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Role of Leader Peptide Synthesis in Tryptophanase Operon Expression in *Escherichia coli* K-12

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We used site-directed mutagenesis to replace the *Escherichia coli* tryptophanase (*tna*) operon leader peptide start codon with AUC. This change greatly decreased the uninduced rate of *tna* operon expression, and it also lowered the response to inducer. We conclude that leader peptide synthesis plays an essential role in *tna* operon expression.

Tryptophanase catalyzes a variety of substitution and β-elimination reactions involving tryptophan, indole, and related compounds. Expression of the *Escherichia coli* tryptophanase (*tna*) operon is induced by tryptophan and is subject to catabolite repression (reviewed in reference 18). The *tna* operon has been cloned and sequenced (4). In vitro studies indicate that the *tna* promoter lies approximately 300 base pairs proximal to the tryptophanase (*tnaA*) initiation codon (5). The resultant transcribed leader region (*tnaL*) contains several sites for ρ-dependent transcription termination (17). The proximal portion of the leader region contains a coding region, *tnaC*, for a 24-residue leader peptide. Gene fusion studies indicate that *tnaC* is translated in vivo (18). Thus, the first 100 nucleotides of the leader region encode the translation initiation and coding segments for the leader peptide, and the remaining 220 nucleotides contain strong ρ-dependent termination sites (see Fig. 1).

Three lines of evidence have suggested a hypothesis for *tna* operon regulation (18). First, *rho* mutants are constitutive for *tna* operon expression, implicating ρ-dependent transcription termination in operon regulation. In vitro studies demonstrating ρ-dependent transcription termination in the *tna* leader region are consistent with this conclusion (17). Second, measurements of in vivo mRNA synthesis indicate that transcription from the *tna* promoter is constitutive, sensitive only to catabolite repression. However, *tnaA* mRNA synthesis is induced by tryptophan, implying that induction relieves the transcription termination that occurs between the promoter and *tnaA* under noninducing conditions (18). Third, most *cis*-acting *tna* constitutive alterations are single-base changes within the leader region. These changes define a sequence, 5′-CGCCCTTGA-3′, that is similar to a sequence (*boxA*) thought to be important for the action of the transcription factor NusA (6, 7, 10, 15; but see also reference 20). The *boxA*-like sequence overlaps the end of the *tnaC* coding region (18).

We therefore hypothesized that, in the absence of tryptophan, constitutive transcription from the *tna* promoter is subject to ρ-dependent transcription termination in the leader region. This termination requires the *boxA*-like sequence, at which NusA presumably acts to facilitate efficient termination. We also suggested that in the presence of tryptophan, a tryptophan-responsive protein acts as an antitermination factor that allows transcription to proceed into the structural genes of the operon (18).

The role of the leader peptide in *tna* regulation is not addressed by this hypothesis (18). We used site-directed mutagenesis to inactivate the *tnaC* initiation codon. We found that *tnaC* translation was required both for setting the basal level of expression in the absence of tryptophan and also for allowing the maximal rate of expression in the presence of tryptophan.

Site-directed mutagenesis. The site-directed changes, designated *tnaC262*, are shown in Fig. 1. This alteration introduces a *Cla*I restriction site. Restriction fragment *Alu*I-*Rsa*I619, which contains the *tna* promoter and leader and the first 21 codons of *tnaA* (4), was ligated to *Bam*HI molecular linkers and cloned into bacteriophage M13mp10 (12). A 22-residue synthetic oligodeoxyribonucleotide complementary to a portion of the coding strand of *Alu*I-*Rsa*I619 was kindly provided by Karl Pope and Gerard Zurawski (DNAX Research Institute, Palo Alto, Calif.). This oligomer contained the two nucleotide changes of *tnaC262* at positions 11 and 12 (Fig. 1).

