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REGULATION OF THE ESCHERICHIA COLI TRYPTOPHAN OPERON BY READTHROUGH OF UGA TERMINATION CODONS

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SUMMARY. The regulation of the synthesis of trp operon enzymes was studied in streptomycin-resistant Escherichia coli mutants temperature-sensitive for UGA suppression by normal $tRNA^{Trp}$. Our mutants carry a $trpR^+$ allele that when transferred to a different genetic background causes repression of trp operon enzyme synthesis at both low (35°C) and high (42°C) temperatures; however, in our mutants with an excess of tryptophan and at increased temperatures trp enzyme synthesis is derepressed. Based on our results and the sequence data of the trpR gene [Singleton et al. (1980) Nucleic Acids Res., 8, 1551-1560], we offer a model for the involvement of the limited misreading of UGA codons by normal charged $tRNA^{Trp}$ in the autogenous regulation of the trpR gene expression. The UGA readthrough process may be a regulatory amplifier of the effect of tryptophan starvation.

INTRODUCTION. The tryptophan (trp) operon of Escherichia coli consists of five structural genes (1) preceeded by two regulatory loci, a promoter-operator (2-4) and an attenuator (5-7). According to the present model of trp operon expression, the intracellular concentration of free tryptophan regulates the initiation of transcription, and the level of charged $tRNA^{Trp}$ regulates the termination of transcription. The initiation of transcription is regulated by the interaction of the operator, at the promoter-operator locus, with a tryptophan-activatable Trp aporepressor, the product of the trpR gene (8-11). At the attenuator site, located within the transcribed leader region of the operon, transcription either proceeds into the structural genes or is terminated (5-7). The availability for protein synthesis of charged $tRNA^{Trp}$ governs whether transcription will proceed or will be terminated: an increase of charged $tRNA^{Trp}$ causes termination (7,12).

In E. coli, normal charged $tRNA^{Trp}$ is a low frequency suppressor of UGA termination codons (13). Here we examine the possibility that the trp operon is also regulated by the suppression of UGA termination by charged $tRNA^{Trp}$. We studied trp operon enzyme regulation in streptomycin-resistant

ABBREVIATIONS: 5-MT, 5-methyltryptophan; ASase, anthranilate synthetase; TSase, tryptophan synthetase.

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Table 1. Escherichia coli strains used in this study

Strains	Relevant genotype	Source and comments	Temperature-sensitivity of phage propagation in the bacterial strains[¶]				
			Qβ	f1	λ[§]	f2	T4D
Q13	*str*^{S}, HfrH, *trpR*$^+$; suppressor free	CGSC[†]	-	-	-	-	-
LD1	Derivative of Q13, *str*^{r}	(20) Spontaneous Str^R mutant of Q13	+	+	+	-	-
LD2	Derivative of Q13, *str*^{r}	This study; *str*^{r} P1 transductant from LD1 back to Q13	+	+	+	-	-
M4131	*trpR*$^-$, *thr*$^-$	J.F. Gardner	NT	NT	NT	NT	NT

[¶] The ratio of the efficiency of plating at 42°C versus that at 35°C: values of about 1.0 are designated as -, and values $<10^{-4}$ are designated as +; NT=not tested.

[†] E. coli genetic stock center, Yale University.

[§] λ*imm*434*cII* was used because all these bacterial strains are lysogenic for λ.

E. coli mutants temperature-sensitive for suppression of UGA termination codons by normal tRNA^{Trp}. In *trpR*$^+$ strains, an excess of tryptophan represses *trp* enzyme synthesis (14-16). Though our mutants carry a functional *trpR* gene, they exhibit derepression of *trp* enzyme synthesis at increased temperatures in the presence of an excess of tryptophan. This suggests that the limited misreading of UGA termination codons by charged tRNA^{Trp} is a mechanism involved in the regulation of the *trp* operon of E. coli. Here we discuss the possible involvement of this mechanism in various stages of *trp* operon regulation.

MATERIALS AND METHODS.

Bacterial strains, media and growth conditions. E. coli strains and bacteriophages are listed in Table 1, and see ref. 17 for phage methods. VB liquid minimal medium, supplemented with 1.0% glucose and 0.05% casamino acids, is described by Vogel and Bonner (18). 5-MT* resistance was tested on VB agar plates containing 100 μg/ml 5-MT. Bacteria were routinely grown on TB plates (17).

