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Control features within the *rplJL–rpoBC* transcription unit of *Escherichia coli*

(gene fusion/attenuator/terminator/RNA polymerase/ribosomal proteins)

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ABSTRACT **Gene fusions constructed *in vitro* have been used to examine transcription regulatory signals from the operon which encodes ribosomal proteins L10 and L7/12 and the RNA polymerase β and β′ subunits (the *rplJL–rpoBC* operon). Portions of this operon, which were obtained by *in vitro* deletions, have been placed between the *ara* promoter and the *lacZ* gene in the gene-fusion plasmid pMC81 developed by M. Casadaban and S. Cohen. The effect of the inserted DNA segment on the expression of the *lacZ* gene (in the presence and absence of arabinose) permits the localization of regulatory signals to discrete regions of the *rplJL–rpoBC* operon. An element that reduces the level of distal gene expression to one-sixth is located on a fragment which spans the *rplL–rpoB* intercistronic region. This strongly supports the idea that there is an attenuator in this region. The terminator for the operon is located on a fragment which spans the 3′ end of the *rpoC* gene. The major promoter for the operon precedes the *rplJ* gene [Yamamoto, M. & Nomura, M. (1978) *Proc. Natl. Acad. Sci. USA* 75, 3891–3895 and Linn, T. & Scaife, J. (1978) *Nature (London)* 276, 33–37] and was not examined in this study. However, a weak promoter is observed on the fragment that spans the *rplJ–rplL* intercistronic region. Other regions of the operon may also contain weak promoters. The contribution of these elements to the regulation of this complex operon is discussed.**

Gene fusions provide a means of examining regulatory mechanisms in systems in which the gene product is difficult to measure. In particular, restriction endonuclease and plasmid techniques permit the assembly of different genetic elements in a directed fashion. Thus, it is possible to characterize the regulatory regions of one operon or gene by measuring the product of a second fused gene. We have used this approach to study the regulation of RNA polymerase synthesis.

The gene-fusion plasmid pMC81, developed by Casadaban and Cohen (see ref. 1; M. Casadaban, and S. N. Cohen, personal communication), has been constructed to detect the presence of both termination signals and promoter regions in cloned restriction fragments. This plasmid carries a DNA segment in which portions of the arabinose, tryptophan, and lactose operons are fused, placing synthesis of β-galactosidase solely under arabinose control. Thus, *lacZ* expression is mediated through the *araI* region. The plasmid contains a single *Hin*dIII site in the *trpB* gene between *araI* and *lacZ*. Control elements can be detected and evaluated when they are inserted into this *Hin*dIII site.

The synthesis of *Escherichia coli* RNA polymerase (EC 2.7.7.6) α, β, and β′ subunits is regulated together with many ribosomal proteins under various physiological conditions (2, 3). Recent reports that the genes for the α subunit (*rpoA*) and those for the β and β′ subunits (*rpoBC*) are in two separate transcription units, each of which also includes a number of ribosomal protein genes, suggest how this type of coordinate regulation could occur (4–7). Yet under certain conditions, coordinate expression of the RNA polymerase subunits and ribosomal proteins is not observed. This is especially true of the *rplJL–rpoBC* transcription unit which encodes the ribosomal proteins L10 and L7/12 and the RNA polymerase subunits β and β′. For example, only the ribosomal proteins are modulated by the stringent regulation system (8, 9) whereas a transient stimulatory effect of rifampicin is specific for the RNA polymerase subunits (10, 11). In addition, different amounts of mRNA hybridize to the *rpl* and *rpo* regions of the *rplJL–rpoBC* transcription unit (12) and the relative amounts of mRNA that hybridize to the two gene clusters can be altered either by the inactivation of temperature-sensitive RNA polymerase molecules (13) or by the elicitation of the stringent response (9). The observed differential regulation of these cotranscribed genes has led to suggestions of attenuation or of mRNA processing as extra control devices (6, 7, 14, 15). It has also been proposed that *rpoBC* may in fact have a second promoter that functions independently of the primary promoter for the *rplJL–rpoBC* transcript (7, 13).

