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The region of the IncN plasmid R46 coding for resistance to β-lactam antibiotics, streptomycin/spectinomycin and sulphonamides is closely related to antibiotic resistance segments found in IncW plasmids and in Tn*21*-like transposons

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ABSTRACT

The nucleotide sequence of a 2.5 kb segment of the pKM101 (R46) genome has been determined. The 1.3 kb from a *BamHI* site at 153 to base 1440 differs by only 2 bases from a part of the published sequence (1) of the *aadB* (gentamicin resistance) gene region including the coding region for the N-terminal 70 amino acids of the predicted *aadB* product. The same sequence has been found (1) 5'-to the *dhfrII* gene of R388 and to the *aadA* gene of Tn*21* (R538-1). Three open reading frames are located in this region, two on the same strand as the resistance genes and one on the complementary strand. The latter predicts a polypeptide of 337 amino acids, whose N-terminal segment is 40% homologous to the predicted product of an open reading frame of 179 amino acids located next to the *dhfrI* gene of Tn*7* (2). The *oxa2* (oxacillin resistance) gene predicts a long polypeptide commencing with (the N-terminal) 70 amino acids of the *aadB* product. A similar arrangement is found in the *aadA* gene of R538-1 (3). The N-terminal segment of an *aadA* gene is located 3'- to *oxa2*, separated by 36 bases. Sequences surrounding the *BamHI* site are identical to sequences 5'- to the *tnpM* gene of Tn*21* (4,5) and homology ceases where homology between Tn*21* and Tn*501* commences (4). The possibility that this antibiotic resistance segment is a discrete mobile DNA element is discussed.

INTRODUCTION

The IncN plasmid R46 encodes resistance to several antibiotics. Three of these determinants, coding for resistance to β-lactam antibiotics (β–lactamase OXAII), to streptomycin and spectinomycin (Sm^R/Sp^R) and to sulphonamide (Su^R), are clustered in a region with a restriction site distribution (6) reminiscent of that found in the Su^R regions of the IncW plasmids pSa and R388 (7,8), in Tn*21* and related transposons (9-14) and in at least two transposons Tn*2620* (15) and Tn*1696* (16) which appear to be unrelated to Tn*21*. This region is characterized by the presence of the Su^R determinant and one or more further antibiotic resistance determinants (e.g. trimethoprim resistance (Tp^R, *dhfrII*) in R388; Sm^R/Sp^R (*aadA*) in Tn*21*). The variable antibiotic resistance (Ant^R) region is flanked on one side by a 0.6 kb *Pvu*II-*Bam*HI fragment and on the other by a 0.16 kb *Hind*III fragment and the Su^R determinant (see Fig. 1 and ref.1). The relationship between the regions in Tn*21* (R538-1), R388 and pSa has been confirmed by sequence comparison and a model for the evolution of the variable resistance (Ant^R) region presented (1).

This region is important in the dissemination of antibiotic resistance genes, as the variable segment can include up to three antibiotic resistance determinants e.g. in Tn*2424*

which codes for chloramphenicol resistance, (Cm^R, *cat*), amikacin resistance (Ami^R, *aac*A), streptomycin/ spectinomycin resistance (Sm^R/Sp^R, *aad*A) (14), and a fourth, gentamicin/tobramycin resistance (Gm^R/Tm^R, *aad*B) has been introduced by recombination (17). Furthermore a large number of antibiotic resistance determinants have been found in this location. These include *aadA, aadB, cat, aacA* and *dhfrII* and also determinants for resistance to the distinct β-lactamases OXAI (12), OXAII (10,18,6) , OXAIII, OXAV, PSE-4, CARB-3 and CARB-4 (19,15). The wide dissemination of the $Su^R(Ant^R)$ region suggests that the DNA segment may be a mobile element.

Here we report the sequence of the region surrounding the *oxa2* gene in pKM101, a deletion derivative of R46 (20) which has lost the Su^R and Sm^R/Sp^R determinants. A region of 2.5 kb was sequenced. The sequence of the *oxa2* region coding for mature OXAII β-lactamase is identical to the previously reported sequence (21). The N-terminal portion (63 amino acids) of the Sm^R/Sp^R (*aadA*) determinant is located 3'- to *oxa2* and separated by 36 base pairs. A 1.3 kb region 5'- to *oxa2* is virtually identical to the sequence of part of the *aadB* gene and its 5'-flanking sequences (1). The DNA sequence includes a further 150 bases beyond the 5' end of the *aadB* region sequenced previously (1) and this sequence was compared to published sequences of the right end of Tn*21* and *Tn501* (4,5). The sequence of Tn*21* beyond its point of divergence from the Tn*501* sequence is identical to sequences found near the *Bam*HI site. This finding is consistent with a model for evolution of Tn*21* by insertion of the antibiotic resistance segment into an ancestral mercury resistance transposon (13,22,23). Evidence supporting the notion that the Su^R (Ant^R) region may be a mobile DNA segment is discussed.

