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# *trp* aporepressor production is controlled by autogenous regulation and inefficient translation

(tryptophan repression/repressor concentration/promoter efficiency/*lac* gene fusion)

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**ABSTRACT** We constructed a *trpR-lacZ* gene fusion that specifies a hybrid protein that has full β-galactosidase activity. The gene fusion was associated with the unaltered *trpR* transcription and translation control region; thus, hybrid β-galactosidase production was an indicator of expression of the *trp* aporepressor (*trpR*) operon. To facilitate *in vivo* expression studies, a DNA segment containing the *trpR-lacZ* gene fusion and the *trpR* controlling region was transferred to bacteriophage λ and subsequently inserted into the bacterial chromosome. Analyses of hybrid β-galactosidase production showed that the *trpR* operon is regulated autogenously but that the rate of synthesis of aporepressor varies only 4- to 5-fold in response to changes in the intracellular concentration of tryptophan. Under comparable conditions, the *trp* operon is regulated by *trp* repressor ≈70-fold. Therefore, the operators of the *trp* operon and the *trpR* operon must have very different affinities for *trp* repressor *in vivo*. The promoter controlling *trpR* expression was found to be moderately active. Nevertheless, there are only about 50–300 molecules of *trp* aporepressor per cell. The low aporepressor level appears to be due to inefficient translation of *trpR* mRNA.

The *trp* repressor of *Escherichia coli* regulates at least three unlinked operons: *trp*, *aroH*, and *trpR* (1–4). The *trp* operon codes for the five polypeptides that catalyze the terminal sequence of reactions in tryptophan formation (5), *aroH* codes for one of three isozymes that perform the initial reaction of the common pathway of aromatic amino acid biosynthesis (6), and *trpR* codes for the *trp* aporepressor itself (7). The *trpR* operon thus is autogenously regulated, and its polypeptide product controls transcription initiation in two biosynthetic operons. The promoters of the three operons contain homologous operators, as expected (4).

Previous indications that the *trpR* operon was autogenously regulated were based on *in vitro* analyses of *trp* repressor binding to *trpR* operator DNA (4). In this report, we examine regulation of the *trpR* operon *in vivo*. Using a *trpR-lacZ* gene fusion that specifies a fully active hybrid β-galactosidase, we show that the *trpR* operon is autoregulated 4- to 5-fold in response to changes in the intracellular tryptophan concentration. We also demonstrate that, although the *trpR* promoter is moderately active, inefficient translation of *trpR* mRNA results in low cellular levels of *trp* aporepressor.

## METHODS

**Growth Conditions.** All cells were grown in minimal medium (8) supplemented with 0.2% glucose, 0.2% lactose, 0.3% glycerol, 0.1–0.5 mM isopropyl-β-D-thiogalactoside, L-tryptophan at 50 μg/ml, L-proline at 50 μg/ml, kanamycin at 30 μg/ml, or ampicillin at 50 μg/ml as indicated. All temperature-sensitive lysogens were grown at 30–32°C.

**DNA Manipulations.** Methods for cloning DNA fragments have been described (9). Restriction enzymes (New England BioLabs and Bethesda Research Laboratories) were used according to the supplier's instructions. Plasmid DNA was isolated from transformants as described (10). DNA sequence analyses were carried out by using the method of Maxam and Gilbert (11).