Preparation of cells for determination of ASase and TSase activities. Cells were grown on TB plates overnight at 37°C, suspended at $2X10^8$ cells/ml in VB medium in the absence or in the presence of 50 μg/ml L-tryptophan, and grown at various temperatures with vigorous shaking to a concentration of $5X10^8$ cells/ml. Cultures with tryptophan were chilled rapidly and collected by centrifugation at 4°C. Cultures without tryptophan were collected by centrifugation at room temperature, washed once with 2 volumes of growth medium lacking tryptophan, sedimented, and resuspended in 1 volume of this medium plus 3-indole acrylic acid (10 μg/ml) (12); tryptophan starvation was induced by shaking at the respective growth temperature for 1.5 hours before

harvesting at 4°C. Sonicates from harvested cells were prepared according to Yanofsky and Soll (12). ASase and TSase activities were assayed according to Creighton and Yanofsky (19).

RESULTS.

E. coli mutant LD1 is streptomycin-resistant and temperature-sensitive for suppression of termination codons (17,20-22). In this work we continued its characterization and have summarized its relevant features in Table 1. Both LD1 and its str^S parental strain Q13 are suppressor free (Su^-); in such Su^- strains of E. coli normal $tRNA^{Trp}$ is a weak suppressor of the termination codon UGA (13). This suppression process is required by E. coli RNA phage Qβ, DNA phage λ, and the filamentous phages because UGA suppression permits them to synthesize vital UGA readthrough proteins (17), such as the Qβ capsid protein IIb (23,24) and the λO' protein (25). We used this fact to demonstrate the temperature-sensitivity of mutant LD1 for UGA suppression by normal $tRNA^{Trp}$: 1) Qβ, λ and the filamentous phages do not grow in LD1 at 42°C (Table 1); 2) when LD1 is infected by Qβ at high temperatures, the UGA readthrough protein IIb is not synthesized, and non-infectious Qβ particles are formed (22). The temperature-sensitivity of mutant LD1 for nonsense suppression is not limited to UGA codon suppression. We previously showed that LD1 ribosomes contain an altered S12 protein, and that in vitro, and in the presence of an amber (UAG) suppressor tRNA, they are temperature-sensitive for UAG suppression as well (21). In a future publication we shall report our results confirming the in vivo sensitivity of LD1 for UAG suppression, and further, our demonstration by P1 transduction from LD1 to several other E. coli strains that the phenotypes of streptomycin-resistance and temperature-sensitivity for nonsense suppression are caused by a single mutation. LD2 is such a streptomycin-resistant P1 transductant from LD1 back to Q13. As in LD1, Qβ, λ, and the filamentous phages do not propagate in LD2 at 42°C (Table 1) because LD2 is also temperature-sensitive for UGA suppression by normal $tRNA^{Trp}$.

Of the five trp operon enzymes, we studied the regulation of the synthesis of two, ASase and TSase in our mutants LD1 and LD2. Figure 1 shows the levels of ASase activity in extracts from the mutants and the parent strain Q13. (Since we found similar results for TSase we do not show these data). The cells were grown at various temperatures under conditions of derepression or repression of the formation of the tryptophan synthesizing enzymes. The condition of derepression was caused by growing the cells in the absence of tryptophan and subsequently starving them for tryptophan as described in Materials and Methods. Under these conditions, similar levels of ASase activity were found in extracts of LD1, LD2, and Q13 after growth at temperatures ranging from 33°C to 42°C, suggesting that when the cells are derepressed, the synthesis of ASase

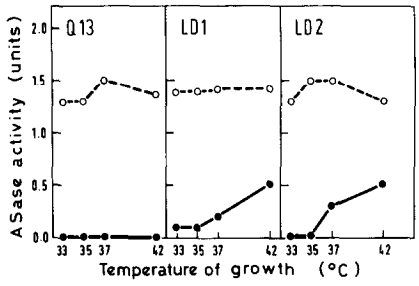

Figure 1. Effect of growth temperature on ASase activity in E. coli strains Q13, LD1 and LD2. E. coli strains Q13, LD1 and LD2 were grown at various temperatures in the absence (o) or in the presence (•) of 50 µg/ml tryptophan. Cell extracts were prepared and ASase activities were determined as described in Materials and Methods. One unit of ASase activity corresponds to the conversion of 0.1 µmol of chorismate to anthranilate in 20 minutes at 37°C per mg of protein.

is not affected by the growth temperature. The condition of maximal repression was caused by growing the cells with an excess of tryptophan. Under this condition, in LD1 and LD2, but not in Q13, an increase in the growth temperature causes an increase in ASase activity: by 42°C the level of ASase activity had reached about 30% of the level of activity under the condition of derepression (Fig 1). This indicates that when the mutants are under conditions of repression the synthesis of ASase is affected by the growth temperature: there is derepression of synthesis at high temperatures. This effect is not due to a change in the growth rates as, during the time of this experiment, at all temperatures tested, the changes in the growth rates were similar in both the mutants and the parent strain.

