Facilitated Transfer of IncPβ R751 Derivatives from the
Chromosome of Bacteroides uniformis to Escherichia coli Recipients by a Conjugative Bacteroides Tetracycline Resistance Element

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The broad-host-range IncPβ plasmid R751 can mobilize itself from Escherichia coli to Bacteroides spp, but it is not maintained in Bacteroides spp. If R751 carries the Bacteroides transposon Tn4351, it can be integrated into the Bacteroides chromosome. Previously we showed that R751, integrated in the chromosome of Bacteroides uniformis, cannot mobilize itself out of B. coli or isogenic B. uniformis strains. In this report, we showed that if the Bacteroides conjugative tetracycline resistance element Tc' ERL was coresident with the R751 insertion in B. uniformis, derivatives of R751 were transferred to E. coli, where they were recovered as plasmids. The most common derivatives were R751::Tn4351 and R751::IS4351, but some strains transferred R751 derivatives, containing additional DNA segments ranging in size from 10 to 23 kilobases. These DNA inserts cross-hybridized with chromosomal DNA from B. uniformis which did not carry the Tc' ERL element. Therefore, the inserts appeared to be segments of the wild-type B. uniformis chromosome and were not associated with the Tc' ERL element. The transfer of integrated R751 from B. uniformis was independent of the RecA phenotype of the E. coli recipients and did not appear to be due to transfer of B. uniformis chromosomal DNA, followed by RecA-dependent recombination between homologous IS4351 sequences to form the resultant R751 plasmid derivatives. Consistent with this, no transfer of Tn4351 (associated with the cointegrated R751) from B. uniformis donors to isogenic B. uniformis recipients was detected (<10⁻⁸). Our data support the hypothesis that R751 excises from the B. uniformis chromosome by recombination involving flanking Tn4351 or IS4351 sequences and forms nonreplicating circles. The mobilization of these circular forms out of B. uniformis to E. coli is then facilitated by the Tc' ERL element.

Human colonic Bacteroides species are only distantly related phylogenetically to Escherichia coli (17). Several laboratories have demonstrated that antibiotic resistance genes from Bacteroides spp. apparently do not confer resistance on E. coli and vice versa (5, 6, 11, 14, 16). Similarly, naturally occurring plasmids from Bacteroides spp. do not replicate in E. coli, and the broad-host-range IncP and IncQ plasmids that replicate in E. coli and related gram-negative bacteria are not maintained in Bacteroides spp. (6, 14-16). However, Bacteroides spp. and E. coli can exchange DNA by conjugal transfer. Chimeric Bacteroides-E. coli shuttle vectors have been constructed that can be mobilized by IncP plasmids (RK2 and R751) from E. coli donors to Bacteroides recipients at frequencies as high as 10⁻³ per recipient (6, 15, 16). The IncP plasmids can mobilize these chimeric vectors either from the mobilization regions contained on the E. coli plasmid portion of the chimeric shuttle vectors (RK2 or RSF1010) or from mobilization regions contained on the component Bacteroides plasmids (pBFTM10 or pBB8-51 [15]). In addition, both pBFTM10 and pBB8-51 contain regions that allow chimeric vectors containing them to be mobilized from B. uniformis donors either to other Bacteroides strains or to E. coli by a chromosomally located conjugative Bacteroides tetracycline resistance element, Tc' ERL (15). Thus, reciprocal genetic exchange by conjugation is possible between E. coli and Bacteroides spp.

Previously, we have shown that derivatives of the IncPβ plasmid R751 which contain the composite Bacteroides transposon Tn4351 can be transferred from E. coli into Bacteroides uniformis and integrated into the Bacteroides chromosome by a transposon-mediated insertion event and that 0.1 to 1% of the transconjugants were auxotrophs (14). R751::Tn4351, integrated in the B. uniformis chromosome, was not transferred by conjugation, but some were able to mobilize a coresident shuttle vector, pE5-2, out of the Bacteroides host into E. coli or Bacteroides recipients (14). In this study, we show that plasmid derivatives of R751 from R751::Tn4351 integrated in the B. uniformis chromosome can be mobilized out of B. uniformis to E. coli recipients if the Bacteroides conjugative tetracycline resistance element Tc' ERL is coresident in the B. uniformis donors.