MATERIALS AND METHODS

Bacterial Strains.

E. coli strain RR1 F^-, *ara-14, proA2, lacY1, rpsL20, hds20, galK2, xyl-5, mtl-1, supE44* was used as a host for plasmids and strain TG1 Δ(*lac-pro), supE, thi, hsdD5,* F',*traD, proA*$^+$*B*$^+$, *lacI*^{Q}, *lacZ ΔMI5* as host for M13.

Chemicals and enzymes

Restriction enzymes, T4 DNA ligase and Calf Intestinal Phosphatase were purchased from either New England Biolabs or Boehringer Mannheim and were used according to the manufacturer's instructions. DNA polymerase I, large fragment (Klenow) was obtained from Boehringer Mannheim and used for dideoxy sequencing. [α–32P] dATP (1800 Ci/mmol) was from BRESA, Adelaide, S.A. Other chemicals were of analytical grade.

DNA Sequencing

DNA was from plasmid pRMH105, an *in vitro* generated deletion of pKM101 (24). The 1.65 kb *Bam*HI-*Eco*RI fragment was separated on a low melting agarose gel and purified over a NACS PREPAC column (BRL, Ma.) according to the manufacturer's instructions.

DNA from pRMH105 was digested with *Bam*H1 and *Eco*R1, *Bam*H1 and *Bgl* II, *Bgl* II and *Eco*RI, *Hinc*II, *Rsa*I,*Sau*3A, *Pvu*II or *Alu*I. The *Bam*HI *Eco*RI fragment was digested with *Sau*3A, *Taq*I or *Hpa*I. Fragments were ligated into suitably digested M13 mp8 or M13 mp9 (New England Biolabs) or M13 mp8, *Sma*I cut and phosphatased (Amersham, U.K.). Sequencing was performed by the dideoxy chain termination procedure (25).

Computing

The DNA sequence was compared to sequences in the GenBank Nucleic Acids database (release No. 49.0) using the programme "SEQF" (26). Predicted protein product sequences were compared to the translated GenBank sequences using the programme "SEQFT" (26).

RESULTS

Nucleotide sequence of the *oxa2* gene region

A restriction map of the *oxa2* region of R46 and pKM101 and the sequencing strategy

