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Nucleotide sequence of the AAD(2'') aminoglycoside adenylyltransferase determinant *aadB*. Evolutionary relationship of this region with those surrounding *aadA* in R538-1 and *dhfrII* in R388

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ABSTRACT

The nucleotide sequence of the *aadB* gene which confers resistance to kanamycin, gentamicin, and tobramycin has been determined. The size of the longest reading frame is 747 bases encoding a protein of predicted size 27,992 daltons. A segment of the *aadB* gene sequence (including the promoter region) was found upstream of the *aadA* gene in R538-1 (1) and of the *dhfrII* gene in R388 (2,3) and the proposed promoters for these genes coincide with the *aadB* promoter region. The sequence homology extends upstream to the end of the sequenced regions of R388 and R538-1. Almost perfect homology was also found between the sequences 3'- to the *aadB* gene and 3'- to the *aadA* genes of R538-1 and pSa (4). This segment includes a 59 base element previously found flanking the Tn*7* *aadA* gene (5). A model is presented for the evolution of this region of the plasmid genomes in which the 59- base element functions as an insertional "hot spot" and the possibility that this region is analogous to the *aadA*/*aadB* region of the Tn*21*-like transposon family is considered.

INTRODUCTION

The gentamicin, kanamycin and tobramycin resistance phenotype in bacteria is mediated by a plasmid borne gene *aadB* coding for the adenylyltransferase (AAD(2")) which modifies the antibiotics by adenylylating the 2"-hydroxyl group. The *aadB* gene has been found on a number of genetic elements, including IncFII plasmids (6,7,8), IncC plasmids (9) and on a multi-resistance plasmid whose incompatibility group could not be determined (10). The *aadB* gene has been found associated with transposable genetic elements such as Tn*732* (8), and Tn*4000*, which is closely related to Tn*21*(6) and also associated with transposition-defective elements related to Tn*21* (6, 11).

We have previously reported the localization of the *aadB* gene from an IncC multi-resistance plasmid (pDGO100) to a 2 kb *Bam*HI-*Hin*dIII fragment (9). Here we report the nucleotide sequence of this fragment and the further localization of the *aadB* gene within this sequence. The DNA sequence of the *aadB* gene region was compared with DNA sequences in the Genbank database and with the published sequences of three *aadA* genes which code for adenylyltransferase AAD (3")(9) and confer resistance to streptomycin and spectinomycin. No homology was detected between the *aadA* and the *aadB* coding regions. However sequences highly homologous to the 5'- flanking sequences and a portion of the *aadB* coding region were found 5'- to the coding region of the *aadA* gene of R538-1(1) and the *dhfrII* gene of R388 (2, 3).

Sequences highly homologous to the 3'- flanking sequences of the *aadB* gene were found located immediately 3'- to the *aadA* genes of R538-1(1) and pSa (4). The significance of these findings is discussed with reference to the evolution of multi-resistance plasmid genomes.

MATERIALS AND METHODS

Bacterial strains

E. coli K12 JP777 *thr1, leu1, lacY1, gal351, supE44, tonA2, hsdR4, rpoB364* (12) was used as a host for plasmids and strain TGI *Δ(lac-pro), supE, thi, hsdD5*, F', *traD36, proA⁺B⁺, lacIᵠ, lacZ ΔM15* was used as a 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 end-filling and dideoxy sequencing. [α -^{32}P] dATP (1800 Ci/mmol) was from BRESA, Adelaide, S.A. Other chemicals were of analytical grade.

DNA sequencing

The 2 kb *Bam*HI-*Hin*dIII fragment of pDGO103 (9), was separated on low melting agarose gels and purified over a NACS PREPAC column (BRL, Ma.), according to the manufacturer's instructions. The DNA was digested with restriction endonucleases *Xho*I, *Sau*3A, *Hpa*II, *Taq*I or *Pvu*II and fragments were cloned into suitably cut M13 vectors mp8, mp9, mp10w, mp11w (New England Biolabs) or M13 mp8 *Sma*I cut and phosphatased (Amersham). Sequencing was performed by the dideoxy chain termination procedure (13).

