 2C). The five plasmids that retained the *Eco*RI site had deletions extending rightward from the γδ end plus point mutations or IS element insertions in *tet*. Although the Tet^s complex deletion plasmids have not been studied in detail, it is probable that they resulted from two independent deletion events.

Isolation and characterization of Str^r mutants of CBK884 (pIF279, pXRD4043). Transposition products could be selected because inactivation of *strA* (*rpsL*) results in streptomycin resistance (9).

Transposition was induced in the 13.2-kb plasmid pIF279 (Fig. 3A), and cells were plated on LA plus kanamycin and streptomycin. Survivors were found at a frequency of about 10^{-5}. Of the 40 survivors analyzed, 22 were Amp^r Tet^r, indicating that they had point mutations or inversions in *strA* or deletions that ended before *tet* (deletions of between 2.8 and 7.6 kb), while 18 were Amp^r Tet^s, indicating that they had deletions that extended into or beyond *tet* (deletions of at least

TABLE 2. Structures of pIF275 derivatives that had mutations in *thyA* or *sacB*[a]

Colony no.	No. of mutations							
	thyA[b]				*sacB*			
	Pt	Ins	Inv	Del	Pt[c]	Ins[d]	Inv[c]	Del[e]
1	0	1	0	0	0	2	0	0
2					0	1	0	0
3	0	0	0	1	0	1	0	0
4					0	1	0	0
5					1	0	0	0
6					0	0	1	0
7					0	0	0	1
8					1	0	0	0
9	0	0	0	1	1	0	0	0
10					0	1	0	0
11					0	1	0	0
12					0	0	1	0
15					0	1	0	0
16					0	1	0	0
17					0	0	1	0
18					0	0	1	0
19					0	1	0	0
21	0	0	6[f]	3[g]	2	13	0	2
Total	0	1	6	5	5	23	4	3

[a] Only colonies that yielded plasmids with mutations in *thyA* or *sacB* are listed (deletions with endpoints beyond *sacB* are excluded). Data are from Table 1 and Fig. 2 to 4. Pt, point mutation; Ins, IS element insertion; Inv, inversion; Del, deletion.

[b] TMP^r Suc^s colonies.

[c] TMP^s Suc^r colonies.

[d] Most colonies were TMP^s Suc^r, but one (from colony 1) was TMP^r Suc^r and 1 kb larger than pIF275. The plasmid in this colony probably had a point mutation in one gene and an IS element insertion in the other.

[e] TMP^r Suc^r colonies.

[f] Some colonies may be siblings, but mapping data indicate at least two independent inversion plasmids (see Results and Fig. 2).

[g] Plasmid size indicates that at least two independent deletions occurred.

