JOURNAL OF BACTERIOLOGY, Nov. 1995, p. 6362–6370
0021-9193/95/$04.00+0

Vol. 177, No. 22

trp RNA-Binding Attenuation Protein (TRAP)-*trp* Leader RNA Interactions Mediate Translational as well as Transcriptional Regulation of the *Bacillus subtilis trp* Operon

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Received 27 June 1995/Accepted 1 September 1995

Expression of the *Bacillus subtilis trpEDCFBA* operon has been shown to be regulated by transcription attenuation in response to the availability of L-tryptophan. Regulation is mediated by the tryptophan-activated *trp* RNA-binding attenuation protein, TRAP, the product of *mtrB*. Formation of mutually exclusive RNA antiterminator and terminator structures within *trp* leader RNA determines whether transcription will terminate in the leader region of the operon. Previous studies suggested that transcripts that escape termination are subject to translational regulation via the formation of a secondary structure that blocks ribosome access to the *trpE* ribosome-binding site. To assess the relative importance of these postulated events in *trp* operon regulation, we used site-directed mutagenesis to alter the putative elements involved in transcriptional and translational control. Using a *trpE′-′lacZ* reporter, we measured translational yield and specific mRNA levels with various leader constructs, in both *mtrB*$^+$ and *mtrB* strains, during growth in the presence and absence of excess tryptophan. To verify that the altered regulatory regions behaved as expected, we carried out in vitro transcription assays with the wild-type and altered leader region templates and performed oligonucleotide competition assays with an oligonucleotide complementary to a segment of the transcription terminator. Our results establish that binding of TRAP to leader RNA regulates of transcription termination in the *trp* operon over about an 88-fold range and regulates translation of *trpE* over about a 13-fold range. The roles played by different *trp* leader RNA segments in mediating transcriptional and translational regulation are documented by our findings.

The *Bacillus subtilis trpEDCFBA* operon is regulated in response to the availability of L-tryptophan by an RNA-binding protein called TRAP (*trp* RNA-binding attenuation protein) (6, 25). TRAP, the product of *mtrB*, is an oligomer composed of 11 identical subunits, each with a molecular mass of about 8 kDa (2–5). When activated by L-tryptophan, TRAP binds to a series of (G/U)AG repeats present in *trp* leader RNA and prevents the formation of an RNA antitermination structure, thereby promoting formation of an alternate RNA terminator structure that causes transcription termination (4–6, 19, 25, 31). TRAP also binds to a second series of (G/U)AG repeats, in a folate operon transcript, and translationally regulates synthesis of TrpG, the sole *trp* protein that is not encoded in the *trp* operon (13, 39). The *trp* leader regions of *Bacillus pumilus* (15, 20), *Bacillus caldotenax* (23), and *Clostridium thermocellum* (23) have an appropriately located series of (G/U)AG repeats, suggesting that in each, the *trp* operon is regulated by a TRAP homolog. RNA secondary structure predictions for *trp* leader RNA of *B. subtilis* suggested that when TRAP was bound, transcripts that escaped termination would have the ribosome-binding site for *trpE* sequestered in an RNA stem-and-loop structure that could block translation initiation (19). Preliminary experiments comparing levels of expression in *trpE* transcriptional and translational fusions indicated that translational regulation of *trpE* expression does in fact occur (19).

To assess the extent of transcriptional and translational regulation of the *trp* operon, we measured polypeptide and mRNA levels with a reporter construct under various growth conditions. We also carried out secondary-structure predictions with leader RNA as a basis for designing mutagenesis strategies that would provide altered leader regions that we expected to (i) abolish transcription termination regulation exclusively, (ii) relieve translation regulation exclusively, and (iii) relieve both transcriptional and translational regulation. To abolish transcription termination, the DNA segment corresponding to the RNA terminator structure was deleted. To eliminate translational regulation, multiple point mutations were introduced in the *trp* leader region that altered the sequences flanking the *trpE* ribosome-binding site. The latter changes were predicted to destabilize the putative RNA secondary structure that blocks *trpE* translation. To abolish both transcriptional and translational regulation, we constructed a double mutant carrying the terminator deletion and the altered Shine-Dalgarno (SD) blocking sequence. Chromosomal *trpE′-′lacZ* translational fusions preceded by the *trp* promoter and either the wild-type or mutated leader regions were used to assess the effects of the introduced changes on expression of the operon. β-Galactosidase (β-Gal) activity and *lacZ* mRNA levels were measured by use of these constructs and their parental wild-type construct in either *mtrB*$^+$ or *mtrB* backgrounds. In vitro transcription and oligonucleotide competition assays were performed to provide evidence that deletion of the transcription terminator did eliminate transcription termination in the leader region of the operon. Our findings suggest that TRAP binding to *trp* leader RNA regulates tran-

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TABLE 1. *B. subtilis* strains used in this study

Strains	Genotype and/or phenotype[a]	Source or reference[b]
W168	Prototroph	BGSC[c]
CYBS2199	*amyE*::[*trpP*(−412 to +203) *trpE′-′lacZ* Cm^r]	ptrpBGI tf→W168
CYBS222	*mtrB*(Δ+1207)	11
CYBS223	*mtrB*ΩTc	pTZmtrABΩTc tf→W168
CYBS301	*amyE*::(polylinker-pTZ18R-′lacZ Cm^r)	ptrpBGI-PLK tf→W168
CYBS302	*amyE*::[*trpP*(−412 to +203) Δ(+108 to +133) *trpE′-′lacZ* Cm^r]	ptrpBGIΔT tf→CYBS301
CYBS303	*amyE*::[*trpP*(−412 to +203) SD^{mod} *trpE′-′lacZ* Cm^r]	ptrpBGIS/D tf→CYBS301
CYBS304	*amyE*::[*trpP*(−412 to +203) Δ(+108 to +133) SD^{mod} *trpE′-′lacZ* Cm^r]	ptrpBGIΔTS/D tf→CYBS301
CYBS305	*mtr-222 amyE*::[*trpP*(−412 to +203) *trpE′-′lacZ* Cm^r]	CYBS222 tf→CYBS2199
CYBS306	*mtrB*ΩTc *amyE*::[*trpP*(−412 to +203) *trpE′-′lacZ* Cm^r]	CYBS223 tf→CYBS2199
CYBS307	*mtr-222 amyE*::[*trpP*(−412 to +203) Δ(+108 to +133) *trpE′-′lacZ* Cm^r]	CYBS222 tf→CYBS302
CYBS308	*mtrB*ΩTc *amyE*::[*trpP*(−412 to +203) Δ(+108 to +133) *trpE′-′lacZ* Cm^r]	CYBS223 tf→CYBS302
CYBS309	*mtr-222 amyE*::[*trpP*(−412 to +203) SD^{mod} *trpE′-′lacZ* Cm^r]	CYBS222 tf→CYBS303
CYBS310	*mtrB*ΩTc *amyE*::[*trpP*(−412 to +203) SD^{mod} *trpE′-′lacZ* Cm^r]	CYBS223 tf→CYBS303
CYBS311	*mtr-222 amyE*::[*trpP*(−412 to +203) Δ(+108 to +133) SD^{mod} *trpE′-′lacZ* Cm^r]	CYBS222 tf→CYBS304
CYBS312	*mtrB*ΩTc *amyE*::[*trpP*(−412 to +203) Δ(+108 to +133) SD^{mod} *trpE′-′lacZ* Cm^r]	CYBS223 tf→CYBS304

[a] *trpP* denotes the *trp* operon promoter; prime indicates truncation of the gene. −412 to +203 denotes the DNA fragment containing the *trp* promoter and neighboring regions that was incorporated. Δ(+108 to +133) designates the segment of the leader region that was deleted. SD^{mod} refers to the altered leader segment containing the SD sequence. The alterations introduced were as follows: C181→T, C182→G, T183→C, C186→T, T196→C, A198→C, G199→T, A200→T, G201→C, and A203→C. Numbers are relative to the transcription start site.

