
Organization and nucleotide sequence of a new ribosomal operon in *Escherichia coli* containing the genes for ribosomal protein S2 and elongation factor Ts

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ABSTRACT

We report the nucleotide sequence of the four min region of the *Escherichia coli* genetic map that includes the genes for ribosomal protein S2 (rpsB) and translation elongation factor EF-Ts (tsf), and the possible location of regulatory sites within this two gene operon. The data indicate that the gene order is: rpsBp-rpsB-tsf-tsft. One potential regulatory site is a 16 nucleotide sequence in the rpsB leader region encompassing the ribosome binding site and the translation initiation codon. This has a high degree of homology with nucleotides 8 through 23 on the 5' end of 16S ribosomal RNA, and might signify a sequence that is necessary for post-transcriptional control of rpsB expression. The data allow one to infer the amino acid sequences of S2 and EF-Ts.

INTRODUCTION

Studies of the organization of essential genes in *Escherichia coli*, especially those for components of the transcription and translation apparatus, have revealed the expected close linkage of related functions, such as among the structural genes for the ribosomal proteins (r-proteins) (3). Rather more unexpected is the location within the same operon of genes whose functions are associated, but not closely related. Thus the genes for β and β' subunits of RNA polymerase, rpoB and rpoC, are located in the same operon with the genes for r-proteins L10 and L12, rplJ and rplL (4-6). rpoA, the gene for RNA polymerase subunit α , is surrounded by r-protein structural genes (7). Among the genes for translation elongation factors one finds a similar situation; fus and tufA, genes for elongation factor G (EF-G) and elongation factor Tu (EF-Tu), are located as the distal-most two genes of a four gene operon that also includes two r-protein genes (8). We have recently reported the analysis of a group of genes in the four minute region of the *E. coli* linkage map (9). Among these were the genes for r-protein S2 (rpsB) and elongation factor EF-Ts (tsf) (10). The evidence suggested that these two genes lie side-by-side in a single transcriptional unit. As an extension of this study we report in this

communication the nucleotide sequence of the region and the location of possible regulatory sites within it.

MATERIALS AND METHODS

The *E. coli* strain used as a recipient for plasmids was MC1000 (araD139, araA(ABOIC), leu7697, lacA(IPOYZ-X74), galU, galk, strA) (11). The structure of plasmids pDB9 (10) and pGA39 (12) have been described earlier. The method of determining resistance to tetracycline on L-broth agar has been described (13).

Restriction endonuclease digestion, ligation with T4 DNA ligase, analysis of DNA fragment size by gel electrophoresis and preparation of plasmid DNA have been described previously (12).

The positions of the restriction endonuclease sites, determined in preparation for nucleotide sequence analysis, were on the basis of analyzing the size of DNA fragments from singly- or doubly-digested pDB9 DNA, or on the basis of radioactively end-labelling a DNA fragment and measuring by gel electrophoresis and autoradiography the fragment size after partial digestion.

DNA fragments were radioactively end-labelled with ^{32}P -deoxyribonucleotide triphosphates using the large fragment of *E. coli* DNA polymerase I (14). DNA fragments were purified by electrophoresis on 5% polyacrylamide gels followed by electroelution. Nucleotide sequence analysis was carried out by the method of Maxam and Gilbert (14).

RESULTS AND DISCUSSION

Restriction Endonuclease Map of the rpsB-tsf Region

An earlier publication reported a genetic analysis of the four min region of the *E. coli* genetic map (15,16). Recently, we have cloned restriction endonuclease fragments of a specialized transducing bacteriophage, $\lambda\text{polC-9}$ (15), on a number of different plasmid cloning vehicles (10). A determination of which proteins were encoded on these plasmids enabled us to locate with a fair degree of precision rpsB and tsf, the structural genes for r-protein S2 and for EF-Ts. One of the plasmids that was analyzed is shown in Fig. 1. Plasmid pDB9 (10) carries a 3.3 Kb (kilobase) PstI fragment that was derived from $\lambda\text{polC-9}$ and which was cloned on pGA46 (12). Fig. 1 shows a detailed restriction endonuclease map of this plasmid along with the location of the two genes that concern us here. It has previously been shown (10) that this plasmid is capable of expressing rpsB and tsf, and that these two genes were probably in a single transcription unit whose promoter (rpsBp) lies to the left of rpsB, and whose direction of transcription is from left to right, as is indicated in Fig. 1