Computing

The sequence was compared to the *aadA* gene region DNA sequences using the programme "DBCOMP" (14) and to all available sequence data in the Genbank database using the programme "MATCH" (15).

Subcloning

The plasmid pDGO109 (9) was cut with *Bam*HI and religated. Gentamicin resistant transformants were selected on nutrient agar plates containing gentamicin (Essex Laboratories, 2ug/ml) and screened for the presence of a single 2 kb *Bam*HI-*Hin*dIII insert using a miniprep procedure (16). A subclone of this type was designated pDGO117. Plasmid pDGO117 was digested with *Bam*HI and *Pvu*II, ligated and gentamicin resistant transformants analysed. A representative plasmid (pDGO119) had lost the *Pvu*II fragment containing the *Bam*HI site and retained the two remaining *Pvu*II fragments religated in the original direction.

RESULTS

Nucleotide sequence of the *aadB* gene region

The gentamicin resistance determinant was initially cloned on a 7.7 kb *Bam*HI fragment

(pDGO101) which also included sulphonamide and trimethoprim resistance determinants, then localized to a 2 kb *Bam*HI-*Hin*dIII fragment (9). The plasmid pDGO117 which includes only this fragment was constructed and a restriction map is shown in Fig. 1. The fragment was sequenced on both strands using the dideoxy chain termination procedure and the sequencing strategy shown in Fig. 1. The sequence of this fragment and the adjacent 160 base pair *Hin*dIII-*Hin*dIII fragment is shown in Fig. 2.

As several open reading frames were included in this sequence, the *aadB* gene was further localized. Deletion of the 580 bp *Bam*HI-*Pvu*II left-end fragment (plasmid pDGO119) did not lead to a loss of gentamicin resistance. Introduction of a frameshift mutation by cleavage of pDGO117 with *Xho*I, end-filling and ligation led to loss of the gentamicin resistance phenotype indicating that the *aadB* coding sequence includes the *Xho*I site at position 1466. The longest reading frame within this region is from an ATG codon at 1082 to a termination codon at 1829. Sequences with homology to the *E. coli* -10 and -35 promoter sequences found at position 1060 (TAAACT) and 1037 (TTGACA) are optimally spaced 17 bases apart (17). The molecular weight of the predicted polypeptide is 27,992 daltons. However, the only possible ribosome binding sequence (AAG) at position 1073 is weak and lies very close to the predicted 5'- terminus of the transcript. It is therefore possible that the second ATG codon, at position 1151, represents

