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Relative Activities of the Transcriptional Regulatory Sites in the *rplKAJLrpoBC* Gene Cluster of *Escherichia coli*

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The pattern of transcription of the *rplKAJLrpoBC* gene cluster of *Escherichia coli* appears to be complex. At least four different promoters and a transcriptional attenuator have been identified. To compare the relative effect of each of the putative promoters and the attenuator on transcription of these genes, we fused these regulatory sites to *lacZ*. These transcriptional fusions were constructed on lambda transducing phages so a single copy of each could be stably integrated into the chromosome. The level of β-galactosidase in a lysogen of each phage reflects the activity of the transcriptional regulatory site. We find that the promoters preceding *rplK* (*rplKp*) and *rplJ* (*rplJp*) are indeed the major promoters of this gene cluster. The minor promoter before *rplL* (*rplLp*) is much weaker and contributes little to the transcription of the downstream genes. Under these conditions, we find no evidence of a promoter (*rpoBp*) in the *rplL-rpoB* intercistronic region. The attenuator (*atn*) terminates ca. 70% of the transcripts initiated at the promoters preceding it. Although we cannot rule out that some transcripts from *rplKp* may read through into *rplJLrpoBC*, we find that *rplJp* alone is sufficient for high-level expression of these genes.

The genes for the subunits of *Escherichia coli* RNA polymerase are cotranscribed with ribosomal protein genes. The genes for β and β′ (*rpoB* and *rpoC*) are cotranscribed with at least the L10 and L7/12 ribosomal protein genes (*rplL* and *rplJ*, respectively) (26, 33, 42), whereas the α gene (*rpoA*) is part of an operon containing four ribosomal protein genes (23). More recently, it has been shown that the gene for σ (*rpoD*) is cotranscribed with the S21 gene (*rpsU*) and the gene for DNA primase (*dnaG*) (10).

The transcription pattern of the *rplKAJLrpoBC* gene cluster located at 90 min on the *E. coli* chromosome is complicated. Initial experiments demonstrated that the genes for the β and β′ subunits of RNA polymerase are cotranscribed with at least the *rplJ* and *rplL* ribosomal protein genes from a promoter upstream of *rplJ* (*rplJp*) (26, 33, 42). A variety of experiments subsequently suggested that there are also two internal promoters within *rplJLrpoBC*, one preceding *rplL* (*rplLp*) and the other before *rpoB* (*rpoBp*) (20, 25, 26, 28, 33). In addition, a transcriptional attenuator (*atn*) was located between *rplL* and *rpoB* and accounts for the lower frequency of *rpoBC* transcription compared with that of *rplJL* (5, 6, 14, 26). A promoter responsible for the cotranscription of *rplK* and *rplA* was identified upstream of *rplK* (*rplKp*) (2, 15, 26, 43). Recent S1 nuclease mapping of in vivo transcripts suggests that transcription initiated at *rplKp* is not necessarily terminated after *rplA*, but can read through into the *rplJL* genes (9).

To further understand the regulation of this complex operon, we have directly compared the relative effect of these different promoters and the attenuator on *rplKAJLrpoBC* transcription. These transcriptional regulatory sites were fused, both separately and in combination, to the *lacZ* gene carried on a lambda transducing phage. The synthesis of β-galactosidase in lysogens of these transducing phages reflects the level of transcription from the attached sites.

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We find *rplKp* and *rplJp* to be the major promoters in the *rplKAJLrpoBC* gene cluster. When these two promoters are cloned separately, *rplKp* initiates transcription ca. 35% more frequently than *rplJp*. The minor promoter *rplLp* is much weaker than *rplJp*, contributing little to the expression of *rpoBC*. We find no evidence for the putative *rpoBp* under these growth conditions. The attenuator terminates ca. 70% of the transcripts initiated at all promoters preceding it. Finally, *rplJp*, without *rplKp* upstream, is sufficient for high-level expression of *rplJLrpoBC*.

MATERIALS AND METHODS

Bacterial strains and lambda and plasmid vectors. The *E. coli* strains used in this study are described in Table 1. All of the recombinant phages described in this paper utilize the left arm of the *Hin*dIII vector λJDW36, a derivative of λJDW19 (41) (Fig. 1). The right arms of the recombinant phages are supplied by λNM515 (40), λNM540 (7), or λKV1 (this study). Lysogens are of the host strain GR50-7 (Table 1), a *recA56* derivative of CSH50. All plasmid subcloning was done in pBR325 (Fig. 2).

Cloning. Cloned fragments originated from λd*rif*^{d}18 (24). The *rpoB* gene carried on this phage contains a single point mutation that confers a dominant rifampin resistance phenotype (36). In the in vitro constructions described, restriction fragments and lambda vector arms were separated by electrophoresis on agarose gels and recovered by electroelution in dialysis tubing (38) or by phenol extraction from low-melting temperature agarose (29), both of which were followed by ethanol precipitation. Recovered fragments were ligated in vitro with T4 DNA ligase (29) into suitably restricted pBR325 or between lambda vector arms prepared in the same fashion. Transformation into HB101 and transfection into TGL72 was accomplished by a $CaCl_2$ cell shock procedure (13). Some ligations were packaged in vitro (18) and then plaqued on C600. Transformed colonies were screened by restriction analysis by the rapid boiling method (19). Plaques were picked, grown as lysates on C600, and screened by restriction analysis, using a rapid DNA isolation

protocol (13). To confirm the fragment orientations and overall structure of the recombinant DNA molecules, large-scale preparations of plasmid or phage constructions were made, and an extensive restriction analysis was performed.

Lysogens. Once the structure of a recombinant phage was confirmed, a high-titer lysate was grown, and a dilution of this was used to infect GR50-7 at a multiplicity of infection of 0.05. The infected culture was plated on MacConkey or Luria plates containing 5-bromo-4-chloro-3-indole-β-D-galactoside (X-gal) (30). Lysogens expressing large amounts of β-galactosidase turn red on MacConkey plates after 24 h at 37°C. Lysogens expressing little β-galactosidase require 48 h to develop any blue color on X-gal plates and remain white on MacConkey plates.

