Intermolecular Transposition of IS10 Causes Coupled Homologous Recombination at the Transposition Site

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ABSTRACT

Interplasmid and chromosome to plasmid transposition of IS10 were studied by assaying inactivation of the phage 434 *cl* gene, carried on a low copy number plasmid. This was detected by the activity of the *tet* gene expressed from the phage 434 P_R promoter. Each interplasmid transposition resulted in the fusion of the donor and acceptor plasmids into cointegrate structure, with a 9-bp duplication of the target DNA at the insertion site. Cointegrate formation was abolished in $\Delta recA$ strains, although simple insertions of IS10 were observed. This suggests a two-stage mechanism involving IS10 conservative transposition, followed by homologous recombination between the donor and the acceptor. Two plasmids carrying inactive IS10 sequences were fused to cointegrates at a 100-fold lower frequency, suggesting that homologous recombination is coupled to and stimulated by the transposition event. Each IS10 transposition from the chromosome to the acceptor plasmid involved replicon fusion, providing a mechanism for IS10-mediated integration of extrachromosomal elements into the chromosome. This was accompanied by the formation of an additional copy of IS10 in the chromosome. Thus, like replicative transposition, conservative transposition of IS10 is accompanied by cointegrate formation and results in duplication of the IS10.

THE ends of the bacterial transposon Tn10 are inverted repeats of the insertion sequence IS10. IS10-right is a fully functional insertion sequence that can transpose as an individual unit. It encodes a single protein, the transposase, which acts to promote Tn10 and IS10 transposition and other types of rearrangement (FOSTER *et al.* 1981; HALLING *et al.* 1982).

Tn10 and IS10 transpose by a nonreplicative "cut and paste" mechanism, in which the transposable element is excised from the donor by double-strand breaks and is then inserted between a pair of staggered breaks in the target. Only limited repair synthesis at the new transposon/target junctions is involved in this process (KLECKNER *et al.* 1984). This model is based on the observation that the genetic information on both strands of the transposon's DNA is recovered after transposition (BENDER and KLECKNER 1986). Further support for Tn10 conservative transposition is provided by the identification of Tn10 transposon circles in which the transposon has been excised from the donor molecule and then circularized as a consequence of intramolecular transposition (BENJAMIN and KLECKNER 1989). In addition, certain IS10 transposase mutants promote a product that consists of the transposon excised from the donor molecule as a linear fragment (HANIFORD *et al.* 1989). Recently, two protein-DNA complexes that are reaction intermediates in the Tn10

cut and paste transposition process were described: a cleaved donor complex, whose DNA component consists of transposon sequences cleanly excised from flanking donor DNA, and a strand transfer complex, whose DNA component contains transposon termini specifically joined to a target site (HANIFORD *et al.* 1991). The kinetics of formation of the first species suggest that it is an early intermediate in the transposition reaction. These two Tn10 complexes are closely analogous to complexes formed during replicative cointegrate formation by bacteriophage Mu (CRAIGIE and MIZUUCHI 1987; SURETTE *et al.* 1987), and thus they represent intermediates that may be common to both nonreplicative and replicative transpositions.

The fate of the donor molecule that undergoes IS10 conservative transposition is not clear. The failure to find IS10 transposition-related excision products ruled out efficient rejoining of the donor chromosome during transposition (BENDER *et al.* 1991). In addition, the finding that Tn10 transposition or a closely related event induces the SOS stress response is consistent with the notion that Tn10 transposition leaves behind a donor molecule gap (ROBERTS and KLECKNER 1988). Three possible fates for such molecules were suggested (BENDER *et al.* 1991): (1) the donor molecule may be lost. Because bacteria frequently contain more than a single copy of their chromosome, such a loss could be tolerated. (2) Host function may completely repair the gap using the homologous region of another chromosome in the cell as a template (double-strand gap repair). (3) Host function may reseal the donor molecule

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TABLE 1
Bacterial strains

Strain	Genotype	Source
AB1157	<i>argE3 his4 leuB6 proA2 thr1 ara14 galK2 lacY1 mtl1xyl5 thi1 tsx33 rpsL31 supE44</i>	Lab stocks
AB2463	AB1157 <i>recA13</i>	Lab stocks
ABE10	AB1157 $\Delta(\text{recA-srlR})$ 306::Tn10	Lab stocks
ABN101	AB1157 $\Delta\text{recA Tet}^S$	This study
ABN102	AB1157 $\Delta\text{recA Tet}^S$	This study
DM49	AB1157 <i>LexA3</i>	Lab stocks
MC4100	$\Delta(\text{argF-lac})$ 205 <i>araD139 rpsL150 thiA1 relA1 ffb5301 deoC1 ptsF25 rbsR</i>	C. GROSS
MG1655.10	W1485 <i>recA56</i> $\Delta(\text{lacIPOZYA})$	M. CASADABAN
NR3883	$\Delta(\text{lac pro})$ <i>ara thi trpE9777 recA56 F' pro-lac</i>	
RK4936	<i>araD139 (argF-lac)</i> 205 <i>ffbB5301 non-9gyrA219 relA1 rpsL 150 metE70 btu:: Tn10</i>	B. AMES
RKZ2	RK4936 <i>Tet</i> ^S	This study
RKZ3	RK4936 <i>Tet</i> ^S	This study
TK701	<i>proA2 his4 thi1 lacY1</i>	T. KATO
TK702	TK701 <i>umuC36</i>	T. KATO
WB535	$\Delta(\text{lac pro})$ <i>thi ara met</i> $\Delta(\text{recA-srlR})$ 306: Tn10 (λ 200 <i>imm21 ind</i>)F' <i>lacI^qZ⁻Y⁺pro⁺</i>	Our lab

after extensive degradation of sequences on both sides of the original transposable element. Such process would generate a "double-ended" deletion; however, despite the searches for such deletions, they have not been detected after transposition of either Tn10 or IS10.

In the present study we show that intermolecular transposition of IS10 greatly stimulates and is coupled to homologous recombination between the donor and the acceptor molecules at the transposition site. This process resulted in the formation of a fused replicon structure, indistinguishable from the cointegrate structure that is formed by replicative transposition.

