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A Cryptic 65-Kilobase-Pair Transposonlike Element Isolated from *Bacteroides uniformis* Has Homology with *Bacteroides* Conjugal Tetracycline Resistance Elements

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A 65-kilobase-pair element, XBU4422, which has some transposonlike characteristics but carries no known antibiotic resistance genes, has been isolated from *Bacteroides uniformis* 0061. XBU4422 was trapped on *Bacteroides-Escherichia coli* shuttle vectors during experiments in which one of the conjugal *Bacteroides* tetracycline resistance (Tc^r) elements was being used to mobilize the shuttle vectors to *Bacteroides* recipients. Results of Southern hybridization experiments showed that XBU4422 is normally integrated in the *B. uniformis* 0061 chromosome and is found only in some strains. Insertion of XBU4422 in the shuttle vectors was site specific and orientation specific. Nonmobilizable vectors that had acquired XBU4422 became transmissible and could be transferred to *Bacteroides* or *E. coli* recipients. In *B. uniformis* transconjugants, the XBU4422 insertion in the vectors was usually intact, but XBU4422 was always lost in matings with *E. coli*, *Bacteroides thetaiotaomicron*, or *B. ovatus*. The loss of XBU4422 did not visibly alter the vector; in the case of *E. coli*, the loss of the insertion appeared to be RecA dependent. Although XBU4422 carried no antibiotic resistances, it shared regions of homology with six conjugal *Bacteroides* Tc^r elements; this homology was strongest with the ends of XBU4422. Using a strain of *B. thetaiotaomicron* that contains no XBU4422-hybridizing sequences, we showed that the ends of XBU4422 were probably reacting with the ends of the Tc^r elements. These results provide the first direct evidence that the Tc^r elements, like XBU4422, are integrated in the chromosome and that insertion of at least some Tc^r elements, such as Tc^r Em^r DOT, is relatively site specific.

Conjugal tetracycline resistance (Tc^r) elements are found in many human colonic *Bacteroides* species (9, 12). These conjugal Tc^r elements may play a major role in the spread of antibiotic resistance genes among colonic bacteria. In addition to the Tc^r gene, many of the elements also carry a gene for the resistance to clindamycin and erythromycin (Em^r). Moreover, all of the Tc^r elements so far tested are able to mobilize coresident plasmids to *Bacteroides* and *Escherichia coli* recipients (16, 22).

The conjugal *Bacteroides* Tc^r elements are believed to be integrated in the host chromosome, because transfer of Tc^r is not associated consistently with transfer of a plasmid (9, 12). However, there has been no conclusive proof of their chromosomal location. Recently, we obtained for the first time a set of overlapping cosmid clones that covered most of a conjugal Tc^r Em^r element, which is designated Tc^r Em^r DOT (14). Hybridization experiments with the cloned DNA demonstrated that most of the *Bacteroides* Tc^r and Tc^r Em^r elements shared large regions of homology with the Tc^r Em^r DOT element and were virtually identical in the region near the Tc^r gene (14; N. Shoemaker, unpublished data). Thus the characteristics of the Tc^r Em^r DOT element are likely to be shared by the other conjugal *Bacteroides* Tc^r elements. The restriction map derived from the Tc^r Em^r DOT clones was linear, as would be expected if the element were located in the chromosome. But this was not conclusive proof of chromosomal location, since we could not be sure that we had cloned the entire element.

We attempted to determine whether the element was inserted in the *Bacteroides* chromosome by testing DNA from the outermost ends of the cloned region for hybridization to chromosomal DNA from *Bacteroides uniformis* 0061, a Tc^s strain we have used as a recipient in our conjugation experiments. Since we had obtained some of our clones from a transconjugant of *B. uniformis* 0061 that had received the Tc^r Em^r DOT element, we expected that if these outermost clones contained junction fragments, i.e., fragments that had both element DNA and host chromosomal DNA, they should hybridize with *B. uniformis* 0061 chromosomal DNA. We found that clones containing the outermost regions of the element did in fact hybridize with *B. uniformis* 0061 DNA. However, some clones containing internal regions of the Tc^r Em^r DOT element also hybridized with *B. uniformis* 0061 DNA. This finding raised the question of whether *B. uniformis* 0061 harbored an element that was related to the Tc^r Em^r elements but did not carry any resistance genes.

In this paper, we show that *B. uniformis* 0061 does indeed contain such a cryptic element, which is about 65 kilobase pairs (kb) in size. We also show that this element is able to invade coresident plasmids and that nontransmissible plasmids that acquire the cryptic element become transmissible. Additionally, we have found that the ends of this cryptic element share homology with the ends of the Tc^r Em^r DOT element. Since the regions of Tc^r Em^r DOT that hybridized with the ends of XBU4422 were not fully represented in any of our cosmid clones, we have used ends of XBU4422 as probes to show that the Tc^r Em^r DOT element is located in the chromosome and appears to have a preference for one integration site.

MATERIALS AND METHODS

Strains and plasmids. The *Bacteroides* and *E. coli* strains are listed in Table 1. The *Bacteroides* strains were grown on prereduced Trypticase (BBL Microbiology Systems, Cockeysville, Md.)-yeast extract (TYE) broth or agar, and *E. coli* strains were grown on Luria broth (LB) or agar (LA) as

