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Conduction of Nonconjugative Plasmids by F′ *lac* Is Not Necessarily Associated with Transposition of the γδ Sequence

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A nonconjugative kanamycin-resistant (Km^r) recombinant plasmid, pNR5311, transferred at a low frequency from an Hfr or F′ *lac Escherichia coli* donor to an F$^-$ *lac*$^-$ recipient. Among the transconjugants, two types of Km^r plasmids were found: one was indistinguishable from pNR5311 (type A), and the other was a recombinant between pNR5311 and the γδ sequence (type B). When the F′ *lac* strain was used as a donor, 5% of lactose-fermenting (Lac$^+$) and 75% of lactose-nonfermenting (Lac$^-$) transconjugants had type A plasmids. A kinetic study revealed that type A plasmids were transferred more readily in short mating periods than were type B plasmids. Involvement of Tn*903*, which is present in pNR5311, in transfer of type A plasmids was unlikely since there was no discernible change in the F′ *lac* molecule coexisting with the type A plasmid in the transconjugant cells. The non-γδ-associated conduction of pNR5311 by F′ *lac* did not require the *recA*$^+$ function of the donor. Conduction of pBR322 by F′ *lac* was also carried out, and two types of plasmids with and without γδ were found, as with pNR5311. These findings suggest that the transfer of nonconjugative plasmids is conducted by a novel pathway which is not associated with translocation of transposable elements into either plasmid.

Conjugational transmission of nonconjugative plasmids by the aid of conjugative plasmids has been elucidated by either of two mechanisms: conduction or donation (9). Conduction is defined as the process whereby a mobilizable plasmid causes transmission of a nonmobilizable plasmid by physical association with it. Donation, by contrast, is defined as the process whereby a nonconjugative mobilizable plasmid is transferred via the effective contact determined by a conjugative plasmid, without physical association of the two plasmids (5, 9). Although the donated plasmid is transferred as efficiently as the conjugative plasmid, the transfer frequency of a conducted plasmid is much lower than that of a mobilizable plasmid probably because the recombination between the two plasmids is rather rare (9). Guyer (18) has reported that conduction of the chimeric plasmid pBR322 by the F plasmid is invariably (>99%) associated with an insertion of the γδ sequence into pBR322. The possible role of γδ in conduction of pBR322 has been interpreted by two hypotheses: (i) a cointegrate F::pBR322::γδ is formed, transferred, and dissociated in the recipient, giving rise to a transconjugant carrying both F and pBR322::γδ, and (ii) γδ carries a sequence which can function as an origin of conjugational transfer. On the other hand, Crisona et al. have reported that when pML31, a derivative plasmid of the F plasmid in which the *oriT* region is deleted, is conducted by the conjugative plasmid pNC21, a recombinant plasmid composed of pNC21 and pML31, the latter flanked by two copies of Tn*3*, is found in the transconjugants (11). They have postulated that this recombinant plasmid is formed as an intermediate in the transposition of Tn*3* from pNC21 to pML31 and is the vehicle for conjugational transmission of pML31 genes by conduction. Since the γδ sequence and Tn*3* are similar transposons in some respects, it is probable that the role of γδ in F conduction of pBR322 is also that of cointegrate formation (17, 29, 30).

During a series of experiments in which we tried to transfer a nonconjugative plasmid, pNR5311, from either an F′ *lac* or an Hfr donor, we noticed two different types of plasmids in the transconjugants. Although most of the transconjugants carried plasmids larger than the original pNR5311, some had plasmids similar to those in the donor. Further studies revealed that the larger plasmids were pNR5311 plasmids which contained an insertion of the γδ sequence, but the others were indistinguishable from pNR5311. Since the latter plasmid was not expected on the basis of the reports cited above, we examined the mode of transfer of the latter plasmid and found that transfer of this type of plasmid could be detected more readily in transconjugants into which cotransfer of F′ *lac* had not occurred and which were obtained after short mating periods. The transfer was *recA*$^+$ independent. Although the mechanism is not yet clear, we propose several possible hypotheses for the non-γδ-mediated conduction of plasmids by F′ *lac*.

MATERIALS AND METHODS

Bacterial strains and growth of cultures. Bacterial strains used in this study are listed in Table 1. Routine cultures of the strains were made in L-broth (26). All incubations were performed at 37°C.

Plasmids. pNR5311 is a kanamycin-resistant (Km^r) recombinant plasmid composed of a 3.2-kilobase-pair (kb) *Eco*RI fragment of Rts1 and a 7.2-kb *Eco*RI fragment of R6-5 containing Tn*903* (15) (Fig. 1). The former fragment is responsible for replication, and the latter confers kanamycin resistance. Subcloning of pNR5311 after digestion with *Mlu*I yielded pNR5411 which has a 4.25-kb deletion (Fig. 1).

