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Translational control of transcription termination at the attenuator of the *Escherichia coli* tryptophan operon

(regulatory mutants/leader peptide synthesis/attenuation)

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ABSTRACT **We have isolated two regulatory mutants altered in the leader region of the *Escherichia coli* tryptophan (*trp*) operon. In one mutant, *trpL29*, the AUG translation start codon for the *trp* leader peptide is replaced by AUA. The other mutant, *trpL75*, has a G→A change at residue 75, immediately after the UGA translation stop codon for the *trp* leader peptide. *In vivo*, *trpL29* and *trpL75* increase the efficiency of transcription termination at the *trp* attenuator 3- to 5-fold. *trpL29* and *trpL75* also fail to respond fully to tryptophan starvation and other conditions that normally relieve transcription termination at the *trp* attenuator. The *trpL29* mutation, which presumably reduces synthesis of the *trp* leader peptide, is *cis* dominant. The effect of starvation for a number of the amino acids in the *trp* leader peptide was determined. Only starvation for tryptophan and arginine, amino acids that occur at residues 10, 11, and 12 of the 14-residue *trp* leader peptide, elicits relief of transcription termination. Our findings suggest that translation of *trp* leader RNA is involved in regulation of transcription termination at the attenuator. A model is discussed in which the location of the ribosome synthesizing the leader peptide is communicated to the RNA polymerase transcribing the leader region.**

RNA polymerase molecules that have initiated transcription at the promoter of the tryptophan (*trp*) operon of *Escherichia coli* may either terminate transcription at the attenuator, or *a* site, in the 160-base-pair leader region of the operon or continue transcription into the structural genes (1). Termination of transcription at *trp a* is regulated, and varies in response to changes in the levels of charged vs. uncharged $tRNA^{Trp}$ (2). We define attenuation as the regulation of this termination (3).

The short RNA molecules produced by transcription termination at an attenuator are termed leader transcripts. The known leader transcripts of amino acid biosynthetic operons code for short peptides containing at least two tandem amino acid residues that are the end product of expression of that operon (refs. 4–7; unpublished results). This fact and the analyses presented here with a mutant that is presumed to be deficient in synthesis of the *trp* leader peptide lead us to suggest that translation of the transcript of the *trp* leader region is involved in attenuation.

MATERIALS AND METHODS

Isolation and Mapping of 5-Methylanthranilic Acid-Resistant Mutants. *E. coli* strain W3110 *trpR tna2 trpB9579* was infected with hydroxylamine-treated P1 *clr* phage grown on W3110 (8), and *trp*+ transductants were selected. About 1% of the *trp*+ transductants were resistant to 5-methylanthranilic acid (5-MA) (100 μg/ml) in the presence of indole (5 μg/ml). Approximately 10% of the *trp*-linked 5-MA-resistant clones had reduced levels of the *trp* enzymes, and, unlike the parental *trpR* strain, their growth was inhibited by 5-methyltryptophan in the absence of indole. To identify those mutations located in the *trp* leader region we mapped the mutations relative to two deletions—ΔtrpED24, which removes most of *trpE* and *trpD* but leaves the *trp* leader region intact (9), and *ΔtrpLD102*, which removes most of *trpL*, *trpE*, and *trpD* but leaves the *trp* operator–promoter intact (10). P1 *clr* lysates prepared from the above mutants were UV irradiated to increase the frequency of recombination (11) and used to transduce *trpR tna2 ΔtrpED24* or *trpR tna2 ΔtrpLD102* to prototrophy. Of 10 mutants examined, 3 produced 7.5–10% recombination between the mutation and *ΔtrpED24* but no recombination between the mutation and *ΔtrpLD102*. Recombination was monitored by scoring for the prototrophic 5-methyltryptophan-resistant recombinant class.

Messenger RNA Experiments. Procedures for the growth of cells, pulse labeling with [^{3}H]uridine for 30 sec, extraction of RNA, and hybridization to denatured, immobilized DNA have been described (2). Amino acid starvation prior to pulse labeling of RNA was for 5 min at 37°C. The DNAs used for hybridization are described in the legend to the appropriate table or figure.