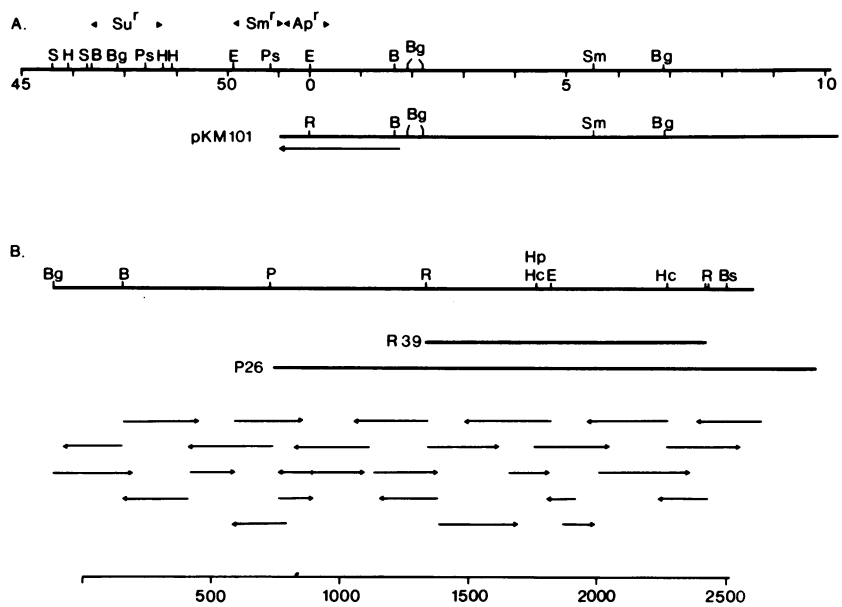
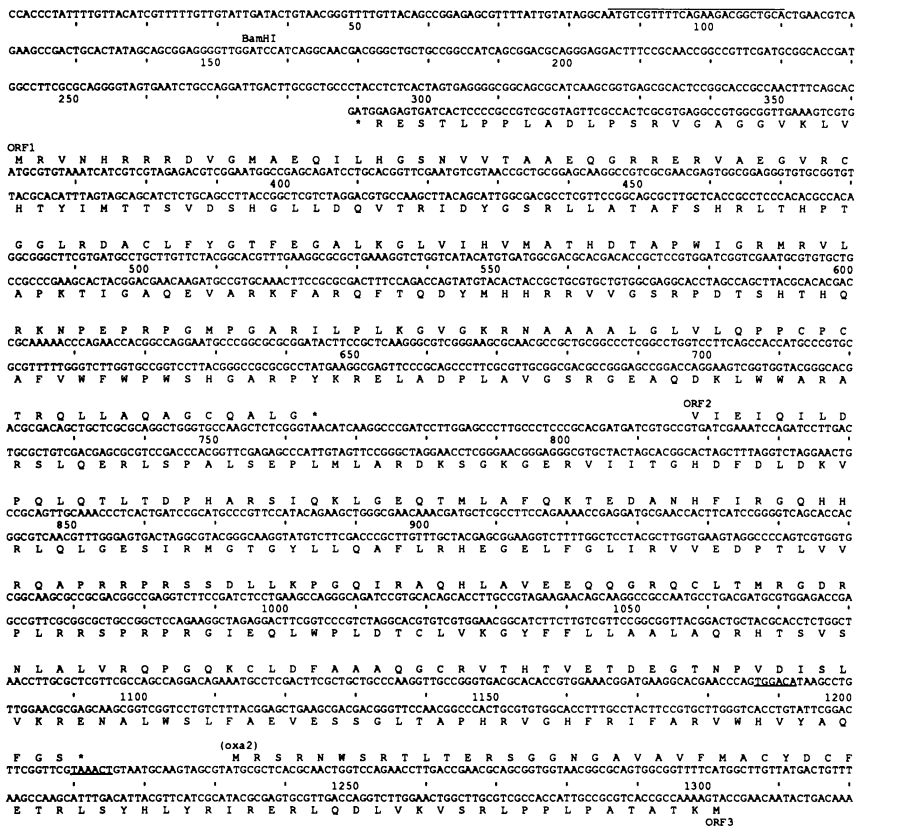


Figure 1. Restriction endonuclease map of R46 and pKM101, and sequencing strategy.
A. Restriction map of the $Su^R Sp^R Ap^R$ region of R46 is after Brown and Willets (6) and the segment of this region retained in pKM101 is shown. The arrow indicates the region sequenced.
B. Detailed map of the sequenced region. Note this map is in the opposite orientation to A. Regions retained in M13 clones P26 and R39 are indicated by heavy lines. Arrows indicate the direction and extent of the sequenced regions. Restriction sites shown are B = *Bam*HI, Bg = *Bgl* II, Bs = *BstE*II, E = *Eco*RI, H = *Hind*III, Hp = *Hpa*I, Hc = *Hinc*II, P = *Pvu*II, Ps = *Pst* I, R = *Rsa*I, Sm = *Sma*I, S = *Sal* I.


BamHI
ORF1
ORF2
(oxa2)
ORF3
oxa2
aadA

is shown in Fig. 1. The DNA sequence of this region is shown in Fig. 2. The *oxa2* gene was completely included in the clone P26 (a 2.1 kb *Pvu*II fragment cloned in the *Sma*I site of M13 mp8) as cells infected with P26 are able to grow on plates containing ampicillin (25 ug/ml). The longest open reading frame within this region extends from an ATG codon at 1232 to a TAA codon at 2285. However this reading frame predicts a polypeptide of MW 40,261 daltons, whereas the OXAII β-lactamase is a dimer with a subunit molecular weight of 28,000-32,000 (27,28). Furthermore the N-terminus of the mature protein has been determined (28) and is overlined in Fig. 2. The mature protein predicted from this point has a molecular weight of 29,570 daltons and the amino acid composition corresponds well with published values (see 21). The DNA sequence from base 1339 to 2384 of the sequence presented here has been reported previously (21), and is identical except for the first base and several differences in the last 70 bases. These authors designated the translation start site as the first AUG codon upstream of the mature end (marked *oxa2* in Fig. 2). However, the longer reading frame has a further N-terminal extension of 74 amino acids, the first 70 of which are identical to the N-terminus of the product predicted from the *aadB* gene sequence (1; see below). A further 6 amino acids fuse this segment with the predicted OXAII precursor. The M13 clone R39 contains an *Rsa*I fragment which extends from a site at 1325 to a site at 2414, and thus completely includes the predicted coding region for the mature OXAII β-lactamase and its predicted precurser. However R39 does not express ampicillin resistance. Thus sequences between the *Rsa*I site at 1325 and the *Pvu*II site at 727 which are present in P26 appear to be required for expression of the *oxa2* gene. The only strong promoter, predicted on the basis of homology with *E. coli* promoters (29), is located in this region, and consists of a -35 sequence TGGACA separated by 17 bases from a -10 sequence TAAACT (underlined in Fig. 2). This putative promoter lies upstream of the start codon for the longer open reading frame, and is probably required for expression of *oxa2*. Therefore the possibility that the longer polypeptide is translated and subsequently processed cannot be excluded.

