JOURNAL OF BACTERIOLOGY, Sept. 1982, p. 1086–1094
0021-9193/82/091086-09$02.00/0

Vol. 151, No. 3

Cointegrate Formation Between Homologous Plasmids in *Escherichia coli*

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Received 28 December 1981/Accepted 17 May 1982

Conjugation experiments were performed in which the donor was *Escherichia coli* K-12 strain KP245 containing either R plasmid NR1 plus an ampicillin-resistant derivative of ColE1 (ColE1::Tn*3*, called RSF2124) or NR1 plus RSF2124 carrying a cloned *Eco*RI fragment of NR1. The recipient was the *polA* amber mutant JG112, in which RSF2124 cannot replicate. Ampicillin-resistant transconjugants can arise only when the genes for ampicillin resistance are linked to NR1 or are transposed to the host chromosome. When *Eco*RI fragment A of NR1 (20.5 kilobases) was cloned to RSF2124, the frequency of cotransfer of ampicillin resistance with tetracycline resistance was 25 to 60%. Plasmid DNA from these ampicillin-resistant transconjugant cells was analyzed by gel electrophoresis and was shown to be a cointegrate of NR1 and the RSF2124 derivative. Analysis of plasmid DNA isolated from donor cultures showed that the cointegrates were present before conjugation, which indicates that the mating does not stimulate cointegrate formation. When the cloned fragment was *Eco*RI fragment H of NR1 (4.8 kilobases), the frequency of cotransfer of ampicillin resistance with tetracycline resistance was about 4%, and the majority of the ampicillin-resistant transconjugants were found to contain cointegrate plasmids. When the donor contained NR1 and RSF2124, the frequency of cotransfer of ampicillin resistance was less than 0.1%, and analysis of plasmid DNA from the ampicillin-resistant transconjugants showed that Tn*3* had been transposed onto NR1. These data suggest that plasmids which share homology may exist in cointegrate form to a high degree within a host cell.

Some bacterial strains found in nature and many produced in the laboratory contain more than one plasmid. These plasmids could be multiple copies of the same plasmid or they could be different types of plasmids. When two or more plasmids reside in the same cell, they may coexist stably without affecting one another, or they may interact in some way. One possible interaction is the physical exchange of segments of DNA. This can occur by transposition or recombination. Transposition of DNA from one segment of DNA to another is widely observed (e.g., reference 4), and since it can occur in the absence of the *recA* function in a host cell, it is therefore not by homologous recombination. Recombination between plasmids can result in the formation of cointegrate molecules in a cell. Cointegrate formation has been seen in *Escherichia coli* (12, 39), *Bacillus subtilis* (18), *Salmonella typhimurium* (14, 24), and *Agrobacterium* species (15, 16, 37). Cointegrate formation has also been observed between plasmids and bacteriophages (17, 29). In addition, in some cases, *recA*-independent cointegrate formation may be involved in the mobilization of nonconjugative plasmids (3, 19, 36).

In this study, we observed *recA*-dependent cointegrate formation between R plasmid NR1 and RSF2124 derivatives which carried a segment of DNA homologous to NR1. Cointegrates were detected by mating from a donor containing the two plasmids to a *polA* recipient, in which RSF2124 cannot replicate (20). The cointegrate nature of the plasmids was confirmed by genetic characterization of the ampicillin-resistant (Ap^r) transconjugants and by physical analysis of the plasmid DNA. We showed that the frequency of recombination is directly related to the amount of homology between the two plasmids, although other factors may also be involved. The frequency of cointegrate formation was quite high when the plasmids had extensive homology to one another, indicating that plas-

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Marker[a]	Genotype or description	Reference or source
E. coli			
KP245		*met his trp thy lac gal*	25
KP246		*met thy lac gal recA*	25
JG112		*met thy rpsL polA*	26
ML1410		*met gyrA*	33
Plasmid			
NR1	Cm^r, Fa^r, Sm/Sp^r, Su^r, Hg^r, Tc^r	Natural isolate	28
RSF2124	Ap^r	Col E1::Tn*3*	31
pRR138	Ap^r	RSF2124 + *Eco*RI fragment A of NR1	25
pRR134	Ap^r	RSF2124 + *Eco*RI fragment H of NR1	T. Miki, unpublished data
pRR129	Ap^r	RSF2124 + *Eco*RI fragment G of NR1	T. Miki, unpublished data
pRR301	Cm^r, Fa^r, Sm/Sp^r, Su^r, Hg^r, Tc^r, Ap^r	NR1::pRR134 cointegrate	This paper
pRR302	Cm^r, Fa^r, Sm/Sp^r, Su^r, Hg^r, Tc^r, Ap^r	NR1::pRR138 cointegrate	This paper
pRR303	Cm^r, Fa^r, Sm/Sp^r, Su^r, Hg^r, Tc^r, Ap^r	NR1::Tn*3* (insertion into *Sal*I fragment D)	This paper
pRR304	Cm^r, Fa^r, Sm/Sp^r, Su^r, Hg^r, Tc^r, Ap^r	NR1::Tn*3* (insertion into *Sal*I fragment C or *Eco*RI fragment B)	This paper
pRR305	Cm^r, Fa^r, Sm/Sp^r, Su^r, Hg^r, Tc^r, Ap^r	NR1::Tn*3* (insertion into *Sal*I fragment C or *Eco*RI fragment H)	This paper