The effect of arginine starvation on the ratio of plasmid *trp mRNA* to chromosomal *trp* mRNA was determined in two strains: W3110 *trpR his pro ilv argE trpL*+ *ΔtrpED24/colVB trpL*+ *trpE10220 trpD*+ *Δ(tonBtrpAC)* and a strain isogenic except for ΔtrpLD102 as the chromosomal marker. Plasmid- and chromosomal-specific *trp* mRNAs were determined by measuring *trpB* mRNA and *trpE* mRNA. DNA of phages $i^{\lambda}h^{\phi 80}$*trpE* and $i^{\lambda}h^{\phi 80}$*trpBA15* were used for these measurements.

Enzyme Assays. Cells were grown in minimal medium (12) containing 0.05% acid casein hydrolysate and 50 μg of L-tryptophan per ml. Extracts were prepared, and the specific activities of *trpE*, *trpD*, and *trpB* polypeptides were determined as described (2).

RESULTS

Isolation of Mutants in the Leader Region of the *trp* Operon That Increase the Efficiency of Transcription Termination. *E. coli* strains that lack a functional Trp repressor protein (*trpR*) are growth inhibited when plated on a medium containing 5-MA plus a low concentration of indole. This inhibition is presumably due to the conversion of the 5-MA to toxic levels of 5-methyltryptophan by the high *trp* enzyme levels present in the *trpR* strains. *E. coli trpR*+ strains have much lower levels of the *trp* enzymes (13) and are resistant to 5-MA under the same conditions. Thus 5-MA is suitable for the selection in *trpR* strains of mutations that decrease the ex-

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Abbreviation: 5-MA, 5-methylanthranilate.

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pression of the *trp* operon. We have selected such mutants and then screened for those with alterations that map in the leader region of the *trp* operon and result in increased termination of transcription at the *trp* attenuator.

To facilitate isolation of the desired mutants, we mutagenized P1 *clr* phage grown on W3110 and transduced W3110 *trpR tna2 trpB9579* to prototrophy. 5-MA-resistant mutants were identified among the transductants, and three were found to have mutations in the *trp* leader region that resulted in increased termination of transcription at the *trp* attenuator. Table 1 shows that the levels of *trp* operon enzymes and structural gene mRNAs in two of the mutants, *trpL29* and *trpL75*, were reduced to 20–40% of those of the *trpL*$^+$ parental strain. We cloned the *trp* leader regions of *trpL29* and *trpL75* onto the multicopy *trpP*$^+$*O*$^+$*L*$^+$*E*$^+$*D*$^+$ plasmid pVH153 (15) by genetic exchange (unpublished results) between the *trp* regions of the chromosome and the *trp* deletion plasmid pVH153 Δ*trpLE1417* [designated pGM3 (16)]. The *Hpa* II$_{570}$ DNA restriction fragment, which carries the *trpPOL* region (17), was isolated from the plasmids carrying the *trpL* mutations and transcribed *in vitro* (17). RNA sequence analysis of the isolated leader transcripts (18) was used initially to identify the mutational changes. *trpL29* and *trpL75* were found to have G→A base changes at residues 29 and 75, respectively (Fig. 1). The above single base changes were confirmed by sequencing leader region DNA in both directions from the *Hha* I site at base pairs 54–62 (21). Similarly, a third mutant was found to be identical to *trpL75*.

To demonstrate that the phenotype of decreased operon expression is due to the observed base pair changes in *trpL29* and *trpL75*, we isolated spontaneous 5-methyltryptophan-resistant mutants from both strains that have a level of operon expression identical to that of the W3110 *trpR tna2* parent strain (Table 1). The leader region of one such 'revertant' from each was cloned by genetic exchange and sequenced from the *Hin*fI site at base pair 180. Both revertants had a wild-type leader sequence. This confirms the conclusion that the observed base pair changes are responsible for the phenotypes of *trpL29* and *trpL75*.