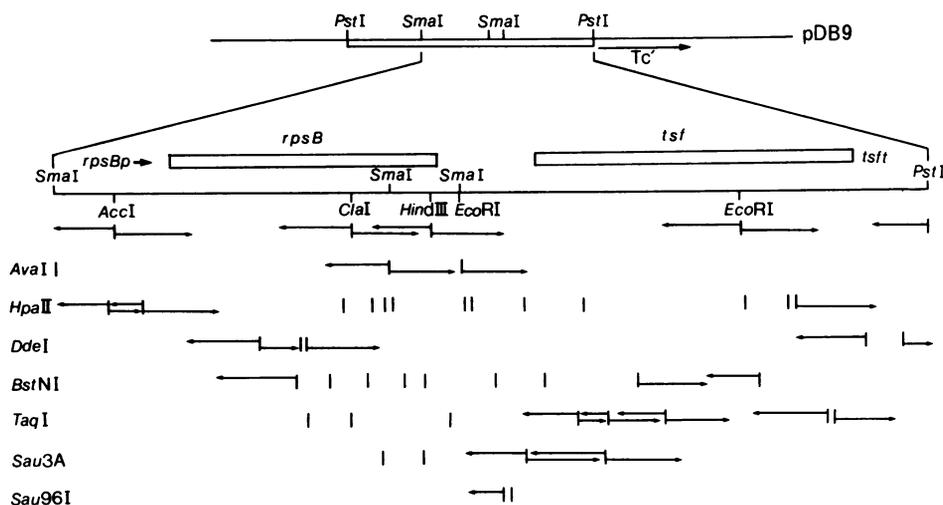


Fig. 1. A restriction map of pDB9 and of the *rpsB-tsf* region, including the tactics for obtaining its nucleotide sequence. The 3.3 KB *Pst*I fragment (double horizontal lines on pDB9) was derived directly from λ polC-9 and was cloned on plasmid pGA46 (single horizontal line on pDB9). pDB9 is shown in linear form as if it were opened at the *Eco*RI site in the chloramphenicol acetylase gene. The positions and sizes of the genes indicated by the open rectangles in the middle region of the figure are based on the nucleotide sequence data shown in Fig. 2. *rpsBp* symbolizes a promoter and *tsft* symbolizes a transcription termination site for the *rpsB-tsf* operon. The horizontal arrows represent the site of radioactive end-labelling and the direction and extent of reading in individual sequence analysis experiments.

Nucleotide Sequence of *rpsB* and *tsf*

In order to obtain a better picture of the genetic organization and of the control mechanisms that operate in this gene cluster we have determined the nucleotide sequence of the *rpsB-tsf* region. Fig. 1 shows the tactics used in this study. The sequence itself is shown in Fig. 2.

Several points are noteworthy:

1) The amino acid sequence of S2 as inferred from the nucleotide sequence of *rpsB* matches the complete amino sequence determined by Liebold-Wittmann and Wittmann (personal communication). There are 240 amino acids in S2 and its calculated molecular weight is 26,582. The inferred amino acid sequence of EF-Ts contains matches the sequence of three peptide fragments (including the N-terminal peptide of 18 amino acids) that have been determined by A. Wittinghofer (personal communication). There are 282 amino acids in EF-Ts and its calculated molecular weight is 30,257.