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant phenotypes[a]	Source or description
E. coli		
SF8	Str^r RecA$^+$	W. Reznikoff
HB101	Str^r RecA$^-$	2
Bacteroides clinical isolates		Contain conjugal Tc^r elements
BF ERL	Tc^r Em^r	Contains Tc^r ERL and Tc^r Em^r ERL (18)
BF 12256	Tc^r Em^r	Contains Tc^r Em^r 12256 (18)
BF V479C	Tc^r	Contains Tc^r V479, cured of pBF4 (18)
BT DOT	Tc^r Em^r	Contains Tc^r Em^r DOT (18)
B. uniformis 0061		Virginia Polytechnic Institute, Blacksburg, type strain
BU1001	Rif^r	Spontaneous Rif^r mutant of *B. uniformis* 0061 (16)
BU1004	Rif^r Tc^r	BU1001 Tc^r ERL transconjugant from BF ERL (16)
BU1100	Thy$^-$ Tpm^r	Spontaneous Thy$^-$ mutant of *B. uniformis* 0061 that causes the strain to be Tpm^r (16)
B. ovatus 0038		Virginia Polytechnic Institute type strain
BO2001	Rif^r	Spontaneous Rif^r mutant of *B. ovatus* 0038
B. thetaiotaomicron 5482		Virginia Polytechnic Institute type strain
BT4001	Rif^r	Spontaneous Rif^r mutant of *B. thetaiotaomicron* 5482
BT4002	Rif^r Tc^r	BT4001 Tc^r V479 transconjugant from BF V479
BT4004	Rif^r Tc^r	BT4001 Tc^r ERL transconjugant from BF ERL
BT4007	Rif^r Tc^r Em^r	BT4001 Tc^r Em^r DOT transconjugant from BT DOT
BT4008	Rif^r Tc^r Em^r	BT4001 Tc^r Em^r 12256 transconjugant from BF 12256
Plasmids		
pB8-51		Cryptic 4.4-kb *Bacteroides* plasmid, cloned in several *Bacteroides-E. coli* shuttle vectors, necessary for replication in *Bacteroides* hosts (16)
pEG920	Ap^r *Tc^r Mob$^{+/-}$ (Em^r Mob$^{+/-}$)	Shuttle vector containing pUC19, pB8-51, and the *Eco*RI fragment from Tn*4400* (16)
pE5-2	Su^r *Tc^r Mob$^+$ (Em^r Mob$^-$)	Shuttle vector containing RSF1010, pB8-51, and the *Eco*RI fragment from Tn*4351* (16)
pNIL51	Ap^r Su^r *Tc^r Mob$^+$ (Em^r Mob$^-$)	Shuttle vector pE5-2 with pBR322 derivative, pDG5 (5), added (16)
pEG920::XBU4422	Unstable (Em^r Tra$^+$)	pEG920 with 65kb insertion, XBU4422; loses XBU4422 in *E. coli* BT4001 and BO2001 transconjugants; Tra$^+$ in *B. uniformis* hosts (this study)
pE5-2::XBU4422	Unstable (Em^r Tra$^+$)	pE5-2 with 65kb insertion, XBU4422; loses XBU4422 in *E. coli* BT4001 and BO2001 transconjugants; Tra$^+$ in *B. uniformis* hosts (this study)
pNIL51::XBU4422	Rep$^-$ (Em^r Tra$^+$)	Rep$^-$ in *E. coli*; loses XBU4422 in BO2001 transconjugants, Tra$^+$ in *B. uniformis* hosts (this study)
pXBU1	Ap^r *Tc^r (Rep$^-$)	*Bam*HI deletion of pEG920::XBU4422; contains the right junction of pEG920 and XBU4422 (this study)
pNFD13-2	Ap^r Mob$^+$ (Tc^r Mob$^+$)	2.5-kb segment of Tc^r Em^r DOT which contains the Tc^r gene, cloned in the *Sst*I site of pFD160 (19) (this study)

[a] Abbreviations for bacterial phenotypes: Str^r, streptomycin resistance; Rif^r, rifampin resistance; Thy$^-$, thymidine requiring; Tpm^r, trimethoprim resistance due to Thy$^-$ phenotype. Abbreviations for plasmid, transposon, or inserted conjugal element phenotypes: Ap^r, ampicillin resistance; Em^r, erythromycin resistance; Su^r, sulphonamide resistance; *Tc^r, aerobic tetracycline resistance carried on the *Bacteroides* transposons (Tn*4351* and Tn*4400*) but no expression in *Bacteroides* hosts; Mob$^+$, can be mobilized; Mob$^-$, cannot be mobilized; and Mob$^{+/-}$, mobilization deficient; Rep$^+$, can replicate; Tra$^+$, capable of self-transfer. Plasmid phenotypes within parentheses are the phenotypes that are expressed in *Bacteroides* recipients, whereas phenotypes not inside the parentheses are the phenotypes expressed in *E. coli*.

described previously (17). The plasmids used in this study are also described in Table 1. Plasmid preparations from both organisms were made by the Ish-Horowitz method (8).

Nitrocellulose filter matings. The filter matings were done as described previously (15, 22). The mating filters were incubated anaerobically if the donors were *Bacteroides* species and aerobically if the donors were *E. coli*. The *E. coli* transconjugants were selected aerobically on LA containing ampicillin (100 μg/ml) or tetracycline (10 μ/ml). BU1100 transconjugants were selected anaerobically on TYE agar containing trimethoprim (100 μg/ml), thymidine (100 μg/ml), and erythromycin (10 μg/ml) or tetracycline (3 μg/ml). BU1001 transconjugants were selected on TYE agar containing rifampin (10 μg/ml) and erythromycin (10 μg/ml) but no added thymidine. Gentamicin (200 μg/ml) was used to select against *E. coli* when necessary.

Agarose gels and Southern blot analysis. Chromosomal DNA was isolated by the method of Saito and Miura (11). The restriction enzymes used to digest the DNA samples were from Bethesda Research Laboratories, Inc., or Boehringer Mannheim Biochemicals. Restriction fragments from plasmids to be used for probes for Southern blots were isolated from 0.8% Seaplaque low-melting-point agarose gels (FMC BioProducts, Rockland, Maine) by freeze-thaw phenol extraction. The extracted piece of agarose was mashed, an equal volume of phenol saturated with buffer was added,

and the sample was frozen at −80°C for 20 min. The unthawed sample was spun for 10 min in a microfuge, and the aqueous layer was removed, extracted with phenol-chloroform (50:50), and then ethanol precipitated. For Southern blots or routine analysis the DNA samples were run on 0.8 to 1% agarose gels in 1× or 4× GGB buffer (1× GGB is 0.04 M Tris−0.02 M sodium acetate–0.002 M EDTA [pH 8.3]). For Southern blot analysis the DNA was transferred to Millipore HAHY nitrocellulose paper by capillary action. The probes were labeled with [^{32}P]dCTP by the nick-translation protocol provided by Bethesda Research Laboratories. The hybridization and washes were done as previously described (15). Briefly, the hybridizations were done at 42°C in 50% formamide, and the final washes were in 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 60°C. In some cases ^{32}P-labeled λ DNA was added as a probe to hybridize to the λ standards on the blot. Occasionally, the blots were stripped by washing them twice for 30 min each at 80°C in distilled water, and the stripped blot was then rehybridized with a second probe.

Colony hybridizations were done as described previously (23), and the filters were then treated the same as Southern blots for hybridization and development of the autoradiograms.

TABLE 2. Insertion of the 65-kb element XBU4422 into shuttle vectors

Shuttle vector in BU1004 donor[a]	Recipient	Frequency of transfer[b]	Vectors with 65-kb insertion/total[c]
pEG920	SF8	3.0×10^{-8}	0/40
	HB101	1.3×10^{-7}	0/40
	BU1100	6.0×10^{-8}	32/40
pE5-2	SF8	$<5 \times 10^{-9}$	
	HB101	$<1 \times 10^{-9}$	
	BU1100	5.0×10^{-9}	1/2[d]
pNIL51	SF8	$<4 \times 10^{-9}$	
	HB101	$<2 \times 10^{-9}$	
	BU1100	3.0×10^{-9}	1/1[d]

[a] The donor cells were grown in 1 μg of tetracycline per ml to induce the transfer functions of Tc^r ERL (16). The Tc^r transferred to BU1100 at frequencies of 10^{-7} to 10^{-6}, independent of the transfer of the Em^r on the vectors.

[b] Frequency of transfer is calculated as the number of transconjugants per recipients at the end of the conjugation period.

[c] The insertions were detected by doing plasmid preparations on the transconjugants and comparing the restriction digests, usually *Hin*dIII, of the original shuttle vector with those of the plasmid isolated from the transconjugants. The total numbers of plasmid preparations made are shown.

[d] Five experiments resulted in only two pE5-2 transconjugants and one pNIL51 transconjugant.

