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Role of Regulatory Features of the *trp* Operon of *Escherichia coli* in Mediating a Response to a Nutritional Shift

CHARLES YANOFSKY* AND VIRGINIA HORN

Department of Biological Sciences, Stanford University, Stanford, California 94305-5020

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Physiological studies were performed under nutritional stress and nonstress conditions to assess the relative importance of the various regulatory mechanisms that *Escherichia coli* can use to alter its rate of tryptophan synthesis. Mutants were examined in which the *trp* repressor was inactive, transcription termination at the *trp* attenuator was altered, transcription initiation at the *trp* promoter was reduced, or feedback inhibition of anthranilate synthase was abolished. Strains were examined in media with and without tryptophan, phenylalanine and tyrosine, or acid-hydrolyzed casein and following shifts from one medium to another. Growth rates and anthranilate synthase levels were measured. In media lacking tryptophan, each of the mutants showed relief of repression and/or attenuation and maintained a near-normal growth rate. Following a shift from a medium containing tryptophan to a tryptophan-free medium containing phenylalanine and tyrosine or acid-hydrolyzed casein, mutants with abnormally low *trp* enzyme levels exhibited an appreciable growth lag before resuming growth. The wild-type strain displayed termination relief only under one extreme shift condition, upon transfer from a minimal medium containing tryptophan to minimal medium with only phenylalanine and tyrosine. A promoter down-mutant had difficulty adjusting to a shift from high tryptophan to low tryptophan levels in a medium containing acid-hydrolyzed casein. In all media tested, anthranilate synthase levels were lower in a feedback-resistant mutant than in the wild type. These studies demonstrate the capacity of *E. coli* to adjust its rate of tryptophan synthesis to maintain rapid growth following a shift to stressful nutritional conditions.

Escherichia coli has the ability to adjust its rate of tryptophan biosynthesis over a several-thousand-fold range. This impressive regulatory capacity emanates from the use of a relatively strong promoter to drive *trp* operon expression, repression and attenuation to regulate *trp* operon transcription, and feedback inhibition to regulate entry of the common aromatic precursor, chorismate, into the tryptophan biosynthetic pathway (36). In addition, tryptophan influences the flow of carbon into and through the common aromatic pathway that culminates in chorismate production (23). Repression in the *trp* operon is mediated by the tryptophan-activated *trp* repressor (30), and hence, transcription initiation at the *trp* promoter is regulated in response to changes in the intracellular concentration of tryptophan; repression regulates *trp* operon expression over a ca. 100-fold range. Synthesis of the *trp* repressor is autoregulated over about a threefold range, with the repressor level at a minimum when cells have excess tryptophan (8, 12). Transcription attenuation is modulated by changes in the concentration of charged $tRNA^{Trp}$ (13, 15). Cells that have charged $tRNA^{Trp}$ levels that are adequate to support protein synthesis generally terminate transcription at or near bp 140 in the transcribed leader region of the operon. Cells that have insufficient charged $tRNA^{Trp}$ do not terminate transcription at the attenuator; therefore, transcription proceeds into the structural genes of the operon. The cellular level of charged $tRNA^{Trp}$ is sensed by attempted translation of tandem Trp codons located in the coding region for the 14-residue *trp* leader peptide. The presence of only two Trp codons in this leader peptide coding region renders attenuation relatively insensitive to tryptophan starvation; i.e., only an extreme deficiency of charged $tRNA^{Trp}$ prevents transcription termination at the attenuator. Attenuation regulates transcription of the structural genes of the *trp* operon ca. six- to eightfold (11, 13). There is one additional regulatory feature of transcriptional attenuation in the *trp* operon. Cells that are incapable of initiating synthesis of the *trp* leader peptide experience five times greater termination at the attenuator than cells that can perform this function (24, 33, 43). This phenomenon has been termed superattenuation (33). Events that prevent or decrease synthesis of the *trp* leader peptide could cause superattenuation and lead to an additional fivefold increase in termination at the attenuator. Feedback inhibition of anthranilate synthase (ASase), the enzyme catalyzing the first reaction of tryptophan biosynthesis, plays a major role in tryptophan formation by regulating the flow of carbon into the tryptophan biosynthetic pathway (5, 22, 41).

Other structural features of the *trp* operon have significant effects on the rate of tryptophan synthesis. An inefficient constitutive promoter, *trpP2*, is located near the distal end of the second gene of the operon, *trpD* (10, 16). In cells growing with excess tryptophan, expression from this promoter is responsible for most of the synthesis of the TrpC, TrpB, and TrpA polypeptides. It is believed that elevated levels of these three polypeptides aid the organism to synthesize tryptophan when cells are shifted from a medium containing a high tryptophan concentration to a medium containing little or no tryptophan. An additional regulatory feature modulating the rates of synthesis of *trp* polypeptides is translational coupling (21, 29). Two pairs of *trp* operon products, the TrpE and TrpD polypeptides and the TrpB and TrpA polypeptides, function as enzyme complexes (36). Translational coupling renders efficient translation of *trpD* mRNA and *trpA* mRNA dependent on completion of translation of *trpE* mRNA and *trpB* mRNA, respectively. Coupling ensures that there is synthesis of equimolar levels of polypeptides that are components of the same enzyme complex (6, 21). One additional feature of

* Corresponding author. Phone: (415) 725-1835. Fax: (415) 725-8221.

regulatory importance is the absence of tryptophan from the TrpE polypeptide. The consequence of this deficiency is that cells deprived of tryptophan continue to synthesize this polypeptide, albeit at a lower rate (39). The increased TrpE level presumably helps the bacterium sequester the branch point intermediate, chorismate, into the tryptophan biosynthetic pathway. *E. coli* produces three permeases that can transport exogenous tryptophan: AroP, a general aromatic amino acid permease, and Mtr and TnaB, tryptophan-specific permeases (3, 27, 35). The *mtr* operon is negatively regulated by tryptophan and the *trp* repressor and positively regulated by tyrosine and the TyrR protein (9, 28). TnaB, a product of the *tna* operon, transports tryptophan when tryptophan is used as a carbon or nitrogen source. Expression of the *tna* operon is subject to tryptophan-induced transcription antitermination and catabolite repression (7, 32).

Since repression and attenuation involve different molecules and events, the effectiveness of each in regulating *trp* operon expression necessarily reflects the sensitivity of that regulatory mechanism to changes in growth conditions. In previous studies, we and others analyzed *trp* operon expression by measuring changes in the levels of *trp* mRNA and/or *trp* enzymes under several growth conditions (14, 17, 18, 24). Particular attention was directed towards determining the relative contributions of repression and attenuation as a function of the severity of tryptophan starvation and as a function of changes in the temperature of growth (35, 40). It was observed that repression was more sensitive than attenuation to changes in the intracellular concentration of tryptophan (40), consistent with the affinity of aporepressor for tryptophan and the presence of only two Trp codons in the leader peptide coding region. It was also observed that repression decreased and attenuation increased as the temperature of growth was increased (35). The effect of inactivating each of the tryptophan permeases, and various combinations thereof, on tryptophan transport was also examined (38).