Transcription Termination in *trpL29*. In wild-type cells growing in the presence of excess tryptophan, about 85% of the RNA polymerase molecules that initiate transcription at the *trp* promoter terminate transcription at the *trp* attenuator (1). In *trpR trpL29* strains growing in the presence of excess tryptophan, the levels of *trp* leader RNA are normal (Table 1). Thus the 65–85% reduction in the level of structural gene mRNA found in *trpL29* (Table 1) probably results from an increase in the efficiency of transcription termination from the wild-type level of 85% to about 95%.

Table 1. *trp* operon expression in *trpL29* and *trpL75* strains

Strain	Relative mRNA levels			Relative enzyme levels		
	trpL	*trpD*	*trpBA*	*trpE*	*trpD*	*trpB*
trpL$^+$	100	100	100	100	100	100
trpL29	127	23	35	26	29	41
trpL75	—	18	22	13	18	40
trpL29→*trpL*$^+$	—	—	—	115	—	—
trpL75→*trpL*$^+$	—	—	—	101	—	—

Values presented are percent relative to an appropriate W3110 *trpR trpL*$^+$ control. Strains *trpL29*→*trpL*$^+$ and *trpL75*→*trpL*$^+$ are revertants of *trpL29* and *trpL75*, respectively. The procedures used for cell growth and enzyme and mRNA measurements are given in *Materials and Methods*. DNA from the *trp* transducing phages i$^{\lambda}$h$^{\phi 80}$ *trpD2*, i$^{\lambda}$h$^{\phi 80}$ *trpBA15*, and *φ80trpP*$^+$*O*$^+$ Δ*trpLC1419* Δ(*tonB-trpA905*) were used to measure *trpD*-, *trpBA*-, and *trpL*-specific mRNA, respectively. To determine *trpL* mRNA, we constructed *trpL*$^+$ Δ*tonBtrpAD* and *trpL29* Δ*tonBtrpAD* strains. DNA of *φ80trpP*$^+$*O*$^+$ Δ*trpLC1419* Δ(*tonBtrpA905*) measures transcription of the leader region exclusively in strains that have *tonBtrp* deletions extending through *trpC*, because only the first 138 base pairs of the leader region are in common (14). All values are the average of duplicate determinations.

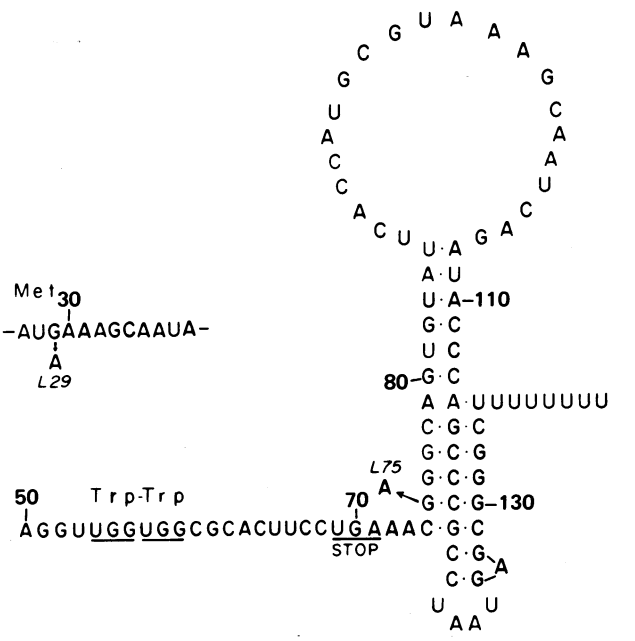

FIG. 1. The nucleotide sequence of two portions of the leader transcript from the *E. coli trp* operon. The residues are numbered with respect to the 5′ end. Residues 27–38 include the AUG translation initiator codon for the *trp* leader peptide. Residues 50–71 code for the COOH-terminal part of the leader peptide, which includes the tandem Trp residues shown. The UGA translation stop codon for the leader peptide is at residues 69–71. Residues 74–134 are presented as the secondary structure proposed by Lee and Yanofsky (17). Residues 114–119 can participate in two alternative stem and loop structures: the first stem and loop includes residues 74–119; the second stem and loop includes residues 114–134. The *trpL29* and *trpL75* residue changes are indicated. The *trpL75* mutation changes the expected free energy of formation of the first stem and loop from $\Delta G \cong -10$ kcal to $\Delta G \cong -2$ kcal (1 cal = 4.184 J) (17, 19, 20).