**Construction of *trpR-lacZ* Fusion.** Plasmid pMC931 (12) was digested with *Bam*HI and ethanol precipitated, and the 3′ ends were filled in with DNA polymerase I Klenow fragment (13). Plasmid pRPG16 was cut with *Hin*cII and both fragments were subjected to electrophoresis through 0.6% agarose. The 6.8-kilobase (kb) pMC931 fragment containing *lacZY* and the 2.4-kb pRPG16 fragment containing the initial portion of *trpR* were eluted from the gel and blunt-end ligated together as shown in Fig. 1. Cells of strain M182 were transformed with the ligation mixture and one blue colony was isolated after 2 days growth on minimal medium/glucose/proline/kanamycin plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside at 40 μg/ml. The transformant contained a plasmid, pTRL1, that gave the predicted restriction fragments around the *trpR-lacZ* fusion junction but contained a large (3.4-kb) deletion at the other junction that was apparently an artifact of cloning. The 3.4-kb *Bam*HI/*Eco*RI fragment of pTRL1 was substituted for the analogous region in pMC931 to yield pTRL2 (Fig. 1). The 7.2-kb *Bam*HI fragment of pTRL2 containing the entire *trpR-lacZ* fusion operon was ligated into the *Bam*HI site of pSIBV to yield pTRL3 (Fig. 1). The DNA sequence of pTRL3 from −375 to +160 relative to the *trpR* transcription start site was determined to confirm that the fusion had occurred as designed and that no point mutations had been selected during the cloning procedure (data not shown).

**Recombination of the *trpR-lacZ* Fusion onto Bacteriophage λ.** To study expression of the hybrid gene in single-copy form, the fused operon was recombined into the temperature-sensitive λ derivative λSm4Cm$^r$ *in vivo* by using the system shown in Fig. 2 (unpublished). This was done by lytically infecting M182/pTRL3 cells with λSm4Cm$^r$ and using the resulting lysate to transduce M182 to Lac$^+$ on minimal medium/lactose at 30°C. A double crossover occurred as shown in Fig. 2 because pSIBV, and therefore pTRL3, contains DNA sequences flanking the *Bam*HI site that are also present flanking the chloramphenicol-resistance gene in the phage. During recombination, any sequence inserted into the *Bam*HI site of pSIBV can substitute for the Cm$^r$ gene of the phage. The recovered lysogens were screened for sensitivity to kanamycin, chloramphenicol, and high temperature. One such recombinant phage was

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Abbreviation: kb, kilobase(s).

used in all later experiments and is designated λSm4TRL3.

**Labeling and Quantifying *lac* mRNA.** RNA was isolated from [$^3$H]uridine-labeled cultures as described (14) and hybridized to denatured linear DNA immobilized on Schleicher & Schuell BA85 nitrocellulose filters. After 15–18 hr of hybridization at 66°C, the filters were washed, treated with ribonuclease, dried, and assayed. The DNA sources used for labeled RNA extracted from strains containing λSm4TRL3 were pMC931 (a plasmid containing *lacZY*; 2.5 μg of DNA per filter) and PACYC177 (the parental plasmid; 2.5 μg of DNA per filter). The DNA sources used for labeled RNA from strains containing pTRL3 were λ*plac5* (15) and λCI857 (10 μg of DNA per filter).

**Quantifying the Amount of β-Galactosidase and Fusion Protein in Whole Cell Extracts.** Cultures (10 ml) were grown with shaking at 32°C for 3 generations to a cell density of $5 \times 10^8$/ml. [$^{14}$C]Leucine (20 μg/ml) was added at the time of inoculation. The *trpR-lacZ* culture contained kanamycin (30 μg/ml). Excess (500 μg/ml) unlabeled leucine was added to each culture 15 min before harvesting the cells. Samples were taken for β-galactosidase assay and for total incorporation determination and the remaining 8 ml was sedimented and washed three times. The labeled cells were lysed by boiling in Laemmli sample buffer (16) and the proteins were separated by electrophoresis in a 7–12% gradient polyacrylamide/$NaDodSO_4$ gel. The gel was stained with Coomassie blue and the β-galactosidase bands were cut out and dissolved in 100 μl of 30% $H_2O_2$ at 65°C. The molecular weight of the fusion polypeptide is 118,000 compared with 116,000 for the native β-galactosidase polypeptide. The dissolved acrylamide/protein was mixed with 5 ml of Aquasol (New England Nuclear) and assayed in a Packard Scintillation Spectrometer. Control experiments were carried out to determine the extent of quenching.