MATERIALS AND METHODS

Bacterial strains and plasmids. E. coli strains HB101 (F⁻, hsdS20 [rB⁻ mB⁻] recA13 ara-14 proA2 lacY galK2 rpsL20 [Str²] xyl-5 mtl-1 supE44) (2) and SF8 (hsdR mfd recB recC lop-11 supE44 gal-96, rpsL20 [Str²] leuB6 thi-1 thr) (4) were used as donors or recipients in conjugal matings with B. uniformis. The Bacteroides strains used were all derivatives of the B. uniformis type strain, 0061. BU1001 is a spontaneous rifampin-resistant (Rff) mutant of 0061. BU1004 is a tetracycline-resistant (Tc') transconjugant of BU1001 from B. fragilis ERL and contains the integrated conjugative tetracycline resistance element Tc' ERL. Tc' ERL transfers itself between Bacteroides strains and mobilizes several chimeric shuttle vectors to both Bacteroides and E. coli recipients (15).

The plasmids used in this study are described in the text with the exception of pBR328::IS4351. pBR328::IS4351 contains one copy of IS4351 and is a resolution product from a cointegrate of R751::Tn4351/18 (14; see Fig. 1) and pBR328 (3). The IS4351 in pBR328::IS4351 mediates plasmid cointe-
TABLE 1. Mating conditions for transfer of R751 derivatives from B. uniformis (BU1004) donor (D4-3) to E. coli (SF8) recipients

<table>
<thead>
<tr>
<th>Incubation condition*</th>
<th>Growth in tetracycline+</th>
<th>Frequency of SF8 transconjugants*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*Tc' *Tp'</td>
<td>Tp'</td>
</tr>
<tr>
<td>Aerobic</td>
<td>1.0 × 10^8</td>
<td>&lt;1.5 × 10^-9 (6/70)</td>
</tr>
<tr>
<td>+</td>
<td>4.6 × 10^-7</td>
<td>5 × 10^-9 (2/160)</td>
</tr>
<tr>
<td>Anaerobic</td>
<td>8 × 10^-9</td>
<td>&lt;4 × 10^-9 (9/22)</td>
</tr>
<tr>
<td>+</td>
<td>4.2 × 10^-6</td>
<td>&lt;2 × 10^-8 (2/211)</td>
</tr>
</tbody>
</table>

* Tp', Trimethoprim resistance from R751 (Tn4351); *Tc', tetracycline resistance on Tn4351 that expresses only in aerobically grown E. coli (6, 16).
+ The donors and recipients were grown to 5 × 10^9 CFU/ml. Donor/recipient ratios for aerobic and anaerobic matings were 5:1 and 1:1, respectively.
+ The filters were incubated overnight at 37°C and the mating mixtures were treated as previously described (15).
+ Donors were grown with (+) or without (−) 1 μg of tetracycline per ml in the medium prior to filter mating.
+ Frequency is the number of transconjugants per recipients at the end of the mating. E. coli recipients were 1.3 × 10^9 CFU/ml after aerobic mating and 1.5 × 10^9 CFU/ml after anaerobic mating.
+ Given in parentheses is the number of Tp' only colonies/total number of Tp' plus *Tc' Tp' colonies.

Grate formation in RecA- and RecA+ E. coli (N. B. Shoemaker, unpublished data). pBR328::IS4351 was used as the probe in Southern blots to detect the location and numbers of IS4351 sequences in B. uniformis chromosomal DNA and in the R751 derivatives transferred from B. uniformis to E. coli.

Growth and mating conditions. B. uniformis strains were grown on prereduced Trypticase (BBL Microbiology Systems)-yeast extract-glucose (TYG) broth or TYG agar plates as described previously (14). E. coli strains were grown in Luria broth or on Luria broth or TYG agar plates.

Nitrocellulose mating filters (Millipore Corp.) for matings between E. coli donors and B. uniformis recipients were incubated at 37°C, aerobically, on TYG agar, at donor/recipient ratios of 1:5 as described previously (16). The mating conditions for transfer of R751 derivatives out of B. uniformis BU1004 donors to E. coli recipients are shown in Table 1. The concentrations of the antibiotics used in the selection of the transconjugants were as follows: for E. coli transconjugants selected on Luria broth agar and incubated aerobically, tetracycline (10 μg/ml) for Tn4351 (*Tc') and trimethoprim (200 μg/ml) for R751 derivatives (Tp'); for B. uniformis transconjugants selected on TYG agar and incubated anaerobically, tetracycline (3 μg/ml) for Tc' ERL (Tc'), erythromycin (10 μg/ml) for Tn4351 (Em'), and gentamicin (400 μg/ml) to select against E. coli donors (Gen').