[b] tf, transformation.

[c] BGSC, *Bacillus* Genetic Stock Center, Ohio State University, Columbus.

scription termination in the leader region of the operon approximately 88-fold and blocks translation of *trpE*, resulting in an additional 13-fold regulation. Our data support the model proposed by Kuroda et al. (19) that TRAP binding to *trp* leader RNA can regulate both transcription and translation in the *trp* operon.

MATERIALS AND METHODS

Site-directed mutagenesis of the *trp* leader region and plasmid construction. Plasmid pTZtrpE was constructed by subcloning the 730-bp *Eco*RI-*Hin*dIII fragment of ptrpBGI (30), containing the *trp* promoter-leader region and a segment of *trpE*, into the *Eco*RI-*Hin*dIII sites of pTZ18R (United States Biochemical Co.). This plasmid was used as a template for single or combinatorial oligonucleotide-directed PCR mutagenesis by the protocol described by Merino et al. (22). PCRs were carried out with Vent DNA polymerase (New England Biolabs) in an Ericomp thermocycler. Three different mutagenesis procedures were applied to the *trp* leader region, as follows. (i) The transcription terminator region, nucleotide (nt) +108 to +133 (see Fig. 1A), was deleted by use of the oligonucleotide 5′-GGATAAAATACTATATAACAAATAAAGAATAAACATAAT GTCTCAGC-3′, complementary to nt 86 to 107 and nt 134 to 158. (ii) The RNA hairpin structure that blocks the *trpE* SD sequence was disrupted. The nucleotide substitutions introduced were as follows: C-to-T substitution at position 181 (C181→T), C182→G, T183→C, C186→T, T196→C, A198→C, G199→T, A200→T, G201→C, and A203→C (see Fig. 2). Mutagenesis was carried out in two steps, i.e., first, with oligonucleotide 5′-GGGAAGCGCTCCTTATGGAC AGCAAGAATGAGAAGATGGCATGA-3′, and second, with oligonucleotide 5′-GCGGAAATGTTTGATTGGAAATTCATGGGAAGCGCTCCTTAT GG-3′. (iii) A combination of the two previous alterations, i.e., deleting the transcription terminator and disrupting the SD hairpin structure, was used. Each of the products of PCR mutagenesis was identified by restriction analysis. Loss of an *Xmn*I restriction site from the DNA region corresponding to the terminator structure was used to detect the terminator deletion. A new *Hae*III restriction site was created during mutagenesis of the hairpin structure that blocks the *trpE* SD sequence. The final products were verified by sequencing by the dideoxy chain termination method of Sanger et al. (28). The PCR products of the three mutagenesis procedures were digested with *Eco*RI and *Hin*dIII and cloned into the same restriction sites of ptrpBGI-PLK to yield plasmids ptrpBGIΔT, ptrpBGISD, and ptrpBGIΔT/SD, respectively. ptrpBGI-PLK is a derivative of ptrpBGI in which the *Eco*RI-*Hin*dIII region containing the *B. subtilis trp* promoter-leader region has been replaced by the polylinker of pTZ18R, leaving a promoterless *′lacZ* gene. This change in ptrpBGI-PLK makes it easier to select transformants because colonies plated on X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) agar will be blue only if they carry the *trp* promoter and mutated *trp* leader.

Other plasmids employed included plasmid pTZtrpE2ΔT, which is a pTZ18R derivative that contains the *Eco*RI-*Hin*dIII fragment from ptrpBGIΔT subcloned into its polylinker. Plasmid pJH101-trpE2 carries the wild-type *trpE* leader region and was described previously (8). These plasmids, pTZtrpE2ΔT and pJH101-trpE2, were used in in vitro transcription assays. pmtrABΩTc is a derivative of pTTmtrAB (11) in which *mtrB* has been disrupted by the insertion of the *Sma*I-*Dra*I fragment carrying the tetracycline resistance gene of plasmid pHY300PLK (17). *Escherichia coli* DH5α [*supE44* Δ*lacU169* (Φ80 *lacZ*Δ*M15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] was used as the host for plasmid constructions. Small-scale preparations of plasmids were obtained as described by Birnboim and Doly (9).

Bacterial strains and transformations. The *B. subtilis* strains used in this study are listed in Table 1. Plasmid ptrpBGI-PLK linearized with *Pst*I was used to transform *B. subtilis* W168, to give strain CYBS301 (*amyE*::[polylinker-pTZ18R-′lacZ Cm^r]). Transformation was carried out by natural competence (1); selection was for chloramphenicol resistance (5 μg/ml). Integration of this plasmid DNA was confirmed by testing amylase production by iodine staining as described by Sekiguchi et al. (29). This strain was further transformed with *Pst*I-linearized ptrpBGI, ptrpBGIΔT, ptrpBGISD, or ptrpBGIΔT/SD, to yield strains CYBS2199 {*amyE*::[*trpP*(−412 to +203) *trpE′-′lacZ* Cm^r]}, CYBS302 {*amyE*::[*trpP* (−412 to +203) Δ(+108 to +133) *trpE′-′lacZ* Cm^r]}, CYBS303 {*amyE*::[*trpP*(−412 to +203) SD^{mod} *trpE′-′lacZ* Cm^r]}, and CYBS304 {*amyE*::[*trpP*(−412 to +203) Δ(+108 to +133) SD^{mod} *trpE′-′lacZ* Cm^r]}, respectively (see Table 1, footnote *a*). Isogenic *mtrB* strains CYBS305, CYBS307, CYBS309, and CYBS311 were derived from CYBS2199, CYBS302, CYBS303, and CYBS304, respectively, by transformation with chromosomal DNA of strain CYBS222 (*mtrB222*) (11) and selecting for 5-fluorotryptophan resistance (200 μg/ml) on plates containing 0.2% glucose, 0.2% acid-hydrolyzed casein, and 1× minimal salts (32). The CYBS222 *mtrB* mutant strain carries a single base-pair deletion (nt +1207 of the wild-type sequence) near the end of *mtrB* that allows reading past the natural *mtrB* stop codon. Plasmid pmtrABΩTc, carrying the mtrBΩTc gene disruption, was used to transform W168 to yield strain CYBS223 (*mtrB*ΩTc). Transformants were selected on Luria broth agar plates supplemented with tetracycline at 10 μg/ml; they were subsequently assessed for 5-fluorotryptophan resistance. Chromosomal DNA of this strain was used to transform CYBS2199, CYBS302, CYBS303, and CYBS304 to yield the *mtrB* strains CYBS306, CYBS308, CYBS310, and CYBS312, respectively. These strains were tested to verify that they were tetracycline and 5-fluorotryptophan resistant.