RESULTS

Insertion of a large cryptic element into three shuttle vectors. Previous hybridization experiments had shown that *B. uniformis* 0061, a strain which is Tc^s and Em^s and was thus thought to have no resident Tc^r or Tc^r Em^r element, nonetheless contained DNA that cross-hybridized with cloned internal segments of the Tc^r Em^r DOT element (14). One explanation of this finding was that *B. uniformis* contained an element that shared homology with the Tc^r Em^r DOT element but did not carry the antibiotic resistances usually associated with the Tc^r elements. Direct evidence for the existence of such an element in *B. uniformis* 0061 was obtained when the element was trapped on three of our *Bacteroides-E. coli* shuttle vectors.

A number of chimeric shuttle vectors have been constructed which can be mobilized by IncP plasmids from *E. coli* to *Bacteroides* recipients at high frequencies (5, 16, 17, 22). Some of these shuttle vectors can also be mobilized from *Bacteroides* donors to *Bacteroides* or to *E. coli* recipients by the conjugal *Bacteroides* Tc^r elements (22). However, other shuttle vectors, such as pE5-2, pNIL51, and pEG920, are either not transferred at all by the Tc^r elements or are transferred at a level that is barely detectable (16). To determine whether the low frequency of mobilization of pE5-2, pNIL51, and pEG920 by the Tc^r elements was true mobilization or represented some cointegrative or recombinational event involving the Tc^r element, we analyzed plasmid preparations of *Bacteroides* and *E. coli* transconjugants. In these experiments, we used a Tc^r element designated Tc^r ERL. Tc^r ERL was used rather than the Tc^r Em^r DOT element because the Tc^r ERL element is Em^s and the Em^r phenotype could thus be used to monitor plasmid transfer. Also, the Tc^r ERL element is closely related to the Tc^r Em^r DOT element (14).

Only pEG920 was transferred to *E. coli* and it was apparently transferred unaltered. However, about 60% of the *Bacteroides* pEG920 transconjugants contained pEG920 with a 60- to 65-kb insertion (Table 2). The single *B. uniformis* pNIL51 transconjugant and one of the two *B. uniformis* pE5-2 transconjugants also contained the vector with a large insertion (Table 2). Transfer, with acquisition of the 60- to 65-kb insertion, was only detected when the donor strain, containing Tc^r ERL and one of the vectors, was pregrown in tetracycline to induce the Tc^r ERL transfer functions. No transconjugants were detected when *B. uniformis* 0061, containing the shuttle plasmids but no Tc^r element, was the donor (transfer frequency, $<10^{-9}$ per recipient). Thus, either the initial insertion of the 60- to 65-kb element into the shuttle vectors or the subsequent transfer step appeared to require some *trans*-acting factor(s) from the Tc^r element. Although the Tc^r ERL element stimulated insertion and/or transfer of the cryptic element, transfer of plasmids containing the cryptic element appeared to occur independently of transfer of the Tc^r ERL element, because none of the Tc^r transconjugants was Em^r and vice versa.

All but one of the large insertions in the vectors appeared to be identical by restriction analysis. The exception was one pEG920 isolate, which contained an additional 1 to 2 kb of DNA. A comparison of *Hin*dIII digests of some of the different insertions is shown in Fig. 1. Restriction digests with other enzymes (data not shown) also indicated that the insertions in the different shuttle vectors were identical. The DNA inserted in the different shuttle vectors cross-hybridized on Southern blots. This is shown in Fig. 1B for two insertions in pEG920 but was also seen in other pEG920 insertions and in the pNIL51 and pE5-2 insertions (data not shown). The Southern blot in Fig. 1B also shows that the insertion that had the extra 1 to 2 kb of DNA did not involve an element that was different from the element responsible for the other insertions. Thus the same element appeared to be responsible for all of the insertions. We will refer to this element as XBU4422. A partial restriction map of pEG920 with a XBU4422 insertion, pEG920::XBU4422, is shown in Fig. 2 (the *Hin*dIII sites that have been mapped are indicated in parenthesis; there are four other sites that have not been mapped).

Source of XBU4422. DNA from *B. uniformis* 0061, which did not contain the Tc^r ERL element, cross-hybridized with XBU4422, and the *Hin*dIII pattern was the same as that of the XBU4422 insertion except for junction fragments (Fig.

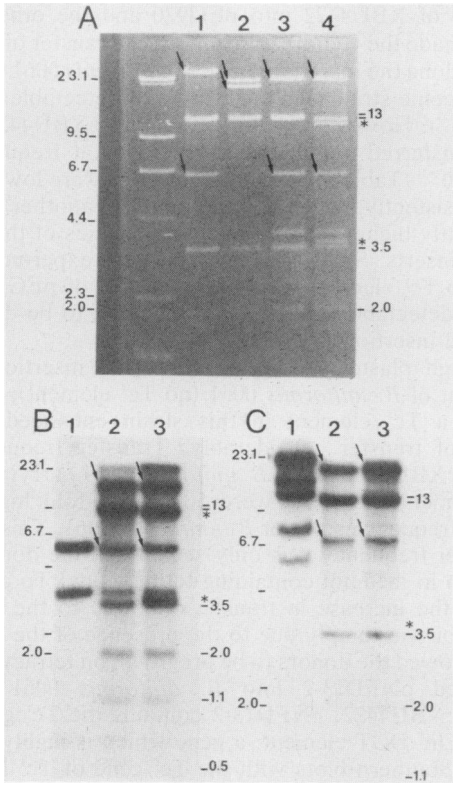

FIG. 1. XBU4422 insertions in the plasmid vectors and in the *B. uniformis* chromosome. (A) Agarose gel of *Hin*dIII digests of insertions in three shuttle vectors: pE5-2 (lane 1), pNIL51 (lane 2), and pEG920 (lanes 3 and 4). The insertion in lane 4 has an extra 1- to 1.5-kb segment, Ω10. The *Hin*dIII lambda standards and their sizes in kilobase pairs are shown on the left. The XBU4422 internal *Hin*dIII fragments common to all four of the insertions are indicated by lines and sizes (in kilobase pairs) on the right of the gel. The 13-kb fragment is a doublet, indicated by a double line. The extra *Hin*dIII site in pEG920Ω10 separates one of two 13-kb fragments into 11- and 3.7-kb fragments not seen in the other insertions, indicated on the right (*). The plasmid vector XBU4422 junction fragments are indicated by arrows in each lane. (B) Autoradiogram of the Southern blot of pEG920 (lane 1), pEG920::XBU4422 (lane 2), and pEG920::XBU4422Ω10 (lane 3) digested with *Hin*dIII and hybridized to ^{32}P-labeled pEG920::XBU4422. The 6-kb bands, which are marked by arrows in lanes 2 and 3, appeared to comigrate with a similar-sized band in lane 1. However, this was shown not to be the same band by digestion with other restriction enzymes (data not shown). (C) Autoradiogram of total DNA from the clinical *B. fragilis* ERL (lane 1), *B. uniformis* BU1001 (lane 2), and a BU1001 Tc^r ERL transconjugant, BU1004 (lane 3). DNA was digested with *Hin*dIII, and the Southern blot was hybridized with the pEG920::XBU4422 probe. The locations of internal XBU4422 bands in pEG920::XBU4422 (A) are indicated on the right side of the autoradiogram. The probable XBU4422-chromosome junction fragments are indicated by arrows in lanes 2 and 3. The agarose gel for the autoradiogram shown in panel C was run longer than the gels in panels A and B to allow better separation of the large fragments (>23 kb).