Chemicals. Restriction endonucleases were purchased from Takara Shuzo Co., Ltd. Nalidixic acid was a gift of Daiichi Pharmaceutical Co., Ltd. Kanamycin sulfate and ampicillin were commercially obtained from Meiji Seika Kaisha, Ltd. Tetracycline was purchased from Japan Lederle Ltd. The detergents Sarkosyl NL-97, Brij 58, and sodium deoxycholate were purchased from Kasho Co., Ltd., ICI America Inc., and Difco Laboratories, respectively. Agarose (type I), RNase A, and lysozyme were purchased from Sigma Chemical Co.

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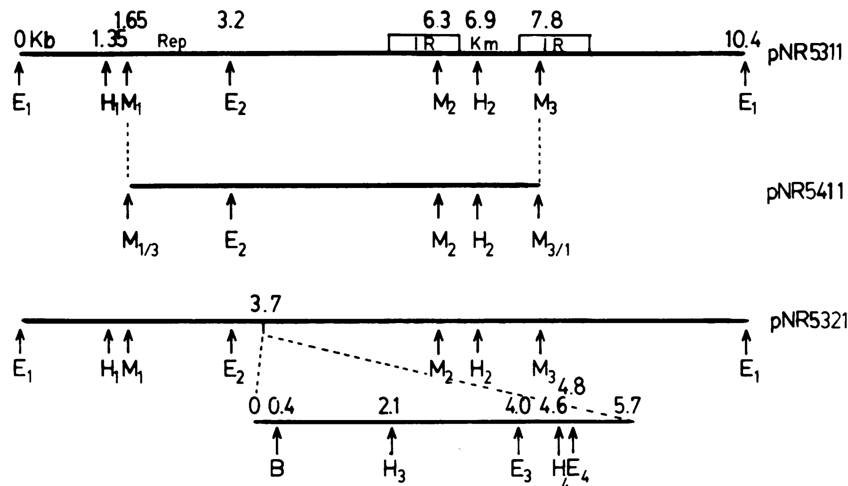

FIG. 1. Physical maps of pNR5311 and its derivatives pNR5411 and pNR5321. B, E, H, and M are the restriction sites of *Bam*HI, *Eco*RI, *Hin*dIII, and *Mlu*I, respectively. IRs are the inverted repeats, IS*903*, flanking the kanamycin resistance gene (Km) of Tn*903*. Numbers on the maps are the coordinates scaled by kilobase pairs (kb) from one of the *Eco*RI sites (E_1). Coordinates on the γδ sequence in pNR5321 are from the δ end.

Preparation of plasmid DNA. Plasmid DNA was isolated from cleared lysates prepared by a modification of the method of Clewell and Helinsky (10). This procedure was especially useful for purification of DNA of such large plasmid as F' *lac*. Cells from 250 ml of overnight culture were harvested and washed with 50 mM Tris-chloride (pH 8.0). The cell suspension was then mixed with 0.1 ml of RNase (5 mg/ml, pretreated with heat) and 2 ml of lysozyme (about 8,000 U/ml) and incubated for 5 min at 0°C. A volume of 2.5 ml of 0.25 M EDTA (pH 8.0) was added, and the mixture was incubated for 5 min at 0°C. Of the detergent mixture (1% Brij 58, 0.4% sodium deoxycholate, 62.5 mM EDTA, 50 mM Tris [pH 8.0]), 16 ml was gently overlaid, mixed with rapid shaking, and kept on ice for 10 min. The mixture was then centrifuged at 15,000 rpm for 30 min at 2°C with a Hitachi RPR-20 rotor. After the volume of the nonviscous supernatant was measured, this fraction (cleared lysate) was transferred to a centrifuge tube and kept on ice. The cleared lysate was treated with Sarkosyl (1%) for 10 min at 30°C and chilled. DNA in the Sarkosyl-lysate was precipitated with 10% polyethylene glycol and 0.5 M NaCl as described by Humphreys et al. (23). After the mixture was kept overnight in a refrigerator, the polyethylene glycol precipitate was collected, resuspended in 2 ml of TES buffer (50 mM Tris, 0.5 mM EDTA, 50 mM NaCl [pH 8.0]) to which 0.2 ml of 25% Sarkosyl solution and 0.2 ml of 0.25 M EDTA (pH 8.0) were then added. The lysate was applied to the mixture for cesium chloride-ethidium bromide centrifugation, and plasmid DNA was obtained by a standard procedure (26).

