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Regulation of Tryptophanyl-tRNA Synthetase Formation

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A previously constructed *trpS-lacZ* fusion encoding a hybrid protein with β-galactosidase activity was subcloned from a multicopy plasmid onto a λ vector. Single-copy lysogens of λ *trpS-lacZ* were used to determine whether *trpS* was regulated in a manner similar to that of other aminoacyl-tRNA synthetases. *trpS* regulation was found to resemble that of the majority of synthetases, in that expression of the lysogen-encoded hybrid β-galactosidase varied with growth rate; β-galactosidase activity increased 2.5-fold as the generation time decreased from 150 to 37 min. This regulatory response was confirmed by DNA/RNA hybridization experiments, which also suggested that this form of metabolic regulation occurred at the transcriptional level. No alteration in the level of hybrid β-galactosidase was observed, however, when cells were starved for tryptophan.

The primary function of tryptophanyl-tRNA synthetase within the cell is to catalyze the aminoacylation of $tRNA^{Trp}$ (26). In addition, it has an indirect role, through its product-charged $tRNA^{Trp}$, in the regulation of transcription of the tryptophan biosynthetic operon (*trp*) (31). Our interest in this secondary regulatory role of tryptophanyl-tRNA synthetase led us to examine the regulation of the formation of the enzyme itself.

Tryptophanyl-tRNA synthetase belongs to a class of 20 aminoacyl-tRNA synthetases. Regulation of the synthesis of these enzymes is accomplished in at least two ways. One mechanism, metabolic regulation, which affects all the aminoacyl-tRNA synthetases studied to date (19), results in two- to threefold increased rates of enzyme formation, concomitant with an increase in growth rate. Superimposed on metabolic regulation is an individual regulatory response to limiting amounts of the cognate amino acid. At least half of the aminoacyl-tRNA synthetases are synthesized more rapidly in response to starvation for the respective amino acid (17, 20). However, this response is variable, and the mechanisms involved in regulating the formation of at least two of the aminoacyl-tRNA synthetases appear to differ (14, 22). To determine whether regulation of tryptophanyl-tRNA synthetase formation falls into either of the classes mentioned, we used a *trpS-lacZ* fusion (10) as an indicator of regulatory behavior. We subcloned the *trpS-lacZ* fusion onto a λ vector and subsequently isolated lysogens containing a single copy of the fused gene. We analyzed hybrid β-galactosidase formation in different lysogenic strains under a variety of growth conditions. The level of hybrid β-galactosidase was found to be subject to metabolic regulation. However, no alteration of β-galactosidase levels was observed when cells were starved of tryptophan.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains employed are described in Table 1.

Construction and use of hybrid phage λ *trpS-lacZ*. The procedure employed is outlined in Fig. 1. pCH22 was digested with *Bam*HI and *Bgl*II. The staggered ends of the resulting 8.9 and 5.6-kilobase (kb) fragments were filled in with DNA polymerase I in the presence of the four deoxynucleoside triphosphates (27). The 8.9-kb fragment was isolated from an 0.8% agarose gel and synthetic *Hin*dIII linkers (dCCAAGCTTGG; Collaborative Research) ligated to its blunt ends (25). In a separate reaction, λ 634 DNA (4) was digested with *Hin*dIII to yield three fragments, the left and right arms of λ and a fragment coding for *supE*. After inactivation of the *Hin*dIII restriction endonuclease by ethanol precipitation, the *Hin*dIII-digested λ 634 DNA was ligated (9) with the 8.9-kb fragment from pCH22 bearing *Hin*dIII ends, and the mixture was used to transfect the *lac* deletion strain, M182 (6). Blue (Lac^+) colonies were selected on Xgal(5-bromo-4-chloro-3-indolyl-β-D-galactoside)-containing minimal agar. Several blue colonies were isolated, and the recombinant phage was recovered (21). One recombinant phage, λ *trpS-lacZ* was chosen for the studies presented here. To insure the production of single lysogens, phage were added at a multiplicity of infection of 0.05 to recipient strains (see below). Serial dilutions of the cell-phage mixture were plated on Xgal-containing minimal agar, and blue colonies were picked and purified.

