The *Bacteroides* Mobilizable Insertion Element, NBU1, Integrates into the 3' End of a Leu-tRNA Gene and Has an Integrase That Is a Member of the Lambda Integrase Family

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NBU1 is a 10.3-kbp integrated *Bacteroides* element that can be induced to excise from the chromosome and can be mobilized to a recipient by trans-acting functions provided by certain *Bacteroides* conjugative transposons. The NBU1 transfer intermediate is a covalently closed circle, which is presumed to be the form that integrates into the recipient genome. We report here that a 2.4-kbp segment of NBU1 was all that was required for site-specific integration into the chromosome of *Bacteroides thetaiotaomicron* 5482. This 2.4-kbp region included the joined ends of the NBU1 circular form (*attN1*) and a single open reading frame, *intN1*, which encoded the integrase. Previously, we had found that NBU1 integrates preferentially into a single site in *B. thetaiotaomicron* 5482. We have now shown that the NBU1 target site is located at the 3' end of a Leu-tRNA gene. The NBU1 integrase gene, *intN1*, was sequenced. The predicted protein had little overall amino acid sequence similarity to any proteins in the databases but had limited carboxy-terminal similarity to the integrases of lambdoid phages and to the integrases of the gram-positive conjugative transposons Tn916 and Tn1545. We also report that the *intN1* gene is expressed constitutively.

A family of mobilizable insertion elements called NBUs (nonreplicative *Bacteroides* units) has been found in human colonic *Bacteroides* species. Four members of the NBU element family (NBU1, NBU2, NBU3, and Tn4555) have been described (3, 22, 24, 26). All of these elements have in common that they are about 10 to 12 kbp in size and their transfer intermediate is a covalently closed circle (13, 15). This circle does not replicate but can be transferred by conjugation, starting from an internal transfer origin (*oriT*). Recent studies have shown that the NBU-type elements all share a region of high sequence similarity that includes *oriT*, a mobilization gene (*mob*) that lies immediately downstream of *oriT*, and a 900-bp region upstream of *oriT* (15, 24).

NBUs are not self-transmissible, nor can they excise on their own. Both excision and mobilization of NBUs require trans-acting functions provided by a *Bacteroides* conjugative transposon (CT) (22, 27, 28). *Bacteroides* CTs are large (>60 kbp) self-transmissible integrated elements that are usually located in a part of the chromosome different from the location of the NBU they excise and mobilize (3). A regulatory protein provided by the CT, RteB, is needed to stimulate excision and mobilization (29).

The conditions for filter matings have been described previously (20, 30). The mating filters (GN-6; Gelman) were incubated at 37°C aerobically on TYG agar and, the donor-to-recipient ratio was 1:3. *Bacteroides* transconjugants were selected on TYG agar plates containing gentamicin (200 µg/ml) and erythromycin (10 µg/ml).

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** Bacterial strains and plasmids used in this study are described in Table 1. *Escherichia coli* strains were grown in Luria broth (LB) or on LB agar plates. *Bacteroides* strains were grown in prereduced Trypticase (BBL Microbiological Systems, Cockeysville, Md.)-yeast extract-glucose (TYG) (9) medium under an atmosphere of 80% nitrogen–20% carbon dioxide or on TYG agar plates in BBL GasPak jars.

DNA extractions and Southern blot analysis. Plasmids were isolated by using the Ish-Horowitz modification of the alkaline lysis procedure (19). Total DNA was isolated by using a modification of the method of Saito and Miura (18). Restriction enzyme digests of the DNA and cloning procedures were done according to the manufacturers' suggestions (GIBCO-BRL; New England Biolabs; or Promega, Madison, Wis.) or as described by Sambrook et al. (19).