TABLE 1. Bacterial strains used[a]

Strain	Relevant genotype	Source (reference)
W1895	Hfr_2 *met*	M. Yoshikawa (2)
JC3273	F42 *thi lac his trp lys str*	M. Achtman (1)
JC1557	*lac leu his arg met str*	M. Yoshikawa (4)
JC1569	Same as JC1557 but *recA1*	M. Yoshikawa (4)
MD67	*lac thi thr leu* Nal^r	Nalidixic acid-resistant mutant of W677 isolated in this laboratory (2)

[a] All strains are derivatives of *Escherichia coli* K-12.

Estimation of the size of plasmid DNA. Cells from 1 ml of an overnight culture were lysed, and plasmid DNA was extracted as described by Kado and Liu (24). After extraction with phenol-chloroform, the aqueous layer was analyzed by agarose gel electrophoresis as described below. The mobility of the plasmid DNA was compared with those of pNR5311 (10.4 kb), pNR5321 (16.1 kb), and pBR322 (4.36 kb) DNAs to estimate its size.

Restriction endonuclease digestion. Reaction conditions for all restriction enzymes were those specified by the supplier. The reaction was terminated by being heated at 65°C for 5 min followed by addition of one-half of a volume of "blue juice" (40% sucrose in 20 mM EDTA [pH 8.0] with 0.04% bromphenol blue).

Agarose gel electrophoresis. Agarose gel electrophoresis was carried out as described previously by Tanaka and Weisblum (32), using 0.7 to 1.0% gel in a horizontal tray. Conditions for estimation of plasmid DNA size were 4 V/cm for 3 h, and for analysis of restriction fragments, conditions were 1 V/cm for 18 h. The gels were stained with 1.5 μg of ethidium bromide per ml and photographed by using a long-wave UV lamp.

Conjugation and isolation of transconjugants. Donor cells were grown overnight, diluted 10-fold with fresh broth, and mixed with an equal volume of an overnight culture of the recipient (MD67). The mixture was then incubated at 37°C for 3 h or as indicated, diluted, and plated on BTB lactose agar plates (Eiken Chemical Co.), containing 25 μg of each of nalidixic acid and kanamycin sulfate per ml to select Km^r transconjugants. To select lactose fermenting (Lac^+) transconjugants, minimal agar (26) supplemented with 1% lactose, 20 μg of leucine and threonine per ml, and 1 μg of thiamine per ml was used.

Determination of the (G+C) content of DNA fragments. About 30 μg of plasmid DNA was digested with *Eco*RI or *Mlu*I or both and applied for use in preparative agarose gel electrophoresis on a 0.7% gel. The fragments were extracted

TABLE 2. Transfer frequency of the plasmids carried by Hfr and F′ *lac* strains

Plasmid	Transfer frequency with[a]:		F′ *lac*/Hfr ratio
	Hfr	F′ *lac*	
pNR5311	6.1×10^{-7}	6.1×10^{-6}	10
pNR5411	8.1×10^{-7}	5.3×10^{-6}	6.6

[a] Values are the number of transconjugants per input donor cell. Donor strains used were W1895 for Hfr and JC3273 for F′ *lac*. Overnight cultures of the donor were diluted 10-fold, mixed with an equal volume of the recipient culture (MD67), and incubated at 37°C for 3 h. The transconjugants were selected as described in the text.

from the gels after destruction of the gel matrix by freezing and thawing and then precipitated with ethanol. CsCl density gradient centrifugation was carried out as described previously (16) with *Micrococcus lysodeikticus* DNA used as a reference density (1.731 g/cm^3). Buoyant densities and the guanine-plus-cytosine (G + C) content were calculated as described previously by Schildkraut et al. (31).

RESULTS

Conduction of the nonconjugative plasmids. Transfer of Km^r plasmids to MD67 was carried out with either an F′ *lac* or an Hfr donor, and the transfer frequencies of kanamycin resistance were calculated as the numbers of Km^r transconjugants per input donor cell. pNR5311 and pNR5411 were transferred at relatively low frequencies, which suggests that they were transferred not by donation but by conduction (Table 2). The F′ *lac* donor could transfer both plasmids about 10 times more efficiently than the Hfr. pNR5311 was then transferred from *recA*$^+$ and *recA* F′ *lac* donor. Since the *recA* donor could not transfer F′ *lac* as efficiently as the *recA*$^+$ donor, the conduction frequency was calculated to compare the efficiencies of conduction by these donors. The conduction frequency was defined as the ratio of the transfer frequency of kanamycin resistance to that of F′ *lac*. pNR5311 was conducted from the *recA* donor as efficiently as from the *recA*$^+$ donor (Table 3), which implied *recA*$^+$ functions are not required for the conduction of pNR5311 by the F′ *lac* plasmid.