We have constructed hybrid plasmids between DNA fragments derived from selected segments of the *rplJL–rpoBC* region and the plasmid pMC81. These hybrid plasmids have been used to study the regulatory features contained in this region.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Enzymes. Strain HB133 (*endoI*$^-$, $r_B^-m_B^+$, *ara*$^-$, *strA*, *lac*$^-$) and plasmid pBR322 (*amp*^{r}, *tet*^{r}) (16) were obtained from H. Boyer; strain MO1000 ($r_K^+m_K^+$, *recB*, *recC*, *sbcA*, *gal*$^-$, *thi*$^-$, Δ*trp-tonB*) was obtained from M. Oishi. Strain MC1000 ($r_K^+m_K^+$, Δ*lacIPOZY*, *galK*, *galU*, Δ*ara-leu*, *strA*) and plasmid pMC81 (*colE1:tn3:araIO-trpB-lacZ*) (1) were obtained from M. Casadaban. DNA derived from the phage λ*drif*^{d}*18* (17) was a generous gift of H.-L. Yang.

Hybrid Plasmid Construction Techniques. Vectors (pBR322 and pMC81) were digested with *Hin*dIII and then treated with bacterial alkaline phosphatase to decrease self-ligation (18). Ligations were performed by the method of Selker *et al.* (19). Transformations were done by the method of Cohen *et al.* (20), with plating on appropriate selective medium containing ampicillin (20 μg/ml) or rifampicin (50 or 100 μg/ml) or both.

Specific pieces of the plasmid DNA were deleted by carrying out partial digestions with the desired restriction endonuclease. Cells were transformed with approximately 1–5 μg of the partially digested DNA without *in vitro* ligation. L-Broth cultures (5 ml) were grown until stationary (37°C overnight) and screened for plasmids by the method of Meagher *et al.* (21). The content and order of restriction fragments in the deleted

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Abbreviation: kbp, kilobase pair(s).

plasmids were established by restriction analyses using various endonucleases (not all data shown, see legends to Figs. 1 and 3).

Growth Conditions and β-Galactosidase Assay. Strains containing plasmids were grown in preconditioned (22) glycerol/Luria broth (containing ampicillin at 20 μg/ml and 0.4% glycerol) to decrease the effects of catabolite repression on the *ara* control system and to give reproducible differential rates of β-galactosidase synthesis under all experimental conditions. Where indicated, arabinose was added to logarithmic phase cultures to a final concentration of 0.4%. The differential rate of β-galactosidase synthesis was determined by using samples taken between OD_{420} of 0.35 and 0.75 (23, 24).

All experiments using recombinant DNA were performed in accordance with the guidelines of the National Institutes of Health.

RESULTS

Cloning the *rplJL–rpoBC* Region. Initial experiments to clone a DNA fragment carrying the *rplJL–rpoBC* region used the vector pBR322. Because this system may result in vector-initiated promotion of the inserted genes, it was abandoned in favor of the newly developed vector pMC81.

λ*drif*^{d}*18* DNA was digested with *Hin*dIII, ligated to cleaved pBR322, and used to transform strain HB133. Ampicillin- and rifampicin-resistant transformants were selected on Penassay plates plus the antibiotics. All resistant transformants checked were found to contain hybrid plasmids of pBR322 and only the 9.74-kilobase-pair (kbp) *Hin*dIII fragment from λ*drif*^{d}*18*. This fragment has been shown to contain part of the *rplJ* gene and all of the *rplL* and *rpoBC* genes (6, 7, 15, 25, 26) but is not thought to have the major promoter for these genes (6, 7, 27). It is interesting that the 10 pBR322 recombinants examined all had the 9.74-kbp fragment inserted in an orientation opposite to that of the *tet*^{r} gene in the vector. Pertinent restriction sites in one of these plasmids, pGB218, are shown in Fig. 1*C*. These 10 clones were selected for resistance to a high concentration of rifampicin (100 μg/ml). This high-level resistance in the absence of the major *rplJ* promoter and the unique orientation suggest that the inserted genes are transcribed from a promoter in the vector. The possibility that pBR322 contains a promoter strong enough to read backward through the *tet*^{r} gene casts doubt on the utility of this vector in gene fusion studies. Therefore, pGB218 was cycled through strain MO1000 to obtain K-modified DNA. The 9.74-kbp *Hin*dIII fragment was then subcloned from pGB218 into pMC81, selecting only for ampicillin- and colicin E1-resistant transformants in strain MC1000. A recombinant in which the *rpo* genes are fused in the same orientation as the *lac* genes is illustrated by pGB81218 in Fig. 1*B* (see also Fig. 3). Strain MC1000 transformants of this recombinant plasmid are phenotypically lactose negative on MacConkey agar plates both with and without arabinose. However, because these transformants are resistant to low concentrations (50 μg/ml) of rifampicin, it was tentatively

