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Evidence for Transcription Antitermination Control of Tryptophanase Operon Expression in *Escherichia coli* K-12

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Tryptophanase, encoded by the gene *tnaA*, is a catabolic enzyme distinct from the enzymes of tryptophan biosynthesis. Tryptophanase synthesis is induced by tryptophan and is subject to catabolite repression. We studied the mechanism of *tna* operon induction. Mutants with altered ρ factor were partially constitutive for *tna* expression, implicating ρ-dependent transcription termination in the control of *tna* expression. Measurements of mRNA synthesis from the transcribed leader region preceeding the *tna* operon suggested that the *tna* promoter was constitutive and that in the absence of inducer, transcription terminated in the leader region. Upon induction, this transcription termination was relieved. *Cis*-acting constitutive mutants had genetic alterations in the *tna* leader region. These lesions defined a site that is homologous to the bacteriophage λ *boxA* sequence, which is thought to play a role in antitermination control of λ lytic gene expression. We propose that *tna* expression is subject to transcription antitermination control. We hypothesize that a tryptophan-activated antiterminator protein mediates induction by suppressing the ρ-dependent termination sites in the leader region, thus allowing transcription to proceed into the *tna* operon structural gene region.

Tryptophanase is a multifunctional enzyme found exclusively in bacteria. The structure, enzymatic activities, and reaction mechanisms of *Escherichia coli* tryptophanase are well understood (34, and references therein). One tryptophanase reaction, a β-elimination, involves degradation of tryptophan to form indole, pyruvate, and ammonia. Other β-elimination substrates include cysteine, *S*-methylcysteine, and serine (44). Tryptophanase also catalyzes replacement reactions, including the condensation of cysteine or serine with indole to form tryptophan (44). The elimination reaction itself is reversible: under appropriate conditions, tryptophanase can synthesize tryptophan from indole, pyruvate, and ammonia (61).

The *tna* operon contains at least two genes. The promoter-proximal gene, *tnaA*, encodes tryptophanase (17). The second gene, *tnaB*, is required for low-affinity tryptophan permease activity (13, 17, 20; V. Stewart and C. Yanofsky, unpublished observations). Expression of the *E. coli tna* operon is induced by tryptophan and is subject to catabolite repression (see below). Thus, tryptophanase probably serves a catabolic role in vivo. In the laboratory, *E. coli* will grow with tryptophan as the sole source of carbon and energy (46).

Some of the major effects of catabolite repression are believed to be mediated through cyclic AMP (cAMP) plus the cAMP receptor protein (cAMP-CRP). The cAMP-CRP complex makes up a positive-acting element required for efficient transcription initiation at catabolite-sensitive promoters; mutants deficient in CRP or adenylate cyclase are incapable of inducing the *tna* operon (reviewed in reference 16). However, other factors, independent of cAMP, probably also play significant roles in the catabolite repression of *tna* operon expression (10, 11).

Newton and Snell (43, 45) developed a selection for *tnaA* expression based on two observations: first, that *E. coli* can use tryptophanase to synthesize tryptophan from indole plus cysteine, and second, that 5-methyltryptophan (5MT) is an efficient inducer of tryptophanase synthesis. Thus, a mutant lacking tryptophan synthetase (encoded by genes *trpB* and *trpA*) will grow on a tryptophan-free medium that contains both indole and 5MT. Newton and Snell extended this selection to isolate a regulatory (constitutive) mutant by selecting for growth on indole medium lacking 5MT.

This selection for constitutive mutants has been used in other laboratories (18, 21, 26). However, few of these mutants have been characterized in detail. No *trans*-acting regulatory alterations have been described, and the genetic basis of *tna* regulation has remained unknown. Others have found that the kinetics of *tna* induction are very similar to those for *lac*, but these observations have not been pursued (7).

The *tna* operon has been cloned and sequenced (17; V. Stewart and C. Yanofsky, unpublished observations). In vitro transcription experiments located a cAMP-CRP-dependent promoter 319 base pairs proximal to the *tnaA* initiation codon (18). It has been postulated that this is the *tna* promoter and that the intervening transcribed leader region is involved in regulating *tna* expression (18).