[a] Resistance markers: Cm, chloramphenicol; Fa, fusidic acid; Sm/Sp, streptomycin/spectinomycin; Su, sulfonamide; Hg, mercuric ions; Tc, tetracycline; Ap, ampicillin.

mids sharing base sequence homology may interact to a great extent when present in the same host cell and may often exist as cointegrates, at least transiently. The mating technique used to select cointegrates did not appear to affect the frequency of their formation.

(This work was presented in part at the 81st Annual Meeting of the American Society for Microbiology, Dallas, Tex., 1 to 6 March 1981.)

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains used are *E. coli* K-12 derivatives. They are listed in Table 1 along with the plasmids used. KP245(NR1)(pRR134), KP245(NR1)(pRR138), and KP245(NR1)(RSF2124) were obtained from T. Miki. pRR138 and pRR134 are recombinant plasmids consisting of RSF2124 plus *Eco*RI fragment A or H, respectively, of pRR12, a copy mutant of NR1 (27). Other strains containing pairs of plasmids were constructed by transformation. Cells were cultured at 37°C in Penassay broth (Difco Laboratories) supplemented with thymine (20 μg/ml). Minimal agar medium (5) containing thymine and either sodium ampicillin (20 μg/ml) or tetracycline hydrochloride (5 μg/ml) was used to select transconjugants in most of the matings. Resistance to other antibiotics was tested as previously described (25).

Transformation. Strains containing pairs of plasmids were constructed by transforming an appropriate NR1-containing recipient with an RSF2124 derivative DNA. Transformation was performed as described by Lederberg and Cohen (22).

Conjugation. Cells from an overnight culture were diluted 1:100 into fresh medium and were grown to a concentration of about 3×10^8 cells per ml (2.5 h). Equal volumes of donor and recipient cells were then mixed and incubated with gentle shaking for 4 h, after which appropriate dilutions were spread on selection plates.

Isolation and characterization of plasmid DNA. Plasmid DNA was isolated from stationary-phase cells and purified by the Triton X-100 cleared-lysate procedure (21). Cointegrate plasmid DNA was isolated from cells grown in the presence of ampicillin to select for their cointegrate structure. The small-lysate procedure of Birnboim and Doly (2) was used when many plasmid DNAs were to be examined at a time.

Restriction endonucleases *Sal*I and *Bgl*II were purchased from Bethesda Research Laboratories, Inc. *Eco*RI was purchased from Bethesda Research Laboratories or Miles Laboratories, Inc. Digestions of plasmid DNA were performed in 0.1 M Tris–0.05 M NaCl–0.005 M $MgCl_2$ (pH 7.4).

Agarose gel electrophoresis was carried out by the method of Helling et al. (13). Electrophoresis of digested DNA was generally carried out at 10 V for 15 to 20 h or at 50 V for 4 to 5 h with 0.7% agarose gels. Undigested DNA was electrophoresed on 0.5%

HGT(P) agarose gels (Miles Laboratories; ultrapure high-gelling-temperature agarose) at 50 V for 5 to 6 h. DNA was made visible by staining with ethidium bromide (1 μg/ml) and illuminating the gel with a short-wave UV transilluminator.