**Bacterial Strains, Plasmids, and Phage.** Strain M182 has the entire *lac* operon deleted (17). The three strains used to prepare lysogens were W3110 *tnaA2 ΔlacU169*, W3110 *tnaA2 trpR ΔlacU169*, and W3110 *tnaA2 trpA46PR9 ΔlacU169* (18, 19). The *lacU169* deletion, removing the entire *lac* operon, was introduced by cotransduction with the tightly linked *proC*:Tn5 marker of MB82 (kindly supplied by M. Benedik). pSIBV is a derivative of pITS18 (unpublished) containing a *Bam*HI linker in place of the *Eco*RI site and lacking the *Bam*HI site of the vector. pRLK10 is a pBR322 derivative containing a promoterless/operatorless *trpR* gene in the *Bam*HI site (see Fig. 3). The aporepressor is apparently constitutively produced by using the *tet* promoter of pBR322 that is located upstream. This supplies the cell with ≈20 times the haploid amount of aporepressor. pBN60 is a pBR322 derivative containing the *trp* promoter/operator on a 490-base pair *Sau*3A fragment. pRPG16 contains *trpR* on a 1.3-kb chromosomal *Bam*HI fragment in pACYC177. Phage λ*plac5* contains *lacZ* and *lacY* (15).

**Enzyme Assays.** Anthranilate synthetase from permeabilized cells was measured as described (20). Cultures (5 ml) were grown to a Klett value of 80 and pelleted, and the pellets were washed in the saline, repelleted, and suspended in 0.6 ml of 0.1 M Tris base, pH 7.8/0.1% Triton X-100. The cells were frozen 15 min on dry ice/acetone and thawed. β-Galactosidase assays (21) and protein assays (22) have been reported.

## RESULTS

**Autogenous Regulation of *trpR*.** A *trpR-lacZ* fusion and the adjacent *trpR* regulatory region was inserted into the promoter-cloning plasmid pSIBV to form pTRL3 (Fig. 1) and then transferred to the promoter-cloning λ derivative λSm4Cm$^r$ to produce λSm4TRL3 (Fig. 2). Expression of structural genes cloned into the appropriate sites in pSIBV and λSm4Cm$^r$ is totally dependent on the associated promoter (unpublished). Phage λSm4TRL3 was used to lysogenize three similar *lac* deletion strains; one was *trpR*$^+$, the second was *trpR*, and the third was a *trpR*$^+$ tryptophan bradytroph carrying the *trpA46PR9* mutation in the structural gene for the tryptophan synthetase α