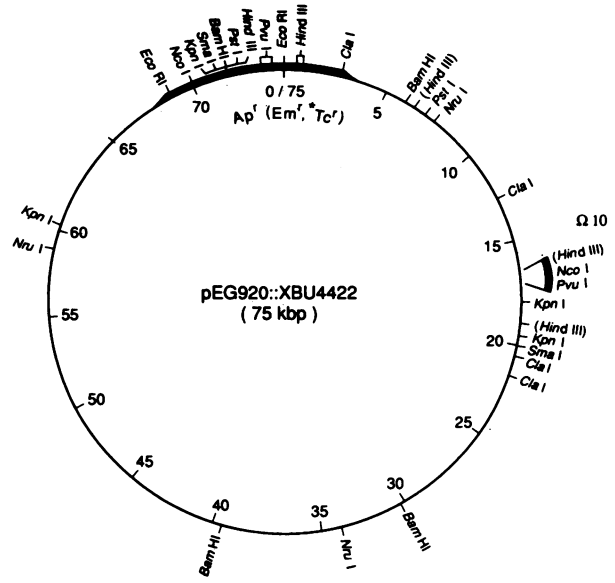

FIG. 2. Partial restriction map of pEG920 containing an XBU4422 insertion. The heavy line between 67 and 3 kb indicates the extent of pEG920. The smaller heavy line at 17 kb represents the small 1- to 1.5-kb insertion and the added restriction sites found in one isolate, pEG920Ω10. The *Hin*dIII sites that have been mapped in XBU4422 and pEG920Ω10 are shown within parenthesis. There are four other *Hin*dIII sites, between 20 and 50 kb on the map, that are not yet placed.

1C). This indicated that XBU4422 was already in *B. uniformis* 0061 before the Tc^r ERL was introduced into this strain and was not part of the Tc^r ERL element. There was no evidence of the extra DNA segment seen in the one pEG920 insertion, so this extra piece was presumably acquired in this one case and not deleted in all of the other insertion events.

Further evidence that XBU4422 was not part of the Tc^r ERL element came from the finding that hybridization of XBU4422 with the Tc^r ERL element was much weaker than hybridization with the DNA in *B. uniformis* 0061 (Fig. 1C, lanes 2 and 3). Introduction of the Tc^r ERL element into *B. uniformis* 0061 (lane 3) did not change the major bands in the hybridization pattern. The bands corresponding to DNA on Tc^r ERL, which has homology with XBU4422, are visible in lane 3, but are faint compared with the bands corresponding to DNA already in *B. uniformis* 0061 and are only seen clearly when the autoradiogram is exposed for longer periods (data not shown).

Interestingly, XBU4422 hybridized strongly with DNA in *Bacteroides fragilis* ERL, the clinical isolate from which the Tc^r ERL element was originally obtained, although the pattern was not the same as that seen in *B. uniformis* 0061 (Fig. 1C, lane 1). The *B. fragilis* ERL strain is known to carry a second Tc^r element, Tc^r Em^r ERL (14), which has stronger homology with XBU4422 than Tc^r ERL (data not shown). However, we cannot rule out the existence of a third element in the *B. fragilis* ERL strain.

B. uniformis 0061 carries a low-copy-number 50-kb plasmid. Southern blot analyses (data not shown) demonstrated that there was no detectable homology between XBU4422 and the 50-kb plasmid. Nor was there any cross-hybridization between XBU4422 and the two integrated elements of *B. uniformis*, which are excised as plasmidlike forms, NBU1, and NBU2, by the Tc^r ERL element (18).

XBU4422 insertion sites in the vectors. XBU4422 insertions in the shuttle plasmids appeared to be site specific. A total of 32 insertions in pEG920, from four separate matings, were analyzed. Several attempts were made to isolate XBU4422 insertions in pNIL51 and pE5-2, but only one insertion in each of these vectors was ever detected. All of the insertions in pEG920 were within the same 0.9-kb *Cla*I-to-*Eco*RI fragment, and all were in the orientation shown in Fig. 2.

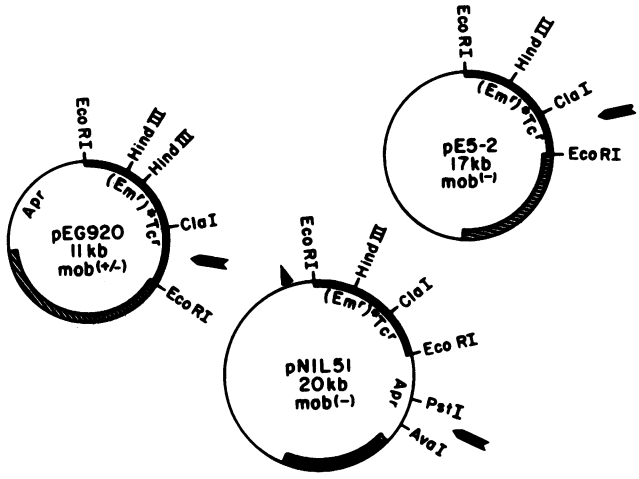

FIG. 3. Locations of XBU4422 insertions in three *Bacteroides-E. coli* vectors are indicated by arrows. The heavy solid line denotes the region containing the *Bacteroides* Em^r and *Tc^r genes. The shaded regions represent *Bacteroides* plasmid pB8-51, which is also shared by all three vectors. The small spontaneous deletion in a RSF1010 replication gene of pNIL51::XBU4422 is indicated by a solid triangle. Because of the deletion, this vector can no longer replicate in *E. coli*.

This 0.9-kb fragment lies within a *Bacteroides* transposon, Tn*4400* (10). It is upstream of an aerobic tetracycline resistance gene (4), which we have designated *Tc^r (21), and it does not contain any known insertion sequence element. In Fig. 3 the XBU4422 insertion sites in pEG920, pE5-2, and pNIL51 are indicated by arrows. The insertion in pE5-2 was within a 0.95-kb *Cla*I-*Eco*RI fragment in the same orientation relative to the two restriction sites as seen for pEG920. This 0.95-kb fragment comes from a second *Bacteroides* transposon, Tn*4351* (15), and is highly homologous but not identical to the 0.9-kb fragment in pEG920 (20). The insertion into pNIL51, a derivative of pE5-2, was the only one that was not within the *Cla*I-*Eco*RI fragment. This insertion occurred near the origin of replication of the pBR322 derivative, pDG5 (5), between the *Pst*I in the ampicillin resistance gene and the *Ava*I site. The pNIL51::XBU4422 plasmid also contained a spontaneous deletion (Fig. 3), which interrupted a replication function on the RSF1010 portion of the vector. The same region of XBU4422 was involved in all the insertions tested.