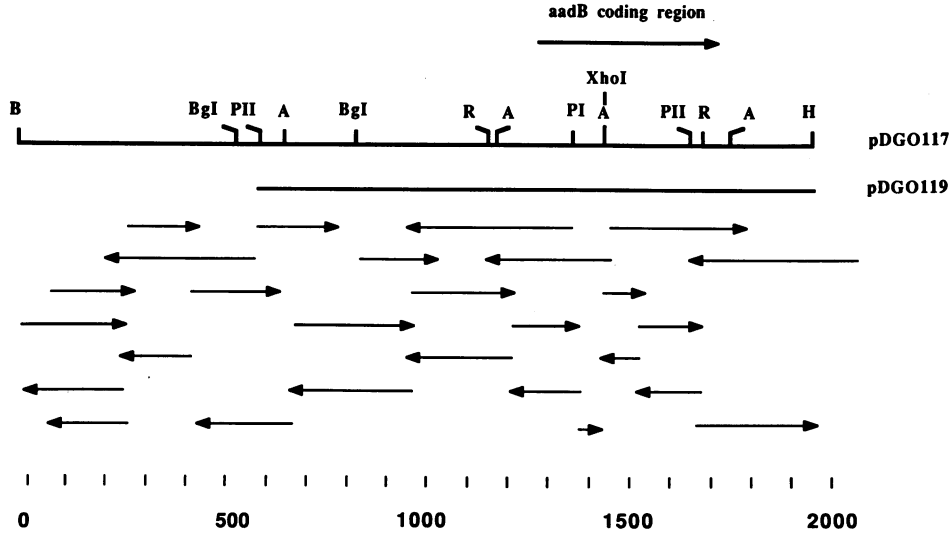

Figure 1. Restriction endonuclease map of the *Bam*HI-*Hin*dIII fragment of pDGO117 and nucleotide sequencing strategy. The pBR322 vector sequences are not shown. Heavy lines represent the region retained in plasmids pDGO117 and pDGO119. The location of the *aadB* gene is indicated by the heavy arrow. The light arrows show the direction and extent of sequenced regions. Restriction sites used for cloning into M13 included *Taq*I, *Hpa*II, *Pvu*II, *Xho*I, *Sau*3A, and *Hin*dIII. Restriction sites shown are B = *Bam*HI, BgI = *Bgl*I, PI = *Pvu*I, PII = *Pvu*II, A = *Ava*I, R = *Rsa*I, H = *Hin*dIII.

the initiation codon. This codon is preceded by a possible ribosome binding sequence GGCGG at position 1141, and translation from this point would yield a product of molecular weight 25,458 daltons. It had been concluded from maxicell analysis that the M.W. of the adenylyltransferase (AAD(2")) was 35,000 daltons (10). However the plasmid used in that analysis, which included only an ampicillin resistance gene and the *aadB* gene, produced two polypeptides (35,000 and 27,000 daltons) in addition to the ampicillin resistance gene products. The 35,000 dalton polypeptide may represent the product of a fusion with the truncated tetracycline resistance gene from pBR322 and the polypeptide of M.W. 27,000 daltons most probably represents the *aadB* gene product. The *aadB* gene described here and the *aadB* gene of Tenover *et al* (10) are highly

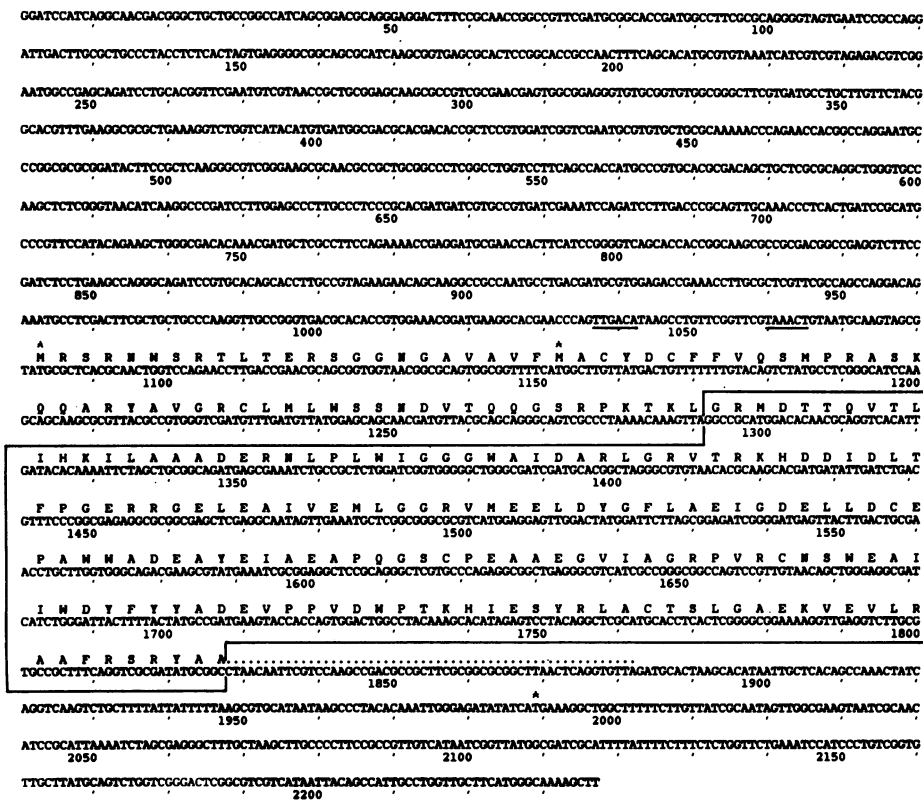

Figure 2. Nucleotide sequence of the *aadB* gene region. The sequence of the BamHI-HindIII fragment (*Bam*HI restriction site at position 1 to a *Hin*dIII restriction site at position 2072) and of the adjacent HindIII-HindIII fragment (2072-2237) is shown. The derived amino acid sequence for the aadB gene is shown and the two possible N-terminal methionine residues are indicated (*). Sequences with homology to the -10 and -35 consensus promoter sequences are underlined. The boxed region is the sequence found to be unique in a comparison with the sequence of the aadA gene region from R538-1 (see text).