**Construction of minimal NBU1 insertion vectors.** To determine the minimum region of NBU1 needed to insert in *B. thetaiotaomicron*, the insertion vector
**TABLE 1. Bacterial strains and plasmids used in this study**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant characteristics</th>
<th>Description and/or source (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DH5αMCR</td>
<td></td>
<td><em>GIBCO-BRL</em></td>
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<tr>
<td>S17-1</td>
<td>RecA</td>
<td>IncP RP4 inserted in chromosome</td>
</tr>
<tr>
<td></td>
<td>RecA Tra- Tp' Sm'</td>
<td>(1RPl4-Tc-Mu-Kn:TN7); furnishes transfer functions (25)</td>
</tr>
<tr>
<td><strong>B. thetaiotaomicron 5482 strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT4001</td>
<td>Rif'</td>
<td>Spontaneous Rif' mutant of BT5482</td>
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<tr>
<td>BT4004</td>
<td>Rif' Tc'</td>
<td>BT4001 containing Tc' ERL</td>
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<tr>
<td>BT4100</td>
<td>Thy' Tp'</td>
<td>Thymidine-requiring spontaneous mutant of BT5482</td>
</tr>
<tr>
<td>BT4100N1-S1</td>
<td>Thy'</td>
<td>BT4100 containing NBU1 in attB, BT1-1 (24)</td>
</tr>
<tr>
<td>BT4104</td>
<td>Thy' Tp' Tc'</td>
<td>BT4104 containing CT, Tc' ERL</td>
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<tr>
<td>BT4104N1-3</td>
<td>Thy</td>
<td>BT4104 containing NBU1 in attB, BT1-1 (24).</td>
</tr>
<tr>
<td><strong>Plasmids and vectors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R751</td>
<td>Tp' Tra+</td>
<td>IncP plasmid used to mobilize vectors in conjugal matings (20)</td>
</tr>
<tr>
<td>pUC19</td>
<td>Ap'</td>
<td><em>E. coli</em> cloning vector (31)</td>
</tr>
<tr>
<td>pVAL1</td>
<td>Ap' Tc' Mob+ (Em') (Mob- Rep+)</td>
<td>*E. coli-Bacteroides shuttle vector (30)</td>
</tr>
<tr>
<td>pNV19</td>
<td>Ap' Mob+ (Em') (Mob- Rep+)</td>
<td></td>
</tr>
<tr>
<td>pNVIN3</td>
<td>Same as pNV19</td>
<td>5.6-kbp <em>PvuII-PstI</em> fragment of NBU1 in pUC19 (Fig. 1) (this study)</td>
</tr>
<tr>
<td>pLA06</td>
<td>Same as pNVIN3</td>
<td>Deletion clone of pNVIN3 (this study)</td>
</tr>
<tr>
<td>Y5D and Y11D</td>
<td>Ap' Tc' Mob+ (Em') (Mob- Rep+ Int+)</td>
<td>pEG920:NBU1 cointegrates, Y5 and Y11, with the pB8-51 replication removed. Integrate and excise in <em>Bacteroides</em> hosts (21, 24)</td>
</tr>
<tr>
<td>Y11DP</td>
<td>Same as Y11D except Exc-</td>
<td>3-kbp <em>PstI</em> deletion of Y11D (24).</td>
</tr>
<tr>
<td>pMJJF2</td>
<td>Ap' Mob+ (Em') (Rep+ Mob- GUS+ )</td>
<td>Replicative GUS (uidA) transcriptional fusion vector (8)</td>
</tr>
<tr>
<td>pCOW1</td>
<td>Same as pMJJF2</td>
<td>Insertional GUS (uidA) transcriptional fusion vector (8)</td>
</tr>
<tr>
<td>pMFJ::PintN1</td>
<td>Ap' Mob+ (Em') (Rep+ GUS- )</td>
<td>850-bp region of NBU1 containing promoter region of <em>intN1</em> cloned in correct orientation in front of GUS reporter gene in pMJJF2 (this study)</td>
</tr>
<tr>
<td>pCOW::intN1</td>
<td>Same as pCOW1</td>
<td>346-bp fragment in N-terminal end of <em>intN1</em> cloned in correct orientation in front of GUS to measure activity of <em>PintN1</em> in inserted NBU1 form (this study)</td>
</tr>
</tbody>
</table>