β-galactosidase assay. Lysogens were grown at 37°C in AB medium (11) supplemented with B1 (2 μg/ml), glucose (0.4%), and proline (40 μg/ml). β-galactosidase was assayed at Klett values of 7 and 15 (optical density values at 600 nm of 0.1 and 0.3) according to Miller (30).

Recombinant phage: details of constructions (Fig. 3). λGR1 (*rplJp*). A 1,874-base-pair (bp) fragment obtained by restriction of λdri*f*^{d}18 with *Hin*dIII and *Eco*RI and extending from within *rplK* to within *rplJ* was purified and ligated as described above between the *Hin*dIII left arm of λJDW36 and the *Eco*RI right arm of λNM616 (Fig. 3a).

λGR2 (*atn* + *rpoBp*). A 9.6-kilobase-pair (kbp) fragment produced by *Hin*dIII digestion of λdri*f*^{d}18 was purified and ligated into the *Hin*dIII site of pBR325 to yield a plasmid pTL3 (Fig. 2). A complete *Bgl*II digest of pTL3 was religated to yield pGR1 (Fig. 2), which lacks both *rpoC Bgl*II fragments.

A partial *Eco*RI digest of pGR1 was separated on a gel, and the 7.0-kbp fragment was purified and religated to yield a plasmid pGR4 (Fig. 2). This plasmid has lost the two *Eco*RI fragments of *rpoB* but retains the 1,083-bp fragment extending from within *rplL* to within *rpoB* and carries the attenuator (5) and putative promoter *rpoBp* (26, 33). The only sequences remaining distal to the 1,083-bp fragment are a 218-bp *Eco*RI-*Bgl*II fragment from *rpoC* and an 83-bp *Bgl*II-*Hin*dIII fragment downstream of *rpoC* (32, 35). This 301-bp adapter is used in many of the constructions to be described and will be referred to as the 301-bp *Eco*RI-*Hin*dIII adapter (Fig. 2).

A complete *Hin*dIII with a partial *Eco*RI digest of pGR4 yields a 1,384-bp fragment which extends from the *Eco*RI site in *rplL* to the *Hin*dIII site of the 301-bp *Eco*RI-*Hin*dIII adapter. This 1,384-bp fragment was purified and ligated between the *Hin*dIII left arm of λJDW36 and the *Eco*RI right arm of λNM616 (Fig. 3c).

λGR3 (*rplJp* + *rplLp* + *atn* + *rpoBp*). This construction was performed in two steps. First, a 1,674-bp *Hin*dIII fragment extending from within *rplJ* and the *Hin*dIII site of the *Eco*RI-*Hin*dIII adapter was purified from a *Hin*dIII digest of pGR4 and ligated between the *Hin*dIII arms of λJDW36 to yield λUT1 (not illustrated). A *Hin*dIII partial digest of this phage yielded the left arm of λJDW36 fused to the 1,674-bp fragment. This was cut from a gel, purified, and ligated in the presence of the 1,874-bp *Eco*RI-*Hin*dIII fragment of λGR1 and the *Eco*RI right arm of λNM616 to yield λGR3 (Fig. 3c).

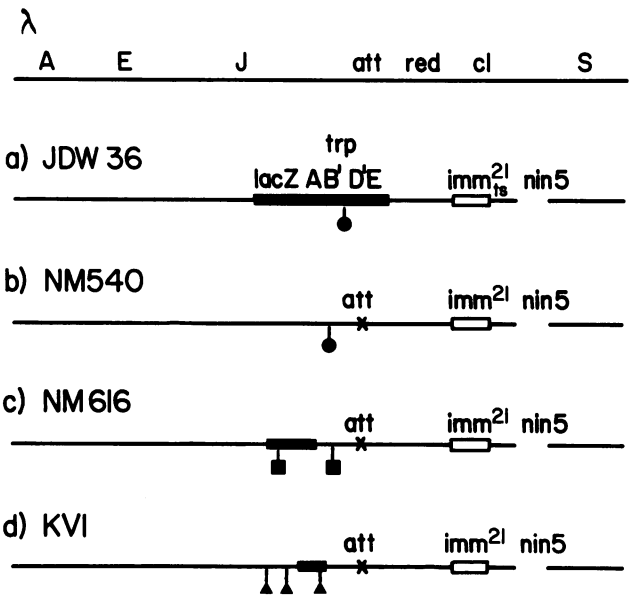

FIG. 1. Lambda vectors used in the construction of *rplrpo-lacZ* fusions. All fusions made use of the left arm of λJDW36 produced by *Hin*dIII digestion. This supplies the essential lambda genes located on the left arm and the W205 *trp-lac* deletion severed at the *Hin*dIII site in *trpB*. The right arm of the lambda genome, including the phage attachment site, was provided by either *Hin*dIII digestion of λ540, *Eco*RI digestion of λ616, or *Kpn*I digestion of λKV1. Heavy lines indicate bacterial DNA substitutions, open boxes indicate a substitution of the immunity region from phage 21, and spaces indicate deletions. Symbols for restriction endonuclease cleavage sites: ●, *Hin*dIII; ■, *Eco*RI; ▲, *Kpn*I.

λGR4 (*rplLp*). A partial *Eco*RI digest of pGR1 was separated on an agarose gel, and the 6.0-kb fragment excised and religated. This plasmid, pGR3, has lost all of the *rplLrpoBC Eco*RI fragments and carries only the *Hin*dIII-*Eco*RI fragment of *rplJ* to *rplL* fused to the *Eco*RI-*Hin*dIII adapter fragment. A complete *Hin*dIII digest of pGR3 yields a 591-bp fragment which was purified and ligated between the *Hin*dIII left arm of λJDW36 and the *Hin*dIII right arm of λNM540 to yield λGR4 (Fig. 3b).

λGR6 (λp*lac5* cI857 × λ*imm*^{21}). A genetic cross of these two phages yielded a phage that would form stable lysogens at 37°C and express β-galactosidase from the p*lac5* promoter.

