Tn2440, a Composite Tetracycline Resistance Transposon with Direct Repeated Copies of IS160 at its Flanks

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The tetracycline resistance region of the multi-resistance plasmid pBP16 is flanked by direct repeats of the insertion sequence IS160. The tetracycline resistance region plus the flanking IS elements can transpose as a discrete unit. The composite transposon, designated Tn2440, has a size of 4.0 kb.

INTRODUCTION

According to Kleckner (1981), transposons (Tns) can generally be divided into two main classes, class II elements, like Tn3 and Tn21, with short inverted repeats at their ends, and composite class I transposons that carry insertion sequences (IS elements) at their flanks in either direct or inverted orientation, like Tn9, Tn10, Tn5 or Tn903. In most transposons that are flanked by direct repeated copies of IS elements, IS1 has been identified as the element providing transposition functions; some of these transposons seem to be derivatives of Tn9 (Iida *et al.*, 1981; Rosner & Gottesmann, 1977; Reif, 1980). Two composite transposons with direct repeats of IS elements that share no homology with IS1 are the kanamycin resistant transposons Tn1525 (Labigne-Roussel *et al.*, 1983) and Tn2680 (Iida *et al.*, 1982). The flanking IS elements in these cases (IS15 and IS26, respectively) are homologous to the recently isolated insertion sequences IS46 from R46 (Brown *et al.*, 1984) and IS160 from pBP16, a multi-resistance plasmid related to R46 (Nies *et al.*, 1985). In pBP16, four copies of IS160 have been identified, two of them flanking the tetracycline resistance region as direct repeats. In this paper we describe the transposability of tetracycline resistance from pBP16.

METHODS

All methods, including plasmid DNA preparations, cloning experiments, transformation and conjugation have been described before (Meyer *et al.*, 1983). The bacterial strains and plasmids used are listed in Table 1.

Resistance towards different drugs are abbreviated as follows: Ap^r (ampicillin), Su^r (sulphonamides), Sm^r (streptomycin), Tc^r (tetracycline), Tp^r (trimethoprim), As^r (arsenate), Km^r (kanamycin) and Nx^r (nalidixic acid). Transfer functions and replication region are abbreviated to Tra and Rep, respectively.

RESULTS AND DISCUSSION

Plasmid pBP16 and its derivative pBP18 contain four copies of the insertion sequence IS160 in inverted and direct orientation. Via a recombination using two direct repeated copies of IS160, pBP15 (size 12.0 kb) can dissociate from either pBP16 (Fig. 1) or pBP18. In pBP15, as in pBP16, the tetracycline resistance region (2.3 kb) is flanked by two IS160 elements (0.85 kb) in direct repeat (Fig. 1; Nies *et al.*, 1985). Since the two IS160-related elements IS15 (Labigne-Roussel *et al.*, 1983) and IS26 (Iida *et al.*, 1982) are components of the composite Km^r transposons Tn1525 and Tn2680 it was reasonable to assume that the tetracycline resistance region of pBP15 (and pBP16) might also be transposable.

Table 1. *Bacterial strains and plasmids*

Bacterial strains	Relevant phenotypes*	Reference
<i>Escherichia coli</i> JC2926	Sm ^r RecA ⁻ Thi ⁻ Thr ⁻ Arg ⁻ His ⁻ Leu ⁻ Lac ⁻	Bachmann (1972)
<i>Escherichia coli</i> C600	Sm ^r Nx ^r RecA ⁻ Thi ⁻ Thr ⁻ Leu ⁻ TonA ⁻ Lac ⁻	Bachmann (1972)
Plasmids		
R751	Tp ^r Tra ⁺	Meyer & Shapiro (1980)
pNO1523†	Ap ^r Sm ^s	Dean (1981)
pBP16‡	Ap ^r Su ^r Tc ^r As ^r Sm ^r Tra ⁺	Nies <i>et al.</i> (1985)
pBP18‡	Ap ^r Su ^r Tc ^r As ^r Tra ⁺	Nies <i>et al.</i> (1985)
pBP15‡	Tc ^r	Nies <i>et al.</i> (1985)
pBP153‡	Ap ^r Tc ^r	This paper
pBP1531	Tc ^r Tp ^r	This paper
pBP1532	Tc ^r Tp ^r	This paper

* See Methods for abbreviations.

† pNO1523 confers streptomycin sensitivity by the allele *rpsL-31*.

‡ Streptomycin resistance is only mediated by pBP16, although the resistance gene (*aph*) is also present in pBP18, pBP15 and pBP153; in the latter cases the *aph* gene is not expressed (Nies *et al.*, 1985).

