Direct Repeats Flanking the *Bacteroides* Transposon Tn4351 Are Insertion Sequence Elements

VIVIAN HWA,* NADJA B. SHOEMAKER, AND ABIGAIL A. SALYERS

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Received 15 June 1987/Accepted 30 September 1987

The clindamycin-erythromycin resistance (C^c Em^r) region of the *Bacteroides* transposon Tn4351 is flanked by direct repeats. This study showed that the direct repeats are insertion sequence (IS) elements. Although both IS elements can mediate transfer of the chloramphenicol (Cm^r) marker on pBR328 by cointegrate formation with the conjugal IncW plasmid R388, IS4351_R-mediated transfer of Cm^r occurred at a consistently lower frequency than did the transfer mediated by IS4351_L. Analysis of plasmids from the resultant transconjugants revealed IS-mediated activities such as deletions, tandem duplication of IS4351_L, and excision of IS4351_R.

Three *Bacteroides* transposable elements have been characterized: Tn4351 (7), Tn4400 (5), and Tn4551 (10). All three carry a gene which codes for resistance to both clindamycin and erythromycin (C^c Em^r). Tn4351 and Tn4400 also contain a tetracycline resistance gene (*Tc^r) which confers resistance on aerobically grown *Escherichia coli* but not on anaerobically grown *E. coli* or on *Bacteroides* spp. (2). All three transposons are flanked by homologous direct repeats of approximately 1.2 kilobases (kb) (8, 9). Evidence suggesting that these direct repeats are insertion sequences (IS) has been obtained by Robillard et al. (5). They showed that replicon fusion between two *E. coli* plasmids mediated by Tn4400 could result in cointegrates flanked by a copy of either one of the direct repeats and a copy of Tn4400. This is the structure expected if one of the direct repeats had mediated replicon fusion. They also obtained structures suggestive of inverse transposition events. However, the proportion of cointegrate formation to inverse transposition was not determined, nor were the relative activities of the two direct repeats and the intact transposon compared. Recently, Rasmussen et al. (4) sequenced a homologous direct repeat from Tn4351. This direct repeat, designated IS4351_R, was able to transpose and activate a promoterless chloramphenicol acetyltransferase gene on a target plasmid in *E. coli*. The activity of the other direct repeat was not investigated.

In this study, the independent transposition activity of each direct repeat on Tn4351 was substantiated, and their relative activities were assessed. Each of the direct repeats was cloned into a Tc^s derivative of pBR328 (deletion of the *Bam*HI site of pBR328, 4.7 kb) (Fig. 1). Since pBR328 (11) is a nonmobilizable plasmid, it can be used to detect transfer due to cointegrate formation or other transposon-related activities. By using a Tc^s derivative of pBR328, we were able to select for *Tc^r on Tn4351.

A mobilization assay was used to detect the activities of Tn4351 and the direct repeats. In these conjugation experiments, *E. coli* HB101 (RecA⁻; 1) or EM24 (RecA⁻; supplied by J. Cronan) was used as a donor, and the recipient was a nalidixic acid-resistant, rifampicin-resistant derivative of EM24, EM24NR. Nalidixic acid-resistant, chloramphenicol-resistant (Nal^r Cm^r) or Nal^r Tc^r transconjugants or both were selected as indicated. The conjugal assay measured the frequency of transfer of markers on pBR328 resulting from

cointegrate formation with the conjugal IncW plasmid R388 (32.2 kb; 12). R388 was used because it transfers between *E. coli* strains at high frequencies and contains no known IS elements.