* Phenotypes in parentheses are expressed only in *Bacteroides* spp., and phenotypes not in parentheses are expressed in *E. coli*. Abbreviations: Ap', ampicillin resistant; Rif', rifampin resistant; Sm', streptomycin resistant; Tp', trimethoprim resistant; Thy', thymidine requiring; Tra, conjugal transfer functions; Mob, mobilizable by Tra function of IncP plasmids or (if in parentheses) by *Bacteroides* conjugative transposons; Rep, replication; Int, integration; Exc, excision.

pNVIN3 was constructed. Steps in the construction of pNVIN3 are shown in Fig. 1. pNVIN3 was mobilized by IncP plasmids (R751 or R4P in S17-1) from *E. coli* to *B. thetaiotaomicron* BT4001. BT4001 transconjugants that contained the secondary *intN1* site for NBU1 in BT4001 (attB1), BT1-2, have been previously described (24). In the present study, more extensive sequencing of the chromosomal region adjacent to the left end of the integrated NBU1 was done to determine the identity of the locus that contained the primary integration site. The only observed secondary attB site for NBU1 in BT4001, BT1-2, was cloned as described for BT1-1 (24). The sequences of the junctions were obtained by using the left and right junction primers from NBU1 (24). The chromosomal sequence obtained was used to design primers for PCR amplification of the intact secondary NBU1 integration site, BT1-2. The resulting 370-bp fragment was cloned into pUC19 (pUC::BT1-2) and sequenced.

**DNA sequencing and sequence analysis.** Double-stranded template sequencing was performed on plasmids by using the dideoxy-chain-terminating reaction with T7 polymerase as provided in the Sequenase 2.0 kit (U.S. Biochemical, Cleveland, Ohio). More extensive sequencing was performed at the University of Illinois Biotechnology Genetic Engineering Facility in Urbana with synthesized primers and the Applied Biosystems model 373A version 2.0.1S dye terminator automated sequencer. Computer analyses of the nucleotide and amino acid sequences were done by using the Genetics Computer Group software systems (7). The amino acid sequence alignment of the integrases of NBU1, Tn1545, and lambda was done by using the BESTFIT program. The BLAST program (1) was used to search databases for nucleotide and amino acid sequence similarities.

**Transcriptional fusions to the NBU1 integrase gene, *intN1*.** To determine if the NBU1 integrase was regulated at the transcriptional level, while in the circular transfer intermediate form or in the inserted preexcision form, two transcriptional fusion constructs were made. To test the circular form of the promoter region of *intN1*, a segment containing the joined ends of the circular form of NBU1 (bp 1 to 840) was cloned upstream of the reporter group, uidA (β-glucuronidase) (GUS), on pMJJF2 to form pMJJF2::*PintN1. pMJJF2 is an *E. coli-Bacteroides* shuttle plasmid that replicates in *Bacteroides* spp. as well as in *E. coli* (8). A GUS fusion to the *intN1* gene in the inserted form of NBU1 was made by cloning an internal 346-bp region near the N terminus of *intN1* (bp 767 to 1113) in the correct orientation relative to the GUS gene in plasmid pCOW1 (8).
TcrERL was involved in expression of the NBU1 but not integrated NBU1 (BT4104). To determine whether RteB (from the CT was also transferred to a strain that contained the CTTc rERL as follows: 2,446-bp NBU1 attN1-intN1 except that the probe used was one that hybridized only with the ends of NBU1, not to the entire NBU1.

