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Location and Characteristics of the Transfer Region of a *Bacteroides* Conjugative Transposon and Regulation of Transfer Genes

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Many *Bacteroides* clinical isolates contain large conjugative transposons, which excise from the genome of a donor and transfer themselves to a recipient by a process that requires cell-to-cell contact. It has been suggested that the transfer intermediate of the conjugative transposons is a covalently closed circle, which is transferred by the same type of rolling circle mechanism used by conjugative plasmids, but the transfer origin of a conjugative transposon has not previously been localized and characterized. We have now identified the transfer origin (*oriT*) region of one of the *Bacteroides* conjugative transposons, Tc^{r}Em^r DOT, and have shown that it is located near the middle of the conjugative transposon. We have also identified a 16-kbp region of the conjugal transposon which is necessary and sufficient for conjugal transfer of the element and which is located near the *oriT*. This same region proved to be sufficient for mobilization of coresident plasmids and unlinked integrated elements as well as for self-transfer, indicating that all of these activities are mediated by the same transfer system. Previously, we had reported that disruption of a gene, *rteC*, abolished self-transfer of the element. *rteC* is one of a set of *rte* genes that appears to mediate tetracycline induction of transfer activities of the conjugative transposons. On the basis of these and other data, we had proposed that RteC activated expression of transfer genes. We have now found, however, that when the transfer region of Tc^{r}Em^r DOT was cloned on a plasmid that did not contain *rteC* and the plasmid (pLYL72) was tested for transfer out of a *Bacteroides* strain that did not have a copy of *rteC* in the chromosome, the plasmid was self-transmissible without tetracycline induction. This and other findings suggest that RteC is not an activator of transfer genes but is stimulating transfer in some other way.

Conjugative transposons are integrated elements that can excise themselves from the genome in which they are integrated, transfer themselves by conjugation into a recipient cell, and integrate into the recipient's genome (1, 3, 14, 18). Conjugative transposons have been found in a variety of species of gram-positive and gram-negative bacteria. The best studied of these conjugative transposons are Tn*916*, the closely related Tn*1545*, and the *Bacteroides* conjugative transposons. The *Bacteroides* conjugative transposons are much larger than Tn*916* (18 kbp) and Tn*1545* (25 kbp). Although the *Bacteroides* conjugative transposons range in size from 65 to over 150 kbp, most of them are in the 70- to 80-kbp range (1). All but one of the *Bacteroides* conjugative transposons characterized to date carry a tetracycline resistance (Tc^r) gene, *tetQ*, and some also carry an erythromycin resistance (Em^r) gene, *ermF* (16, 18). In the case of Tn*916* and Tn*1545*, the transposition intermediate has been shown to be a covalently closed circle (14, 15). It has been suggested that this circular form is also the transfer intermediate (15), but the transfer origin (*oriT*) has not yet been located on either Tn*916* or Tn*1545*. There is indirect evidence that the *Bacteroides* conjugative transposons also have a circular transfer intermediate (1, 16), but the circular form has not been isolated. We have now localized the *oriT* region of *Bacteroides* conjugative transposon Tc^{r}Em^r DOT and have shown that it is internal, as expected if the transfer intermediate is a circle and is transferred by a rolling-circle-type mechanism.

In addition to transferring themselves, *Bacteroides* conjugative transposons can mobilize coresident plasmids, both in *trans* and in *cis*, and they can excise and transfer in *trans* unlinked integrated elements called NBUs (stands for nonreplicating *Bacteroides* units) (9, 17, 19, 21, 27). The transfer intermediate of the NBUs is a covalently closed circle with an internal *oriT* (9). Although it seemed likely that coresident plasmids and NBU circle forms are transferred through the same mating pore as that used by the conjugative transposon itself, the large size of the *Bacteroides* conjugative transposons raised the possibility that there might be more than one set of transfer genes, one for coresident plasmids and NBU circle forms and one for the conjugative transposon itself. In this report, we present evidence that there is a single transfer region, which is located near the *oriT* and mediates transfer of the conjugative transposon, coresident plasmids, and NBU circle forms.

Unexpectedly, as a result of work to localize the transfer region of the conjugative transposon, we uncovered new information about how transfer genes on the conjugative transposon are regulated. Previously, we had found that transfer activities of the *Bacteroides* conjugative transposons are stimulated 100- to 1,000-fold by tetracycline and that tetracycline enhancement of these activities is mediated by at least three regulatory genes called *rte* genes (stands for regulation of Tc^r element activities) (24, 26). Two of these regulatory genes, *rteA* and *rteB*, have amino acid sequence similarity to components of known two-component regulatory systems, with RteA being the putative sensor and RteB being the putative response regulator (24). Since disruption of *rteB* abolishes all of the transfer activities of the conjugative transposon, RteB is clearly essential for NBU excision and circularization and for expression of essential transfer. Previous work has shown that RteB probably acts directly on excision genes of the NBUs, but it was not clear

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whether RteB was acting directly on transfer genes or was acting through another regulatory protein. RteB activates a downstream gene, *rteC*. Disruption of *rteC* eliminates self-transfer of the conjugative transposon but does not eliminate mobilization of coresident plasmids or excision and mobilization of NBUs (26). This observation raised the possibility that the effect of RteB on transfer gene expression was mediated entirely through RteC. In this report, we present evidence that RteB is acting through RteC and that RteB may also have other regulatory roles. We also present evidence that RteC is probably not an activator of genes involved in transfer of the circular intermediate, as we had suggested previously, but affects transfer at some other level.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The strains and plasmids used in this study are listed in Table 1. Two *Bacteroides* conjugative transposons, Tc^{r}Em^r DOT and Tc^r ERL, were used in this study. To differentiate between different *Bacteroides* conjugative transposons, individual elements are designated by the antibiotic resistances they carry and by the name of the strain from which they were first obtained. The conjugative transposon Tc^{r}Em^r DOT, for example, carries both a Tc^r (tetracycline resistance) gene and an Em^r (erythromycin resistance) gene and was originally found in the strain *Bacteroides thetaiotaomicron* DOT (16). Tc^r ERL and Tc^{r}Em^r DOT are related enough to cross-hybridize on high-stringency Southern blots, although there are some restriction site differences between them (16).

Escherichia coli strains were grown in Luria broth or on Luria broth agar plates. *Bacteroides* strains were grown in prereduced trypticase-yeast extract-glucose broth or agar (8, 16). Antibiotics were provided in the following concentrations: ampicillin, 100 μg/ml (*E. coli*) or 25 to 50 μg/ml (*Bacteroides* strains); chloramphenicol, 15 to 25 μg/ml; erythromycin, 10 μg/ml; gentamicin, 200 μg/ml; kanamycin, 100 μg/ml; rifampin, 10 μg/ml; streptomycin, 100 μg/ml; tetracycline, 1 μg/ml (induction) and 3 μg/ml (selection); trimethoprim, 100 μg/ml.