After electrophoresis, plasmid DNA was partially depurinated in the gel by shaking the gel very gently in 0.25 M HCl for 15 min at room temperature. This denatures and fragments larger molecules to facilitate their transfer to nitrocellulose filters (35). The DNA was transferred onto nitrocellulose filters by the method of Southern (32). RSF2124 which was labeled with [α-^{32}P]ATP (New England Nuclear Corp.) by the nick translation procedure of Maniatis et al. (23) was used as a probe. Filters were prepared by first incubating them for 4 h at 65°C in sealed plastic bags containing a solution of 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0), 0.1% sodium dodecyl sulfate, 0.1 mg of denatured calf thymus DNA (heated for 10 min at 100°C) per ml, and 0.2% each of Ficoll (average molecular weight, 400,000), polyvinylpyrrolidine (average molecular weight, 360,000), and bovine serum albumin (7). The solution was then removed, and 500,000 cpm of denatured probe, heated for 10 min at 100°C in 15 ml of the hybridization solution (without calf thymus DNA), was added. Hybridization was carried out for 15 to 20 h at 65°C. After hybridization, the filters were washed two times at 65°C for 30 min in the hybridization solution (without added probe or calf thymus DNA) and then three times in 2× SSC–0.1% sodium dodecyl sulfate. Autoradiograms were then prepared by exposing X-ray film (Kodak XR-5) to the filters for 15 to 48 h.

RESULTS

Initial conjugation experiments. Conjugation experiments were performed with *E. coli* K-12 strain KP245 carrying various combinations of compatible plasmid pairs used as a donor. The plasmid pairs consisted of the conjugative plasmid NR1 and either RSF2124 (a ColE1 derivative which carries the ampicillin resistance transposon Tn*3*) or RSF2124 to which had been cloned an *Eco*RI fragment from NR1. The recipient used in the conjugation experiments was the *polA* amber strain JG112, in which ColE1 and ColE1 derivatives cannot replicate (20). Colonies arising on selection plates containing tetracycline must contain NR1. Colonies arising on plates containing ampicillin must contain NR1 carrying the ampicillin resistance (β-lactamase) gene of Tn*3*. The β-lactamase gene could be attached to NR1 in one of two ways. First, Tn*3* could be transposed onto NR1. Second, the entire RSF2124 plasmid could be recombined with NR1 to form a cointegrate plasmid molecule. Although Ap^r JG112 transconjugants could also arise by transposition of Tn*3* onto the host chromosome, in no case was the ampicillin resistance of RSF2124 seen to be transferred to JG112 without the concomitant transfer of the tetracycline resistance of NR1.

The frequency at which ampicillin resistance is simultaneously transferred along with tetracycline resistance gives the frequency of transposition, cointegrate formation, or both. With RSF2124, less than 0.1% of tetracycline-resistant (Tc^r) transconjugants were Ap^r; with pRR134 (RSF2124–*Eco*RI-H), cotransfer of ampicillin resistance with tetracycline resistance was 3.5 ± 1.1% (five experiments); with pRR138 (RSF2124–*Eco*RI-A), the frequency was 38 ± 11% (five experiments). These data show that the cotransfer frequency is greater when RSF2124 carries a cloned fragment homologous to NR1 and that there is a higher frequency of cotransfer when that fragment is *Eco*RI fragment A. This would be expected if cointegrate formation is involved in associating the β-lactamase gene of RSF2124 with the NR1 replicator.

Genetic evidence for homologous recombination. The initial matings suggested that homologous recombination may be involved in the cotransfer of ampicillin resistance with tetracycline resistance. To test whether recombination is required in the donor, matings to JG112 were performed with the closely related *recA* strain KP246 as a donor carrying the various plasmid pairs. When the *recA* strain carrying plasmids with shared homology was used as the donor, the cotransfer frequency was reduced to 0.03 and 0.01% with pRR138 and pRR134, respectively, approximately the level observed for NR1 plus RSF2124 (0.08%) and for RSF2124 in the *recA*$^+$ donor. This suggests that homologous recombination in the *recA*$^+$ donor is involved in the higher frequency of cotransfer which occurs when RSF2124 carries a cloned fragment homologous to NR1.

Recombination between homologous sequences within the cointegrate should result in the formation of the two circular parent plasmids. Since RSF2124 cannot replicate in a *polA* host, it would be lost from cells which would therefore become ampicillin sensitive. To examine this, an Ap^r transconjugant was picked from each mating and was repeatedly subcultured in a drug-free medium. Appropriate dilutions of stationary-phase cultures were plated on media with and without ampicillin to test for the presence of the β-lactamase gene of Tn*3* (Fig. 1). An Ap^r transconjugant (pRR302) from a mating in which pRR138 was in the donor with NR1 was found to lose ampicillin resistance very rapidly. This would be expected since, in the cointegrate molecule, RSF2124 is flanked by direct repeats of the *Eco*RI fragment common to both parent plasmids. This homology could serve as endpoints for intramolecular recombination. An Ap^r transconjugant from a mating in which pRR134 was in the donor with NR1 (pRR301) lost ampicillin resistance much more slowly than the transconjugant from the NR1-pRR138 mating.