In this paper, we extend these physiological studies on *trp* operon expression. We focus on the response of the organism in media lacking or containing aromatic amino acids. We examine nutritional shifts to and from media containing or lacking tryptophan in the presence and absence of the other aromatic amino acids. We ask what the effects on growth rate and in vivo *trp* operon expression are when we increase, decrease, or prevent a change in transcription termination at the attenuator. We also examine the effects of eliminating repression of the trp operon and eliminating feedback inhibition of ASase. We also determine the consequences of reducing *trp* promoter strength to 5% of the wild-type level and the effects of inactivating the internal promoter. Our findings document the utility to the organism of the ability to use alternate mechanisms to adjust *trp* enzyme levels and *trp* enzyme activity in response to a shift to a different medium.

MATERIALS AND METHODS

Bacterial strains employed and their characteristics. The various strains employed in this investigation are listed in Table 1. Strain CY15507 contains a cI^+ derivative of λTL constructed by Robert Landick of Washington University. λTL contains a *trpL'-'trpE'-'lacZ'* triple coding region translational fusion driven by the *trp* promoter-operator (40). Since this construct lacks the attenuator, transcription regulation is strictly by *trp* repressor-operator interaction. The strain containing this prophage is W3110 Δ*lacU169 tnaA2*. The nature of the mutational alterations in these strains and their effects on strain behavior are described as follows. *trpL29* mutant strain has a mutation of A to G at bp 29 in the leader region of the *trp* operon. This change replaces the leader peptide start codon by GUG and decreases operon expression in cells growing in the presence or absence of tryptophan (42, 43). *trpL75* mutant strain has a mutation of G to A at bp 75 in the leader region of the *trp* operon. This change decreases the stability of the transcript antiterminator structure and decreases operon expression in cells growing in the presence or absence of tryptophan (42, 43). *trpL117* mutant strain has a mutation of C to T at bp 117 in the leader region of the *trp* operon. This change decreases the stability of the transcript terminator and causes a three- to fourfold increase in operon expression in cells growing in the presence of excess tryptophan (31). *trpLAGG2* mutant strain has an insertion of two AGG codons preceding the tryptophan codons in the *trp* leader region. Introduction of these rare codons prevents relief of termination upon tryptophan starvation (25). *trpLAGG4* mutant strain has an insertion of four AGG codons preceding the tryptophan codons in the *trp* leader region. This change prevents relief of termination upon tryptophan starvation (25). *trpP156* mutant strain has a mutation of C to T at bp −32 of the *trp* promoter. This change reduces transcription initiation at the promoter to about 5% that of the wild-type promoter and does not affect repression. *tnaA2* mutant strain has a mutation in *tnaA*, the structural gene for tryptophanase, that results in inactivation of the enzyme (4). *trpR2* mutant strain has a mutation in the structural gene for the *trp* repressor that abolishes all repressor activity (37). *trpE19* mutant strain has a mutation in *trpE* that eliminates feedback inhibition of anthranilate synthase by tryptophan (5, 22). This mutation has no effect on the activity of the enzyme. *trpP2* mutant strain has an altered internal promoter (in *trpD*) that has no promoter activity. The wild-type −35 and −10 promoter sequences in *trpD*, CGTGAC and TTACAA, were replaced by CGCGAT and CTCCAG, respectively (the underlined nucleotides of the wild-type promoter were replaced) (40a).

TABLE 1. Bacterial strains

Strain	Relevant genotype[a]	Reference
CY15000	Wild type	37
CY15001	*trpR*	37
CY15006	*trpR trpL29*	37, 43
CY15007	*trpR trpL75*	37, 43
CY15008	*trpR trpL117*	31, 37
CY15501	*trpL75*	This study
CY15502	*trpL29*	This study
CY15503	*trpL117*	This study
CY15504	*trpP156*	This study
CY15505	*trpR trpP156*	This study
CY15506	*trpE19*	This study
CY15507	*trpR trpP156* (λTL)	This study
CY15508	*trpLAGG2*	25
CY15509	*trpLAGG4*	25
CY15510	*trpP2*	This study

[a] All strains are derivatives of *E. coli* W3110 and contained the *tnaA2* allele (CY15000). They are isogenic, and most were prepared by P1-mediated transduction. CY15507 is a *trp* promoter-operator reporter strain. It is lysogenic for a cI^+ derivative of λTL. In λTL, the *trp* promoter-operator drives expression of a triple coding region fusion composed of the *trp* leader peptide coding region, *trpL*, fused to *trpE*, which is fused to *lacZ* (40). See text for a description of each mutant allele.

Growth curve determinations. Vogel and Bonner minimal medium (34) was used throughout. Single colonies picked from minimal agar plates were used to inoculate tube cultures containing minimal medium plus 0.2% glucose with or without

one or more of the following supplements: (i) L-tryptophan (100 μg/ml), (ii) 0.05% acid-hydrolyzed casein, or (iii) L-phenylalanine (100 μg/ml) and L-tyrosine (50 μg/ml). Cultures were grown with shaking overnight at 37°C. The next morning, cultures were diluted in fresh medium of the same composition to a cell density of 10 Klett units (660-nm filter) and were grown with shaking at 37°C to 40 Klett units. Each culture was divided in half, and the cells were collected by centrifugation for 5 min at 5,000 rpm (Sorvall GLC-1 centrifuge) at room temperature. One pellet was resuspended and washed in the same medium; the second pellet was washed in the same or a different (shift) medium. Each suspension was centrifuged, and the wash cycle was repeated. Pellets were resuspended, and each culture was diluted to 20 Klett units. All wash media and growth media were prewarmed at 37°C. Cultures (10 ml in 125-ml Erlenmeyer flasks) were grown at 37°C in a New Brunswick rotary shaker. Cell density was measured at 10-min intervals. Duplicate cultures were taken throughout each procedure.

Samples for specific activity determinations. (i) Nutritional shift experiments. Initial cultures prepared as described above were grown with shaking at 37°C to 40 Klett units. A zero-time sample of 10 ml (equivalent to 400 Klett units of cells) was removed and chilled, and chloramphenicol was added (to 20 μg/ml) to stop protein synthesis. The remainder of each culture was pelleted by centrifugation at room temperature for 5 min at 5,000 rpm. Each pellet was washed twice in the same or a different medium without tryptophan and diluted to a density of 20 Klett units in fresh medium lacking tryptophan. Cultures were reincubated with shaking at 37°C. Cell density was recorded at frequent intervals, and samples were removed at appropriate times, usually 30-min intervals. Samples equivalent to 400 Klett units were removed and chilled, and chloramphenicol was added. Collected samples were centrifuged at 5°C for 10 min at 5,000 rpm, each pellet was washed in 5 ml of cold 0.85% NaCl, the samples were centrifuged again, and the pellets were stored overnight at −80°C.