In an initial set of experiments, the donor contained R388 and pVOH1, R388 and pVOH2, or R388 and pVOH3. Results are shown in Table 1. The frequency of Cm^r transfer mediated by IS4351_L (pVOH2) was 10-fold lower than that mediated by Tn4351 (pVOH1). The transfer of Cm^r on pVOH3 (carrying IS4351_R) occurred at a 100-fold-lower frequency than did the transfer associated with IS4351_L. To verify the independent activity of the IS elements, retransfer experiments were carried out with derivatives of R388 into which the cloned Tn4351 or one of the direct repeats had transposed. Table 2 shows the frequency of transfer of Cm^r on pBR328 by R388::Tn4351, R388::IS4351_L, and R388::IS4351_R. The transfer frequencies associated with either Tn4351 or IS4351_L, carried on R388, were comparable to those associated with pVOH1 and pVOH2, respectively (Table 1). However, IS4351_R carried on R388 was 40-fold more active than it was when carried on pBR328 (pVOH3). The increase in activity could be due to differences in the adjacent DNA segments. The effect of adjacent DNA on the activity of the IS elements was not further investigated.

The results clearly demonstrate that both direct repeats were able to mediate the transfer of a marker on pBR328 (Tc^s) through replicon fusion, but IS4351_R was consistently less active than IS4351_L. Neither IS element was as active as the intact transposon. This apparent difference in activity between Tn4351 and the IS4351 elements could have been due in part to the fact that inverse transposition involving the ends of Tn4351 (expected phenotype of transconjugants is *Tc^s Cm^r) or direct transposition of Tn4351 (expected phenotype is *Tc^r Cm^s) could also occur, whereas only cointegrate formation would have been detected in the case of the IS elements.

Tn4351-mediated cointegrate formation should have resulted in the cotransfer of Cm^r and *Tc^r. However, in transfers involving pVOH1, a 98% loss of *Tc^r was detected when chloramphenicol was the selective antibiotic (Table 1). This implied a higher frequency of inverse transposition events (10⁻⁵) than of cointegrate formation (10⁻⁷). Similarly, when tetracycline was the selective antibiotic, cotransfer of Cm^r and *Tc^r was at the same low frequency of 10⁻⁷ (Table 1). In this case, direct transposition of Tn4351 seems to predominate over cointegrate formation events. When the

* Corresponding author.

FIG. 1. Construction of plasmids pVOH1, pVOH2, and pVOH3, carrying *Tn4351*, *IS4351_L*, and *IS4351_R*, respectively. —→, Position of the *Tc^r* gene in pBR328; —, pBR328 sequences; ▨, R751 sequences; ■, *Tn4351* sequences. R751::*Tn4351* (6) was digested with *SalI* to produce a 6.2-kb *SalI* fragment containing the whole transposon *Tn4351*. This *SalI* fragment was then cloned into the *SalI* site on pBR328 (*Tc^s*), and the resulting plasmid was designated pVOH1 (approximately 10.2 kb). The removal of a *HindIII* fragment from pVOH1 resulted in pVOH3, a plasmid of 6.5 kb which contains *IS4351_R*. Plasmid pVOH2 (8.4 kb) contains the **Tc^r* gene and *IS4351_L* from a *HindIII-SalI* digest of R751::*Tn4351* cloned into their respective sites on pBR328 (*Tc^s*).

frequency of *Tn4351*-mediated cointegrate formation was compared with that of *IS4351*-mediated events (Table 1, pVOH2), the frequency of *Tn4351*-mediated cointegration was actually 10-fold lower. In contrast, when *Tn4351* was carried on R388, the frequency of cointegrate formation due to *Tn4351* was 10-fold higher than that due to *IS4351_L*; 26% of *Tn4351*-mediated events cotransferred *Cm^r* and **Tc^r* (Table 2). A possible explanation for the apparent inconsistency in the frequency of *Tn4351*-mediated cointegrate formation could be that some of the events in Table 1 which appear to involve inverse transposition may have resulted from cointegrate formation followed by an IS-mediated deletion of DNA inside *Tn4351*.

Evidence for IS-mediated deletions came from matings in which the donor carried R388 and pVOH2. The resultant transconjugants were expected to contain cointegrates and therefore to be **Tc^r Cm^r*. However, most of these transconjugants carried at least two plasmids; one was usually larger than R388, and the smaller plasmids varied in size. Moreover, of the *Cm^r* transconjugants, nearly one-fourth were tetracycline sensitive (Table 1). Loss of *Tc^r* reflected the deletion of the **Tc^r* region, an event which could occur either because of an unusually high deletion-forming activity of *IS4351_L* or because the instability of the cointegrates provided a selection for deletions and rearrangements.