Computer analysis of *trp* RNA leader secondary structures. Folding of wild-type and mutated *trp* leader RNA was predicted by use of the FOLDRNA program of the Genetics Computer Group (GCG) sequence analysis software package (versions 7 and 8; April 1991 and September 1994) based on that of Zuker and Stiegler (41). Two different conditions were considered, i.e., cells grown with and without added tryptophan. With added tryptophan, TRAP presumably would bind to the (G/U)AG repeats in *trp* leader RNA and prevent antiterminator formation; therefore, this segment of *trp* leader RNA (nt +36 to +91) was excluded from this analysis. Without tryptophan, TRAP does not bind to RNA; thus, intact *trp* leader RNA, from nt +1 to the *trpE* AUG, was considered in the folding analysis.

β-Gal activity measurements. *B. subtilis* cells were grown in minimal-CH medium (27) containing 0.2% acid-hydrolyzed casein, 0.2% glucose, and minimal salts (32) supplemented with 5 μg of chloramphenicol per ml in the presence or absence of 50 μg of tryptophan per ml. One milliliter of the culture was harvested at the late log phase (reading of 110 in a Klett colorimeter, filter no. 54) by

centrifugation, washed with cold 10 mM Tris hydrochloride (pH 7.5), and resuspended in 1 ml of Z buffer (24). Ten microliters of fresh lysozyme solution (10 mg/ml) was added, and the tubes were incubated for 5 min at 37°C. Ten microliters of 10% Triton X-100 was added, and the mixture was assayed for β-gal activity by the method of Miller (24). β-Gal activity is reported in Miller units and represents the average of three or more independent experiments.

***lacZ* mRNA measurements.** Cells were grown under the same conditions used for β-Gal assays and harvested at late log phase (Klett 110). The cells were poured into frozen killing mixture, and RNA was extracted as described by Ulmanen et al. (36). Briefly, each cell lysate was treated with RNase-free DNase I, extracted with phenol-chloroform, precipitated with ethanol, and dissolved in Tris-EDTA buffer. Quantification of specific *lacZ* mRNA was performed by slot blot hybridization by the following protocol: 4, 10, and 20 μg of total RNA from each culture were incubated in 2% formaldehyde–2.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C for 15 min, chilled on ice, and loaded onto the slot blotter (Hoefer). After filtration, membranes (MagnaGraph nylon membrane) were baked in a vacuum oven at 80°C for 1 h. Prehybridization was performed at 65°C for 1 h in a buffer containing 5× SSC, 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), formamide at a final concentration of 50%, and 150 μg of calf thymus DNA per ml. Hybridization was carried out at 42°C for 12 h with the prehybridization buffer mixed with the probe. An internal 2,121-bp *Bgl*I-*Bgl*I fragment of *lacZ* was labeled by nick translation as described by Rigby et al. (26) and used as a probe. The filters were washed successively twice for 10 min with 2× SSC–0.1% SDS at room temperature, twice for 10 min with 1× SSC–0.1% SDS at 65°C, and twice for 10 min with 0.1× SSC–0.1% SDS at 65°C. The filters were exposed to X-ray film for 4 h. Subsequently, slots were excised from the filters, and the radioactivity was quantified in a liquid scintillation counter (Beckman). In comparisons of *lacZ* mRNA levels, the counts per minute in *lacZ* mRNA isolated from strain CYBS305 grown in the absence of exogenous tryptophan was set at 100%, and all other values were related to this value. As a negative control for *lacZ* mRNA measurements, wild-type W168 and its *mtrB* derivative CYBS223 (both strains lack *lacZ*) were grown with or without tryptophan, and the average of the blank values obtained with these strains was subtracted from the values for *lacZ*-containing strains grown in the same medium.

In vitro transcription attenuation assays. In vitro transcription attenuation assays and TRAP purification were performed as described previously (6). Transcription reaction mixtures contained *B. subtilis* vegetative (σ^{43}) RNA polymerase, DNA template, TRAP, L-tryptophan, and ribonucleoside triphosphates. Reaction conditions were described previously (6). *Eco*RI-*Hin*dIII restriction fragments that contain the *B. subtilis trp* promoter and leader regions from plasmids pJH101-trpE2 and pTZtrpE2ΔT were used as DNA templates in transcription reactions. Modifications of the standard assay are described in the text or figure legends.

RESULTS

Construction of *B. subtilis* strains carrying mutated *trp* leader regions. Secondary-structure predictions were performed with *trp* leader RNA to identify the changes in leader RNA that should abolish transcription termination in the leader region without affecting translational regulation or should abolish translational regulation without affecting transcriptional regulation. To eliminate transcription termination, we deleted nt +108 to +133 (relative to the transcription start site) (Fig. 1); this deletion would remove essentially the entire terminator hairpin structure. As shown in Fig. 2, the predicted folding of this ΔT*trpL* RNA would be almost identical to the folding of WT*trpL* RNA, with the exception that the stem and loop of the terminator structure would be missing and the antiterminator structure would have some changes (Fig. 2). Predicted folding of *trp* leader RNA in the presence of L-tryptophan-activated TRAP was also analyzed. This analysis suggested that deletion of the terminator would have no effect on the formation of the hairpin structure proposed to regulate translation of *trpE* (Fig. 1B and Fig. 2).

A similar approach was used to predict mutational changes that would eliminate translational regulation without affecting transcription termination (Fig. 2). These mutational changes should destabilize the hairpin structure that is assumed to sequester the *trpE* SD sequence; the predicted Δ*G* for the mutated structure containing the *trpE* SD segment was −2.6 kcal/mol (ca. −11 kJ/mol) compared with −12.1 kcal/mol (ca. −50.6 kJ/mol) for the wild-type structure. The terminator deletion and the set of mutational changes around the SD region