(ii) Steady-state experiments. A log-phase culture at 40 Klett units was prepared as described above. A zero-time sample equivalent to 400 Klett units was taken. The remaining culture was centrifuged, washed twice in the same medium, and resuspended in the same medium at 20 Klett units. Cultures were incubated with shaking, samples equivalent to 400 Klett units were removed, generally at 30-min intervals, and chilled, and chloramphenicol was added. Samples were collected and processed as described above.

Enzyme assays. (i) ASase assay. Each frozen cell sample was thawed and suspended in 200 μl of permeabilization buffer (0.1% Triton X-100 in 100 mM Tris buffer [pH 7.8]). ASase activity was determined fluorometrically by measuring the conversion of chorismate plus glutamine to anthranilate (12). Specific activity was calculated as fluorometer units produced per 0.1 ml of a permeabilized cell preparation with an optical density of 12.5 (at 600 nm), following a 20-min incubation at 37°C. Fluorometer units were based on the following standard. Five microliters of a 1 mM solution of anthranilic acid was added to a tube containing the standard 0.5-ml reaction mixture, and the tube was incubated and processed with the assay tubes. Following acidification and extraction of anthranilate with ethyl acetate, the ethyl acetate extract of the standard tube was set at 100 fluorometer units. A blank reaction tube was treated similarly, and the ethyl acetate extract was set at zero. The fluorometer units in each assayed sample were then determined. The permeabilized cell assay was found to be unreliable for measurements of very low ASase levels. To determine the ASase levels of *trpR*$^+$ cultures grown in the presence of tryptophan, cell extracts were prepared and assayed. The assay components and conditions were as described above, and the values obtained were related to the values obtained with permeabilized cells.

(ii) β-Galactosidase assay. Samples for β-galactosidase assay were prepared and treated as described by Miller (20).

RESULTS

Steady-state *trp* operon expression in *trpR* and *trpR*$^+$ mutant strains growing in the three test media containing or lacking tryptophan. Mutations affecting repression, attenuation, promoter strength, and feedback inhibition were examined in this study. The characteristics of the various mutations examined and their effects are summarized in Materials and Methods. Two mutational changes, *trpL75* and *trpP156*, were studied most extensively. The *trpL75* mutation prevents the six- to eightfold increase in *trp* operon expression that is typically obtained upon severe tryptophan starvation. Rather, an approximately twofold increase is observed (42). The *trpL75* mutation has a second effect, namely, it increases transcription termination at the attenuator about fivefold in cells growing with excess tryptophan (42). The *trpP156* promoter down-mutation reduces transcription initiation to about 5% that of the wild type. This level of transcription initiation is slightly higher than in the wild type under maximal repression.

In our initial examination of the effects of these and other mutations on *trp* operon expression, we determined the steady-state ASase levels of log-phase *trpR* and *trpR*$^+$ cultures growing in six test media: minimal medium, minimal medium plus excess phenylalanine and tyrosine, and minimal medium plus 0.05% acid-hydrolyzed casein, each with or without tryptophan. The results of these analyses are presented in Fig. 1. The ASase levels of *trpR* cultures (Fig. 1A) were essentially identical in all the media, with or without tryptophan. The specific activity values were lower in the acid-hydrolyzed casein medium than in the other two media. This was noted previously (26) and is presumably largely due to the more rapid growth rate in this medium and the fact that the *trp* operon is near the terminus of chromosomal replication. The ASase levels of *trpR trpL75* cultures were also similar in all three media and were one-third to one-fifth that of the *trpR* strain. The growth rates of *trpR trpL75* and *trpR* cultures were identical in all media (data not shown), indicating that the lower enzyme levels in the *trpR trpL75* cultures were adequate to support maximal growth rates in the absence of added tryptophan. The *trpR trpP156* strain had very different ASase levels when grown with and without tryptophan in any of the media (Fig. 1A). The maximum levels attained were below those of the *trpR trpL75* strain. The greatest difference was observed in acid-hydrolyzed casein media, in which the absence of tryptophan resulted in about a fivefold increase in ASase specific activity. This increase is most likely due to severe tryptophan starvation imposed by the presence of the weak *trpP156* promoter. This presumed tryptophan starvation probably resulted in reduced transcription termination at the attenuator. Previous studies suggested that severe tryptophan starvation could lead to as much as a six- to eightfold increase in expression of the *trp* operon (1, 11). Most likely, transcription initiation at the mutant *trpP156* promoter cannot provide levels of the *trp* enzymes sufficient for rapid growth in the absence of tryptophan unless both repression and transcription termination at the attenuator are largely relieved. Consistent with this indication of nutritional stress, the doubling time of *trpR trpP156* cultures growing in acid-hydrolyzed casein medium lacking

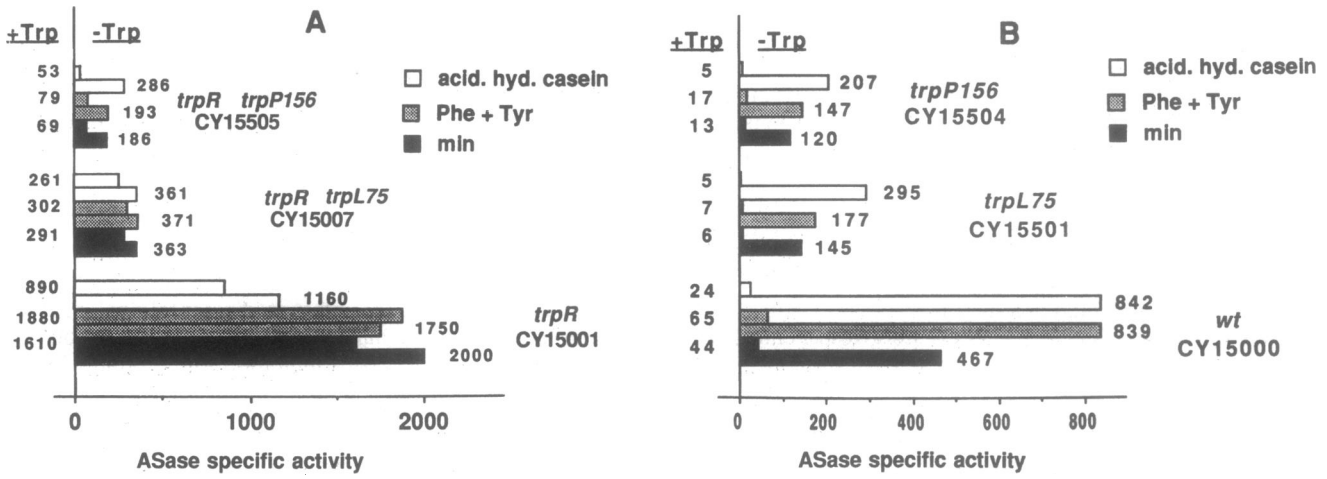

FIG. 1. Steady-state ASase levels of log-phase cultures of wild type (wt) and strains carrying the *trpL75* or *trpP156* mutation growing in the three test media with and without tryptophan. The test media included minimal medium (min), minimal medium plus excess phenylalanine and tyrosine (Phe + Tyr), and minimal medium plus 0.05% acid-hydrolyzed casein (acid. hyd. casein). Log-phase cultures growing in the media indicated were diluted into fresh medium of the same composition, and samples were taken at 30-min intervals for turbidity determination. At 80 Klett units, samples were removed for ASase assays. The bars and adjacent numbers indicate the average ASase specific activities for duplicate cultures grown in the same medium at the same time. (A) *trpR* cultures; (B) *trpR*$^+$ cultures. Note that the ASase specific activity scales differ in panels A and B. For additional details, see Materials and Methods.

tryptophan was slower than that of the corresponding wild-type culture, 56 versus 51 min (data not shown).