Determination of *trpS* mRNA. Hybridization of DNA fragment *Hae*III-*Hin*cII$_{500}$ to total *Escherichia coli* RNA was performed as described elsewhere (2, 3). The relative location of the DNA fragment in pCH22 is shown in Fig. 1. After treatment with S1 nuclease, the

TABLE 1. Bacterial strains

Strain	Genotype[a]	Characteristics
W3110		Wild type
CH1	Δ*lac-169*	*lac* deletion[b]
CH2	Δ*lac-169 trpR*	*lac* deletion, inactive *trp* repressor[b]
CH3	Δ*lac-169 trpA46PR9*	*lac* deletion, *trp* synthetase α chain with lower activity than wild type[b]
CH4	*trpS003*	Tryptophanyl-tRNA synthetase with reduced affinity for tryptophan; mutation results in tryptophan auxotrophy[c]
CH5	*trpS10343*	
CH6	*trpS10160*	
CH7	*trpR miaA*	Altered $tRNA^{Trp}$, inactive *trp* repressor (8)
M182	Δ(*lacIPOZY*)*74 galK galU rpsL*	*lac* deletion (6)

[a] All strains except M182 are derivatives of W3110 and carry the same *tnaA* allele.
[b] Constructed by R. Kelley
[c] W. F. Doolittle, Ph.D. thesis.

hybrids were electrophoresed on Tris-borate-EDTA-polyacrylamide gels (13), which were then dried and autoradiographed. The relative amount of label in each hybrid band was determined by densitometry of the autoradiogram.

Media. Vogel and Bonner minimal medium (30) was used in all experiments. The carbon sources utilized were 0.4% sodium acetate, 0.4% glucose, or 0.4% glucose plus 0.5% casein hydrolysate. Tryptophan was added at 30 μg/ml.

β-Galactoside assays. For the assays shown in Table 2, exponentially growing cells were harvested, washed with saline, and suspended in 0.1 M Tris-hydrochloride, pH 7.5. A cell extract was prepared by sonication, and β-galactosidase activity was determined by the method of Miller (15). Protein concentration was determined using Bradford reagent (5) with bovine serum albumin as a standard.

For the assays presented in Table 3, cells which had been at 37°C were directly assayed for β-galactosidase activity as described by Miller (15).

RESULTS

Construction of a λ phage containing a *trpS-lacZ* fusion. To maintain a constant copy number for regulatory studies, we subcloned the (*trpS-lacZ*$^+$) gene fusion onto a λ vector, as outlined in Fig. 1 and subsequently isolated lysogens. The DNA from one recombinant phage, λ *trpS-lacZ*, was analyzed by restriction mapping, using several enzymes (data not shown). The results demonstrated that no large deletions or obvious rearrangements had occurred during cloning. In addition, the orientation of the DNA insert within the *Hin*dIII site of the λ vector was determined from the restriction data (Fig. 1). We also examined the structure of the *trpS* regulatory