The oligomer was annealed to single-stranded template DNA, and the templates were converted to double-stranded DNA in vitro essentially as previously described (1). The resulting double-stranded phage DNAs were transfected into *E. coli* JM105 (23) in the presence of the β-galactosidase indicator X-Gal (12). Colorless plaques were picked and used to prepare replicative-form DNA (2, 12). Twenty-four isolates were screened by *Cla*I digestion and agarose gel electrophoresis (3). Six isolates contained the expected *Cla*I site. Two independent isolates were retained, and both gave identical results in all experiments reported in this paper. The DNA sequence (11, 18) of approximately 50 base pairs to either side of the site-directed changes was identical to the wild-type sequence (data not shown).

Gene fusions. We cloned the *tnaC262*-containing *Alu*I-*Rsa*I619 fragment into plasmid pRLK15 to construct a *tnaC262 tnaA′-lacZ*$^+$ gene fusion, which was then transferred to bacteriophage λ RL1 by in vivo recombination to produce bacteriophage λ SVS47 (18). *E. coli* SVS1100 [W3110 *bglR551* Δ(*lac-argF*)*U169*; 18] was infected with λ SVS47, and a lysogen carrying a single copy of the prophage was retained and designated SVS1147. (Prophage copy number was determined as previously described [18].)

Expression of *tnaA′-lacZ*$^+$. Strains lysogenic for either the *tnaC*$^+$ or the *tnaC262* derivatives of λ*tnaA′-lacZ*$^+$ (SVS1144 and SVS1147, respectively) were cultured at 37°C in 15 ml of MOPS (morpholinepropanesulfonic acid)-AHC medium as

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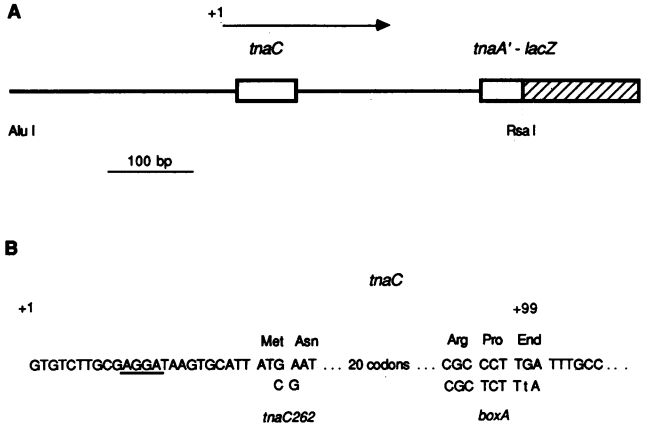

FIG. 1. (A) Schematic diagram of the *tnaA'-lacZ*$^+$ gene fusion constructed with restriction fragment *Alu*I-*Rsa*I619. Transcription initiates at +1 and proceeds to the right (5). This fragment contains the leader peptide coding region (*tnaC*), the 219-base-pair (bp) untranslated region, and the first 21 codons of *tnaA*. For the gene fusion, *tnaA* codon 21 is fused in frame to *lacZ* codon 8 (4, 18). (B) Nucleotide sequence of the *tnaC* translation initiation and termination sites. Transcription in vitro initiates at either of the G residues at positions +1 and +3 (5). The putative Shine-Dalgarno sequence is underlined. The two-base change introduced by site-directed mutagenesis to produce *tnaC262* is shown. These changes introduced a *Cla*I site. The *boxA*-like sequence in *tnaC* overlaps the translation termination site. One version of the *boxA* consensus sequence (7, 18) is shown.

previously described (18). L-Tryptophan was added, as indicated in Fig. 2, to a final concentration of 0.5 mM. At defined intervals, 0.5-ml samples were mixed with 0.5 ml of prewarmed MOPS-AHC medium containing 100 μg of chloramphenicol per ml, incubated at 37°C for 5 min, and placed on ice (8). β-Galactosidase activity was measured in permeabilized cells, and activities were calculated without correction for culture density (13). The results obtained (Fig. 2) are plotted as the total enzyme activity per milliliter of culture vs culture density (Klett units); the slopes correspond to the differential rates of enzyme synthesis (dZ/dX; 14). In the wild type, the uninduced rate of hybrid β-galactosidase synthesis was approximately 12.5 U/Klett unit, and the induced rate was approximately 250 U/Klett unit; this corresponds to an induction ratio of 20. The *tnaC262* lesion had two effects (Fig. 2). First, it depressed the uninduced rate of synthesis 25-fold to roughly 0.5 U/Klett unit. Second, it depressed the induction ratio approximately fivefold; the induced rate of synthesis was roughly 2.0 U/Klett unit. Essentially identical results were obtained with the other independent isolate of the *tnaC262*-containing fusion. Further, *tnaC262* acted strictly in *cis*; tryptophanase induction was normal in both SVS1144 and SVS1147 (data not shown).