MATERIALS AND METHODS

Sources of materials: *Escherichia coli* DNA polymerase I (Klenow fragment), T4 DNA polymerase, T4 DNA ligase, and bacterial alkaline phosphatase were purchased from US Biochemical Corporation. Restriction endonucleases were bought from New England Biolabs and from Pharmacia P.L. Biochemicals. Multiprime labeling kit, nylon membranes (Hybond-N), (γ -³²P)ATP, and (α -³²P)dCTP at 3000 Ci/mmol were the products of the Radiochemical Center, Amersham. Nitrocellulose and DEAE membranes were from Schleicher & Schull, antibiotics were from Sigma, and bacterial media were from Difco.

Media, bacterial strains, and growth condition: The medium used in this study was LB. Kanamycin (70 mg/l), ampicillin (100 mg/l), tetracycline (5 mg/l), and chloramphenicol (30 mg/l) were supplemented as required. Dilution of bacteria was done in buffer PS (10 mM NaH₂PO₄ pH 7.4 and 150 mM NaCl).

The strains used in this study are listed in Table 1. ABE10 is a ΔrecA derivative of AB1157. ΔrecA was introduced by transducing AB1157 to tetracycline resistance (*Tet*^R) with P1 grown on P1.AC1335 (NUSSBAUM and COHEN 1988). ABN101 and ABN102 are derivatives of ABE10 that lost their Tn10. These strains were obtained by selecting ABE10 for the loss of its tetracycline resistance as described (MALOV and NUNN 1981). Only colonies that showed no reversion (frequency < 5 × 10⁻⁹) were chosen for further analysis. The loss of Tn10

was verified by Southern blot analysis of chromosomal DNA using an IS10-specific probe. Strains RKZ2 and RKZ3 are *Tet*^S derivatives of RK4936 obtained by the same procedure. However, they do contain IS10 sequences in their genome, as revealed by Southern blot analysis (see RESULTS and Table 5 for details).

Enzymatic reactions: Cloning procedures were generally carried out as described (SAMBROOK *et al.* 1989). DNA sequence analysis was carried out by the dideoxynucleotide chain-termination method (SANGER *et al.* 1977) using a mutant T7 DNA polymerase (sequenase version 2.0) DNA sequencing kit from US Biochemicals.

Preparation, fractionation, and hybridization of DNA: Large-scale plasmid preparation was done as described (DAVIS *et al.* 1980). For rapid preparations of plasmid DNA from small-volume cultures, the boiling method was used (HOLMES and QUIGLEY 1981). Purification of chromosomal DNA was done on a CsCl gradient (WEEKS *et al.* 1986) or by a rapid procedure (KEMPTER and GROSSBADERN, 1992).

DNA samples were fractionated on agarose gel by electrophoresis, after which the DNA was transferred bidirectionally (dry transfer) onto Hybond-N nylon membranes and hybridized (SMITH and SUMMERS 1980).

Construction of plasmids: Plasmid pZP48 carries the *cat* gene, conferring resistance to chloramphenicol, the *cl* repressor gene of the lambdaoid phage 434 (*cl*434), and the tetracycline resistance gene (*tet*) fused to the O_RP_R control region of the phage 434. It was constructed by ligating the 2.4-kb *Ava*I-*Aad*I fragment of pNS1 (Nikolnikov *et al.* 1984) that contained the *cl*(434) and the *tet* genes to a 2.55-kb *Hinc*II-*Hind*III fragment of pACYC184 (CHANG and COHEN 1978) that carried the origin of replication and the *cat* gene.

Plasmid pZF42 carries the *cat* and *cl*(434) genes, and the *tet* gene fused to the O_RP_R operator-promoter of phage 434. It was constructed by partial digestion of pZP48 with *Pvu*II followed by full digestion with *Xba*I. The 4.04-kb fragment, which carried the *cat*, *cl*(434), and *tet* genes, was ligated to the 6.75-kb *Eco*RI fragment of pMF21 (KLINE and TRAWICK 1983), which contained the origin of replication of the F episome (Figure 1).

Plasmid pZFM is a pZF42-derivative that has a deletion of 150 bp in its *cl*(434) gene, thus conferring a *Tet*^R phenotype on the cells harboring it. The deletion was introduced at the unique *Hind*III site by cleavage with the restriction nuclease

TABLE 2
Subcloning of IS10/*cl(434)* junctions from cointegrates 1 and 2

Subclone name	Parental cointegrate	Subcloned fragment	IS10/ <i>cl(434)</i> junction	Sequencing primer
pCO1L	1	<i>XhoI</i> - <i>Bam</i> HI (6652 bp)	Left	pL(IS10-in)
pCO1R	1	<i>Bam</i> HI- <i>Pst</i> I (6015 bp)	Right	pR(IS10-out)
pCO2L	2	<i>XhoI</i> - <i>Bam</i> HI (7072 bp)	Left	pL(IS10-in)
pCO2R	2	<i>Bam</i> HI- <i>Pst</i> I (5595 bp)	Right	pR(IS10-out)

followed by digestion with *Ba*31 nuclease, filling in, and self-ligation.

Plasmid pMVIS10 is a mutant of plasmid pMV2 (SKALITER *et al.* 1992) with an IS10 insert in the λ *cro* gene (Figure 2). In addition, it carries the *amp* and *kan* genes, conferring resistance to ampicillin and kanamycin, respectively, and the origin of replication of plasmid pBR322. Plasmid pMV05 is a derivative of plasmid pMV2 carrying a mutant *cro* gene (EICHENBAUM 1987).

Plasmids pMV Δ Tnp and pMV Δ IR are pMVIS10-derivatives that have deletions in the IS10 sequence. Plasmid pMV Δ Tnp has a 291-bp deletion in the transposase gene generated by digestion with *Nco*I, isolating the 5.63-kb fragment, and self-ligating it. Plasmid pMV Δ IR has a IS10 sequence in which the flanking inverted repeats were deleted. It was constructed by cloning the 0.94-kb *StuI*-*NdeI* IS10 fragment from pNK290 (SIMONS and KLECKNER 1983) into the *Bgl*II site of plasmid pMV2. Plasmids pZFIS10 Δ and pZF Δ IR are derivatives of pZF42 that contain the same inactivated IS10 elements in the *cl(434)* gene. Plasmid pZF Δ Tnp was constructed by cloning the 1.19-kb *Bgl*II-*Cla*I fragment from pMV Δ Tnp into the *Hin*dIII site of pZF42. Plasmid pZF Δ IR was constructed by cloning the 0.94-kb *StuI*-*NdeI* fragment from pNK290 (SIMONS and KLECKNER 1983) into the *Hin*dIII site of pZF42.