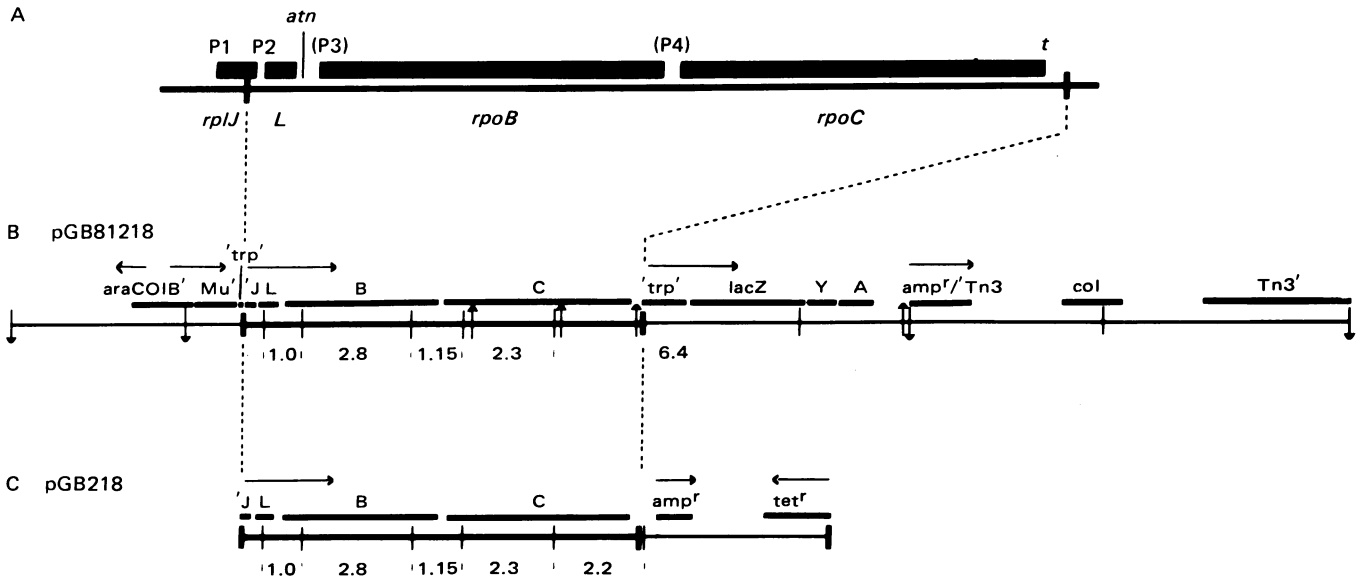

FIG. 1. Gene and restriction enzyme maps of hybrid plasmids containing the *rplJL–rpoBC* region. (*A*) Genetic map. The solid rectangles above the continuous line indicate the extent of the four genes in the operon. The precise distance between *rpoB* and *rpoC* is unknown. P1 is the major promoter for the operon (6, 7) and is not carried on the 9.74-kbp *Hin*dIII fragment examined in this study. Minor promoter P2, attenuator-like element *atn*, and terminator *t* are shown by this work. The presence of promoters P3 and P4 is tentative.