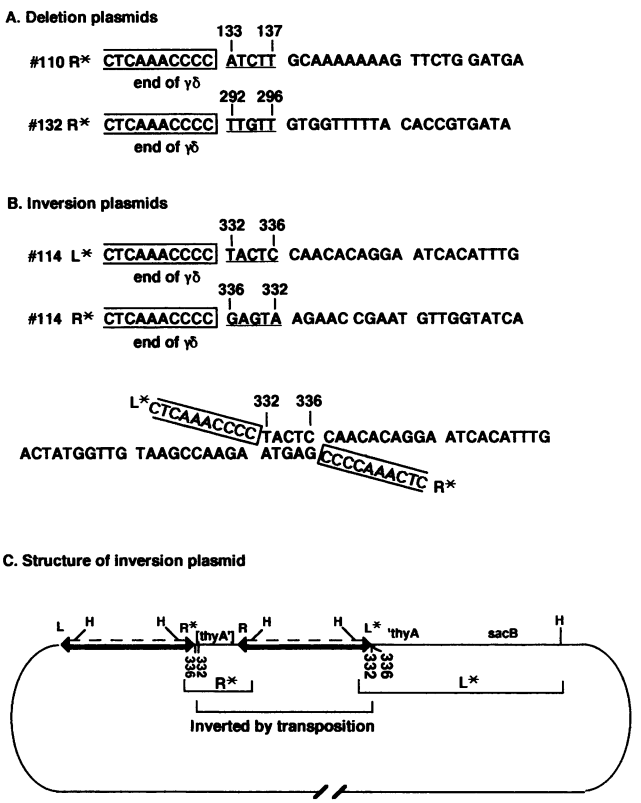


FIG. 4. (A and B) Sequences at intramolecular transposition target sites in the *thyA* gene of pIF275. The *thyA* numbering scheme (18) was used. Symbols: boxed letters, ends of mini-γδ; underlined sequences, five-base target site duplication (A and B) and presumed duplications (only one copy recovered) (A); L and R, left and right ends of mini-γδ, respectively; L* and R*, left and right ends of mini-γδ, respectively, at a new site; thyA', *thyA* gene with clockwise truncation; 'thyA, *thyA* gene with counterclockwise truncation. (A) Sequences at the new γδ-*thyA* juncture in two deletion plasmids; (B) sequences at the new γδ-*thyA* junctures of inversion plasmid 114 (lower panel, target sequences aligned to show the 5-bp target site duplication); (C) reconstruction of plasmid 114 (see Fig. 1, lower right) showing the inverted 0.44-kb R*-containing and the noninverted 2.8-kb L*-containing *Hin*dIII fragments.

7.1 kb) (Fig. 3). One plasmid had a point mutation and two had IS element insertions in *strA*. The deletion plasmids ranged in size from 3.8 to 10 kb (Fig. 3). The 37 independent deletion plasmids had at least 18 different deletion endpoints. No inversions were found, and no colonies were Amp^s, probably because of the small number analyzed.

Sequence characterization of deletion and inversion plasmids. The transposition insertion sites in *thyA* were determined for two pIF275 deletion derivatives (Fig. 1 [left], 2B, 2D, and 4A) and one inversion derivative (Fig. 1 [right], 2D, 4B, and 4C). The insertion site in the inversion plasmid could not be sequenced directly because the mini-γδ component was duplicated and could not provide a unique primer binding site. Therefore, plasmid 114 was digested with *Hin*dIII, and fragments were subcloned into the *Hin*dIII site of pSPORT. Clones containing the 0.44- and 2.8-kb inserts were sequenced by using vector-specific primers. As predicted (Fig. 1), the DNA sequence between the transposon copies was inverted and the five-base target site was duplicated (Fig. 4B and C). In contrast to the situation found after intermolecular transposition and resolution, each γδ copy has one end at a new site and one end at the old site (with the 5-bp duplication adjacent to one end of each γδ copy).

DISCUSSION

Intramolecular transposition of the engineered γδ element used here was found to be efficient. It occurred at frequencies of 10^{-5} to 10^{-4}. Over 90% of the mutants selected for resistance to a single contraselectable agent had undergone a γδ-catalyzed inversion or deletion (Tables 1 and 2; Fig. 2 to 4). Deletions far outnumbered inversions because of the relative sizes of the targets. In pIF275, for example, selection for TMP^r colonies yields both inversions and deletions if transposition was into a site in *thyA* (Fig. 1) but only deletions if transposition was into a site beyond *thyA*. Therefore, estimates of the relative frequencies of inversions and deletions were obtained by focusing on transposition events that occurred into the contraselected gene. In pIF275, six insertions in *thyA* were inversions and five were deletions, while four insertions in *sacB* were inversions and three were deletions (Table 2). In pIF279, both insertions in *strA* were deletions (Fig. 3). Therefore, of a total of 20 mutants that had an insertion in one of the contraselectable genes, 10 had deletions and 10 had inversions, in accord with the model for intramolecular transposition depicted in Fig. 1 (21). In contrast to the equal frequency of deletions and inversions generated by intramolecular transposition of γδ in this study, an unrelated element, Tn*5*, generates an excess of deletions that can be attributed to the recovery of aberrant, partially degraded transposition products (23).