Plasmids pCO1L and pCO1R are subclones of cointegrate 1, and plasmids pCO2L and pCO2R are subclones of cointegrate 2. Both cointegrates are natural isolates that were obtained during this study (see RESULTS for details). Each of the subclones is carrying the pBR322 origin of replication and one of the two IS10/*cl(434)* junctions of the cointegrate. Subcloning was performed by self-ligation of the appropriate fragments as described in Table 2. Cointegrate 1 is presented in Figure 4. Cointegrate 2 has the same structure; however, insertion of IS10 into *cl(434)* occurred at a different position.

DNA probes and oligonucleotide primers: The IS1-specific probe was the 0.64-kb fragment of plasmid pBRG36 (BIEL *et al.* 1984), obtained upon digestion with *Pvu*II, *Tth*111I, and *Eco*RI. As IS10-specific DNA we used the 0.94-kb *StuI*-*NdeI* fragment obtained from plasmid pNK290 (SIMONS and KLECKNER 1983). As a DNA specific for the plasmids pMV2 (the donor plasmid without IS10 sequence) and pZF42, we used the plasmids after digestion with *Pst*I, *Xho*I, and *Bgl*II (pMV2) or with *Bgl*II (pZF42).

The synthetic oligonucleotides primers for determining the IS10/*cl(434)* junctions were pR(out), 5'-GTATCCACCTTAACCT-3', complementary to the "outside end" of IS10R, and pL(in), 5'-ACTCGTGGCTGCAACCC-3', complementary to the "inside end" of IS10R. They were synthesized by the Chemical Services Unit of the Weizmann Institute by phosphoramidate coupling.

Transposition assay: The interplasmid transposition assay was performed with cells harboring both the donor and the

acceptor plasmids (pMVIS10 and pZF42, respectively). Cultures were grown at 37°C on LB supplemented with chloramphenicol and kanamycin to O.D.₅₉₅ 0.5. The cells were washed, resuspended in 1:5 vol of buffer PS, and spread on LB plates containing chloramphenicol and kanamycin for determination of the total number of cells and on LB plates containing chloramphenicol, kanamycin, and tetracycline for determination of Tet^R mutant colonies. Tet^R mutants were analyzed by plasmid DNA preparation, fractionation on agarose gel, and hybridization with radiolabeled probes.

Intermolecular IS10 transposition from the bacterial chromosome was performed with Tet^S cells harboring IS10 elements in their chromosome. The assay was performed essentially the same as for intermolecular transposition; however, the cells contained only plasmid pZF42. Tet^R mutants were analyzed by Southern hybridization of plasmid and chromosomal DNA preparations.

The selection for Tet^R mutants was performed at cell densities of 10⁷ to 3 × 10⁸ cells per plate. No influence of the cell densities on the production of Tet^R mutants was observed except for *recA* strains. These strains needed to be spread at densities below 2 × 10⁸ cells/plate, because massive death of Tet^R mutants occurred at higher densities, leading to an underestimation of mutation frequency. Because tetracycline resistance is achieved by events in the cell membrane that result in a decreased intracellular accumulation of the drug (McMURRY *et al.* 1980), we presume that the Tet^R mutants might be more permeable to DNA damaging metabolites due to changes in their membrane. The *recA* strains may be more sensitive to such agents because of their reduced DNA repair ability.

RESULTS

The *cl(434)* transposition assay system: This system is based on a low copy miniF-derived plasmid carrying the *cl* repressor gene of phage 434 as a reporter gene. Its inactivation is detected via the activity of the tetracycline resistance gene (*tet*) expressed from the phage 434 P_R promoter present on the plasmid (Figure 1). Cells harboring the target plasmid, termed pZF42, are sensitive to tetracycline because the *cl(434)* gene product represses the expression of *tet* from P_R. Mutations in *cl(434)* that have altered the repressor so that its binding to O_R is reduced enable expression of *tet* and lead to the formation of Tet^R colonies.

To test whether the expression of the *tet* gene in this plasmid is under the control of the *cl(434)* repressor, we constructed a control plasmid, termed pZFM, which

FIGURE 1.—Plasmid pZF42. Plasmid pZF42 carries the *cI(434)* repressor, the *cat* gene, and the *tet* gene fused to the O_{RPR} operator-promoter of phage 434. In addition, pZF42 carries a segment of the mini-F plasmid pMF21, containing the origin of replication of the F episome. The segment contains the origin of replication *OriS*, the *repE* gene, which codes for the initiator protein, the *ccd* operon, which encodes proteins required for the coupling between F plasmid replication and cell division, and the *sop* operon, which carries the complete genetic information required for correct partitioning of plasmid DNA.

carried an inactivated repressor gene. Cells harboring pZFM were Tet^R as expected and were able to grow on tetracycline concentrations of 5–7 mg/l. The generation time of cells harboring the mutated pZF42 was 31 ± 1 min, somewhat slower than the growth rate of cells harboring the parental plasmid pZF42 (28 ± 3). This means that cells harboring a mutant pZF42 plasmid have a slight growth disadvantage over cells carrying the wild-type plasmid.

The newly developed system was used to assay spontaneous mutagenesis in *E. coli*. Tet^R mutants from exponentially growing cells harboring pZF42 were selected and analyzed. In three different *E. coli* strains, AB1157, TK701, and MC4100, the spontaneous mutation frequency was similar, $(4 \pm 0.1) \times 10^{-7}$, $(10 \pm 1) \times 10^{-7}$, and $(5 \pm 0.2) \times 10^{-7}$, respectively. Tetracycline resistance was conferred on the cells because of a mutation in plasmid pZF42, because secondary transformations using plasmids extracted from Tet^R colonies yielded only Tet^R colonies. Analysis of mutant pZF42 plasmids extracted from cultures of AB1157 (pZF42) revealed they were maintained at a low copy number similar to the parental pZF42. Restriction endonucleases analysis of 24 independent mutants revealed three insertions and 21 “point mutations.” These are mutations such as frameshifts or base substitutions that did not alter significantly the plasmid migration on agarose gels. Thus, in our system ~10% of all spontaneous mutations appeared to be chromosome to plasmid transposition events.