To verify that attenuation was relieved in the *trpR trpP156* culture, an experiment was performed with a reporter strain that allowed us to distinguish between effects at the promoter and relief of attenuation. This strain was similar to one used previously to distinguish between repression relief and attenuation relief (40); it contains an integrated copy of lambda carrying a *trp* promoter-operator *trpL'-'trpE'-'lacZ* triple coding region translational fusion that eliminates attenuation control. This strain grows slightly slower than its lambda-free parent and shows normal repressor-operator responses. We prepared a *trpR trpP156* derivative of this strain (CY15507) and measured ASase and β-galactosidase levels in cultures grown in acid-hydrolyzed casein medium with and without tryptophan (Table 2). It can be seen that the β-galactosidase level of this strain was unaffected by the absence of tryptophan from the growth medium, whereas the ASase level was elevated when the medium lacked tryptophan. The doubling times for this strain in the presence and absence of tryptophan (Table 2) confirmed that the *trpP156* mutation does result in a reduced growth rate in acid-hydrolyzed casein medium lacking tryptophan. These findings demonstrate that transcription termination at the attenuator can be reduced appreciably with only a modest effect on cell growth rate.

TABLE 2. Steady-state growth of CY15507 (*trpR trpP156* λTL) on acid-hydrolyzed casein medium with or without tryptophan[a]

Medium	Doubling time (min)	ASase sp act[b]	β-Galactosidase activity[c]
ACH + Trp	60 ± 2	119	15,800
ACH − Trp	74 ± 1	398	14,200

[a] Steady-state cultures were grown at 37°C in acid-hydrolyzed casein medium (ACH) with (+) and without (−) tryptophan as described in Materials and Methods.
[b] For units, see Materials and Methods.
[c] Miller units (20).

The *trpR*$^+$ derivatives of the various mutants were also grown in the six test media and assayed for ASase activity (Fig. 1B). As expected, there were enormous differences in ASase levels for cultures grown with and without tryptophan. In wild-type cultures grown without added tryptophan, the presence of phenylalanine and tyrosine or acid-hydrolyzed casein led to an appreciable increase in the ASase level. The presence of these two aromatic amino acids, as such or as components of acid-hydrolyzed casein, apparently partially starve the cultures of tryptophan. Expression of the operon in the wild-type and *trpL75* strains, in the absence of tryptophan, was somewhat below that of the corresponding *trpR* strains. It seems likely, therefore, that the wild-type and *trpL75* strains can attain their maximum growth rates without relieving repression completely. The *trpP156* strain had lower ASase levels than those of the *trpR trpP156* strain. We suspect that both *trpP156* and *trpR trpP156* strains experience relief of repression and attenuation when they are grown without tryptophan. Comparison of the ASase levels of *trpL75* and *trpP156* strains suggest that they can attain a similar level of operon expression during growth in the absence of tryptophan, although these strains differ in the defect that limits operon expression. It is interesting that the *trpL75* and *trpP156* strains have similar growth rates in tryptophan-free media (see Tables 3 and 4). These data indicate that the capacity of wild-type *E. coli* to express the *trp* operon greatly exceeds the needs of the organism under the growth conditions examined.

In culture media containing tryptophan, the three *trpR*$^+$ cultures had lower ASase levels in the acid-hydrolyzed casein medium than in the other two media; the ASase levels were lower in the *trpL75* and *trpP156* cultures than in the wild-type culture. The values presented for the *trpR*$^+$ cultures grown with tryptophan were determined with cell extracts. ASase measurements with cell extracts versus permeabilized cells of cultures grown with excess tryptophan indicated that very low values obtained with permeabilized cells were unreliable.

Doubling times of *trpR*$^+$ cultures growing in different media with and without a shift to media lacking or containing

TABLE 3. Doubling times following transfer to and from various media without tryptophan

Relevant genotype	Doubling time (min) (± SD) after transfer from medium[a] lacking Trp[b]					
	Minimal to:		ACH to:		Phe + Tyr to:	
	No Trp	Trp	No Trp	Trp	No Trp	Trp
wt[c]	72 (±1)	73 (±1)	51 (±1)	49 (±1)	79 (±2)	77 (±1)
trpL29	75 (±2)	74 (±1)	54 (±0)	49 (±1)	76 (±1)	81 (±0)
trpL75	72 (±0)	75 (±1)	54 (±4)	51 (±4)	78 (±2)	75 (±2)
trpL117	71 (±3)	73 (±2)	52 (±0)	49 (±1)	77 (±1)	78 (±2)
trpP156	78 (±1)	75 (±1)	56 (±2)	51 (±1)	78 (±3)	75 (±4)

[a] Media included minimal medium (Minimal), minimal medium plus excess phenylalanine and tyrosine (Phe + Tyr), and minimal medium plus 0.05% acid-hydrolyzed casein (ACH).

[b] Duplicate cultures grown in the medium indicated were washed and transferred to the same medium with (Trp) or without (No Trp) tryptophan. Doubling times were calculated from log plots of the growth data. The values presented are the averages for two to six cultures. For additional details, see Materials and Methods.

[c] wt, wild type.

tryptophan. To assess the effects of various mutations on the ability of the organism to respond to a nutritional shift, we transferred cultures to and from tryptophan-containing media, in the presence or absence of phenylalanine and tyrosine or acid-hydrolyzed casein. Cultures growing in the indicated media were harvested by centrifugation, washed, and diluted into fresh media of the compositions indicated. The resuspended cultures were then incubated with shaking, and changes in turbidity were measured. The doubling times calculated from log plots of these growth data are presented in Tables 3 and 4. In these studies, we examined the three test stains, the wild type, *trpL75*, and *trpP156*, plus two additional strains altered in attenuation responses, *trpL29* and *trpL117*. The *trpL29* mutant is defective in relieving attenuation (termination), whereas the *trpL117* mutant does not terminate transcription at the attenuator (see above). When the inoculum had been grown in media without tryptophan (Table 3), the doubling times of cultures growing with or without tryptophan were similar, even when the shift was to a medium containing phenylalanine and tyrosine or acid-hydrolyzed casein. Only *trpP156* cultures consistently showed a slightly lower growth rate in media without tryptophan. Apparently, the *trp* enzyme levels of mutant cultures growing without tryptophan are sufficient to support near-maximum growth rates. The doubling times of these cultures were lowest in media containing the two aromatic amino acids (Table 3).