1 ATTCTGAATTTGCCAAACGTCCACTGAAAGTTTTCTAATAACAAAAATTGACGCTCTGACTGTACACAGCGCCAACAATATTGGTGTCCACGACG

101 TATTTTGGTATAAAGCGCCGGGACTTCCGATCCATTTTCGTATACACAGACTGGACGGAAAGCCACAATCTCACTTTGTGTAAACAACACACAGCTATCGG

201 CACATATTCCGGGTTCCCTTTGGGTCGGTAATATCGGATAGCTGCAGGCATAACCCCAACTTTTATATAGAGGTTTAAATC ATG GCA ACT GTT
 ala thr val

286 TCC ATG CCC GAC ATG CTC AAG GCT GGT GTC TCC GGT CAC CAG ACC CGT TAC TGG AAC CCG AAA ATG AAG CCG
 ser met arg asp met leu lys ala gly val his phe gly his gln thr arg tyr trp asn pro lys met lys pro

361 TTC ATC TTC GGT CCG CGT AAC AAA GTT CAC ATC AAC CTT GAG AAA ACT GTA CCG ATG TTC AAC GAA GCT CTG
 phe ile phe gly ala arg asn lys val his ile ile asn leu glu lys thr val pro met phe asn glu ala leu

436 GCT GAA CTG AAC AAG ATT GCT TCT CCG AAA GGT AAA ATC CTT TTC GTT GGT ACT AAA CCG GCT GCA AGC GAA GCG
 ala glu leu asn lys ile ala ser arg lys gly lys ile leu phe val gly thr lys arg ala ala ser glu ala

510 GTG AAA GAC GCT GCT CTG AGC TGC GAC CAG TTC TTC GTG AAC CAT CCG TGG CTG GCG GGT ATG CTG ACT AAC TGG
 val lys asp ala ala leu ser cys asp gln phe phe val asn his arg trp leu gly gly met leu thr asn trp

586 AAA ACC GTT CGT CAG TCC ATC AAA CGT CTG AAA GAC CTG GAA ACT CAG TCT CAG CAG GCT ACT TTC GAC AAG CTG
 lys thr val arg gln ser ile lys arg leu lys asp leu glu thr gln ser gln asp gly thr phe asp lys leu

661 ACC AAG AAA GAA GCG CTG ATG CCG ACT CGT GAG CTG CAG AAA CTG GAA AAC AGC CTG GCG GGT ATC AAA GAC ATG
 thr lys lys glu ala leu met arg thr arg glu leu glu lys leu glu asn ser leu gly gly ile lys asp met

736 GGC GGT CTG CCG GAC GCT CTG TTT GTA ATC GAT GCT GAC CAC GAA CAC ATT GCT ATC AAA GAA GCA AAC AAC CTG
 gly gly leu pro asp ala leu phe val ile asp ala asp his glu his ile ala ile lys glu ala asn asn leu

811 GGT ATT CCG GTA TTT GCT ATC GTT GAT ACC AAC TCT GAT CCG GAC GGT GTT GAC TTC GTT ATC CCG GGT AAC CAC
 gly ile pro val phe ala ile val asp thr asn ser asp pro asp gly val asp phe ala phe ala ser gly asn asp

886 GAC GCA ATC CGT GCT GTG ACC CTG TAC CTG GGC GCT GTT GCT GCA ACC GTA CGT GAA GCG CGT TCT CAG GAT CTG
 csp ala ile arg val thr leu thr leu gly ala val ala ala thr val arg met arg gly arg ser gln asp leu

961 GCT TCC CAG CCG GAA GAA AGC TTC GTA GAA GCT CAG TAA TAA GGCTTGATAACCTCCCCCAAAATAGTTCGAGTTCGACGAAAGCCCG
 ala ser gln ala glu glu ser phe val glu ala glu ter ter