This would be expected since there is less homology flanking RSF2124, which would result n a lower frequency of intramolecular recombination. Chloramphenicol resistance was stably maintained (data not shown), indicating that NR1 itself was not lost. The ampicillin resistance of the transconjugant from the KP245(NR1)(RSF2124) mating (pRR303) was stably maintained, as expected when ampicillin resistance is acquired by transposition.

Physical analysis of plasmid DNA isolated from Ap^r transconjugants. Plasmid DNA was isolated from Ap^r transconjugants, digested with appropriate restriction endonucleases, and electrophoresed to resolve the resulting fragments (Fig. 2). Plasmid DNA from 10 to 20 Ap^r transconjugants from each mating was examined. Comparison of the *Sal*I restriction pattern of the plasmid DNA isolated from the Ap^r transconjugant from the KP245(NR1)(pRR138) mating (Fig. 2, lane 2) with that of NR1 (Fig. 2, lane 1) shows that *Sal*I fragment A has increased in size from 47 kilobases (kb) to about 79 kb. This is the result expected from the insertion of pRR138 into NR1 through a crossover mediated by *Eco*RI fragment A of each plasmid (Fig. 3). In a comparison of *Bgl*II-digested plasmid DNA from an Ap^r transconjugant of a KP245(NR1)(pRR134) mating (Fig. 2, lane 9) with *Bgl*II-digested NR1 DNA (Fig. 2, lane 8), it can be seen that the band corresponding to *Bgl*II fragment B of NR1 is missing in the transconjugant plasmid DNA and the band corresponding to *Bgl*II fragment A of NR1 is brighter than expected from its stoichiometry with respect to the other bands in that lane. *Bgl*II fragment B (17.7 kb) has presumably increased to a size close to that of *Bgl*II fragment A (34.4 kb) (Fig. 3), making that band brighter. These are the results expected for the integration of pRR134 into *Eco*RI fragment H of NR1. Ap^r transconjugants in these matings were a result of cointegrate formation in 9 of the 13 transconjugants tested. The remaining Ap^r transconjugants arose as a result of transposition of Tn*3* onto *Sal*I fragment D or *Sal*I fragment C of NR1. These plasmids have restriction patterns like those shown in Fig. 2, lanes 3 and 4.

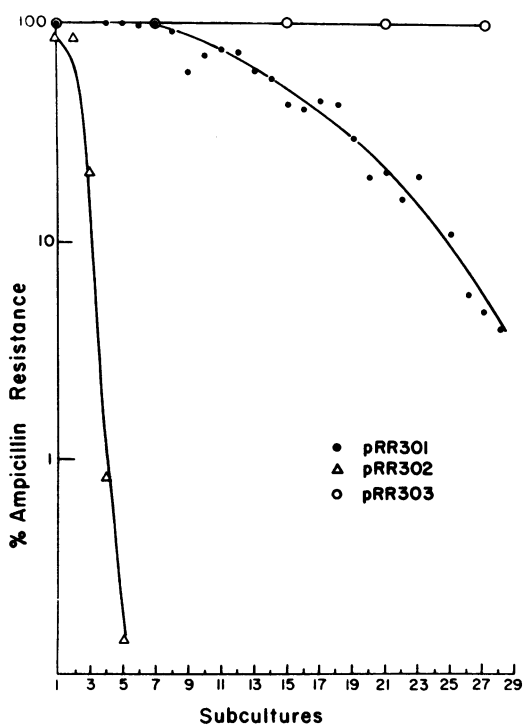

FIG. 1. Dissociation of cointegrate plasmids as determined by loss of ampicillin resistance. Cultures were diluted by a factor of 10^6 daily into fresh Penassay broth containing 20 μg of thymine per ml. The percentage of the cells that were ampicillin resistant was determined by plating dilutions of the overnight culture onto nutrient agar (containing 20 μg of thymine per ml) with or without 20 μg of ampicillin per ml.

Analysis of plasmid DNA from 10 Ap^r transconjugants from the KP245(NR1)(RSF2124) matings showed that in all cases Tn*3* had transposed to *Sal*I fragment D of NR1. Southern blots confirmed that all of the relevant fragments of interest discussed above had homology to RSF2124 (Fig. 2, b lanes).

Plasmid DNA from six Ap^r transconjugants of each *recA* mating was also analyzed by restriction digests. All of the samples of plasmid DNA which were examined were found to have Tn*3* transposed onto either *Sal*I fragment D (6 kb) or *Sal*I fragment C (15.7 kb) of NR1, as indicated by the increased sizes of these fragments and their homology to RSF2124 (Fig. 2, lanes 3 and 4). The Tn*3* elements transposed onto *Sal*I fragment C were shown to be in either *Eco*RI fragment B (12 kb, pRR304; Fig. 2, lane 6) or *Eco*RI fragment H (4.8 kb, pRR305; Fig. 2, lane 7).