λGR7 (*rplKp* + *rplJp*). A 2.8-kb fragment derived from a *Kpn*I-*Hin*dIII double digest of λdri*f*^{d}18 extends from a *Kpn*I site 600 bp before *rplK* to the *Hin*dIII site within *rplJ* and carries both *rplKp* and *rplJp* (2, 15, 26, 43). This fragment was cloned between the *Hin*dIII left arm of λJDW36 and the *Kpn*I right arm of λKV1 (Fig. 3g).

λKV1 was made to function as a *Kpn*I right arm donor. The 1,083-bp *Eco*RI fragment of pGR4 has a *Kpn*I site 161 bp

TABLE 1. Bacterial strains

Strain	Genotype	Source of reference
CSH50	*ara* Δ(*lac-pro*) *rpsL thi*	30
GR50-7	*ara* Δ(*lac-pro*) *rpsL thi recA56* ΔTn*5*::*srb*	From CSH50, this study by P1 transduction
HB101	*hsm hsr recA gal pro rpsL leu lac*	H. Boyer; 29
C600	*thi thr leu tonZ lacY supE*	3
TGL72	Δ(*gal-bio-uvrB*) *his rpsL trpLD102 lacZMI5*	N. E. Murray, strain AA125

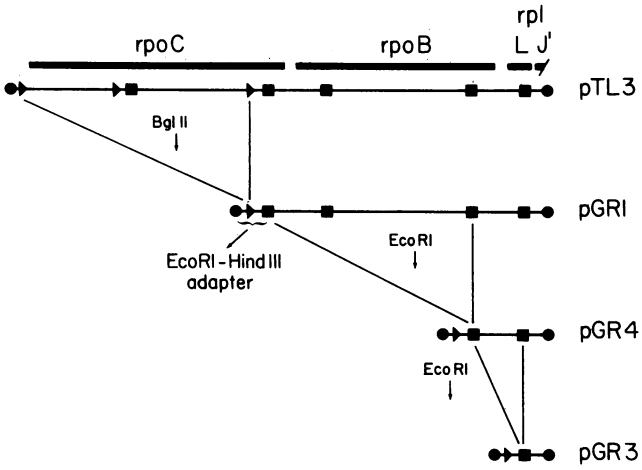

FIG. 2. Deletion derivatives of pTL3 used in the construction of *rplrpo-lacZ* fusions. The 10.1-kbp DNA fragment produced by *Hin*dIII digestion of λ d*rif*^{d}18 was cloned into the *Hin*dIII site of pBR325 to yield pTL3 (the pBR325 sequences are not shown). This DNA contains the 3′ end of *rplJ* and all of *rplL*, *rpoB*, and *rpoC*. pTL3 was digested with *Bgl*II and ligated to produce pGR1. This creates the *Eco*RI-*Hin*dIII adapter used in many of the transducing phage constructions. pGR1 in turn was subjected to limited digestion with *Eco*RI and ligated to yield pGR3 and pGR4. Symbols for restriction endonuclease cleavage sites: ●, *Hin*dIII; ■, *Eco*RI; ▶, *Bgl*II.

from one end. This 1,083-bp fragment was cloned between the right and left arms of λNM616. A *Kpn*I digest of this phage yields a right arm terminated by a *Kpn*I site (Fig. 1).

λGR8 (*rplKp*). A 760-bp *Kpn*I-*Eco*RI fragment from λGR7 extending from 600 bp before *rplK* to the *Eco*RI site within rplK was ligated between an *Eco*RI left arm from λGR15 and a *Kpn*I right arm of λKV1. A partial *Eco*RI digest of λGR15 was done to obtain a left arm consisting of the left arm of λJDW36 carrying the 301-bp *Eco*RI-*Hin*dIII adapter (Fig. 3f).

λGR11 (*rplKp* + *rplJp* + *rplLp* + *atn* + *rpoBp*). A double restriction digest of λd*rif*^{d}18 with *Sal*I and *Kpn*I yields a 3.3-kbp fragment which extends from a *Kpn*I site ca. 600 bp before *rplK* to a *Sal*I site located just before *rpoB*. This fragment was ligated between a *Sal*I left arm from a *Sal*I digest of λGR2 and a *Kpn*I right arm from λKV1. Fusing the *Sal*I sites reconstructs the *rpoB* sequence carried on λGR2 and yields λGR11 (Fig. 3h).

λGR12 (*rplLp* + *atn*). For The construction of λGR3, an intermediate phage λUT1 was used (described earlier). A partial *Hin*dIII digest of λUT1 yields the left arm of λGR12 which carries a fragment extending from the *Hin*dIII site in *rplJ* through the *Eco*RI site in *rpoB* and fused to the left arm of λJDW36 by the 301-bp *Eco*RI-*Hin*dIII adapter. This left arm was ligated to the *Hin*dIII right arm of λNM540 to yield λGR12 (Fig. 3d).

λGR15 (λJDW36-adapter-λNM616). Many of the constructions described utilize a 301-bp *Eco*RI-*Hin*dIII adapter derived from *rpoC* and DNA distal to *rpoC*. This fragment was ligated between vector arms to yield λGR15 (Fig. 3i) which is used here to establish the background level of β-galactosidase expression.

