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NOTES

Transposition of the Gram-Positive Transposon Tn*917* in *Escherichia coli*

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The streptococcal transposon Tn*917* was demonstrated to transpose in *Escherichia coli* from the *Bacillus subtilis-E. coli* shuttle plasmid pHK1207 into an F′ plasmid derivative. Subsequently, a second round of transposition from the F′ plasmid into pACYC184 could be readily demonstrated. These results represent the initial demonstration of the transposition of a gram-positive transposon in a gram-negative bacterium at a relatively high frequency.

Naturally occurring transposition elements in a wide variety of both gram-positive and -negative bacteria have been described (6). When directly selectable markers are normally present or are engineered into these elements, it has been possible to utilize some of the transposons for genetic mapping or for the construction of gene fusions (1, 3). At present, gram-negative transposons have been demonstrated to transpose only in related gram-negative organisms. In addition, transposition of gram-positive transposons in gram-negative bacteria at relatively high frequencies has not yet been reported. However, such a transposition at extremely low frequencies was recently reported for the gram-positive transposon Tn*916* in *Escherichia coli* under special conditions (4).

Since many gram-positive genes can be expressed in *E. coli* (12), it was of interest to investigate the possibility that some naturally occurring gram-positive transposons might be capable of transposition in these organisms. The naturally occurring streptococcal transposon Tn*917* (11) has been shown to share many similarities with gram-negative transposons of the Tn*3* family (5, 9). Recent results (10) have further demonstrated significant homology between regions of the Tn*917* and Tn*3* transposons. Therefore, it was of interest to directly examine the possibility of Tn*917* transposition in *E. coli*.

The inducible erythromycin resistance (Em^r) gene present in Tn*917* cannot be readily utilized to select for the presence of the transposon in *E. coli* since these organisms are normally quite resistant to high concentrations of the antibiotic (7). However, the *lacZ* gene has been recently introduced into Tn*917* (14) and can be utilized to demonstrate the presence of the transposon in a suitable *lacZ* background. Therefore, a shuttle plasmid containing Tn*917-lacZ* capable of replicating in *E. coli* was constructed and transformed into an F′ kanamycin-resistant (Km^r) recipient. Transposition of Tn*917* into the F′ plasmid could then be detected by demonstrating the transfer of the putative Tn*917* F′ by conjugation into an F$^-$ *lacZ*$^-$ recipient.

Shuttle plasmid pHK1207 was constructed (Fig. 1) following ligation of *Eco*RI-cleaved plasmids pACYC184 and pTV32 (15). This latter plasmid was originally constructed to produce operon fusions in *Bacillus subtilis* and was kindly provided by P. Youngman (University of Pennsylvania, Philadelphia). Since a promoter capable of expressing *lacZ* activity was present just outside of the transposon (15), both gram-positive and gram-negative cells harboring pTV32 appear as blue colonies on 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; Bachem Chemical Co.)-containing agar plates. Plasmid pHK1207 was initially transformed into *E. coli* MD106 (*recA* Δ*lac-169* lacking δγ sequences, and containing the F′ Km^r plasmid pOX38; 13) constructed by M. Ditto and M. Casadaban (University of Chicago, Chicago, Ill.), and transformants were selected on M63–X-gal–tetracycline (12.5 μg/ml) agar plates. One blue colony, HK328, containing both pHK1207 and the F′ plasmid, was selected for further manipulation.

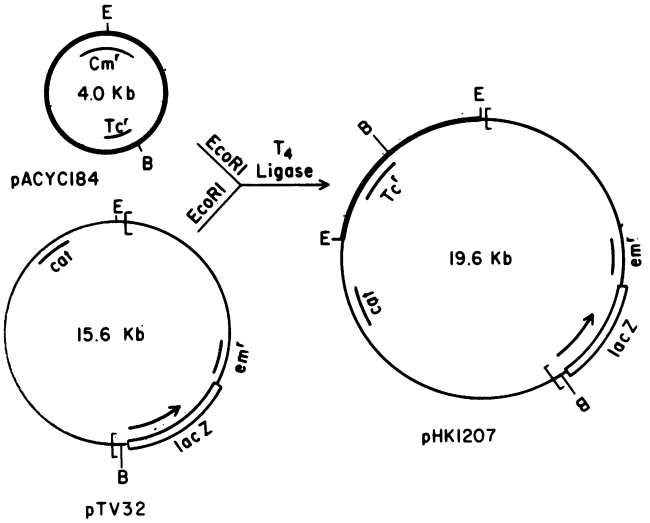

FIG. 1. Construction of pHK1207. Plasmids pACYC184 and pTV32 were cleaved with *Eco*RI, ligated, and transformed (8) into *E. coli* MC1040-2 (2) with selection for Tc^r *lacZ* transformants. The plasmids are drawn to approximate scale with only the relevant restriction sites shown: E, *Eco*RI; B, *Bam*HI. [———], Tn*917*. kb, Kilobases.