We report here an analysis of *tna* regulation. We found that strains with altered ρ factor were partially constitutive for *tna* expression. Measurements of mRNA synthesis provided evidence for regulated transcription termination in the leader region preceeding the *tnaA* gene. Constitutive mutants harbored single-base-pair changes in the *tna* leader region. These alterations were *cis*-acting and were located in a short coding region, which we designate *tnaC*. The region defined by most of these *tnaC* lesions is homologous to a sequence *boxA*, that is important for antitermination control of bacteriophage λ gene expression (23, 24, 48, 49). We propose a model for transcription antitermination control of *tna* operon expression that is analogous to that for λ lytic gene expression. We hypothesize that a tryptophan-responsive antitermination protein mediates *tna* operon in-

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TABLE 1. Bacterial strains, bacteriophages, and plasmids

Strain	Genotype	Reference[a]
E. coli K-12 strains		
W3110	F^- λ^- prototroph	2
LE392	*hsdR514 supE44 supF58 lacY1 galK2 galT22 metB1 trpR55*	22
MX397	*supD43,74 sueA sueC leu*(Am) *lac*(Am) *galK*(Am) *galE rpsL metB relA*	47
SVS1	As LE392 but Δ(*trpEA*)*2 tnaA2*[b] *bglR551 recA56*	
SVS3	As LE392 but Δ(*lac-argF*)*U169 recA56*	
SVS4	As MX397 but Δ(*trpEA*)*2*	
Derivatives of W3110		
SVS7	*rho-15*	31
SVS8	*rho-101*	37
SVS9	*rho-102*	37
SVS10	*rho-103*	37
SVS100	*bglR551*	
SVS102	*bglR551 tnaA2*[b]	13
SVS113	*bglR551 bgl-552*::Tn*10*	
SVS120	*bglR551 trpR2*	64
SVS121	*bglR551 trpS10343*	19
SVS124	*bglR551 nusA1*	24
SVS300	*bglR551* Δ(*trpEA*)*2*	
SVS1100	*bglR551* Δ(*lac-argF*)*U169*	
SVS1142	*bglR551* Δ(*lac-argF*)*U169* (λSVS42)	
SVS1144	*bglR551* Δ(*lac-argF*)*U169* (λSVS44)	
SVS1500	*bglR551* Δ(*lac-argF*)*U169* (λSVS40)	
SVS1600	*bglR551* Δ(*lac-argF*)*U169 recA1* (λSVS40)	
SVS1650	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC250* (λSVS40)	
SVS1651	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC251* (λSVS40)	
SVS1652	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC252* (λSVS40)	
SVS1653	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC253* (λSVS40)	
SVS1654	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC254* (λSVS40)	
SVS1655	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC255* (λSVS40)	
SVS1656	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC256* (λSVS40)	
SVS1657	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC257* (λSVS40)	
SVS1658	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC258* (λSVS40)	
SVS1659	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC259* (λSVS40)	
SVS1660	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC260* (λSVS40)	
SVS1661	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC261* (λSVS40)	
SVS1744	*bglR551* Δ(*lac-argF*)*U169 recA1* (λSVS44)	
SVS1746	*bglR551* Δ(*lac-argF*)*U169 recA1* (λSVS46)	
SVS1844	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC255* (λSVS44)	
SVS1846	*bglR551* Δ(*lac-argF*)*U169 recA1 tnaC255* (λSVS46)	
SVS1910	*bglR551* Δ(*lac-argF*)*U169 recA1* (F′*lacI*^{Q}) (pMY231)	51
SVS1911	*bglR551* Δ(*lac-argF*)*U169 recA1* (F′*lacI*^{Q}) (pSWC101)	51
Bacteriophages		
P1 *kc*		40
λgt*S*m4 Cm^r		35
λRL1	as λgt*S*m4 Cm^r but *cI*$^+$	R. Landick
λSVS40	pSVS40 × λRL1 recombinant	
λSVS42	pSVS42 × λRL1 recombinant	
λSVS44	pSVS44 × λRL1 recombinant	
λSVS46	pSVS46 × λRL1 recombinant	
Plasmids		
pMD4		17
pMD6		17
pRLK15		64
pMC931		14
pMY231	*trpT*$^+$	51
pSWC101	*trpT176* (Su7-UGA)	51
pSVS40	*tnaC*$^+$ Φ(*tnaA′-lacZ*$^+$) (pRLK15 with *Rsa*I910 from pMD6)	
pSVS42	Φ(*tnaC′-lacZ*$^+$) (pRLK15 with *Alu*I-*Sau3A*I325 from pSVS1)	
pSVS44	*tnaC*$^+$ Φ(*tnaA′-lacZ*$^+$) (pRLK15 with *Alu*I-*Rsa*I619 from pSVS1)	
pSVS46	*tnaC255* Φ(*tnaA′-lacZ*$^+$) (pRLK15 with *Alu*I-*Rsa*I619 from pSVS1-55)	

[a] For all SVS bacterial strains, the literature citations refer to the allele.
[b] *tnaA2* is the allele from strain T_3C (13).