Role of IS*1* in cointegrate formation. Insertion sequences are known to mediate recombination between plasmids (17). Since *Eco*RI fragments A and H of NR1 both contain IS*1*, the question arose as to whether it was essential for recombination. A KP245 strain containing both NR1 and pRR129 (RSF2124–*Eco*RI-G) was constructed. *Eco*RI fragment G (5.3 kb) is about the same size as *Eco*RI fragment H (4.8 kb), but fragment G has no IS*1*. This strain was used as a donor in a mating with the *polA* amber mutant JG112. The resulting cotransfer frequency was about 1%, about the same as that for the KP245(NR1)(pRR134) mating. From this we conclude that IS*1* probably does not play a role in cointegrate formation except as a segment of homology common to both plasmids.

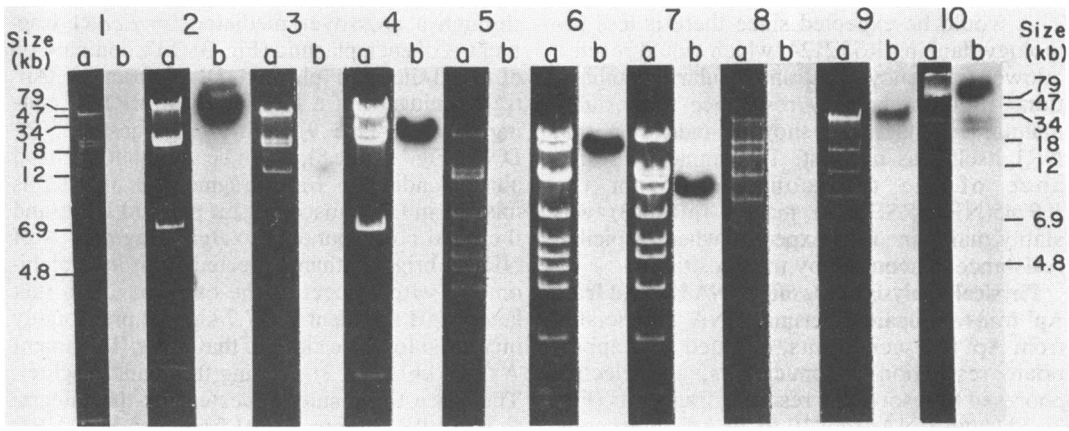

FIG. 2. Agarose gel electrophoresis and DNA-DNA hybridization of restriction fragments of plasmids from Ap^r transconjugants. Plasmid DNA was isolated from stationary-phase cultures by a cleared-lysate procedure (21), digested, and electrophoresed on a 0.7% agarose gel. The gel shows *Sal*I digests of NR1 (1a), pRR302 (2a), pRR303 (3a), and pRR304 (4a); *Eco*RI digests of NR1 (5a), pRR304 (6a), and pRR305 (7a); and *Bgl*II digests of NR1 (8a) and pRR301 (9a). Lane 10a contains undigested pRR138. Autoradiograms of the gel lanes, made with ^{32}P-labeled RSF2124 as the probe, are shown in the b lanes. Size standards correspond to NR1 bands in the various lanes (see text).

Involvement of *finO* inactivation in determination of some mating frequencies. The *finO* gene product found on some plasmids, including NR1, represses conjugal transfer of that plasmid (8, 9). Inactivation of *finO* by the insertion of Tn*3* would greatly increase the transfer frequency of a plasmid. The use of mating in our experiments could therefore lead to an overestimation of the number of transposed molecules of NR1 in the population if Tn*3* were inserted into the *finO* gene of NR1.

In some matings, the cotransfer frequency from KP245(NR1)(RSF2124) to JG112 was 4 to 7%, which is higher than would be expected for the transposition of Tn*3* to NR1. Analysis of plasmid DNA from five Ap^r transconjugants derived from a mating with a 6.5% cotransfer frequency showed that Tn*3* had been inserted into *Sal*I fragment D of NR1. Previous experiments in this laboratory and others (6, 34) have indicated that *Sal*I fragment D of NR1 carries the *finO* gene.