RESULTS AND DISCUSSION

NBU1 primary and secondary integration sites in B. thetaiotaomicron. Previously we found that NBU1 inserts site specifically in B. thetaiotaomicron, in an attB site designated BT1-1. A comparison of the sequence in the region containing the joined ends of NBU1 (attN1) with the sequence of BT1-1 revealed that attN1 and BT1-1 had two regions of high similarity, region I and region II (Fig. 2A). Region I was a 14-bp sequence that was identical in both the NBU1 attN1 and BT1-1. Region II was a 10-bp sequence found in attN1 that had partial identity to a corresponding region in BT1-1 and was located within a region that contained inverted repeats (Fig. 2A). Therefore, when the circular intermediate of NBU1 inserts into BT1-1, as shown in Fig. 2B, there is a copy of the 14-bp region I sequence and a copy of a 10-bp region II sequence at both ends of the inserted NBU1. The inverted-repeat region, within which the 10-bp region II structure is located, is depicted as a stem-and-loop structure in Fig. 2B. In only one instance has NBU1 been seen to insert into a secondary site (24). This site has been designated BT1-2. BT1-2 was cloned from the BT4001 chromosome and sequenced. This site had identity of 10 of 14 bp in region I but lacked region II of attN1 (Fig. 2A).

The primary site in B. thetaiotaomicron lies at the 3’ end of a tRNA gene. To determine the identity of the locus into which NBU1 was integrating, an extended region of chromosomal DNA adjacent to attL and attR was sequenced. This sequence was compared with GenBank sequences, by using the BLAST program (1). An 86-bp stretch of the sequence in the left junction region, including the 14-bp sequence in region I of BT1-1, was 60 to 68% identical to Leu-tRNA sequences from a variety of organisms, including the Leu-tRNA4 (TAA) of E. coli K-12 (12). The E. coli Leu-tRNA4 had 63% identity along its entire length with the Leu-tRNA sequence of B. thetaiotaomicron. Eleven of the 14 bp in region I and 9 of the 10 bp in region II of attN1 were identical to the corresponding regions in the E. coli Leu-tRNA sequence. However, the 10-bp sequence of region II of the E. coli gene was not within a region containing inverted repeats. The sequence of the putative B. thetaiotaomicron Leu-tRNA is shown in Fig. 3, along with its E. coli Leu-tRNA4 homolog. The anticodon of the Bacteroides Leu-tRNA (CAA) differs from that of the E. coli Leu-tRNA4 (UAA). The 14-bp region I sequence was in the acceptor stem, within 2 nucleotides of the 3’ ends of the Leu-tRNAs (shown boxed in Fig. 3). The 10-bp region II sequence of BT1-1 (GAAAGCTAAG) started 1 nucleotide from the 3’ CA of the B. thetaiotaomicron Leu-tRNA. In E. coli Leu-tRNA, the region II sequence (GAAAGCTAAG) started 3 nucleotides after the 3’ CA. Several bacteriophages are known to integrate into the 3’ ends of tRNA genes (6). tRNA genes are also the targets of the actinomycete plasmids that integrate into the chromosomes of some hosts (4, 5). Thus, NBU1 falls into this family of elements that integrate into tRNA genes.

The overall DNA-DNA homologies among different Bacteroides species are 45% or lower, so that sequences similar enough to be detected by Southern blot analysis (>70%) are the exception rather than the norm (10). Southern hybridization with a probe derived from the attL region demonstrated that the tRNA gene into which NBU1 was integrating was found in all Bacteroides spp. tested (data not shown). Thus, the NBU1 target is found widely in Bacteroides spp., as expected from the fact that NBU-hybridizing elements have been found to produce pCW1::intN1. pCW1 replicates in E. coli but not in Bacteroides spp. This plasmid was transferred by conjugation from E. coli S17-1 to B. thetaiotaomicron recipients with selection for Genr (gentamycin-resistant) Emr tetracycline per ml to induce synthesis of RteB (27). GUS assays were done as described by Feldhaus et al. (8).

Excision assay. A Southern blot approach was used to determine whether NBU1 or portions of it were capable of excision from the B. thetaiotaomicron chromosome. This was done as described previously by Stevens et al. (27, 28) except that the probe used was one that hybridized only with the ends of NBU1, not to the entire NBU1.