Plasmid constructions and cloning. pLYL7, a derivative of pFD160 that is not mobilized by the *Bacteroides* conjugative transposons, has been described previously (9, 21). In this earlier study (9), we were cloning DNA into pLYL7 that allowed IncP plasmids to mobilize the constructs from *E. coli* to *Bacteroides*, but in the present studies the DNA we were cloning did not allow pLYL7 to be mobilized by IncP plasmids. To make a form of pLYL7 that could be transferred from *E. coli* to *Bacteroides* strains to test for *oriT* or transfer activity in *Bacteroides* spp., the large *Afl*III-*Sph*I fragment of pLYL7 was ligated with the 1.1-kbp fragment of pFD288 (22), which provides the RK2 *oriT*, to produce pLYL7$_{oriT(RK2)}$. This plasmid was mobilized from *E. coli* donors to *Bacteroides* recipients, either by plasmid RP4 (also known as RK2) or by the integrated transfer region of RP4 in *E. coli* S17-1, at frequencies of 10^{-3} to 10^{-4} transconjugants per recipient. Plasmid pLYL72, which carries the transfer region of *Bacteroides* conjugative transposon Tc^{r}Em^r DOT, was constructed via two intermediates, pLYL72$_{UC}$ and pLYL72$_{JRD}$ (Table 1). Plasmid pLYL52, which carries a constitutively expressed *rteC* gene, was a subclone of pLYL51B (Table 1).

Construction of disruption mutants of Tc^r ERL. The cloned conjugative transposon DNA came from Tc^{r}Em^r DOT (16). Insertional mutations were made in Tc^r ERL so that the Em^r gene could be used as the selectable marker. So far, in our hands, the Ap^r (ampicillin resistance) (13) and Cm^r (chloramphenicol resistance) (23) genes that work in *Bacteroides* spp. have proved to be adequate for selecting for plasmid acquisition but not for insertions in the chromosome. BT4104ΩAMS15 (a strain which contains a mutant Tc^r ERL with a single crossover disruption in *rteC*), BT4104ΩRDBT (a strain which contains a mutant Tc^r ERL with a single crossover disruption in *tetQ*), and BT4104ΩRDB1 (a strain which contains a mutant Tc^r ERL with a disruption in *rteA*) have been described previously (24–26). BT4104Ω*rteC* is a strain that contains a mutant Tc^r ERL, in which the disruption in *rteC* was made with a 127-bp fragment from near the amino-terminal end of *rteC*. To construct this disruption mutant, the *Bst*UI-*Xmn*I fragment of pLYL52 was cloned into pLYL03, a suicide vector, and the resulting clone (pΩ*rteC*) was introduced into *B. thetaiotaomicron* 4104 (BT4104), which contains a copy of Tc^r ERL, with selection for the Em^r gene on pLYL03. All constructs were checked by Southern blotting to confirm that the insertion had occurred in the correct place. Southern analysis and cloning were done as described previously (16).

Transfer and mobilization tests. Filter matings were done as described previously (17, 27). For tetracycline induction, donors were grown overnight in tetracycline (1 μg/ml) and then subcultured into the same medium prior to mating. Self-transfer of the conjugative transposon and its derivatives was determined by mating derivatives of *B. thetaiotaomicron* 4100 (BT4100), which carried a conjugative transposon, with *B. thetaiotaomicron* 4001 (BT4001). In all of these matings, the selection was for either Tc^r or Em^r (markers on the conjugative transposon). The selective medium also contained rifampin and no thymidine, to select against BT4100 (which requires thymidine) and for BT4001 (which is Rif^r [rifampin resistant]). Mobilization of coresident plasmids was determined by mating derivatives of BT4100 with *E. coli* HB101. In general, the selective medium contained ampicillin (to select for the plasmid) and was incubated aerobically (to select against *Bacteroides* donors). To test for mobilization of the NBU1 circle form, we used a mobilization-deficient derivative of pFD160, into which the *oriT-mob* region of NBU1 had been cloned (pLYL20) (9). To transfer plasmid constructs into BT4100, *E. coli* S17-1, which carries the transfer region of RP4 (RK2) in its chromosome (20), was used as the donor. In all experiments, the transconjugants were tested to ascertain that they contained the plasmid being transferred and that the plasmid had sustained no deletions or rearrangements.

In initial experiments to locate the *oriT* of Tc^{r}Em^r DOT, p6T3 (Tc^r) was tested for mobilization out of strain BT4104ΩRDBT (Tc^s), because the Tc^r gene was the only selectable marker on p6T3. The insertion in Tc^r ERL in BT4104 ΩRDBT eliminates expression of the *tetQ-rteA-rteB* operon. p6T3 contains this entire region and thus provides these genes in *trans*. In other experiments to locate the *oriT* of Tc^{r}Em^r DOT, BT4104 was used and plasmids carrying DNA segments from Tc^{r}Em^r DOT had a selectable Ap^r gene. In all these experiments, *E. coli* HB101 was the recipient.

Transcriptional fusions. In one set of experiments, the *E. coli* β-glucuronidase (GUS) gene was fused to DNA segments to test for promoter activity. GUS assays were done as described previously (5).

Construction of pLYL52, a plasmid that constitutively produces RteC. In the process of subcloning *rteC*, we obtained two plasmids, pLYL51A and pLYL51B (Table 1), which contained a 2.6-kbp *Sca*I-*Pvu*II fragment of p6T3 cloned in different orientations in pLYL7$_{oriT(RK2)}$. This fragment contained only 64 bp of DNA upstream of the first possible start codon of *rteC*, a segment that previous results had indicated was too small to contain the *rteC* promoter. Yet one of these plasmids, pLYL51B, complemented a transfer-minus mutant of Tc^r ERL caused by a disruption in *rteC* to transfer proficiency (data not shown). A subclone of the region cloned in pLYL51B was created, which eliminated about 1.5 kbp of DNA downstream from *rteC*. This plasmid was designated pLYL52. Results of GUS fusion experiments showed that a new promoter had been created during the cloning process. That is, a GUS fusion to the site in the vector where the *rteC* region had been cloned in pLYL52 showed no GUS activity (<0.1 U/mg of protein), whereas a GUS fusion within the *rteC* open reading frame (ORF) on pLYL52 had a GUS specific activity of about 1 U/mg of protein, regardless of whether cells were grown in the presence of tetracycline.

RESULTS

Localization of the *oriT* region of conjugative transposon Tc^{r}Em^r DOT. Previously, two cosmid clones carrying Tc^{r}Em^r DOT DNA had been shown to be capable of self-transfer from *Bacteroides* donors to *Bacteroides* or *E. coli* recipients (p6T5 and pD2T1 [16]). All of these clones contained *tetQ*, a gene which is located near the center of the conjugative transposon. The fact that these clones were capable of self-transfer in the absence of a chromosomal copy of the conjugative transposon indicated that they contained the *oriT* of Tc^{r}Em^r DOT as well as the genes necessary for conjugal transfer. p6T5 and pD2T1 both contained about 40 kbp of Tc^{r}Em^r DOT DNA, approximately 30 kbp of which was common to the two clones. Two smaller clones, p6T3 and p6TE, contained only a portion of this overlapping region and were not capable of self-transfer (Fig. 1). p6T3 was mobilizable from *Bacteroides* donors to *E. coli* recipients by Tc^{r}Em^r DOT, but p6TE was not (Fig. 1). This result suggested that the *oriT* of the element was located within the 3.2-kbp region present on p6T3 but missing on p6TE. This entire region has been sequenced previously (GenBank accession numbers L02419, M81881, and X58717 and unpublished data). Further subclones of the region were tested for mobilization by cloning them into a plasmid, pLYL7$_{oriT(RK2)}$, which can be mobilized from *E. coli* to *Bacteroides* spp. by RK2 but is not mobilizable by the *Bacteroides* conjugative transposons. The results of these experiments (Fig. 2) suggested that the *oriT* might be located between bp 8189 and 8456, because this region was common to the two smallest mobilizable subclones, pLYL63 and pLYL65. However, a subclone that extended from bp 8039 to 8583 (pLYL67A/B; Fig. 2) was not mobilizable. Thus, DNA outside this region is essential for full *oriT* functioning.