IS10 interplasmid transposition results in cointegrate formation: Interplasmid transposition of IS10 was assayed in cells harboring two plasmids: a donor plasmid, pMVIS10 (Figure 2), which carried a single copy of

FIGURE 2.—Plasmid pMVIS10. Plasmid pMV2 carries an insertion of IS10R in the phage λ *cro* gene. In addition, it carries the genes conferring resistance to ampicillin and kanamycin, the pBR322 origin of replication and the intergenic region (IG) of phage M13 that carries its origin of replication.

IS10R, and the acceptor plasmid, pZF42, with the *cI(434)* gene as the target for transposition. Because the host cells do not carry IS10 element in their chromosome, its sole source is the donor plasmid.

The plasmids contents of 51 Tet^R mutants from several *E. coli* strains were analyzed by restriction endonucleases digestion and Southern blot hybridization with IS-specific probes. A significant portion of 25–60% of the mutants carried IS10 insertion (Table 3), and those were analyzed in detail. As can be seen (Figure 3, lanes 10, 12, and 15), whenever pZF42 plasmid had gained an insert of IS10, a very slow migrating plasmid, present at a high copy number, was formed. Plasmid pZF42 from mutants that did not receive an IS10 sequence was maintained at a low copy number, similar to wild-type pZF42 (e.g., lanes 4, 5, and 11 in Figure 3). When the slow migrating plasmids were isolated and reintroduced into a new host, they always conferred resistance to both donor and acceptor markers (Kan^R, Cap^R, and Tet^R). Upon digestion with restriction nucleases *Bam*HI and *Xho*I, each of these structures produced two bands

TABLE 3

Restriction nuclease digestion and hybridization analysis of spontaneously occurring Tet^R mutants in the *cI(434)* biplasmidic system

Strain	Mutation frequency	Mutants analyzed	Point mutations	Insertions	
				IS10	Other IS
TK701	3.0×10^{-7}	12	2	3	7
TK702	2.0×10^{-7}	12	2	7	3
AB1157	1.0×10^{-7}	14	7	4	3
KM159	4.0×10^{-7}	13	5	3	5

FIGURE 3.—Southern blot analysis of plasmid DNA from Tet^R mutants of TK702 (pZF42; pMVIS10) cells. Plasmid DNA was prepared and the undigested forms (lanes 10, 12, and 15) or the *Bam*HI and *Xho*I digest of the plasmids (lanes 1–9, 11, 13, 14, 16, and 17) were fractionated by agarose gel electrophoresis. DNA was then bidirectionally transferred onto nylon membranes and probed in parallel with a pZF42-specific probe (A) or with an IS10-specific probe (B). U, uncut plasmid; C, *Bam*HI- and *Xho*I-cut plasmid. The arrow in the figure points to the uncleaved form of cointegrates.

(6.65 and 3.62 kb) that cohybridized with both pZF42 and IS10 probes (e.g., lanes 1–3, 6–9, 13, 14, 16, and 17 in Figure 3). Because neither *Bam*HI nor *Xho*I cleave IS10, this indicates the presence of two copies of IS10 on each of the slow migrating plasmids. These data suggest that the IS10 transposition had promoted the fusion of pMVIS10 and pZF42 into a structure that contains a duplication of IS10 at each donor-target junction (Figure 4).

Determination of the DNA sequence at the sites of insertion of IS10 into *cl(434)* gene in two independent fused plasmids confirmed the presence of IS10 elements (Figure 5). In addition, the DNA sequence contained duplications of 9 bp of the target site (Figure 5), which is typical of IS10 transposition (KLECKNER 1989). In one case, transposition of IS10 had occurred after bp 186 of *cl(434)* and in the other at position 567

FIGURE 4.—A Cointegrate structure resulting from fusion of plasmids pMVIS10 and pZF42. See text for details.

FIGURE 5.—The DNA sequence of *IS10/cI(434)* junctions in two cointegrates. (a) The left junction in cointegrate 1. (b) The right junction in cointegrate 1. (c) The left junction in cointegrate 2. (d) The right junction in cointegrate 2. The duplicated insertion sites are underlined.

[the sequence of *cI(434)* is given in NIKOLNIKOV *et al.* (1984)]. Both positions are consistent with the insertion sites that were deduced at a lower resolution by restriction analysis. The fused replicon structures in the *cI(434)* system are indistinguishable from the cointegrate structures that are formed by replicative transposition (IIDE *et al.* 1983; GRINDLEY and REED 1985; DERBYSHIRE and GRINDLEY 1986). The fusion of the plasmids was specific for *IS10* insertion mutants, because pZF42, which carried mutated *cI(434)* genes with either point mutations or insertion of other IS elements (originating from the bacterial chromosome), was not fused to pMVIS10 (*e.g.*, lanes 1–3, 5–6, and 8–20 in Figure 6). In addition, no fusion was observed in Tet^R mutants of cells harboring plasmids pZF42 and a control plasmid pMV05, which does not contain any IS element (data not shown).

Each pZF42-pMVIS10 fused molecule carried two origins of replication: the pBR322 origin from plasmid pMVIS10 and the *OriS* of plasmid pZF42. The dominant origin is likely to be that of pBR322, as indicated by the high copy number of the cointegrates (*e.g.*, lanes 4 and 7 in Figure 6). In many cases the cointegrates were observed along with pMVIS10 or its dimer. These resulted from the resolution of the cointegrate structure, which yielded plasmid pZF42-*IS10* as well as pMVIS10. The absence of pZF42-*IS10* is probably the consequence of its loss by segregation facilitated by its low copy number.