mRNA production. We measured the rates of *lacZ* mRNA synthesis in strains SVS1144 and SVS1147 to determine whether the reduced expression observed in SVS1147 was due to decreased transcription. Parallel cultures, growing exponentially at 37°C in MOPS-AHC medium with or without 0.5 mM tryptophan, were pulse-labeled with [^{3}H]uridine for 30 s. Labeled RNA was extracted, hybridized to denatured *lacZ* DNA on nitrocellulose filters, and quantitated exactly as previously described (18). *lacZ* mRNA synthesis was induced normally by tryptophan in the *tnaC*$^+$ strain. Of the total labeled RNA, 0.41% was *lacZ* mRNA. (This level was set as the arbitrary 100% level.) In the absence of tryptophan, the *lacZ* mRNA level was only 23% of that measured when tryptophan was added. The magnitude and ratio of induction under the conditions employed were similar to previously observed values (18). Very low levels of mRNA were present in the *tnaC262* strain cultured in the absence of tryptophan (1.2% of the arbitrary 100% level), and these levels were only slightly induced by tryptophan (1.7% of the 100% level). This experiment confirms the results shown in Fig. 2 and indicates that *tnaC262* interferes with the production of *tnaA* mRNA.

The growth conditions used for the mRNA measurements involved relatively high cell densities coupled with limiting concentrations of the inducer tryptophan (18; V. Stewart and C. Yanofsky, unpublished data). This resulted in only a four-to fivefold induction of *tnaA* mRNA synthesis in wild-type cells (18; see above). The data presented in Fig. 2, showing 20-fold induction in the wild type, were obtained with what we believe were conditions for optimal *tna* operon induction (V. Stewart and C. Yanofsky, unpublished data).

Conclusions. The *tnaC262* lesion changed the *tna* leader peptide start codon to AUC and presumably eliminated leader peptide systhesis (9). This alteration was strictly *cis* acting, in that expression of the resident *tna* operon was unaffected by the presence of *tnaC262* in *cis* to a *tnaA'-lacZ*$^+$ gene fusion (data not shown). The major effect of the *tnaC262* lesion was to lower the uninduced rate of hybrid β-galactosidase synthesis approximately 25-fold (Fig. 2) by affecting the rate of *tnaA'-lacZ*$^+$ mRNA production (see above). Presumably this decrease in distal gene expression was due to increased transcription termination in the *tna*