TABLE 1. Bacterial strains and plasmids used in this study

Plasmid or strain	Relevant characteristics[a]	Description (reference and/or source)
Plasmids		
p6T5	Km^r (Tc^r Tra^+)	Self-transmissible (Tra^+) cosmid clone from Tc^rEm^r DOT (16)
pD2T1	Km^r (Tc^r Tra^+)	Tra^+ cosmid clone from Tc^rEm^r DOT (16)
p6T3	Km^r (Tc^r Tra^-)	Tra^- cosmid clone of Tc^rEm^r DOT DNA (16)
p6TE	Km^r (Tc^r Em^r Tra^-)	Tra^- cosmid clone of Tc^rEm^r DOT DNA that overlaps DNA in p6T3 (16)
pLYL7	Ap^r (Ap^r) Mob^- (Mob^-)	Plasmid that replicates in *E. coli* and *Bacteroides* spp. but is not mobilized by IncP plasmids or *Bacteroides* conjugative transposons (9)
pLYL7$_{oriT(RK2)}$	Ap^r (Ap^r) Mob^+ (Mob^-)	365-bp *Afl*III-*Sph*I fragment of pLYL7 replaced by 1.12-kbp *Afl*III-*Sph*I fragment of pFD288 (this study); can be mobilized by IncP plasmid RP4 but not by *Bacteroides* conjugative transposons
pFD288	Sp^r (Em^r)	Plasmid carrying the *oriT* of RK2 (22)
pLYL01	Ap^r (Tc^r) Mob^+	2.6-kbp *Sst*I fragment (containing the *tetQ* gene) from pNFD13-2 (25) cloned into the *Aat*II site of pFD160R (21; this study)
pLYL03	Ap^r (Em^r) Mob^+	Religation of 6-kbp *Sma*I-*Sst*I fragment of pCQW-1 (5; this study)
pLYL20	Ap^r (Ap^r) Mob^+ (Mob^+)	Mob^+ subclone of NBU1 containing both the *oriT* and *mob* genes (10)
pLYL31	Ap^r (Em^r)	302-bp internal *Ssp*I fragment of ORF5 cloned into the *Hin*cII site of pLYL03 (this study)
pLYL34	Ap^r (Em^r)	685-bp internal *Bst*UI fragment of ORF6 amino terminus cloned into the *Hin*cII site of pLYL03 (this study)
pLYL50	Ap^r (Ap^r)	3.3-kbp *Xmn*I fragment of p6T3 cloned into the *Sma*I site of pLYL7$_{oriT(RK2)}$ (this study)
pLYL51A/B	Ap^r (Ap^r)	2.6-kbp *Sca*I-*Pvu*II fragment of p6T3 cloned into the *Sma*I site of pLYL7$_{oriT(RK2)}$ (this study); A and B are different orientations of the same fragment.
pLYL52	Ap^r (Ap^r)	1.1-kbp *Eco*RI fragment of pLYL51B cloned into the *Sma*I site of pLYL7$_{oriT(RK2)}$ (this study)
pLYL63	Ap^r (Ap^r)	1.36-kbp *Pst*I-*Rsa*I fragment of pLYL50 cloned into pLYL7$_{oriT(RK2)}$ digested with *Pst*I and *Sma*I (this study)
pLYL64	Ap^r (Ap^r)	2.48-kbp *Pst*I-*Pvu*II fragment of pLYL50 cloned into pLYL7$_{oriT(RK2)}$ digested with *Pst*I and *Sma*I (this study)
pLYL65	Ap^r (Ap^r)	1.38-kbp *Dra*I-*Sst*I fragment of pLYL64 cloned into pLYL7$_{oriT(RK2)}$ digested with *Sma*I and *Sst*I (this study)
pLYL67A/B	Ap^r (Ap^r)	545-bp *Ssp*I fragment of pLYL64 cloned into the *Sma*I site of pLYL7$_{oriT(RK2)}$ (this study)
pLYL68	Ap^r (Ap^r)	686-bp *Ssp*I-*Sst*I fragment of pLYL64 cloned into pLYL7$_{oriT(RK2)}$ digested with *Sma*I and *Sst*I (this study)
pLYL72$_{UC}$	Ap^r	18-kbp *Eco*RV-*Pst*I fragment of pD2T1 cloned into pUC19 digested with *Sma*I and *Pst*I (this study)
pLYL72$_{JRD}$	Km^r Cm^r	18-kbp *Hin*dIII-*Sst*I fragment of pLYL72$_{UC}$ cloned into pJRD215 (4) digested with *Hin*dIII and *Sst*I (this study)
pLYL72	Km^r Cm^r (Cm^r)	18-kbp *Cla*I fragment of pLYL72$_{JRD}$ cloned into the *Cla*I site of pNJR24 (this study)
pNJR24	Km^r Cm^r (Cm^r)	2.8-kbp *Nar*I-*Xba*I fragment of pFD342 (23) cloned into the *Sal*I site of pNJR1 (16; A. M. Stevens).
pΩ*rteC*	Ap^r (Em^r)	127-bp *Bst*UI-*Xmn*I fragment of pLYL52 cloned into the *Hin*cII site of pLYL03 (this study)
E. coli strains		
HB101	*recA* Str^r	(2)
S17-1	*recA* Str^r Tp^r	RP4 integrated in the chromosome (20)
B. thetaiotaomicron 5482A derivatives		
BT4001	(Rif^r)	Rif^r derivative of *B. thetaiotaomicron* 5482A (17)
BT4004	(Rif^r Tc^r)	BT4001 with a copy of Tc^rERL (18)
BT4100	(Thy^- Tp^r)	Thy^- Tp^r derivative of *B. thetaiotaomicron* 5482A (26)
BT4104	(Thy^- Tp^r Tc^r Em^r)	BT4100 with a copy of Tc^rERL (16)
BT4104ΩRDB1	(Thy^- Tp^r Tc^r Em^r Ω*rteA*)	Chromosomal disruption of *rteA* in BT4104 (this study); a similar disruption in Tc^rERL in a strain of *Bacteroides uniformis* was described previously (25)
BT4104ΩRDBT	(Thy^- Tp^r Em^r Ω*tetQ*)	Chromosomal disruption of *tetQ* in BT4104 (this study); a similar disruption in Tc^rERL in a strain of *B. uniformis* was described previously (25)
BT4104ΩAMS15	(Thy^- Tp^r Tc^r Em^r Ω*rteC*)	Chromosomal disruption of *rteC* using a 500-bp fragment of the gene (26)
BT4104Ω*rteC*	(Thy^- Tp^r Tc^r Em^r)	Chromosomal disruption of *rteC* by integrating pΩRteC into Tc^rERL in BT4104 (this study)
BT4107	(Thy^- Tp^r Tc^r Em^r)	BT4100 carrying a copy of Tc^rEm^r DOT (16)
BT4104ΩORF5	(Thy^- Tp^r Tc^r Em^r)	Insertion of pLYL31 into *orf5* of a chromosomal copy of Tc^rERL in BT4104 (this study)
BT4014ΩORF6	(Thy^- Tp^r Tc^r Em^r)	Insertion of pLYL34 into *orf6* of a chromosomal copy of Tc^rERL in BT4104 (this study)

[a] Phenotypes in parentheses are expressed in *B. thetaiotamicron*, and phenotypes not in parentheses are expressed in *E. coli*. Ap, ampicillin; Cm, chloramphenicol; Em, erythromycin; Km, kanamycin; Rif, rifampin; Sp, spectinomycin; Tc, tetracycline; Thy^-, thymidine auxotroph; Tp, trimethoprim; Tra^+, self-transmissible; Mob^+, mobilizable by IncP plasmid or (if in parentheses) by *Bacteroides* conjugative transposons.