Intrigued by the high frequency and stability of cointegrates in our system, we examined their maintenance under different selection conditions. When cointegrates were isolated and introduced into a RecA⁺ host, they were resolved into pZF42-*IS10* and pMVIS10. This was observed after an overnight incubation, regardless of the presence of tetracycline selection. After five passages in the absence of tetracycline, the cointegrates were completely resolved. When tetracycline was present, a considerable fraction of colonies still contained cointegrates even after five passages. This suggests that in the presence of tetracycline, cells harboring the high

FIGURE 6.—Plasmid pZF42 from Tet^R mutants of a *lexA3* strain. Plasmid DNAs from Tet^R mutants of DM49(pZF42; pMVIS10) cells were introduced into competent MG1655.10- (*recA56*) cells. Transformants were selected on LB plates containing chloramphenicol and tetracycline and then were checked for their resistance to kanamycin. Plasmid DNAs, each from an independent transformation test tube, were prepared and fractionated by agarose gel electrophoresis. Lanes 1–3, 5, 6, and 8–20 carry plasmid DNA from Kan^S Tet^RCap^R transformants, and lanes 4 and 7 carry plasmid DNAs from Kan^RTet^RCap^R transformants. Cointegrate B (lane 3) is the fusion product of plasmids pZF42 and pMVIS10; the free pMVIS10 that is observed in lane 3 is the resolution product of this DNA structure. Cointegrate A (lane 7) is the fusion product of plasmids pZF42 and the dimer of pMVIS10. Also seen in this lane is a dimer of pMVIS10, which migrates similar to pZF42. It is the resolution product of the cointegrate structure.

copy number cointegrates have an advantage over cells harboring the low copy *tet*-plasmid that is formed upon resolution of the cointegrates, most probably because of stronger expression of *tet*. In *recA* strains cointegrates were usually not resolved. Occasionally, they yielded plasmids having sizes different than the size of pZF42-*IS10* and pMVIS10. This suggests that the normal resolution requires RecA protein and that it occurred via homologous recombination between the two *IS10* sequences. The “abnormal” resolution of cointegrates in a *recA* background may occur by intraplasmid transposition (BENJAMIN and KLECKNER 1989).

Interplasmid transposition of *IS10* is *recA*-independent and enhances homologous recombination at the transposition site: Because there is strong evidence suggesting that *IS10* transposes by a cut and paste mechanism (BENDER and KLECKNER 1986; KLECKNER 1989), it

FIGURE 7.—Southern blot analysis of pZF42 plasmids from Tet^R mutants of a *recA56* strain. The plasmid content from Tet^R mutants of MG1655.10(pZF42; pMVIS10) cells was extracted and pZF42 was separated from pMVIS10 by a secondary transformation. Plasmid DNA extracted from independent transformation test tubes were fractionated by agarose gel electrophoresis, bidirectionally transferred onto nylon membranes, and probed in parallel with a pZF42-specific probe (A) or with an IS10-specific probe (B). Lanes 1 and 3–20 carry plasmid DNAs from Kan^RTet^RCap^R transformants, and lane 2 carries plasmid DNA from a Kan^RTet^RCap^R transformant.

is likely that cointegrates were produced in our system by a mechanism other than replicative transposition. In an effort to explore further the mechanisms of cointegrate formation, we analyzed spontaneous Tet^R mutants of *recA56* and Δ *recA* cells harboring plasmids pZF42 and pMVIS10. The analysis of 90 Tet^R mutants from two *recA56* strains revealed that most of the IS10 transpositions yielded simple IS10 insertion (e.g., lanes 3, 5, 8, 9, 13, and 14 in Figure 7 and Table 4), although some cointegrates were observed too (e.g., lane 2 in Figure 7 and Table 4). The overall frequency of IS10 insertion mutants was similar to that of wild-type cells (Table 4).

The *recA* dependence of cointegrate formation was further confirmed in experiments performed with the Δ *recA* strains ABN101, ABN102, and WB535. Analysis of 85 independent mutants revealed that all of the 13

IS10 insertions into pZF42 were simple, and no cointegrate formation was observed (Table 4). Thus, RecA protein is needed for the IS10-mediated cointegrate formation in our biplasmidic system. The fact that some cointegrates were observed in the *recA56* background suggests that the *recA56* point mutation is somewhat leaky.

The formation of cointegrates could have resulted from transposition of a dimeric donor molecule. Although most of the donor plasmid molecules are monomeric, each preparation contains also a small fraction of dimers that might have been responsible for cointegrate formation. The *recA*-dependence could have then been explained by a reduced fraction of plasmid dimers in *recA* strains. To examine this possibility, we assayed plasmid to plasmid transposition starting with a dimer of the donor plasmid pMVIS10 (dimeric plasmids are obtained occasionally during regular plasmid preparations). The structure of Tet^R mutants obtained in cells harboring plasmid pZF42 and a dimer of pMVIS10 are shown in Figure 8. As can be seen, IS10 transposition in this case led to the production of cointegrates larger than with monomeric pMVIS10. Resolution products and restriction endonuclease analysis revealed that these DNA structures were cointegrates of the pMVIS10 dimer and pZF42 (Figure 8). Thus, the cointegrates formed from monomeric pMVIS10 and pZF42 were not formed by a small fraction of dimers in the monomeric pMVIS10 preparation.

The data presented above are consistent with a mechanism in which every transposition of IS10 into *cl*(434) was accompanied by a *recA*-dependent homologous recombination event involving two IS10 elements (Figure 9). The question arises as to whether this remarkably high recombination frequency is a result of a coupling between the transposition and recombination events or does interplasmid recombination occur with high frequency between two IS10 sequences regardless of transposition? To answer this question, we examined homologous recombination between pZF42 and pMV2 plasmids carrying mutated IS10 sequences that were unable to promote transposition. In IS10 Δ Tnp we deleted 219 bp from the middle of the transposase gene that introduced a frameshift in its remaining coding region. In IS10 Δ IR we deleted the flanking inverted repeats of IS10 and 97 codons from the 3' C-terminus of the transposase gene. Each of the inactivated IS10 elements was cloned into pMV2 and pZF42 [inside *cl*(434)] to form a pair of plasmids with a homologous mutated IS10-segment. We then measured the frequency of their fusion into cointegrates, a process that can occur via homologous recombination. This was done by analyzing the plasmid DNA of transformants harboring either both pMV Δ Tnp and pZF Δ Tnp or pMV Δ IR and pZF Δ IR. In 3 of 155 cells harboring pMV Δ Tnp and pZF Δ Tnp and in 2 of 102 cells carrying pMV Δ IR and pZF Δ IR plasmids, we observed the pro-

TABLE 4
IS10 interplasmidic transposition in *recA* strains.