The sequence 3'- to the *oxa2* gene of pKM101 (R46) contains a fragment of an *aadA* streptomycin/ spectinomycin resistance determinant. The remainder of the *aadA* gene is absent in pKM101, having been lost in the deletion events which gave rise to pKM101 from R46 (20). The *aadA* gene commences at 2325 and extends to the end of the sequence presented in

Figure 2. Nucleotide sequence. The complete sequence of one strand is shown; the complementary strand is shown only for the region encoding ORF3. The derived amino acid sequences of the three open reading frames ORF1, 2 and 3, the long open reading frame which includes the *oxa2* coding region preceded by the N-terminal of the *aadB* coding region, and the segment of *aadA* are shown. The N-terminal amino acids of the mature OXAII β-lactamase (28) are overlined. The start of the probable OXAII precursor and of the longer reading frame are marked *oxa2* and *(oxa2)* respectively.The predicted promoter -10 and -35 sequences for *oxa2* and ORF3 are underlined. The *Bam*HI site referred to in the text is also shown and the 25 base sequence found at the ends of the Tn*21* insert (see Text) is overlined. Sequences unique to the *oxa2* gene region commence at base 1441.

```
aadA  R46   CAGTCAACTCGGACGCTGCGCGATAAAACCGCGCAGCGCCGG-TTACTTCAA-CGTTAAACATCATG
              : :      : :    ::: :   :  ::: ::: ::: ::: ::::   ::::::::::::
aadA  Tn7   GCCTAACAATTCATTCAAGCCGACACCGCTTCGCGGCG-CGGCTTAATTCAGGAGTTAAACATCATG
```

Figure 3. Comparison of sequences 5'- to the coding region of the *aad*A genes of pKM*101* and Tn*7*. The Tn*7* sequence is from Fling et al. (30). The ATG underlined is the *aadA* initiation codon, the TAA underlined is the *oxa2* termination codon. The sequence of the 59 base element (1) is highlighted.

Fig.2. Sequences beyond this point are from IS*46* which is homologous to IS*26* and IS*15* (30). The *aadA* DNA sequence is identical to the sequence for the *aadA* gene from Tn*7* (31). The 36 bases between the *oxa2* and *aadA* genes in pKM101 were compared to the 5'-flanking sequence of Tn*7* (Fig. 3). This comparison shows that 32 of the 36 bases between *aadA* and *oxa2* can be aligned with the 5'-flanking sequence of *aadA* from Tn*7*. In Tn*7* the *aadA* gene is flanked by direct repeat sequences. Further copies of this element are located 3'- to the *aadB* gene (1) and 3'- to the *aadA* genes of R538-1 (Tn*21*)(2) and pSa (32). A consensus sequence for this 59-base element has been presented (1) and the 59 base element 5'- to *aadA* in Tn7 is highlighted in Fig. 3. One half of the 59 base element has been lost from R46 but 21 bases are identical to those found in the remaining half (28 bases) of the consensus sequence and two of the differences are also present in the 5'-element of *aadA* from Tn*7*. The *aadA* genes from Tn7 and from R46 (pKM101) clearly share a recent common evolutionary origin. The short distance between the *oxa2* and *aadA* genes contains no promoter sequence and *aadA* is presumably co-transcribed with *oxa2*.

Comparison of the DNA sequence with other DNA sequences.