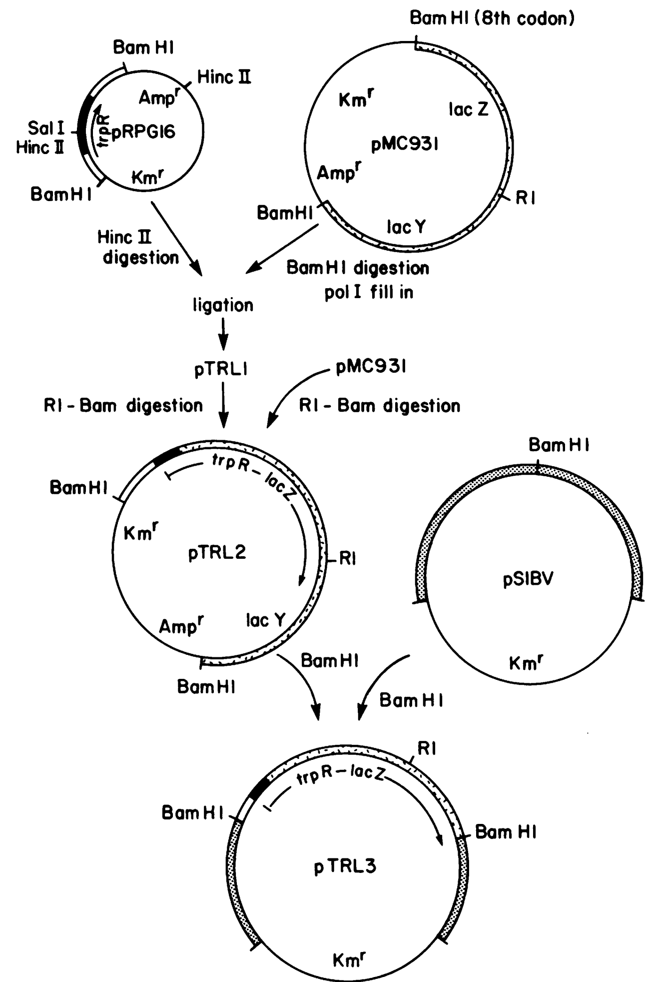


FIG. 1. Construction of plasmid pTRL3 containing the *trpR-lacZ* fusion. ▬, *trpR* structural gene; ▭, chromosomal DNA flanking *trpR*; ▒, portion of the *trp* operon of *Serratia marcescens*; ▒, segment of the *lac* operon.

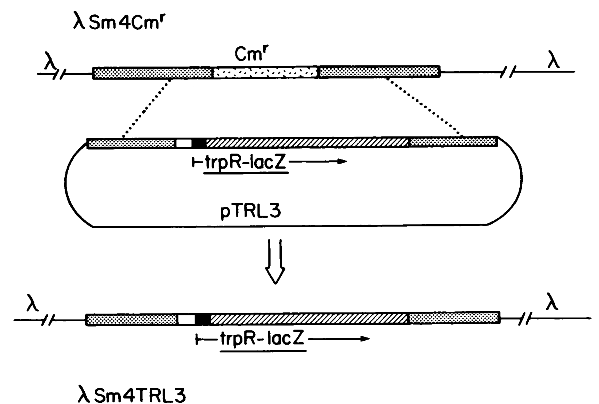


FIG. 2. Construction of λSm4TRL3. Transformed cells containing pTRL3 were lytically infected with λSm4Cm$^r$. Recombination occurred in the homologous regions to give λSm4TRL3 containing the *trpR-lacZ* fusion.

Table 1. *lac* enzyme and mRNA levels in various strains grown under different conditions

| Exp. | Strain* | Growth medium | β-Galactosidase units (a) | % labeled RNA as *lac* mRNA (b) | (a/b) × 100 |
|---|---|---|---|---|---|
| 1 | *trpR*$^+$ (λSm4TRL3) | Glucose | 91 | 0.04 | 22 |
| 2 | *trpR*$^+$ (λSm4TRL3) | Glucose/tryptophan | 40 | 0.02 | 20 |
| 3 | *trpR* (λSM4TRL3) | Glucose | 163 | 0.05 | 32 |
| 4 | *trpR* (λSM4TRL3) | Glucose/tryptophan | 160 | 0.05 | 32 |
| 5 | *trpR*$^+$ *trpA46PR9* (λSm4TRL3) | Glucose | 142 | 0.04 | 36 |
| 6 | *trpR*$^+$ *trpA46PR9* (λSm4TRL3) | Glucose/tryptophan | 36 | 0.011 | 33 |
| 7 | *trpR*$^+$ (Lac$^+$) | Lactose | 1,370 | 0.11 | 125 |
| 8 | *trpR*$^+$ (Lac$^+$) | Glycerol/iPr-β-S-Gal | 5,830 | 0.58 | 100 |
| 9 | M182 (Δ*lac*) (pTRL3) | Glycerol | 5,410 | 2.3 | 23 |
| 10 | *trpR* Δ*lac* (pTRL3) | Glycerol | 14,550 | 8.0 | 18 |

Cultures were grown with shaking at 32°C (experiments 1–7) or 37°C (experiments 8–10) to *ca.* $5 \times 10^8$ cells/ml. Samples were removed for β-galactosidase assay and the remainder was pulse labeled with [$^3$H]uridine for 1 min. RNA was isolated and hybridized to plasmid *lac* DNA (cultures 1–7) or phage *lac* DNA (cultures 8–10) and appropriate control DNAs and the percent labeled RNA that is *lac* mRNA was determined. Comparable values were obtained in repeat experiments. iPr-β-S-Gal, isopropyl-β-D-thiogalactoside.