Properties of the vectors containing the XBU4422 insertions. Insertion of XBU4422 into pEG920 and the other shuttle vectors made the vectors transmissible. Transfer of the three vectors alone (no insertion) from *B. uniformis* 0061 (BU1001) to an isogenic strain (BU1100) was not detectable (frequency, $<10^{-9}$). However, vectors containing XBU4422 inserts were transferred out of the same donor at frequencies of 10^{-7} to 10^{-8} (Table 3). These frequencies were low but were seen consistently from one experiment to another and were significantly higher than transfer frequencies of the vectors with no inserts. Since the donor in these experiments contained no Tc^r element, and since transfer of pEG920 alone was not detectable, the transfer appeared to be due to the XBU4422 insertion.

Although plasmids carrying XBU4422 insertions transferred out of *B. uniformis* 0061 (no Tc^r element), the presence of a Tc^r element in this strain enhanced the frequency of transfer considerably. Transfer frequencies of pEG920::XBU4422 out of *B. uniformis* 0061 carrying the Tc^r ERL element (BU1004) were 50- to 100-fold higher than transfer frequencies out of *B. uniformis* 0061. This increase in transfer frequency was only seen when the donors were pregrown in medium containing tetracycline. To determine whether the increase in transfer was due to the Tc^r ERL element or was simply due to the presence of the Tc^r gene, which allowed the donors to be pregrown on tetracycline, we introduced pNFD13-2 into *B. uniformis* 0061 carrying pEG920::XBU4422. pNFD13-2 contains the Tc^r gene from the Tc^r Em^r DOT element, a gene which is highly homologous on Southern blots with the Tc^r gene of Tc^r ERL. We found that pNFD13-2 could be mobilized from *E. coli* to *B. uniformis* with R751 and replicated in *B. uniformis*, with a copy number slightly lower than that of the pB8-51-based vectors such as pEG920. In *B. uniformis*, pNFD13-2 was compatible with pEG920::XBU4422 and with all other vectors containing pB8-51. *B. uniformis* 0061 carrying both pNFD13-2 and pEG920::XBU4422 transferred pEG920::XBU4422 to *E. coli* SF8 (where it was recovered as pEG920) at the same frequency as *B. uniformis* containing only pEG920::XBU4422. Pregrowth of donors on tetracycline did not affect the transfer frequency of pEG920::XBU4422. Thus it appears that some other *trans*-acting genes of Tc^r ERL are needed to give the 50- to 100-fold increase in transfer of pEG920::XBU4422 that was seen when donors carried Tc^r ERL and were pregrown on tetracycline. Interestingly, pEG920::XBU4422 was apparently able to transfer pNFD13-2 to *E. coli* at a high frequency (1×10^{-5} to 3×10^{-5}).

Interpretation of these transfer experiments was compli-

TABLE 3. Transfer of the shuttle vectors containing the 65-kb element XBU4422 from *B. uniformis* donors to *E. coli* or *B. uniformis* recipients

Shuttle vector in BU1100 donor	Frequency of transfer[a]		
	E. coli SF8[b]	*E. coli* HB101	*B. uniformis* BU1001[c]
pEG920::XBU4422	0.2×10^{-7}–3×10^{-7}	$<1 \times 10^{-9}$	1×10^{-7}–3×10^{-7}
pEG920::XBU4422Ω10	0.2×10^{-7}–10×10^{-7}	$<1 \times 10^{-9}$	0.8×10^{-7}–10×10^{-7}
pE5-2::XBU4422	0.1×10^{-7}–2.6×10^{-7}	$<1 \times 10^{-9}$	1×10^{-7}–3×10^{-7}
pNIL51::XBU4422	–[d]	–	2×10^{-7}–8×10^{-7}
pEG920[e]	$<1 \times 10^{-9}$	$<1 \times 10^{-9}$	$<5 \times 10^{-9}$

[a] The conjugations were performed as described previously (16). Ranges given here represent three different experiments.
[b] All of the SF8 transconjugants contained vector without the XBU4422 insertion.
[c] Of the BU1001 transconjugants, 60 to 80% contained vectors with the XBU4422 insertion; the remainder contained vector without the XBU4422 insertion.
[d] –, pNIL51::XBU4422 did not replicate in *E. coli* due to a deletion in the replication region which is recognized in *E. coli*.
[e] pEG920 is not transferred from BU1100 donors at a detectable frequency, although it is mobilized at low frequencies by the Tc^r ERL element to both SF8 and HB101 recipients (16) (Table 2). pE5-2 and pNIL-51 are also not transferred out of BU1100 (transfer frequency, $<10^{-9}$), nor are they mobilized by the Tc^r ERL element (16).

cated by the fact that the *B. uniformis* 0061 donors contained a chromosomal copy of XBU4422 as well as the XBU4422 insertion in the shuttle vector. Also, it is possible that there are other, as yet undiscovered conjugal elements in this strain. Accordingly, we attempted to mate the vectors with the XBU4422 insertions into *Bacteroides* strains that we found by hybridization experiments to be free of XBU4422-hybridizing DNA. These strains included *Bacteroides thetaiotaomicron* 5482 (BT4001), *B. ovatus* 0038 (BO2001), and *B. fragilis* 2044R. When *B. uniformis* 0061 was the donor, the only transfer detected was to *B. ovatus* 0038, at a frequency of 10^{-8}. None of the vectors recovered from these transconjugants contained XBU4422 insertions. When *B. uniformis* 0061 containing the Tc^r ERL element (BU1004) was the donor and the donor was pregrown in medium containing tetracycline, transfer to *B. thetaiotaomicron* was also detected (1×10^{-4} to 7×10^{-4} per recipient), but all of the transconjugants contained vectors that had lost XBU4422.

pEG920::XBU4422 and pE5-2::XBU4422 transferred reproducibly from *B. uniformis* 0061 to *E. coli* SF8 recipients at low frequencies (10^{-9} to 10^{-7}), but no transfer was detected if the recipient was RecA$^-$ (*E. coli* HB101). Although higher transfer frequencies from *B. uniformis* to *E. coli* SF8 (2×10^{-5} per recipient) were obtained when the *B. uniformis* donor carried a Tc^r element, there was still no detectable transfer to *E. coli* HB101 RecA$^-$ recipients under the same conditions. All of the *E. coli* SF8 transconjugants contained only the vector pEG920 or pE5-2, without any evidence of XBU4422. Thus loss of the XBU4422 inserts during transfer to *E. coli* appeared to be RecA dependent. Also, loss of the XBU4422 insertion appeared to be necessary for recovery of the transconjugant. We tried to introduce pEG920::XBU4422 and pE5-2::XBU4422 into *E. coli* SF8 by transformation. All of the transformants contained pEG920 or pE5-2 without the XBU4422 insertion, and the transformation frequency was very low.

Although the XBU4422 insertion was lost from the plasmid when pEG920::XBU4422 was transferred to *E. coli*, *B. thetaiotaomicron*, or *B. ovatus*, it was possible that XBU4422 had entered the chromosomes of these recipients. Since XBU4422 contains no known markers, it was necessary to screen for such events by colony hybridization with an internal *Bam*HI fragment of XBU4422 that was isolated from an agarose gel. Eighty *B. ovatus* 0038 transconjugants, 400 *B. thetaiotaomicron* transconjugants, and over 400 *E. coli* SF8 transconjugants were tested and all were negative. Therefore, if XBU4422 does insert in the recipient chromosome, it does so at frequencies of less than 10^{-3} to 10^{-2}.