The sequence presented here was compared with the published sequence of the *aadB* gene and 5'-flanking sequences (1). The pKM101 sequence from the *Bam*HI site at 153 to position 1440 was virtually identical to the *aadB* region sequence from *Bam*HI at 1 to position 1290. The pKM101 sequence differs by 2 single base substitutions (C→T at 266, T→G at 1188) and two single base deletions (-C between 445-6 and 896-7). We have checked the *aadB* sequence in these regions and found that the two extra C residues represent errors in that sequence. The region of homology includes the coding sequence for the first 70 amino acids of the predicted *aadB* gene product (1) and this arrangement resembles that found 5'- to the *dhfrII* coding region of plasmid R388 (33,34) and 5'- to *aadA* in R538-1 (Tn*21*) (3). A detailed discussion of the comparisons between the *aadB*, *aadA* and *dhfrII* regions has been presented previously (1). The predicted promoter upstream of *oxa2* and underlined in Fig.2 is also the predicted promoter for *aadB* and *aadA* gene expression and has been shown to be required for expression of the *dhfrII* gene (34). In R538-1 a portion of the *aadB* coding region also forms a reading frame continuous with that of *aadA* gene (3), though frame-shifts are present in the *aadB* coding region. In R388 the *aadB* coding segment is separated by 100 bases from the *dhfrII* gene and the two reading frames are not fused (33,34). The extremely high homology

```
pKM101   GACGTTCAGTGCAGCCGTCTTCTGAAAAGCACATTGCCTATACAATAAAACGCTCTCCGGCTG
         :::::::::::::::::::::::::::::::::: ::    :   :    : : : :
Tn21     GACGTTCAGTGCAGCCGTCTTCTGAAAACGACAATGGAGGTGGTAGCCGAGGGTGTGGAAACA
          ::       :    : :   :       :   ::::: ::::: :: :: :: :::::::::
Tn501    CACACCGTGATTCAGCTTGCGCACGGGCTGGGCATGGATGTGGTGGCTGAAGGCGTGGAAACA
```

Figure 4. Comparison of bases 1-63 of the sequence of Tn*501* and Tn*21* (Diver et al. 1983) with bases 57 - 119 of the pKM*101* sequence as the complement. The 25 base sequence found flanking the Tn*21* insert (22) is overlined.

between the sequences upstream of these four antibiotic resistance genes suggests that they are all recently derived from a common ancestor.

The Su^R (Ant^R) segment, as defined by restriction map or DNA sequence comparisons, appears in several different locations and it is possible that this segment is mobile. Also, comparisons of the transposon Tn*21* which encodes mercury resistance as well as an Su^R *aadA* segment with transposons Tn*501* and Tn*2613* which encode only mercury resistance (13,22,23) has led to the proposal that Tn*21*-like transposons arose by insertion of extra DNA segments into an ancestral mercury resistance transposon. The sequence of the transposase gene region for Tn*501* and Tn*21* has been determined (4) and the two sequences show considerable homology over most of their length followed by a region (bases 1-33) of non-homology. The junction is close to the *Bam*HI site of Tn*21* which corresponds to the *Bam*H1 site of the Su^R (Ant^R) segment. A region completely homologous to bases 1-33 of the Tn*21* sequence was found at 87-119 in the pKM101 sequence (Fig. 4). The homology extends for a further 144 bases to the end of the Tn*21* region sequenced by Hyde and Tu (5). The last 25 bases of the region of homology between pKM*101* and Tn*21*, overlined in Fig. 2, are found in an inverted repeat configuration (23/25 bases identical) in Tn*21* beyond the junction of Tn*501* and Tn*21* homologies in the mercury resistance region (22). Brown *et al* have pointed out that the inverted repeats of the Tn*21* insert are flanked by a 5 base direct repeat which represents a duplication of the sequence of Tn*501* at the point of insertion (22) and that these features are consistent with a model for evolution of Tn*21* from a Tn*501*-related transposon by transpositional insertion of the 11.2 kb insert found in Tn*21*. However the insert in Tn*21* is 11.2 kb in length, which is larger than the Su^R *aadA* resistance segment (see Discussion).