DNA isolation, agarose gels, and Southern blots. Plasmids were isolated from E. coli and B. uniformis by the Ish-Horowitz modification of the alkaline lysis procedure as described by Maniatis et al. (8). Chromosomal DNA was prepared by the procedure of Saito and Muira (12).

Restriction endonuclease digestions of plasmids and chromosomal DNA were done according to manufacturers' suggestions or as described in Maniatis et al. (8). Agarose gel electrophoresis and Southern blot analysis was done as described previously (16).

RESULTS

Transfer of R751 derivatives out of the B. uniformis chromosome into E. coli. The salient features of R751::Tn4351/Δ8, are shown and described in Fig. 1. R751::Tn4351*Δ4 contains a partially duplicated Tn4351, including a second copy of the drug resistance determinants (Em' and *Tc') and an additional IS4351 in the same SalI fragment as R751Δ8 (14). R751::Tn4351*Δ4 is unstable in RecA+ E. coli strains, where it is converted to R751Δ8, but it is apparently stable in RecA- hosts. Both R751Δ8 and R751*Δ4 function as suicide vectors which form R751::Tn4351 insertions in the B. uniformis chromosome (14). We will designate R751::Tn4351Δ8.

FIG. 1. Tn4351 inserted in IncP8 R751, R751::Tn4351/Δ8. The salient features of Tn4351 are indicated at the top. Two 1.1-kb directly repeated sequences sequenced by J. Rasmussen (unpublished results) are designated IS4351 due to their ability to cause cointegrate formation in Bacteroides spp. (14; this study) and in E. coli (Shoemaker, unpublished data). The two IS4351 elements flank two drug resistance determinants: a chloramphenicol-erythromycin resistance (Co'-Em') which expresses only in Bacteroides spp. (6, 10, 16) and a tetracycline resistance (*Tc') which is cryptic in Bacteroides spp. but is expressed in aerobically grown E. coli (5, 11, 16). The two EcoRI sites which define a 3.8-kb fragment containing 98% of one IS4351, the drug resistance determinants, and the HindIII site on Tn4351 are shown. Tn4351 is inserted in a 0.7-kb SalI fragment of R751 (14) as indicated by the arrow. R751::Tn4351*Δ4 contains a partial duplication of Tn4351 inserted in the same SalI fragment (14). The modified restriction map of R751 and the tentative locations of the R751 genes are from Meyer and Shapiro (9) and Smith and Thomas (submitted).
insertions in the Bacteroides chromosome as \( \Omega R751::Tn4351 \).

Although R751::Tn4351 insertions in the chromosome of B. uniformis appear to be rare (14), we wanted to know if the transfer defects of the R751 insertions could possibly be complemented by one of the conjugal Bacteroides elements. A logical candidate to try was the integrated, conjugative tetracycline resistance element Tc\(^r\) ERL. We have shown previously that this element mobilizes certain Bacteroides- E. coli chimeric shuttle vectors from Bacteroides donors to Bacteroides or E. coli recipients (15). The Tc\(^r\) ERL element appears to be integrated in the Bacteroides chromosome, but it is not known whether or not the insertion site, if it exists, is unique. Therefore, we made the different R751::Tn4351 insertions in the same B. uniformis transconjugant containing Tc\(^r\) ERL, BU1004, to provide as isogenic a background as possible.

The R751::Tn4351 insertions were confirmed by Southern blot analysis of the BU1004 transconjugants, and all appeared to have different junction fragments mediated by IS4351 and Tn4351. Several of the transconjugants were auxotrophic (14; described below). Thus, the insertions appear to be in different regions of the chromosome.

Nine BU1004::R751::Tn4351 donors were mated with isogenic B. uniformis recipients by methods described previously (14–16). No transfer of Em\(^r\) (from Tn4351) was detected (<10\(^{-8}\)), although the transfer frequencies of Tc\(^r\) ERL itself were normal (10\(^{-6}\) to 10\(^{-7}\)). Since the trimethoprim resistance (Tp\(^r\)) on R751 does not function in Bacteroides spp., we could not follow the transfer of R751 directly.