Nucleotide sequence accession numbers. The GenBank accession numbers are as follows: 2,446-bp NBU1 attN1·intN1 region, no. 451917; primary NBU1 inte-
FIG. 2. (A) Comparison of NBU1 attN1 and the primary and secondary attB sites in *B. thetaiotaomicron*. The NBU1 attN1 is shown at the top with the 14-bp sequence of region I in bold capital letters, and the 10-bp region is shown, boxed, in a region containing inverted repeats, which are depicted as a stem-and-loop structure. The primary NBU1 attB site in *B. thetaiotaomicron*, BT1-1, has 100% identity to the 14-bp sequence of region I and partial identity to the 10-bp sequence of region II. BT1-2, the only known secondary attB site in *B. thetaiotaomicron*, had partial identity to region I but no region II sequence or inverted repeats. The identical nucleotides in the target sequences in region I are shown as capital letters. The bold capital letters indicate where the crossover occurred to give the hybrid attR and attL sequences at the NBU1-target junctions shown in panel B. The crossover occurred in BT1-2 within the four bases, TGCG. (B) Integration of NBU1 into the primary attB site, BT1-1, in *B. thetaiotaomicron* 5482. NBU1 is shown in the excised circular intermediate form which can either be mobilized from an internal origin of transfer (oriT) and mobilization (mob) region by a conjugative transposon (13, 24) or integrate into an attB site. For integration of NBU1 into its primary *B. thetaiotaomicron* attB site, BT1-1, the crossover occurs within or at either end of the 14-bp region of identical sequence contained on both att sites shown as filled (NBU1 attN1) and open (attB, BT1-1) rectangles. Depending on the location of the actual crossover in the 14-bp region, the 14-bp sequences at the left junction, attL, and the right junction, attR, would be hybrid sequences with part of the sequence coming from attB and the remainder coming from attN1. These hybrid sequences are identical for attN1 and BT1-1, but they would be different if the insertion was in a secondary site with only partial sequence identity, as seen for the secondary attB, BT1-2 (see panel A). There is a second region on both attN1 and BT1-1 which includes a 10-bp sequence of partial identity within an inverted-repeat inverted. The inverted repeats are represented by the corresponding closed (attN1) and open (attB, BT1-1) lollipop symbols. The location of the site of insertion of the shuttle vector pEC920 into NBU1 to form Y11 (21, 24) is indicated by the arrows in both the circular and integrated forms of NBU1, and the PsrI deletion in Y11D derivative Y11DP is also indicated. Y11 and its derivatives were used to monitor integration and transfer of NBU1 in *Bacteroides* hosts (24). The NBU1 gene required for integration, intN1, is also indicated. The highlighted (grey) portion of NBU1 is the region that contains sequences homologous to other NBU elements (15, 24). The enlarged *HincII* (Hc) sites flank a 1.5-kbp fragment in the NBU1 circular intermediate which includes attN1 and part of the IntN1 region. In the integrated form of NBU1 the same Hc sites were used to clone NBU1-chromosomal right and left junction fragments to use as probes on Southern blots. The attB sequence at the left junction, attL, was identical to attN1, and the sequence at the right end, attR, was identical to the respective attB sequence, BT1-1 or BT1-2. Restriction site abbreviations are as follows: H, *HindIII*; Hc, *HincII*, P, *PstI*, Pv, *PvuII*.

widespread in the *Bacteroides* group. The DNA sequence of the attR region had no similarity to any known genes, and a probe from this region did not detect any cross-hybridizing segments in *Bacteroides* spp. other than *B. thetaiotaomicron* (data not shown).

When the secondary site, BT1-2, was used as a probe in Southern blot experiments, it hybridized only to *B. thetaiotaomicron* DNA (data not shown). Approximately 370 bp of sequence was obtained from the right and left chromosomal regions adjacent to BT1-2. No significant homologies to genes in the database were seen when a BLAST search was performed with these sequences. Thus, BT1-2, in contrast to BT1-1, is not located at the end of a conserved gene.