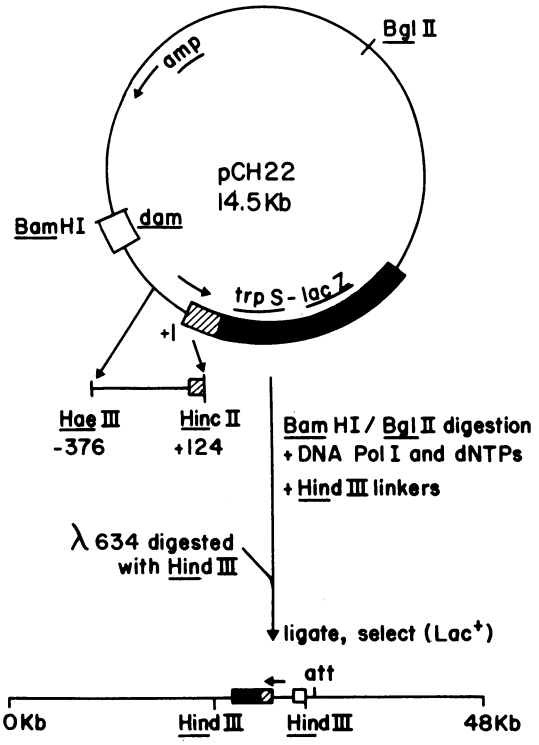

FIG. 1. Construction of a λ phage containing the *trpS-lacZ* fusion. pCH22 was digested with *Bam*HI and *Bgl*II, the resulting cohesive ends were filled in with DNA polymerase I and the four deoxynucleoside triphosphates (dNTPs), and ligated to *Hin*dIII linkers. λ 634 was digested with *Hin*dIII. After ligation and transfection, Lac$^+$ colonies were selected. One phage, λ *trpS-lacZ* was characterized, and the orientation of the 6.7-kb insert fragment on the phage was determined to be as shown. Arrows indicate the direction of transcription. *trpS*, *dam*, and *lacZ* are represented by a hatched bar, an open bar, and a solid bar, respectively. The *Hae*III/*Hin*cII$_{500}$ fragment from pCH22 is enlarged to show a more detailed map. The base pair coordinates are relative to the start of the structural gene at +1. The asterisk represents the 5′ ^{32}P label at the *Hin*cII site.

TABLE 2. Growth rate dependence of hybrid β-galactosidase activity

Carbon source	Doublings/h	μmol of ONPG hydrolyzed per min per mg of protein[a]
Acetate	0.4	43
Glucose	1.0	76
Glucose + casein hydrolysate	1.6	101

[a] Each number is the average of four or more determinations. Analyses were performed with CH1 lysogenized with λ*trpS-lacZ* as described in the text. ONPG, *O*-Nitrophenyl-β-D-galactopyranoside.

TABLE 3. β-galactosidase activity in different λ*trpS-lacZ* lysogens

Strain lysogenized with λ*trpS-lacZ*	Relevant mutation	Medium[a]	β-galactosidase activity[b]
CH1	Δ*lac*	Trp	28.7
			31
CH2	Δ*lac trpR*	Trp	25.8
			25.5
CH3	Δ*lac trpA46PR9*	Trp	27.4
			27.8
CH4	*trpS003*	Trp	27.3
CH5	*trpS10343*	Trp	27.7
CH6	*trpS10160*	Trp	26
CH7	*trpR miaA*		35

[a] Cells were grown in 0.2% glucose minimal medium. Trp indicates that the medium was supplemented with 30 μg of L-tryptophan per ml.

[b] Assays were performed as described by Miller (15).

region more closely by a modification of the procedure of Southern (24, 28; data not shown) and could detect no differences in this region (−1,000 to 500 bp; Fig. 1) between pCH22 and λ *trpS-lacZ*.

Growth-rate-dependent regulation of *trpS*. We determined the effect of growth rate on the expression of *trpS* by measuring hybrid β-galactosidase activity levels in a λ *trpS-lacZ* lysogen of CH1 grown in several media. Three different carbon sources, acetate, glucose, and glucose supplemented with casein hydrolysate, gave growth rates ranging from 0.4 to 1.6 doublings per h. We detected a 2.5-fold increase in the specific activity of β-galactosidase as the growth rate increased fourfold (Table 2).

The different levels of β-galactosidase activity in the λ *trpS-lacZ* lysogen grown in different media suggest that *trpS* is metabolically regulated. To determine whether this response was at the transcriptional level, we measured the relative amount of *trpS* message in two nonlysogenic strains, M182 and W3110. Each strain was grown in glucose-minimal medium. Total cellular RNA, isolated from each culture, was hybridized to an excess of single ^{32}P-end-labeled *Hae*III-*Hin*cII$_{500}$ DNA fragment under conditions favoring DNA/RNA hybridization (Fig. 2). Resulting DNA/RNA hybrids were treated with S1 nuclease to eliminate single strands and then separated on a polyacrylamide gel. The autoradiograms were quantitated by densitometry. The amount of *trpS* message was approximately twofold higher in each strain grown in glucose-minimal medium (Fig. 2, lanes A and C) as compared with acetate-minimal medium (Fig. 2, lanes B and D). Thus, a 2.5-fold increase in growth rate (acetate compared with glucose) results in both a twofold increase in hybrid β-galactosidase activity and *trpS* mRNA.