(*B*) Map of pGB81218 including the organization of the pertinent vector genes (M. Casadaban & S. N. Cohen, personal communication). The continuous horizontal line indicates the DNA of the plasmid: the thick part indicates the extent of the inserted *E. coli* sequences; the thin part indicates vector sequences. The discontinuous thick lines indicate the extent of pertinent genes. Horizontal arrows above the genes indicate the direction but not the extent of transcription (1, 15). Restriction endonuclease sites are marked as follows: ▌, *Hin*dIII; |, *Eco*RI; ↑, *Bgl* II; ↓, *Bam*HI. The numbers below the continuous line indicate the size of the *Eco*RI fragments in kbp. *Eco*RI digestion of the plasmid results in seven fragments (Fig. 3). The orientation of the *Hin*dIII fragment within pGB81218 is determined with respect to the *Eco*RI site in the *lacZ* gene of the vector. The distance from the *Hin*dIII site to the *Eco*RI site in *lacZ* is 4.2 kbp. Thus, if the *rplJ* end of the inserted *rplJL–rpoBC* fragment were closest to *lac*, a 4.5-kbp *Eco*RI fragment would be expected. If the *rpoC* end of the fragment were closest to *lac*, then a 6.4-kbp *Eco*RI fragment would be expected. *Eco*RI digests contain the 6.4-kbp fragment (Fig. 3, lane 6, third band from top) and thus the *ara–trp–lac* sequence of the vector and the *rpl–rpo* sequence of the insertion have the same orientation.

(*C*) Map of pGB218. The 9.74-kbp *Hin*dIII fragment from λ*drif*^{d}*18* is inserted into the *Hin*dIII site of pBR322 (16). The convention for marking restriction sites is the same as in *B* but only *Eco*RI and *Hin*dIII sites are shown. The orientation of the *rpl–rpo* operon in this plasmid (and in nine other identical plasmids tested) is opposite to that of the *tet*^{r} gene of the vector. This is concluded on the basis of *Eco*RI digests (data not shown) which give rise to a 2.2-kbp fragment and an approximately 4.5-kbp vector-containing fragment. The reverse orientation would give rise to *Eco*RI fragments of 0.3 kbp and 6.5 kbp. The sizes of the other four *Eco*RI fragments remain the same in either orientation.

concluded that the 9.74-kbp fragment contains a minor promoter for the *rpoB* gene but that a terminator at the end of the *rpoBC* region prevents readthrough into the *lacZ* gene.

***In Vitro* Deletions of the *rplJL-rpoBC-lacZ* Fusions.** The 9.74-kbp *Hin*dIII fragment can be divided into nine segments by *Eco*RI and *Bgl* II digestion. There are five *Eco*RI sites distributed throughout the fragment (15) and three *Bgl* II sites within and beyond *rpoC* (28). Control features in this region can be localized by successive *in vitro* deletion of these *Eco*RI and *Bgl* II fragments from pGB81218. Two *Bgl* II deletion plasmids were isolated, represented by pGBΔB1 and pGBΔB2 in Fig. 2. Various *Eco*RI deletion plasmids were subsequently isolated from pGBΔB2. A second set of deletion plasmids was constructed by reversing the order of partial digestion. One *Eco*RI deletion plasmid, pGBΔR1, was chosen, and the remaining *Bgl* II fragment was removed to produce plasmid pGBΔRB1. Fig. 2 displays these various *Eco*RI and *Bgl* II deletion plasmids. Induced and noninduced rates of β-galactosidase synthesis for transformants containing these plasmids were determined.