The minimal region required for integration of NBU1 consists of the joined ends plus 2 kbp of the right end of the element. Generally, integrases are located near an end of the element that carries them (6). In our initial investigations of NBU1 integration, we had found that clones of NBU1 that lacked NBU1 sequences between the Y11 insertion site and the *PvuI* site (Y11DP, Fig. 2B) were still able to integrate (24), but a clone with a deletion to the *HindIII* site was not able to integrate. These results suggested that the NBU1 integrase was located near the right end of the element, as depicted in Fig. 2B. To test this hypothesis and to determine what sequences near the left end of NBU1 may be required, a 5.6-kbp *PvuII* fragment of NBU1, which contained the closed ends of the circular form of NBU1 plus a sizable portion of the left end of NBU1, was cloned into the *Smal* site of the insertional vector, pNV19, to produce pNVIN3 (Fig. 1). pNVIN3 replicates in *E. coli* and is mobilizable by IncP plasmids from *E. coli* donors to *Bacteroides* recipients, but it does not replicate in *Bacteroides* spp. pNVIN3 was mobilized into BT4001 or BT4004 recipients with selection for the erythromycin resistance (*ermF*) marker on pNVIN3. pNVIN3 inserted into the chromosome of BT4001 at a frequency of about 10^-6 transconjugants per recipient. BT4004 differs from BT4001 in that BT4004 carries the conjugative transposon, Te' ERL. BT4004 was used as a recipient in some experiments to determine whether the presence of a CT would stimulate integration. pNVIN3 integrated into the BT4004 chromosome at the same frequency as it did in BT4001 (10^-6 transconjugants per recipient). Thus, integration of NBU1, unlike excision and mobilization, was not dependent on functions provided by Te' ERL.

In all of the transconjugants tested, pNVIN3 integrated into the primary attB site, BT1-1. Integrated pNVIN3 could not be induced to excise and transfer out of the BT4004 transconjugants to *E. coli* recipients, even when the transconjugants were grown in the presence of tetracycline to induce functions on
Tc<sup>+</sup> ERL (<10<sup>-9</sup> transconjugants per recipient). A possible explanation for the failure of integrated pNVIN3 to excise was that the large segment of plasmid DNA inside the integrated NBU1 ends of pNVIN3 somehow interfered with excision, making the distance between the NBU1 ends too great for efficient excision. This was not the case, however, because in previous experiments we had shown that the NBU::plasmid chimeras Y11D and Y5D, which had integrated into the BT4004 chromosome, were capable of excision and transfer out of BT4004 (24). Both Y11D and Y5D carry a segment of non-NBU1 DNA as large as the plasmid DNA segment in pNVIN3. Thus, the failure of the integrated pNVIN3 to transfer out of BT4004 suggests that the portion of NBU1 cloned on pNVIN3 is unable to excise and transfer because it is missing one or more genes necessary for excision. This was confirmed by the finding that no circular intermediate of pNVIN3 could be detected on Southern blots of DNA from tetracycline-induced BT4004 hosts or by PCR amplification of the joined ends of NBU1 (data not shown). The excised circular forms of Y11D and Y5D were detectable under the same conditions, either by Southern blotting or by PCR amplification. Thus, although pNVIN3 contained all of the DNA necessary for integration, it lacked one or more genes necessary for excision.

pNVIN3 contained about 2.5 kbp of DNA from the right end of the element, as well as 2 kbp of DNA from the left end. To localize the integration region further, unidirectional deletions were made from the PvuII end of the NBU1 fragment in pNVIN3 and tested for the ability to integrate site specifically in B. thetaiotaomicron 4001. The smallest integration-proficient deletion clone was pLAl<sub>al</sub> pLAl<sub>bo</sub> contained both region I and region II, plus 200 bp upstream of the inverted repeat of region II and about 2 kbp of DNA downstream from region I. The integration frequency for pLAl<sub>bo</sub> was the same as that for Y11D, which carried the entire NBU1 sequence (about 10<sup>-6</sup> transconjugants per recipient), and insertion occurred only in the primary site, BT1-1 (data not shown).