Strain	Allele	Mutation frequency	Mutants analyzed	Point mutations	IS10-transposition		Other insertions
					Simple insertion	Cointegrate	
MG1655.10	<i>RecA56</i>	4.0×10^{-7}	68	56	7	2	3
NR3883	<i>RecA56</i>	4.5×10^{-7}	22	17	4	1	0
ABN101	$\Delta recA$	26×10^{-7}	14	7	3	0	4
ABN102	$\Delta recA$	22×10^{-7}	14	6	6	0	2
WB535	$\Delta recA$	13.3×10^{-7}	57	43	4	0	10

duction of fused replicons. Thus, homologous recombination between inactivated IS10 sequences residing on different plasmids occurred with a frequency in the range of 10^{-2} and was not dependent on the presence of the inverted repeats of IS10.

To find whether the high occurrence of interplasmid homologous recombination after IS10 transposition requires or involves general induction of the SOS system in the cell, we examined the production of cointegrate in a *lexA3* strain, in which the SOS system cannot be induced. We analyzed 26 spontaneously occurring Tet^R mutants in DM49(*lexA3*) cells harboring pZF42 and pMVIS10 plasmids. Of nine IS10 transposition mutants, six were cointegrates and three contained simple insertions of IS10 into *cl(434)*. Thus, although the production of cointegrates was not dependent on general induction of the SOS system, it was less efficient in its absence, maybe due to a reduced level of the RecA protein in the *lexA3* strain.

IS10 transposition from the bacterial chromosome to plasmid pZF42 results in integration of pZF42 into the chromosome: If a similar mechanism operates for chromosome to plasmid transposition and if resolution is not very fast, one would expect to see integration of the plasmid into the chromosome. This possibility was addressed by studying transposition of IS10 from the bacterial chromosome to *cl(434)* residing on pZF42.

To construct Tet^S cells carrying IS10, we selected Tet^S derivatives of RK49(*butB::Tn10*) cells (MALOY and NUNN 1981). Six mutants did not show Tet^R reversion ($<5 \times 10^{-9}$), indicating deletions or rearrangements. One of the mutants, termed RKZ2, contained two IS10 sequences residing each on a smaller *EcoRI* fragment as compared with the parental strain, indicating the deletion of *Tn10* sequences during the curing procedure (Table 5). Another isolate, termed RKZ3, underwent a more complex rearrangement, which led to a net increase of one IS10 element (three copies of IS10 instead of two) (Table 5). A high proportion of such complex rearrangements among Tet^S derivatives of *Tn10* harboring strains was reported previously (RALEIGH and KLECKNER 1984).

Spontaneous Tet^R mutants from exponentially growing RKZ2 and RKZ3 cells harboring plasmid pZF42

were selected and their plasmid content was analyzed by fractionation on agarose gels (Figure 10), followed by Southern blot hybridization (Figure 11). Eighteen of 31 mutant colonies contained plasmid pZF42 in wild-type size (pZF42 with a point mutation, e.g., lanes 1–3, 5–10, 12–17 in Figure 10), and one mutant carried an insertion (Table 6). In the remaining 12 Tet^RCap^R mutant colonies, free plasmid pZF42 could not be detected by either ethidium bromide staining (e.g., lanes 4, 11, and 18 in Figure 10) or by Southern blot hybridization after short exposures (6 h) of the radiolabeled blots. Long exposures (96 h) revealed that some of these mutants have a reduced level of a pZF42 plasmid carrying an IS10 insert (e.g., lanes 4, 11, and 18 in Figure 11), whereas others showed no free pZF42-related plasmid. The chromosomal DNA bands in both cases were lit up by the pZF42-specific probe. Whenever a strong hybridization band of free pZF42 was present, there were no pZF42 sequences in the chromosomal DNA (i.e., lanes 1–3, 5–10, 12–17 in Figure 11). This suggested that pZF42 was integrated into the chromosome after the initial IS10 transposition into the *cl(434)* gene. Similar results were obtained using the other IS10-carrying strain RKZ3 (Table 6).

We performed a more detailed analysis of 13 “integrated pZF42” mutants, by digestion of chromosomal DNA with restriction endonucleases (which do not cleave IS10) and hybridization to radiolabeled probes. As expected, in three mutants that carried non-IS10 insertion, we did not observe any variation in either the size or the number of the chromosomal DNA bands that hybridize with IS10, indicating integration at site unrelated to IS10 sequence (e.g., mutant 4 in Figure 12).

In nine mutants that were suspected to have plasmid pZF42 integrated into the chromosome via an IS10-related pathway, the numbers and sizes of the DNA bands that hybridized to the IS10-specific probe were altered (e.g., mutants 1–3 in Figure 12). In all of these mutants usually one band cohybridized with both IS10- and pZF42-specific probes (bands A in mutant 3 and band B in mutant 4 in Figure 12). In addition to these strong hybridization bands, pZF42 integration was accompanied by the production of extra IS10 copies,

FIGURE 8. Cointegrates formed in cells harboring pZF42 and dimeric pMVIS10. The plasmid content from Tet^R mutants of AB1157 (pZF42; dimeric pMVIS10) cells was extracted and analyzed by agarose gel electrophoresis (A). Plasmid pZF42 was separated from pMVIS10 by a secondary transformation, and plasmid DNA was extracted from independent transformation test tubes and fractionated by agarose gel electrophoresis (B). The DNA was then bidirectionally transferred to nylon membranes, and probed in parallel with a pZF42-specific probe (C) or with a pMV2-specific probe (D). Each lane represents an independent mutant.