Continued on following page

duction by suppressing transcription termination in the leader region.

MATERIALS AND METHODS

Strains. The *E. coli* K-12 strains employed in this study are listed in Table 1. All strains except SVS1 through SVS4 were derived from the prototroph W3110. For simplicity, only the final strains are listed. Genetic crosses were performed by bacteriophage P1 *kc*-mediated generalized transduction (40). In most cases, strains were constructed with the aid of transposon insertions (15, 36). For example, *recA* alterations were introduced by a two-step procedure: first, the strain was transduced to *srl-300*::Tn*10*; and second, the *srl* derivative was transduced to *srl*$^+$ *recA*.

The tryptophanase (*tna*) operon is closely linked to cryptic genes (termed *bgl*) for utilization of β-glucosides such as salicin (3). A spontaneous Bgl$^+$ (*bglR551*) strain was isolated as a red papillus from a colony of W3110 growing on MacConkey-salicin medium (53) to provide a convenient selectable marker for introducing *tna* alleles into Bgl$^-$ strains.

rho alleles were cotransduced with *ilv*$^+$ into strain W3110 *lacZU118 ilv*::Tn*10*. Ilv$^+$ transductants were screened for suppression of the LacY$^-$ phenotype by the donor *rho* lesion (37). The allele *rho-15* is generally considered to confer a temperature-sensitive phenotype (31). However, strain SVS7 (*rho-15*) was not temperature sensitive, in agreement with the findings of others (5, 32).

Media. Media for routine use, including LB, λ, and supplemented minimal media, were prepared from standard

recipies (15). E minimal medium was used for preparing defined agar media (57). Medium for selecting *tna*$^+$ was E medium supplemented with 0.2% potassium succinate, 0.2% acid-hydrolyzed casein (AHC), 10 μg of indole per ml, 50 μg of 5MT per ml, and 20 μg of cysteine per ml. This formula is adapted from that of Newton and Snell (45). 5MT is poorly incorporated into protein and thus is nontoxic under these conditions (19). Medium for selecting tryptophanase constitutive mutants was identical except that 5MT was omitted. Solidified media contained 1.6% Bacto-Agar (Difco Laboratories, Detroit, Mich.). Vitamin-free, salt-free AHC was from ICN Nutritional Biochemicals, Cleveland, Ohio. (Acid hydrolysis destroys the tryptophan present in casein.) Other dehydrated media and agar were from Difco Laboratories.

3-[*N*-Morpholino]propanesulfonic acid (MOPS)-based minimal medium (42) was prepared from a modified formula (9), except that it contained 13.2 mM K_2HPO_4 (42). 1% AHC was added as a source of carbon and energy. Additional carbon sources used to elicit catabolite repression were filter sterilized and added, as indicated, at 20 mM.

Eosin-methylene blue base supplemented with 1% salicin was used for selecting *bglR* (Bgl$^+$) in genetic crosses. Transductants were visible above the background growth after overnight incubation at 37°C. MacConkey agar base supplemented with 1% salicin was used for scoring the Bgl phenotype.

Culture conditions. Cultures for measurements of enzyme specific activity were inoculated by diluting 0.02 ml of an overnight MOPS-AHC culture into 10 ml of fresh medium. Cultures contained in 125-ml sidearm flasks were incubated at 37°C with vigorous shaking in gyratory water baths. Culture densities were monitored with a Klett-Summerson photoelectric colorimeter equipped with a number 66 (red) filter.

Enzyme assays. Cultures were harvested at approximately 3×10^8 CFU/ml (about 60 Klett units), washed once with cold 150 mM NaCl, and suspended in 5 ml of assay buffer. Cell extracts were prepared by passing the cell suspensions through a French pressure cell at 8,000 lb/in^2 (54). Unbroken cells were removed by centrifugation at 3,500 × *g* for 5 min. Protein concentrations were measured with the Bio-Rad protein assay reagent (12). Enzyme activities were determined at room temperature, approximately 23°C. Each sample was assayed in triplicate by varying the volume of extract used. Activities reported in the text are the averages of these values.