homologous on the basis of restriction maps and of hybridization analysis with specific internal gene sequences (data not shown).

Comparison of the *aadB* gene region with *aadA* and other resistance gene regions

It has been reported (10), that some sequences from the *aadB* gene region hybridize to plasmids which contain the *aadA* gene, and it has been proposed that the *aadA* and *aadB* sequences show some homology and may have a common evolutionary origin (10, 18). The *aadA* gene codes for an adenylyltransferase, AAD(3")(9), which confers resistance to streptomycin and spectinomycin. The DNA sequence presented here was compared with the published sequences for the *aadA* gene regions of plasmid R538-1 (1), of transposon Tn*7* (5, 19) and of plasmid pSa (4). High homology is observed between the coding regions of the three *aadA* genes (4, 5, 19), and we have assumed that the *aadA* coding region of R538-1 commences at met-61 where homology between the three gene regions commences (see 5, 19, 4). No homology was observed between the coding regions of the *aadA* genes and the *aadB* gene described here. It is important to note that the open reading frame originally reported for the R538-1 sequence (1) predicts a polypeptide 60 amino acids longer than those predicted for the *aadA* genes of Tn*7* and pSa. The N-terminal amino acid sequence of the protein from Tn*7* has recently been reported and confirms the start of this *aadA* gene (19). We have therefore also assumed in the comparisons which follow, that the R538-1 *aadA* coding region commences at the ATG codon corresponding to met-61 in the predicted polypeptide.

The sequences flanking the *aadA* gene in plasmid R538-1 were found to be highly homologous to the flanking sequences of the *aadB* gene. The position and the extent of these two regions of homology are shown in Fig. 3. The unique segment of the *aadB* gene, which lies between the two regions homologous to the R538-1 sequence, is boxed in Fig. 2. In the R538-1 sequence the boxed region is replaced by the complete *aadA* coding region. The sequence which lies upstream of the R538-1 *aadA* gene (bases 1-396) is 95% homologous to a region (bases 908-1291) of the pDGO100 sequence presented here. This region includes the putative promoters for both *aadA* and *aadB* and extends for 210 bases into the proposed coding region of the *aadB* gene. The probable start codon of *aadA* lies 6 bases beyond the region of homology. Sequences immediately 3'- to the *aadB* gene (bases 1828-2077) also showed 96% homology to the 3'-flanking sequences of the R538-1 *aadA* gene (bases 1196-1443). This region extends to the *Hin*dIII site at 2077 in the *aadB* sequence and to the end of the sequenced region of R538-1. The presence 3'- to the R538-1 *aadA* gene of an element homologous to the 54 base repeat which flanks the Tn*7* *aadA* gene has been noted previously (5). This element is also present 3'- to *aadB* (see below). The sequences downstream of this element differ by only a single base deletion in the R538-1 sequence.

The region (bases 944-1188) immediately 3'- to the *aadA* gene of pSa (4) is also homologous to the regions downstream of *aadB* and *aadA* (R538-1) (Fig. 3). A segment homologous to the 54 base element is present in the pSa sequence, though in this case the