In addition to analyzing the amount of *trpS* message in nonlysogenic strains, we determined the amount of *trpS* message present in a λ *trpS-lacZ* lysogen of CH1. The presence of the prophage presumably doubled the number of copies of the *trpS* regulatory region and the first 500 bp of *trpS* (Fig. 1). Although the chromosomal *trpS*

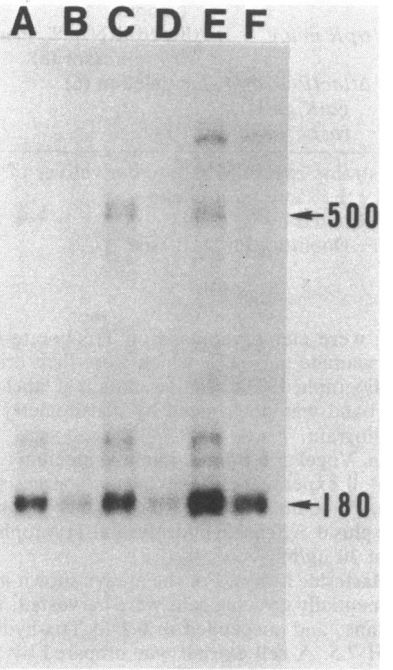

FIG. 2. Amount of *trpS* message in cells grown in acetate-minimal medium versus glucose-minimal medium. An excess of 5′ end-labeled *Hae*III/*Hin*cII$_{500}$ DNA fragment (Fig. 1) was hybridized with 100 μg of M182 RNA (lanes A and B); 100 μg of W3110 RNA (lanes C and D); 100 μg of CH1 lysogenized with λ *trpS-lacZ* RNA (lanes E and F). The RNA in lanes A, C, and E was isolated from cells grown in glucose-minimal medium, and the RNA in lanes B, D, and F was isolated from cells grown in acetate-minimal medium. The DNA/RNA hybrids were treated with endonuclease S1 and electrophoresed on a 7% TBE-polyacrylamide gel. The size of the bands are indicated to the right in base pairs. The 500-bp band corresponds to the *Hae*III/*Hin*cII$_{500}$ fragment, and the 180-bp band has been previously identified as the hybrid band (10). A nick-translated *Hin*fI digest of pCH6 was used to provide size markers (data not shown). The autoradiogram was traced by densitometry, the peaks were cut out and weighed, and the values were normalized to the amount of *trpS* message in 100 μg of M182 RNA from cells grown in acetate-minimal medium to give ratios of: lane A = 1.9, lane B = 1.0, lane C = 1.8, lane D = 0.9, lane F = 4.2, and lane F = 2.6.

and λ *trpS-lacZ* messages certainly differ due to the insertion of *lacZ* sequences in one, the DNA fragment used to quantitate *trpS* message spans only the first 124 bp of *trpS*; consequently hybridization of this fragment with either the chromosomal *trpS* or λ *trpS-lacZ* messages, followed by S1 nuclease treatment, yielded the same length of hybrid (Fig. 2, lanes E and F). With the lysogenic strain, we detected the same change in relative messenger levels at different growth rates as with nonlysogenic strains. In addition, the amount of *trpS* message detected in cultures of the lysogenic strain reflected the increase in copy number; i.e., twice as much *trpS* message was present in glucose- or acetate-grown lysogenic cells compared with nonlysogenic cells.

Effect of tryptophan limitation. To determine whether *trpS* expression is increased when cells are starved of tryptophan, we lysogenized CH3, a *lac* deletion strain carrying the *trpA46PR9* mutation, with λ *trpS-lacZ1*. β-galactosidase activity was measured in cells grown in glucose-minimal medium with or without tryptophan. The tryptophan synthetase α chain with the *trpA46PR9* mutation has much lower activity than the wild-type α chain, resulting in growth-limiting synthesis of tryptophan (32). In the absence of exogenous tryptophan, the *trp* operon was derepressed in strains with the *trpA46PR9* mutation, but the β-galactosidase activity of a *trpA46PR9* strain lysogenic for λ *trpS-lacZ1* remained unchanged (Table 3). We conclude that *trpS* is not derepressed in a strain that is starved for tryptophan.

Regulation of *trpS* and the *trp* operon. To determine whether *trpS* expression is influenced by mutations demonstrated to affect the expression of the *trp* operon, we constructed λ *trpS-lacZ1* lysogens of several such mutants. *trpR* mutations failed to produce active *trp* repressor protein and cause derepression of the *trp* operon. Mutations in *miaA* which result in undermodification of certain tRNAs, including $tRNA^{Trp}$ (8), and mutations in *trpS*, both lead to decreased transcription termination at the *trp* attenuator (18, 33). The amount of hybrid β-galactosidase synthesized by the lysogen was unaffected by the presence of these mutations (Table 3).