To determine whether these transposed molecules were transferred more frequently than wild-type NR1, Ap^r JG112 transconjugants from the previous matings were used as donors to a nalidixic acid-resistant recipient, *E. coli* ML1410 (33). The donor cells were cultured overnight in medium containing ampicillin so that most of the cells would contain cointegrate molecules. Transconjugants were selected on plates containing nalidixic acid and tetracycline. When JG112 carrying pRR302 (NR1::pRR138) was used as the donor, tetracycline resistance transferred at a frequency of 5.4×10^{-3}. For JG112 carrying pRR301 (NR1::pRR134), the frequency was 2.5×10^{-3}. However, for JG112 carrying pRR303 (NR1::Tn*3* in *Sal*I fragment D), the frequency was 2.0×10^{-1}. The transfer frequency of NR1 in similar experiments was 2.0×10^{-2}. Thus, NR1 carrying Tn*3* in *Sal*I fragment D (pRR303) has an increased transfer frequency, presumably due to the inactivation of the *finO* gene.

Another possible explanation for a high transfer frequency could be that an unusually high proportion of transposed molecules preexist in the donor population owing to an early transposition event in the growth of the population. If that is the case, for a cotransfer frequency of 6.5%, most cells should still contain NR1 molecules without a Tn*3* element. To test this possibility, isolated single colonies from a population of KP245(NR1)(RSF2124) showing a high cotransfer frequency (6.5%) were grown in liquid medium to be used as donors. Five single colonies of KP245(NR1)(pRR138) were also used to inoculate donor cultures for mating experiments. The results of these matings are given in Table 2. All cloned donors of KP245(NR1)(RSF2124) showed a cotransfer frequency of <0.2%. These data support the idea that the KP245(NR1)(RSF2124) donor showing a high cotransfer frequency is a heterogeneous population; a few cells carry NR1::Tn*3*, and most cells carry NR1 without a transposed Tn*3*. If that had not been the case, each isolate should also have a cotransfer frequency of 4 to 7%.

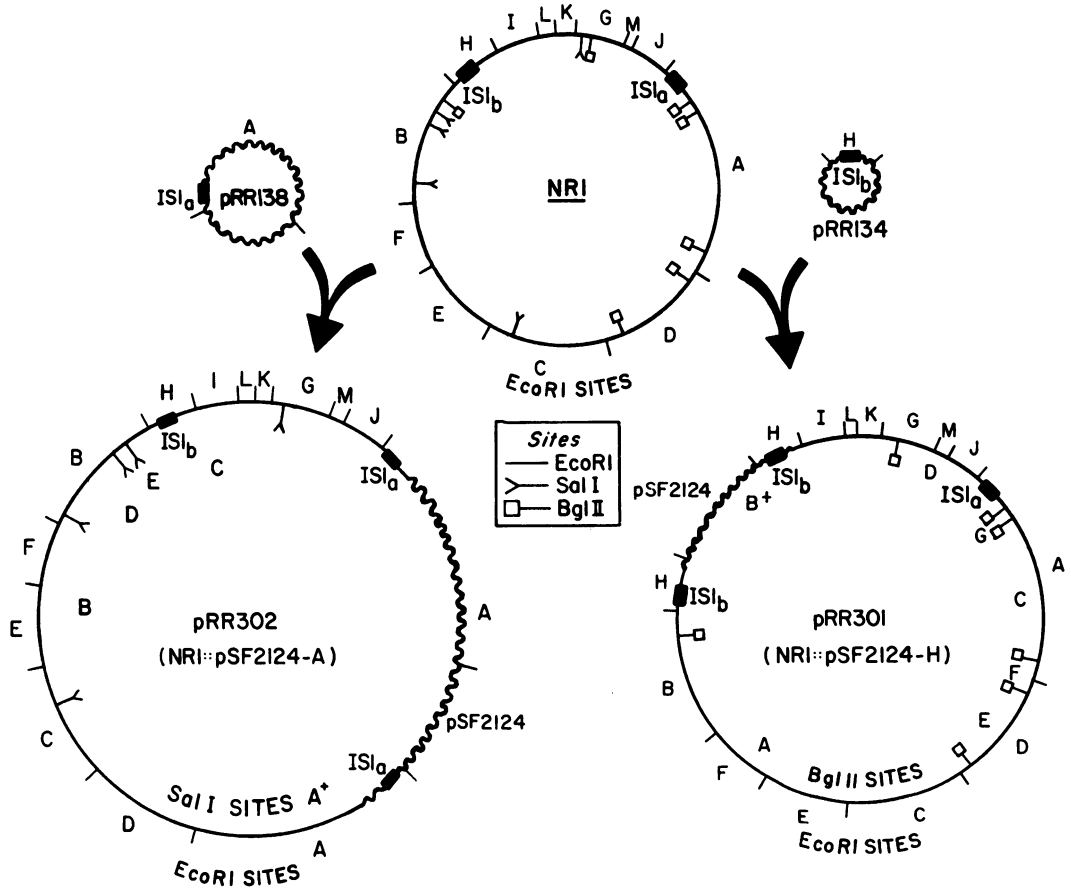

FIG. 3. Restriction maps of NR1 and the cointegrate plasmids pRR301 and pRR302. The locations of the *Eco*RI (33), *Sal*I (1), and *Bgl*II (30) restriction sites of NR1 have been previously described.