When a DNA restriction fragment carrying the wild-type *trpPOL* region is transcribed *in vitro*, 95% of the transcripts terminate at the attenuator (17). When a DNA fragment carrying the *trpL29* mutation was transcribed *in vitro* the efficiency of transcription termination was identical to that of a wild-type fragment (results not shown). Thus one effect of the *trpL29* mutation is to increase the efficiency of transcription termination *in vivo* to the level observed *in vitro*.

***trpL29* and *trpL75* Are Unable to Fully Relieve Transcription Termination in Response to Tryptophan Starvation.** We have examined the *trpL29* and *trpL75* strains under conditions that normally relieve transcription termination at the *trp* attenuator. In the experiments summarized in Table 2 tryptophan starvation of the *trpL*$^+$ strain increased the rate of synthesis of *trp* structural gene mRNA 10-fold. When the *trpL29* and *trpL75* strains were starved of tryptophan, the rate of structural gene *trp* mRNA synthesis increased only 2-fold and 4-fold, respectively, to levels comparable to those of unstarved *trpL*$^+$ cells. Similarly, experiments with a *trpT*^{ts} allele that specifies a tRNA^{Trp} defective in charging (2) indicate that the mutants do not respond to tryptophan starvation properly (Table 3). In the presence of excess tryptophan *trpL29 trpT*^{ts} and *trpL75 trpT*^{ts} strains have *trp* enzyme levels well below those of the *trpT*^{ts} *trpL*$^+$ control. The *trpX*$^-$ allele, which results in undermodification of tRNA^{Trp} (S. Eisenberg, L. Soll, and M. Yarus, personal communication), presumably relieves

Table 2. Tryptophan and arginine starvation of *trpL29* and *trpL75* strains

Strain	Synthesis of *trpE* or *trpD* mRNA, % of input labeled RNA hybridized: Trp excess	Trp starved	Arg starved
trpL$^+$	0.18	1.8	*
trpL29	0.09	0.17	0.071
trpL75	0.06	0.25	0.14
trpL29/trpL$^+$ Δ*trpED24*	0.16	0.21	—
trpL29/trpL$^+$ Δ*trpLD102*	0.05	0.05	—

trpE and *trpD* mRNA were determined by hybridization to i$^\lambda$h$^{\phi 80}$*trpE* and i$^\lambda$h$^{\phi 80}$*trpD2* DNA, respectively (2). To facilitate tryptophan starvation, we made the first three strains *trpA9761am* by crossing *trpL*$^+$, *trpL29*, or *trpL75* into W3110 *trpR trpE9829 trpA9761am* by P1 *clr* transduction with selection for growth on indole. *trpE* enzyme assays were used to identify *trpL trpE*$^+$ *trpA9761am* recombinants. To facilitate arginine starvation, we crossed *trpL*$^+$, *trpL29*, and *trpL75* into W3110 *trpR his pro ilv argE* Δ*trpEA2* by P1 *clr* transduction and selection for *trp*$^+$ transductants. We constructed the merodiploid strains by mating W3110 *trpR trpL29 trpA9761am* with donor strains containing either colVB *cysB*$^+$ *trpL*$^+$ Δ*trpED24 trpC*$^+$*B*$^+$*A*$^+$ or colVB *cysB*$^+$Δ*trpLD102 trpC*$^+$*B*$^+$*A*$^+$ and *pro cysB* Δ(*tonBtrpAE12*) as chromosomal markers. *trp*$^+$ *pro*$^+$ recombinants were selected. Cells were starved for tryptophan or arginine for 5 min before [^{3}H]uridine was added. Tryptophan starvation of the merodiploid strains was accomplished by the addition of indoleacrylic acid to 20 μg/ml (22). Pulse labeling in all cases was for 30 sec at 37°C. The slightly higher values for the Δ*trpED24* diploid are due to the contribution of transcription from the transcription start site to the deletion end-point in Δ*trpED24* at about base pair 40 of *trpE*. (For other details see Fig. 2.)
* See Fig. 2.

transcription termination by reducing the rate at which charged undermodified tRNA^{Trp} can be utilized in translation (2, 23). This allele has no apparent effect on the *trpL29* and *trpL75* mutants. These results show that the *trpL29* and *trpL75* mutations prevent cells with these alterations from responding fully to conditions that normally signal relief of transcription termination.