FIGURE 9.—The production of a cointegrate structure by a two-step mechanism of transposition followed by interplasmid homologous recombination.

some of which showed a weaker hybridization signal (e.g., arrows a and b in Figure 12). These results can be explained by the integration pZF42 into the chromosome via a two-step mechanism involving a simple insertion of *IS10* into *cl(434)* followed by homologous recombination, similar to the two-plasmid situation; however, at this point other mechanisms cannot be ruled out. The production of the additional copies of chromosomal *IS10* elements can be explained by secondary events stimulated by the *IS10* elements. The fact that the hybridization intensity of the extra *IS10*-containing bands is weaker and variable indicates that they are present in only a subpopulation of the cells that were used for the analysis, representing continuously ongoing processes. A similar phenomena of an increase in the number of *IS10*-containing DNA fragments was observed before, although the mechanism underlying this change is not clear yet (SKALITER *et al.* 1992). The low amounts of episomal pZF42-*IS10* that were observed in some of the integrated mutants resulted most probably from the resolution of the chromosome-plasmid cointegrate structures.

DISCUSSION

In several *E. coli* strains, each *IS10* transposition from plasmid pMVIS10 into the *cl(434)* gene on plasmid pZF42 resulted in the fusion of the donor and the acceptor plasmids into a cointegrate structure that existed at a high copy number. No simple insertions of *IS10* were observed, although other IS sequences that originated from the chromosome did give rise to simple insertions in the reporter plasmid. This high efficiency formation of cointegrates by *IS10* was surprising because in two other studies it was reported that *IS10* did not to produce cointegrate structure at a significant frequency (WEINERT *et al.* 1984; NAVAS *et al.* 1985).

Cointegrates are usually produced by replicative transposition, whereas *Tn10* and *IS10* are considered the classical examples of the nonreplicative cut and paste transposition. This led us to suggest that the *IS10* cointegrates in our system were produced by a two-stage mechanism: transposition by a cut and paste mecha-

TABLE 5

Hybridization patterns of genomic *IS10* element in *E. coli* RK49 and its derivatives

Strain	Restriction fragments that hybridized to an <i>IS10</i> -probe (kbp)		
	<i>Xho</i> I	<i>Bam</i> HI	<i>Eco</i> RI
RK49::Tn10 Tet ^R			7.25, 3.7
RKZ2 Tet ^S		8.2, 6.8	5.5, 2.7
RKZ3 Tet ^S	14	12.8, 9.5	5, 2.45, 1.85

Chromosomal DNA was isolated and purified on CsCl gradients as described in MATERIALS AND METHODS. The DNA was then digested with either *Xho*I, *Bam*HI, or *Eco*RI and examined by Southern blot analysis using a radiolabeled *IS10*-specific probe.

nism followed by coupled homologous recombination between the donor and acceptor molecules. This is supported by the findings that cointegrate formation was abolished by a $\Delta recA$ mutation, whereas the more leaky *recA56* allele caused a reduction in the frequency of cointegrates, and a parallel appearance of simple insertion of *IS10*.

A second possible mechanism is "plasmid-transposition" mediated by *IS10* from a "head to tail" dimer of pMVIS10. Such a mechanism was suggested for the rare formation of cointegrates mediated by *IS50* (BERG 1983; LICHENS-PARK and SYVANEN 1988). The dependence on *recA* in that case was indirect and resulted from the reduction in copy number of donor plasmid dimers in the *recA* background (JAMES *et al.* 1982; DOHERTY *et al.* 1983). Several lines of evidence and reasoning argue that the *IS10*-promoted cointegrate structures were formed by the two-step mechanism of insertion followed by homologous recombination, rather than plasmid-transposition. (1) *IS10R* transposition was reported to be ≥ 10 times more frequent than any other events promoted by *Tn10* or *IS10R* (SHEN *et al.* 1987). (2) In general, the transposition rates of a long DNA elements such as the entire pZF42 plasmid are much lower than simple insertions of the short *IS10* sequence (MORISATO *et al.* 1983; WAY and KLECKNER 1985). (3) The formation of cointegrate is not a reflection of the inability to produce simple insertion or to propagate such mutants under the Tet selection. This is evident from the observation that simple insertions occur at a significant frequency in *lexA* and *recA* strains. It might have been speculated that the Tet selection picks up cointegrates because they have a growth advantage under the selection. However, the assay condition were optimized to allow growth of cells harboring the low copy plasmid with point mutations in *cl(434)*. Indeed, a significant portion of the mutants obtained contained point mutations. (4) When experiments were performed with a dimeric form of pMVIS10, cointegrates of the pMVIS10 dimer and pZF42 were obtained. The lack of cointegrates of monomeric pMVIS10 and pZF42 argues

FIGURE 10.—Plasmid DNAs from spontaneous Tet^R mutants of RKZ2(pZF42) cells. Plasmid DNA was prepared and fractionated by electrophoresis on an agarose gel containing ethidium bromide.

against the “plasmidic transposition model,” which predicts that such structures will be formed and in fact with higher frequency than the dimer-pZF42 cointegrate. The homologous recombination model, on the other hand, predicts formation of the larger cointegrates only.

The frequency of cointegrate formation via homologous recombination between mutated *IS10* sequences that were cloned into *cl(434)* (on plasmid pZF42) and into *cro* (on plasmid pMV2) was found to be $\sim 10^{-2}$. Therefore, the presence of *IS10* sequences on the two plasmids is not sufficient to promote their fusion into cointegrate structure with high efficiency. Based on this observation, we suggest that *IS10* transposition and the homologous recombination are not independent of each other, rather *IS10* transposition greatly stimulates and is coupled to interplasmid homologous recombination at the transposition site.

Tn10 transposition can induce the expression of the bacterial SOS system, via a mechanism in which the degradation of the donor molecule probably provides the activating signal (ROBERTS and KLECKNER 1988). Although SOS induction will elevate the cellular capac-

ity for homologous recombination (due to an increase in RecA production), this is unlikely to be the major cause responsible for the stimulated homologous recombination after *IS10* transposition. This is indicated by the fact that in *lexA3* cells, in which RecA and other SOS functions cannot be induced, still a majority of the *IS10* transposition events (67%) leads to cointegrate formation.