* Genetic background of the strains in experiments 1–6 and 10 was W3110 *tnaA2* Δ*lacU169 proC*::Tn*5* and that of the strains in experiments 7 and 8 was W3110 *tnaA2*.

chain. These strains were assayed for β-galactosidase activity after growth under different conditions (Table 1). As shown, a *trpR*$^+$ culture growing in minimal medium contains ≈100 units of hybrid β-galactosidase. If exogenous tryptophan is supplied, the level decreases to ≈40 units. In contrast, if the culture is *trpR*, the fused *trpR-lacZ* operon is fully derepressed and 160 units of hybrid β-galactosidase are observed, with or without excess tryptophan. The hybrid β-galactosidase levels of lysogens containing the *trpA46PR9* mutation in a *trpR*$^+$ background show that, despite the presence of the *trpR*$^+$ allele, derepressed levels of hybrid β-galactosidase are obtained when cells are starved of tryptophan.

We also compared expression of the *trp* operon and the *trpR-lacZ* operon as a function of tryptophan availability (Table 2). As shown, the *trp* operon is appreciably repressed in minimal medium (compare *trpR* and *trpR*$^+$ values) whereas the *trpR-lacZ* operon is repressed only slightly. Furthermore, addition of tryptophan leads to much greater repression of the *trp* operon than of the *trpR-lacZ* operon. Thus, over the normal *trp* aporepressor concentrations that exist *in vivo*, the *trp* operon is

Table 2. Expression of the *trp* and *trpR-lacZ* operons in strains that have excess aporepressor or excess *trp* operators

| Strain characteristics | *trpR* operon expression* | | *trp* operon expression† | |
|---|---|---|---|---|
| | −Trp | +Trp | −Trp | +Trp |
| *trpR* | 150 | 152 | 1,020 | 920 |
| *trpR*$^+$ | 90 | 33 | 100 | 13 |
| *trpR*$^+$/pRLK10 | 20 | 0.62 | 75 | 7.3 |
| *trpR*$^+$/pBN60 | 117 | 96 | 171 | 118 |

Expression of the *trpR-lacZ* fusion operon and the *trp* operon are compared under various conditions. Results are averages of two experiments, each with duplicate cultures. Growth was in minimal medium/0.2% glucose/proline with (+Trp) or without (−Trp) added tryptophan. The strain used is W3110 *tnaA2* Δ*lacU169 proC*::Tn*5* (λSm4TRL3) with the noted differences.

* *trpR* expression is measured from the *trpR-lacZ* fusion operon and is presented as units of β-galactosidase.

† *trp* operon expression is measured by anthranilate synthetase (*trpE*) polypeptide production and is expressed as percent specific activity relative to that of wild-type cells grown in minimal medium (set at 100). Although λSm4TRL3 contains a promoterless *S. marcescens trpE* downstream from the *trpR-lacZ* fusion, it does not contribute measurable amounts of anthranilate synthetase. This was shown by also measuring phosphoribosyltransferase (*trpD*) activity, which was indistinguishable from the *trpE* activities shown.

clearly more responsive to changes in the availability of tryptophan. When the aporepressor level is increased (by providing a plasmid, pRLK10, that constitutively overproduces the aporepressor), *trpR-lacZ* expression is reduced in minimal medium and dramatically so in the presence of tryptophan. Note that the high *trp* aporepressor level has only a slight effect on *trp* operon expression. These findings suggest that the aporepressor concentration limits *trpR* repression and that the *trp* operon operator has a higher affinity for *trp* repressor than does the *trpR* operator. When a multicopy plasmid containing the *trp* operator, pBN60, was introduced into the same strains, and the strains were grown with excess tryptophan, expression of both the *trpR* and the *trp* operons was elevated appreciably. Apparently, in cells that have a single copy of *trpR*$^+$, autoregulation cannot increase *trp* aporepressor production significantly in response to additional *trp* operon operators. In minimal medium, the extra copies of the *trp* operator have only a slight effect on expression of the two operons. These findings establish that both tryptophan and aporepressor limit repression of the *trpR* operon while the *trp* operon is near maximal repression when the haploid level of *trp* repressor is present.