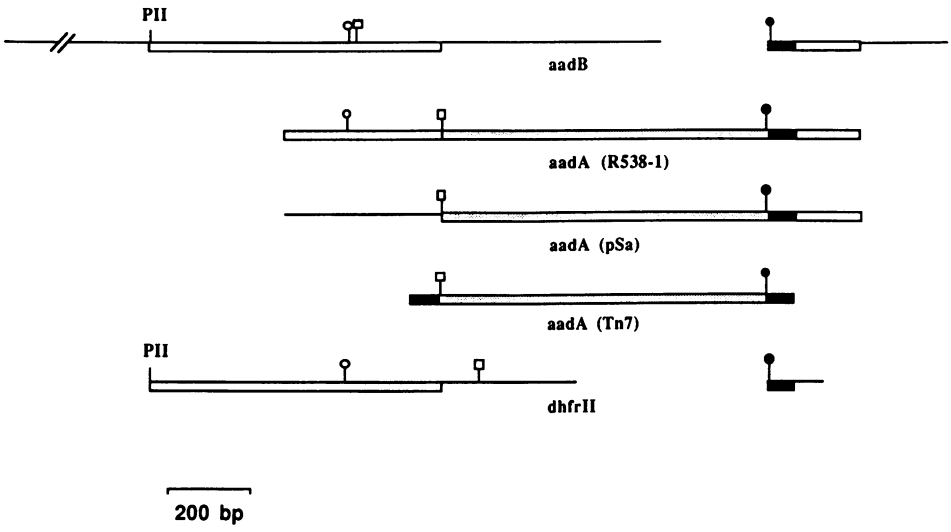

Figure 3. Comparison of the *aadB* region with *aadA* and *dhfrII* regions. The published sequences of R538-1 (1), pSa (4), Tn7(5) and R388(2,3) have been aligned with sequence of the *aadB* gene region to show the positions of regions of homology of the sequence. Gaps have been introduced into the *aadB* and *dhfrII* regions to allow alignment of homologous regions flanking the 3'- ends of the genes. Lines represent unique sequence, boxed areas represent the regions of homology. The hatched boxes represent the coding sequences of the *aadA* genes and the solid boxes represent the Tn*7* 54 (59) base element. The position of the predicted promoter regions (), initiation codons () and termination codons () for the *aadA*, *aadB* and *dhfrII* genes are shown.

sequence is less well conserved (Fig. 4). The region downstream of this element is 97% homologous to the equivalent region in the *aadB* sequence and in the R538-1 sequence. We considered the the possibility that this region may include a segment of the sulphonamide resistance gene which has been localized 3'- to each of the three genes. In the sequence reported here an open reading frame commencing at base 1988 extends to the end of the sequenced region. However the single base deletions in the equivalent regions of both the R538-1 and the pSa sequences preclude the tentative assignment of this reading frame to the sulphonamide resistance gene.

The sequence of the *aadB* gene region was also compared with the DNA sequences in the Genbank database. This analysis revealed a region of 98% homology between bases 576-1291 of *aadB* and a region 5'- to the coding region for dihydrofolate reductase II (*dhfrII*) in R388 (2 ,3). We have used the sequence of Swift (2) for detailed comparison, as this sequence includes the longer region of homologous flanking sequence (bases 1-709). This region completely includes the region of homology located 5'- to R538-1 and ends at essentially the same point within the *aadB* gene sequence (Fig. 3). In the case of the *dhfrII* gene, the region proposed here as the

	aadB	GCG	GC	CTAACAATTCGTCCAAGCCGACGCCGCTTCGCGG-CGCGGCTTAACTCAGGTG-TT	AGA TGCACTAA
Tn7 5'-	*aadA*	GAC	GC	CTAACAATTCATTCAAGCCGACACCGCTTCGCGG-CGCGGCTTAATTCAGGAG-TT	AAA CATCATG
Tn7 3'-	*aadA*	AAT	GT	CTAACAATTCGTTCAAGCCGACGCCGCTTCGCGG-CGCGGCTTAACTCAAGCG-TT	AGA GAGAGC
R538-1	*aadA*	AAT	GT	CTAACAATTCGTTCAAGCCGACGCCGCTTCGCCG--GCG--TTAACTCAAGCGATT	AGA TGCACTAA
pSa	*aadA*	GAT	GT	CTAACAATTCGTTCAA-CCGAC---TCATCGCGCCGGCGGCTTAACTCCGGCG-TT	AGA TGCACTAA
R388	*dhfrII*	GTG	GC	CTAACAATTCGCTCCAGCGGACG--GCTTCGCCG-C-CCGCTGAGCTTTATCG-TT	AGG CGTCA
CONSENSUS		G[C/T]		CTAACAATTCGTTCAAGCCGACGCCGCTTCGCGG-CGCGGCTTAACTCA[A/G]GCG-TT	AGA