When cultures growing with tryptophan were washed and transferred to tryptophan-free media (Table 4), the wild-type and *trpL117* cultures had no difficulty adjusting to this shift. However, when the *trpL29*, *trpL75*, and *trpP156* cultures were treated identically, there was about a 30-min growth lag in each following transfer to tryptophan-free media containing either acid-hydrolyzed casein or phenylalanine and tyrosine. In addition, in the acid-hydrolyzed casein medium, the growth rates of the three mutant cultures just mentioned were lower than that of the wild-type culture over the time course of the experiment. The lag and reduced growth rates were abolished when identical experiments were performed with *trpR* derivatives of the mutant strains (data not shown). These findings demonstrate that the defects in the *trpL29*, *trpL75*, and *trpP156* mutant strains restrict the ability of cultures grown with tryptophan to respond to a shift to a medium containing either acid-hydrolyzed casein or phenylalanine and tyrosine and lacking tryptophan. This limitation must be due to the low *trp* enzyme levels that are present in tryptophan-grown cultures of these mutants and the reduced ability of these strains to respond to a tryptophan deficiency. It was shown above that in minimal medium lacking phenylalanine and tyrosine, the mutant cultures experienced no apparent difficulty adjusting to the nutritional shift. These findings support the interpretation that the presence of phenylalanine and tyrosine in a growth medium lacking tryptophan imposes a requirement for increased expression of the *trp* operon to sustain rapid growth. Assays of ASase formation following comparable nutritional shifts (Fig. 2 and 3) demonstrate this directly.

ASase levels following shifts from media containing tryptophan to media lacking tryptophan. To determine the effects of nutritional shifts on *trp* operon expression directly, ASase levels were measured following shifts of cultures of wild-type, *trpL75*, and *trpP156* strains (Fig. 2) and wild-type, *trpL29*, and *trpL117* strains (Fig. 3). Cultures growing in tryptophan-containing media were collected, washed, and diluted into the corresponding tryptophan-free media. Cultures were incubated with shaking, and samples were taken immediately following the shift and at appropriate intervals thereafter and assayed for ASase activity. It can be seen in Fig. 2 and 3 that the ASase level of each culture was very low prior to the shift and increased rapidly following the shift. The ASase levels attained by the *trpL75* and *trpP156* mutant strains were well below those of the wild-type strain. In addition, in acid-hydrolyzed casein medium, the response of the mutants was somewhat slower. As time progressed, the ASase level of each culture declined and approached the steady-state level characteristic of that strain growing in that medium.

The ASase level undoubtedly would have continued to decrease to the steady-state level if growth had continued. It is evident from these data that the *trpL75* and *trpP156* mutations severely limited operon expression relative to that of the wild

TABLE 4. Doubling times following transfer to and from various media with tryptophan[a]

Relevant genotype	Doubling time (min) after transfer from medium containing Trp					
	Minimal + Trp to:		ACH + Trp to:		Phe + Tyr + Trp to:	
	No Trp	Trp	No Trp	Trp	No Trp	Trp
wt	73 (±1)	74 (±1)	50 (±2)	50 (±2)	77 (±0)	73 (±1)
trpL29	75 (±1)	73 (±1)	Lag[b]; 60 (±1)	51 (±0)	Lag; 75 (±1)	75 (±1)
trpL75	71 (±0)	69 (±1)	Lag; 60 (±3)	50 (±2)	Lag; 77 (±1)	73 (±2)
trpL117	73 (±1)	71 (±0)	53 (±0)	52 (±2)	75 (±3)	75 (±1)
trpP156	76 (±1)	75 (±1)	Lag; 59 (±1)	51 (±1)	Lag; 81 (±1)	73 (±1)

[a] For additional information and abbreviations, see Table 3, footnotes *a* to *c*.

[b] Lag, ca. 30-min. growth lag following transfer to tryptophan-free medium.

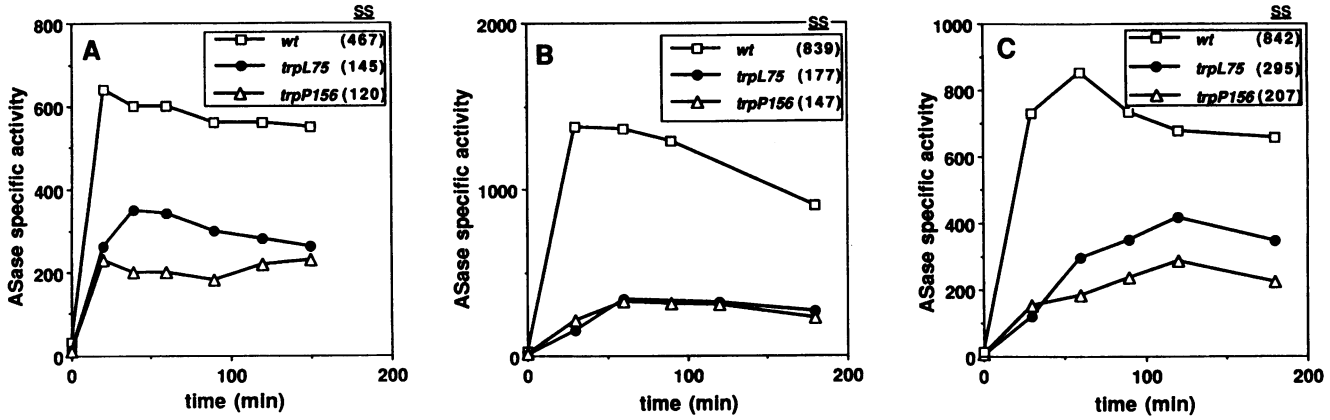

FIG. 2. Nutritional shifts of wild-type (wt) and mutant strains *trpL75* and *trpP156* from tryptophan-containing media to tryptophan-free media. Log-phase cultures growing in the first medium plus tryptophan were harvested, washed, and transferred to the same medium lacking tryptophan and reincubated. Samples were taken at 30-min intervals, with their turbidities determined at 10-min intervals, and cells were harvested for ASase assays. (A) Shift from tryptophan minimal medium to the same medium lacking tryptophan; (B) shift from minimal medium containing tryptophan, phenylalanine, and tyrosine to the same medium lacking tryptophan; (C) shift from minimal medium containing tryptophan and acid-hydrolyzed casein to the same medium lacking tryptophan. The values given are the averages for duplicate cultures. The steady-state ASase levels (SS) for the strains examined are given in the inset box in each panel. Note that the ASase specific activity scales differ for panels A, B, and C. For additional details, see Materials and Methods.

type. In addition, the response of these mutant cultures was slightly slower. Recall that these mutants experience a growth lag when shifted to media containing phenylalanine and tyrosine or acid-hydrolyzed casein (Table 4). For the wild-type culture, minimal medium containing phenylalanine and tyrosine appeared to cause the most severe tryptophan deficiency since the ASase levels were highest following the shift in this medium. The ASase levels of the wild type were slightly higher in acid-hydrolyzed casein medium than in minimal medium (Fig. 2 and 3).