Since pBP15 is mobilizable by complementation of defective *tra* functions, it is not particularly suitable as a transposon donor molecule. Accordingly, the tetracycline resistance region plus the flanking IS elements was cloned, using *Sma*I, into the *rpsL* gene of pNO1523 (Dean, 1981), a plasmid which confers ampicillin resistance (Fig. 1). The recombinant plasmid (designated pBP153) was transferred to *Escherichia coli* JC2926 (RecA⁻) harbouring the conjugative trimethoprim resistance plasmid R751 (Meyer & Shapiro, 1980). In matings with *E. coli* JC2926 (R751, pBP153) as donor and *E. coli* C600 (Nx^r) as recipient, Nx^r Tp^r Tc^r transconjugants were obtained at a frequency of 5×10^{-6} , compared to the number of Nx^r Tp^r transconjugants (R751 transconjugants) obtained. To distinguish cointegrate mobilization from transposition of the presumptive Tc^r transposon, 400 colonies were replicated to test for ampicillin resistance; 395 of these clones were Ap^r. Plasmid analysis of some representative clones revealed that all contained a single, large plasmid. These plasmids were identified as cointegrates of R751 and pBP153, mediated by IS160 (data not shown).

Analysis of the plasmid content of the five remaining Ap^s clones showed that three of them contained two plasmids each, one the size of R751 (about 50 kb) and one of approximately 20 kb. The smaller plasmid in each isolate possibly represents pBP153 with Tn402 (from R751; Meyer & Shapiro, 1980) inserted into the *bla* gene of pBP153.

Two of the Ap^s clones harboured plasmids (pBP1531 and pBP1532) slightly larger than R751. The *Pst*I restriction pattern of these plasmids (Fig. 2) revealed that the tetracycline resistance region and both the flanking IS160 copies had, in each case, inserted into *Pst*I fragment C of R751. Due to this insertion pBP1531 and pBP1532 contain three additional *Pst*I fragments compared to R751. The smallest one resembles the internal *Pst*I fragment of the insertion, located between the *Pst*I sites within the IS160 copies (identical with the largest *Pst*I fragment of pBP153, fragment a). The other two fragments comprise the sequence located between these *Pst*I sites and the sites which define R751 *Pst*I fragment C. The different sizes of these fragments in pBP1531 and pBP1532 indicate the transposition of the IS160-flanked tetracycline resistance region of pBP15 into different sites of R751. In agreement with the plasmid reference centre the IS160-flanked tetracycline resistance region has been designated Tn2440. To our knowledge Tn2440 is the first Tc^r transposon isolated with direct repeated copies of an IS element at its flanks. Although mediating tetracycline resistance, Tn2440 is more closely related to the Km^r transposons Tn1525 and Tn2680 than to the composite Tc^r transposon Tn10 (Foster *et al.*, 1981), on the basis of the properties of the flanking elements and their direct orientation.

From the results available for IS160 (Nies *et al.*, 1985) and the related elements IS46, IS15, IS15Δ, IS26 and IS140 (Brown *et al.*, 1984; Labigne-Roussel *et al.*, 1983; Labigne-Roussel & Courvalin, 1983; Iida *et al.*, 1982; Bräun & Piepersberg, 1983) it is probable that these insertion

Fig. 1. Isolation of Tn2440. The solid boxes represent copies of *IS160* and the arrows indicate their orientation. Plasmid pBP15 was formed by dissociation of the tetracycline resistance and Rep2 regions from pBP16. The tetracycline resistance region of pBP15 was cloned with *Sma*I (S) into pNO1523 to generate plasmid pBP153. Mobilization of tetracycline resistance from pBP153 by R751 was due to two mechanisms, cointegrate formation via *IS160* or transposition of Tn2440. In the representations of pBP153, R751 and pBP1531 the *Pst*I sites (P) and fragments (Fig. 2) are indicated. Streptomycin resistance is mediated only by pBP16, although the resistance gene (*aph*) is also present in pBP15, pBP153 and in cointegrates of pBP153 and R751; in the latter cases the *aph* gene is not expressed (Nies *et al.*, 1985).

Fig. 2. *Pst*I cleavage pattern of pBP153, pBP1531, pBP1532 and R751 (lanes 1, 2, 3 and 4, respectively). The size standard in lane 5 was produced by cleaving λ DNA with *Pst*I. The R751 fragments A and B and the fragments C and D (lane 4) form one band each, because of their similar size. Due to their small size, fragments e and f of pBP153 (lane 1) are not visible.

sequences, when they are in direct orientation, can transpose any DNA sequence if the intervening sequence is not too large. The influence of the size of the sequence between the IS elements on transposition frequency has been reported by Chandler *et al.* (1982) for IS1 mediated transposons. The observation that the tetracycline resistance region of the plasmid N3 (about 11 kb) does not transpose at a detectable frequency ($< 3 \times 10^{-9}$; Brown *et al.*, 1984) is consistent with these findings. The lack of transposons flanked by inverted copies of IS160 and related elements may reflect an inability of such segments to transpose, as demonstrated by the tetracycline and arsenate resistance region of R46 (Brown *et al.*, 1984) and the arsenate resistance region of pBP16 (Nies *et al.*, 1985), which do not transpose at a detectable frequency. Another possible reason for the inability of these segments to transpose efficiently is the size of the intervening sequence; the arsenate resistance region of pBP16 is 6.5 kb while the tetracycline and arsenate resistance region of R46 is more than 11 kb.

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