DISCUSSION

λ *trpS-lacZ* lysogens were used to determine the effect of growth rate on *trpS* expression. Metabolic regulation has been found for other aminoacyl-tRNA synthetases, for proteins involved in protein synthesis, and for ribosomal RNAs (12, 16). The expression of these diverse genes appears to depend on the growth rate of the cell: higher growth rates lead to increased expression. By measuring β-galactosidase levels in a λ *trpS-lacZ* lysogen grown in several different media, β-galactosidase activity was found to increase approximately 2.5-fold as the growth rate increased from 0.4 to 1.6 doublings per h. The extent of the increase was similar to that found for other aminoacyl-tRNA synthetases analyzed over a similar range of growth rates (19, 23).

To confirm that the levels of β-galactosidase in the λ *trpS-lacZ* lysogen were a true measure of *trpS* expression, the *trpS* mRNA level was measured in two nonlysogenic strains grown either in glucose-minimal medium or acetate-minimal medium. Quantitation of the relative amounts of *trpS* RNA from each strain established that the amount present in cells grown in glucose was twofold higher than that in cells grown in acetate. These results confirm those obtained with the *trpS-lacZ* fusion and indicate that growth rate regulation affects the transcription of *trpS*. In these experiments, *trpS* expression was measured when the gene was at its normal location, 73 min on the *E. coli* chromosome, and also when it was integrated at the λ attachment site at 17 min on the chromosome. Metabolic regulation was observed with both arrangements, demonstrating that chromosomal location relative to the origin of replication was not responsible for the observed metabolic effect.

The genes encoding a number of aminoacyl-tRNA synthetases exhibit either transient or continuous derepression during starvation for their cognate amino acid. A *trpS-lacZ* lysogen was starved for tryptophan by the introduction of a missense mutation, *trpA46PR9*, which reduces the activity of the tryptophan synthetase α chain. Levels of the hybrid β-galactosidase remained unchanged in the lysogenic *trpA46PR9* strain compared with the wild type, suggesting that *trpS* expression does not respond to changes in the levels of intracellular tryptophan.

Sequence comparisons between the *trpS* and *trp* biosynthetic operon regulatory regions revealed no similarities which would suggest a common regulatory mechanism. Nonetheless, three additional chromosomal mutations were tested which affect regulation of the *trp* operon: mutations in *trpS* which increase the enzyme's K_m for tryptophan, a mutation in *trpR* which inactivates the *trp* repressor, and a mutation in *miaA* which affects a tRNA modifying enzyme. Mutations in each of these three loci resulted in derepression of the *trp* operon. However, none of these mutations affected the level of β-galactosidase produced by the *trpS-lacZ* gene fusion. In addition, Doolittle (W. F. Doolittle, Ph.D. thesis, Stanford University, Stanford, Calif., 1968) has examined the effect of the tryptophan

analog indole-3-acrylic acid on the level of tryptophanyl-tRNA synthetase activity. This analog, which cannot be charged on to $tRNA^{Trp}$ by tryptophanyl-tRNA synthetase, decreases termination at the *trp* operon attenuator, but has no effect on tryptophanyl-tRNA synthetase formation.

Putney and Schimmel (22) have recently presented evidence suggesting that alanyl-tRNA synthetase represses the transcription of its gene, *alaS*, in vitro. Preliminary experiments in which a DNA fragment containing the *trpS* promoter was transcribed in vitro in the presence or absence of purified tryptophanyl-tRNA synthetase plus tryptophan showed no evidence of repression of *trpS* transcription (unpublished data). Furthermore, when the intracellular levels of tryptophanyl-tRNA synthetase are increased by the introduction of a multicopy *trpS* plasmid into a *trpS-lacZ* lysogen, the level of hybrid β-galactosidase activity remains unaltered.

We have examined several potential regulatory molecules that conceivably might affect *trpS* expression and have found none to be effective. Thus, we believe that *trpS* expression is constitutive. For leucyl-, valyl-, and seryl-tRNA synthetases (1, 7, 11), operator-like mutations which suggest the existence of a repressor protein have been isolated. In addition, mutations that increase the expression of leucyl-tRNA synthetase or seryl-tRNA synthetase have been mapped at sites distant from each synthetase structural gene and may encode repressor proteins (11, 29). The *trpS-lacZ* gene fusion we have constructed should prove useful for the selection of *trpS* regulatory mutants.

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