Comparable shift experiments were performed with strains containing the *trpL29* and *trpL117* mutations (Fig. 3). Following a shift in minimal medium, the wild-type and *trpL117* ASase levels were nearly identical, while following the shift in the other two media, the *trpL117* strain had much higher enzyme levels than the wild-type strain. The *trpL29* strain behaved like the *trpL75* strain, as expected. We interpret these data as follows. We assume that in medium containing phenylalanine and tyrosine or in medium containing acid-hydrolyzed casein, *trpL117* cells are incapable of increasing repression sufficiently to maintain an ASase level comparable to that of the wild-type strain, a level that we assume is sufficient to support maximum growth. In contrast, in minimal medium, formation of ASase is identical in the wild-type and *trpL117* strains. This result suggests that in minimal medium the *trpL117* strain uses repression more extensively than the wild-type strain in regulating *trp* operon expression. Apparently, growth and nutritional conditions dictate the level of ASase that a cell must maintain to allow active growth, and this level reflects the use of the various regulatory mechanisms that can be called upon by the organism.

Two additional strains were examined in shift experiments in minimal medium containing acid-hydrolyzed casein, *trpLAGG2* and *trpLAGG4* (Fig. 3C). The genetic changes in these mutants (addition of rare codons prior to the Trp codons in the leader peptide coding region) prevent the normal relief of transcrip-

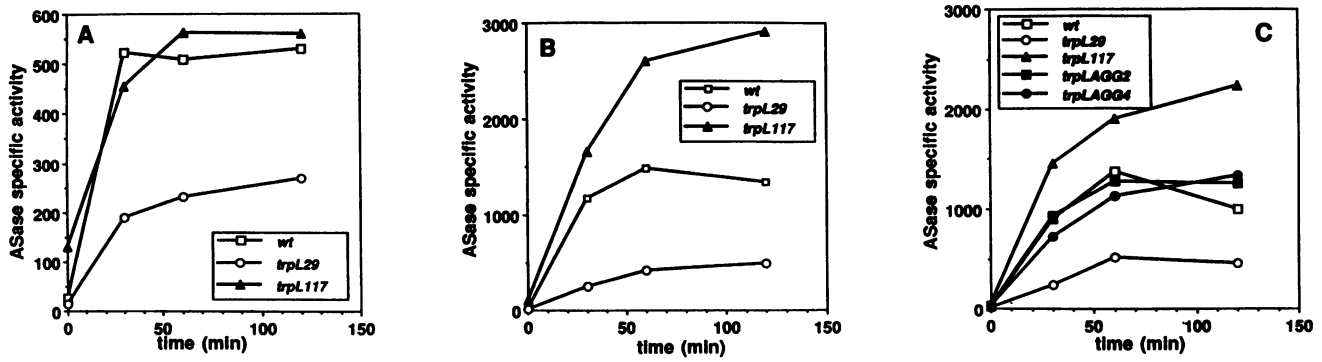

FIG. 3. Nutritional shifts of wild-type (wt) and mutant strains *trpL29* and *trpL117* from tryptophan-containing media to tryptophan-free media. (A) Shift from minimal medium containing tryptophan to the same medium lacking tryptophan; (B) shift from minimal medium containing tryptophan, phenylalanine, and tyrosine to the same medium lacking tryptophan; (C) shift from minimal medium containing tryptophan and acid-hydrolyzed casein to the same medium lacking tryptophan. In the latter medium, two additional strains were examined, *trpLAGG2* and *trpLAGG4*. The values given are the averages for duplicate cultures. Note that the ASase specific activity scale differs in panels A, B, and C. For other details, see Materials and Methods.

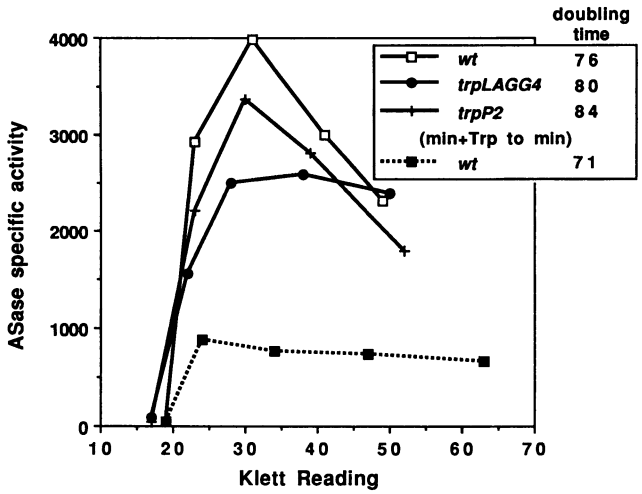

FIG. 4. Shift of wild-type (wt) and mutant strains *trpLAGG4* and *trpP2* from minimal medium plus tryptophan to minimal medium containing phenylalanine and tyrosine. Also shown for comparison is a shift of a wild-type culture from minimal medium containing tryptophan to minimal medium lacking tryptophan (min+Trp to min). Turbidity was measured every 10 min, and samples were taken at 30-min intervals. Asase specific activity is plotted versus culture density (Klett reading). The values given are the averages for duplicate cultures. The average doubling times (in minutes) given are for the entire period of the experiment. For other details, see Materials and Methods.

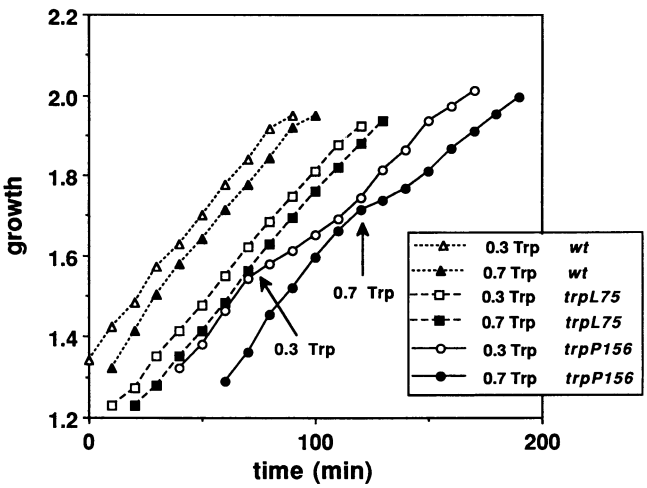

FIG. 5. Growth of wild-type (wt), *trpL75*, and *trpP156* strains following transfer to medium containing acid-hydrolyzed casein and a low level of tryptophan (Trp). Log-phase cultures growing in acid-hydrolyzed casein medium plus 100 μg of L-tryptophan per ml were collected by centrifugation, washed in acid-hydrolyzed casein medium lacking tryptophan, and resuspended in acid-hydrolyzed casein medium containing either 0.3 μg or 0.7 μg of tryptophan per ml. The values presented are the averages for duplicate cultures. The curves shown in the figure were displaced on the time axis to make the changes in growth rates more obvious. The log of the Klett reading (growth) is plotted. For additional details, see Materials and Methods. Arrows in the figure indicate where growth slowed in *trpP156* cultures in media with low concentrations of tryptophan.

tion termination that is observed in the wild type upon severe tryptophan starvation (Table 2). Their responses were very similar to that of the wild-type strain (Fig. 3C). This finding suggests that if attenuation (termination) is relieved initially in the shifted wild-type strain, this relief must be brief.