Comparison with plasmid, NBU, and Tn*4399 oriT*s. The nick site of Tc^rEm^r DOT is most likely to be located within the 544-bp region of overlap between the two smallest *oriT*-active subclones. The sequences of *oriT*s from a number of self-transmissible or mobilizable plasmids and from the ends of T-DNA have been compared, and the nick sites were found to fall into two major consensus groups: RBHYATCCTGYM, represented by IncP plasmids and T-DNA ends, and AGGG CGCAMTTA, represented by IncQ plasmids (11, 28). None of these consensus sequences was found within the 544-bp region of Tc^rEm^r DOT that might contain the *oriT*. We also compared the sequence of the *oriT* region of NBU1 (9) with the

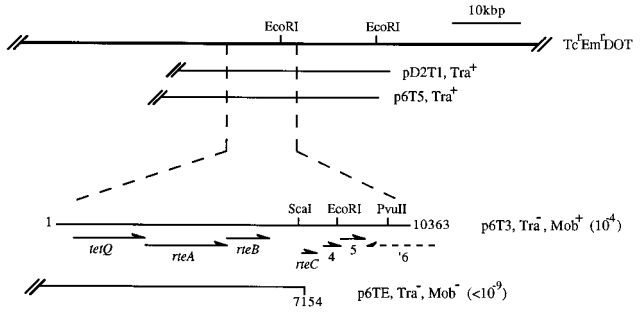

FIG. 1. Portions of Tc^rEm^r DOT cloned into pD2T1, p6T5, p6T3, and p6TE. Tc^rEm^r DOT is indicated by the horizontal line at the top of the figure, and the ends of the cloned regions are indicated by horizontal lines under Tc^rEm^r DOT. //, the cloned region extends beyond regions so far mapped, so the exact endpoint is still uncertain. The regions cloned in p6T3 and p6TE are shown in expanded form to show the region of Tc^rEm^r DOT for which sequence information is available and to illustrate what genes are missing from p6TE. The locations, extent, and direction of transcription *tetQ*, *rteA*, *rteB*, *rteC*, and some ORFs of unknown function are indicated by lines with arrowheads. *orf6* ('6) is shown as a dotted line to emphasize that the sequence of this ORF is incomplete and the location of the amino terminus has not yet been determined. Tra$^+$, self-transmissible; Tra$^-$, not self-transmissible; Mob$^+$, mobilizable by Tc^r ERL in *trans*; Mob$^-$, not mobilizable by Tc^r ERL. Numbers in parentheses are transfer frequencies expressed as transconjugants per recipient.

544-bp region of Tc^{r}Em^r DOT. No sequence similarities were detected. Tn*4399* is a mobilizable transposon (10) that is mobilized by the Tc^{r}Em^r DOT family of conjugative transposons. Tn*4399*, like the NBUs, has an internal *oriT*. The nick site of Tn*4399* has been identified and falls in a region that has some sequence similarity to the *oriT* regions of plasmids (9). We have found recently that there is some sequence similarity between Tn*4399* and NBU1, which lies upstream of the *oriT*s of both of these elements (9), but we were unable to detect any similarity between the putative *oriT* region of Tc^{r}Em^r DOT and the *oriT* site of Tn*4399*.

Mobilization proteins of different *Bacteroides* conjugative transposons are cross-functional. The *Bacteroides* conjugative transposons Tc^r ERL and Tc^{r}Em^r DOT are closely related but are not identical. Since we had originally been using Tc^r ERL to mobilize subclones containing the *oriT* of Tc^{r}Em^r DOT, so that the Em^r gene could be used as a selectable marker (see later sections), the question of whether Tc^r ERL could mobilize this *oriT* as efficiently as Tc^{r}Em^r DOT itself arose. The recent availability of selectable markers other than *ermF*, such as *cfxA*, which confers resistance to ampicillin (13), has made it possible to test the mobilization of the Tc^{r}Em^r DOT *oriT* by Tc^{r}Em^r DOT itself. pLYL50, a plasmid which contained the *oriT* region of Tc^{r}Em^r DOT and the Ap^r gene, was mobilized out of *B. thetaiotaomicron* 4107 (BT4107), which contains Tc^{r}Em^r DOT. The mobilization frequency of pLYL50 was identical to the mobilization frequency of this same plasmid out of strain BT4104, which contains Tc^r ERL (10^{-5} to 10^{-6} per recipient in both cases). Thus, Tc^r ERL and Tc^{r}Em^r DOT appear to have similar if not identical *oriT*s, and their relaxase proteins are cross-functional.

Cloning and localization of the transfer region of Tc^{r}Em^r DOT. To locate the transfer region of Tc^{r}Em^r DOT, we subcloned the overlapping region of the two large self-mobilizing cosmid clones of Tc^{r}Em^r DOT DNA into pNJR24 and tested for the ability to transfer from *Bacteroides* donors to *E. coli* recipients. (Fig. 2). The smallest subclone capable of self-transfer (pLYL72) contained an 18-kbp segment (Fig. 2). The transfer of a chromosomal copy of Tc^{r}Em^r DOT is stimulated 1,000- to 10,000-fold when donors are exposed to tetracycline. Since pLYL72 did not carry a Tc^r gene, the effect of tetracycline on its transfer frequency could not be determined using *B. thetaiotaomicron* 4001 (BT4001) donors. Accordingly, we provided a small plasmid, pLYL01, which carries the *tetQ* gene but no other genes from the conjugative transposon. pLYL72 was transferred to *E. coli* recipients by tetracycline-stimulated donors at the same level as by donors that had not been exposed to tetracycline (Table 2). The transfer frequency of pLYL72 to