Table 3. The effect of the *trpT*^{ts}, *trpX*$^-$, and *trpL*$^+$ alleles on *trp* operon expression in the *trpL29* and *trpL75 strains*

Strain	Relative *trpE* enzyme levels
trpL$^+$ *trpT*$^+$ *trpX*$^+$	100
trpL29 trpT$^+$ *trpX*$^+$	26
trpL75 trpT$^+$ *trpX*$^+$	13
trpL$^+$ *trpT*^{ts}	540
trpL29 trpT^{ts}	70
trpL75 trpT^{ts}	70
trpL$^+$ *trpX*$^-$	200
trpL29 trpX$^-$	27
trpL75 trpX$^-$	8
trpL29/trpL$^+$ Δ*trpED24*	17
trpL29/Δ*trpLD102*	16

Conditions for growth of cells and assay of *trpE* enzyme activity were as described in *Materials and Methods*, except that the *trpT*^{ts} strains were grown at 34°C and the *trpX*$^-$ strains were grown at 41°C. The *trpT*^{ts} strains were constructed by crossing the leader region from the donor *trpL*$^+$, *trpL29*, and *trpL75* strains into W3110 *trpR* Δ*trpLE1417 his29 metE trpT*^{ts}*90* by P1 *clr* transduction and selection for *trp*$^+$ transductants. The *trpX* strains were similarly constructed except that the recipient was W3110 *trpR trpX lacZU118*. The construction of the merodiploid strains is described in Table 2. Two isolates of each strain were assayed.

Arginine Starvation Relieves Transcription Termination at the *trp* Attenuator. The *trpL29* mutation replaces the AUG translation initiation codon for the *trp* leader peptide by AUA. As mentioned, this mutation prevents full relief of transcription termination in response to tryptophan starvation. This result and the presence of tandem tryptophan residues at positions 10 and 11 of the leader peptide (Fig. 1) suggest an essential role for synthesis of a part of the leader peptide in the tryptophan starvation response. To examine the specificity of the starvation response we have determined the effect of starvation of amino acids other than tryptophan on the expression of the *trp* operon. Fig. 2 shows that starvation for methionine, isoleucine, leucine, glycine, and threonine, each of which is present in the leader peptide, and histidine and proline, which are not, has little or no effect on expression of the *trp* operon. However, starvation for arginine elicits a response approaching that of starvation for tryptophan. We also found that the ratio of plasmid *trp* operon mRNA to chromosomal *trp* operon mRNA in a *trpR argE* Δ*trpLD102*/colV*BtrpL*$^+$Δ*trpCBA* merodiploid strain increased 3- to 4-fold upon starvation for arginine (see *Materials and Methods*). However, in an isogenic *trpR argE trpL*$^+$ Δ*trpED24*/colVB*trpL*$^+$ Δ*trpCBA* merodiploid strain the ratio of plasmid *trp* operon mRNA to chromosomal *trp* operon mRNA did not change upon starvation for arginine. These results support the conclusion that arginine starvation relieves transcription termination at the *trp* attenuator.

To determine whether the response to arginine and tryptophan starvation involves the same events we compared arginine starvation with tryptophan starvation in strains with the *trpL29* and *trpL75* mutations. Table 2 shows that the *trpL29* and *trpL75* mutations also prevent relief of transcription termination in response to arginine starvation.