RESULTS

Construction of *rplrpo-lacZ* transcriptional fusions. All fusions were assembled, using the W205 *trp-lac* deletion (4, 31) carried on λJDW36 (41). This phage has a single *Hin*dIII cleavage site within the 5′ region of the structural gene for *trpB* (12) (Fig. 1). The *trp* DNA between this *Hin*dIII site and *lacZ* contains no promoters or terminators, therefore expression of *LacZ* is dependent on the transcriptional signals connected to *trpB* via the *Hin*dIII site. In several of the phage constructions, a *Hin*dIII site was conveniently located downstream of the promoter(s) to be analyzed and could be ligated directly to the *Hin*dIII site in *trpB*. Other promoters were fused, either separately or in combination with the attenuator, to *trpB* by utilizing *Eco*RI sites downstream of these transcriptional sites. A 301-bp *Eco*RI-*Hin*dIII DNA fragment which originates from the *rpoC* region (see above; Fig. 2) and contains no promoter (see λGR15, Fig. 3i) or terminator (5) was used as an adapter. So that stable

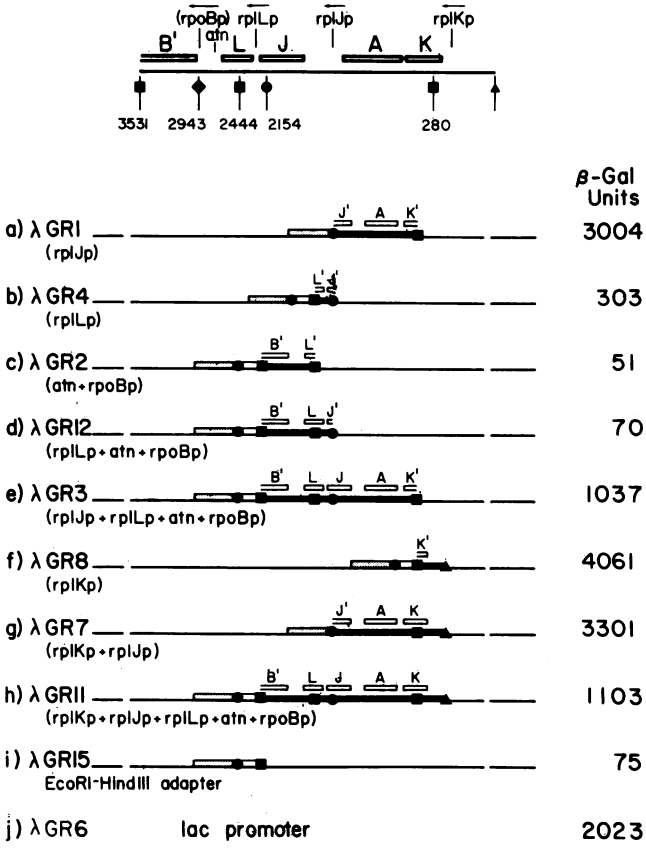

FIG. 3. Structure of the lambda transducing phages carrying *rplrpo-lacZ* fusions and transcriptional activity of *rplKAJLrpoBC* regulatory sites. In the upper panel, the *rplKAJLrpoBC* region is drawn to scale. Positions of the promoters and attenuator are indicated. The restriction sites are numbered according to the sequence determined by Post et al. (37) (nucleotide no. 1 is located 175 bp upstream of the start of *rplK*; the *Kpn*I site is ca. 600 bp upstream of *rplK*). In the lower panel, the transducing phages are diagrammed. The thick lines represent bacterial DNA from the *rplKAJLrpoB* region, the open region represents the *Eco*RI-*Hin*dIII adapter, the stippled region represents the *lacZ* gene, and the thin lines represent phage DNA. The phage arms are not drawn to scale as indicated by the breaks. The bacterial genes carried by each phage are diagrammed above the line drawings. A prime (′) indicates a partial gene sequence severed at a restriction endonuclease cleavage site. The amount of β-galactosidase produced in a lysogen of each transducing phage is shown. Symbols for restriction endonuclease cleavage sites: ●, *Hin*dIII; ■, *Eco*RI; ▲, *Kpn*I; ◆, *Sal*I.

lysogens of each of these fusions could be isolated, the right arm of the lambda genome carrying a functional phage attachment site was supplied. The DNA upstream of the promoters to be analyzed was cleaved with either *Eco*RI, *Hin*dIII, or *Kpn*I, and the right arm of λ616, (40), λ540 (7), or λKV1 (Fig. 1), respectively was ligated to the fragments.

rplJp. Previous work demonstrated that a major promoter for *rplJLrpoBC* transcription (*rplJp*) is found on the DNA fragment extending from the *Eco*RI site in *rplK* to the *Hin*dIII site in *rplJ* (2, 26, 39, 42, 43). To measure the efficiency of *rplJp*, this DNA fragment was ligated to the *Hin*dIII site in *trpB*. An exponentially growing lysogen of the transducing phage carrying this *rplJp-lacZ* fusion yielded 3,004 U of β-galactosidase (λGR1, Fig. 3a). This is a vigorous promoter, as it produces a higher β-galactosidase level than the derepressed *lac* promoter (2,023 U, Fig. 3j).

rplLp. A variety of experiments have indicated that there may be an internal promoter within the *rplJLrpoBC* gene cluster for the independent expression of *rplL* (2, 5, 8, 15, 17, 28). To assess the strength of this promoter, we fused the *rplJ-rplL* intercistronic region extending from the *Hin*dIII site in *rplJ* to the *Eco*RI site in *rplL* to *lacZ*. A lysogen of the transducing phage containing this fusion gave 303 U of β-galactosidase (Fig. 3b). This amount is clearly above the background level of λGR15 (Fig. 3i), but was much lower than the β-galactosidase produced from *rplJp*.

atn + rpoBp. In addition to an attenuator, several experiments have suggested that a weak promoter is also located in the *rplL-rpoB* intercistronic region (2, 5, 20, 28). Thus, to determine whether the *rpoBC* genes are transcribed at a significant level from a promoter in this region, the DNA encompassed by the *Eco*RI sites in *rplL* and *rpoB* was joined to *lacZ* (Fig. 3). A lysogen carrying this fusion synthesized only 51 U of β-galactosidase. This is no higher than that synthesized by the control phage carrying only the *Eco*RI-*Hin*dIII adapter (Fig. 3i).