Plasmid products from six randomly selected *Cm^r* trans-

TABLE 1. Mobilization frequencies associated with *Tn4351*, *IS4351_R*, and *IS4351_L* cloned into pBR328 (*Tc^s*)

Plasmids carried by donor strain	Frequency of transfer of ^a :	
	<i>Cm^r</i>	<i>*Tc^r</i>
R388, pBR328 (<i>Tc^s</i>)	$<2.9 \times 10^{-9}$	NA ^b
R388, pVOH1 (<i>Tn4351</i>)	0.5×10^{-5} – 2.5×10^{-5c}	0.2×10^{-6} – 2.8×10^{-6d}
R388, pVOH2 (<i>IS4351_L</i>)	0.8×10^{-6} – 3.4×10^{-6e}	1.5×10^{-6} – 2.1×10^{-6}
R388, pVOH3 (<i>IS4351_R</i>)	1.6×10^{-8} – 2.3×10^{-8}	NA

^a Number of transconjugants per recipient.

^b NA, Not appropriate.

^c 13 of 789 (2%) events cotransferred **Tc^r* and *Cm^r*.

^d 56 of 641 (9%) events cotransferred **Tc^r* and *Cm^r*.

^e 215 of 281 (76%) events cotransferred **Tc^r* and *Cm^r*.

TABLE 2. Transfer frequencies associated with Tn4351, IS4351_R, and IS4351_L transposed into R388

Plasmids carried by donor strain	Frequency of transfer of Cm ^r ^a
R388, pBR328	$<2.9 \times 10^{-9}$
R388::Tn4351, pBR328 (Tc ^r)	0.4×10^{-5} – 4.0×10^{-5} ^b
R388::IS4351 _L , pBR328	0.2×10^{-6} – 1.9×10^{-6}
R388::IS4351 _R , pBR328	0.8×10^{-7} – 8.0×10^{-7}

^a Number of transconjugants per recipient.^b 40 of 156 (26%) events cotransferred *Tc^r with Cm^r.

conjugants were analyzed. Because these transconjugants contained more than one plasmid, the plasmid mixtures were digested with *Eco*RI, and a Southern blot (3) was probed with the ³²P-labeled *Eco*RI fragment of Tn4351. Since this fragment contains most of IS4351_R, the number of cross-hybridizing *Eco*RI fragments gives an estimate of the number of IS elements in the plasmid mixture. Also, the labeled *Eco*RI fragment contained the *Tc^r gene and should hybridize to the corresponding 3.4-kb fragment of pVOH2 (Fig. 1). Deletions in this region would result in a smaller cross-hybridizing fragment.

The *Eco*RI digest of plasmids R388 and pVOH2 generated three fragments (Fig. 2A, lane a). The 5- and 3.4-kb fragments, corresponding to pBR328-IS4351_L and pBR328-*Tc^r (Fig. 1), respectively, hybridized to the probe (Fig. 2B, lane a). The largest fragment (>21 kb) corresponded to R388 and did not hybridize to the probe. The plasmid products from each of the six transconjugants generated at least three *Eco*RI fragments which hybridized to the Tn4351 probe. The 5-kb fragment was conserved, except in one case (Fig. 2, lane f) in which the fragment was slightly smaller. In two cases in which the transconjugants were *Tc^s (Fig. 2, lanes b and e), the 3.4-kb *Eco*RI fragment was missing. These results indicate that IS4351_L may be able to mediate the deletion of adjacent DNA sequences.

The isolation and restriction enzyme analysis of two smaller plasmids from a *Tc^s transconjugant supported this

possibility. Both plasmids, essentially the same as the original pVOH2 plasmid, retained the two cloned *Hind*III sites (Fig. 1) but lost the *Cla*I site located in the middle of the *Tc^r gene (data not shown). Furthermore, one of the plasmids also carried extra *Eco*RI and *Ava*I sites, corresponding to a second copy of IS4351_L in tandem duplication. Tandem duplication of the IS element may be a frequent event, since all six Cm^r transconjugants examined (Fig. 2) also contained a 1-kb *Eco*RI fragment (size equivalent to that of the IS element) which hybridized to the Tn4351 probe.