Figures 2 and 3 show that the various mutant and wild-type cultures have very different ASase specific activities over the course of these experiments. Note that the horizontal axes in these figures represent elapsed time following the shift and not increase in cell mass. If these data are plotted as Asase specific activity versus increase in cell mass, then the differences between the wild type and the various slow-responding mutants are not as great as pictured. The data were plotted versus time because growth rates and lags differed for the different strains.

Detection of attenuation relief in the wild-type strain. The experiments described in the preceding sections indicate that wild-type cultures can adapt to the nutritional shifts employed without relieving attenuation in the *trp* operon significantly. It is also apparent from these findings that shifts in media containing phenylalanine and tyrosine impose the most severe demands on tryptophan-synthesizing capacity since the wild-type ASase levels are highest following shifts in this medium (Fig. 2 and 3). To determine if there are nutritional shift conditions that require the wild-type strain to relieve attenuation, we performed shifts from minimal medium containing tryptophan (but lacking phenylalanine and tyrosine) to minimal medium supplemented with phenylalanine and tyrosine (but not tryptophan). We compared the wild-type strain and the *trpLAGG4* strain (Fig. 4). Following the shifts, there were no growth lags and only slightly different growth rates. A differential plot is shown of Asase specific activity versus cell mass (Klett reading). The wild-type culture had the highest ASase specific activity we have seen, which was well above that of the mutant defective in attenuation relief, *trpLAGG4*. It seems likely from these results that under these shift conditions, the wild-type strain does increase expression of the *trp* operon by relieving attenuation. The response of a wild-type culture following a shift from minimal medium plus tryptophan to minimal medium lacking tryptophan is shown for comparison (Fig. 4, dashed line). We also examined a strain in which the internal promoter, *trpP2*, was inactivated by mutations in its −35 and −10 regions. This strain responded almost as well as the wild-type strain despite the expectation that the *trpC*, *trpB*, and *trpA* enzyme levels in the inoculum would be one-fifth that of the wild-type culture.

Shifts to low-tryptophan media. When the *trpP156* and *trpL75* cultures were shifted to tryptophan-free media containing either acid-hydrolyzed casein or phenylalanine and tyrosine, there was about a 30-min growth lag (Table 4). This lag could have two causes, the abnormally low *trp* enzyme levels in the inoculum (Fig. 1) and/or the inability to synthesize *trp* enzymes rapidly. No growth lag was observed under comparable conditions with the wild-type strain. This finding demonstrates that in the wild type, a combination of slightly higher enzyme levels in the inoculum and the capacity to synthesize *trp* enzymes faster allowed a rapid metabolic adjustment that provided sufficient tryptophan for continued rapid growth.

When *E. coli* is growing in a natural habitat, tryptophan is probably depleted gradually, not at once as it was in the preceding experiments. To examine the abilities of mutants *trpL75* and *trpP156* to adjust their tryptophan biosynthetic capacities during transfer to a medium containing a low level of tryptophan, we shifted cultures growing in acid-hydrolyzed casein medium plus a high level of tryptophan to acid-hydrolyzed casein medium containing either 0.7 or 0.3 μg of tryptophan per ml. In Fig. 5, it can be seen that under these conditions the growth rate of the wild-type culture did not

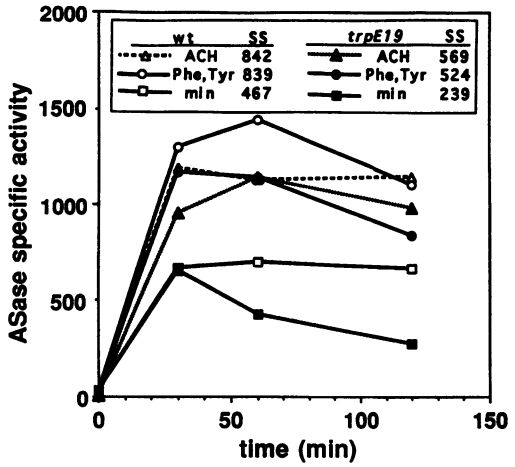

FIG. 6. Nutritional shifts of the feedback-sensitive wild-type (wt) strain and feedback-resistant mutant *trpE19* from tryptophan-containing media to tryptophan-free media. Abbreviations: min, shift from tryptophan minimal medium to minimal medium; Phe, Tyr, shift from minimal medium containing tryptophan, phenylalanine, and tyrosine to the same medium lacking tryptophan; ACH, shift from minimal medium containing tryptophan and acid-hydrolyzed casein to the same medium lacking tryptophan. The values presented are the averages for duplicate cultures. The steady-state ASase levels (SS) for the strains examined in the indicated media are given in the enclosed rectangle. The growth rates for feedback-sensitive and -resistant cultures grown in each medium were identical (data not shown). For other details, see Materials and Methods.

change as the low level of exogenous tryptophan was being depleted. Thus, the wild-type strain is capable of adjusting its tryptophan biosynthetic capacity as a limited exogenous tryptophan supply is being exhausted. The *trpP156* culture could not adjust to this change; its growth slowed appreciably, presumably when the 0.3 or 0.7 μg of added tryptophan per ml was at or near exhaustion. Subsequently, the strain was able to adjust, since rapid growth was resumed. The attenuation-defective strain *trpL75*, although capable of producing *trp* enzyme levels only slightly higher than those of the *trpP156* strain under tryptophan starvation conditions, did not exhibit a growth lag following shifts into the same media. The slight difference in *trp* operon expression (Fig. 1) between the *trpL75* and *trpP156* mutants must account for this difference in behavior.

The effect of feedback inhibition on *trp* operon expression. The ASase enzyme complex is sensitive to tryptophan-mediated feedback inhibition (5, 22, 41). The TrpE subunit of the complex contains a tryptophan binding site; tryptophan bound at this site results in inhibition of the catalytic activities of both the TrpE and TrpD subunits (5). The affinity of ASase for tryptophan is similar to the affinity of the *trp* repressor for tryptophan (19, 22). Thus, as the intracellular tryptophan concentration decreases or increases, this change will affect both repression and feedback inhibition. Tryptophan inhibition of ASase is antagonized by chorismate, the principal substrate of the enzyme, and hence, the effectiveness of tryptophan as a feedback inhibitor is influenced by all of the events that affect the intracellular concentration of chorismate (23).