However, when the BU1004::R751::Tn4351 donors were mated with an E. coli recipient, SF8 (RecA\(^+\)) or HB101 (RecA\(^-\)), several of them transferred Tp\(^r\) or Tc\(^r\) Tp\(^r\) to the recipients. The results of an experiment with donor BU1004 D4-3 and recipient SF8 to determine the optimal conditions for the transfer of Tp\(^r\) or Tc\(^r\) are shown in Table 1. The frequency of HB101 (RecA\(^-\)) transconjugants was slightly lower (two- to threefold), but it was similar to the difference observed for the transfer of shuttle vectors from BU1004 donors (15) and is apparently not due to the RecA\(^-\) phenotype of the recipient. The Tc\(^r\) Tp\(^r\) phenotype was transferred 100 times more frequently than Tp\(^r\) alone and both phenotypes were associated with large (>50-kilobase [kb]) plasmids. This indicated that Tn4351 (Tc\(^r\)) cotransferred >99% of the time with R751 (Tp\(^r\)). The analysis of the transconjugants is given below. Pregrowth of BU1004 D4-3 in tetracycline (1 \(\mu\)g/ml) enhanced the transfer frequencies at least 50-fold. We have shown previously that pregrowth in tetracycline also increases both the transfer of Tc\(^r\) ERL to Bacteroides recipients and the mobilization of shuttle vectors to E. coli or Bacteroides recipients by 50- to 100-fold (15). Anaerobic mating conditions also increased the transfer frequency (transconjugants per recipients) 10-fold, although the absolute number of transconjugants per milliliter was nearly the same for anaerobic and aerobic conditions (Table 1). All subsequent matings were done with BU1004 donors pregrown in 1 \(\mu\)g of tetracycline per ml and the mating filters were incubated anaerobically.

We compared the transfer frequencies and the patterns of the drug resistances transferred from nine independently isolated donors which, by Southern blot analysis, had R751::Tn4351 inserted in different sites. BU1004 donors DI-R5 and XK-1 were formed from R751::Tn4351 and the other seven R751::Tn4351 insertions were from R751::Tn4351. Four of the strains are insertional auxotrophs: DI-R5 and DI-29 are methionine requirers, DI-2 requires additional filtered yeast extract, and DI-3 requires several amino acids (14). The results of matings to SF8 are shown in Table 2.

Not only the frequency of transfer, but also the pattern of the drug resistances transferred varied among the strains (Table 2). Three of the strains (D4-1, D4-3, and DI-R5) transferred Tc\(^r\) Tp\(^r\) at much higher frequencies than the other strains. Four strains (D4-6, DI-2, DI-3, and DI-29) transferred Tc\(^r\) Tp\(^r\) at low but detectable frequencies. One strain (D4-2) did not transfer either of the drug resistances at detectable frequencies, and one strain (XK-1) transferred Tc\(^r\) but not Tp\(^r\), at a very low frequency.

Analysis of the R751 derivatives isolated from the E. coli transconjugants. All of the E. coli Tp\(^r\) transconjugants tested contained plasmids larger than R751. Structures of different classes of these plasmids were determined by restriction enzyme and Southern blotting analysis. Some typical results are shown in Fig. 2. With one exception (DI-2) the Tc\(^r\) Tp\(^r\) plasmids, which comprised the largest transconjugant class, were indistinguishable from R751::Tn4351 (Fig. 1; Fig. 2A and B, lane 1). The second largest group of transconjugants were Tp\(^r\) only and contained R751, with one IS4351 insertion.
removing the \( \Omega \)8 site of R751 (Fig. 2A and B, lane 7). Neither the \(*Tc\)' \( Tp \)' plasmids nor the \( Tp \)' plasmids of these two groups had any apparent deletions of R751 sequences or additions of \( B. \) \( uniformis \) DNA. \( B. \) \( uniformis \) DI-3 transferred predominantly R751::\( IS4351 \), whereas four of the donors in Table 2 transferred R751::\( IS4351 \) at higher frequencies than R751::\( IS4351 \). Two donors, D-4-1 and X-1, transferred \(*Tc\)' (\( Tn4351 \)) with no detectable plasmid DNA. The \(*Tc\)' on \( Tn4351 \) was inserted in the chromosome of the \( E. \) \( coli \) recipients and was detectable by Southern blot analysis of \( EcoRI \)-digested DNA (data not shown). In one experiment, \( E. \) \( coli \) HB101 containing \( RSF1010 \) (1) was used as the recipient in matings with \( B. \) \( uniformis \) X-1, and \( Tn4351 \) was found inserted in the sulfonamide resistance gene of \( RSF1010 \) (data not shown).