Figure 4. Comparison of the sequences of regions homologous to the Tn*7* 54 base sequence. Sequences immediately 3'- to the coding regions of *aadB*, *aadA* (R538-1), *aadA* (pSa), and *dhfrII* are compared to the 54 base repeat which flanks the Tn*7* *aadA* gene (5). Sequences have been aligned to give maximum homology. Boxes enclose the 54 base sequence and the extended 59 base sequence proposed here. The consensus sequence is shown and bases which differ from the consensus are underlined.

aadB promoter has been shown to be essential for expression of the *dhfrII* gene (2). Significant homology to the Tn*7* 54 base repeat sequence is also detected 3'- to the *dhfrII* coding region (Fig.3,4), but no homology is observed downstream of this element.

It appears that the *aadA* coding region of R538-1 and the *dhfrII* coding region have become joined to the same point within the *aadB* gene (and replaced the remainder of the *aadB* coding region) up to the 54 base repeat sequence. These events have led to the coupling of the *aadA* and the *dhfrII* coding regions to the *aadB* promoter.

The 59 base element

We have compared (Fig. 4) the sequences of all the elements identified in this study which show homology to the 54 base element which flanks the Tn*7* *aadA* gene as a direct repeat (5). We suggest that the 54 base sequence identified by comparison of the two Tn*7* elements (5) should be extended to include a further 5 bases. The consensus sequence for this 59 base element is shown. Fling *et al* (5) have noted that this element is a palindromic sequence which can form a hairpin structure. The 3'- flanking element of *aadA* in Tn*7* conforms to the consensus and the 5'- flanking element differs from the consensus at 5 positions. The element located 3'- to *aadB* differs at 2 positions. The R538-1 element is also highly homologous to the consensus except that it has an insertion of an A residue and the 11 residue GC rich region contains only 8 residues and differs somewhat in sequence. The latter difference probably represents incomplete resolution of the strong compression in this region in sequencing gels (5, our observations).

The element present in pSa has diverged somewhat more from the consensus and is only 56 bases long. When aligned to give maximum homology it differs from the consensus by 4 base deletions, a single base addition in the GC rich region and 5 base substitutions. This element has lost the potential to form a hairpin structure. The degree of divergence of the pSa element is consistent with the fact that the *aadA* coding region of pSa shows only 88% homology to the corresponding R538-1 region (4), while the latter shows almost perfect homology to the Tn*7* *aadA* sequence (5).