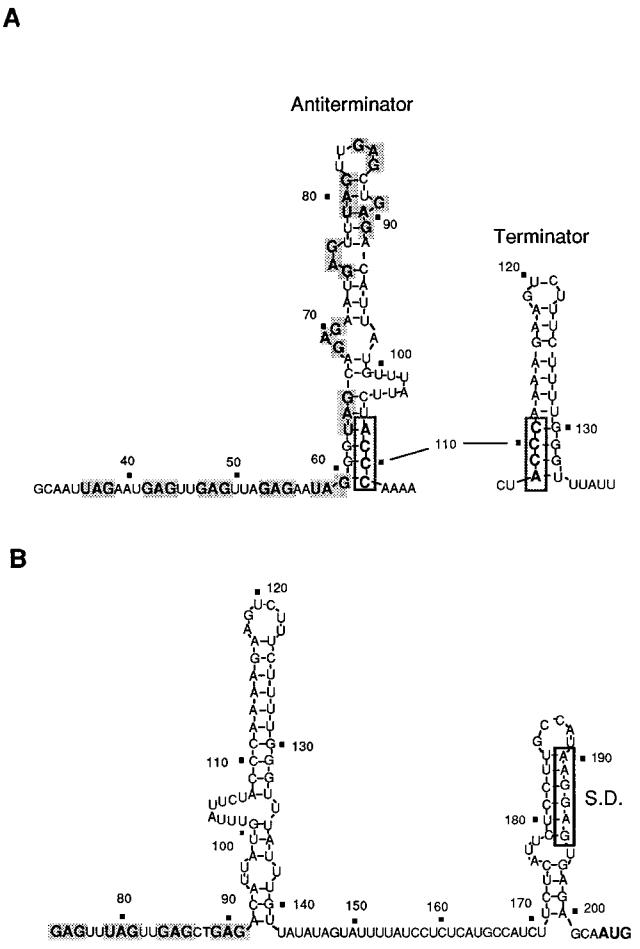

FIG. 1. Putative secondary structures involved in transcriptional and translational regulation of the *B. subtilis trp* operon. Numbers indicate the nucleotide positions relative to the transcription start site (+1). The (G/U)AG repeats proposed to be TRAP recognition sites are indicated in shaded boxes. (A) Presumed mutually exclusive antiterminator and terminator structures involved in transcription termination. The boxed sequence at positions +108 to +111 (5′-ACCC-3′) indicates the participation of these critical nucleotides in formation of alternate antiterminator and terminator structures. (B) Predicted secondary structure of the *trp* leader transcript segment involved in *trpE* translational regulation by tryptophan-activated TRAP. In this case, TRAP presumably would bind to the (G/U)AG repeats in *trp* leader RNA; therefore, only the remaining segment of the leader transcript (nt +92 to +206) is included in the folding analysis. The *trpE* SD site at positions +190 to +195, 5′-AAGGAG-3′, is boxed. The *trpE* start codon is in boldface type.

were also combined in a single construct. The predicted secondary structures in this ΔT/SD*trpL* RNA, in the presence or absence of active TRAP, suggest that this leader RNA should be free of secondary structures that would cause termination or interfere with translation (Fig. 2).

trpE′-′lacZ translational fusions were used to assess the relative effects of these changes on gene expression. PCR-mediated mutagenesis (22) was used to introduce desired changes in the *trp* leader region preceding a *trpE′-′lacZ* translational fusion. The products of independent mutagenesis strategies were cloned into the single-copy integration vector ptrpBGI-PLK and separately integrated into the *amyE* locus of *B. subtilis* strain W168 (*mtrB*$^+$). The resulting strains contain both an intact *trp* operon and a *trpE′-′lacZ* translational fusion under *trp* promoter control, with either a wild-type or a mutant *trp* leader region (Table 1). Four different strains were construct-

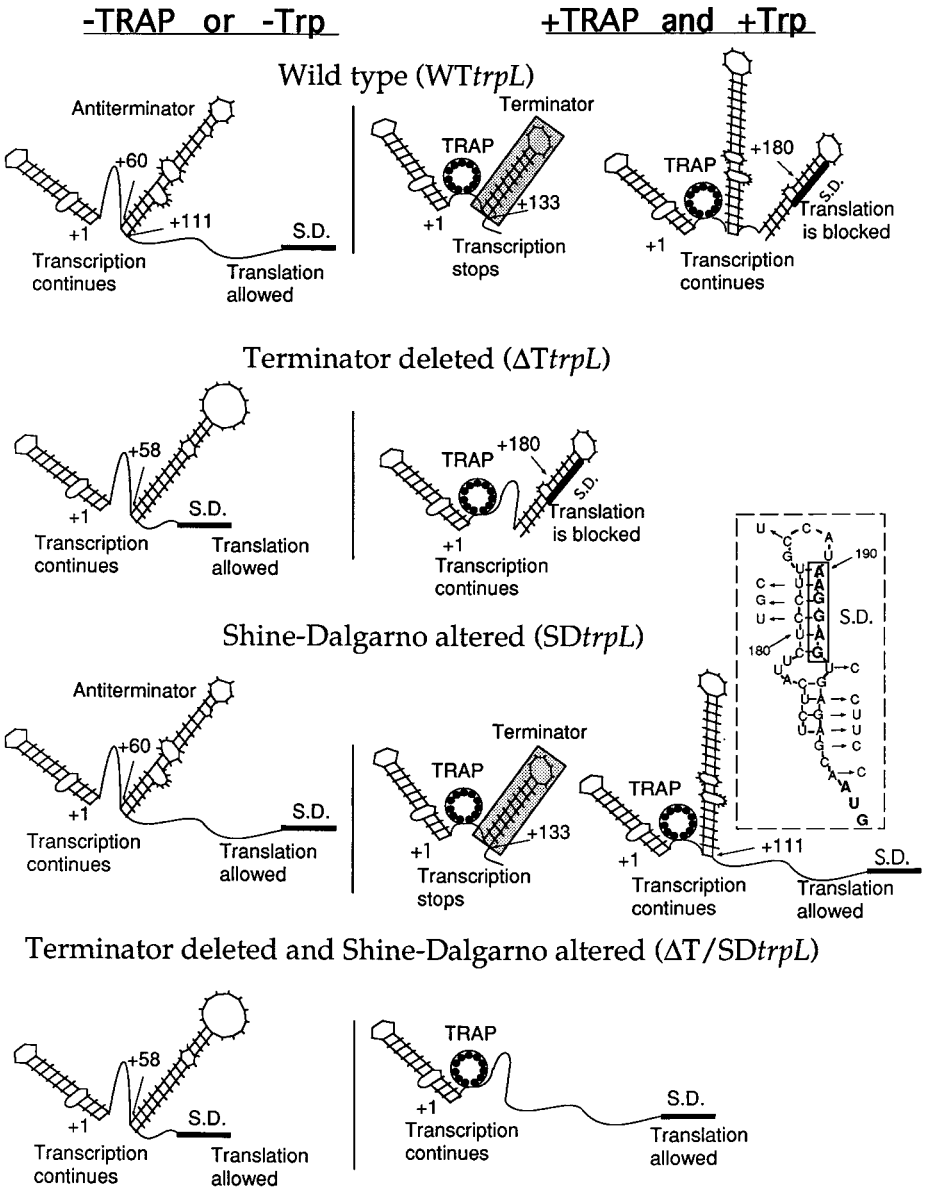

FIG. 2. Schematic representation of structures involved in transcriptional and translational regulation of the *B. subtilis trp* operon. Numbers indicate the nucleotide position relative to the transcription start site (+1). The relevant elements involved in *trp* operon regulation are represented as follows. TRAP protein is represented by an 11-circle ring. The putative transcription antiterminator structure is numbered +60 to +111. The transcription terminator structure is pictured in a shadowed box. The *trpE* SD site is indicated by a black bar labeled S.D. The hairpin structure proposed to be involved in translation regulation and nucleotide substitutions introduced by site-directed mutagenesis are shown enlarged in the dashed box. Our regulatory model is based on secondary structures predicted with the Genetics Computer Group versions of the computer programs of Zuker and Stiegler (41). Note that our schematic representations do not show the exact number of base pairs in the stems nor bases in the loops. See Materials and Methods for experimental details.

ed: CYBS2199, containing the wild-type *trp* leader region (WT*trpL*); CYBS302, carrying the terminator deletion in the *trp* leader (Δ*trpL*); CYBS303, containing nucleotide substitutions flanking the *trpE* SD sequence that presumably would abolish *trpE* translational regulation (SD*trpL*); and CYBS304, carrying both sets of mutational changes (ΔT/SD*trpL*). To eliminate TRAP regulation, the *mtrB222* point mutation (11) was introduced by transformation into CYBS2199, CYBS302, CYBS303, and CYBS304. The *mtrB*ΩTc gene disruption from strain CYBS223 was also introduced into the same group of strains (see Materials and Methods and Table 1).