Therefore, the high cotransfer frequencies in some matings are due to both the location of the insertion of Tn*3* and the relative number of NR1::Tn*3* derivatives in the population.

The data in Table 2 also indicate that cloned donors of KP245(NR1)(pRR138) have widely varying cotransfer frequencies. This suggests that the KP245(NR1)(pRR138) used as a donor in the initial matings is also a heterogeneous population of cells with respect to their degree of cointegrate plasmid content. If this is the case, and some sort of distribution of cointegrate frequency results in an average for the population as a whole, the clones showing 60 or 6% cotransfer frequency might be expected to approach the observed steady-state average after further growth. Two cultures, one having a cotransfer frequency of 60% (culture 1) and one having a cotransfer frequency of 6% (culture 2), were subcultured three times. These cultures, along with the original cultures that had not been subcultured, were then used as donors to JG112. Culture 1 showed a cotransfer frequency of 54% without subculturing and a frequency of 34% with subculturing. Culture 2 showed a frequency of 3.4% without subculturing and 11% with subculturing. These data support the idea that the population consists of individual cells which differ in their degree of cointegrate formation, which is most likely a dynamic state.

Analysis of plasmid DNA from a donor strain. To determine whether the plasmids in the donor strains were always recombined or whether the mating in some way stimulated recombination, samples of undigested plasmid DNA isolated from cultures 1 and 2 discussed above were electrophoresed on 0.5% high-gelling-temperature agarose gels (Fig. 4). Plasmid DNA isolated from culture 1 (Fig. 4, lane 4a) has, in addition to bands corresponding to RSF2124, a wide band, presumably a doublet of NR1 and the cointegrate. The lanes labeled b in Fig. 4 show autoradiograms of the gel with ^{32}P-labeled RSF2124 used as a probe. The intensities of the bands for

TABLE 2. Cotransfer frequency with purified donors[a]

Culture	Cotransfer frequency (%) with donor:	
	KP245(NR1)(pRR138)	KP245(NR1)(pRR134)
Original	31	6.5
Isolate 1	61	0.07
Isolate 2	60	0.06
Isolate 3	62	0.08
Isolate 4	44	0.19
Isolate 5	6	0.09

[a] Five isolated colonies of the original culture were used as donors in matings to JG112 to test for population heterogeneity.

culture 1 (Fig. 4, lane 4b) were measured with a recording microdensitometer. The band corresponding to the cointegrate molecule accounted for about 15% of the radioactivity in the labeled bands. This would correspond to 1 copy of the cointegrate molecule for every 6.5 copies of pRR138. Since there are about four copies of pRR138 per NR1 in the cell (as determined by the procedure of Womble et al. [38]), this would indicate a cointegrate frequency of 55%, which agrees with all of our other results. Any band corresponding to a cointegrate molecule in culture 2 would be too faint to detect under these conditions since we would expect a low fraction of cointegrate molecules. We conclude from this that the plasmids are recombined in the donor to about the extent expected from the cotransfer frequencies, regardless of whether mating is taking place.

Recombination in the absence of replication. Strains JG112 and JG112(NR1) were transformed with RSF2124 and pRR138, selecting for ampicillin resistance. Ap^r transformants could not be detected in JG112, with a transformation frequency limit of $<1 \times 10^{-9}$ per μg of DNA. JG112(NR1) gave rise to Ap^r transformants at a frequency of 5×10^{-9} per μg of DNA when RSF2124 was the transforming DNA and at a frequency of 1.7×10^{-7} per μg of DNA when pRR138 was the transforming DNA. In contrast, the *polA*$^+$ strain KP245(NR1) was transformed to ampicillin resistance at a frequency of 4×10^{-5} per μg of DNA for each plasmid.

DISCUSSION

We have examined the extent of cointegrate formation when two plasmids sharing homology are present within the same cell. Genetic evidence of various sorts suggested that homologous recombination was involved in the cotransfer of ampicillin resistance with tetracycline resistance. Cotransfer was greater from *recA*$^+$ donors when there was more homology between the two plasmids. Matings from a *recA* donor harboring plasmids which share sequence homology resulted in cotransfer frequencies which were low compared with the *recA*$^+$ matings and were independent of the size of the cloned homology. This is evidence that homologous recombination is involved in the cotransfer of ampicillin resistance with tetracycline resistance. Where no homology existed between the two plasmids, cotransfer of ampicillin resistance was usually low and was shown to be mediated by transposition of Tn*3* onto NR1. If that transposition was into the *finO* gene of NR1, the conjugation frequency of that plasmid was elevated due to the derepression of the plasmid transfer system.

