Transposition of a Gene Encoding OXA-2 β -Lactamase

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A *bla* gene encoding OXA-2 β -lactamase has been transposed from the *Salmonella typhimurium* R plasmid R1767. This transposable element which was designated Tn2410 encoded sulphonamide and mercury resistance in addition to the *bla* gene. It had a size of 18.5 kb and appeared to be flanked by small inverted repeats. Restriction enzyme analysis resulted in a detailed map of Tn2410, which showed a high degree of similarity with the published map of Tn2603, a transposon encoding OXA-1 β -lactamase. The ampicillin and sulphonamide resistance genes on Tn2410 were mapped in a cluster on the region between the centre and the right end of the map.

INTRODUCTION

Plasmid mediated resistance to penicillins and cephalosporins is due mainly to the formation of β -lactamases. Eleven types of plasmid specified β -lactamases have been identified (Matthew, 1979). They differ in their ability to hydrolyse β -lactam antibiotics, in their isoelectric points, immunological properties, susceptibility to inhibition and molecular weights (Dale & Smith, 1974; Matthew & Harris, 1976; Matthew *et al.*, 1979).

The TEM-type enzyme is the most frequently found bacterial β -lactamase (Matthew, 1979). Other lactamases like the OXA-type enzyme are less common but have been reported in *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Salmonella typhimurium* (Simpson *et al.*, 1980; Sykes & Matthew, 1976). The OXA-enzymes hydrolyse the semi-synthetic oxacillin, that is resistant to hydrolysis by the TEM-type enzyme (Dale & Smith, 1974). There are three different groups of β -lactamases that hydrolyse oxacillin: OXA-1, OXA-2, and OXA-3. These enzymes are distinct from one another in molecular weights and isoelectric points (Dale & Smith, 1974; Matthew *et al.*, 1979).

TEM-1 and 2 are often carried by transposons such as Tn1, Tn2 and Tn3 (Benedick *et al.*, 1977; Hedges & Jacob, 1974; Heffron *et al.*, 1975; Kopecko & Cohen, 1975; Yamada *et al.*, 1979). Transposition is thought to play an important role in the rapid epidemiological spread of resistance genes (Kopecko, 1980), but there has been only one report of a transposon encoding an OXA β -lactamase (Yamamoto *et al.*, 1981). That transposon (Tn2603) carries genes for OXA-1 enzyme, and for resistance to streptomycin, sulphonamides and mercury. In this paper we describe a transposon (Tn2410) derived from plasmid R1767 originally isolated from *S. typhimurium* (Schmidt *et al.*, 1982). It is 18.5 kb in size, mediates resistance to ampicillin (by OXA-2 enzyme), sulphonamides, and mercury. This paper reports the physical and genetical map of Tn2410.

METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Plasmid R1767 was originally isolated from a clinical strain of *Salmonella typhimurium* and codes for resistance to chloramphenicol, tetracycline, ampicillin, sulphonamides, streptomycin, spectinomycin, mercury, and ColI production (Schmidt *et al.*, 1982).

Table 1. *Bacterial strains and plasmids*

Nomenclature is according to the recommendations of Demerec *et al.* (1966) and Novick *et al.* (1976).

Strain*	Relevant characteristics	Source or reference
<i>E. coli</i> WA 921 NaI ^r	<i>lac thr leu met thi hsd</i>	Wood (1966)
<i>E. coli</i> SK 1592	<i>gal thi T1^r sbc B15 hsr4 hsm</i>	Kushner (1978)
<i>E. coli</i> JC 2926 Rif ^r	<i>recA thi thr arg his leu mal</i>	Bachmann (1972)
<i>Proteus mirabilis</i> PM13		Coetzee (1963)
Plasmid		
R1767	Ap ^r Su ^r Tc ^r Cm ^r Sm ^r Sp ^r Hg ^r Coll Tra ⁺	Schmidt <i>et al.</i> (1982)
pUB307	Km ^r Tc ^r Tra ⁺	Bennett <i>et al.</i> (1977)
pBR322	Ap ^r Tc ^r	Bolivar <i>et al.</i> (1977)
pPB12	Ap ^r Su ^r	This paper
pBP401	Ap ^r Tc ^r Su ^r Hg ^r	This paper: pBR322::Tn2410
pBP402		
pPB403		

* The *E. coli* strains derived from *E. coli* K12.