***trpL29* Is a *cis* Dominant Mutation.** It is conceivable that the leader peptide acts as a diffusable regulatory factor. Because the *trpL29* mutation probably reduces the level of the *trp* leader peptide (see *Discussion*), the abnormal regulatory behavior of strains with this mutation could be due to the lack of

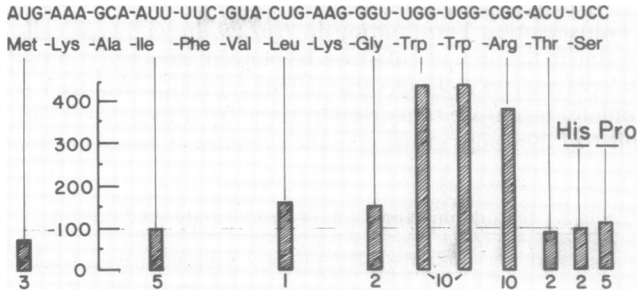

FIG. 2. The effect of starvation for various amino acids on read-through transcription of the structural genes of the *trp* operon. The height of the bar under each amino acid indicates the average structural gene (*trpCA*) mRNA level in Δ*trpED24* cultures starved for the particular amino acid, relative to the value obtained with a nonstarved culture (100%). The number under each bar indicates the number of separate experiments that were averaged to give the value represented by the height of the bar. Isoleucine was added to the threonine-starved culture and isoleucine starvation was imposed by either removing isoleucine and valine or adding inhibitory levels of L-valine. Strain W3110 *trpR his pro ilv argE* or *metB* Δ*trpED24* and Δ*trpLD102* and strain W3110 *trpR leu, gly,* or *thr* Δ*trpED24* and Δ*trpLD102* were grown in minimal medium containing the required amino acids, and samples were filtered, washed, and starved for individual amino acids for 5 min before being pulse-labeled for 30 sec at 37°C with [^{3}H]uridine. The labeled RNA was hybridized to φ80*trpCA* DNA and φ80*tonB* DNA in duplicate. The difference was taken as structural gene mRNA, a measure of read-through transcription beyond the attenuator. Occasionally, we pulse labeled parallel *trp*Δ*LD102* cultures to ensure that the values obtained were due to effects on attenuation.

the leader peptide. To test this hypothesis we examined the ability of the *trpL*$^+$ allele to complement the *trpL29* alteration *in trans*. We constructed merodiploid strains by introducing *trpL*$^+$ Δ*trpED24* or Δ*trpLD102* carried on the low copy number plasmid colVB‡ into the *trpL29* strain (see legend to Table 2). *trpL*$^+$ Δ*trpED24 in trans* did not restore *trpE* enzyme levels to the *trpL*$^+$ level (Table 3). Also, the rate of synthesis of structural gene mRNA specific for the chromosomal *trpL29 trp* operon was not increased appreciably by tryptophan starvation (Table 2). Thus the leader peptide does not act *in trans* on *trpL29* to overcome either of the defects associated with the *trpL29* mutation.

DISCUSSION

We believe that the findings presented in this report implicate translation of the *trp* leader transcript as a determinative event in the regulation of transcription termination at the *trp* attenuator. The *trp* leader regions of four Enterobacteria (refs. 4, 5; unpublished results), and the *phe* and *his* leader regions of *E. coli* (6) and *Salmonella typhimurium* (7), respectively, have sequences that code for short peptides. The transcript corresponding to each has a potential translation start codon near its 5′ end, and, in the *E. coli trp* leader transcript this start codon is centered in a ribosome binding site that is known to function *in vitro* (24) and *in vivo* (15, 25). Each of the predicted leader peptides contains at least two tandem amino acids that are synthesized by the enzymes under regulation. The *trp* operons of *E. coli*, *Shigella dysenteriae*, and *Salmonella typhimurium* specify 14 residue leader peptides that contain the sequence Trp-Trp-Arg at positions 10, 11, and 12 (4, 5). The *trp* operon of *Serratia marcescens* codes for a leader peptide either 19 or 28 residues long that also contains the sequence Trp-Trp-Arg (unpublished results). The *phe* operon of *E. coli* specifies a 15-residue leader peptide with seven Phe residues at positions 6, 7, 8, 10, 11, 12, and 14 (6), and the *his* operon of *Salmonella typhimurium* codes for a 16-residue leader peptide with 7 contiguous His residues at positions 8–14 (7). The presence in these leader peptides of amino acids that are the end product of the operon being regulated and the demonstration that transcription termination varies in response to changes in the levels of charged or uncharged tRNA specific for that amino acid (2, 3, 26) suggest a role for synthesis of the leader peptide in the regulation of transcription termination. This role is to allow the cell to monitor the availability of the particular amino acid that is the biosynthetic product of the operon's enzymes. Thus synthesis of the *entire* leader peptide would reflect a bountiful supply of the amino acid and therefore most transcription would be terminated at the operon's attenuator. Alternatively, if the amino acid were scarce, synthesis of the leader peptide could not be completed and transcription would not be terminated at the attenuator.