rplLp + atn + rpoB. The simplest model of attenuator function proposes that it should reduce transcription from all promoters preceding it. However, it has been shown for lambda transcription that appropriate modification of the transcribing complex at *nut* sites on the DNA allows it to ignore normal termination signals. We have shown that *rplLp* is only 8% as strong as *rplJp*. If, however, transcription initiating at *rplJp* was reduced at the attenuator, whereas transcription from *rplLp* was not, then initiation at *rplLp* would lead to a significant fraction of *rpoBC* transcription. To test this directly, the DNA carrying *rplLp* plus the downstream attenuator was joined to *lacZ*. This DNA extending from the *Hin*dIII site in *rplJ* to the *Eco*RI site in *rpoB* also contains the putative *rpoBp*, but as demonstrated by the previous fusion (*atn + rpoBp*), this region does not initiate any transcription under these conditions. *rplLp* plus the attenuator gave only 70 U of β-galactosidase (Fig. 3d), a substantial reduction from *rplLp* alone (303 U).

rplJp + rplLp + atn + rpoBp. The frequency of readthrough of the attenuator from transcription initiated at *rplJp* was measured by fusing the DNA fragment encompassed by the *Eco*RI sites in *rplK* and *rpoB* to *lacZ*. This region also carries *rplLp* and *rpoBp*, but the previous construction demonstrates that they yield no significant transcription into *rpoB*. The fusion carrying all these sites produced 1,037 U of β-galactosidase (Fig. 3e), demonstrating that the attenuator terminates transcription of ca. 70% of the RNA polymerase molecules initiating at *rplJp*.

rplKp. Earlier experiments demonstrated that a promoter preceding *rplK* is used for the cotranscription of *rplK* and *rplA* (2, 15, 26, 27, 43). The efficiency of *rplKp* was measured by ligating the DNA extending from the *KpnI* site preceding the gene to the *Eco*RI site within *rplK*. *rplKp* was found to be a strong promoter yielding 4,061 U of β-galactosidase.

rplKp + rplJp. Recent S1 nuclease mapping experiments have suggested that transcription initiating at *rplKp* is not terminated past *rplA*, but continues into *rplJLrpoBC* (9). The fusions described above demonstrate that *rplKp* is ca. 35% stronger than *rplJp*. To determine whether transcription of *rplJ* was higher when placed downstream of both *rplKp* and *rplJp*, rather than only *rplJp*, the DNA encompassed by the *Kpn*I site preceding *rplK* and the *Hin*dIII site in *rplJ* was attached to *lacZ*. These two promoters, assembled in their usual orientation, resulted in the synthesis of 3,301 U of β-galactosidase. This is only 10% higher than with *rplJp* alone.

rplKp + rplJp + rplLp + atn + rpoBp. Although the transcription of *rplJL* is only slightly greater when it is preceded by both *rplKp* and *rplJp*, it is not clear what fraction of transcription results from each promoter. If a significant amount of this transcription is initiated at *rplKp* and if these transcribing RNA polymerase molecules terminated more or less frequently at the attenuator than molecules initiated at *rplJ*, they would lead to an altered expression of *rpoBC*. We tested this directly by measuring transcription from the DNA containing all these transcriptional sites. The DNA fused to *lacZ* extended from the *Kpn*I site preceding *rplK* to the *Eco*RI site in *rpoB*. This DNA also carries *rplLp* and *rpoBp*, but recall that they initiate no significant β-galactosidase synthesis (Fig. 3d). This construction produced 1,103 U of β-galactosidase (Fig. 3h), which is only slightly higher than *rplJp* alone followed by the attenuator (Fig. 3e).

DISCUSSION

This collection of gene fusions has allowed us to determine the effect of the regulatory sites within the *rplKAJLrpoBC* gene cluster on the level of transcription of these ribosomal protein and RNA polymerase genes. Although such gene fusions could have been more easily constructed on plasmid vectors, they were assembled on lambda transducing phages so that a single copy of each gene fusion could be stably integrated into the bacterial chromosome. This circumvents any problem with differences of plasmid copy number between the individual fusions, which would alter the amount of β-galactosidase and be misinterpreted as reflecting promoter or attenuator efficiency or both.

A second reason for constructing these specific fusions on lambda stems from the nature of regulation of the *rplKAJLrpoBC* gene cluster. Biochemical and genetic evidence has shown that synthesis of each of these gene products is under autogenous regulation (for a review, see reference 25). The expression of *rplK* and *rplA* is regulated by the concentration of free L1 in the cell, whereas the level of expression of *rplJ* and *rplL* is controlled by the amount of free L10-L7/12 complex. The synthesis of the β and β′ subunits of RNA polymerase appears to be negatively regulated by the amount of RNA polymerase holoenzyme. In each case, this feedback repression appears to be exerted primarily at the translational level. Thus, when these genes are cloned on multicopy plasmids, the synthesis of the proteins increases only slightly compared with the haploid parent. The level of mRNA synthesis, however, increases approximately with the gene dosage, although at high copy numbers less is synthesized than expected. Hence, when these genes are carried on multicopy plasmids, their normal expression is altered.

Those fusions we have constructed which carry the complete structural genes for *rplA* and for *rplJ* and *rplL* attached to their promoters would be expected to increase feedback repression of translation of the ribosomal protein mRNA. However, because these fusions were assembled with the W205 *trp-lac* deletion, this translational feedback repression should have little effect on β-galactosidase synthesis. This deletion removes the normal promoter for *lacZ*, but leaves the AUG start codon and ribosomal binding site intact (4, 31). Therefore transcription of *lacZ* is dependent on a promoter attached upstream of the *Hin*dIII site in *trpB*, but β-galactsidase is independently translated from the *lacZ* mRNA. The addition of a second copy of the ribosomal protein genes carried on the fusions would be expected to reduce the frequency of translation of the ribosomal sequences on the hybrid mRNA. It is uncertain whether this decrease would cause a reduction of transcription into *lacZ* as a result of polarity. The observation that at low copy numbers the level of ribosomal protein mRNA synthesis increases in approximate proportion to gene dosage would argue against this (25). Ideally, one would like to fuse *lacZ* directly to these genes on the bacterial chromosome as has been done in the study of other operons. However, because these genes are essential for growth, such fusions would be lethal. Therefore, by adding a single extra copy of the *rplKAJLrpoBC* transcriptional sites (and in some cases the structural genes) to the cell on a transducing phage, we are causing the minimal possible perturbation of the normal transcription of these genes.