Plasmids found in Km^r transconjugants. We examined plasmid DNA from the transconjugants to determine whether insertion of any DNA sequence into the original plasmid had occurred. A total of 84 independent Km^r transconjugants from separate matings between JC3273(pNR5311) and MD67 were isolated. As many lactose-nonfermenting (Lac$^-$) transconjugants were isolated as possible to avoid the complexity of dealing with both F′ *lac* and Km^r plasmid DNA. Of the transconjugants, 44 were Lac$^+$ and 40 were Lac$^-$. Cell lysates were prepared from them, and plasmid sizes were determined by agarose gel electrophoresis as described above. Some transconjugants contained larger plasmids than the original pNR5311 (Fig. 2). Subsequent purification of the plasmid DNAs followed by restriction cleavage analysis revealed that the larger plasmids (16.1 kb) were recombinants between pNR5311 (10.4 kb) and the γδ sequence (5.7 kb). Of 84 transconjugants, 52 contained such recombinant plasmids (type B, see below), whereas the remainder carried plasmids which were proven by the restriction cleavage patterns to contain no γδ insertion and to be identical to pNR5311 (type A, see below).

TABLE 3. Effect of *recA* mutation on conduction of pNR5311 by F′ *lac*

Genotype of donor[a]	Transfer frequency with[b]:		Conduction frequency[c]
	Km^r	Lac$^+$	
recA$^+$	4.6×10^{-5}	1.8×10^{-1}	2.6×10^{-4}
recA	2.7×10^{-7}	6.8×10^{-4}	4.0×10^{-4}

[a] *recA*$^+$, JC1557(F′ *lac*) (pNR5311); *recA*, JC1569(F′ *lac*) (pNR5311).

[b] Conjugation was carried out with indicated selective markers as described in the footnote to Table 2, except for the length of conjugation time (overnight for this experiment).

[c] Ratio of Km^r transconjugants to Lac$^+$ transconjugants.

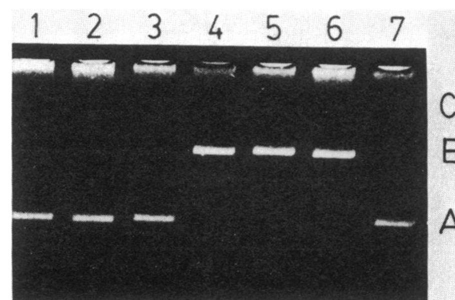

FIG. 2. Agarose gel electrophoretic patterns of the cell lysates from Lac Km^r transconjugants. Electrophoresis was carried out in 1% agarose gel as described in the text. Lanes 1, 2, and 3, strains carrying the same plasmid as pNR5311 (type A); lanes 4, 5, and 6, strains carrying the plasmid with increased size (type B); lane 7, the donor JC3273(pNR5311) as a size marker. C indicates contaminated chromosomal DNA. F *lac* DNA could not be seen under these conditions.

Although 95% of Lac$^+$ and 25% of Lac$^-$ transconjugants contained the type B plasmid, 5% of Lac$^+$ and 75% of Lac$^-$ clones had the type A plasmid (Table 4). Kinetic experiments demonstrated that, in the earlier period of matings, most of Km^r transconjugants were Lac$^-$, and Km^r Lac$^-$ transconjugants increased to the maximum at 20 min, whereas the total Km^r transconjugants still increased at 90 min (Fig. 3). A number of independent transconjugants were isolated after the mating for 10 and 60 min and were tested for plasmid sizes. Regardless of the length of conjugation time, type A plasmids were found more frequently in Lac$^-$ transconjugants (Table 5).

Restriction cleavage analysis of the conducted plasmids. A total of 28 type A and 21 type B plasmids were screened by agarose gel electrophoresis of cell lysates of independent transconjugants. The plasmid DNAs were purified and subjected to the restriction cleavage analysis by *Eco*RI,

TABLE 4. Incidence of γδ transposition in pNR5311 in Lac$^+$ and Lac$^-$ transconjugants[a]

Phenotype[b]	No. of transconjugants tested	γδ transposition[c]	
		+	−
Lac$^+$	44	42	2
Lac$^-$	40	10	30

[a] Conjugation was carried out between JC3273(pNR5311) and MD67 for various times. Independent transconjugants were selected, and the presence of the γδ sequence in pNR5311 was determined by agarose gel electrophoresis of transconjugant lysates, using pNR5311 and pNR5411 (pNR5311::γδ) as molecular-size references.

[b] Phenotype of transconjugants.

[c] Transposition of γδ into kanamycin resistance plasmid was detected by agarose gel electrophoresis of the cell lysate. +, Transposed; −, not transposed.