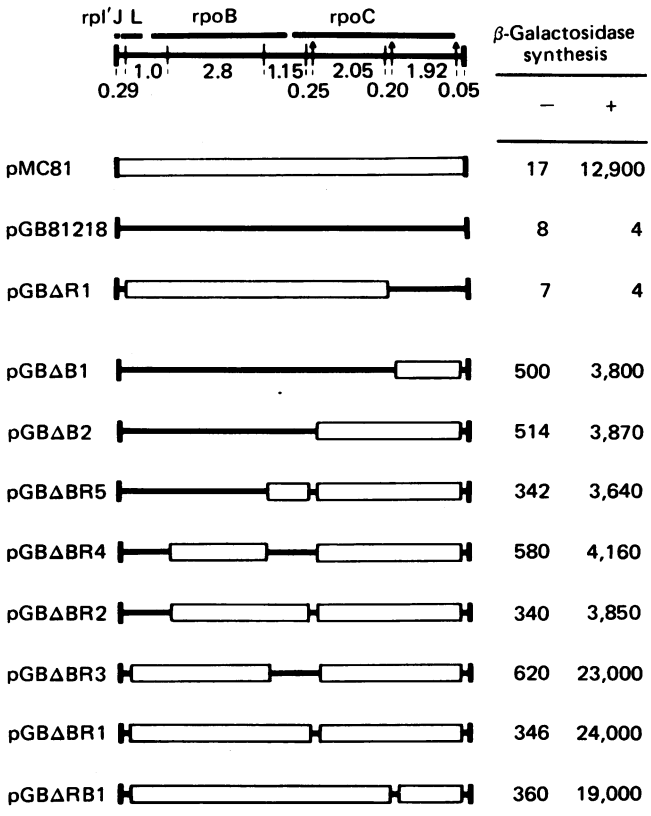

FIG. 2. Structure and β-galactosidase activity of *Eco*RI and *Bgl* II deletion plasmids derived from pGB81218. Plasmids pMC81 and pGB81218 are included as controls. The top of the figure shows the genetic map of the 9.74-kbp *Hin*dIII fragment from λ*drif*^{d}*18*. The convention for marking restriction sites is the same as that used in Fig. 1*B*. The numbers indicate the approximate size, in kbp, of the nine fragments generated by cleavage with *Hin*dIII, *Eco*RI, and *Bgl* II (15, 28). We have confirmed the sizes and relative positions of these sites (data not shown). The various *Eco*RI and *Bgl* II deletion plasmids are shown below the controls. Multiple independent isolates of each plasmid type were obtained. Segments of the operon removed by *in vitro* deletion are represented by open bars. The designations ΔBR and ΔRB refer to the order in which the fragments were deleted—e.g., *Bgl* II first or *Eco*RI first. The differential rate of β-galactosidase synthesis is shown for each plasmid both with (+) and without (−) arabinose. Each rate was calculated from five or more samples taken during exponential growth. Values shown are the averages of two or more independent experiments. Replicate rate determinations did not vary by more than 10%.

Comparison of the β-galactosidase activity of pGB81218 and pGBΔR1 with that of pGBΔB1 places the terminator for the *rplJL-rpoBC* operon in the 1.92-kbp *Bgl* II fragment. When this fragment was removed (e.g., pGBΔB1) the rate of β-galactosidase synthesis without arabinose was 30 times that of pMC81 but, equally surprising, with arabinose the rate was significantly lower than that for pMC81. The regions responsible for these effects on β-galactosidase production can be further localized by examination of the various *Eco*RI and *Bgl* II deletion plasmids shown in Fig. 2.

The rates of β-galactosidase synthesis with arabinose permit the deletion plasmids to be divided into two groups. The first group, represented by pGBΔBR2, pGBΔBR4, and pGBΔBR5, shows induced rates similar to those of pGBΔB1 and pGBΔB2. The second group, composed of pGBΔBR1, pGBΔBR3, and pGBΔRB1, shows induced rates 5–6 times higher than the first group. Comparison of these groups indicates that the decreased rate correlates with the presence of the 1.0-kbp *Eco*RI fragment. This allows the assignment of the element responsible for this decreased rate to the DNA segment that spans the *rplL-rpoB* intercistronic region.