Regions of DNA homology between the conjugal Tc^r elements and XBU4422. Hybridization experiments had shown that XBU4422 hybridized with DNA from a number of the Tc^r and Tc^r Em^r elements, including the Tc^r Em^r DOT element. Although our clones of DOT element DNA cover at least 55 kb, it was not clear whether any of these clones carry the ends of the DOT element. Thus, information about the extent of homology between the DOT element and XBU4422 would not only indicate how related these elements were but might also provide information about the DOT element itself. In particular, if there was homology between the ends of the Tc^r elements and the ends of XBU4422, the ends of XBU4422 could be used as probes to determine whether the Tc^r elements are inserted in the *Bacteroides* chromosome and, if so, whether insertions are site specific. To determine the extent of the homology between XBU4422 and the Tc^r Em^r DOT element, two large cosmid clones of Tc^r Em^r DOT element DNA, which span

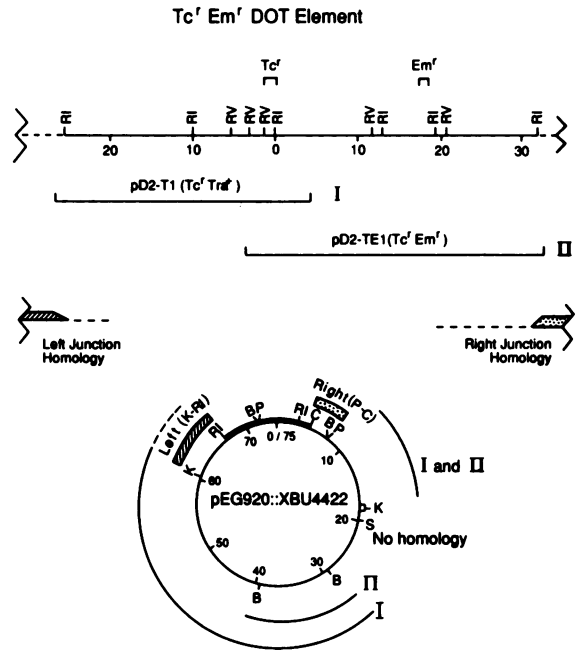

FIG. 4. Regions of homology between XBU4422 and the cloned Tc^r Em^r DOT element. A partial restriction map, showing the *Eco*RI (RI) and the *Eco*RV (RV) sites, derived from overlapping cosmid clones (14), is at the top of the figure. The vertical zig-zags at the ends represent the junctions between the element and the recipient chromosome. Two of the cosmids, cloned from the clinical BT DOT strain, which together cover the entire cloned regions of the Tc^r Em^r DOT, are shown below the map. pD2-T1 (I) contains the Tc^r region and enough of the transfer functions to allow apparent self-transfer (Tra$^+$) of the cosmid (14). pD2-TE1 has both the Tc^r and Em^r regions, it is Tra$^-$. An abbreviated circular map of pEG920::XBU4422 is shown at the bottom. Regions of XBU4422 that hybridized to pD2-T1 (I) or to pD2-TE1 (II) on Southern blots are indicated in the outside arcs. There was a small region of homology between D2-T1 and the K-R left junction fragment (segment with diagonal lines) of XBU442, which appears to hybridize with one of the ends of the Tc^r Em^r DOT element. The *Pst*I-*Cla*I right junction probe (segment with dots) appears to hybridize with the other end of the Tc^r DOT element, but this cross-hybridizing DNA was not on any of our clones.

the portion of the element that has been cloned, were used as probes in Southern blots containing XBU4422 digested with different restriction enzymes (Fig. 4). Regions homologous to the two DOT probes were distributed throughout XBU4422, although there was a 12-kb region on XBU4422 that had no homology with either of the DOT clones.

We made numerous attempts to clone the ends of XBU4422 separately in *E. coli*. We were successful in cloning a large region containing the right end (pXBU1), but we were not successful in cloning the left end, even when we tried to construct the clones by deleting large regions of pEG920::XBU4422 and religating. Accordingly, to determine whether the ends of XBU4422 were homologous to the ends of the DOT element, we isolated end regions of XBU4422 from gels and labeled the isolated fragments for use as probes. A right-junction *Pst*I-*Cla*I fragment from pEG920::XBU4422, which contained an end of XBU4422 plus some pEG920 DNA, hybridized, as expected, to the Em^r region of pD2TE1, because sequences in the *Cla*I-*Eco*RI fragment of pEG920 are known to share homology with the Em^r region. However, there was no additional homology between the end of XBU4422 and either pD2TE1

or pD2T1. By contrast, a left-junction *Kpn*I-*Eco*RI clone that contained DNA from the other end of XBU4422 hybridized with the outermost *Eco*RI fragment of the DOT clone, pD2T1. The *Pst*I-*Cla*I and *Kpn*I-*Eco*RI regions from XBU4422 did not hybridize with each other or with any internal regions of the DOT element except the Em^r region. From these results, it appeared that the *Kpn*I-*Eco*RI segment of XBU4422 hybridizes with an end of the DOT element. However, it was not possible to ascertain from these data whether the *Pst*I-*Cla*I segment of XBU4422 hybridized with the right end of the DOT element. Either the *Pst*I-*Cla*I segment did not hybridize at all with the right end of the DOT element, or our clones did not contain this end of the DOT element.

If the right-junction (*Pst*I-*Cla*I) segment of XBU4422 had homology with DNA at or near an end of the Tc^r Em^r DOT element, it should hybridize with different-sized restriction fragments of chromosomal DNA from different *Bacteroides* species containing the DOT element. Since these other species have at most 30 to 40% DNA homology with *B. uniformis* 0061 (7), the sizes of restriction fragments containing the end of an inserted element would be likely to be different even if the element was inserted in the same site. By contrast, an internal segment (e.g., the Em^r region) should hybridize to the same-sized fragment regardless of strain background. To test this, we used the *Pst*I-*Cla*I fragment as a probe to detect cross-hybridizing DNA in digests of DNA from *B. uniformis* 0061, *B. uniformis* 0061 containing Tc^r Em^r DOT, the original *B. thetaiotaomicron* DOT strain, *B. thetaiotaomicron* 5482, and *B. thetaiotaomicron* 5482 containing Tc^r Em^r DOT (Fig. 4). *B. thetaiotaomicron* 5482 contains no XBU4422 sequences.

A new band was apparent in a transconjugant of *B. uniformis* 0061 that had acquired the Tc^r Em^r DOT element (Fig. 5), and this band did not comigrate with the strongest cross-hybridizing band in DNA from *B. thetaiotaomicron* 5482 carrying the DOT element. The lighter band was the internal fragment of the DOT element near the Em^r gene, which hybridized with the pEG920 portion of the *Pst*I-*Cla*I probe and was the same in all three strains. Although the hybridizing band from the *B. thetaiotaomicron* DOT strain appeared to comigrate with the band in the *B. uniformis* transconjugant in Fig. 5A (*Eco*RI digest), it is clear from the pattern in Fig. 5B (*Hin*dIII digest) that the band in the original *B. thetaiotaomicron* DOT strain is different from that in the *B. uniformis* 0061 or *B. thetaiotaomicron* 5482 transconjugants. Thus the *Pst*I-*Cla*I fragment containing the right end of XBU4422 hybridized with DNA from the Tc^r Em^r DOT. This hybridizing DNA was not an internal segment of the DOT element and was probably an end of the DOT element. Similar results were seen when the *Kpn*I-*Eco*RI (left-end) fragment was used as the probe (data not shown).