Polarity is a potential problem with any gene fusion. If the DNA downstream of the fusion joint is in a different reading frame than the attached gene, the occurrence of normally out-of-frame termination codons could lead to premature termination of transcription. Therefore the level of β-galactosidase would not accurately reflect the frequency of transcription of the attached genes. This is a serious problem with the *trp-lac* deletion, for the *Hin*dIII site occurs near the beginning of the *trpB* gene, and nonsense mutations in *trpB* are known to be polar (44). In these experiments, we addressed this problem by constructing all the fusions such that the *trpB* gene is in the same reading frame as the upstream gene. The DNA sequences for the *rplKAJLrpoBC* region (32, 34, 35) and *trpB* (12) are known, therefore we can deduce the reading frame of the downstream *trpB* in these fusions. Those constructions which make use of the *Hin*dIII site in *rplJ* join this gene to *trpB* in the same reading frame. The remaining fusions utilize an *Eco*RI site in the gene of interest. These sites were ligated to the *Hin*dIII site in *trpB* via an *Eco*RI-*Hin*dIII adapter. Although three different *Eco*RI sites were used, they all sever the respective genes in the same reading frame, which is different than that of *trpB*. The *Eco*RI-*Hin*dIII fragment not only functions as a restriction site adapter but also realigns the reading frame correctly into *trpB*. Therefore the level of β-galactosidase produced by any of this collection of transcriptional fusions will not be reduced by polar effects resulting from a translational frameshift.

The *rplKAJLrpoBC* gene cluster appears to have an elaborate pattern of transcription. Early experiments demonstrated that the *rplJ*, *rplL*, *rpoB*, and *rpoC* genes were cotranscribed from a major promoter upstream of *rplJ* (26, 42). This conclusion was based on the finding that the *rpoBC* genes were expressed fully in the UV-irradiated cell system from a lambda transducing phage only when the DNA fragment also carried the preceding *rplJ* and *rplL* genes. In vitro evidence suggests that this promoter is located ca. 380 nucleotides upstream of *rplJ* (37, 39). However, when segments of the operon were analyzed either by cloning them upstream of *lacZ* on a plasmid (2, 5, 21) or transducing phage (20) or directly by cloning onto a lambda transducing phage and examining the synthesis of the gene products in the UV-irradiated cell system (28), it was suggested that there were internal promoters preceding *rplL* and *rpoB*. Most of these experiments indicated that these promoters were relatively weak. Yet other experiments suggested that the *rplLrpoBC* genes could be transcribed at a high level separately from *rplJ*. Such experiments analyzed either the proteins synthesized in vitro by different subsets of this DNA (19), or the in vivo expression of the gene products from these DNA segments cloned onto plasmids (15). These different techniques have not lead to a coherent understanding of the scheme of *rplJLrpoBC* transcription.

The collection of gene fusions constructed for this study has allowed us to assess the relative effect each of these proposed sites has on *rplKAJL* and, in particular, on *rpoBC* transcription. They allow a direct comparison of the efficiency of the promoters and attenuator in an in vivo steady-state system that causes minimal alteration of the normal regulation of these genes.

We find that *rplJp* is an efficient promoter. Under these growth conditions, it directs a higher level of transcription (3,004 U of β-galactosidase) than does the derepressed *lacZ* promoter (2,023 U). Under the same conditions, we find no evidence of a promoter in the *rplL-rpoB* intercistronic region (*rpoBp*). If such a promoter exists, it is extremely inefficient under these conditions and yields no higher β-galactosidase synthesis than the control phage carrying only the *Eco*RI-*Hin*dIII adapter fragment. The *rplJ-rplL* intercistronic region does contain a weak promoter (*rplLp*) that is only 8% as effective as *rplJp*. Transcription from this promoter cannot account for the fourfold higher rate of synthesis of L7/12 compared with all other ribosomal proteins, unless translation is much more efficient from this transcript.

Even though the *rplJLrpoBC* genes are cotranscribed from a promoter upstream of *rplJ*, RNA-DNA hybridization has shown that the *rplJL* genes are transcribed more frequently than *rpoBC* (14; T. Linn, unpublished data). This dichotomy is resolved by an attenuator in the *rplL-rpoB* intercistronic region (5, 6). The level of β-galactosidase synthesized by the *rplJp-lacZ* fusion compared with that synthesized by the fusion that carries the attenuator downstream of *rplJp* indicates that ca. 70% of the transcripts initiated at *rplJp* are terminated at this site. The termination efficiency of the attenuator for transcripts initiated at *rplLp* is at least as great, for it reduces the level of β-galactosidase synthesis from a fusion carrying both these sites to the background level. Therefore *rplLp* does not lead to significant transcription of *rpoBC*.

The original experiments in which UV-irradiated cells were infected with transducing phages carrying different segments of the *rplKAJLrpoBC* region argued that *rplJp* is sufficient for high-level expression of *rplJLrpoBC* (26, 42). When the DNA sequence of this region was determined, Post et al. (37) found no canonical terminator sequence between the end of *rplA* and the proposed sequence for *rplJp*. Although a terminator site cannot be unambiguously identified from the DNA sequence alone, they speculated that transcription from *rplKp* might read through into *rplJLrpoBC*. Biochemical evidence supporting this idea was later provided by S1 nuclease mapping of in vivo mRNA. This analysis showed that most transcripts of these genes extend from *rplK* through *rplL* (9). A more complex genetic

argument is also consistent with the idea that at least *rplJL* is cotranscribed with *rplKA* (16).