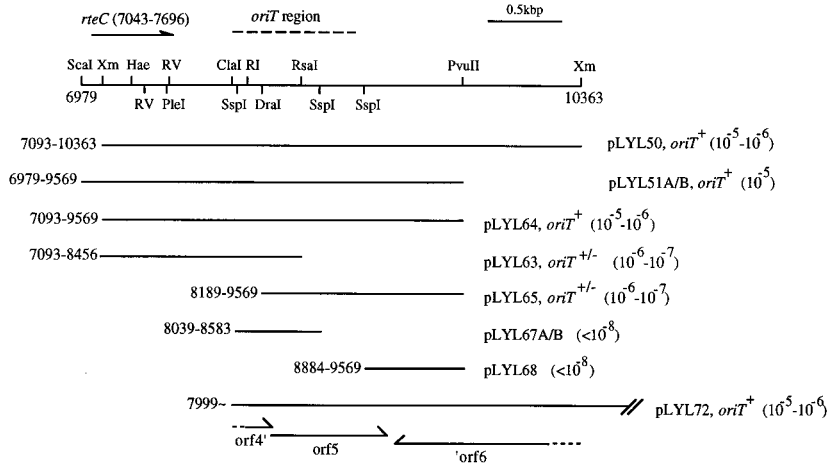

FIG. 2. Localization of the *oriT* region. A restriction map of the region of Tc^{r}Em^r DOT containing *rteC* and downstream DNA is shown at the top of the figure. The extent and direction of transcription of *rteC* are indicated by the arrow above the restriction map. The numbers in parentheses indicate the base pairs of the first possible start codon and stop codon, respectively. Horizontal lines below the restriction map indicate the DNA segments cloned into pLYL7$_{oriT(RK2)}$ (Table 1). The numbers at the left of each line indicate the base pairs of the endpoints of the cloned region. To the right of each line is the name of the plasmid carrying the cloned DNA, whether the clone had *oriT* activity (*oriT*$^+$) or not (*oriT*), and the range of frequencies of mobilization (in parentheses). At the bottom of the figure, the extent of the DNA cloned into the self-transmissible clone, pLYL72, is shown. The number at the left indicates the base pair of that side of the cloned region. // (at the other end), the endpoint lies outside the region for which DNA sequence information is available. The extent and direction of transcription of *orf4*, *orf5*, and *orf6* are indicated under this line. *orf4* is shown as a dashed line to indicate that only part of it is present on the cloned region. *orf6* is shown as a dashed line because only a part of it falls within the sequenced region, so the precise endpoint of the ORF is still unknown. Abbreviations: RV, *Eco*RV; RI, *Eco*RI; Hae, *Hae*III; Xm, *Xmn*I.

TABLE 2. Localization of the transfer region of Tc^{r}ERL

Strain[a]	Mobilizing element[b]	Element being transferred[c]	Frequency of transfer[d]	
			−Tc	+Tc
BT4001(pLYL01[e], pLYL72)	pLYL72	pLYL72	10^{-5}–10^{-6}	10^{-5}–10^{-6}
BT4104	Tc^r ERL	Tc^r ERL	$<10^{-9}$	10^{-5}–10^{-6}
BT4001(pLYL72, pLYL01[f])	pLYL72	pLYL01	ND[g]	10^{-5}–10^{-6}
BT4001(pLYL72, pLYL20[f])	pLYL72	pLYL20	10^{-4}–10^{-6}	NA[h]
BT4104(pLYL01) or BT4104(pLYL20)	Tc^r ERL	pLYL01 or pLYL20	$<10^{-9}$	10^{-5}–10^{-6}
BT4104ΩORF5(pAFD2)	Tc^{r}ERLΩ*orf5*	pAFD2[i]	$<10^{-8}$	10^{-5}–10^{-6}
BT4104ΩORF5(pLYL20)	Tc^{r}ERLΩ*orf5*	pLYL20	$<10^{-9}$	10^{-4}–10^{-5}
BT4104ΩORF5	Tc^{r}ERLΩ*orf5*	Tc^{r}ERLΩ*orf5*	$<10^{-9}$	10^{-5}–10^{-6}
BT4104ΩORF6(pAFD2)	Tc^{r}ERLΩ*orf6*	pAFD2	$<10^{-9}$	10^{-5}–10^{-6}
BT4104ΩORF6(pLYL20)	Tc^{r}ERLΩ*orf6*	pLYL20	$<10^{-9}$	10^{-5}–10^{-6}
BT4104ΩORF6	Tc^{r}ERLΩ*orf6*	Tc^{r}ERLΩ*orf6*	$<10^{-9}$	10^{-5}–10^{-6}

[a] Strain plus plasmid(s) carried by the strain. BT4001 carries no conjugative transposon, and BT4104 carries a single chromosomal copy of Tc^r ERL. Disruption mutants of Tc^{r}ERL are indicated by Ω and the ORF number following BT4104. In cases where plasmid transfer is being monitored, the recipient is *E. coli* HB101, with selection for Ap^r (pLYL01 or pLYL20) or Cm^r (pLYL72 or pAFD2). In cases where transfer of Tc^{r}ERL or its derivatives is being monitored, the recipient is BT4001.

[b] Plasmid or conjugative transposon that is providing the transfer functions.

[c] Plasmid or conjugative transposon, whose transfer is being monitored.

[d] Frequencies are expressed as transconjugants per recipient cell observed when the donors were induced (+Tc) or not induced (−Tc) by tetracycline. The range of values indicates the experiment-to-experiment variation and represents at least three separate mating experiments.

[e] pLYL01 (*tetQ*) supplied to allow tetracycline induction.

[f] pLYL01, pAFD2, and pLYL20 are not transferred ($<10^{-9}$ per recipient) out of BT4001 or B4100.

[g] ND, not done because tetracycline selection was needed to keep pLYL01 in the strain.

[h] NA, not appropriate because the strain is not Tc^r.

[i] pAFD2 is a Cm^r derivative of pFD160 (26). This plasmid contains the same *Bacteroides* origin and mobilization region as that found in pLYL01 and pLYL20 (Table 1). The choice of which of these plasmids to use was dictated by markers on other plasmids in the same strain.

E. coli recipients was comparable to that obtained when donors containing Tc^{r}Em^r DOT were tetracycline induced and mated with *Bacteroides* recipients (Table 2). An evident difference between pLYL72 transfer and Tc^{r}Em^r DOT transfer was that the transfer frequency of pLYL72 was not affected by tetracycline. We saw no indication of deletions or rearrangements in pLYL72 when it was transferred from *Bacteroides* donors to *E. coli* recipients. pLYL72$_{UC}$, a derivative of pLYL72 that lacked the RSF1010 mobilization region (Table 1), could not transfer itself from *E. coli* to *Bacteroides* spp., however, nor was it mobilized by IncP plasmids (data not shown).

Bacteroides conjugative transposons can mobilize coresident plasmids and NBU circle forms. Thus, it seemed reasonable that the transfer region cloned on pLYL72 should be able to mobilize plasmids and NBU circle forms. Nonetheless, it was also possible that there is more than one transfer region within the 70-kbp conjugative transposon and that another transfer region was actually mobilizing coresident plasmids and NBUs. We tested pLYL72 for the ability to mobilize plasmid pLYL01, which has the replication and mobilization regions of the cryptic *Bacteroides* plasmid pBI143, and plasmid pLYL20, which carries the *mob-oriT* region of NBU1. pLYL72 was able to mobilize both pLYL01 and pLYL20 (Table 2). Mobilization frequencies for pLYL01 and pLYL20 were comparable to those obtained when a single copy of Tc^r ERL in the chromosome was the mobilizing element and donors were grown in tetracycline (Table 2). This suggests that the same region of Tc^{r}Em^r DOT that mediates self-transfer of the element also mediates the mobilization of coresident plasmids and NBUs.

pLYL72 contained the *oriT* region of Tc^{r}Em^r DOT plus about 18 kbp of downstream DNA. DNA sequence information was available for nearly 3 kbp of DNA downstream of the *oriT*. This region contained three possible ORFs (*orf4*, *orf5*, and *orf6*; Fig. 2). None of these ORFs had any homologs in the databases. Previously, we had shown that a disruption in *orf4* had no effect on any of the transfer activities of Tc^r ERL (26). To determine if *orf5* or *orf6* encoded essential transfer genes, we created single crossover disruptions in each of them and tested the mutant conjugative transposons for self-transfer, for the ability to mobilize a shuttle vector that contained a mobilizable cryptic *Bacteroides* plasmid (pAFD2), and for the ability to mobilize a plasmid carrying the *mob-oriT* region of NBU1 (pLYL20). Neither of these disruptions affected self-transfer, mobilization of plasmids, or mobilization of the NBU1 *mob-oriT* clone (Table 2). Thus, the region containing essential transfer genes is located within the 16-kbp region that extends from the end of the sequenced region of DNA to the end of the DNA segment cloned in pLYL72.