1047 CAAGCTCGAAGTTCGCCGAGCTTACATCAGTAACTGACCGGGATGACGCGGCAAGATAACCGCATCTGGCGCGCAAAATATCAAGGGGAGAGCCCTT

1147 ATAGACAGGTAGTACACGTTTGGTATAGGGGGCTGCATATGGGGCCCTTTTTCACCTTTTATATCTGTGGGTTTAATCGCCGGCAGATCACATCTCCGA

1247 GGATTTTAG. ATG GCT GAA ATT ACC GCA TCC CTG GTA AAA CAG CTG CGT CAG CGT ACT GGC GCA GGC ATG ATG GAT
 ala glu ile thr ala ser leu val lys glu leu arg glu arg thr gly ala gly met met asp

1323 TGC AAA AAA GCA CTG ACT GAA GCT AAC GGC GAC ATC CAG CTG GCA ATC GAA AAC ATG CGT AAG TCC GGT GCT ATT
 lys lys ala leu thr glu ala asn gly asp ile glu leu ala ile glu met arg lys ser gly ala ile

1398 AAA GCA GCG AAA AAA GCA GGC AAC GTT GCT GCT GAC GGC GTG ATC AAA ACC AAA ATC CAC GCG AAC TAC GGC ATC
 lys ala ala lys lys ala gly asn val ala ala asp gly val ile lys thr lys ile asp gly asn tyr gly ile

1473 ATT CTG GAA GTT AAC TCC CAG ACT CAC TTC GTT GCA AAA CAC GCT GGT TTC CAG GCG TTC GCA GAC AAA GTT CTG
 ile leu glu val asn cys gln thr asp phe val ala lys asp ala gly phe gln ala phe ala asp lys val leu

1548 GAC GCA GCT GTT GCT GGC AAA ATC ACT GAC GTT GAA GTT CTG AAA GCA CAG TTC GAA GAA GAA CGT GTT CCG CTG
 asp ala ala val ala gly lys ile thr asp val glu val leu lys ala gln phe glu glu arg val ala leu

1623 GTA GCG AAA ATT GGT GAA AAC ATC AAC ATT CCG CCG GTT GCT GCG CTG GAA GGC GAC GTT CTG GGT TCT TAT CAG
 val ala lys ile gly glu asn ile asn ile arg arg val ala ala leu glu gly asp val leu gly ser tyr gln

1698 CAC GGT GCG CGT ATC GGC GTT CTG GTT GCT GCT AAA GGC GCT GAC GAA GAG CTG GTT AAA CAC ATC GCT ATG CAC
 his gly ala arg ile gly val leu val ala ala lys gly ala asp glu glu leu val lys his ile ala met his

1773 GTT GCT GCA AGC AAG CCA GAA TTC ATC AAA CCG GAA CAC GTA TCC GCT GAA GTG GTA GAA AAA GAA TAC CAG GTA
 val ala ala ser lys pro glu phe ile lys pro glu asp val ser ala glu val val glu lys glu tyr gln val

1848 CAG CTG GAT ATC CCG ATG CAG TCT GGT AAG CCG AAA GAA ATC GCA GAG AAA ATG GTT GAA GCG CCG ATG AAG AAA
 gln leu asp ile ala met gln ser gly lys pro lys glu ile ala glu lys met val glu gly arg met lys lys

1923 TTC ACC GCG GAA GTT TCT CTG ACC GGT CAG CCG TTC GTT ATG GAA CCA AGC AAA ACT GTT GGT CAG CTG CTG AAA
 phe thr gly glu val ser leu thr gly gln pro phe val met glu pro ser lys thr val gly gln leu leu lys

1998 GAG CAT AAC GCT GAA GTG ACT GGC TTC ATC CCG TTC GAA GTG GGT GAA GGC ATC CAG AAA GTT CAG ACT CAC TTT
 glu his asn ala glu val thr gly phe ile arg phe glu val gly glu gly ile glu lys val glu thr asp phe

2073 GCA GCA GAA GTT CCT CCG ATG TCC AAG CAG TCT TAA TTATCAAAAAGGAGCGGCTCAGCGGCTTTTGTGGCCATCTGTAAATTCGA
 ala ala glu val ala ala met ser lys gln ser ter

2161 CCTAACCCCTGTGGGCTCCGC

Figure 2. The DNA nucleotide sequence of the *rpsB-tsif* region. As is indicated in Fig. 1, for almost the entire region both DNA strands were analyzed. Possible RNA polymerase binding sites are underlined. Regions of inverted repeat are indicated by horizontal lines immediately above the relevant sequences.