Other Open Reading Frames

A search for open reading frames in the region between the junction (base 87) and the beginning of the unique segment of the *oxa2* coding sequence (base 1440) revealed three open reading frames. Two of these are encoded on the same strand as the *oxa2* and *aadA* genes and extend from an ATG at 361-3 to a TAA at 763-5 (ORF 1, 134aa) and from a GTG at 817-9 to a TAA at 1210-2 (ORF 2, 131aa). The third open reading frame (ORF3, 337 amino acids) lies on the complementary strand and extends from an ATG at 1302-1300 to a TAG at 291-289.

```
                                                    50                                                  100
R46         MKTATAPLPPLRSVKVLDQLRERIRYLHYSLRTEQAYVHWVRAFIRFHGVRHPATLGSSEVEAFLSWLANERKVSVSTHRQALAALLFFYGKVLCTDLPW
Tn7                 MSNSPFLNSIRTDMRQKGYALKTEKTYLHWIKRFILFHKKRHPQTMGSEEVRLFLSSLANSRHVAINTQKIALNALAFLYNRFLQQPLGD
Consensus           ------L---R---R---Y-L-TE--Y-HW---FI-FH--RHP-T-GS-EV---FLS-LAN-R-V---T---AL-AL-F-Y---L---L--


                                                   150                                                  200
R46         LQEIGRPRPSRRLPVVLTPDEVVRILGFLEGEHRLFAQLLYGTGMRISEGLQLRVKDLDFDHGTIIVREGKGSKDRALMLPESLAPSLREQLSRARAWWL
Tn7         IDYIPASKP-RRLPSVISANEVQRILQVMDTRNQVIFTLLYGAGLRINECLRLRVKDFDFDNGCITVHDGKGGKSRNSLLPTRLIPAIK*LIEQARLIQQ
Consensus   ---I----P-RRLP-V----EV-RIL-----------LLYG-G-RI-E-L-LRVKD-DFD-G-I-V--GKG-K-R---LP--L-P--------AR----


                                                   250                                                  300
R46         KDQAEGRSGVALPDALERKYPRAGHSWPWFWVFAQHTHSTDPRSGVVRRHHMYDQTFQRAFKRAVEQAGITKPATPHTLRHSFATALLRSGYDIRTVQDL
Tn7         DDNLQG-VGPSLPFALDHKYPSAYR
Consensus   -D---G--G--LP-AL--KYP-A--


R46         LGHSDVSTTMIYTHVLKVGGAGVRSPLDALPPLTSER*
```

Figure 5. Comparison of the predicted amino acid sequences of ORF3 and of the ORF in Tn*7* left end (3).

All three predicted polypeptide sequences were compared to polypeptides predicted from DNA sequences in the GenBank data base. No significant homologies were found for the ORF1 and ORF2 polypeptides. The N-terminal region of ORF3 polypeptide exhibited a homology of 40% with the predicted protein product (179 amino acids) of an open reading frame which lies leftward of the *dhfrI* gene of Tn*7* (2). The homology continues at the same level (40%) beyond the stop codon of the Tn*7* ORF for a further 35 amino acids to the end of the published sequence for this region of Tn*7* (2). The alignment of these two putative polypeptide sequences is shown in Fig. 5. Potential promoter -10 (TAGACT) and -35 (TTGCTG) sequences upstream of ORF3 in pKM101 are located at 1334-1329 and 1357-1352, and separated by 17 bases. The homology between the ORF3 and the Tn*7* ORF favours the notion that these polypeptides are functional, even though no function has been identified for either open reading frame. The coding sequence for the N-terminal 23 amino acids of the ORF3 overlaps the coding region for the predicted *aadB* gene segment, and the divergent putative transcripts for *oxa2* and for ORF3 thus overlap. If ORF3 is a functional reading frame this arrangement may well explain why a segment of the *aadB* coding region is retained in front of *oxa2* in R46, of *dhfrII* in R388 (33,34) and of *aadA* in R538-1 (Tn*21*) (3).

DISCUSSION

The segment of pKM101 (R46) sequenced was similar to Su^R (Ant^R) regions found in several other plasmids and transposons. The *oxa2* gene represents the third antibiotic resistance gene which has been found by sequence analysis to be flanked on its 5'- side by a segment of the *aadB* gene. In the case of the *dhfrII* gene in R388 the promoter region upstream of the *aadB* gene coding region has been shown to be essential for expression of trimethoprim resistance (34) and it seems likely that this promoter is also required for expression of *oxa2*.

The *oxa2* gene is fused in frame with the *aadB* gene and it remains to be determined if the *aadB* fragment is translated in vivo. A strong translation start signal (AAGGAA) is located upstream of the first AUG codon in the sequence unique to *oxa2*, and it is possible that this signal can direct translation to commence at the following AUG. However as the sequences upstream of the *aadB* gene are identical to those found where the complete gene is present and gentamicin/tobramycin resistance is expressed (1), the possibility that the longer polypeptide is translated cannot be excluded. The *aadA* gene in R538-1 (Tn*21*) also shows a long reading frame consisting of amino acids translated from the *aadB* region fused to the predicted *aadA* polypeptide (3).