Rates of β-galactosidase synthesis without arabinose were increased for most of the deletion plasmids relative to the rate obtained for pMC81. This increase in rate, although low in comparison to the induced rate for pMC81, was consistently 20 or 40 times the noninduced rate for pMC81 depending on the particular deletion plasmid. Two regions contributed to this increased expression. Comparison of the rates without arabinose for pGBΔBR1 and pGBΔRB1 with that of pMC81 rules out the possibility that any of the internal *Eco*RI or *Bgl* II fragments is solely responsible for this effect. This leaves the 0.29-kbp *Hin*dIII/*Eco*RI fragment at the left and the 0.05-kbp *Bgl* II/*Hin*dIII fragment at the right. Because the 0.05-kbp fragment shows no increase over pMC81 in plasmid pGB81218 or pGBΔR1, it is concluded that the 0.29-kbp fragment must be responsible for the increase in rate of synthesis. This fragment spans the *rplJ-rplL* intercistronic region. The second region that appears to contribute to the low-level increase in the absence of arabinose is the 1.15-kbp *Eco*RI fragment (e.g., compare pGBΔBR1 with pGBΔBR3).

Plasmid Copy-Number Is Not Involved. An alternative explanation for some of the different rates of β-galactosidase expression between plasmids is that mutations have been selected that affect the number of plasmid copies per cell. The frequency of occurrence of copy-number mutations would be expected to be much less than the frequency with which the deletion plasmids were isolated (>1% of total transformants). Direct evidence that copy number does not form the basis for differences in synthesis rates was obtained by standardized procedures used in screening transformants grown on preconditioned Luria broth. The purified DNA, displayed on an agarose gel (Fig. 3), showed no major differences in plasmid yield.

DISCUSSION

Selected segments of the *rplJL-rpoBC* operon have been fused to the *lacZ* gene in the plasmid pMC81. The DNA segment that spans the *rplL-rpoB* intercistronic region decreases β-galactosidase synthesis by a factor of 5–6, and synthesis is also blocked by DNA from the distal end of the *rpoC* gene. In addition, two fragments that span the *rplJ-rplL* and *rpoB-rpoC* intercistronic regions increase β-galactosidase expression. We conclude, on the basis of our analysis of these results, that the indicated regions contain an attenuator-like control element, a terminator, and at least one minor promoter (Fig. 1*A*).