Media and drugs. L broth (LB) and agar (LA) (Lennox, 1955) were used for the growth of bacterial strains; Iso-Sensitest agar (Oxoid) for the determination of sulphonamide resistance. Antibiotics were added at the following concentrations: sulphonamide 300 µg ml⁻¹; kanamycin 50 µg ml⁻¹; ampicillin 15 µg ml⁻¹; tetracycline 10 µg ml⁻¹; nalidixic acid 100 µg ml⁻¹; rifampicin 50 µg ml⁻¹; streptomycin 10 µg ml⁻¹; chloramphenicol 25 µg ml⁻¹, and HgCl₂ 20 µg ml⁻¹.

Identification of β-lactamases. Analytical isoelectric focusing of crude cell extracts on polyacrylamide gels (LKB) was used for characterization of plasmid-mediated β-lactamases (Matthew *et al.*, 1975), with β-lactamases TEM-1, TEM-2, OXA-1, OXA-2, and OXA-3 as references.

Isolation of plasmid DNA. Isolates from 5 ml cultures were screened for plasmid content by the procedure of Klein *et al.* (1980). Plasmid DNA from 500 ml cultures were prepared according to the Triton X-100 cleared lysate technique described by Kuperszock-Portnoy *et al.* (1974), and centrifuged in ethidium bromide-CsCl gradients.

Genetic transfer. Conjugation was carried out on membrane filters. For this procedure 1 ml donor cells and 4 ml recipient cells of exponentially growing cultures were mixed and filtered through membrane filters (pore size 0.45 µm). The filters were placed on LA plates for 2 h at 37 °C, then the cells were washed from the filters with LB, diluted and plated on selection plates. *Escherichia coli* strain SK1592 was transformed with plasmid DNA as described by Kushner (1978).

Electron microscopy. Heteroduplex molecules were prepared by the formamide technique of David *et al.* (1971). For calculations of length, double and single stranded DNA's of phage φX174 were used as internal standards (5357 bp) (Sanger *et al.*, 1977). Contour lengths were measured with a Numonics digitizer.

Restriction enzyme analysis. Digestion of plasmid DNA and agarose gel electrophoresis in a horizontal gel apparatus (BRL, Neu-Isenburg, F.R.G.) was performed as described by Schmidt *et al.* (1982).

RESULTS

Transposition of OXA-2 gene on to plasmid pUB307

Plasmid R1767, originally isolated from *Salmonella typhimurium* (Schmidt *et al.*, 1982) is not transmissible to *Proteus mirabilis* PM13. This enabled us to demonstrate the transposition of an OXA-2 resistance gene from R1767 to plasmid pUB307, which transfers efficiently to *P. mirabilis* PM13. *Escherichia coli* *recA* strain JC2926 carrying R1767 and pUB307 was mated with *P. mirabilis* PM13. Fifty ampicillin resistant *P. mirabilis* transconjugants were used as donors to *E. coli* WA921. All *E. coli* transconjugants selected on ampicillin showed the resistance pattern of pUB307 (Km^r, Tc^r) and in addition, the markers Ap^r, Su^r, Hg^r, but not Cm^r or Sm^r, hence the plasmid carried at least part of R1767. In further conjugation experiments with two of these clones, all resistance markers cotransferred irrespective of which one was used for selection. This is consistent with the transposition of a genetic element of R1767 carrying resistance genes for ampicillin, sulphonamides and mercury to plasmid pUB307. The presumptive transposon was designated Tn2410.