Transfer gene expression is controlled by a repressor that is encoded on the conjugative transposon. The finding that pLYL72 was self-transmissible in a strain that contained no *rteB* or *rteC* was unexpected. We had found previously that a disruption in *rteC* completely abolished element self-transfer. Since RteB was required for *rteC* expression, this had led us to expect that both *rteB* and *rteC* would have to be provided in *trans* to allow pLYL72 to transfer. Results shown in Table 2 show clearly that this was not the case. Moreover, transfer was constitutive, in contrast to the transfer of Tc^r ERL itself, which is stimulated at least 1,000-fold by tetracycline (Table 2). These results suggested the hypothesis that transfer genes carried on pLYL72 are normally controlled by a repressor, which had been lost in the process of subcloning the *tra* region, and that the role of RteB and/or RteC was to counter the action of this repressor.

This hypothesis led to two predictions. First, *rteC* provided in *trans*, in the absence of the rest of the conjugative transposon, should not affect pLYL72 transfer frequency. We were able to test this prediction, because in the process of subcloning *rteC*, we obtained a construct (pLYL52) in which *rteC* was placed under the control of a constitutive promoter that was created during the cloning process (see Materials and Methods). According to the GUS fusion results, expression of *rteC* from pLYL52 was comparable to tetracycline-induced expression from the normal *rteC* promoter. Moreover, pLYL52 was capable of complementing a mutant of Tc^r ERL with a disruption in *rteC* (Tc^{r}ERLΩAMS15, an earlier form of Tc^{r}ERLΩ*rteC*) to

TABLE 3. Role of RteB and RteC in transfer of the conjugative transposon

Strain[a]	Mobilizing element[b]	Element being transferred[c]	Frequency of transfer[d]	
			−Tc	+Tc
BT4104ΩAMS15	Tc^{r}ERLΩAMS15	Tc^{r}ERLΩAMS15	$<10^{-9}$	$<10^{-9}$
BT4104ΩAMS15(pLYL52)	Tc^{r}ERLΩAMS15	Tc^{r}ERLΩAMS15	10^{-5}–10^{-6}	10^{-4}–10^{-5}
BT4001(pLYL52, pLYL72)	pLYL72	pLYL72	10^{-5}–10^{-6}	NA[e]
BT4104(pLYL72)	Tc^r ERL and pLYL72	pLYL72	10^{-6}–10^{-7}	10^{-2}–10^{-3}
BT4104Ω*rteC*(pLYL72)	Tc^{r}ERLΩ*rteC* and pLYL72	pLYL72	10^{-7}–10^{-8}	10^{-5}–10^{-6}
BT4104ΩRDB1(pLYL72)	Tc^{r}ERLΩRDB1 and pLYL72	pLYL72	10^{-7}–10^{-8}	10^{-6}–10^{-7}
BT4104Ω*rteC*(pLYL72)	Tc^{r}ERLΩ*rteC* pLYL72	Tc^{r}ERLΩ*rteC*	$<10^{-9}$	$<10^{-9}$
BT4104ΩRDB1(pLYL72)	Tc^{r}ERLΩRDB1 pLYL72)	Tc^{r}ERLΩRDB1	$<10^{-9}$	$<10^{-9}$

[a] Strain plus plasmid or conjugative transposon, whose transfer is not being monitored.
[b] Plasmid and/or conjugative transposon that is providing the transfer functions.
[c] Plasmid or conjugative transposon, whose transfer is being monitored.
[d] Frequencies are expressed as transconjugants per recipient cell observed when the donors were induced (+Tc) or not induced (−Tc) by tetracycline. The range of values indicates the experiment-to-experiment variation and represents at least three separate mating experiments.
[e] NA, not appropriate because the strain is Tc^s.

full transfer proficiency (Table 3), and transfer of the mutant element was now nearly constitutive rather than tetracycline stimulated. pLYL52 had no effect on the transfer frequency of pLYL72 (Table 3). Thus, providing RteC in *trans* did not stimulate pLYL72 transfer, as would be expected if RteC were activating transcription of transfer genes on pLYL72.

A second prediction was that the transfer frequency of pLYL72 should be decreased in a strain that contained Tc^{r}ERLΩ*rteC* (BT4104Ω*rteC*), because Tc^{r}ERLΩ*rteC* produces the repressor but not RteC. In fact, the uninduced frequency of transfer of pLYL72 dropped by 100-fold in BT4104Ω*rteC* (Table 3). The fact that Tc^{r}ERLΩ*rteC* did not eliminate pLYL72 transfer completely can be explained by the fact that pLYL72 is a multicopy plasmid (about 10 to 15 copies per cell), and the amount of repressor produced by single-copy chromosomal Tc^{r}ERLΩ*rteC* is insufficient to repress all expression of the plasmid-encoded genes. The effect of the repressor was seen in the absence of tetracycline but not when donors were exposed to tetracycline (Table 3). This tetracycline-induced derepression was further decreased in donors carrying Tc^{r}ERLΩRDB1, a mutant form of Tc^r ERL that did not produce RteB, suggesting that RteB may be acting to counter the effect of the repressor.

If the only effect of RteC is to counter the effect of a repressor that normally represses expression of the transfer genes carried on pLYL72, two predictions can be made about the effect of wild-type Tc^r ERL on the transfer frequency of pLYL72. First, since Tc^r ERL produces a low basal level of RteC in the absence of tetracycline stimulation, the depression of pLYL72 under noninducing conditions should not be as great as that due to Tc^{r}ERLΩ*rteC*. This was the case (Table 3). Second, under inducing conditions, when RteC is being expressed by the chromosomal copy of Tc^r ERL, pLYL72 should transfer at the same frequency as that for transfer out of a strain carrying no conjugative transposon (BT4001). In fact, the frequency of pLYL72 transfer out of BT4104 was 1,000-fold higher under tetracycline-induced conditions than that of transfer of pLYL72 out of BT4001, suggesting that Tc^r ERL is stimulating pLYL72 transfer in some tetracycline-dependent manner. One explanation of this observation is that RteC not only counteracts the effect of a repressor but also stimulates expression of genes outside the region encoded on pLYL72, whose products somehow stimulate transfer of pLYL72. That is, the genes encoded on pLYL72 are sufficient for transfer but do not produce the highest level of transfer possible.

Previously, we had shown that RteB stimulates expression of *rteC* by at least 20-fold. This raises the question of whether RteB acts through RteC or whether RteB also acts on other genes carried on the conjugative transposon. If RteB acts through RteC, providing constitutively expressed *rteC* on pLYL52 in *trans* should allow a mutant element that cannot produce RteB to transfer. Tc^{r}ERLΩRDBT is a mutant form of Tc^r ERL in which expression of the entire *tetQ-rteA-rteB* operon has been eliminated. Tc^{r}ERLΩRDBT is completely transfer deficient ($<10^{-9}$ transconjugants per recipient) (25). pLYL52 restored the ability of Tc^{r}ERLΩRDBT to transfer at frequencies of 10^{-4} to 10^{-5} transconjugants per recipient under uninduced conditions. Thus, RteC can replace RteB.