TABLE 5. Correlation of γδ transposition with transfer of F' *lac* in early and late transconjugants

Conjugation time (min)	F' *lac* transfer[a]	No. (%) of transconjugants[b]	
		$\gamma\delta^+$	$\gamma\delta^-$
10	+	19 (95)	1 (5)
10	−	3 (30)	7 (70)
60	+	14 (100)	0 (0)
60	−	3 (15)	17 (85)

[a] Independent Km^r transconjugants were isolated from BTB lactose plates supplemented with kanamycin sulfate. As many Lac^- transconjugants as possible were picked.
[b] Transposition of γδ into Km-resistance plasmid was detected by agarose gel electrophoresis of the cell lysate. $\gamma\delta^+$, Transposed; $\gamma\delta^-$, not transposed.

*Hin*dIII, and *Mlu*I. A total of 27 strains carrying type A plasmid and 10 strains carrying type B plasmids were Lac^-, and no plasmid other than type A or type B was detected in these strains.

Of 28 type A plasmids, 25 were the same as the original plasmid pNR5311, and three were found to differ only in that they carried Tn*903* in an inverted orientation (Fig. 4, lane 2). Only one strain was Lac^+ and carried F' *lac* DNA in addition to the type A plasmid DNA.

Type B plasmids carried an insertion of 5.7 kb in the pNR5311 molecule. The inserted sequences all had the same restriction pattern as the γδ sequence (18), but were inserted at different sites on pNR5311 (Fig. 4, lane 3, 7–10). We, therefore, concluded that the inserted sequence is γδ. One of the type B plasmids was named pNR5321, and its restriction cleavage map is shown in Fig. 1.

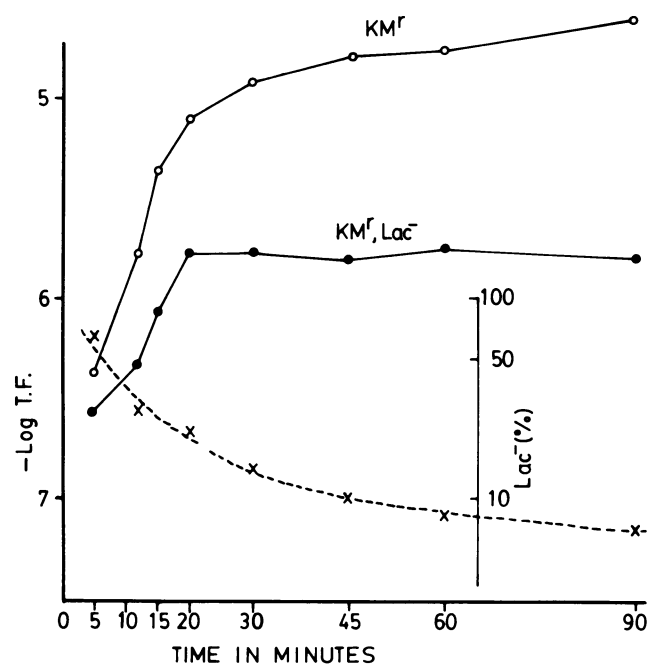

FIG. 3. Kinetics of transfer of kanamycin resistance. Conjugation mixture was withdrawn at indicated time and plated on BTB lactose plates supplemented with kanamycin sulfate. The transfer frequency (T.F.) was calculated as the number of total (○) and Lac^- (●) transconjugants per input donor cell. The percentages of Lac^- transconjugants (x) are shown against the Lac^-(%) axis.

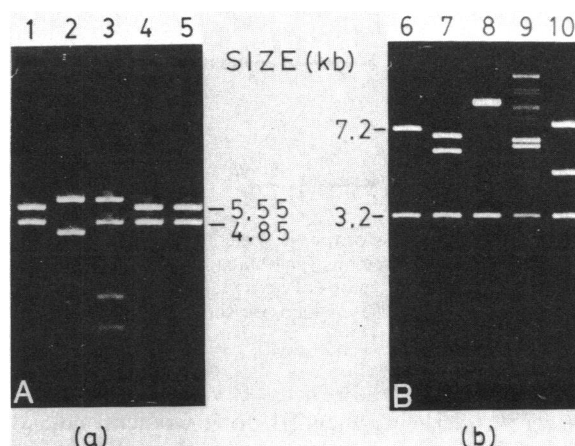

FIG. 4. Agarose gel electrophoretic patterns of (A) *Hin*dIII-digested and (B) *Eco*RI-digested fragments of purified plasmid DNAs. Lane 1 and 6, pNR5311 as a size marker; lane 2, plasmid with Tn*903* in inverted orientation; lane 3, 7, 8, and 10, type B plasmids (pNR5311::γδ); lane 4 and 5, type A plasmids (same as pNR5311). Lane 9, type B plasmid plus F' *lac*. The other DNAs were all from Lac^- strains.