Tryptophanase activities were determined by measuring the hydrolysis of *S*-*o*-nitrophenyl-L-cysteine to *o*-nitrothiophenolate. The assay procedure was adapted from that of Kazarinoff and Snell (34). Extracts in T buffer (0.1 M KPO_4 [pH 7.8], 1 mM EDTA, 0.2 mM dithiothreitol, 20 μM pyridoxal phosphate [34]) were incubated at 37°C for 30 min to ensure full activation of tryptophanase by pyridoxal phosphate. A sample of cell extract was mixed with T buffer to a final volume of 1 ml. The assay was started by adding 1 ml of 1.2 mM *S*-*o*-nitrophenyl-L-cysteine in water, and it was stopped by adding 0.2 ml of 1 N NaOH. The optical density was measured at 470 nm, and activity was calculated by assuming an extinction coefficient for *o*-nitrothiophenolate of 626 M^{-1} cm^{-1} (34). This assay was linear with time for at least 2 h and with protein to at least 250 μg/ml. However, the assay was nonlinear at optical density (470 nm) values greater than 0.22. *S*-*o*-Nitrophenyl-L-cysteine was synthesized in the Department of Chemistry, Stanford University.

β-Galactosidase activities were determined by measuring the hydrolysis of *o*-nitrophenyl-β-D-galactoside to *o*-nitrophenol. The assay procedure closely followed the one described by Miller (40). A sample of cell extract in Z buffer (40) was mixed with Z buffer to a final volume of 1 ml. The assay was started by adding 0.2 ml of 4-mg/ml *o*-nitrophenyl-β-D-galactoside in Z buffer and stopped by adding 0.5 ml of 1 M Na_2CO_3. The optical density was measured at 420 nm, and activity was calculated by assuming an extinction coefficient for *o*-nitrophenol of 4,500 M^{-1} cm^{-1} (40).

Mutant isolation. Tryptophanase constitutive mutants (TnaC$^-$) were isolated from strains SVS4 and SVS300. Ethyl methanesulfonate and 2-aminopurine mutageneses were performed exactly as described by Miller (40). Samples of cultures were plated on the indole-selective medium (lacking 5MT) described above. After 2 days of incubation at 42°C (SVS4) or 37°C (SVS300), colonies were picked and purified by single-colony isolation on selective medium. Each allele described in this paper represents an independent mutational event, because only a single isolate from each culture was retained for analysis. Genetic alterations were mapped by generalized transduction with SVS113 (*bgl-552*::Tn*10*) as the donor. Alleles analyzed in this study were cotransduced into strain SVS300 by linkage to *bgl-552*::Tn*10*. These strains were then crossed back to Bgl$^+$ by transduction from SVS300.

Strain MX397 contains a temperature-sensitive allele of *supD*, so that strains carrying amber lesions have the mutant phenotype at 42°C but show the wild-type (i.e., suppressed) phenotype at 30°C (47). A derivative of MX397, SVS4, allowed the selection of TnaC$^-$ mutants. More than 500 mutants were selected at 42°C, but none appeared to be wild type at 30°C.

Replica plating was used to screen pools of random chromosomal Tn*10* or Tn*5* transposon insertions for TnaC$^-$ or Bgl$^-$ mutants (15, 36, 62). No TnaC$^-$ mutants were recovered after mutagenesis with Tn*5* (approximately 100,000 colonies screened) or Tn*10* (over 60,000 colonies screened).

Molecular cloning. Standard methods were used for routine cloning, including restriction endonuclease digestion, ligation, transformation, gel electrophoresis, etc. (15). DNA-modifying enzymes were either purchased from commercial suppliers or purified in our laboratory by published procedures. Plasmid DNA was isolated by the alkaline lysis procedure (8). Large-scale plasmid preparations were purified by CsCl-ethidium bromide equilibrium density gradient ultracentrifugation (15).

tnaC alleles were cloned by recombination in vivo, following the method of Stauffer et al. (56). Plasmid pMD4 carries DNA from the *tna* region of *E. coli*, except that it contains an internal deletion removing the *tna* promoter, leader, and N-terminal coding sequence of *tnaA* (17). pMD4 was transformed into *tna*C strains, and transformants were clutured for several generations to allow the chromosomal *tna* region to recombine onto pMD4. Isolated plasmid DNA was used to transform strain SVS1, and the cells were plated on indole-5MT-tetracycline medium. Tna$^+$ transformants contained recombinant plasmids carrying the intact *tna* region from the donor strain, including the donor *tnaC* lesion.

Gene fusions. Published methods were used to construct gene fusions (35, 64) (Fig. 1). Plasmid pRLK15, a derivative of pMC931, contains an engineered *lacZ* gene that replaces the coding region for the first seven amino acid residues with a *Bam*HI restriction site (14, 64). Fragment *Alu*I-*Rsa*I619 from the pMD4-recombinant plasmids (described above) and fragment *Rsa*I910 from the *tnaA*$^+$ plasmid pMD6 (17) both contain the *tna* promoter and leader and the amino-terminal