**Regulation Is at the Level of Transcription Initiation.** The level of *lac* mRNA produced by the various lysogens was measured to confirm that the modulation of hybrid β-galactosidase production was due to regulation of transcription initiation. The percentage of total labeled RNA that hybridized to *lac*-specific DNA is shown in Table 1. It can be seen that mRNA levels vary directly with hybrid β-galactosidase activity. If we calculate the ratio of enzyme specific activity to percent *lac* mRNA, it is evident that, under all growth conditions, approximately the same proportion is obtained regardless of whether haploid lysogens or strains with high copy number plasmids are examined.

**The *trpR* Transcript Is Translated Inefficiently.** When wild-type β-galactosidase activity and *lac* mRNA were measured as controls in the above experiments, a different ratio of enzyme activity to *lac* mRNA was observed (Table 1). This difference is subject to several interpretations. The transcript of the fusion operon might be degraded more rapidly than *lac* mRNA and thus be available for translation for a shorter period. This possibility was ruled out by measuring the half-lives of the two messages, which were essentially identical. A second explanation is that both mRNAs are translated equally to yield the same number of protein molecules, but the hybrid protein is only 20% as active enzymatically as wild-type β-galactosidase. This possibility was eliminated by quantifying the amount of β-galac-

Table 3. Comparison of β-galactosidase and the *trpR-lacZ* hybrid β-galactosidase

| Strain characteristics | Amount loaded, cpm | β-Gal band, cpm | Quench corrected net β-gal band,* cpm | % total protein as β-gal (a) | β-Gal, units (b) | β-Gal b/a |
|---|---|---|---|---|---|---|
| *lac* deletion | 540,000 | 1,024 | 0 | 0 | 0 | — |
| Wild-type *lac*⁺ | 330,000 | 8,244 | 8,765 | 2.65 | 7,240 | 2,732 |
| *trpR-lacZ* fusion | 291,000 | 7,829 | 8,364 | 2.87 | 7,200 | 2,509 |

The quantity of wild-type β-galactosidase (β-gal) or *trpR-lacZ* fusion protein is compared with the corresponding enzymatic specific activities. The three strains used were W3110 *tnaA2* Δ*lacU169 proC*::Tn5 (*lac* deletion), W3110 *tnaA2* (wild-type *lac*⁺), and W3110 *tnaA2* Δ*lacU169 proC*::Tn5 λSm4TRL3 (*trpR-lacZ* fusion). The cultures had the following $^{14}C$ specific activities (cpm/μg of total cell protein): *lac* deletion, 5,000; wild-type *lac*⁺, 6,750; fusion, 7,200.

* Net cpm in β-gal band (corrected for cpm loaded) = gross cpm in β-gal band − (1,024/540,000) cpm loaded. Samples containing acrylamide and $H_2O_2$ had 87% as much radioactivity as protein samples alone.

tosidase and hybrid β-galactosidase isotopically in extracts of whole cells and comparing these values with the corresponding enzyme specific activities. As shown in Table 3, equal amounts of protein correspond to equal β-galactosidase specific activities. A third interpretation, which seems most likely, is that ribosomes translate the *trpR* message inefficiently. This explanation is consistent with expectations based on examination of the nucleotide sequence preceding the start codon for the *trpR* protein (Fig. 3). This region has essentially no homology to the consensus Shine–Dalgarno sequence believed necessary for efficient translation initiation (23). *lacZ* mRNA contains such a sequence (24).