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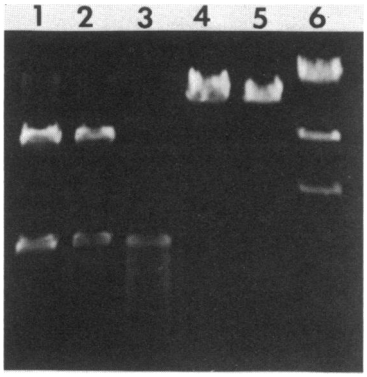

FIG. 2. Agarose gel patterns of restriction endonuclease-cleaved Tn*917* insertions into pACYC184. After restriction digestion, the samples were analyzed on 0.4% agarose gels. Lanes: 1, pHK914-1 cleaved with *Bam*HI; 2, pHK914-2 cleaved with *Bam*HI; 3, pACYC184 cut with *Bam*HI; 4, pHK914-1 cleaved with *Eco*RI; 5, pHK914-2 cleaved with *Eco*RI; 6, *Hin*dIII-cleaved lambda DNA standards (23.1, 9.4, and 6.6 kilobases, from top to bottom).

To demonstrate the transposition of Tn*917* into the F′ Km^r plasmid of HK328, cotransfer of the episome containing potential Tn*917* inserts was detected following conjugation of the latter organisms with *E. coli* M182 (F$^-$ Δ*lacIPOZYA* Str^r). Conjugal transfer of the Km^r marker occurred at a frequency of approximately 40% under these conditions. Cotransfer of the putative cointegrate at a frequency of approximately 1.5×10^{-3} was detected in a typical experiment by screening for Km^r blue colonies on X-gal–kanamycin (50 μg/ml) agar plates. Only 1.4% of the *lacZ*$^+$ colonies were Tc^r, indicating that cotransfer of F′ Km^r and intact pHK1207 into M182 was a relatively rare occurrence from the *recA* donor strain. Restriction analysis of F′ Km^r pOX38 and the episome from one of the *lacZ*$^+$ Km^r transconjugants, HK425, following alkaline-sodium dodecyl sulfate extraction (8) of the cells suggested that Tn*917* insertion into the mini-F′ plasmid had occurred (data not shown).

To demonstrate that Tn*917* present in F′ Km^r was capable of further transposition in *E. coli* into a smaller, more readily analyzable target, conjugation of HK425 with strain VA850 (*hsdR met thi gal* Rif^r Nal^r; provided by F. Macrina, Virginia Commonwealth University, Richmond) containing *E. coli* plasmid pACYC184 was carried out. After selection of Cm^r Km^r transconjugants, two independently isolated Tc^s colonies were selected as candidates for Tn*917* insertion into the Tc^r gene of pACYC184. Restriction analysis of the plasmids, pHK914-1 and pHK914-2 (Fig. 2), was compatible with insertion of Tn*917* into the Tc^r gene of pACYC184 near the single *Bam*HI site of both plasmids (Fig. 1). Since the 8.3-kilobase Tn*917-lacZ* transposon lacks an *Eco*RI site, the presence of a 12.7-kilobase fragment following restriction of the plasmids with *Eco*RI indicated a cointegrate of the transposon with the pACYC184 plasmid. The detection of two *Bam*HI fragments of 4.2 and 8.5 kilobase indicated that the transposon is inserted in the Tc^r gene between the *Hin*dIII and *Bam*HI sites of the gene (Fig. 1) with the *lacZ* gene transcribed in the same direction as the Tc^r gene. This was also confirmed by using *Hin*dIII and *Sal*I digestion of the plasmids (data not shown). In addition, the presence of Tn*917* in pACYC184 resulted in *lacZ* expression since colonies with a *lacZ* background harboring each plasmid appeared as blue colonies on X-gal agar plates. Based on the results from the restriction analysis, it was not possible to determine whether or not both plasmids were identical or represented Tn*917* insertions at two nearby sites on pACYC184.

The present results have demonstrated that the gram-positive transposon Tn*917* is capable of transposition in *E. coli* strains. The similarity of Tn*917* to the gram-negative transposons of the Tn*3* family may allow for both the expression and functioning of Tn*917* transposition elements (transposase, *res* site, etc.) in *E. coli*.

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