**R751 derivatives containing \( B. \) \( uniformis \) DNA.** Two of the BU1004\( \Omega \)8R751::\( Tn4351 \) donors, D-4-6 and D-2, transferred R751 derivatives which contained 6 to 22 kb of additional DNA in the \( Tn4351 \)\( \Omega \)8 site, the 0.7-kb \( SalI \) fragment, of R751 (Fig. 1; data not shown). Four R751 (\(*Tc\)' \( Tp \)') derivatives with inserts of approximately 6, 12, 14, and 22 kb, according to \( SalI \) digestions, were isolated from \( E. \) \( coli \) transconjugants from donor D-4-6. The \( EcoRI \) digest of the plasmids in these transconjugants are shown in Fig. 2A, lanes 3, 4, 5, and 6, respectively. The Southern blot of the gel shown in Fig. 2A was probed with \( ^{32}P \)-labeled pBR328::\( IS4351 \) to determine the location and the number of \( IS4351 \) copies (Fig. 2B). \( EcoRI \)-digested R751::\( IS4351 \), shown in lane 7, has one \( IS4351 \) copy in a 5-kb \( EcoRI \) fragment. Note that all of the R751 derivatives have a 5-kb fragment. Three of the four R751 + insert derivatives, isolated from donor D-4-6, have an additional \( EcoRI \) fragment which hybridizes to the \( IS4351 \) probe. One isolate (lane 3) may have two large additional hybridizing bands. The R751 + inserts in lanes 4 to 6 also have an additional \( EcoRI \) band which does not hybridize to the \( IS4351 \) probe and which comigrates with either a 12-kb \( EcoRI \) band (lane 4) or a 9.2-kb \( EcoRI \) band (lanes 5 and 6) fragment that is also present in the R751::\( IS4351 \) derivatives (lane 7). Thus, the extra DNA inserted in the \( \Omega \)8 site of R751 appears to be flanked by \( IS4351 \) (two copies) and to have one \( EcoRI \) site between the two \( IS4351 \) copies.

The donor DI-2 transferred R751 derivatives (\(*Tc\)' \( Tp \)') which had 10-kb inserts in the \( \Omega \)8 site and which still contained \( Tn4351 \) (\(*Tc\)'). The \( EcoRI \) digestion of this R751::\( Tn4351 \) + insert (lane 2) resembled the R751::\( Tn4351 \)\( \Omega \)8 digestion pattern (lane 1). However, there were three \( EcoRI \) bands that hybridized to the \( Tn4351 \) probe: the 3.8-kb \( EcoRI \) band of \( Tn4351 \) (Fig. 1), the 5-kb \( IS4351 \) band seen for both R751::\( Tn4351 \)\( \Omega \)8 (lane 1) and R751::\( IS4351 \) (lane 7), and a third 12-kb \( EcoRI \) band that comigrates with the 12-kb \( EcoRI \) fragment of R751 (lane 7). Thus, the inserted material is probably flanked by \( IS4351 \) and \( Tn4351 \).

**Source of the extra DNA inserted in the R751 derivatives.** The largest R751 + insert derivative (Fig. 2, lanes 6) from D-4-6, designated R751-INS1, was nick translated and used to probe a Southern Blot containing (i) \( HindIII \) digests of two of the smaller R751 + insert derivatives from D-4-6 (Fig. 2, lanes 4 and 5) and (ii) \( EcoRI \) and \( HindIII \) digests of the chromosomal DNA of \( B. uniformis \) (BU1001) and of BU1001 containing the conjugalative \( Tc ' ERL \) element (BU1004) (Fig. 3). The smaller R751 + 10- to 12-kb insert derivatives gave \( HindIII \) banding patterns which were different from each other (Fig. 3, lanes 7 and 8) and from R751-INS1 (Fig. 3, lane 4). All of the \( HindIII \) bands of the smaller insert derivatives
hybridized to the probe. A duplicate Southern blot probed with \(^{32}\)P-labeled R751 (not shown) identified bands which were entirely inserted material and did not contain R751 sequences. These bands are indicated by arrows.