**Promoter Strength.** If the amount of *lac* mRNA produced from the wild-type *lac* promotor is compared with that from the *trpR-lacZ* promoter (Table 1), the fully derepressed *trpR* promoter is ≈10% as active as the *lac* promoter induced with isopropyl-β-D-thiogalactoside. This level of promoter activity is unexpectedly high for a gene whose product is apparently needed in small amounts. By using a different measuring system, the strength of the *trpR* promoter has been related to many other promoters and found to be essentially the same as reported here (unpublished).

**Calculation of the Number of Repressor Molecules Per Cell.** By using the data in Tables 1 and 3, we can estimate the number of repressor molecules (RM) in each cell. If 2.87% of the total cellular protein corresponds to 7,200 units (U) of hybrid β-galactosidase (β-gal), and there is ≈150 μg of protein per $10^9$ cells (21, 25), then

$$\mathrm{RM} = \frac{0.0287}{7{,}200\ \mathrm{U\ of\ }\beta\text{-gal}} \times \frac{150\ \mu\mathrm{g\ of\ protein}}{10^9\ \mathrm{cells}} \times \frac{1\ \mu\mathrm{mol\ of\ }\beta\text{-gal}}{118 \times 10^3\ \mu\mathrm{g\ of\ protein}} \times \frac{6.02 \times 10^{17}\ \mathrm{monomers}}{\mu\mathrm{mol}} = \frac{3\ \mathrm{monomers}}{\mathrm{cell{\cdot}U\ of\ }\beta\text{-gal}}$$

Thus, a wild-type cell growing in minimal medium has ≈300 repressor monomers or 150 dimers. This value is twice the estimate made years ago on the basis of *in vitro* repressor binding studies (26). Although previous reports suggested that the *trp* repressor was a tetramer (4), recent crosslinking experiments indicate that it is actually a dimer (A. Joachimiak and P. Sigler, personal communication).

Our estimate of three monomers per unit of β-galactosidase activity is one-third that of others (27). We do not know the source of this discrepancy.

## DISCUSSION

Purified *trp* aporepressor, when activated by L-tryptophan, can protect restriction sites in the *trpR* operator and inhibit transcription initiation at the *trpR* promoter (4). As shown here, the *trp* repressor regulates its own synthesis *in vivo*. Interestingly, we find that *E. coli* modulates synthesis of the *trp* aporepressor over only a 5-fold range; the aporepressor level depends on whether the cell is tryptophan starved, synthesizing tryptophan, or provided with an exogenous supply of the amino acid. Apparently the ability to realize this small regulatory change affecting this minor protein component was sufficiently important to the bacterium to have led to the development of an appropriate regulatory mechanism.

The direction of regulatory change is opposite to what might be expected; i.e., less *trp* aporepressor is synthesized when the intracellular tryptophan concentration is high—when the cell needs the repressor to shut off its two target biosynthetic operons, the *trp* and *aroH* operons. However, our results indicate that in the presence of excess tryptophan the aporepressor level is adequate, since providing more aporepressor causes only a modest increase in *trp* operon repression. We believe that a reasonable explanation for the narrow range and direction of *trpR* expression is that the bacterium is attempting to maintain a level of active *trp* repressor sufficient to control transcription initiation in its target operons at all tryptophan concentrations. We suggest that, in the presence of excess tryptophan, essentially all *trp* aporepressor molecules are actively complexed with tryptophan and available for operator binding. Under such conditions, a small number of aporepressor molecules would suffice since every molecule would be active. However, when cells are growing in minimal medium and synthesizing their own tryptophan, they also require active repressor since the *trp* operon is ≈90% repressed; i.e., *trp* operon enzyme levels in *trpR*⁺ cultures growing in minimal medium are ≈1/10th those of *trpR* mutants in the same medium (see Table 2). To accomplish this regulation, the cell must form an appropriate number of active repressor molecules despite a low intracellular tryptophan concentration. To maintain this level of active repressor molecules during growth in minimal medium, we believe that the bacterium increases the aporepressor concentration 2- to 3-fold. A prediction of our proposal is that the repressed level of *trp* aporepressor would be inadequate to control *trp* operon expression in minimal medium.