Table 2. Sizes of *Hind*II fragments (kb) of hybrid plasmids pBP401, pBP402 and pBP403 (pBP322::Tn2410)

pBP401	pBP402	pBP403	pBP322
3.46	3.61		
3.38	3.46	3.46	
			3.26
		3.23	
3.15	3.15	3.15	
3.00	3.00	3.00	
2.54	2.54	2.54	
2.23	2.23	2.23	
1.85	1.85	1.85	
		1.5	
1.35			
	1.15		
<u>1.10</u>	<u>1.10</u>	<u>1.10</u>	<u>1.10</u>
0.52	0.52	0.52	
0.25	0.25	0.25	

Identification of the β -lactamases

From two clones of *E. coli* WA921 (pUB307::Tn2410) a crude enzyme preparation was prepared and the isoelectric point of the β -lactamase was determined by isoelectric focusing. For comparison, crude lysates of several strains producing known β -lactamases were isofocused simultaneously. The bands of the β -lactamase produced by WA921 (pUB307::Tn2410) were at a pH of 7.7 and 7.45 and were identical with the bands of crude extracts from an *E. coli* strain containing R1818, a plasmid coding for OXA-2 enzyme (Matthew *et al.*, 1979).

Transposition of Tn2410 on to pBR322

Plasmid DNA from the *E. coli* *recA* strain JC2926 harbouring pBR322 and pUB307::Tn2410 was prepared by the method of Klein *et al.* (1980) and used to transform *E. coli* SK1592. A high concentration of ampicillin (2 mg ml⁻¹) was used to select for the multicopy plasmid pBR322 that encodes TEM-1 β -lactamase. Strains harbouring plasmids coding only for OXA-2 enzyme are unable to grow under these conditions, as the TEM-type enzyme is about 11 times more efficient than the OXA-type enzyme (Hedges *et al.*, 1974). Sulphonamide was used to select for Tn2410. Transformants were checked for sensitivity to kanamycin to discriminate hybrid plasmids from cells containing both parent plasmids. Twenty-four of 200 clones tested were Ap^r, Tc^r, Su^r, Hg^r, but Km^s. In agarose gel electrophoresis, DNA from these clones showed only one band with a size of 23 kb and no bands corresponding to pBR322 or to pUB307::Tn2410. Purified DNA's of three plasmids, pBP401, pBP402, and pBP403, together with pBR322 DNA were digested with *Hind*II endonuclease and fragment sizes were determined by agarose gel electrophoresis (Table 2). The 1.1 kb pBR322 *Hind*II fragment was present in all three replicons. Eight of the remaining 10 fragments were common to all three plasmids; the two fragments that were different, indicated that the transposon inserted in three different sites in the 3.26 kb *Hind*II fragment of pBR322.

Construction of a restriction endonuclease cleavage map of Tn2410

DNA of one of the recombinant plasmids, pBP403, was completely digested with the restriction endonucleases *Eco*RI, *Bam*HI, *Hind*III, *Hind*II, *Sal*I, *Bgl*II, *Pst*I, or *Sma*I and the resulting DNA digests were electrophoresed on agarose gels of 0.7% (w/v) to 1.2% (w/v) depending on the size of fragments separated. The sizes and number of the resulting fragments are shown in Table 3. The relative locations of restriction enzyme cleavage sites on pBP403 were determined mainly by a series of double digestion experiments and by comparison of the cleavage patterns with that of pBR322 (Sutcliffe, 1978). Fragments smaller than about 0.2 kb

Table 3. DNA fragments generated by digestion of pBP403 (pBR322::Tn2410) DNA with restriction enzymes