Other genetic evidence for the involvement of recombination in the cotransfer of ampicillin resistance was the loss of ampicillin resistance from transconjugants grown without selection for drug resistance. This suggested that there were cointegrate molecules with homology flanking the ampicillin resistance gene of RSF2124. The rate at which the resistance was lost was directly related to the amount of homology between the two plasmids. There was a more rapid loss of ampicillin resistance when there is more homology.

It is interesting to note that the cointegrate plasmid pRR302 (NR1::pRR138) was maintained in a cell even though it contains two copies of *Eco*RI fragment A. Fragment A contains the stability locus of NR1 (30). Evidently, two copies of this locus do not interfere with the

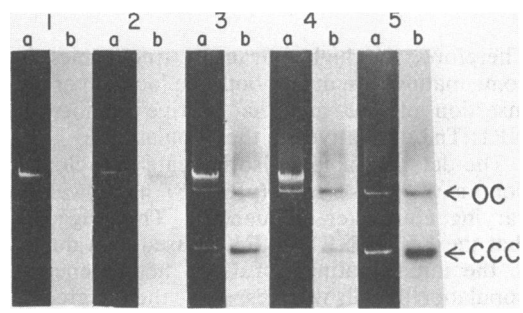

FIG. 4. Agarose gel electrophoresis and DNA-DNA hybridization of plasmids isolated from donor cells. Plasmids were isolated from stationary-phase cultures of donor cells by a cleared-lysate procedure (21) and electrophoresed on a 0.5% agarose (high-gelling-temperature agarose, Miles Laboratories) at 50 V for 6 h. The gel shows NR1 (lane 1), pRR302 (lane 2), plasmid DNA from culture 2 (lane 3) and culture 1 (lane 4), and pRR138 (lane 5). Bands corresponding to open circular (OC) and covalently closed circular (CCC) forms of pRR138 are present. Autoradiograms of the gel, made with ^{32}P-labeled RSF2124 as the probe, are shown in the b lanes.

replication of the plasmid in the same way that two copies of the replication region do (30).

Restriction analysis of plasmid DNA isolated from Ap^r transconjugants confirmed that recombination within the region of cloned homology was the major reason for the cotransfer of ampicillin resistance. No recombination took place in other regions of NR1. Since *Eco*RI fragments A and H both contain IS*1*, recombination could have occurred between pRR134 and IS*1* of *Eco*RI fragment A or between pRR138 and IS*1* of *Eco*RI fragment H of NR1. This was not seen in any of the Ap^r transconjugants tested, although in principle it could occur at a frequency lower than we would detect here.

We tested a number of factors which could potentially affect the frequency of cointegrate formation. Since ampicillin resistance is cotransferred at about the same frequency for pRR134 and pRR129, the presence of IS*1* did not appear to affect the frequency of recombination. In addition, the experiment in which pRR138 and RSF2124 were transformed to JG112 and JG112(NR1) showed that replication of the RSF2124 derivative is not necessary for recombination. The mating event did not seem to play a role in the formation of cointegrates. Cointegrate molecules were detected in the donor cells tested at approximately the frequency expected from the mating results.

Even though it is fairly clear that the degree of cointegrate formation is directly related to the amount of homology between the two plasmids, there may be other factors involved. The degree of cotransfer of pRR138 (38%) as compared with the amount of homology between the two plasmids (20.5 kb) is relatively greater than that for pRR134 (3.5% for 4.8 kb). It may be that some regions of the plasmid are more active in recombination than others. It has been shown that there is a gene on the sex factor F and on NR1 which stimulates recombination (10, 11), although the exact location of this gene on NR1 is not known.

Plasmid interactions can be very important for maintenance and conjugal transfer of the plasmids. For example, recombination and cointegrate formation between plasmids have been implicated in processes such as the mobilization of nonconjugative plasmids (3, 19). Our results suggest that plasmids may physically interact within a cell to a great extent. This implies that multiple plasmids in a cell should be carefully examined to determine their structural integrity and that even a unique plasmid of high copy number may be in a constant state of active molecular interaction.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant GM14398 from the National Institutes of Health. B.C.P. is a predoctoral trainee supported by Public Health Service training grant GM07133-07 from the National Institutes of Health.

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