The R751-INS1 probe hybridized to the chromosomal DNA from both BU1001 and BU1004 (BU1001 containing Tc\(^{r}\) ERL). There was one homologous 14- to 16-kb EcoRI band in the chromosomal DNA of both BU1001 (lane 1) and BU1004 (lane 2). The HindIII-digested chromosomal DNAs of BU1001 (lane 5) and BU1004 (lane 6) have three bands which have strong homology to the inserted material of R751-INS1 (lane 4), but only one of these chromosomal bands appears to be identical in size to the inserted material of R751-INS1. The inserted material in R751-INS1 may contain deletions or rearrangements of the original B. uniformis DNA. Since the cross-hybridizing DNA is present in the wild-type B. uniformis (BU1001), it is not DNA from the Tc\(^{r}\) ERL element.

The SF8 transconjugants isolated from matings with donor DI-2 all contained R751 derivatives with inserts of about 10 kb of additional DNA and had the same restriction enzyme digestion patterns as the example shown in Fig. 2, lane 2. The inserted DNA from one of these R751 derivatives also hybridized to the chromosomal DNA of both BU1001 and BU1004 (data not shown).

Loss of integrated Tn4351 or R751::Tn4351 leaves a copy of IS4351 in the chromosome. Analysis of three BU1001 Em\(^{r}\) transconjugants is shown in Fig. 4A: 4-8 is an insertion of R751::Tn4351 and 1-1 and 2-4 are Tn4351 insertions. The Southern blot of the chromosomal DNAs of the Em\(^{r}\) transconjugants (R) and one to three Em\(^{r}\) isolated (S1 to S3) of each, digested with EcoRI, was probed with pBR328::IS4351 to determine the number of Tn4351 or IS4351 copies. The characteristic 3.8-kb EcoRI fragment, containing the Cc\(^{r}\)-Em\(^{r}\) and *Tc\(^{r}\) determinants and one IS4351 or Tn4351, is indicated at the left of Fig. 4A and by arrows within the R lanes. All of the Em\(^{r}\) isolates have one or more bands that hybridize to the IS4351 probe, indicating that the loss is due to homologous recombination between the directly repeated IS4351 copies. In Fig. 4B, a Southern blot of the 4-8 R and 4-8 (S1-S3) isolates was also probed with \(^{32}\)P-labeled R751 to determine if the 4-8 Em\(^{r}\) isolates still contained the R751 sequences inserted in the chromosome. Approximately 10% of the 4-8 Em\(^{r}\) isolates also lost the R751 sequences (e.g., 4-8 S2 in Fig. 4B). The marks at the left are the locations of the EcoRI bands of the R751 plasmid, and the arrow indicates the location of the 3.8-kb EcoRI internal fragment of Tn4351 which does not hybridize to the R751 probe. The Em\(^{r}\) isolates which still contain R751 sequences have two bands which hybridize to the IS4351 probe and which are presumably IS4351 copies flanking the inserted R751. The isolate which has lost R751 and Tn4351, 4-8 S2, still has one copy of IS4351. The loss of Tn4351 (Em\(^{r}\) to Em\(^{r}\)) or the loss of the entire \(\Omega\)R751::Tn4351 appeared to occur by recombination between the flanking IS4351 copies which should produce a circular intermediate.

Attempts to isolate circular intermediates. Several attempts were made to detect circularized R751::IS4351 or R751::Tn4351 in the BU1004 donors. Plasmid preparations of tetracycline-induced D4-1 and D4-3 were made from 10\(^{10}\)
cells, and the lysates were digested with *SalI* or *HindIII* and probed with R751::Tn4351Ω to detect R751 sequences. R751 sequences were detected. However, the IS4351 chromosomal junction fragments were also detected, indicating chromosomal contamination. All attempts to detect R751 plasmid forms in these lysates by transforming competent *E. coli* or by CsCl gradients were also unsuccessful.