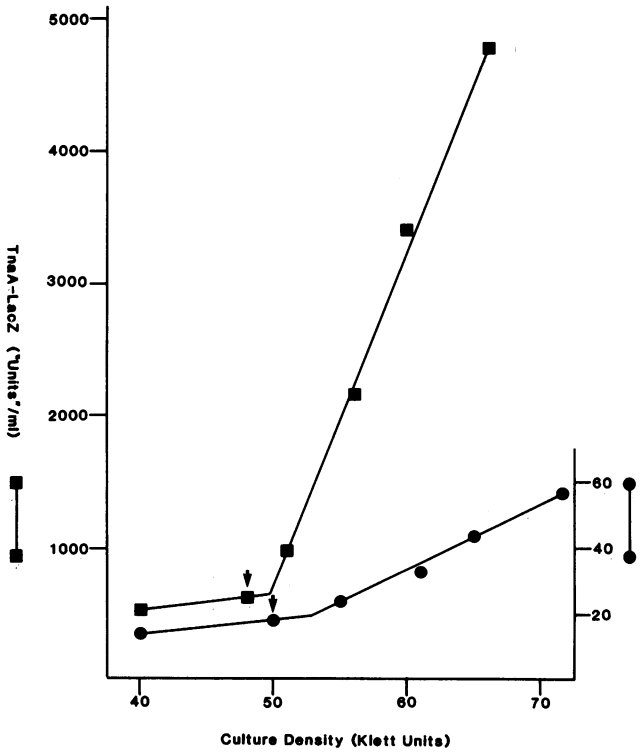

FIG. 2. Expression of *tnaA'-lacZ*$^+$. Strains SVS1144 (λ *tnaC*$^+$ *tnaA'-lacZ*$^+$; ■) and SVS1147 (λ *tnaC262 tnaA'-lacZ*$^+$; ●) were cultured at 37°C and sampled for β-galactosidase assays as described in the text. The arrows mark the addition of tryptophan to 0.5 mM. The slopes correspond to the differential rates of enzyme synthesis (14).

leader region. We envision two explanations to account for this observation.

First, preventing translation of the *tnaC* coding region may have simply enhanced ρ-dependent transcription termination. Untranslated RNA is thought to be the preferred target for ρ-dependent transcription termination (10, 21). Although the *tna* leader region encodes a long (220 nucleotides) stretch of untranslated RNA, it is likely that eliminating translation of the upstream 24-residue *tnaC* coding region would enhance the efficiency of ρ-dependent transcription termination (10, 21). In the wild type, ribosomes engaged in translation probably mask nascent *tnaC* mRNA and thus may reduce the efficiency of ρ-dependent transcription termination. This might account for the rather high uninduced rate of wild-type *tna* expression (Fig. 2).

Second, preventing translation of the leader peptide coding region may have influenced the activity of the *boxA*-like site in the *tna* leader region. It has been proposed that *boxA* provides a recognition site for NusA (6, 7; but see also reference 20). In the *tna* leader region, the *boxA*-like site overlaps the leader peptide coding region, and genetic alterations in this site result in constitutive *tna* operon expression (18). This suggests that NusA is required for efficient transcription termination in the *tna* leader (10, 16, 18) if the *boxA*-like site acts as a NusA recognition sequence. In the wild type, translation of the *tna* leader peptide coding region could alter the presumptive NusA-*boxA* interaction, thereby inhibiting transcription termination. In a strain carrying *tnaC262*, the absence of *tnaC* mRNA translation may have allowed a more efficient NusA-*boxA* interaction, thereby promoting transcription termination. In in vitro transcription experiments we were unable to demonstrate an effect of the *boxA*-like lesions on NusA-promoted transcription pausing in the *tna* leader region. Perhaps leader peptide synthesis normally modulates the activity of the presumed NusA-*boxA* interaction (17). Others have concluded that altered translation affects the NusA-*boxA* interaction in the bacteriophage λ early rightward terminator (15, 22).

Preventing leader peptide synthesis also reduced the induction ratio from approximately 20 in *tnaC*$^+$ to only 4 in *tnaC262* (Fig. 2). Our lack of knowledge concerning the mechanism of tryptophan-mediated transcription antitermination in the *tna* leader precludes a simple interpretation of this observation. However, it is conceivable that translation of the *tnaC* coding sequence facilitates the action of tryptophan-specific or general transcription antitermination factors (6, 7). Regardless of the events normally responsible for transcription antitermination in the *tna* leader region, our results indicate that translation of the *tnaC* coding region is essential for maximal induction of the *tna* operon by tryptophan.

Lesions that interfere with synthesis of the tryptophan (*trp*) biosynthetic operon leader peptide in *Serratia marcescens* cause increased transcription termination at the attenuator, a phenomenon termed superattenuation (19). It was hypothesized that superattenuation may play a physiological role in preventing *trp* operon expression under conditions in which protein synthesis is inhibited. Perhaps under conditions of amino acid limitation the absence of synthesis of the *tnaC* leader peptide similarly reduces *tna* expression. This possibility has not yet been tested.

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