The sites and orientation of the γδ insertions on pNR5311 were not specific. Only 2 of 21 γδ insertions were at locations indistinguishable from each other (Fig. 5). The distribution of the insertion sites was, however, not random. Of 21 insertions, 19 were mapped between the second *Eco*RI (E_2) site and inner end of the left inverted repeat (IR_L). Since pNR5411 had a deletion of the region flanked by the third *Mlu*I (M_3) and first *Mlu*I (M_1) sites of pNR5311 (Fig. 1), the region between the inner end of the right inverted repeat (IR_R) and the M_1 site (fragment I and III* in Fig. 5) is not essential for the plasmid replication and kanamycin resistance. This indicated, therefore, that the observed low frequency of transposition of the γδ sequence did not result from a counterselection for essential genes.

pNR5311 DNA was then digested by relevant restriction enzymes, and the DNAs of fragments II, III, and III + I (Fig. 5) were purified by preparative gel electrophoresis. The buoyant densities of the DNA fragments were determined by CsCl density gradient centrifugation (Fig. 6). The density of the fragment I was calculated from those of the fragments III and III + I, by taking their sizes into consideration. G+C content values of the fragments were calculated as described in the text. The percent G+C content of the fragment designated by the asterisk, which was flanked by the *Mlu*I site and the inner end of IS*903* (Fig. 5), was obtained from the base sequence data of Oka et al. (28). Fragment II*, in which twice as many sites were observed than expected

TABLE 6. Distribution of γδ insertion sites in pNR5311 molecule

Fragments[a]	Molecular size (kb)	No. of γδ insertion sites		%G+C[c]
		Expected[b]	Observed	
I	1.65	4	1	49
II*	3.4	9	19	47
III*	2.9	8	1	57

[a] See Fig. 5.
[b] Expected values were calculated as if the 21 γδ sites were distributed on each fragment randomly.
[c] %G+C was calculated from the buoyant densities of the DNA fragments extracted from the slab gel after electrophoresis.

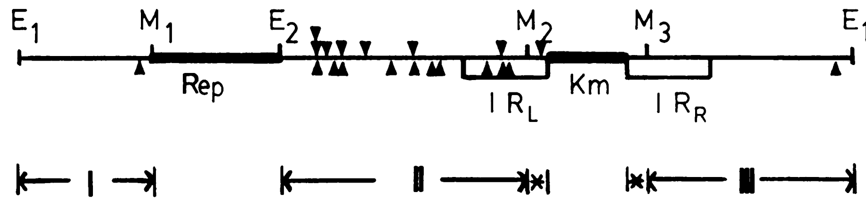

FIG. 5. Insertion sites of the γδ sequence on pNR5311. The triangles above and below the map indicate γ to δ, and δ to γ orientations, respectively. Essential regions for replication (Rep) and kanamycin resistance (Km) are shown by thick lines. Fragments I, II, and III are three major unessential regions of pNR5311, each flanked by restriction sites. Asterisks (*) indicate small fragments flanked by the *Mlu*I site and the inner end of IS*903*s, which are shown as IR_L and IR_R.

from its size, had the lowest G+C value (47%) among the three (Table 6). The fragment III*, on the other hand, having only one-eighth of expected number of sites, had the highest G+C value (57%).

The molecular structure of F′ *lac* plasmid coexisting with type A plasmid in transconjugants. Since pNR5311 contains the transposon Tn*903* which has two IS*903*'s as terminal inverted repeats (28), there is a possibility that one of these transposable elements mediated the formation of a cointegrate between pNR5311 and F′ *lac*. If this happened, the cointegrate would be able to transfer by conjugation and, after the transfer, resolve into pNR5311 (type A plasmid) and either F′ *lac*::Tn*903* or F′ *lac*::IS*903*. As the molecular sizes of Tn*903* and IS*903* are 3.1 and 1.05 kb, respectively (24, 28), an insertion of either element into the F′ *lac* molecule should be detected by restriction analysis. We isolated a spontaneous kanamycin-sensitive (Km^s) segregant from one of the Lac^+ transconjugants carrying a type A plasmid. The plasmid DNA was purified and its *Mlu*I restriction pattern was compared to that of the original F′ *lac* DNA. If either Tn*903* or IS*903* is transposed into the F′ *lac* molecule, there must be an increase of *Mlu*I sites by two or one, respectively (Fig. 1). Since IS*903* has an *Mlu*I site at 787 base pairs (bp) from an end, an insertion of IS*903* into F′ *lac* should produce a new *Mlu*I fragment larger than at least 787 bp. No such change was observed in the F′ *lac* molecule, which implies there is no evidence of transposition of any known transposable elements on both F′ *lac* and pNR5311 in this case (Fig. 7).