The transposition-generated deletion endpoints were generally quite randomly distributed (Fig. 2 and 3). An apparent clustering of deletion endpoints when a number of isolates from one original single colony were screened was due to deletions that arose early during growth of the colony (the transposase gene is partially constitutive in the strain used here; data not shown). There was, however, one bona fide hot region in pIF279 in which 13 of the 37 independent insertions analyzed were in two adjacent clusters (Fig. 3). These insertions were in the vicinity of one of the Tn*3* end sequences (which is similar to γδ's end sequences) in the parental plasmid. However, this association may be coincidental because excess insertions were not found in either of the other *ori-amp* regions of this plasmid (Fig. 3) or in either of the *ori-amp* regions of pIF275 (Fig. 2). The usually quasi-random distribution of deletion endpoints suggested that γδ would be useful for isolating nested deletions for mapping and sequencing of cloned DNAs. This has been demonstrated by using a γδ-based vector designed for isolating deletions that extend in either direction into cloned DNA (5, 25).

Deletions as large as 9.4 kb were isolated in this study (larger deletions remove the replication origin and are inviable) (Fig. 2 and 3). Deletions as large as 210 kb were isolated in an F' factor plasmid in another study (10), suggesting that there is no upper limit to the distance over which γδ will act in intramolecular transposition.

The replicative transposition model predicts that the two new transposon-target junctions formed during intramolecular replicative transposition will be associated with different copies of the transposon (Fig. 1). This was confirmed for an inversion derivative (Fig. 4) but could not be tested for deletion derivatives in this study since only one of the reciprocal products can be recovered (the other one lacks a replication origin). However, the expected reciprocal products were recovered in a minority of the deletion derivatives in another study of an

unrelated transposon, Tn*903*, and the target sites in the reciprocal deletion plasmids were duplicated (27).

E. coli strains that contain thymidylate synthetase (encoded by the *thyA* gene) are sensitive to folic acid inhibitors such as aminopterin and TMP, while *thyA* auxotrophs are not (22). This property has been used to isolate *thyA* auxotrophs in a number of organisms (16). We demonstrate here that the cloned *thyA*$^+$ gene (sometimes called TD) is a useful contraselectable marker (Table 1; Fig. 2).

Although more than 90% of γδ or Tn*3* intermolecular transposition events are replicative, γδ and other members of the Tn*3* family yield simple conservative (apparent break-join) products a few percent of the time when intermolecular transposition is assayed under the appropriate conditions (1, 26, 29). Conservative transposition could not be detected in the plasmids studied here because it would not yield viable products. However, possible conservative γδ intramolecular transposition was found with use of different plasmids (3).

Intramolecular γδ transposition, unlike intermolecular transposition, is a one-step process (Fig. 1), requiring transposase but not resolvase since there is no resolution step. The results presented here indicate that γδ intramolecular transposition generally occurs by the same replicative process as intermolecular transposition, during which the transposon and the characteristically A+T-rich 5-bp target site are duplicated. The data suggest that deletions and inversions occur with similar frequencies. The only critical component of the replicative transposition model that could not be tested in this system is the reciprocity of the deletion products, since one of the two expected products lacked a plasmid origin and was inviable.

ACKNOWLEDGMENTS

We thank Catherine Green for technical assistance and Barbara Bachmann, Fred Bloom, Richard Deonier, Nigel Grindley, Pierre Prentki, Daniel Santi, and Lawrence Wiater for strains and plasmids and for critical discussions. We also thank Paul Betts of the University of Connecticut Biotechnology Center for synthesizing the oligonucleotides used and Perkin-Elmer Instruments for the loan of a model 480 Thermal Cycler.

This work was supported by grants from the Department of Energy (DE-FG02-89ER-60862 and DE-FG02-90ER-610), Life Technologies Inc., the National Institutes of Health (HG00563), the National Science Foundation (DMB8802310), and the University of Connecticut Research Foundation.

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