The phenotype of strains with the *trpL29* mutation, which changes the sequence AUGA at the *E. coli trp* leader RNA translation initiation region to AUAA, provides direct evidence that translation of the leader peptide region is crucial to normal regulation of transcription termination at the *trp* attenuator. Taniguchi and Weissman (27) demonstrated that, when the translational start site for the coat protein gene of the RNA phage Qβ is changed from AUGA to AUAA, the efficiency of ribosome binding at this site is reduced by 93%. This result suggests that the *trpL29* mutation should markedly decrease the rate of synthesis of the *trp* leader peptide. Experimentally, the *trpL29* mutation has two distinct regulatory effects. The most striking of these is that it prevents the full relief of transcription termination normally associated with tryptophan starvation. Relief from termination therefore appears to require translation of the leader transcript, at least until the Trp and Arg codons. Secondly, in cells growing in the presence of excess tryptophan, the efficiency of transcription termination increases from the *trpL*$^+$ value of 85% to 95%. This effect is seen only *in vivo*, because *in vitro* transcription of both *trpL*$^+$ and *trpL29* operons terminates with 95% efficiency. Thus *in vivo* the efficient translation of the leader peptide region probably reduces the extent of transcription termination at the *trp* attenuator to about 30% of the levels found when translation of the leader peptide region is severely impaired (i.e., in *trpL29*). One advantage of having such subtle regulatory control would be to allow the cell to reduce transcription of the structural genes of some amino acid biosynthetic operons when there is a deficiency of another amino acid. We have found that starvation for *trp* leader peptide amino acids other than Trp or Arg does not significantly affect the efficiency of transcription termination at the *trp* attenuator. However, it is possible that the severity of starvation attained in 5 min of amino acid deprivation, the condition we have examined, has a less drastic effect on synthesis of the leader peptide than does the *trpL29* mutation. It should be noted that it has also been shown that a temperature shift in a strain with an altered ribosomal protein that is temperature sensitive for initiation of translation results in an apparent increase in the extent of transcription termination at the *trp* attenuator (28).

The 3′ half of leader transcripts can form two alternative stem and loop structures (e.g., see Fig. 1) (refs. 4–7; unpublished