Conduction of pBR322. pBR322 was transferred from JC3273(pBR322) to MD67 at about a 10-times-lower frequency than was pNR5311. A total of 22 independent transconjugants, of which 6 were Lac^-, were isolated. Their lysates were prepared, and the sizes of the plasmid DNAs were determined so as to monitor the presence of the γδ sequence in the pBR322 molecule conducted. Seven (32%) pBR322 transconjugants contained no γδ insertion in the molecule, whereas the rest of the transconjugant plasmids were recombinants of pBR322 and γδ (Table 7). These results indicate that F′ *lac* could conduct without transposi-

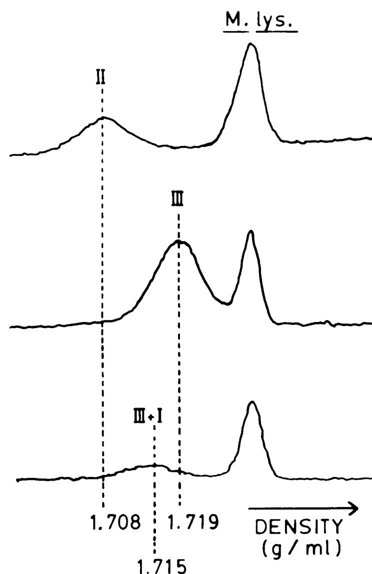

FIG. 6. CsCl density gradient profiles of nonessential fragments of pNR5311 DNA. The fragments were named as illustrated in Fig. 5. *M. lysodeikticus* (M. lys.) DNA was added as an internal reference of buoyant density (1.731 g/cm³).

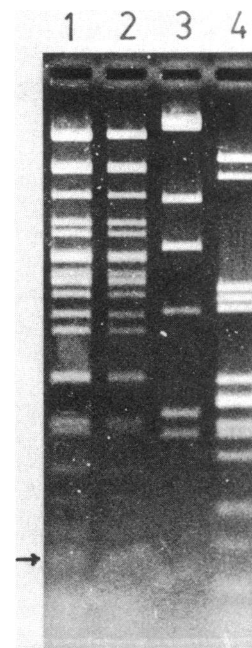

FIG. 7. *Mlu*I-digested patterns of F′ *lac* DNAs before (lane 1) and after (lane 2) the conduction of type A plasmid (same as pNR5311). From a transconjugant harboring both F′ *lac* and type A plasmid, a Km^s derivative was isolated and F′ *lac* DNA was purified to compare the restriction patterns with original F′ *lac* DNA. Lanes 3 and 4 are λ DNA digested with *Hin*dIII and *Pst*I, respectively. The arrow indicates the smallest F′ *lac* fragment whose size was estimated at ca. 0.68 kb.

TABLE 7. Incidence of γδ transposition into pBR322 in Lac^+ and Lac^- transconjugants[a]

γδ transposition	No. of transconjugants which were[b]:	
	Lac^+	Lac^-
+	12	3
−	4	3

[a] An overnight culture of the donor JC3273(pBR322) was diluted 10-fold and mixed with MD67. Conjugation was carried out overnight at 37°C, and transconjugants were selected on minimal agar supplemented with lactose as a sole carbon source. Transposition of γδ into pBR322 after the conjugation was monitored by agarose gel electrophoresis of the transconjugant lysates, using pBR322 and pBR322::γδ DNAs as size references.

[b] Phenotype of the ampicillin- and tetracycline-resistant transconjugants.

tion of γδ not only pNR5311 but also pBR322 which carries no known transposable element.

Conduction of pNR5311 by *recA* donor. As mentioned above, conduction of pNR5311 by F′ *lac* was $recA^+$ independent (Table 3). A total of 24 independent transconjugants, three of which were Lac^-, were obtained from a *recA* donor. The lysates were analyzed by agarose gel electrophoresis to determine whether the γδ sequence was inserted into the pNR5311 molecule. One of Lac^+ and all three Lac^- transconjugants were found to have the type A plasmid (no γδ). To determine whether Tn*903* or IS*903* was transposed into the F′ *lac* molecule, F′ *lac* DNA was purified from the Lac^+ transconjugant carrying the type A plasmid, and examined for its *Mlu*I restriction pattern. It had the same pattern as the original F′ *lac* (data not shown).