We have previously proposed(1) that the Su^R *aadB* gene arrangement found in pDGO100 represents an ancestral sequence and that other antibiotic resistance genes can be inserted in the 59-base element located 3'- to the *aadB* gene. A subsequent deletion event which removes part of the *aadB* sequence would give rise to the sequence arrangement found in the pKM101 *oxa2* region. Indeed the deletion end-point in the *aadB* gene is identical in R46 (*oxa2*), in R388 (*dhfrII*) and in R538-1 (*aadA*). In pKM101, 18 bases separate this point from the first AUG codon which is unique to *oxa2*. It was also proposed(1) that the 59-base element is involved in insertion of new antibiotic resistance genes which were flanked by two such elements, and that this process could be reversed if the two flanking elements remain intact. Removal of, or divergence of the sequence of, at least one 59-base element would be necessary to stabilize the gene arrangement. The presence of only a fragment of the 59-base element sequence between the *oxa2* and *aadA* coding regions in pKM101 may result from such a process. Further sequence from R46 is required to determine if a complete 59 base element is located 3'- to the *aadA* gene.

The possibility that the Su^R (Ant^R) segment is a mobile DNA segment is suggested by several lines of evidence. Firstly the segment is found in several different locations, in the IncN plasmid R46 (6), the IncW plasmids R388 and pSa (7,8), in the Tn*21*-like transposons (9-14) as well as in the unrelated transposons Tn*2610* (15) and Tn *1696* (16). Secondly comparison of the restriction maps and heteroduplex analysis of the IncW plasmids R7K and R388 shows that the Su^R and Tp^R (*dhfrII*) determinants of R388 are located in a DNA segment not found in R7K, though the plasmids are highly homologous elsewhere (7,35). Thirdly comparisons of mercury resistance transposons with Tn*21*-like transposons which include an Su^R (Ant^R) region suggest that the latter region was inserted into an ancestral mercury resistance transposon (13,22,23). Tn*21* carries a DNA segment of 11.2 kb not found in Tn*501*, and both junctions have been sequenced (4,22). These boundaries contain a short inverted repeat (23/25 bases identical) flanked by a 5 base direct repeat which is a duplication of 5 bases found at the point of insertion in the Tn*501* sequence (22). This finding is consistent with the transpositional insertion of the 11.2 kb segment of Tn*21* into an ancestral transposon related to Tn*501*. The finding that the pKM101 sequence diverges from the Tn*21* sequence at

the position where homology between Tn*21* and Tn*501* transposase gene regions commences (see Fig. 3) is consistent with this proposal. This junction then identifies one end of the Su^R (Ant^R) segment. The other end appears to lie close to one end of the Su^R determinant as restriction map homologies cease in this region (see 1). However, the Su^R (Ant^R) segment in R388 is considerably shorter (7,35) than the Tn*21* insert and the Su^R *aadA* region may account for only 4-5 kb of the 11.2 kb Tn*21* insert. At least part of the remaining sequence in the Tn*21* insert could result from a subsequent insertion of sequences between one end of the Su^R *aadA* segment and the Su^R determinant, as closely related transposons which lack either a 2.8 kb segment or a 1.45 kb segment (36),or which lack a 5.5 kb segment (13) have been identified.

Analysis of the open reading frames found in the DNA segment 5'- to the coding region for mature OXAII β-lactamase protein indicates that at least one of the three open reading frames is probably a functional protein. The predicted product of the reading frame, ORF3, is 40% homologous to a shorter ORF found in the left end of Tn*7* , adjacent to the Tp^R (*dhfrI*) determinant. It is tempting to speculate that this polypeptide may be involved in transposition of the Su^R (Ant^R) segment. Although the genes identified as essential for transposition of Tn*7* are located at the right end of Tn*7* (37, and refs therein), the possibility that the ORF at the left end also plays a role has not been rigorously excluded. When the Tn*7* deletions used did not retain the left end ORF, transposition into R388 was assayed (37), and R388 contains a region identical to the ORF3 coding region of pKM101 (see above). The second possible function for ORF3 is in the insertion of antibiotic resistance genes into the 59-base element. Tn*7* includes an *aadA* determinant flanked by two complete 59-base elements, and this gene may also have been inserted into a 59-base element present in an ancestor of Tn*7*. The DNA sequences of the *aadA* genes in Tn*7*, R46 and Tn*21* (R538-1) are nearly identical suggesting that the genes have only recently arisen from either one another or from a common ancestor.

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REFERENCES

1. Cameron, F.H., Groot Obbink, D.J., Ackerman, V.P. and Hall, R.M. (1986). *Nucleic Acids Res.* **14,** 8625-8635.
2. Simonsen, C.C., Chen, E.Y. and Levinson, A.D. (1983). *J. Bacteriol.* **155,** 1001-1008.
3. Hollingshead, S. and Vapnek, D. (1985). *Plasmid* **13,** 17-30.
4. Diver, W.P., Grinsted, J., Fritzinger, D.C., Brown, N.L., Altenbuchner, J., Rogowsky, P. and Schmitt, R. (1983). *Mol. Gen. Genet.* **191,** 189-193.
5. Hyde, D.R., and Tu, C-P.D. (1985). *Cell.* **42,** 629-638.
6. Brown, A.M.C. and Willetts, N.S. (1981). *Plasmid* **5,** 188-201.
7. Ward, J.M., and Grinsted, J. (1982). *Plasmid* **7,** 239-250.