Clues that support the idea that *IS10* transposition enhances homologous recombination can be found in previous studies. One study described the formation of mutants by simple insertion of *IS10* followed by intramolecular homologous recombination of the inserted element with another *IS10* sequence residing nearby (KLECKNER and ROSS 1980). Their high frequency of production implied that these mutants were obtained by coupled events. In another report, in which the mobilization of plasmid pACYC177 by R388::Tn10 were studied by the “mating out” assay, the production of cointegrate structures mediated by *IS10* was observed (HARAYAMA *et al.* 1984). This phenomena was interpreted by the authors as a *recA*-dependent transposition pathway of *IS10* that produced cointegrates. In light

FIGURE 11.—Southern blot analysis of plasmid DNA from spontaneously arising Tet^R mutants of RKZ2(pZF42) cells. The DNA shown in Figure 9 was bidirectionally transferred onto nylon membranes and analyzed by Southern hybridization using a pZF42-specific probe (A) or an *IS10*-specific probe (B).

of the present study, these results can be alternatively interpreted as *IS10* transposition followed by homologous recombination. These observations indicate that the enhancement of homologous recombination by *IS10* transposition is not unique to the *cl(434)* system but represents a general aspect of *IS10* transposition.

Each *IS10* transposition from the bacterial chromosome into plasmid pZF42 resulted in the integration of pZF42 into the chromosome. This integration was accompanied by the formation of an additional copy of chromosomal *IS10* sequence. Assuming that *IS10* does not transpose replicatively, this increase in the copy number of the *IS10* element provides further support for a mechanism of simple insertion followed by homologous recombination. Transposition that involves *IS10* ends each from different neighboring element (similarly to plasmid-transposition) would not have altered the copy number of *IS10* elements.

Interestingly, transposition of *IS10* was usually accompanied by an increase of more than one band that cohybridized with the *IS10* probe. These additional *IS10* sequences did not seem to be related to the initial transposition event that resulted in pZF42 integration, because they

gave signals up to 20-fold less intense than the bands that cohybridized with the *IS10* and pZF42 probes. We interpret these as additional *IS10*-mediated chromosomal events that had occurred in part of the population after the initial transposition-integration event. A similar observation, in which *IS10* intermolecular transposition caused multiple events that led to changes in the number and the distribution of chromosomal *IS10* elements, was made by us in a former study (SKALITER *et al.* 1992). In addition, it was reported that a single chromosomal Tn10 element can frequently mediate complex rearrangements that are the sum of two “classical” *IS10* events (RALEIGH and KLECKNER 1984). Interestingly, only closely linked *IS10* sequences promote such double events. In our experiments the origin of the *IS10* sequences in the bacterial chromosome was a single Tn10 element, suggesting that the *IS10* sequences are close. It should be emphasized that the integration of pZF42 into the bacterial chromosome by itself is not responsible for the multiple events mediated by *IS10*. This is supported by the fact that no changes in the number of *IS10* elements was observed when integration of pZF42 was mediated by an *IS* element other than *IS10*.

TABLE 6

Spontaneous mutagenesis of plasmid pZF42 in cells harboring chromosomal *IS10* elements.

Strain	Mutation frequency	Mutants analyzed	Point mutation	Insertions		Chromosomal integration
				Other IS	<i>IS10</i>	
RKZ2	1.31×10^{-8}	17	13		2	4
RKZ3	0.67×10^{-8}	14	5	1		8

FIGURE 12.—Hybridization patterns of pZF42 and IS10 probes to chromosomal DNA from mutants in which pZF42 had integrated into the bacterial chromosome. Tet^R mutants of RKZ3 (pZF42) in which plasmid pZF42 had integrated into the bacterial chromosome were isolated, and their chromosomal DNA was purified on CsCl gradient and digested with *Xho*I or *Bam*HI. The DNA was then fractionated on an agarose gel, transferred bidirectionally onto nylon membrane, and hybridized in parallel with a pZF42-specific probe (A) or with an IS10-specific probe (B). U, uncut plasmid; X, *Xho*I-digest; B, *Bam*HI-digest. Arrows A and B mark DNA bands that cohybridized with IS10 and pZF42 specific probes. Arrows a and b mark additional copies of IS10 elements.

Does intramolecular IS10 transposition produce cointegrates with the same efficiency? If this was the case, IS10 transposition was expected to efficiently produce chromosomal rearrangement such as “adjacent deletion,” in which a contiguous chromosomal segment adjacent to the transposon is deleted while the element responsible remains intact. Indeed, IS10 can generate adjacent deletions but at a frequency that is approximately 1/30th the frequency of transposition (ROBERTS *et al.* 1991). Therefore it seems that IS10 promotes intramolecular cointegrates less efficiently than intermolecular cointegrates.

The stimulation of homologous recombination can be explained by RecA activation by ssDNA regions exposed during or subsequent to the transposition event. Because the sequences flanking IS10 in the donor plasmid have no homologies to the acceptor DNA, recombination must have occurred via the IS10 sequence itself. If IS10 was excised from the donor, where does the second copy of IS10 come from? Because IS10 transposition is negatively regulated by *dam* methylation, transposition occurs most likely only after the passage of the replication fork, that is, when the DNA is in a hemimethylated state (ROBERTS *et al.* 1985). Therefore, there is always an additional copy of IS10 sequence in close proximity to the transposition complex or product, and it can serve as a donor for homologous recombination. It was previously reported that in >99% of cells that undergo IS10 transposition, IS10 is retained in the orig-

inal donor site (BENDER *et al.* 1991). This observation was explained by the restoration of the original information at the donor site by recombinational repair mechanism analogous to double-strand break repair. Such an event can also provide the homologous sequence that can recombine with the transposed IS10 element.

The mechanism suggested by our studies provides an effective mechanism for replicon fusion and integration of extrachromosomal elements into the chromosome. In addition, such a process causes duplication of IS10, despite its nonreplicative mode of transposition. A stimulation of homologous recombination by Tn7 at the transposition site was recently reported (HAGENMANN and CRAIG 1993). Because Tn7 also transposes by a cut and paste mechanism, it is possible that transposition-coupled homologous recombination is a feature common to nonreplicative transposons.

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