DISCUSSION

In this study, it became evident that the F′ *lac* (F42) plasmid could promote transfer of both pNR5311 and pBR322 without discernible changes in molecular sizes of the nonconjugative plasmids. An F′ *lac* isolated from such transconjugants similarly did not show any change in the restriction pattern, which would indicate that the cointegration of the plasmids mediated by the transposable elements in pNR5311 had occurred. Although we have no direct evidence to prove that the insertion of γδ into pNR5311 and pBR322 resulted from the dissociation of a cointegrate formed between these plasmids and F′ *lac*, the close association of γδ transposition and cotransfer of those plasmids with F′ *lac* may well be interpreted to suggest the formation of a cointegrate in which there are two copies of γδ at the junctions of the component plasmids (6, 19, 21). Another possibility is that type B plasmids resulted from transposition of γδ from F′ *lac* to a type A plasmid in the recipient.

Since the γδ sequence of F42 has been mapped very close to the *lac* operon and proximal to the origin of transfer (12, 22), the cointegrate would transfer the integrated plasmid before the *lac* operon. Failure to transfer the entire cointegrate plasmid molecule to the recipient cell could result in a Lac^- transconjugant carrying a type B plasmid with a γδ insertion in the molecule. As mentioned above, we found no such plasmid that may derive from F plasmid in Lac^- transconjugants, which suggests that such a defective F plasmid, if formed, failed to either circularize or establish itself in the recipient cell.

The presence of the type A plasmid (same as the original plasmid) in the transconjugants may be explained by one of the following hypotheses: (A) F′ *lac* can conduct the nonconjugative plasmids without forming cointegrates, or (B) F′ *lac* can integrate and exise the nonconjugative plasmids without leaving discernible changes in molecular structure of both plasmids. The events of hypothesis A readily lead to low-efficiency donation of the nonconjugative plasmids. Donation is, as mentioned above, the process whereby a nonconjugative plasmid is mobilized by a mobilizable plasmid without physical association. It is believed to require at least one *cis*-acting (*bom*) and one *trans*-acting (*mob*) gene on the donated plasmid such as ColE1 (5, 9, 14). The donated plasmid is thus transferred as efficiently as the mobilizable plasmid. It is possible to postulate that a donated plasmid could be mobilized only at a lower frequency due to an unknown defect in either *bom* or *mob*. It seems unlikely, however, that both pNR5311 and pBR322, which are unrelated to each other, each bear such a defective gene. We should therefore postulate that the transfer system of F′ *lac* erroneously recognizes some sites on these plasmids instead of recognizing *oriT*. The recognition sites might be places where a single strand nick has occurred.

Hypothesis B requires a mechanism which can mediate cointegration and excision with minor sequence alteration. Such process can be accomplished by: (i) a mechanism similar to the λ phage-type recombination system (13), (ii) an internally deleted mini Mu (3, 8), or (iii) a prereplicative intermediate in transposition. Of these mechanisms, (i) seems most unlikely because it requires the presence of specific recognition sites not only on F′ *lac* but also on pNR5311 and pBR322. If, on the other hand, F42 carries terminal transposable sequences, which are too short to be detected by electrophoresis, and one of the transposable elements in the donor can help in their transposition, then F42 could integrate and excise a plasmid leaving only these short sequences. If such sequences locate close to *oriT* of F42, the integrated plasmid will be transferred well before the entire genome of the cointegrate. Mechanism (iii) is that the 3′ end of a transposable sequence on the F plasmid may be ligated to a 5′ end of a nick on the nonconjugative plasmid, and the strand on which this occurs could be displaced and transferred before the replication necessary for duplication of transposable sequence and cointegrate formation. In this case also, only a portion of the F plasmid would be transferred, whereas all of the nonconjugative plasmid could be.

The present data do not support any one of these mechanisms exclusively and some other alternatives may be possible. The type A plasmid, furthermore, may not be transferred by a single mechanism. For example, we cannot exclude the possibility that either IS*903* or Tn*903* of pNR5311 is involved in transfer of the type A molecule at least in part, because we analyzed only two F′ *lac* DNAs from Lac^+ transconjugants that received simultaneously the type A plasmid molecule. The presence of a pNR5311-like plasmid carrying Tn*903* in inverted orientation in some transconjugants may suggest some role of this transposon in the transfer.

The insertion sites of γδ in the pNR5311 molecule were located preferentially in fragment II* which has the highest adenine-plus-thymine content among the dispensible fragments of pNR5311 (Fig. 4). This may suggest that the sequence translocates preferentially into regions rich in adenine plus thymine, as has been reported for IS*1* (7, 9, 25), Tn*9* (27), and Tn*3* (7, 20, 32).

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