**Transfer of R751::IS4351 from Em*<sup>+</sup> isolates of D4-3.** D4-3 transferred R751::Tn4351 10 times more frequently than R751::IS4351. To determine if the intact Tn4351 influenced the transfer frequency of R751::IS4351, three Em<sup>+</sup> isolates of D4-3 which still contained R751 flanked by IS4351 were tested for transfer of R751::IS4351. The transfer frequency of R751::IS4351 from these Em<sup>+</sup> isolates was within the same range (0.3 × 10<sup>-7</sup> to 5 × 10<sup>-7</sup>) as seen for the transfer of R751::IS4351 by the original donor, D4-3 (Tables 1 and 2), but the frequency was still 10-fold lower than the transfer of R751::Tn4351.

**Cause of low transfer frequencies.** All of the BU1004ΩR751::Tn4351 donors, by Southern blot analysis (e.g., Fig. 4B), appeared to have all of the *EcoRI* and *SalI* fragments of R751, which indicated that large deletions or rearrangements of the inserted R751 sequences were not occurring in the donors. Donor D4-6 transferred R751 derivatives at very low frequencies (Table 2) to *E. coli* recipients. Low transfer frequencies could be due to small rearrangements or deletions in R751 not detectable by Southern blot analysis or due to mixed cultures caused by instability of the original R751::Tn4351 insertion. To test these two possibilities, five single-colony isolates of D4-6 were selected and used as donors in matings with *E. coli*. All five D4-6 isolates transferred R751::Tn4351 or R751::IS4351 at very low frequencies (10<sup>-9</sup> to 10<sup>-8</sup>). The R751 derivatives in the *E. coli* transconjugants appeared to be normal in *E. coli* in their conjugal functions and transferred at frequencies of 0.5 to 1.0 to *E. coli* recipients.

**Mobilization of cosmid vectors by R751 integrated in the *B. uniformis* chromosome.** We have shown previously that R751 integrated in the *B. uniformis* chromosome could not mobilize chimeric vectors from the origin of transfer on *E. coli* plasmid RSFl010 (14). We found in this study that pDP1, a chimeric shuttle vector which contains the *Bacteroides* plasmid pBFTM10, also could not be mobilized out of *Bacteroides* donors by ΩR751, although pDP1 is readily mobilized by R751 in *E. coli* hosts and by Tc<sup>c</sup> ERL in *Bacteroides* hosts (15).

**DISCUSSION**

The facilitated transfer of the R751 derivatives by the Tc<sup>c</sup> ERL element to *E. coli* recipients was independent of the RecA phenotype of the recipient and probably did not occur...
due to chromosomal transfer. Plasmid cointegrates formed by IS4351 and Tn4351 in *E. coli*, like those reported by Robillard et al. (11) for the nearly identical Tn4400 and IS4400 (7, 13), resolve very inefficiently if all in RecA+ *E. coli* hosts (N. Shoemaker and V. Hwa, unpublished data). Unless we assume that the Tc\textsuperscript{r} ERL element transfers a functional resolvase to the *E. coli* recipients which recombines the R751 derivatives out of the transferred material, it seems more likely that the R751 derivatives were transferred as intact plasmids.

Our suggested model for the mechanism of transfer of the R751 derivatives by Tc\textsuperscript{r} ERL is that R751::IS4351 or R751::Tn4351 circular intermediates are formed by recombination between the flanking IS4351 copies in the *B. uniformis* donors and these circular intermediates are then transferred to the *E. coli* recipients. Several attempts were made to determine if circular R751 intermediates were in fact made. No circular intermediates were unequivocally detected. The large size of R751 (51 kb) may be part of the problem since chromosomal contamination would interfere with visual detection on agarose gels and large plasmids (>50 kb) do not transform *E. coli* efficiently.

Although no R751 circular intermediates were detected in the *B. uniformis* donors, the resultant R751 derivatives isolated from the majority of the *E. coli* transconjugants, R751::Tn4351 and R751::IS4351, strongly support the circular intermediate model. What the model does not answer is why R751::Tn4351 derivatives are transferred at 100 times higher frequencies than R751::IS4351 from four BU1004 donors (Table 2). One would predict a ratio closer to 1:1. It is also not clear why some of the donors (e.g., D4-2) do not transfer any R751 derivatives to *E. coli*, although all of the R751 sequences by Southern blot analyses appeared to be intact and the coresident Tc\textsuperscript{r} ERL element was capable of self-transfer. The low transfer seen for D4-6 was not due to mixed cultures containing rearrangements or defects in R751, since separate D4-6 single-colony isolates exhibited the same low transfer frequency. The R751 derivatives isolated appeared normal in their transfer functions in *E. coli*. It is possible that the defect is due to some function other than self-transfer genes of Tc\textsuperscript{r} ERL or to the chromosomal insertion site of \(\Delta\)R751::Tn4351 in D4-6.