Do RteB and RteC control excision gene expression? The fact that pLYL72 still transferred itself out of Tc^{r}ERLΩ*rteC*, which produced no RteC, and out of Tc^{r}ERLΩRDB1, which produced no RteB or RteC, allowed us to test indirectly the hypothesis that RteB and/or RteC controls excision genes of the conjugative transposon. If the only role of RteB and RteC is to aid in the expression of genes necessary for transfer of the circular intermediate, both Tc^{r}ERLΩ*rteC* and Tc^{r}ERLΩRDB1 should be able to excise from and integrate into the chromosome. In this case, pLYL72 should be able to partially complement the transfer defect of Tc^{r}ERLΩ*rteC* or Tc^{r}ERL ΩRDB1 by producing the transfer and mobilization proteins necessary to allow the excised circular form of the mutant element to transfer. pLYL72 provided in *trans* in the strain containing Tc^{r}ERLΩ*rteC* or the strain containing Tc^{r}ERL ΩRDB1 did not allow the mutant element to transfer (Table 3). This suggests that RteC or RteB or both control excision gene expression. This result also shows that RteB is not controlling excision genes directly, because RteB is still produced by the *rteC* disruption mutant.

DISCUSSION

This is the first report of the localization of an *oriT* region from a conjugative transposon. Previously, *oriT* regions from a number of self-transmissible and mobilizable plasmids have been identified and characterized. The *oriT* regions of two types of integrated *Bacteroides* elements which are mobilized by conjugative transposons have also been described previously: that of Tn*4399*, a transposon that carries an *oriT* and two mobilization genes, and that of NBU1 and NBU2. Scott et al. (15) have suggested that the gram-positive conjugative transposon Tn*916* had an internal transfer origin, but this has not been proven experimentally. The fact that the *oriT* region of

the *Bacteroides* conjugative transposon Tc^{r}Em^r DOT is located in the middle of the element supports the hypothesis that the transfer intermediate of this conjugative transposon is a covalently closed circle. This hypothesis is also supported by previous analysis of excision and integration events involving XBU4422 (1), a cryptic conjugative transposon that has ends similar to those of Tc^{r}Em^r DOT.

Usually, *oriT*s are less than 200 bp in size (6, 7, 12, 28). In fact, a 220-bp region of the NBU1 circle form was sufficient to provide *oriT* function (9). In the present study, the smallest subclones with *oriT* activity were about 1.4 kbp in size (pLYL63 and pLYL65), and these subclones had a mobilization frequency that was 10-fold lower than that of the smallest subclone that had a wild-type mobilization frequency (the 2.5 kbp subcloned in pLYL64; Fig. 2). The fact that pLYL72 was transferred at high frequencies suggests that the *oriT* region is to the right of bp 7999 in Fig. 2 and that pLYL63 and pLYL64 transferred at lower frequencies because they contained less DNA than is required for full *oriT* function. The large amount of DNA needed for full *oriT* activity may indicate that multiple proteins bind the *oriT* or that *oriT* DNA has to be wrapped around the relaxosome complex to a greater extent than reported for transmissible plasmids (28). Another possible explanation for the large size of the DNA segments needed to provide *oriT* activity is that there is more than one *oriT* in this region. This seems unlikely, because the presence of two *oriT*s should give rise to deletions in this area. Since the two ORFs in this region, *orf4* and *orf5*, are not essential, there would be no selection against such deletions. Yet, we have never seen any evidence of such deletions in all of the matings we have investigated.

The fact that the gene we had designated *rteC* is located adjacent to the *oriT* region raised the question of whether *rteC* was actually a mobilization gene rather than a regulatory gene. Mobilization genes are usually located close to the *oriT* region (9, 10). Moreover, a disruption in *rteC* abolished Tc^{r}Em^r DOT self-transfer but did not affect mobilization of coresident plasmids. This is the phenotype expected for a mutation that abolished expression of an element-specific mobilization gene. Moreover, since disruptions in *orf4*, *orf5*, and *orf6* had no effect on Tc^{r}Em^r DOT self-transfer, there ORFs did not encode mobilization genes. Proof that *rteC* does not encode a mobilization gene comes from the finding that pLYL72 was self-transmissible. If *rteC* encoded a mobilization gene, it should have been essential for pLYL72 self-transfer. Thus, the *Bacteroides* conjugative transposons are unusual in that they have an *oriT* region that is at least 3 kbp from the genes that encode relaxosome proteins.

RteC is evidently a regulatory protein, but its exact function is still unclear. Some possible functions of RteC and RteB are summarized in Fig. 3. We had established previously that RteB is essential for transcription of RteC (26). The fact that pLYL52, a plasmid that constitutively produced RteC, could complement an *rteB* disruption mutant of Tc^r ERL to full transfer proficiency suggests that either RteB acts primarily through RteC or that RteB and RteC have similar enough functions that RteC overproduction can compensate for the lack of RteB.

RteC is essential for Tc^r ERL self-transfer, but it appears not to act directly on genes essential for transfer of the circular intermediate of the conjugative transposon, because providing *rteC* in *trans* on a multicopy plasmid (pLYL52) had no effect on pLYL72 transfer frequency. Yet RteC does have a stimulatory effect on transfer, because a mutant element with a disrupted *rteC* no longer provides the 1,000-fold tetracycline-dependent stimulation of pLYL72 transfer that was seen when a wild-type

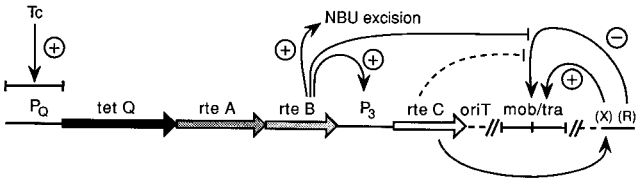

FIG. 3. Possible roles of RteB and RteC in tetracycline-regulated transfer of the *Bacteroides* conjugative transposons. Tetracycline stimulates transcription of the *tetQ-rteA-rteB* operon in a manner that is still not understood, resulting in increased RteB production. RteB activates expression of *rteC* and may counter the effects of a repressor (R) that is encoded outside the region cloned in pLYL72. RteC may also have antirepressor activity (dashed line). The main function of RteC, however, appears to be to increase transfer by stimulating some gene(s), designated X, that lies outside the region cloned on pLYL72. RteC may also control excision and integration genes (not shown).

conjugative transposon was provided in *trans* (Table 3). Also, pLYL52 enhanced the transfer frequency of the intact conjugative transposon by as much as 100-fold, even though it had no effect on the transfer frequency of pLYL72. The simplest explanation for these observations is that RteC acts on some gene(s) located outside the region cloned on pLYL72 (designated X in Fig. 3) and that X somehow stimulates transfer of the circular intermediate.