Our *rplKp-lacZ* gene fusion suggests that *rplKp* is ca. 35% stronger than *rplJp*. Yet when *rplKp* is placed in its normal position upstream of *rplJp*, the level of transcription continuing into *rplJ* is only slightly greater than when this gene is preceded by *rplJp* alone. Since transcription is initiated more frequently at *rplKp*, this argues that at least some transcripts must be terminated before *rplJ*. If the majority of transcripts initiated at *rplKp* continued into *rplJL* but were terminated more or less frequently at the attenuator than transcripts initiated at *rplJp*, then an altered frequency of *rpoBC* transcription would be observed. However, the level of transcription into *rpoB* is essentially the same whether only *rplJp* or both *rplJp* and *rplKp* precede the attenuator. These experiments demonstrate that *rplJp* alone is sufficient for high-level transcription of the *rplJLrpoBC* genes. A similar conclusion was reached by Hui et al. (22) after transposon mutagenesis of this region of DNA. Insertions of Tn*5* downstream of *rplJp* greatly reduced expression of *rplJLrpoBC* due to the polar effects of the transposon. However, insertions upstream of *rplJp* but downstream of *rplKp* had no effect on the expression of these genes.

These results, however, do not directly determine whether *rplKp* or *rplJp* is normally the primary promoter for *rplJLrpoBC* expression. If *rplKp* is the primary promoter for *rplJLrpoBC* transcription, as the S1 nuclease mapping experiments suggest, this may be an interesting example of "promoter occlusion". The best-characterized example of promoter occlusion involves the interaction of the lambda phage P_L promoter and the galactose operon promoter (1). When P_L is placed upstream of P_{gal} it abolishes initiation at P_{gal}. This suggests that transcription through the galactose promoter from the 30-fold-more-powerful P_L inhibits its activity. Transcription from *rplKp* may inhibit initiation at *rplJp*, although it is only 35% stronger. Experiments are required to test this directly as our understanding of promoter occlusion is incomplete.

A more likely candidate for promoter occlusion is *rplLp*. Initiation at this site measured in the absence of the upstream promoters, is much lower than that at *rplKp* or *rplJp*. When connected in its normal fashion downstream of the stronger promoters, there may be little if any initiation at *rplLp* under these steady-state growth conditions. However, there are conditions such as increasing growth rate, nutrient shifts, and amino acid starvation, under which the synthesis of ribosomal proteins and RNA polymerase is discoordinately regulated (25). Activation of weak promoters or modulation of termination at the attenuator may play a role in this differential expression. We are examining such conditions with this series of transcriptional fusions.

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LITERATURE CITED

1. **Adhya, S., and M. Gottesman.** 1982. Promoter occlusion: transcription through a promoter may inhibit its activity. Cell **29**:939–944.
2. **An, G., and J. D. Friesen.** 1980. Characterization of promoter-cloning plasmids: analysis of operon structure in the *rif* region of *Escherichia coli* and isolation of an enhanced internal promoter mutant. J. Bacteriol. **144**:904–916.
3. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39**:440–452.
4. **Barnes, W., R. Siegel, and W. Reznikoff.** 1974. The construction of transducing phages containing deletions defining regulatory elements of the *lac* and *trp* operons in *E. coli*. Mol. Gen. Genet. **129**:201–205.
5. **Barry, G., C. Squires, and C. L. Squires.** 1979. Control features within the *rplJL-rpoBC* transcription unit of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:4922–4926.
6. **Barry, G., C. Squires, and C. L. Squires.** 1980. Attenuation and processing of RNA from the *rplJL-rpoBC* transcription unit of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **77**:3331–3335.
7. **Borck, K., J. D. Beggs, W. J. Brammer, A. S. Hopkins, and N. E. Murray.** 1976. The construction *in vitro* of transducing derivatives of phage lambda. Mol. Gen. Genet. **146**:199–207.
8. **Brot, N., Caldwell, P., and Weissbach, H.** 1980. Autogenous control of *Escherichia coli* ribosomal protein L10 synthesis *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **77**:2592–2595.
9. **Bruckner, R., and H. Matzura.** 1981. *In vivo* synthesis of a polycistronic messenger RNA for the ribosomal proteins L11, L1, L10 and L7/12 in *Escherichia coli*. Mol. Gen. Genet. **183**:277–282.
10. **Burton, Z. F., C. A. Gross, K. K. Watanabe, and R. R. Burgess.** 1983. The operon than encodes the sigma subunit of RNA polymerase also encodes ribosomal protein S21 and DNA primase in *E. coli* K12. Cell **32**:335–349.
11. **Clarke, D. J., and O. Maaloe.** 1967. DNA replication and the division cycle in *Escherichia coli*. J. Mol. Biol. **23**:99–112.
12. **Crawford, I. P., B. P. Nichols, and C. Yanofsky.** 1980. Nucleotide sequence of the *trpB* gene in *Escherichia coli* and *Salmonella typhimurium*. J. Mol. Biol. **142**:489–502.
13. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Dennis, P. P.** 1977. Transcription patterns of adjacent segments of *Escherichia coli* chromosome containing genes coding for four 50S ribosomal proteins and the β and β′ subunits of RNA polymerase. J. Mol. Biol. **115**:603–625.
15. **Fiil, N. P., D. Bendiak, J. Collins, and J. D. Friesen.** 1979. Expression of *Escherichia coli* ribosomal protein and RNA polymerase genes cloned on plasmids. Mol. Gen. Genet. **173**:39–50.
16. **Friesen, J. D., G. An, and N. Fiil.** 1983. The lethal effect of a plasmid resulting from transcriptional readthrough of *rplJ* from the *rplKA* operon in *Escherichia coli*. Mol. Gen. Genet. **189**:275–281.
17. **Goldberg, G., T. Zarucki-Schulz, P. Caldwell, H. Weissbach, and N. Brot.** 1979. Regulation of the *in vitro* synthesis of *E. coli* ribosomal protein L12. Biochem. Biophys. Res. Commun. **91**:1453–1461.
18. **Hohn, B.** 1979. *In vitro* packaging of λ and cosmid DNA. Methods Enzymol. **68**:299–309.
19. **Holmes, D. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114**:193–197.
20. **Holowachuk, E. W., J. D. Friesen, and N. P. Fiil.** 1980. Bacteriophage λ vehicle for the direct cloning of *Escherichia coli* promoter DNA sequences: feedback regulation of the *rplJl-rpoBC* operon. Proc. Natl. Acad. Sci. U.S.A. **77**:2124–2128.
21. **Howe, K. M., A. J. Newman, I. Garner, A. Wallis, R. S. Hayward.** 1982. Effect of rifampicin on expression of *lacZ* fused to promoters or terminators of the *E. coli rpoBC* operon. Nucleic Acids Res. **10**:7425–7438.
22. **Hui, I., K. Maltman, R. Little, S. Hastrup, M. Johnson, N. Fiil, and P. Dennis.** 1982. Insertions of transposon Tn*5* into ribosomal protein RNA polymerase operons. J. Bacteriol. **152**:1022–1032.
23. **Jaskunas, S. R., R. R. Burgess, and M. Nomura.** 1975. Identification of a gene for the α-subunit of RNA polymerase at the *str-spc* region of the *Escherichia coli* chromosome. Proc. Natl. Acad. Sci. U.S.A. **72**:5036–5040.
24. **Kirschbaum, J. B., and E. Konrad.** 1973. Isolation of a specialized transducing bacteriophage carrying the beta subunit gene