Transcriptional and translational regulation of *trpE'-'lacZ* expression. To determine the extent of transcriptional and translational regulation of *trp* operon expression, we measured β-Gal activity and *lacZ* mRNA levels in strains containing a *trpE'-'lacZ* translational fusion controlled by the wild-type *trp* promoter-leader region (Table 2). The strains had either the wild-type *mtrB* allele or either of two mutant alleles, one with an inactivating point mutation and a second with an inactivating insertion. β-Gal levels should reflect the combined effects of transcriptional and translational regulation of the operon, whereas *lacZ* mRNA levels should be indicative of the extent of transcriptional regulation alone. A 1,180-fold difference (average) was observed between the β-Gal levels in strains with *mtrB* inactivated (CYBS305 or CYBS306) and the strain with *mtrB*$^+$ (CYBS2199) (Table 2; 1,740 and 2,000 Miller units

TABLE 2. β-Gal activity and *lacZ* mRNA levels in strains carrying various *trpE'-'lacZ* gene fusions[a]

Strain	Relevant genotype	β-Gal activity (Miller units)[b]			β-Gal −Trp/+Trp ratio	*lacZ* mRNA level (%)[c]			*lacZ* mRNA −Trp/+Trp ratio
		+Trp	−Trp	Avg[d]		+Trp	−Trp	Avg[d]	
CYBS2199	WT*trpL*	**1.6** ± 0.21	314 ± 36		196	**1.0** ± 0.25	21 ± 3.9		21
CYBS302	Δ*trpL*	3.4 ± 0.28	176 ± 21		52	8.4 ± 1.7	20 ± 4.1		2.4
CYBS303	SD*trpL*	23 ± 1.9	192 ± 25		8.3	0.85 ± 0.24	15 ± 3.8		18
CYBS304	Δ*T*/SD*trpL*	34 ± 3.7	136 ± 15		4.0	9.3 ± 1.9	13 ± 2.8		1.4
CYBS305	WT*trpL mtrB222*	**1,740** ± 123	1,900 ± 132	1,880	1.1	**83** ± 16	100 ± 19	88	1.2
CYBS306	WT*trpL mtrB*ΩTc	**2,000** ± 150	1,860 ± 142		0.9	**90** ± 21	77 ± 16		0.9
CYBS307	Δ*trpL mtrB222*	1,150 ± 128	1,160 ± 109	1,090	1.0	78 ± 17	65 ± 15	74	0.8
CYBS308	Δ*trpL mtrB*ΩTc	1,030 ± 113	1,010 ± 92		1.0	73 ± 16	79 ± 18		1.1
CYBS309	SD*trpL mtrB222*	711 ± 82	716 ± 69	714	1.0	56 ± 12	66 ± 16	58	1.2
CYBS310	SD*trpL mtrB*ΩTc	788 ± 91	640 ± 71		0.8	59 ± 13	53 ± 13		0.9
CYBS311	Δ*T*/SD*trpL mtrB222*	330 ± 43	304 ± 49	323	0.9	55 ± 12	46 ± 10	53	0.8
CYBS312	Δ*T*/SD*trpL mtrB*ΩTc	319 ± 37	340 ± 42		1.1	51 ± 10	59 ± 12		1.2

[a] The values shown are the averages (± standard deviations) of three independent experiments. Cultures were grown in minimal-CH medium containing 0.2% glucose, 0.2% acid-hydrolyzed casein, and 1× minimal salts with either no tryptophan (−Trp) or supplemented with 50 μg of tryptophan per ml (+Trp). The calculated values shown were obtained by use of the original values before they were rounded off.

[b] The activity assayed is β-Gal expressed from the *trpE'-'lacZ* fusion and is given in Miller units (24).

[c] The counts per minute per 10 μg of RNA for RNA from strains W168 and its *mtrB* derivative CYBS223 (grown with 50 μg of L-tryptophan per ml), each of which lacks the *trpE'-'lacZ* fusion, provided the blank values and were 40 and 52 cpm, respectively. The average of these values, 46, was subtracted from all other values. The *lacZ* mRNA levels presented are relative to that of strain CYBS305 grown in the absence of exogenous tryptophan (actual value = 31,100 cpm/10 μg), which was set at 100%.

[d] Avg, average of β-Gal values or *lacZ* mRNA levels in the +Trp and −Trp columns for *mtrB* strains with identical leader constructs (WT*trpL*, Δ*trpL*, SD*trpL*, or Δ*T*/SD*trpL*). For example, the average for WT*trpL* β-galactosidase was taken as (1,740 + 1,900 + 2,000 + 1,860)/4 = 1,875, rounded off to 1,880. Some of the rounded-off values presented were calculated with the original values before they were rounded off.

versus 1.6. Miller units [see boldface numbers]). An 88-fold difference (average) was observed between *lacZ* mRNA levels in the same strains (Table 2; 83 and 90% versus 1.0%). The significance of the difference between the *lacZ* mRNA levels depends on the accuracy of the *lacZ* mRNA measurement in the *mtrB*$^+$ strain grown with excess tryptophan. The *lacZ* mRNA value obtained in the presence of excess tryptophan, 1.0, was eight times greater than the control value obtained with a strain lacking the *trpE'-'lacZ* fusion. Thus, this basal value is significant. Since the extent of overall expression of the operon increased 1,180-fold and transcriptional control accounted for 88-fold regulation, the effects of translational control of *trpE* expression presumably added about 13-fold regulation.

Analysis of transcriptional and translational regulation in strains with the terminator deletion and/or the SD sequence alterations. To establish that transcriptional regulation was dependent on the RNA terminator and that translational regulation was due to the presumed RNA pairing that might mask the *trpE* SD region, β-Gal activity and *lacZ* mRNA levels were measured in our four test constructs. Analyses were performed in *mtrB*$^+$ and *mtrB* strains in cultures grown in the presence or absence of exogenous tryptophan. Minimal expression was observed in the *mtrB*$^+$ WT*trpL* strain CYBS2199 grown in the presence of tryptophan (1.6 U of β-Gal activity and 1.0% relative units of *lacZ* mRNA) (Table 2). The effect of exogenous tryptophan on expression of WT*trpL trpE'-'lacZ* can be assessed from the −Trp/+Trp ratios, which were 196 and 21, for β-Gal activity and *lacZ* mRNA levels, respectively (Table 2). The 21-fold difference observed in the *lacZ* mRNA level (i.e., −Trp/+Trp ratio) reflects transcriptional regulation in response to exogenous tryptophan, whereas the 196-fold difference in β-gal activity (i.e., −Trp/+Trp ratio) reflects both transcriptional and translational control. Note that expression of WT*trpL trpE'-'lacZ* in the *mtrB*$^+$ strain grown in the absence of exogenous tryptophan (−Trp) is lower than that in the corresponding *mtrB* strain (314 versus 1,900 Miller units and 21 versus 100% for β-gal activity and *lacZ* mRNA levels, respectively) (Table 2). These differences reflect the fact that there is an intermediate level of tryptophan in cultures growing without a tryptophan supplement.

Comparable experiments were performed with strains carrying *trp* leader alterations. Deletion of the terminator structure decreased the extent of regulation: the −Trp/+Trp ratios for β-gal activity and for *lacZ* mRNA level of strain CYBS302 were 52 and 2.4, respectively (Table 2). Thus, the extent of transcription regulation in response to exogenous tryptophan in this strain (−Trp/+Trp ratio) appears to be ninefold lower than that in strain CYBS2199 (WT*trpL*), and the *lacZ* mRNA −Trp/+Trp ratio, 2.4, approaches unity.