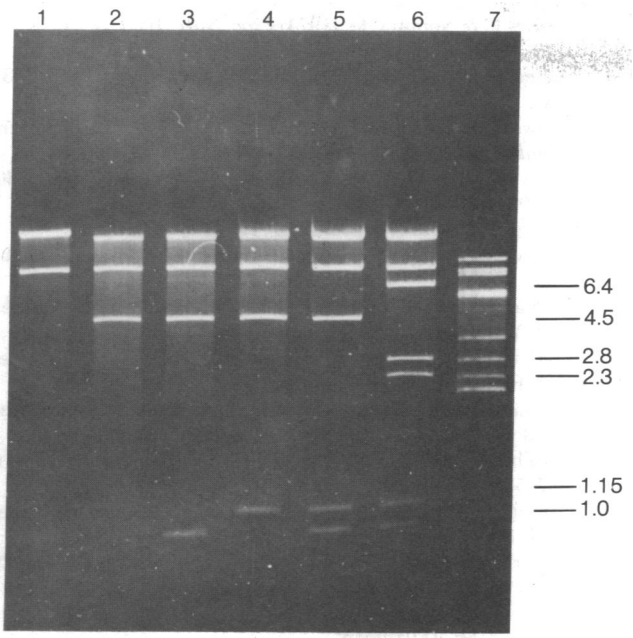

FIG. 3. Analysis of plasmid DNA yield by agarose gel electrophoresis. For lanes 1–6, the DNA was purified from cultures (3 ml) and resuspended in 250 μl. An 8-μl aliquot from each plasmid DNA extract was digested and run on a 0.8% agarose gel; all samples were digested with *Eco*RI. Lanes: 1, pMC81; 2, pGBΔBR1; 3, pGBΔBR2; 4, pGBΔBR3; 5, pGBΔBR4; 6, pGB81218; 7, λ*drif*^{d}*18*. The sizes of the pertinent *rpl* and *rpo* bands in λ*drif*^{d}*18* are indicated (kbp). All λ*drif*^{d}*18 Eco*RI bands that are contained entirely within the 9.74-kbp *Hin*dIII fragment cloned in pGB81218 can be seen in lane 6 (see Fig. 1). Comparison of lane 1 with lanes 2–6 indicates that all of the pGB plasmids contain the second largest *Eco*RI fragment unchanged. This is the fragment spanning the *lacY–Tn3–ColE1* sequences of pMC81. The largest *Eco*RI fragment in lanes 2–6 contains the 0.29-kbp *Hin*dIII/*Eco*RI fragment which spans the *rplJ–rplL* intercistronic region (data not shown). Deletion of the two *Bgl* II fragments from the *rpoC* region of pGB81218 results in the loss of the 6.4-kbp and 2.3-kbp *Eco*RI fragments (see Figs. 1 and 2) and the appearance of a 4.5-kbp *rpo–trp–lac* fragment (lanes 2–5).

β-Galactosidase expression in the fusion strains examined in this study reflect the effects of promoters, attenuators, and terminators. In addition, polarity could result from some of the fusions as a consequence of reading-frame shifts. The nonsense codon could arise within the insert itself or in the following fused *trpB* gene which is known to be very polar (29). The *Hin*dIII site in *trpB* occurs within the first 200 bp of the gene. DNA inserted into this site could be extremely polar. The magnitude of the polarity effect at this site was determined by inserting a 567-bp 16S ribosomal DNA fragment that contained nonsense codons in every reading frame. This resulted in a 90% decrease in induced β-galactosidase synthesis (data not shown). Critical examination of the data presented in Fig. 2 indicates that, for all of the fusion plasmids that lack the 1.92-kbp *Bgl* II fragment, the distal *trpB* sequences must be translated in the correct phase (see below). The most plausible explanation of this observation is that the 0.05-kbp *Bgl* II/*Hin*dIII fragment specifies a ribosome loading site that realigns the reading frame within the *trpB* sequence.

Comparison of enzyme levels resulting from plasmids plus arabinose (compare pGB81218 with pGBΔB1 and pGBΔR1 with pGBΔRB1) indicates that the 1.92-kbp *Bgl* II fragment decreases expression by 99.89–99.98%. This is a greater decrement than that observed for the most polar *trpB* mutation known (29). Such large effects are consistent with termination of transcription but are excessive when compared with known polar effects.

The other region that exerts a substantial effect on the expression of β-galactosidase is the 1.0-kbp *Eco*RI fragment that spans the *rplL–rpoB* intercistronic region. The exceptionally high levels of arabinose-induced β-galactosidase expression observed for deletion plasmids pGBΔBR1 and pGBΔBR3 indicate that ribosomes can be loaded on the mRNAs at the start of either the *rplL* gene or the *rpoC* gene and that no nonsense is encountered in the translation of these messages either before or within the *trpB* gene. Addition of the 1.0-kpb *Eco*RI fragment (compare pGBΔBR2 with pGBΔBR1) reduces the expression of induced β-galactosidase by 83.9%. If pGBΔBR2 were the only example available it would not be possible to rule out polarity as the cause of the intermediate level of synthesis conferred by this fragment. Comparison of pGBΔBR4 with pGBΔBR3, however, suggests that, if the 1.0-kbp *Eco*RI fragment does exert a frame shift polar effect, it would have to be localized to the distal portion of the *rpoB* gene, before the ribosome loading site for *rpoC*. This area, including the *rpoC* gene start, is carried on the 1.15-kbp *Eco*RI fragment. The same intermediate levels of enzyme synthesis are obtained in the presence of arabinose whether this 1.15-kbp fragment is: (*i*) connected directly to the 1.0-kbp *Eco*RI fragment by deletion of the 2.8-kbp *Eco*RI fragment (pGBΔBR4); (*ii*) totally deleted (pGBΔBR5); or (*iii*) read in phase with the *rpoB* gene start (pGBΔB2 and pGBΔBR5). Thus, we conclude that the 1.0-kbp *Eco*RI fragment exerts its major effect not by generating polarity but rather by carrying a sequence that reduces the level of expression by a factor of 5–6. The observed decrease in β-galactosidase production associated with this DNA segment is consistent with the observation that the β and β′ subunits of RNA polymerase are normally present in only one-fifth the molar yield of the ribosomal proteins (15). In addition, the *rplJL* genes are transcribed 5 times more frequently than the adjacent *rpo* genes (12, 13). It has been proposed that this region contains an attenuator (6, 7, 15), and the magnitude of the decreased expression observed here is consistent with the differences in the *in vivo* mRNA levels from *rpl* and *rpo* genes. Post *et al.* (30) have noted that there is a region containing several overlapping dyad symmetries following the gene for *rplL*. One feature that could have significance in the RNA is a very stable (−24.4 kcal) hairpin structure comprised of 11 bp followed by a U-U-U-U sequence [positions 2690–2715 (30)]. The similarity of this structure to that of known terminators (31) and attenuators (32) is noteworthy. However, unlike most attenuators and terminators there appears to be no significant difference in G + C content before and after the hairpin structure.