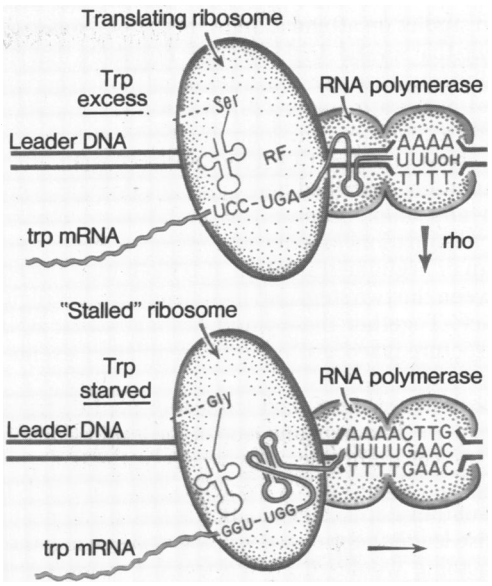

FIG. 3. A proposed model for the regulation of transcription termination at the attenuator of the *E. coli trp* operon. Under conditions of tryptophan excess the first ribosome translating *trp* leader RNA follows closely behind the transcribing polymerase and synthesizes the entire *trp* leader peptide. At the stage shown the ribosome is reading the translation stop codon, UGA, and the polypeptide release factor (RF) is about to release the polypeptide. The second RNA stem and loop has formed, signaling the polymerase to cease synthesis. Rho factor (30) then will promote dissociation of the termination complex. Under conditions of tryptophan starvation the first ribosome stalls at the Trp codons. In this configuration, interactions between the Trp codons or their immediately distal region and a more distal segment of the transcript result in the formation of an alternative RNA secondary structure. The existence of this secondary structure prevents transcription termination, allowing the polymerase to continue transcription into the first structural gene, *trpE*, and beyond.

‡ Fredericq, P. (1963) *Proceedings of the 11th International Congress of Genetics* **1**, 42–43.

results). In studies on attenuation in the *trp* operon of *E. coli*, it was shown that several single base pair mutations that relieved transcription termination at the attenuator *in vivo* and *in vitro* are located in the attenuator (29). All of these mutations destabilize the second stem and loop, and those with the greatest effect *in vivo* destabilize this stem and loop exclusively. The *trpL75* mutation, described here (Fig. 1), destabilizes only the first stem and loop. Because this mutation *increases* transcription termination *in vivo* and *reduces* the response to tryptophan or arginine starvation, it is likely that the equilibrium between the two stem and loop structures has regulatory significance. Together these findings suggest that RNA secondary structure is recognized in the regulatory events at the attenuator.

The proximity of the *E. coli trp* leader peptide translation stop codon to the first stem and loop of the leader transcript has suggested a mechanism (Fig. 3) by which synthesis of the leader peptide could influence RNA secondary structure and thereby the extent of transcription termination at the *trp* attenuator (17). If the entire leader peptide were being synthesized, the translating ribosome would reach the translation stop codon. A ribosome at this position would be expected to mask 10 or more residues downstream from this codon (31) and should prevent the formation of the first stem and loop structure, thereby facilitating formation of the second stem and loop. Alternatively, if a ribosome stalls during synthesis of the leader peptide due to lack of a required charged tRNA, the first stem and loop will be free to form. These alternatives predict that starvation for any amino acid within the leader peptide, except those whose codons are within about 10 residues of the first stem and loop, should elicit equal relief of transcription termination. Clearly, this is not the case (Fig. 2). What additional factor could account for the localization of the relief of transcription termination to ribosome stalling at only certain codons of the leader transcript? The amino acids regulating a particular attenuator are found in the COOH-terminal half of each leader peptide. Ribosomes stalling at codons for the key regulatory amino acids would expose the sequence of nucleotides coding for the COOH-terminal portion of the peptide. In the *phe* leader transcript this sequence of nucleotides could pair with the loop region of the first stem and loop to form a third hydrogen-bonded region, because there is almost perfect complementarity between the two sequences over a stretch of 20 nucleotides (6). In the *trp* leader transcripts of *E. coli* and *S. typhimurium* there are also potential, though weaker, base pairing possibilities between the exposed sequence and distal stem and loop sequences. Interestingly, the *trp* codon regions of the *trp* leader transcripts of *E. coli* and *S. typhimurium* are moderately resistant to ribonuclease attack *in vitro* and therefore presumably are base paired with as yet undesignated sequences (17). We suspect that such base pairings may allow the starvation response to be specific to a limited number of codons in the leader RNA. We have no direct evidence implicating the ribosome as a participant in mediating the postulated regulatory base pairing, however, this appears to us to be a reasonable possibility. Further study of the secondary structure of the *trp* leader transcript and its alteration by mutation should allow us to elucidate the mechanism by which starvation for tryptophan or arginine relieves transcription termination. Regardless of how this is accomplished, the available evidence suggests that translation of *trp* leader RNA is utilized to regulate transcription termination at the *trp* attenuator. Studies on the regulation of the *his* operon of *Salmonella typhimurium* (32, 33) have also implicated translation in the control of transcription of the structural genes of the operon.

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