Alteration of the complementary sequence flanking the SD region also reduced regulation by exogenous tryptophan (Table 2, strain CYBS303). The β-Gal −Trp/+Trp ratio of this strain, 8.3, is significantly lower than the ratio, 196, in strain CYBS2199 (WT*trpL*). On the other hand, the *lacZ* mRNA −Trp/+Trp ratio, 18, is similar to the ratio in CYBS2199, 21. These results suggest that loss or reduction of translational regulation as a consequence of alterations around the SD region appears to leave transcriptional regulation relatively unaffected.

As expected, strain CYBS304, carrying both the terminator deletion and the altered SD region, had the lowest β-Gal activity and *lacZ* mRNA −Trp/+Trp ratios, 4 and 1.4, respectively, compared with strains CYBS2199, CYBS302, and CYBS303 (Table 2). The significant decrease in the extent of transcriptional and translational regulation is in good agreement with the model that there is both transcriptional and translational control of *trp* operon expression, with each having an independent effect on overall expression.

In *mtrB* strains lacking functional TRAP, *trp* operon expression should be maximal, and neither the β-Gal activity nor the level of *lacZ* mRNA should be affected by the addition of tryptophan. This expectation is supported by the data (Table 2) which show −Trp/+Trp ratios close to unity for both β-Gal activity and *lacZ* mRNA levels for strains CYBS305 through CYBS312.

The extent of transcriptional and translational regulation in vivo of different constructs. Differences between maximal *trp*

TABLE 3. Relationship of β-Gal activity to *lacZ* mRNA level in strains carrying different *trpL* constructs

trpL configuration	β-Gal activity[a]			β-Gal *mtrB*/*mtrB*$^+$ ratio	*lacZ* mRNA level[a]			*lacZ* mRNA *mtrB*/*mtrB*$^+$ ratio	β-Gal activity/*lacZ* mRNA		
	mtrB	*mtrB*$^+$			*mtrB*	*mtrB*$^+$			*mtrB*$^+$		*mtrB*
		+Trp	−Trp			+Trp	−Trp		+Trp	−Trp	
Wild type (WT*trpL*)	1,880	1.6	314	1,180	88	1.0	21	88	1.6	15	21
Terminator deleted (ΔT*trpL*)	1,090	3.4	176	320	74	8.4	20	8.8	0.41	8.8	15
SD region altered (SD*trpL*)	714	23	192	31	58	0.85	15	68	27	13	12
SD region altered and terminator deleted (ΔT/SD*trpL*)	323	34	136	9.5	53	9.3	13	5.7	3.7	11	6.1

[a] β-Gal activities (in Miller units) and *lacZ* mRNA levels (in percentages) are compiled from Table 2. For *mtrB* strains, the averages of β-Gal activities and the relative *lacZ* mRNA levels are presented. *mtrB*/*mtrB*$^+$ ratios were calculated by use of the values for *mtrB*$^+$ strains grown with tryptophan.

operon expression in *mtrB* mutant strains and minimal *trp* operon expression in *mtrB*$^+$ strains grown in the presence of exogenous tryptophan should indicate the extent of overall operon regulation for any *trp* leader region configuration. These values are given in Table 3. As mentioned, the extent of overall regulation in the strain with the WT*trpL* region is 1,180-fold, whereas the extent of transcriptional regulation is 88-fold (Table 3, columns 5 and 9). Accordingly, translational regulation can be assumed to account for the difference between the two, or 13-fold regulation (1,180/88 = 13). Translational regulation can also be assessed by comparing β-Gal values for the WT*trpL* strain with those for the SD*trpL* strain (1,180/31 = 38) (Table 3, column 5). Similarly, one can assess translational regulation by comparing the β-Gal values of strains with ΔT*trpL* versus those with ΔT/SD*trpL*. This difference is 320/9.5, or a 34-fold increase (Table 3, column 5). Possible explanations for the unexpected difference between these estimates of translational control, 38- and 34-fold, and the estimate given above, 13-fold, are discussed below. By comparison, the *lacZ* mRNA values for WT*trpL* and SD*trpL* (88/68, or 1.3-fold) and for ΔT*trpL* and ΔT/SD*trpL* (8.8/5.7, or 1.5-fold) (Table 3) suggest that the SD alterations have no appreciable effect on the *lacZ* mRNA level.

The translation yield per unit of *lacZ* mRNA (β-Gal activity/*lacZ* mRNA) is presented in Table 3, columns 10 through 12. This yield was expected to be low when translational regulation occurs and high when there is no translational regulation. In agreement with this expectation, the β-Gal/mRNA ratios in the presence of excess tryptophan (+Trp) for the WT*trpL* and ΔT*trpL* constructs were small, 1.6 and 0.41, respectively, whereas in the SD*trpL* construct in which translation regulation is presumed to be partially relieved, this ratio was 27. In the double mutant ΔT/SD*trpL*, the translation yield also should be high, but it was only 3.7. However, this value is close to the value for its corresponding *mtrB* strain, 6.1, suggesting that some other factor is responsible for the low value. Apparently, introduction of multiple changes in the leader region has unanticipated effects on transcript levels and transcript translation. When cells are grown in the absence of exogenous tryptophan (−Trp), the β-Gal/mRNA ratios for the WT*trpL* and ΔT*trpL* constructs are 10 to 20 times higher than they are in cells grown with tryptophan (+Trp), as shown in Table 3, columns 10 and 11. This could be due to the presence of the SD blocking sequence. In agreement with this interpretation, β-Gal/mRNA ratios for strains carrying the altered SD region (SD*trpL* or ΔT/SD*trpL* constructs) were much less sensitive to the presence of tryptophan in the culture medium. These findings support the conclusion that alteration of the SD flanking region reduces translational regulation. In most cases, the translational yields for *mtrB*$^+$ strains under −Trp conditions were similar to the values for *mtrB* strains (Table 3, columns 11 and 12). Maximum translational yield was expected in *mtrB* strains, and the yield was anticipated to be the same in all *mtrB* strains, regardless of the *trp* leader alteration. This expectation was not realized, and the translational yield varied depending on the *trp* leader construct, as can be seen in Table 3, column 12. This finding is discussed below.

Examination of the effect of the terminator deletion on transcription termination in vitro. In vitro transcription analyses (6) were used to examine the extent of transcription termination with template DNA from which the *trp* leader terminator region had been deleted. Plasmids pJH101-trpE2 and pTZtrp E2ΔT were used as templates in a reaction mixture containing *B. subtilis* vegetative (σ^{43}) RNA polymerase, TRAP, L-tryptophan, and the four ribonucleoside triphosphates, one of which was radioactively labeled. The major transcript produced from the wild-type template in the absence of L-tryptophan, TRAP, or both, was the 320-nt read-through transcript. A faint 140-nt RNA species corresponding to transcripts that terminated at the attenuator was also detected (Fig. 3, lanes 1 to 3). In the presence of L-tryptophan and TRAP, the 140-nt terminated transcript was observed to be the most abundant RNA species (Fig. 3, lane 4). Thus, as demonstrated previously, transcription termination in the wild-type leader region in vitro is dependent on the presence of TRAP and L-tryptophan (6). When