To determine whether the ends of XBU4422 hybridized to DNA from Tc^r elements other than the DOT element and to determine whether the Tc^r Em^r DOT element inserted in a single site, the right- and left-end fragments of XBU4422 were used to probe *Hin*dIII-digested DNA from transconjugants of *B. thetaiotaomicron* 5482 that contained various Tc^r and Em^r elements. All of the transconjugants contained DNA homologous to the XBU4422 end probes (Fig. 6). In the case of the Tc^r elements, Tc^r V479 and Tc^r ERL, only one band hybridized with the end fragments. The extra band in DNA from strains carrying the Tc^r Em^r elements came from internal homology between the Em^r regions of these elements and the region of pEG920 into which XBU4422 inserted (13). In the case of the right-end probe of XBU4422 (Fig. 6B), the cross-hybridizing restriction fragments associated with different Tc^r and Tc^r Em^r elements were clearly different from each other. In the case of the left-end probe from XBU4422 (Fig. 6C), the cross-hybridizing bands were so large that it was difficult to determine whether they were different in size, but other restriction digests (data not shown) indicated that they were not the same. A comparison of five different transconjugants containing the Tc^r Em^r DOT element (Fig. 6, lanes 5a through 5e) showed that in four of the five transconjugants, the DOT element appeared to have inserted in the same site in the chromosome.

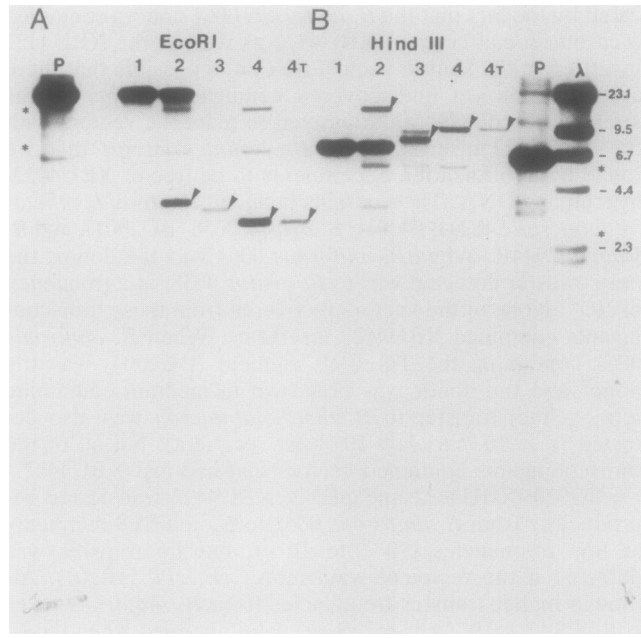

FIG. 5. Autoradiogram illustrating junction fragments with different mobilities in three different *Bacteroides* species containing the Tc^r Em^r DOT element. Total DNA from *Bacteroides* strains with and without the conjugal Tc^r Em^r DOT element was digested with *Eco*RI (A) or *Hin*dIII (B) and run on a 0.8% agarose gel (lanes): 1, BU1001; 2, BU1006; 3, BT DOT; 4, BT4007; 4t, BT4007 grown in tetracycline; P, pEG920::XBU4422 cut with the enzymes. The probe used for the Southern blot was the ^{32}P-labeled 4.2-kb *Pst*I-*Cla*I right-junction fragment between pEG920 and XBU4422 (Fig. 4). The Tc^r Em^r DOT-associated band which hybridizes to the probe and which changes in size in the different host backgrounds, is indicated by arrows in the individual lanes. The dark bands seen in lanes 1 and 2 arise from hybridization of the probe with the copy of XBU4422 which was already in the strain. The faint band seen in lanes 2 and 3 is due to cross-hybridization between a small segment of pEG920, which was cloned along with the right end of XBU4422, and a region near the Em^r gene of the Tc^r Em^r DOT element. The sizes in kilobase pairs of the λ standards are indicated on the right.

Other *Bacteroides* strains with sequences homologous to that of XBU4422. None of the type strains from the human colonic *Bacteroides* species other than *B. uniformis* contained any detectable XBU4422 sequences. We also tested six other *B. uniformis* strains from our collection and found that two of them, BUC7-17 and BUT1-1, had one or two >23-kb *Hin*dIII fragments that hybridized to the XBU4422 probes. Both of these strains were also Tc^r, but we do not know whether the Tc^r is associated with a conjugal element.

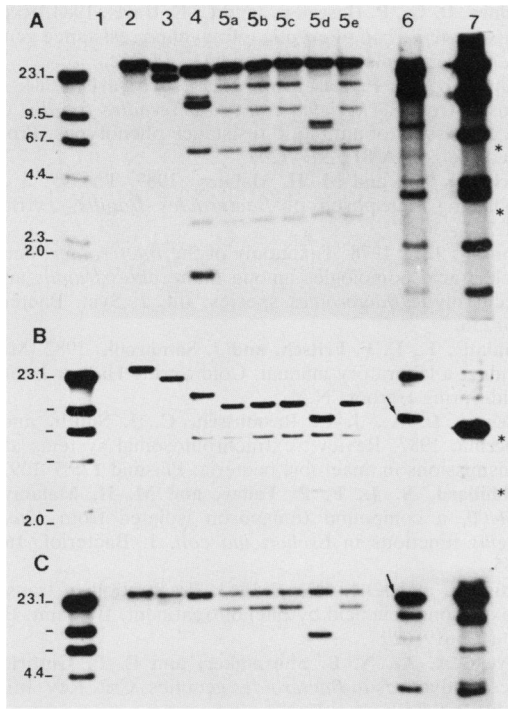

FIG. 6. Autoradiograms indicating the regions of homology between four conjugal Tc^r elements and XBU4422. The total DNA from each of the following strains was digested with *Hin*dIII and run on an 0.8% agarose gel (lanes): 1, BT4001; 2, BU4002 (Tc^r V479); 3, BT4004 (Tc^r ERL); 4, BT4008 (Tc^r Em^r 12256); 5a through 5e, five independently isolated BT4007 strains (Tc^r Em^r DOT); 6, BU1001; 7, pEG920::XBU4422Ω10 digested with *Hin*dIII. *Hin*dIII λ standards are indicated on the left. The Southern blots of the gels were hybridized to ^{32}P-labeled pEG920::XBU4422 (A) and the 4.2-kb *Pst*I-*Cla*I right-junction fragment between pEG920 and XBU4422 (B). The Southern blot in panel B was stripped and hybridized to the 7.6-kb *Kpn*I-*Eco*RI left-junction fragment between pEG920 and XBU4422 for panel C. Labeled λ DNA was added as a probe in all three panels to identify the *Hin*dIII λ standards on the autoradiograms. The *Hin*dIII junction fragments of XBU4422 and the BU1001 chromosome, which correlate to the *Pst*I-*Cla*I right probe and the *Kpn*I-*Eco*RI left probe, are indicated by arrows in lane 6 of panels B and C, respectively. Since the homology between the Tc^r transconjugants and the XBU4422 probes was not 100%, the completely homologous sequences in lane 6 and the plasmid lane are slightly overexposed. Note the altered migration of two of the bands in lane 5d compared with those in lanes 5a, 5b, 5c, and 5e. The locations of common bands due to homology of the probes to the region around the Em^r gene on the elements in panel A and B are indicated to the right (*).