The trpR gene codes for the Trp aporepressor (8,9,26) and mutations in this gene may cause derepression of the synthesis of trp enzymes when in the presence of excess tryptophan (16). We therefore looked for an additional mutation in the trpR gene in mutants LD1 and LD2, and found only the wild type allele. In E. coli, trpR is co-transducible with the threonine (thr) operon; mutations in trpR cause resistance to 5-MT (15,16). Thr^+ transductants were selected from E. coli regulatory constitutive strain M4131 ($trpR^-thr^-$) infected with a P1 lysate from mutants LD1 or LD2. 80% of the Thr^+ transductants were sensitive to 5-MT at both 35°C and 42°C as expected when the donor is $trpR^+$. When several of these transductants were grown at either 35°C or 42°C with an excess of tryptophan there was no ASase activity, further confirming the presence of the wild type allele in mutants LD1 and LD2 (data not shown).

DISCUSSION.

In E. coli, normal charged $tRNA^{Trp}$ is a low frequency suppressor of UGA codons (13). Though this form of suppression may be found in other systems than E. coli (27,28), its importance has been neglected as significant in the regulation of gene expression. The UGA readthrough process facilitates the production of more than one protein from a single gene, and is thus a mechanism for the regulation of gene expression at the level of translation. It is known to participate in the regulation of the gene expression of several E. coli phages: Qβ, λ and the filamentous phages (17,23-25). Though it seems clear that the UGA readthrough process must take place in uninfected bacterial cells, its role has not been clarified.

We studied the involvement of the UGA readthrough process in the regulation of the enzyme synthesis of the E. coli trp operon. For this purpose, we isolated two str^r E. coli mutants, LD1 and LD2, temperature-sensitive for UGA suppression by normal $tRNA^{Trp}$ (Table 1 and Results). Like Q13, LD1 and LD2 both carry a $trpR^+$ allele that when transferred to a different genetic background codes for an active Trp aporepressor at both low (35°C) and high (42°C) temperatures. When complexed with L-tryptophan, the Trp aporepressor binds to the operator within the promoter region of the trp operon, thus repressing transcription initiation (8-11). Mainly due to this process (6) the production of the trp enzymes in $trpR^+$ strains is repressed by an excess of tryptophan, leading us to have expected such behaviour in Q13, LD1, and LD2. Unlike Q13, however, with an excess of tryptophan causing maximally repressing conditions, at increased temperatures LD1 and LD2 exhibit derepression of the synthesis of the trp operon enzymes ASase (Fig. 1) and TSase (data not shown). When our mutants are starved for tryptophan, causing maximally derepressed conditions, formation of the trp enzymes is not affected by increased temperatures. Thus in these mutants increased temperatures selectively affect the repression of trp enzyme production, and not their synthesis per se. These results, obtained in suppressor free strains temperature-sensitive for UGA suppression by normal $tRNA^{Trp}$, suggest that UGA readthrough participates in the regulation of the trp operon. According to the nucleotide sequence data, it is possible that this process might take place in two regulatory steps: 1) the synthesis of an active Trp aporepressor; 2) termination of transcription at the trp attenuator site. Though the present study seems to reflect the first possibility, we shall briefly discuss the second one also.