NBU1 integrase is in the lambda integrase family. A segment of NBU1 which extended from the HincII site (61 bp upstream of the end of pLAl<sub>bo</sub>) to the PstI site at the other end of the DNA cloned in pLAl<sub>bo</sub> (bp 2446) was sequenced. In this sequence, the inverted-repeat region containing the 10-bp sequence of region II of BT1-1 (GAAAAGCTAAG) and BT1-1 (GAAAAGCTAAG), but it is not within an inverted-repeat region. Sequence differences are indicated by underlined bases.

E. coli Leu-tRNA<sub>4</sub> (UAA)

B. thetaiotaomicron Leu-tRNA (CAA)

FIG. 3. NBU1 primary att<sub>B</sub> site in Bacteroides spp. identified as the 3′ end of a Leu-tRNA gene. The sequence adjacent to att<sub>B</sub> had 67% identity with the Leu-tRNA<sub>4</sub> gene of E. coli K-12 (12). The nucleotides in the Leu-tRNA<sub>4</sub> gene that were identical to the putative Bacteroides Leu-tRNA gene are shown as capital letters. The boxed sequences on the acceptor and psistem regions correspond to the 14 bp of region I on attN1 and BT1-1 and the corresponding sequence in the E. coli Leu-tRNA<sub>4</sub>. Immediately 3′ of the ACCA-3′ end of the Leu-tRNA<sub>4</sub> is the 10-bp sequence (GAAAGCTAAG) that has a high degree of identity to the corresponding 10-bp sequences in region II of attN1 (GAAAAGCTAAG) and BT1-1 (GAAAAGCTAAG), but it is not within an inverted-repeat region. Sequence differences are indicated by underlined bases.
Domain I

\[
\text{NBU1:} \quad \begin{array}{c|c|c|c|c|c|c}
\text{Resif} & \text{Fgt} & \text{Lsg} & \text{Vdyl} & \text{Nyl} & \text{KvnG} & \text{vNv} \\
\text{IntN1:} & \text{at} & \text{b} & \text{c} & \text{d} & \text{e} & \text{f} \\
\end{array}
\]

\[
\text{Lambda:} \quad \begin{array}{c|c|c|c|c|c|c}
\text{Resif} & \text{Fgt} & \text{Lsg} & \text{Vdyl} & \text{Nyl} & \text{KvnG} & \text{vNv} \\
\text{IntN1:} & \text{at} & \text{b} & \text{c} & \text{d} & \text{e} & \text{f} \\
\end{array}
\]

\[
\text{Tn9165:} \quad \begin{array}{c|c|c|c|c|c|c}
\text{Resif} & \text{Fgt} & \text{Lsg} & \text{Vdyl} & \text{Nyl} & \text{KvnG} & \text{vNv} \\
\text{IntN1:} & \text{at} & \text{b} & \text{c} & \text{d} & \text{e} & \text{f} \\
\end{array}
\]

\[
\text{NBU1:} \quad \begin{array}{c|c|c|c|c|c|c}
\text{Resif} & \text{Fgt} & \text{Lsg} & \text{Vdyl} & \text{Nyl} & \text{KvnG} & \text{vNv} \\
\end{array}
\]

**Domain II**

\[
\text{NBU1:} \quad \begin{array}{c|c|c|c|c|c|c}
\text{Resif} & \text{Fgt} & \text{Lsg} & \text{Vdyl} & \text{Nyl} & \text{KvnG} & \text{vNv} \\
\end{array}
\]

**FIG. 4.** Similarity of NBU1 integrate to lambdabase integrate. NBU1 integrate has low overall similarity to lambda and related integrates, but it had 26% identity and 48% similarity to lambda Int in the conserved C-terminal domains identified by Argos et al. (2). NBU1 integrate contained the conserved arginine (R) in domain I and the tyrosine (Y) in domain II, indicated by the # symbol. The other highly conserved amino acids shared by most of the lambda family integrates in domain II are in bold letters and are indicated by + symbols. The sequence of the Tn9165 Int, which is nearly identical to Tn9165 Int (16, 29), is shown at the bottom for comparison with the gram-positive conjugal transposons. Identical amino acids are indicated by a | symbol, and amino acid similarities are indicated by a period or a colon.