2) The codon usage for both rpsB and tsf (Fig. 3) is typical of other genes for translation-associated functions (17). Whether this is related to the fact that most of these proteins are relatively abundant in the cell (18-20) or whether there are other more subtle reasons for components of the translation apparatus having a peculiarly skewed codon usage is not known.

3) There are at least two possible sites for promoters in the region upstream from S2. The two acceptable Pribnow sequences (21) are located at positions 38-44 and 231-237. However, neither of these has the typical -35 region that is often associated with the RNA polymerase recognition site (22). Nor is either of the Pribnow sequences followed by a CG-rich region, which has been proposed as necessary for stringent control of expression (23), even though tsf (and one assumes also rpsB) is regulated by the stringent mechanism (24). Nevertheless, data presented below indicate that the promoter for the operon, rpsBp, lies within 300 bp upstream from rpsB and thus presumably involves one of the Pribnow sequences that we have noted.

4) There is a stem-loop structure at 2121-2137 ($\Delta G = -12$ Kcal/mol, approx.) (25) that might signify a rho-independent transcription termination sequence (tsft) following tsf (22). The data presented below also strongly suggest the

Second base

		U a,b	C a,b	A a,b	G a,b		
First base	U	Phe 2,1 10.9	Ser 4,4 3.4	Tyr 0,1 2,2	Cys 0,0 1,2	Third base	
		Leu 0,0 0,0		term 1,1 0,0	term 0,0 3,0		
	C	Leu 2,0 1,0 0,0 18.16	Pro 0,0 0,0 0,2 7,3	His 1,1 5,3	Arg 8,5 5,4 0,0 0,0	U C A G	
		A		Ile 3,5 11,14		Thr 7,7 6,4	Asn 0,0 12,8
Met 0,0 7,9				Lys 14,23 5,5			Arg 0,0 0,0
G	Val 9,20 0,0 5,5 3,4	Ala 16,17 0,0 5,14 4,8	Asp 4,2 13,12	Gly 12,9 5,15 0,0 0,0	U C A G		
		Glu 12,24 4,8					

Figure 3. Frequency of codon usage in rpsB (a) and tsf (b). The number of occurrences of each codon is indicated, including the termination codon, UAA.

presence of an active terminator within 200 bp downstream from tsf.

5) There are several possible internal regulatory sites in the operon. A typical rho-independent termination or attenuation site (22) lies in the intergenic region (1175-1195) between rpsB and rts (Fig. 2). This is preceded by a very large and extremely stable ($\Delta G = -80$ Kcal/mol, approx.) (25) stem-loop structure (999-1148) that, in its RNA form, is reminiscent of an RNaseIII site that occurs at gene 1.0 in the T7 bacteriophage early transcript (26). It is interesting to note that the structures found in the region between rpsB and tsf are similar to those found between the first two and the last two genes of the rplJL-rpoBC operon (27). There is another interesting similarity between these two operons: in both cases distal genes (tsf on the one hand and rpoBC on the other) are expressed about one-third to one-fifth as strongly as the promoter-proximal gene(s) (19,28,29). In the case of the rplJL-rpoBC there is a transcription attenuation of about five-fold (19,29) and an RNaseIII recognition site (27) between the promoter-proximal and promoter-distal genes. We do not yet know whether this holds in the rpsB-tsf operon. The intergenic sequence suggests that it might, however the data presented below failed to reveal any evidence for an attenuator or a terminator between rpsB and tsf. The intergenic region between rpsB and tsf has at least two sequences that could correspond to Pribnow boxes. However, as discussed below, we do not think that either of these is active under normal conditions.