for *Escherichia coli* ribonucleic acid polymerase. J. Bacteriol. **116**:517–526.
25. **Lindahl, L., and J. M. Zengel.** 1982. Expression of ribosomal genes in bacteria. Adv. Genet. **21**:53–121.
26. **Linn, T., and J. Scaife.** 1978. Identification of a single promoter in *E. coli* for *rplJ*, *rplL* and *rpoBC*. Nature (London) **276**:33–37.
27. **Linn, T., M. Goman, and J. G. Scaife.** 1979. Studies on the control of the genes for transcription and translation in *Escherichia coli* K12. I. *tufB* and *rplA*, *K* have separate promoters. J. Mol. Biol. **130**:405–420.
28. **Ma, J., A. J. Newman, and R. S. Hayward.** 1981. Internal promoters of the *rpoBC* operon of *Escherichia coli*. Mol. Gen. Genet. **184**:548–550.
29. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Mitchell, D., W. Reznikoff, and J. Beckwith.** 1975. Genetic fusions defining *trp* and *lac* operon regulatory elements. J. Mol. Biol. **93**:331–350.
32. **Monastyrskaya, G. S., S. O. Gurev, N. F. Kalinina, A. V. Sorokin, I. S. Salomatina, T. M. Shuvaeva, V. M. Lipkin, E. D. Sverdlov, and Yu. A. Ovchinnikov.** 1982. Primary structure of *Eco*RI-D fragment of *rpoC* gene and corresponding fragment of β′subunit of RNA polymerase from *Escherichia coli*. Bioorg. Khim. **8**:130–134.
33. **Newman, A. J., T. G. Linn, and R. S. Hayward.** 1979. Evidence for cotranscription of the RNA polymerase genes *rpoBC* with a ribosomal protein gene of *Escherichia coli*. Mol. Gen. Genet. **169**:195–204.
34. **Ovchinnikov, Y. A., G. S. Monastyrskaya, V. V. Gubanov, S. O. Guryev, O. Y. Chertov, N. N. Modyanov, V. A. Grinkevich, I. A. Makarova, T. V. Marchenko, I. N. Polovnikova, V. M. Lipkin, and E. D. Sverdlov.** 1981. Nucleotide sequence of the *rpoB* gene and amino-acid sequence of the β-subunit. Eur. J. Biochem. **116**:621–629.
35. **Ovchinnikov, Y. A., G. S. Monastyrskaya, V. V. Gubanov, I. S. Salomatina, T. M. Shuvaeva, V. M. Lipkin, and E. D. Sverdlov.** 1981. Primary structure of RNA polymerase from the *Escherichia coli* nucleotide sequence of a DNA fragment containing a part of the *rpoC* gene and the corresponding carboxyl terminal amino acid sequence of the β′-subunit. Bioorg. Khim. **7**:1107–1112.
36. **Ovchinnikov, Y. A., G. S. Monastyrskaya, S. O. Guriev, N. F. Kalinina, E. D. Sverdlov, A. I. Gragerov, I. A. Bass, I. R. Kiver, E. P. Moiseyeva, V. N. Igumnov, S. Z. Mindlin, V. G. Nikiforov, and R. B. Khesin.** 1983. RNA polymerase rifampicin resistance mutations in *Escherichia coli*: sequence changes and dominance. Mol. Gen. Genet. **190**:344–348.
37. **Post, L. E., G. D. Strycharz, M. Nomura, H. Lewis, and P. P. Dennis.** 1979. Nucleotide sequence of the ribosomal protein gene cluster adjacent to the gene for RNA polymerase subunit β in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **76**:1697–1701.
38. **Smith, H. O.** 1980. Recovery of DNA from gels. Methods Enzymol. **65**:371–380.
39. **Taylor, W. E., and R. R. Burgess.** 1979. *Escherichia coli* RNA polymerase binding and initiation of transcription on fragments of λ*drif*^{d}18 DNA containing promoters for λ genes and for *rrnB*, *tufB*, *rplK*, *A*, *rplJ*, *L* and *rpoB*, *C* genes. Gene **6**:331–365.
40. **Wilson, G. G., and N. E. Murray.** 1979. Molecular cloning of the DNA ligase gene from bacteriophage T4. J. Mol. Biol. **132**:471–491.
41. **Windass, J. D., and W. J. Brammer.** 1979. Aberrant immunity behavior of hybrid λ*imm*^{21} phages containing the DNA of *Col*E1-type plasmids. Mol. Gen. Genet. **172**:329–337.
42. **Yamamoto, M., and M. Nomura.** 1978. Cotranscription of genes for RNA polymerase subunits β and β′ with genes for ribosomal proteins in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **75**:3891–3895.
43. **Yamamoto, M., and M Nomura.** 1979. Organization of genes for transcription and translation in the *rif* region of the *Escherichia coli* chromosome. J. Bacteriol. **137**:584–594.
44. **Yanofsky, C., V. Horn, M. Bonner, and S. Stasiowski.** 1971. Polarity and enzyme functions in mutants of the first three genes of the tryptophan operon of *Escherichia coli*. Genetics **69**:409–433.