Singleton et al (29) recently sequenced the DNA of the E. coli trpR gene. They predicted that the trpR mRNA starts at position 384 with AUG and ends at position 650 with UGA. When we examined their data for the DNA sequence in the region downstream from this UGA codon, we found three additional in-phase termina

..AUU[AUG]GCC...//...GCC[UGA]AAG...//...UGC[UGA]AAA...//...GCG[UGA]ACG...//CCG[UAA]
386 650 698 773 803

Figure 2. UGA codons in E. coli trpR mRNA. The trpR mRNA sequence and numbering of the nucleotides is according to the DNA sequencing of Singleton et al (29). The initiation and termination codons which are in-phase with the predicted reading frame of the Trp aporepressor are shown in boxes. The A residues at the 3' side of UGA codons are underlined. An arrow indicates the last nucleotide of the initiation or termination codons.

tion codons: two UGA codons, and one UAA codon following the first UGA codon by 16,41 and 51 codons respectively (Fig. 2). The weak suppression of the UGA termination codon by normal charged $tRNA^{Trp}$ may hypothetically permit the trpR mRNA to direct the synthesis of four polypeptides of which three are UGA readthrough products. Our present study, on the str^r $trpR^+$ mutants LD1 and LD2, is the first suggestive evidence that the UGA readthrough process is required for the synthesis of a fully active Trp aporepressor. This raises several questions: are the four hypothetical trpR readthrough polypeptides actually synthesized; if so, how are they included in the structure of the Trp aporepressor; how might they participate in the repression of transcriptional initiation at the promoter-operator region of the trp operon. It is difficult to answer these questions because our knowledge in this field is based on studies with an only partially purified Trp aporepressor (30-32). It is presently estimated that there are about 20 copies of the active aporepressor per cell (10,16) and that the efficiency of UGA readthrough is only about 3% (13,33). If so, in order to produce 20 copies of the longest polypeptide, the cell would have to produce $7.5X10^5$ copies of the shortest polypeptide. This seems to us an outrageously large number, and leads us either to question the above estimates, or to suggest that only the first two of the four hypothetical polypeptides are synthesized. We therefore suggest that a fully active Trp aporepressor may include at least one UGA readthrough polypeptide. Such a model supports the idea that the synthesis of the Trp aporepressor is autogenously regulated (29) and we suggest that the UGA readthrough process has a role in this regulation.

The UGA readthrough process might also be involved in the regulation of transcription termination at the trp attenuator site of E. coli. Two models have been proposed to account for the regulation of transcription termination at the trp attenuator (34,35). According to both these models, the configuration of the leader RNA, and therefore transcription termination, is mediated through the translation of the leader segment coding for a 14-residue leader peptide including two tandem tryptophan residues. A UGA codon terminates the

leader segment of E. coli (34,35). We observe that eight codons downstream from this UGA codon there is an efficient termination codon UAA and suggest that when there is UGA readthrough, translational runs continue until this efficient stop signal. In this way, charged $tRNA^{Trp}$ can regulate the translation of the trp leader RNA at two levels: by an efficient translation of the two tandem tryptophan codons, and by an inefficient translation of the UGA codon. We believe that UGA readthrough translation affects the configuration of the leader RNA and/or permits the production of a 22-residue readthrough leader peptide. We are presently studying this problem with trpR⁻ derivatives of mutants LD1 and LD2.

An additional support for the idea that the UGA readthrough process is involved in the translation of the E. coli trpR mRNA and of the leader RNA is the presence of the nucleotide adenine (A) at the 3' side of the in-phase UGA codons of these mRNAs (Fig. 2 and ref 35). We have found that there is an A residue adjacent to the 3' side of in-phase UGA codons in several mRNAs of E. coli phages which specify UGA readthrough proteins (to be published elsewhere). We suggest that the codon context effect, that is, the nature of the nucleotide following the UGA codon, determines whether UGA signals inefficiently or efficiently the termination of polypeptide chain synthesis: an A residue at this position permits the UGA readthrough process.

What new regulatory features does the UGA readthrough process add to the known mechanisms involved in the regulation of the trp operon? We suggest that UGA readthrough may amplify the effects of tryptophan starvation. As well as UGA codons, both trpR mRNA and trp leader RNA contain UGG codons which efficiently specify for charged $tRNA^{Trp}$. Because normal charged $tRNA^{Trp}$ is such a weak suppressor of the UGA terminator, translation is nearly always terminated at UGA and only occasionally continues to the full extent of the translational readthrough runs. As a result, when tryptophan is limiting, a certain level of translation until the UGA terminator is possible, but the level of suppression, if there is any at all, will be below the threshold to permit readthrough. Thus, the UGA readthrough mechanism aids the cell to respond to slight changes in the intracellular level of tryptophan. Even when the cell is starved for tryptophan, residual amounts may remain in the cell due to proteolysis. Because the UGA readthrough effect amplifies the effect of limiting the tryptophan, the cell will respond as if there were no tryptophan at all permitting a more sensitive regulation of the operon.

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