DISCUSSION

In this report we describe a 65-kb *Bacteroides* element, designated XBU4422, that was isolated as insertions in three shuttle vectors. This element, which is cryptic in the sense that it contains no known antibiotic resistance genes, is the second transmissible cryptic element to be found in *Bacteroides* species. Recently, Hecht and Malamy (6) described a 9.6-kb transmissible element that carried no antibiotic resistances and behaved like a transposon. This element has been designated Tn*4399*. Transfer frequencies of plasmids that had acquired Tn*4399* were similar in magnitude to those associated with XBU4422, but, unlike XBU4422 insertions, Tn*4399* insertions were stable in *E. coli*.

XBU4422 is an endogenous integrated element in *B. uniformis* 0061, the strain from which it was isolated, but is not found in most other *Bacteroides* strains, including other strains of *B. uniformis*. Nontransmissible plasmids that acquired XBU4422 insertions became transmissible at low frequencies. This transfer frequency was enhanced considerably when one of the Tc^r elements was in the same strain and the donor was pregrown on tetracycline. Thus tetracycline-regulated genes of the Tc^r elements can act in *trans* to aid transfer of XBU4422. Hecht and Malamy (6) did not ascertain whether transfer of plasmids carrying Tn*4399* was enhanced by any of the *Bacteroides* Tc^r elements.

Although it appears that transfer of pEG920::XBU4422 from *B. uniformis* 0061 was mediated by XBU4422 itself, we cannot rule out the possibility that XBU4422 merely provided a mobilization region that is recognized by some other, as yet undiscovered, element in *B. uniformis* 0061. We were unable to transfer plasmids containing XBU4422 to any other *Bacteroides* strain without losing the XBU4422 insertion, and thus we were unable to demonstrate retransfer in a different genetic background.

It appears that loss of XBU4422 from the plasmids occurs in the recipients. First, since pEG290 without XBU4422 is not transferred at a detectable frequency out of *B. uniformis* 0061, we would not have detected pEG920 in the transconjugants if XBU4422 had been lost in the donor. Moreover, the requirement for RecA function in *E. coli* recipients suggests that XBU4422 is excised in the recipient. Possibly the conjugation process causes the same sort of zygotic induction of XBU4422, as seen with the streptococcal conjugal transposon Tn*916* (13) and the *E. coli* transposon Tn*5* (1). The fact that insertions of XBU4422 can be recovered from most *B. uniformis* 0061 transconjugants indicates that such insertions can be transferred and maintained in this strain background. Possibly, the XBU4422 insertions are more stable in *B. uniformis* 0061 than in the other strains we tested, because the chromosomal copy of XBU4422 in *B. uniformis* 0061 stabilizes the XBU4422 insertions in the plasmids.

XBU4422 exhibited some transposonlike properties. It inserted into different plasmids. The same regions of XBU4422 always appeared at the ends of the insertions, indicating that XBU4422 invaded the shuttle vectors rather than vice versa. However, we cannot conclude that XBU4422 is a transposon. First, insertion appears to be relatively site specific. Second, the apparent requirement for RecA in transfers to *E. coli* indicates that loss of XBU4422 was due to a process involving homologous recombination, although we cannot rule out the possibility that loss of XBU4422 occurred differently from insertion. More important, we have been unable to demonstrate movement of XBU4422 from the shuttle vectors in which it was originally isolated to some other replicon. Without the introduction of some selectable marker onto XBU4422, it will be difficult to detect a second insertion event.

XBU4422 is not the first cryptic element we have found integrated in the *B. uniformis* 0061 chromosome. Two other elements, NBU1 and NBU2, are 10- to 11-kb DNA segments that were isolated as insertions in the IncP plasmid R751 (18). However, unlike XBU4422 insertions, NBU1 and NBU2 insertions in R751 appear to have been due to invasion of NBU1 and NBU2 by an insertion sequence on R751 and not to invasion of R751 by NBU1 and NBU2 themselves (18). NBU1 and NBU2 have no detectable homology to XBU4422 by Southern blot analysis. The presence of cryptic elements such as XBU4422 in some *Bacteroides* strains is important for two reasons. First, these

elements could become resistance transfer elements if they acquired resistance genes. Second, their presence can complicate interpretation of experiments involving known Tc^r and Tc^r Em^r elements because of the significant amount of homology. Our findings indicate that *B. uniformis* 0061, a strain that has been used by us and others as a recipient in matings involving the Tc^r and Tc^r Em^r elements, may not be the best strain to use in these experiments. In particular, since there is homology between XBU4422 and the Tc^r elements, it is possible that the majority of the insertions of these elements in the *B. uniformis* chromosome occur by homologous recombination with XBU4422 rather than by the insertion pathway used in strains that do not contain homologous DNA. In fact, we observed previously that the frequency of transfer of some Tc^r elements, including Tc^r Em^r DOT, into and out of *B. uniformis* 0061 was higher than that seen with other *Bacteroides* strains (N. Shoemaker, unpublished).

Although XBU4422 is not part of the conjugal Tc^r ERL element, it does have partial DNA homology to Tc^r ERL and to five other *Bacteroides* conjugal Tc^r or Tc^r Em^r elements. This homology was concentrated near the ends of the XBU4422 element (compare Fig. 6A, in which the whole XBU4422 insertion was used to probe the Tc^r elements, with Fig. 6B and C, in which only the ends of XBU4422 were used as probes). The homology between the XBU4422 element and the conjugal Tc^r elements suggests that all of the elements are members of a family of conjugal elements but are not identical. The homology between the ends of XBU4422 and the ends of the Tc^r elements allowed us to conclude that our clones of the Tc^r Em^r DOT element do not contain either end of that element. However, by using the ends of XBU4422 as probes, we were able to show for the first time that the Tc^r Em^r DOT element is integrated in the chromosome and appears to have a preference for a single region. The fact that we have not been able to capture any of the Tc^r elements on plasmids may indicate that, although the ends of the Tc^r elements have homology, the different elements do not have the same insertion site. We have some evidence that Tc^r ERL can coexist in a *B. uniformis* strain with either Tc^r Em^r DOT or Tc^r Em^r 12256 (18). Since XBU4422 is already in *B. uniformis*, this would mean that there were three large elements in the same host. It has also been observed that some of the integrated 70-kb conjugal Tc^r elements in *Streptococcus pneumoniae* can coexist in the same host (3).

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