It is evident from the results for β-galactosidase expression without arabinose (Fig. 2) that the DNA fragment examined in these experiments contains only minor promoter activity. This observation is consistent with the work of Nomura and coworkers (6, 30) and Linn and Scaife (7) who place the major promoter for the *rplJL–rpoBC* operon before the gene for protein L10 (*rplJ*). Weak promoter activity (P2, Fig. 1A) may be assigned to the 0.29-kbp *Hin*dIII/*Eco*RI fragment that spans the *rplJ–rplL* intercistronic region. Jackson and Yanofsky (33) have shown that the weak promoter within the *trp* operon, which initiates repressed level transcription of the last three genes, is actually located at the distal end of the second gene rather than between the second and third genes. The DNA sequence (30) for the end of *rplJ* and the intercistronic region does not contain elements that are readily associated with promoter activity. However, the sequence T-A-T-T-C-T-G, which differs from the sequence of Pribnow (34) by two bases, is present at position 2259, 19 nucleotides before the start of the *rplL* gene. The relatively high percentage of A + T (72%) in this intercistronic region may be related to the weak promoter

activity observed. It has been noted (35) that protein L7/12 is present in four copies per ribosome whereas the remaining ribosomal proteins are present in only one copy. In addition, L7/12 is synthesized at a rate 2–5 times that of L10 both *in vivo* (36) and *in vitro* (37). A second promoter (P2) could explain these observations. However, the weak activity observed for P2 is insufficient to account for the magnitude of the effect observed. The increased expression attributed to P2 apparently is not affected by the attenuator on the 1.0-kbp *Eco*RI fragment (see below). Post *et al.* (30) suggested that a translational control mechanism might be responsible for the high levels of L7/12 produced.

It has been suggested that the *rpoBC* genes may have a promoter distinct from that for the *rplJL* genes (7, 9, 11, 13, 26). Comparison of the noninduced rates for pGBΔBR4 with those for pGBΔBR3 and for pGBΔBR2 with those for pGBΔBR1 shows no apparent effect for the *rplL–rpoB* intercistronic region. However, the induced rates indicate that this region contains an attenuator. This raises the possibility that the transcription complex initiated in the *rplJ–rplL* intercistronic region (promoter P2) is not affected by this attenuator. Alternatively, the *rplL–rpoB* intercistronic region may also contain a promoter (P3) similar in strength to the one in the *rplJ–rplL* intercistronic region. There is, in fact, a T-A-T-A-C-T-G sequence starting at position 2800 (30) which differs by only one base from the Pribnow sequence (34) and lies within a region of extensive dyad symmetry. The 1.15-kbp *Eco*RI fragment that spans the *rpoB–rpoC* intercistronic region increases the rate of β-galactosidase synthesis by approximately 2-fold. Previous work has shown that *rpoB* and *rpoC* are cotranscribed (38). The effect observed here can be interpreted as a weak promoter (P4 in Fig. 1*A*) or it may be related to the apparent greater stability of *rpoC* message over that of *rpoB* (12).

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