The requirement for a modest level of active *trp* repressor molecules could be met by a constitutively expressed operon. Perhaps this is not what occurs in *E. coli* because there are at least three different operator DNA sequences that must be regulated by the repressor. Each may be regulated differently as the intracellular concentration of repressor is varied. This is clearly so for the *trp* vs. *trpR* operons. We have not yet examined *aroH* expression as a function of repressor concentration.

The *trpR* operon was found to have a moderately active promoter and to specify a transcript that is poorly translated. Inefficient translation can be explained by the absence of a recognizable Shine–Dalgarno region preceding the AUG initiation

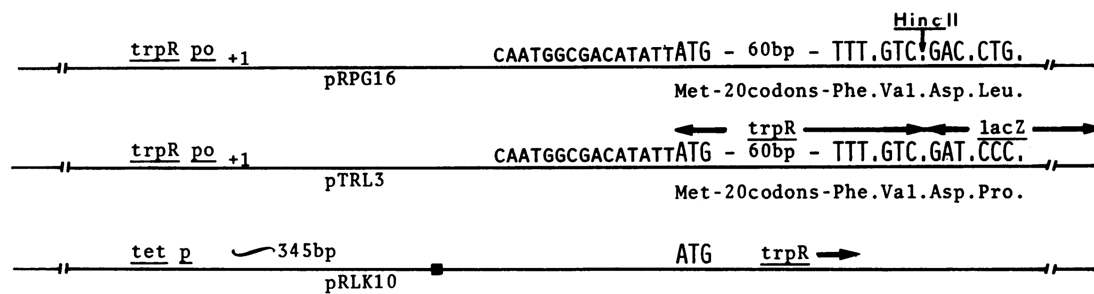


FIG. 3. Fine structure around the *trpR-lacZ* fusion in pTRL3. Plasmid pRPG16 was the source of the *trpR* regulatory region and the initial segment of *trpR*. The sequence preceding the ATG start codon lacks good homology to the 3′ end of 16S RNA. The organization of pRLK10 is shown; in this plasmid, the *trpR* promoter and operator have been replaced by the tetracycline-resistance promoter of pBR322. ■, Location of a *Bam*HI linker used in construction.

codon for the *trpR* protein (see Fig. 3). These characteristics raise the question—is there an advantage to this arrangement over one in which the operon has a weaker promoter and specifies a mRNA that is efficiently translated? We speculate that the existing arrangement reflects the possibility that under certain conditions promoters must compete for RNA polymerase molecules. If the *trpR* promoter had a poor affinity for RNA polymerase, there might be circumstances, such as a shift to rapid growth conditions, in which the *trpR* operon could not compete for polymerase effectively and therefore would not be transcribed. If the aporepressor concentration were low at the start of such a shift, the level might fall below some critical minimal threshold value. By having a good *trpR* promoter, the bacterium may ensure that synthesis of a moderate supply of *trpR* mRNA will occur under all conditions. The requirement for few aporepressor molecules is then satisfied by inefficient translation. Alternatively, the nature of the repressor–operator interaction responsible for autogenous control of transcription initiation in the *trpR* operon may demand a moderately active promoter—i.e., one that is frequently complexed with RNA polymerase. Regardless of the explanation for the present arrangement, our findings provide evidence suggesting that translational efficiency plays a major role in establishing the level of expression of an operon.

Our results do not agree with those of Bogosian *et al.* (28). Using a similar *trpR-lacZ* fusion, they found much higher expression and no autogenous regulation unless repressor was overproduced from a plasmid. We feel that their results are best explained by read-through transcription from the pBR322 tetracycline-resistance promoter in the *Eco*RI-*Sal* I fragment they used in the construction of their *trpR-lacZ* fusion.

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