8. Ireland Valentine, C.R. (1985). *Plasmid* **14,** 167-170.
9. De la Cruz, F. and Grinsted, J. (1982). *J. Bacteriol.* **151,** 222-228.
10. Kratz, J., Schmidt, F. and Wiedemann, B. (1983). *J. Gen. Microbiol.* **129,** 2951-2957.
11. Schmidt, F. 1984. *Mol. Gen. Genet.* **194,** 248-259.
12. Yamamoto, T., Tanaka, M., Baba, R. and Yamagishi, S. (1981). *Mol. Gen. Genet.* **181,** 464-469.
13. Tanaka, M., Yamamoto, T. and Sawai, T. (1983). *J. Bacteriol.* **153,** 1432-1438.
14. Meyer, J.R., Nies, B.A. and Wiedemann, B. (1983). *J. Bacteriol.* **155,** 755-760.
15. Yamamoto, T., Watanabe, M., Matsumoto, K. and Sawai, T. (1983). *Mol. Gen. Genet.* **189,** 282-288.
16. Hirsch, P.R., Wang, C.L. and Woodward, M.J. (1986). *Gene* **48,** 203-209.
17. Wiedemann, B., Meyer, J.F. and Zuhlsdorf, M. (1987). *J. Antimicrobial Chemotherapy* **18,** 85-92.
18. Kratz, J., Schmidt, F. and Wiedemann, B. (1983a). *J. Bacteriol.* **155,** 1333-1342.
19. Levesque, R.C., Medeiros, A.A. and Jacoby, G.A. (1987). *Molec. Gen. Genet.* **206,** 252-258.
20. Mortelmans, K.E. and Stocker, B.A.D. (1979). *Mol. Gen. Genet.* **167,** 317-327.
21. Dale, J.W., Godwin, D., Mossakowska, D., Stephenson, P. and Wall, S. (1985). *Febs Letts.* **191,** 39-44.
22. Brown, N.L., Misra, T.K., Winnie, J.N., Schmidt, A., Seiff, M. and Silver, S. (1986). *Mol. Gen. Genet.* **202,** 143-151.
23. Schmidt, F. and Klopfer-Kaul I. (1984). *Mol. Gen. Genet.* **197,** 109-119.
24. Hall, R.M., Podger, D.M. and Collis, C.M. (1985). *Mutation Res.* **146,** 47-53.
25. Sanger, F., Nicklen, F. and Coulsen, A.R. (1977). *Proc. Natl. Acad. Sci. USA.* **74,** 5463-5467.
26. Kanehisa, M. (1982). *Nucleic Acids Res.* **10,** 183-196.
27. Dale, J.W. and Smith, J.T. (1976). *Biochem. Biophys. Res. Comm.* **68,** 1000-1005.
28. Holland, S. and Dale, J.W. (1984). *Biochem. J.* **224,** 1009-1013.
29. Harley, C.B. and Reynolds, R.P. (1987). *Nucleic Acids Res.* **15,** 2343-2361.
30. Hall, R.M. 1987. *Nucleic Acids Res.* **15.** (In Press).
31. Fling, M.E., Kopf, J. and Richards, C. (1985). *Nucleic Acids Res.* **13,** 7095-7106.
32. Tait, R.C., Rempel, H., Rodriguez, R.L. and Kado, C.I. (1985). *Gene* **36,** 97-104.
33. Zolg, J.W. and Hanggi, U.J. (1981). *Nucleic Acids Res.* **9,** 697-710.
34. Swift, G., McCarthy, B.J. and Heffron, F. (1981). *Mol. Gen. Genet.* **181,** 441-447.
35. Gorai, A.P., Heffron, F., Falkow, S., Hedges, R.W. and Datta, N. (1979). *Plasmid* **2,** 485-492.
36. Meyer, J.F., Nies, B.A., Kratz, J. and Wiedemann, B. (1985). *J. Gen. Microbiol.* **131,** 1123-1130.
37. Rogers, M., Ekaterinski, N., Nimmo, E. and Sherratt, D. (1986). *Mol. Gen. Genet.* **205,** 550-556.