IncP plasmids have been used in other systems to produce plasmids plus chromosomal DNA, called R primes, to clone regions of interest flanking the inserted plasmid. In our case, the prime production was very inefficient. Two strains gave R751 derivatives containing *B. uniformis* DNA, but we did not detect any primes from the other donors (<2% of the transconjugants). If prime production requires a secondary insertion of IS4351 in a site near \(\Delta\)R751::Tn4351, it could be very site specific and could also explain the series of related insertions seen for D4-6, as a cluster of such sites (Fig. 2). At this time, prime production does not offer a reliable method for cloning DNA flanking the R751::Tn4351 insertions, but it still may work in special cases.

The mechanism of transfer of the R751 derivatives out of *B. uniformis* is not yet known. We have shown that the transfer genes on R751 necessary to mobilize the shuttle vector pDP1 are either not functional or not produced in high enough concentrations in the *Bacteroides* hosts and that R751 does not mobilize itself out of *Bacteroides* spp. (14; this study). When the conjugative Tc\textsuperscript{r} ERL element was coresident, however, transfer of R751 derivatives did occur. We could not detect any evidence that would indicate that the R751 derivatives were transferred due to cointegrate formation with the responsible conjugative element. Therefore, at this time we believe the R751 derivatives are being mobilized from regions on the R751 plasmid by Tc\textsuperscript{r} ERL. We are in the process of determining whether the R751 origin of transfer and mobilization genes are being recognized for transfer by Tc\textsuperscript{r} ERL. These genes are only tentatively located on R751 at this time (C. Thomas and C. Smith, submitted for publication).

Formation of Em\textsuperscript{r} (Tn4351) *B. uniformis* transconjugants from BU1004 donors containing integrated R751::Tn4351 would require transfer of the R751::Tn4351 derivatives followed by transposition of Tn4351 or cointegration of R751::Tn4351 into the *B. uniformis* chromosome. Although we did not detect transfer of Tn4351 or R751::Tn4351 from the BU1004 donors to the *B. uniformis* recipients, we assume that it probably does occur, but at frequencies <10\textsuperscript{-6}.

In this study we have shown how an integrated cryptic element, R751, cointegrated in the *B. uniformis* chromosome, under appropriate conditions, can be "conjugated" into *E. coli*, where it can then be replicated and where its genes can be expressed. There appear to be specific interactions between the conjugal transfer systems of certain *E. coli* and *Bacteroides* plasmids. We have shown previously that IncPa, IncPB, and IncX plasmids can mobilize shuttle vectors containing the *Bacteroides* plasmids pB8-51 and pBFTM10 (pCP1) from *E. coli* donors to *E. coli* or *Bacteroides* recipients (15). In this study the *Bacteroides* conjugative element Tc\textsuperscript{r} ERL, which mobilizes shuttle vectors from *Bacteroides* donors to *Bacteroides* or *E. coli* recipients, was instrumental in the transfer of R751 derivatives to *E. coli* recipients. These results provide further support that genetic exchange between *E. coli* and *Bacteroides* spp., which are almost mutually exclusive in plasmid replication and gene expression, could and probably does occur in vivo. *Bacteroides* spp. could function as reservoirs of antibiotic resistances for *E. coli* or other enteric organisms or vice versa. How much genetic exchange actually occurs among the genera in the human colon is of interest but is not yet known. This study does emphasize the possibility of silent antibiotic resistance reservoirs which could be clinically important and very difficult to detect.

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ADDENDUM

We did detect transfer of Em\textsuperscript{r} from several BU1004 \(\Delta\)R751::Tn4351 donors to a *B. uniformis* recipient at frequencies of 1 × 10\textsuperscript{-9} to 3 × 10\textsuperscript{-9}. The Em\textsuperscript{r} transferred independently from Tc\textsuperscript{r} ERL, and only Tn4351 insertions (no R751 sequences) were found inserted in the chromosomes of the recipients.

LITERATURE CITED