Another possible role for RteC is suggested by results that support the hypothesis that a repressor encoded outside the region cloned on pLYL72 controls expression of genes essential for transfer of the circular intermediate (designated R in Fig. 3). RteC might act to counter the effect of this repressor. If so, however, RteC is not the only antirepressor, because an *rteC* disruption mutant of Tc^r ERL suppressed pLYL72 transfer in the absence of tetracycline but not in the presence of tetracycline. This, together with the finding that a mutant that did not produce RteB suppressed pLYL72 transfer under inducing and noninducing conditions, suggests that RteB can act as an antirepressor (Fig. 3). The fact that pLYL52 (constitutively produced RteC) complemented a mutant that could not produce RteB (Tc^{r}ERLΩRDB1) supports the hypothesis that RteC may also have a similar activity. Still another type of activity that could be controlled by RteB and RteC is excision and integration of the element from the chromosome. Evidence that RteB and RteC might be regulating excision and/or integration genes comes from the failure of pLYL72 to restore transfer of Tc^{r}ERLΩ*rteC* (RteC$^-$) or Tc^{r}ERLΩRDBT (RteB$^-$). The genes responsible for excision and integration of Tc^r ERL and related conjugative transposons have not yet been located and characterized, so a direct test of the hypothesis that RteC and/or RteB controls excision and integration genes cannot be made at present. Work to identify and characterize the excision and integration genes of the *Bacteroides* conjugative transposons is under way. If RteB and/or RteC proves to control excision as well as transfer genes, this will establish that there is a linkage between excision, which is the initial step in the transfer process, and conjugal transfer itself. Such a connection has been suggested for Tn*916* but has not yet been demonstrated (3, 14).

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REFERENCES

1. **Bedzyk, L. A., N. B. Shoemaker, K. E. Young, and A. A. Salyers.** 1992. Insertion and excision of *Bacteroides* conjugative chromosomal elements. J. Bacteriol. **174:**166–172.

2. **Boyer, H. B., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41:**459–472.
3. **Clewell, D. B., and S. E. Flannagan.** 1993. The conjugative transposons of Gram-positive bacteria, p. 369–393. *In* D. B. Clewell (ed.), Bacterial conjugation. Plenum Press, New York.
4. **Davison, J., M. Heusterpreute, N. Chevalier, V. Ha-Thi, and F. Brunel.** 1987. Vectors with restriction site banks. V. pJRD215, a wide-host-range cosmid vector with multiple cloning sites. Gene **51:**275–280.
5. **Feldhaus, M. J., V. Hwa, Q. Chang, and A. A. Salyers.** 1991. Use of an *Escherichia coli* β-glucuronidase gene as a reporter gene for investigation of *Bacteroides* promoters. J. Bacteriol. **173:**4540–4543.
6. **Fürste, J. P., W. Pansegrau, G. Ziegelin, M. Kröger, and E. Lanka.** 1989. Conjugative transfer of promiscuous IncP plasmids: interaction of plasmid-encoded products with the transfer origin. Proc. Natl. Acad. Sci. USA **86:** 1771–1775.
7. **Guiney, D. G., and E. Yakobson.** 1983. Location and nucleotide sequence of the transfer region of the broad host range plasmid RK2. Proc. Natl. Acad. Sci. USA **80:**3593–3598.
8. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnical Institute and State University, Blacksburg, Va.
9. **Li, L.-Y., N. B. Shoemaker, and A. A. Salyers.** 1993. Characterization of the mobilization region of a *Bacteroides* insertion element, NBU1, that is excised and transferred by *Bacteroides* conjugative transposons. J. Bacteriol. **175:** 6588–6598.
10. **Murphy, C. G., and M. H. Malamy.** 1995. Requirements for strand- and site-specific cleavage within the *oriT* region of Tn*4399*, a mobilizing transposon from *Bacteroides fragilis*. J. Bacteriol. **177:**3158–3165.
11. **Pansegrau, W., and E. Lanka.** 1991. Common sequence motifs in DNA relaxases and nick regions from a variety of DNA transfer systems. Nucleic Acids Res. **19:**3455.
12. **Pansegrau, W., G. Ziegelin, and E. Lanka.** 1988. The origin of conjugative IncP plasmid transfer: interaction with plasmid-encoded products and the nucleotide sequence at the relaxation site. Biochim. Biophys. Acta **951:**365–374.
13. **Parker, A. C., and C. J. Smith.** 1993. Genetic and biochemical analysis of a novel Ambler class A β-lactamase responsible for cefoxitin resistance in *Bacteroides* species. Antimicrob. Agents Chemother. **37:**1028–1036.
14. **Scott, J. R.** 1993. Conjugative transposons, p. 597–614. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria. American Society for Microbiology, Washington, D.C.
15. **Scott, J. R., F. Bringel, D. Marra, G. V. Alstine, and C. K. Rudy.** 1994. Conjugative transposition of Tn*916*: preferred targets and evidence for conjugative transfer of a single strand and for a double-stranded circular intermediate. Mol. Microbiol. **11:**1099–1108.
16. **Shoemaker, N. B., R. D. Barber, and A. A. Salyers.** 1989. Cloning and characterization of a *Bacteroides* conjugal tetracycline-erythromycin resistance element by using a shuttle cosmid vector. J. Bacteriol. **171:**1294–1302.
17. **Shoemaker, N. B., C. Getty, E. P. Guthrie, and A. A. Salyers.** 1986. Regions in *Bacteroides* plasmids pBFTM10 and pB8-51 that allow *Escherichia coli-Bacteroides* shuttle vectors to be mobilized by plasmids and by a conjugative *Bacteroides* tetracycline resistance element. J. Bacteriol. **166:**959–965.
18. **Shoemaker, N. B., and A. A. Salyers.** 1990. A cryptic 65-kilobase-pair transposonlike element isolated from *Bacteroides uniformis* has homology with *Bacteroides* conjugal tetracycline resistance elements. J. Bacteriol. **172:**1694–1702.
19. **Shoemaker, N. B., G.-R. Wang, A. M. Stevens, and A. A. Salyers.** 1993. Excision, transfer, and integration of NBU1, a mobilizable site-selective insertion element. J. Bacteriol. **175:**6578–6587.
20. **Simon, R., U. Priefer, and A. Pühler.** 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram negative bacteria. Bio/Technology **1:**784–791.
21. **Smith, C. J.** 1985. Development and use of cloning systems for *Bacteroides fragilis*: cloning of a plasmid-encoded clindamycin resistance determinant. J. Bacteriol. **164:**294–301.
22. **Smith, C. J.** 1989. Clindamycin resistance and the development of genetic systems in the *Bacteroides*. Dev. Ind. Microbiol. **30:**23–33.
23. **Smith, C. J., M. Rogers, and M. McKee.** 1992. Heterologous gene expression in *Bacteroides fragilis*. Plasmid **27:**141–154.
24. **Stevens, A. M., J. M. Sanders, N. B. Shoemaker, and A. A. Salyers.** 1992. Genes involved in production of plasmidlike forms by a *Bacteroides* conjugal chromosomal element share amino acid homology with two-component regulatory systems. J. Bacteriol. **174:**2935–2942.
25. **Stevens, A. M., N. B. Shoemaker, and A. A. Salyers.** 1990. The region of a *Bacteroides* conjugal chromosomal tetracycline resistance element which is responsible for production of plasmidlike forms from unlinked chromosomal DNA might also be involved in transfer of the element. J. Bacteriol. **172:** 4271–4279.
26. **Stevens, A. M., N. B. Shoemaker, L.-Y. Li, and A. A. Salyers.** 1993. Tetracycline regulation of genes on *Bacteroides* conjugative transposons. J. Bacteriol. **175:**6134–6141.
27. **Valentine, P. J., N. B. Shoemaker, and A. A. Salyers.** 1988. Mobilization of *Bacteroides* plasmids by *Bacteroides* conjugal elements. J. Bacteriol. **170:** 1319–1324.
28. **Wilkins, B., and E. Lanka.** 1993. DNA processing and replication during plasmid transfer between Gram-negative bacteria, p. 105–136. *In* D. B. Clewell (ed.), Bacterial conjugation. Plenum Press, New York.