	Sizes of fragments (kb) generated by:							
	<i>Hind</i> II	<i>Eco</i> RI	<i>Hind</i> III	<i>Bam</i> HI	<i>Pst</i> I	<i>Sal</i> I	<i>Sma</i> I	<i>Bgl</i> II
A	3.46	4.70	10.30	11.20	13.4	11.7	22.5	22.5
B	3.23	4.45	5.61	7.10	9.2	11.0		
C	3.15	4.15	3.61	4.10				
D	3.00	3.54	2.90	0.22				
E	2.54	2.15	0.30					
F	2.23	2.08	0.18					
G	1.85	1.23						
H	1.50	0.25						
I	1.10	0.22						
J	0.52							
K	0.25

Fig. 1. Physical and functional map of pBR322::Tn2410. Black boxes indicate the inverted repeats, flanking the transposable element. Coordinates are given in kilobases relative to the single *Eco*RI site in the vector plasmid pBR322. The positions of resistance genes on Tn2410 are indicated. The bar indicates the 4.1 kb *Bam*HI fragment which carries the Ap Su genes and is homologous to pBP12.

would not have been detected in the gels used. The final map generated by these studies is presented in Fig. 1.

Heteroduplexes between *Pst*I-cleaved molecules of pBR322 and pBP403 showed that the transposable element was flanked by short inverted repeats. Comparative measurements of the inverted repeat flanking Tn2410 and Tn3 [38 nucleotides (Ohtsubo *et al.*, 1979)] revealed the same order of magnitude for both sequences (data not shown).

The integration site of Tn2410 in pBR322 in the recombinant plasmid pBP403 determined by restriction analysis and measurements of heteroduplex structures was localized on the restriction map of pBR322 at position 1.75 kb clockwise relative to the single *Eco*RI site (Sutcliffe, 1978).

Localization of ampicillin and sulphonamide resistance determinants of Tn2410

Recently we described pBP11, a derivative of R1767 coding for resistance to Ap and Su but not Hg, which confers resistance to ampicillin by OXA-2 β -lactamase (Schmidt *et al.*, 1982). Its genome organization is shown in Fig. 2. The genetic and physical map of pBP11 have been analysed. By cloning the position of its resistance genes has been localized on a 4.1 kb *Bam*HI fragment including restriction sites for *Bgl*II, *Pst*I, *Hind*III, *Hind*II, and *Eco*RI. Both genes can be separated from each other by the single *Eco*RI site on this fragment (Schmidt *et al.*, 1982). On the basis of restriction analysis Tn2410 carries the same 4.1 kb *Bam*HI fragment together with a 0.2 kb *Bam*HI fragment which is also present on pBP11 close to the single *Sma*I site on its DNA (Fig. 1). A heteroduplex formed between pBP403 and pBP12, a deletion derivative of pBP11 (Fig. 2), demonstrates a 4.6 ± 0.2 kb region of homology next to the single *Sma*I site on Tn2410

Fig. 2. Schematic representation of the genome organization of pBP11 and its deletion derivative pBP12. The thick lines represent the position of the large inverted repetition of pBP11 which contains the resistance genes (Ap, Su). *EcoRI* and *BamHI* restriction sites are indicated. The DNA segment of pBP11, forming deletion derivative pBP12, is represented by the outer line.

(pBP12 carries no *SmaI* site at this position) covering the two *BamHI* fragments in both structures (data not shown). On the basis of these restriction and heteroduplex data the position of the Ap and Su gene could be located in the restriction map of Tn2410 (Fig. 1).