(6) There is a significant base sequence homology between the rpsB leader messenger RNA (mRNA) and the 5' end of 16S ribosomal RNA (rRNA) (Fig. 4). The region of homology encompasses the Shine and Dalgarno sequence (30) through the first five nucleotides of rpsB; this region of rpsB leader mRNA had no significant homology with any other region of the 16S rRNA. This might be related to the presence of homologies between rRNA and other r-protein mRNAs, such as rpsD (r-protein S4) (31), rpsG (r-protein S7) (31), rpsT (r-protein S20) (G. Mackie, personal communication), rplJ (r-protein L10) (unpublished observations), and rplK (r-protein L11) (32). However, unlike most of the other cases cited (31,32), there is no significant secondary structure in either the

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      U A U A U A G A G G U U U U A A U C A U G G C A A C   rpsB mRNA
      *   *   * *   * * *   * * * * * * * *
5'pA A A U U G A A G A G U U U G A U C A U G G C U C A   16S rRNA
    
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Figure 4. Sequence homology between the leader region of rpsB-tsf mRNA and 16S rRNA of E. coli. The asterisks indicate homologous nucleotides. The Shine-Dalgarno sequence and the translation start codon for rpsB are underlined.

rpsB leader or the 16S rRNA, in the region of base sequence homology. It has been demonstrated that S4, S7, S8, L1, L4 and L10 are key proteins in the autoregulation at the post-transcriptional level not only of their own synthesis, but also of other genes in the same operon (31,33-38). Moreover, S4, S7, S20, L1 and L10 (as the L10/L7/L12 complex) bind directly to rRNA, as does S2 (weakly) (39,40). It is not yet known whether S20 and S2 are subject to feedback regulation. However, it should be noted that biosynthesis of S2 is subject to regulation, since cells that harbour a rpsB multi-copy plasmid do not over-produce S2 (10). These results fit an emerging picture of autogenous regulation of r-protein production that is governed at the post-transcriptional level by competition between a sequence in its own leader mRNA and a homologous sequence in rRNA for binding of a particular key r-protein.

Location of the Promoter and Terminator for the rpsB-tsf Operon

We have made use of the plasmid cloning system devised by An and Friesen (12) for a further analysis of possible regulatory regions in the rpsB-tsf operon. These plasmids carry the genes for tetracycline resistance (Tc) without their normal promoter; thus Tc can be activated by insertion of a foreign promoter. Plasmid pDB9, shown in Fig.1, is already such a construction. This plasmid was initially constructed by cloning the 3.3 Kb PstI fragment originally derived from λ polC-9 into the recipient plasmid vehicle, pGA46 (12). A promoter on this fragment activated Tc to the extent that the host strain that carried pDB9 became resistant to tetracycline at a concentration of 3 μ g/ml (Fig. 5). However, this is almost 10-fold lower than we would have predicted on the basis of previous results with other r-protein promoters (13). From the structure of pDB9 (Fig. 5) it is not immediately obvious that the low expression of Tc is being driven by the rpsB promoter, rpsBp, or by some other unidentified promoter. This was clarified by the in vitro removal from pDB9 of one or both adjacent SmaI (XmaI) restriction fragments, to yield plasmids pGA207 and pGA209 (Fig. 5). In both cases host cells that carry either of these two derivative plasmids were resistant to tetracycline at a concentration of 1 μ g/ml, suggesting that the promoter had been removed and thus is located in the position indicated in Fig. 1. A corroboration of the inferred promoter position was obtained by cloning the XmaI fragment that contains the proximal region of rpsB plus 300 bp to the 5' side of it. For this purpose another member of the promoter-cloning plasmid set (12), pGA39, was used. The resulting plasmid, designated pGA214 (Fig.5), endowed the host cell with resistance to 24 μ g/ml tetracycline. We conclude that rpsBp lies within the larger of the two SmaI fragments, as is shown in Fig. 1. These results confirm the position of