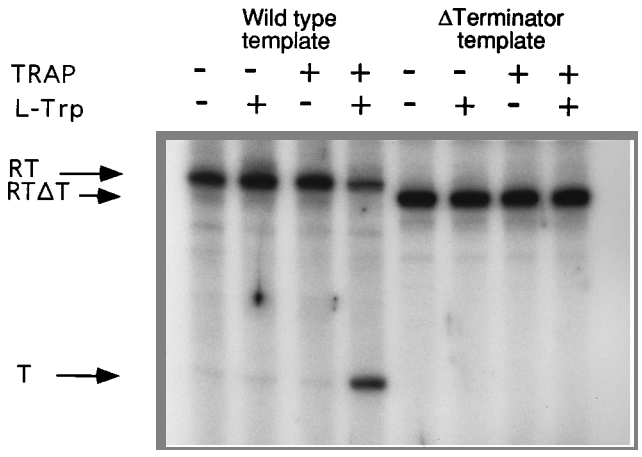

FIG. 3. Deletion of the *trp* leader terminator eliminates transcription termination in vitro. The presence (+) or absence (−) of TRAP (0.25 μg) or L-tryptophan (100 μg/ml) is indicated. Arrows indicate the positions of the 320-nt read-through (RT) and the 140-nt terminated (T) transcripts generated from the wild-type template and the 294-nt read-through (RTΔT) transcripts generated from the terminator deletion template.

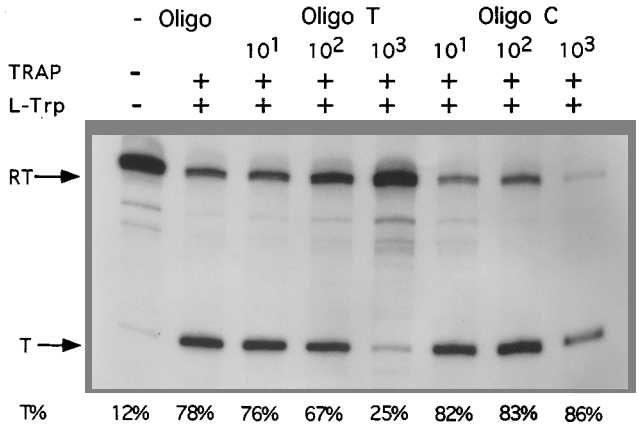

FIG. 4. Oligonucleotide competition in vitro transcription assay. DNA oligonucleotides added to the assay mixture were complementary to nt 106 to 120 (oligo T) or nt 10 to 36 (oligo C) of the *trp* leader transcript. Oligonucleotides were added in 10-fold (10^1), 100-fold (10^2), or 1,000-fold (10^3) molar excess over template DNA. TRAP (0.25 μg) or L-tryptophan (100 μg/ml) was either present (+) or absent (−). Arrows indicate the positions of the 320-nt read-through (RT) and 140-nt terminated (T) transcripts. Gel slices corresponding to the RT and T bands were excised from the gel, and radioactivity was quantified in a liquid scintillation counter. The molar percentage of terminated transcripts is indicated at the bottom of each lane.

pTZtrpE2ΔT DNA, lacking the terminator, was used as the template, the transcripts produced were 294 nt in length, even in the presence of both TRAP and L-tryptophan (Fig. 3, lanes 5 to 8). The difference in length between the read-through transcripts obtained with the wild-type and deletion templates is due to the 26-nt deletion in the latter template. These results demonstrate that deletion of the putative transcription terminator structure abolishes transcription termination in vitro.

An oligonucleotide complementary to a segment of the transcription terminator structure reduces transcription termination in vitro. Previous studies and the in vitro analyses performed with leader deletion DNA described above suggest that a putative stem-and-loop structure in *trp* leader RNA (nt +108 to +133) is required for transcription termination (6, 19, 25, 31). To provide additional support for this model, we performed in vitro transcription assays in the presence of an oligonucleotide complementary to part of the hairpin structure. This approach was used previously with the *E. coli trp* operon (38). Transcription was carried out with a wild-type DNA template in a reaction mixture containing *B. subtilis* vegetative (σ^{43}) RNA polymerase, TRAP, L-tryptophan, and ribonucleoside triphosphates in the presence or absence of a complementary oligonucleotide (Fig. 4). The oligonucleotide used, oligo T, is complementary to the 5′ stem of the hairpin terminator structure (Fig. 1A, nt +106 to +120). We expected that oligo T would base pair with the 5′ half of the terminator stem, which would prevent formation of the structure, thereby preventing termination, even in the presence of activated TRAP. Formation of the 140-nt terminated transcript would indicate that termination had occurred. Different oligonucleotide/template ratios were examined (10^1, 10^2, and 10^3-fold molar excess over that of the template) in the presence of TRAP and L-tryptophan. In Fig. 4, lane 1, it can be seen that in the absence of TRAP and L-tryptophan, the majority of the transcripts were full length (the percent termination, 12%, is given at the bottom of the lane). The addition of TRAP and L-tryptophan (lane 2) increased the percentage of terminated transcripts to 78%. As predicted, the addition of increasing amounts of oligo T to the transcription reaction decreased the percentage of terminated transcripts (lanes 3 to 5). By contrast, a control oligonucleotide, oligo C, complementary to nt +10 to +36 of the *trp* leader transcript, had no effect on the extent of termination (Fig. 4, lanes 6 to 8). These results support the conclusion that formation of the base-paired hairpin terminator structure is essential for transcription termination in the *trp* leader region.

DISCUSSION

Previous studies in which expression of the *trp* operon of *B. subtilis* was measured indicated that there is appreciable regulation of the operon in response to changes in the availability of tryptophan (19, 30, 31). It was also found that mutations in either of two genes, *mtrB* and *trpS*, result in elevated expression of the operon, despite the presence of excess tryptophan in the culture medium (13, 14, 33). Subsequent molecular analyses established that transcription of the *trp* operon is regulated by transcription attenuation at a transcription termination site located in the leader region of the operon (5, 6, 11, 19, 25, 31). Mutational studies and in vivo and in vitro experiments indicated that the *trp* leader transcript could fold to form alternative hairpin structures, one of which would serve as a transcription terminator (6, 19, 25). The terminator presumably forms whenever the L-tryptophan-activated regulatory protein, TRAP, the product of *mtrB*, is bound to a segment of the *trp* leader transcript containing 11 (G/U)AG repeats, 6 of which are present in the antiterminator structure (Fig. 1A) (3–5).

The wide range in the levels of expression of the operon in response to the presence or absence of exogenous tryptophan or active TRAP, approximately 1,000-fold, raises the possibility that the operon is regulated by some mechanism in addition to transcription termination. RNA secondary-structure predictions for *trp* leader RNA suggested that when TRAP was bound to the antiterminator segment, an additional downstream RNA hairpin structure that would block the *trpE* SD sequence could form (19). The initial test of this possibility supported this hypothesis (19).

In the present study, experiments to determine the extent of transcriptional and translational regulation of the *trp* operon by TRAP and L-tryptophan were performed. A *trp* regulatory module, consisting of the intact *trp* promoter-leader-*trpE* region in which *trpE* was fused in frame to *lacZ* as a reporter, was constructed (30). This module was integrated at the *amyE* locus of *B. subtilis*. Using strains with various alterations of this module, we measured transcription levels and translation of *trp* promoter-leader *trpE′-′lacZ* as an indication of the extent of operon regulation. We made no attempt to determine the separate contributions of transcription initiation and mRNA degradation; our *trpE′-′lacZ* mRNA values reflect steady-state transcription levels. We performed analyses in strains with mutant or wild-type *mtrB* following growth in media with or without tryptophan. To assess the role of the terminator hairpin in operon regulation, we deleted the corresponding DNA segment while ensuring that formation of other hairpin structures would not be affected. We performed in vitro experiments that established the role of the terminator hairpin in termination and demonstrated that our deletion of the terminator did prevent termination. We also introduced multiple mutations in the leader region that would reduce or eliminate base pairing in the RNA segment containing the SD sequence for *trpE*.