We examined the impact of feedback inhibition on *trp* operon expression by comparing ASase levels in the feedback-sensitive wild-type strain and a feedback-resistant mutant (*trpE19*) following steady-state growth in the three test media lacking tryptophan and following nutritional shifts from tryptophan-containing media to tryptophan-free media (Fig. 6). Growth rates were found to be identical in paired feedback-sensitive and -resistant strains during steady-state growth in all the media lacking tryptophan (data not shown). The steady-state ASase level was higher in the feedback-sensitive strain than in the feedback-resistant strain in each of the test media (Fig. 6, SS values).

ASase levels were also determined following shifts of feedback-sensitive and -resistant cultures from tryptophan-containing media. ASase levels were somewhat comparable initially in sensitive and resistant strains. However, as time progressed, the ASase level of the feedback-resistant strain approached the steady-state level more rapidly than that of the feedback-sensitive strain (Fig. 6). The presence of phenylalanine and tyrosine or acid-hydrolyzed casein increased the apparent tryptophan deficiency since ASase levels were higher in these cultures than in cultures growing in minimal medium. The growth rates of the paired shifted cultures in each medium were identical (data not shown).

DISCUSSION

E. coli employs a variety of regulatory mechanisms to control tryptophan biosynthesis. These mechanisms allow the organism to monitor its tryptophan needs and to respond by increasing or decreasing *trp* operon expression and the rate of tryptophan synthesis. We have examined the behavior of mutants with alterations affecting the known regulatory processes that influence tryptophan biosynthesis. We were particularly interested in analyzing the importance of each regulatory mechanism under nutritional stress conditions. We compared strains in which repression of the *trp* operon was eliminated or in which transcription initiation at the *trp* promoter was reduced appreciably. We also examined mutant strains in which transcription termination at the attenuator did not occur, was greater than in the wild type, or could not be relieved when cultures were starved of tryptophan. Our findings suggest that greatest stress is placed on the tryptophan-synthesizing capacity of the organism when a culture is shifted to a medium lacking tryptophan that contains phenylalanine and tyrosine. We therefore examined the effects of these conditions on the behavior of the wild-type bacterium. We also determined the effects on enzyme levels of eliminating feedback inhibition of ASase, the enzyme catalyzing the first step in tryptophan biosynthesis.

Our findings demonstrate that relief from the separate or combined negative action of the regulatory processes that the wild-type bacterium employs to limit tryptophan synthesis allows the organism to respond to the stressful nutritional shifts and stressful environments we tested without experiencing a severe growth lag. The wild-type bacterium apparently can increase its rate of tryptophan synthesis appropriately whenever the tryptophan concentration drops to a level that is insufficient to support ongoing protein synthesis. Most importantly, in the wild-type strain, expression of the *trp* operon can be increased appreciably, as needed, ensuring that growth will continue at an unreduced rate in that unfavorable medium. Under the most extreme shift condition we tested, the wild-type bacterium appeared to eliminate repression and reduce termination at the attenuator to maintain rapid growth.

The ability of the organism to respond to a nutritional shift depends on two features of expression of the *trp* operon, namely, the basal levels of ASase and other *trp* enzymes that are maintained during growth in the presence of excess tryptophan and the rate at which the *trp* operon can be

expressed as intracellular tryptophan is being depleted. It is evident from our findings that the bacterium responds immediately to a tryptophan deficiency by increasing *trp* operon expression. It attains levels of *trp* operon polypeptides greatly exceeding those needed for steady-state growth in that medium. Our findings with mutants that exhibit greater-than-normal termination at the attenuator or that have greatly reduced promoter strength further indicate that *trp* enzyme levels well below those of the wild-type strain are sufficient for near-maximal steady-state growth rates in certain media. Appreciable growth lags were observed, however, when these mutant cultures were transferred from tryptophan-containing media to tryptophan-free media when the medium contained phenylalanine and tyrosine or acid-hydrolyzed casein. Interestingly, these growth lags were not observed in minimal medium which did not contain phenylalanine and tyrosine. Even under inhibitory nutritional shift conditions that produced a growth lag, there was a relatively rapid rate of growth following the lag period. We conclude that the established basal level of the *trp* biosynthetic enzymes and the ability to adjust the rate of tryptophan synthesis are adequate to allow wild-type *E. coli* to respond to many of the nutritional imbalances that the organism probably experiences. We presume that the wild-type culture can instantly relax both repression and attenuation when it is shifted from a tryptophan-containing medium to a tryptophan-free medium, particularly if phenylalanine and tyrosine are present in the tryptophan-free medium.

Relief of attenuation in the *trp* operon during steady-state growth was most evident in the *trpR trpP156* culture growing in any of the three media (Fig. 1A). The repressorless strain growing in these media had ASase levels two- to fivefold higher than those of the tryptophan-containing culture. Apparently, transcription initiation in the *trp* operon of *trpR trpP156* cultures is insufficient to support rapid growth in tryptophan-free media unless transcription termination at the attenuator is at least partially relieved. It is important to note that these cultures had only slightly lower growth rates although termination was appreciably relieved. Similarly, wild-type cultures showed only a slightly lower growth rate when subjected to our most severe shift conditions (Fig. 4). The levels of charged $tRNA^{Trp}$ that are maintained under these conditions must be sufficient. Comparisons of *trp* operon expression in feedback-sensitive and -resistant strains support the conclusion drawn previously that the feedback sensitivity of the ASase of wild-type *E. coli* requires the bacterium to produce somewhat elevated *trp* enzyme levels when grown in a tryptophan-free medium (2, 40). Thus, under these growth conditions, some fraction of the ASase molecules of the wild-type bacterium must be inhibited by bound tryptophan.

A strain in which there is no termination at the attenuator, *trpL117*, had higher basal levels of ASase than the wild type in all tryptophan-containing media. This strain experienced no difficulty coping with a nutritional shift in any of the test media. Interestingly, repression seemed to be greater in this mutant than in the wild type when these strains were shifted to minimal medium lacking tryptophan (Fig. 3A). Two strains that had alterations that allowed normal termination but prevented relief from attenuation responded identically to the wild-type strain following a shift from a tryptophan-containing medium to a tryptophan-free medium (Fig. 3C). In these strains, relief of repression appears to be sufficient to satisfy the need for tryptophan under the shift conditions examined. This also appears to be true for the wild-type strain.

The findings described in this paper demonstrate that wild-type *E. coli* has the ability to relax repression or repression and termination, reduce feedback inhibition of ASase, and permit tryptophan synthesis to proceed at a rate sufficient to support rapid growth, regardless of the prior nutritional history. The high basal activity of the principal *trp* promoter and the presence and activity of the internal promoter probably also aid by ensuring that the levels of all of the *trp* enzymes are sufficiently high during growth with tryptophan to allow an adequate rate of tryptophan synthesis when a culture is shifted to a tryptophan-free environment.

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