Transconjugants from transfers mediated by Tn4351 and IS4351_R similarly carried at least two plasmids. Deletions into the *Tc^r region, tandem duplication of an IS element, and other DNA rearrangements were also detected (data not shown). In the case of IS4351_R, restriction enzyme analysis of some putative cointegrates implied an excision event which resulted in the fusion of R388 and pBR328 DNA (data not shown). Clearly, both IS4351_L and IS4351_R are independently active, at least in *E. coli*. Tn4351 in *Bacteroides* spp. has been shown to be active (6), although each of the IS elements has not been demonstrated to be independently active. It is likely, however, that the activity of Tn4351 in *Bacteroides* spp., as in *E. coli*, is mediated by the flanking IS elements.

This work was supported by Public Health Service grant AI22383 from the National Institutes of Health.

LITERATURE CITED

- Boyer, H. W., and R. Roulland-Dussoix. 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. *J. Mol. Biol.* **41**:459–472.
- Guiney, D. G., P. Hasegawa, and C. E. Davis. 1984. Expression in *E. coli* of cryptic tetracycline resistance genes from *Bacteroides* R plasmids. *Plasmid* **11**:248–252.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Rasmussen, J. L., D. A. Odelson, and F. L. Macrina. 1987. Complete nucleotide sequence of insertion element IS4351 from *Bacteroides fragilis*. *J. Bacteriol.* **169**:3573–3580.
- Robillard, N. J., F. P. Tally, and M. H. Malamy. 1985. Tn4400, a compound transposon isolated from *Bacteroides fragilis*, functions in *Escherichia coli*. *J. Bacteriol.* **164**:1248–1255.
- Shoemaker, N. B., C. Getty, J. F. Gardner, and A. A. Salyers. 1986. Tn4351 transposes in *Bacteroides* spp. and mediates the integration of plasmid R751 into the *Bacteroides* chromosome. *J. Bacteriol.* **165**:929–936.
- Shoemaker, N. B., E. P. Guthrie, A. A. Salyers, and J. F. Gardner. 1985. Evidence that the clindamycin-erythromycin resistance gene of *Bacteroides* plasmid pBF4 is on a transposable element. *J. Bacteriol.* **162**:626–632.
- Smith, C. J. 1985. Characterization of *Bacteroides ovatus* plasmid pBI136 and structure of its clindamycin resistance region. *J. Bacteriol.* **161**:1069–1073.
- Smith, C. J., and M. A. Gonda. 1985. Comparison of the transposon-like structures encoding clindamycin resistance in *Bacteroides* R-plasmids. *Plasmids* **13**:182–192.
- Smith, C. J., and H. Spiegel. 1987. Transposition of Tn4551 in *Bacteroides fragilis*: identification and properties of a new transposon from *Bacteroides* spp. *J. Bacteriol.* **169**:3450–3457.
- Soberon, X., L. Covarrubias, and F. Bolivar. 1980. Construction and characterization of new cloning vehicles. IV. Deletion derivatives of pBR322 and pBR325. *Gene* **9**:287–305.
- Ward, J. W., and J. Grinstead. 1978. Mapping of functions in the R-plasmid R388 by examination of deletion mutants generated in vitro. *Gene* **3**:87–95.

FIG. 2. *Eco*RI digests (A) and Southern blot hybridizations (B) of plasmid products from Cm^r transconjugants from matings in which the donor carried R388 and pVOH2. Fragment sizes (in kilobases) are indicated on the left. The probe, pNIL5 (an RSF1010 derivative containing the *Eco*RI fragment of Tn4351; 7), was ³²P labeled by nick translation with a nick translation reagent kit (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). pNIL5 otherwise has no homology with either R388 or pVOH2. Lanes: a, R388 and pVOH2; b and e, plasmid products from *Tc^s Cm^r transconjugants; c, d, f, and g, plasmid products from *Tc^r Cm^r transconjugants.