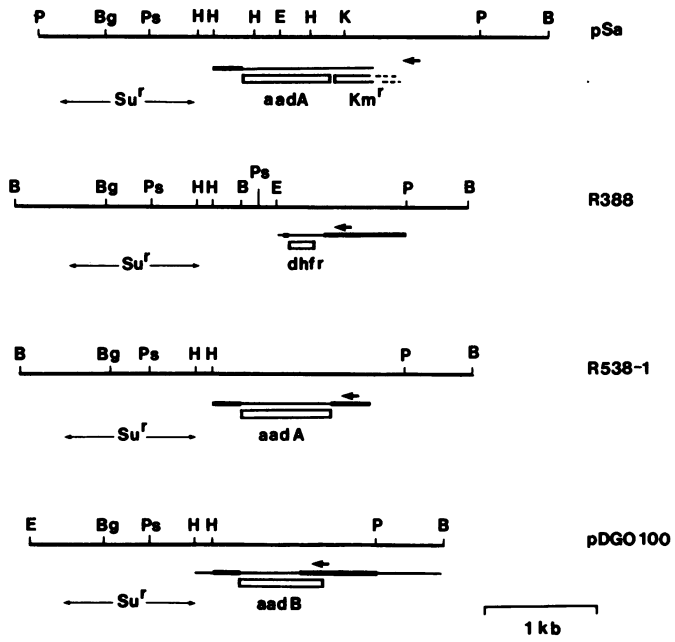

Figure 5. Comparison of the restriction maps of the regions surrounding the sequenced regions of pSa, R388, R538-1 and pDGO100. Restriction maps of pDGO100 (this study), pSa and R388 (19,20) and R538-1(1) have been aligned at the two *Hin*dIII restriction sites. The position and the extent of the sequenced regions are shown by lines; the thicker regions indicate homology with the *aadB* sequence. The coding regions are represented by open boxes and the direction of transcription is indicated by the small arrow. Restriction sites shown are B = *Bam*HI, P = *Pvu*II, E = *Eco*RI, Ps = *Pst*I, H = *Hin*dIII, Bg = *Bgl*II, K = *Kpn*I.

The sequence which lies 3'- to the *dhfrII* gene of R388 is also 56 bases long and shows homology to the 59 base element only at the termini and in the central region. This element has been included because, despite the extensive sequence divergence, it retains the potential to form a hairpin structure similar to that of the 59 base element. It is perhaps pertinent that this element is not located in an equivalent position (with respect to the Su^r determinant) to that of the *aadB*, R538-1 and pSa elements (see Fig. 5 and Discussion).

DISCUSSION

We have determined the nucleotide sequence of the *aadB* gene region from an IncC plasmid isolated during an outbreak of gentamicin resistant *Enterobacteriaceae* at Royal North Shore Hospital in 1978. The *aadB* gene codes for a predicted polypeptide of molecular weight 27,992 daltons, or 25,458 daltons if the second ATG codon is utilized. We also considered the

possibility that the polypeptide is translated from the ATG codon at 1298 which lies just within the region identified as unique when the *aadB* sequence was compared to the sequence of R538–1 (boxed in Fig.2). The polypeptide translated from this point would, however, have a molecular weight of 19,873 daltons, and no protein of this size was observed in the maxicell analysis (10). As the *aadB* gene described here is very closely related to the *aadB* gene used in the maxicell analysis (on the basis of restriction map identity and of hybridization analysis under stringent conditions), we have concluded that initiation occurs at either the first or second ATG codon of the open reading frame.

It has also been reported (10) that two probes isolated from the *aadB* gene region showed different patterns of hybridization to plasmids containing *aadA* and *aadB* genes. These probes, 280 bp and 310 bp *Ava*I fragments, both hybridized to *aadB* containing plasmids but the 280 bp probe also hybridized to *aadA* containing plasmids pIP71a and pSa. On the basis of these results it was concluded that the two adenylyltransferase genes *aadA* and *aadB* may share a region of homologous sequence. Our results do not support this conclusion. Identical *Ava*I fragments are present in the *aadB* gene sequenced in this study (*Ava*I sites at positions 1188, 1466 and 1772) and both fragments are completely included in the *aadB* coding region. Comparison of the *aadB* sequence with the published sequences for *aadA* from Tn*7* (5) and pSa (4) revealed no DNA or protein homology within the coding regions. We are, however, able to provide an explanation for the hybridization data. A region with almost perfect homology to a segment of the *aadB* gene was found 5'- to the *aadA* region of R538-1 (1). The same *aadB* segment was found upstream of the *dhfrII* coding region of R388 (2,3). The 310 bp *Ava*I fragment lies outside this region, but the 280 bp *Ava*I probe includes about 100 bases of this segment. The plasmids pIP71a and pSa which hybridized to this fragment presumably also include this segment of the *aadB* gene. The fact that a segment of the *aadB* coding region can be found in plasmids which do not express this gene indicates that caution must be exercised in selecting probes for epidemiological studies.