Our in vivo findings establish that in strains with the wild-type *trp* leader region, the presence of functional TRAP and tryptophan can regulate transcription of *trpE′-′lacZ* 88-fold and can regulate overall expression of *trpE′-′lacZ* 1,180-fold.

These values could indicate that there is 13-fold regulation of translation of *trpE'-'lacZ* that is independent of transcriptional control; i.e., in the absence of functional TRAP, *trpE'-'lacZ* could be translated more efficiently. In an *mtrB*$^+$ strain grown in a tryptophan-free medium (compared with a medium with tryptophan), transcription increased 21-fold rather than 88-fold and β-Gal activity increased 196-fold rather than 1,180-fold. Therefore, when there is intermediate expression of the wild-type *trp* operon, the level of translational expression is about 10-fold higher than could be accounted for by transcriptional regulation alone.

To establish that translational expression was partially independent of transcriptional control, we examined constructs in which the terminator was deleted and in which the region complementary to the SD region was mutationally altered. It was our expectation that deleting the terminator or mutating the SD pairing region would have little or no effect on expression in strains with an inactive *mtrB* locus. The data in Table 2 show that this was not the case. In *mtrB* strains, overall expression was reduced by a factor of two by the terminator deletion, by a factor of almost three by the SD alterations, and by a factor of six when these alterations were combined (Table 2, β-Gal activity). When transcription alone was measured, the terminator deletion decreased the *lacZ* mRNA level only slightly. When the SD region was altered or the terminator deletion and SD region alterations were combined, the transcript level was reduced to approximately half (Table 2, *lacZ* mRNA level). These findings suggest that the alterations we have introduced in the leader region do affect transcript survival slightly and translation of the surviving transcripts significantly. In agreement with this conclusion, the β-Gal/*lacZ* mRNA ratios in *mtrB* strains (Table 3) indicate that each of our alterations decreases the β-Gal yield slightly. In view of these deviations from expectations, the values obtained with the leader alterations in *mtrB*$^+$ strains must be analyzed cautiously.

Despite these complications, our findings demonstrate that deleting the terminator increases *lacZ* mRNA levels in *mtrB*$^+$ cultures in the presence of tryptophan eightfold, whereas altering the SD region has no effect on the mRNA level under comparable conditions (Table 2). In contrast, altering the SD region increases the β-Gal level in *mtrB*$^+$ cultures in the presence of tryptophan 14-fold without affecting the *lacZ* mRNA level; deleting the terminator results in only a 2-fold elevation of the β-gal level, although it increases the *lacZ* mRNA level 8-fold (Table 2). These findings provide convincing proof that TRAP binding to the *trp* leader transcript affects translation of *trpE* as well as transcription termination in the leader region. We have no explanation for the observation that deleting the terminator increases the *lacZ* mRNA level only 8-fold rather than the expected 88-fold. Either the deletion strain retains some TRAP-dependent transcription termination in vivo or its transcript is subject to some form of TRAP-dependent mRNA degradation. In agreement with this hypothesis, Kuroda et al. (19) showed that the addition of tryptophan to *mtrB*$^+$ strains carrying a *trpE'-'lacZ* gene fusion did increase the rate of *lacZ* mRNA decay. Previous regulatory studies indicated that TRAP does not regulate initiation of transcription of the *trp* operon.

Attenuation in the *trp* operon of *E. coli* and many other bacterial species responds to changes in the level of charged $tRNA^{Trp}$ (42). In addition, in these species, the level of free tryptophan is sensed by a repressor protein. Studies performed to date with the TRAP protein of *B. subtilis* indicate that tryptophan is the ligand that activates this regulatory protein (5–7, 25). However, the existence of *trpS* mutants in which *trp* operon expression is elevated when the synthetase is partially defective (33) implies that in this species, charging of $tRNA^{Trp}$ also is being sensed. Exactly how this is done is currently being investigated.

At its normal chromosomal location, the *trp* operon of *B. subtilis* is immediately downstream from three genes involved in aromatic metabolism, *aroF-aroB-aroH*, and upstream of three additional aromatic genes, *hisH-tyrA-aroE*, in what has been termed a supraoperon (13). Nucleotide sequence analyses revealed that there is no transcription terminator between the three upstream genes and the *trp* promoter (12). Thus, transcription initiated at the *aroF* promoter presumably continues through the *trp* promoter region into the *trp* leader region and, like *trp* promoter-initiated transcripts, can be terminated at the *trp* attenuator by L-tryptophan-activated TRAP. It is known that transcription initiation at the *aroF* promoter is regulated by aromatic amino acids (18), but it is not known what fraction of transcription proceeding over the *trp* leader region is due to transcription initiated at the *aroF* promoter versus the *trp* promoter. Presumably, the presence of aromatic amino acids in the growth medium influences the extent of transcription into the *trp* operon from the *aroF* promoter. Our constructs were designed with the intent of examining expression from the *trp* promoter exclusively. It is likely, therefore, that in vivo regulation of transcription of the *trp* operon is somewhat more complex than that described here.

The number of *B. subtilis* operons known to be controlled by regulatory mechanisms similar to the mechanism operating in the *trp* operon has increased recently. Both the *pur* and *pyr* operons of *B. subtilis* are regulated by transcription attenuation, and the transcripts of both operons can form alternative antiterminator-terminator secondary structures (35, 40). The regulatory regions of these operons resemble that of the *trp* operon, suggesting that RNA-binding proteins may play a role in regulation. Transcription attenuation in the *bgl* operon of *E. coli* also involves the action of an RNA-binding regulatory protein (16). The classic examples of translational regulation by RNA secondary structures involve control of synthesis of the coat protein of phage R17 (34) and regulation of synthesis of the products of the plasmid-borne genes that confer chloramphenicol and erythromycin resistance in bacteria, *cat* and *erm*, respectively (10, 21, 37). In the latter two examples, the presence of the drug leads to disruption of an RNA hairpin structure that blocks the ribosome-binding site that is used for synthesis of the protein that confers drug resistance. In contrast, in the present study, we show that binding of a regulatory protein, TRAP, to the *trp* leader transcript regulates operon expression transcriptionally as well as translationally. Thus, translational control also is used to ensure that tryptophan biosynthesis is effectively shut down when cells have adequate levels of tryptophan.

ACKNOWLEDGMENTS

We are grateful to Virginia Horn for expert technical assistance. We thank Michael Chamberlin for purified *B. subtilis* σ^{43} RNA polymerase. We are indebted to Rosana Sánchez for continued interest in this project, her thoughtful advice, and critical reading of the manuscript. We also thank Kurt Gish, Peter Margolis, M.-C. Yee, and Joseph Sarsero for critical reading of the paper.

E.M. is a postdoctoral member of the Pew Latin American Fellows program. P.B. was a postdoctoral fellow of the National Institutes of Health (grant GM14396). This study was supported by National Institutes of Health grant GM09738.

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