DISCUSSION

The first report of the identification of a transposable OXA gene was by Yamamoto *et al.* (1981). That 20 kb transposon, Tn2603, mediates resistance to ampicillin, streptomycin, sulphonamides, and mercury. The ampicillin resistance is due to the production of an OXA-1 β -lactamase. In its size and resistance determinants Tn2410 shows some similarity to Tn2603, but the ampicillin resistance in Tn2410 is produced by an OXA-2 β -lactamase. OXA-1 and OXA-2 enzymes are distinct in molecular weight (24000 for OXA-1 and 46000 for OXA-2), in isoelectric point (7.3 for OXA-1 and 7.45 and 7.7 for OXA-2) and in their ability to hydrolyse methicillin (Dale & Smith, 1974; Matthew *et al.*, 1979). The OXA-1 enzyme hydrolyses methicillin much more rapidly than benzylpenicillin, whereas the OXA-2 enzyme hydrolyses methicillin poorly (Dale & Smith, 1974). The transposon Tn4, formed by insertion of Tn3 into Tn21 (Kopecko *et al.*, 1976) also codes for resistance to ampicillin, sulphonamide and mercury but carries a TEM-1 β -lactamase gene.

Comparison of the restriction fragments and the resulting map of Tn2603 (Yamamoto *et al.*, 1982) with the data for Tn2410 shows that the ends of both structures seem to be identical. Tn2410 and Tn2603 carry at the left end a 4.1 kb *EcoRI* fragment and at the right a 1.2 kb *EcoRI* fragment contiguous with two small *EcoRI* fragments containing a small *HindIII* fragment. Tn21 (Kopecko *et al.*, 1976) also carries at its ends homologous fragments of similar sizes to those of Tn2410 and Tn2603 (unpublished). In Tn21 and Tn2603 the Hg gene is cleaved by the *EcoRI* site terminating the 4.1 kb *EcoRI* fragment at the left end on these structures (De La Cruz & Grinsted, 1982; Yamamoto *et al.*, 1982). By these data we located the Hg gene on Tn2410 at the same position. The inner part of Tn2410 (7–15 kb, Fig. 1) differs from Tn2603 with respect to the restriction pattern, specifically for *EcoRI*, *HindIII*, and *BamHI* (Yamamoto *et al.*, 1982). We localized the position of the Ap and Su gene on the Tn2410 restriction map by identification of identical *BamHI* fragments on Tn2410 and pBP11, a Ap^r , Su^r , but Hg^s derivative of R1767. The location of the β -lactamase genes in Tn2410, Tn2603 and Tn4 is different. Tn3 inserted at the left end of Tn21 forms Tn4; in Tn2603 the Ap gene is located close to the Sm gene; and in Tn2410 the Ap gene is located beside the Su gene. Despite the different ampicillin genes all three transposons are similar in size and in their physical map, suggesting that these transposons could have been generated from a common ancestor.

Schmidt *et al.* (1982) have identified an origin of replication in pBP11, located between the Ap and Su gene (Fig. 2), the region common in both structures, indicating that Tn2410 carries an origin of replication on its DNA. From our present data it is not known whether this origin is expressed together with the origin present on pBR322 in the hybrid plasmid.

The 4.6 kb region of homology between Tn2410 and pBP11 covering the Ap and Su gene in both structures indicates that Tn2410 can be fragmented by molecular rearrangements of internal sequences. R1767, carrying Tn2410, was found to rearrange its DNA sequence frequently (Wiedemann, 1981). Derivations of pBP11 that were Ap^r, Su^r, but Hg^s were selected from R1767 after conjugation (Schmidt *et al.*, 1982) and transformation with plasmid DNA (unpublished results), indicating the formation of recombinant plasmids from R1767 at low frequency. The mechanisms involved in these rearrangements are not yet known. McCormick *et al.* (1981) reported rearrangements in plasmids carrying Tn3 by deletions stimulated by tnpR between the normal resolution site and similar sites nearby. TnpR- and tnpA-like transposition functions have been identified recently on the Tn2410 related transposons Tn2603 and Tn21 (Tanaka *et al.*, 1982; De La Cruz & Grinsted, 1982). From these results we speculate, that the tnpR recombination system encoded by Tn2410 together with the reciprocal recombination system depending on *recA* functions could stimulate molecular rearrangements of Tn2410 sequences by the formation of recombinant plasmids carrying Tn2410 and R1767 DNA segments.

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