A detailed map showing the relationship between the plasmids pSa and R388 has been published (20,21). The region of R388 containing the trimethoprim determinant (*dhfrII*) replaces the streptomycin/spectinomycin (*aadA*) gene and the kanamycin resistance (Km^r) determinant of pSa. The plasmids are homologous upstream of this region (20,21,22) (see Fig. 5). This comparison allows us to tentatively locate the segment of pSa which hybridizes to the 280 bp *Ava*I *aadB* probe 5'- to the Km^r determinant. An interesting feature which emerges from our comparisons is that we are able to identify the promoter regions proposed for the *aadA* (R538–1) and the *dhfrII* (R388) genes as the *aadB* promoter region. This region has been shown to be essential for the expression of the *dhfrII* gene (2). It is possible that the Km^r and *aadA* genes of pSa which are co-transcribed (4) also utilize the *aadB* gene promoter.

The restriction maps of the areas surrounding the sequenced regions of pSa, R388, R538-1 and pDGO100 (*aadB*) are shown in Fig. 5. This comparison highlights the similarity between the regions surrounding the four sequenced genes compared here. In each plasmid a

*Pvu*II-*Bam*HI fragment of approximately 0.6 kb lies upstream (rightwards) and a Su^r determinant lies downstream in a region characterized by a *Pst*I, a *Bgl*II, and two *Hin*dIII restriction sites. The similarity of the surrounding regions has lead us to propose a simple model for the evolution of these plasmids. In this model the configuration present in the *aadB* gene region which we have sequenced would represent the ancestral sequence. The R538-1 and the R388 sequence configurations are proposed to arise by a two step process. The first step is the insertion of *aadA* or *dhfrII* sequences between Su^r and *aadB* ; the second step is a deletion event which led to loss of part of the *aadB* coding region and coupling of the inserted genes to the *aadB* promoter.

The presence immediately 3'- to the *aadB* gene of a sequence closely related to the 59 base element suggests a possible mechanism for gene insertion. The *aadA* gene in the configuration found in Tn*7* (flanked by two 59 base elements) should be able to insert either by homologous recombination with the *aadB* element or by some other mechanism specific to the 59 base element. Any other gene region which is flanked by two 59 base elements should also be able to insert at the same position. We propose that the configuration present after gene insertion includes two 59 base elements both of which could function as "hot spots" for further insertion events. Stabilization could be achieved either by sequence divergence at critical positions in one or both elements or by a deletion event which removes part or all of one of the elements. In support of this, a short 5/6 base homology (GTTAGG) with the 3'- terminal sequence of the 59 base element (GTTAGA) was found at the junctions of the unique R538-1 and R388 sequences with the region homologous to the *aadB* sequence. We suggest that this sequence homology may be involved in the deletion events which led to fusion of *aadA* and the *dhfrII* genes to the *aadB* sequences. Though the sequence GTTA is found six times in the *aadB* coding region, and four of these lie between the fusion point and the *aadB* promoter, only the sequence at the fusion end point conforms to the consensus for the 3'- end of the proposed extended consensus (see Fig. 4).

It is not necessary to demand that the events leading to plasmid evolution occur at high frequency since the evolution of antibiotic resistance plasmids is favoured by the selective pressure of antibiotic usage. The organisms which would be most successful are those which can express resistance at a high level and are stable within the plasmid. If the promoter region associated with the *aadB* gene is more efficient than the promoters originally associated with the *aadA* and *dhfrII* genes, the configuration of *aadA* in R538-1 and of *dhfrII* in R388 would confer a selective advantage. In this context it is interesting to note that the proposed promoter for *aadA* in Tn*7* shows poor homology with the consensus -10 and -35 regions (5).

Comparison of the physical and functional maps of the transposons and transposition-defective elements related to Tn*21* suggests that they have evolved by insertion of antibiotic resistance genes into an ancestral sequence (23, 24). These elements include a region which shows striking similarities to the regions compared in this study. A "hot spot" for insertion lies between the Su^r determinant and the *aadA* gene of Tn*21*. When *aadB* is present (e.g. Tn*4000*) it lies adjacent to the *aadA* gene and distal to the Su^r determinant (6, 11). It is tempting to speculate

that this region of Tn*21* may be analogous to the regions compared in detail in our study. In this context, we have shown that the original plasmid isolate (pDGO100) from which the *aadB* gene was isolated also confers resistance to mercury. Mercury resistance is frequently associated with Tn*21*-like elements.

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