Values for the wild type. Y11D was not completely deficient in excision, however, because a small number of transconjugants were detected in the assay and low levels of the circular form were detected on Southern blots. Y11DF, a derivative of Y11D which contained a deletion of the region between the Y11 insertion and a PstI site close to the end of IntN1, was completely deficient in excision as measured by the mating-out assay, and no circular form could be detected on Southern blots. Further sequence analysis of the region, which is currently under way, suggests that there are at least three open reading frames in this region. The function of the open reading frames in the region of NBU1 downstream of intN1 remains to be determined. Also, it is not yet clear whether there are still other genes on the NBU1, e.g., between the 3’ end of the mob and the left end of the NBU1, which are required for excision.

**Expression of intN1 is constitutive.** Integration of NBU1 in *B. thetaiotaomicron* appeared to be independent of functions provided by Te’ ERL because pNVIN3 could integrate into a *B. thetaiotaomicron* strain that did not contain Te’ ERL or any other Bacteroides CT. Nonetheless, we have shown previously that the regulatory protein RteB from Te’ ERL is essential for excision and circularization of NBU1. This finding suggested that RteB was regulating expression of one or more excision-integration genes on NBU1. Since int genes of Tn9165-Tn9154 and lambdoid phages are essential for both excision and integration, it was possible that expression of the NBU intN1 gene was regulated at some level by RteB.

To determine whether intN1 was expressed constitutively or was regulated by RteB, we constructed two types of transcriptional GUS fusions to intN1. In one type, a segment from the circular form of attN1 was fused to GUS on a plasmid that replicates in *B. thetaiotaomicron* (pMJF1::PintN1). This plasmid was introduced into BT4100N1-3 (containing a single copy of NBU1), BT4104 (containing Te’ ERL but no NBU1), and BT4104N1-3 (containing NBU1 and Te’ ERL). In the second type of fusion, the Gus gene was inserted into the intN1 gene of an integrated NBU1 to produce BT4100N1-3 (OOCW::intN1) or BT4104N1-3 (OOCW::intN1). These two strains differed in that BT4100N1-3 had no CT and BT4104N1-3 carried Te’ ERL. The GUS specific activity in extracts from strains containing the multicopy plasmid pMJF2::PintN1 was 50 to 60 U/mg of protein regardless of whether the strain contained Te’ ERL and regardless of whether the strain containing Te’ ERL was grown in medium containing low levels of tetracycline (Table 2). GUS expression from fusions to intN1 in the integrated form of NBU1 (OOCW::intN1) was also constitutive and was about eightfold lower than in strains containing pMJF2::PintN1, as expected from the fact that the copy number of pMJF2 is approximately 10 per cell (8).

We also considered the possibility that proteins encoded on NBU1 itself might affect expression of intN1. In pMJF2::PintN1, none of the NBU1 genes was present on the plasmid. With the insertion into the intN1 of the chromosomal copy of NBU1, intN1 was disrupted but the insertion might have exerted a polar effect on downstream genes. To test the hypothesis that proteins encoded on NBU1 might affect expression of intN1, we introduced pMJF2::PintN1 into a strain that contained an integrated copy of NBU1 as well as the CT Te’ ERL. The GUS specific activity in extracts from this strain was the same as that in extracts from the other strains tested. Taken together, these results suggest that intN1 is expressed constitutively and that neither the CT regulatory protein, RteB, nor proteins encoded by NBU1 have any effect on expression of the gene. The requirement for RteB in the NBU1 excision process
must reflect either the interaction of RteB with the promoter of some other gene or a posttranscriptional effect of RteB. The integrated NBU1 that had the GUS gene inserted into intN1 was unable to excise. Results presented above suggested that intN1 was not sufficient for excision. This result suggests that intN1 is essential for excision or that the disruption in intN1 is having a polar effect on an essential excision gene downstream of intN1. Work to identify the gene(s) necessary for excision of NBU1 is currently under way.

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