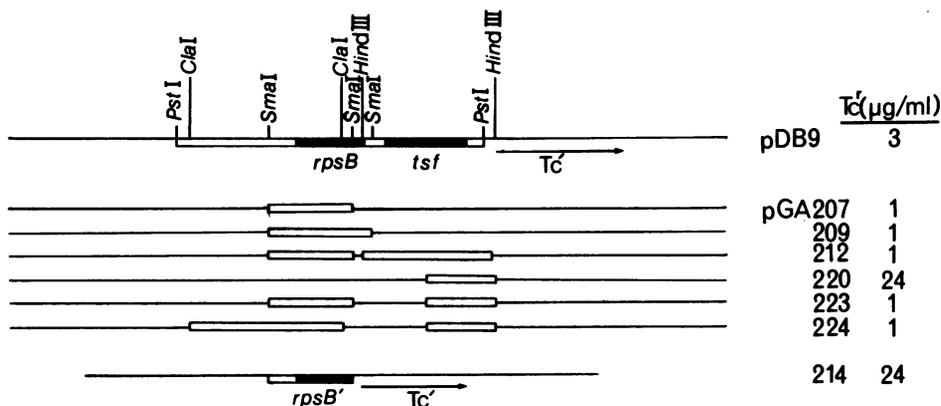


Figure 5. Structure of pDB9 and its derivatives. Bacterial DNA from λ polC-9 is indicated by double horizontal lines on pDB9. The plasmid is shown in linear form derived by opening at the *EcoRI* site in the chloramphenicol acetylase gene. The locations of *rpsB* and *tsf* are indicated by the filled boxes. The structures of several deletion plasmids are also illustrated. The deletions in these plasmids are represented by open boxes. The level of drug resistance bestowed on the host cell by each plasmid is given on the right-hand side of the diagram.

rpsBp that was inferred from the sequence analysis. However, we do not yet know the precise transcription start site. No functional promoter is able to read through from a position upstream from *rpsB* since plasmid pGA212, from which the entire *rpsB*-*tsf* operon has been excised, bestowed only 1 µg/ml tetracycline resistance on the host cell (Fig. 5).

From the characteristics of three other plasmids we conclude that a strong transcription terminator (*tsft*) lies distal to *tsf*. pGA220 is a spontaneous in vivo deletion that was selected as a mutant of pDB9 that bestowed 24 µg/ml resistance to tetracycline (Fig. 5). We infer that this deletion removes *tsft*. The nucleotide sequence indicates a possible stem-loop structure between *rpsB* and *tsf* that might correspond to the site of a transcription attenuator (Fig.2). However, we were unable to observe an attenuator effect since both pGA214 (which carries only *rpsBp*) and pGA220 (which carries *rpsBp* and the intergenic region) drive Tc to a level of 24 µg/ml.

Two in vitro derivatives were constructed from pGA220: pGA223 lacks the *XmaI* fragment that carries the major promoter of the operon (Fig. 5). This plasmid bestowed tetracycline resistance at 1 µg/ml, indicating that there is no significant promoter activity downstream from *rpsB*, particularly in the intergenic region. Similarly, deletion of the *ClaI* fragment (pGA224) reduced

the tetracycline resistant level to 1 $\mu\text{g/ml}$ (Fig. 5); this observation supports our earlier conclusion as to the position of the promoter for the operon.

In conclusion these deletion analyses indicate that there is only one major promoter for the rpsB-tsf operon, and it is located immediately upstream from rpsB. A strong transcription terminator is located downstream from tsf. We were unable to obtain evidence for the action of a possible transcription attenuator that on the basis of sequence analysis (Fig. 2) one might have expected to lie between rpsB and tsf.

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