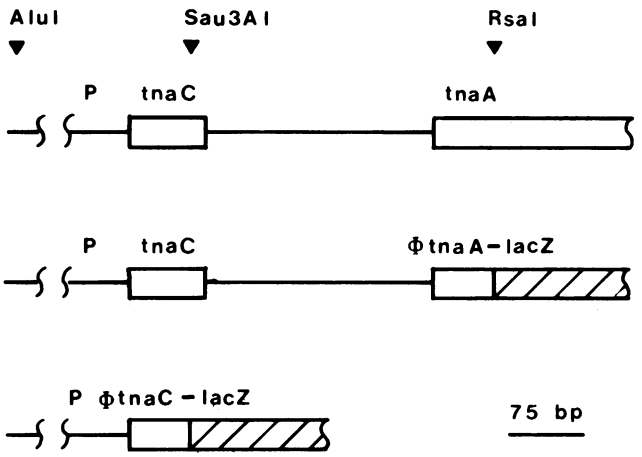

FIG. 1. Schematic representation of *tna'-lacZ*$^+$ gene fusions. Only the N-terminal portions of *tnaA* and *lacZ* are diagrammed. Open boxes represent *tna* translated sequences; crosshatched boxes represent *lacZ* sequences. Transcription initiates at the promoter (P) and proceeds from left to right. See Materials and Methods for details of construction. bp, Base pairs.

coding region for *tnaA* (Fig. 2). The distal *Rsa*I site occurs at *tnaA* codon 21 (17). *Bam*HI molecular linkers (5'-CCGGATCCGG-3'; Bethesda Research Laboratories, Inc., Gaithersburg, Md.) were ligated to the ends of the fragments; the DNA sequence is such that cloning these *Bam*HI fragments into pRLK15 produced in-phase *tnaA'-lacZ*$^+$ gene fusions. Fragment *Alu*I-*Sau*3AI325 was used to construct the *tnaC'-lacZ*$^+$ hybrid gene fusion (17). In this case, the *Sau*3AI site within the *tnaC* coding region overlapped the *Bam*HI site in pRLK15 to directly produce an in-phase gene fusion (Fig. 2). Recombinant plasmids from several transformants were analyzed to identify those with the proper *tna'-lacZ*$^+$ gene fusions.

Rec$^+$ strains carrying selected plasmids were used as hosts to prepare lysates of bacteriophage λRL1, which shares homology with the regions flanking the *lacZY* sequence in pRLK15. Thus, the resulting phage lysates contained recombinant phage which had acquired the *tna'-lacZ*$^+$ fusions from the plasmid (35). Strain SVS1100 was transduced with fusion-bearing specialized transducing phages isolated from these lysates (15). All gene fusion experiments reported in this paper were done with single-copy lysogens (4).

DNA sequencing. The chemical cleavage procedure of Maxam and Gilbert was used for all DNA sequence analyses (39). The reaction products were resolved on thin 8 or 20% acrylamide–7 M urea gels (55) or on 6% acrylamide–7 M urea buffer gradient gels (6).

mRNA levels. Cultures (10 ml) growing in MOPS-AHC medium were pulsed with [^{3}H]uridine for 30 s, and RNA was isolated by previously described methods (65). The RNA was hybridized to 2 μg of plasmid DNA that had been immobilized on nitrocellulose filters (BA85; Schleicher & Schuell, Inc., Keene, N.H.). Conditions for hybridization and quantitation procedures have been described previously (64).

tnaA mRNA was measured by hybridization to *Hin*dIII-digested pMD6 plasmid DNA, less background hybridization to the vector, pBR322. *lacZ* mRNA was measured by hybridization to *Bam*HI-digested pMC931 DNA, less background hybridization to the vector, pACYC177. The data reported in the text are the averages of two independent experiments.

RESULTS

Culture conditions. We measured tryptophanase production in cultures after growth in MOPS medium supplemented with AHC as a source of carbon and energy (9, 42). Strain W3110 grew with a doubling time of approximately 70 to 75 min in this medium, and its growth was exponential to a density of greater than 5 × 10^8 CFU/ml (over 100 Klett units). Tryptophanase specific activity increased with the level of tryptophan in the medium up to 0.5 mM. Specific activity was further increased only slightly by tryptophan concentrations as high as 20 mM, so we usually added 0.5 mM tryptophan for induction.

In several studies it has been shown that catabolite repression has a more severe effect on tryptophanase formation than on β-galactosidase formation (10, 11; and references therein). We observed similar effects in MOPS-AHC medium supplemented with both 20 mM tryptophan and 20 mM of any of several carbon sources (data not shown). Glucose, lactose, and arabinose each repressed tryptophanase synthesis to less than 1% of the control value. Even relatively poor carbon sources, such as glycerol or pyruvate, resulted in catabolite repression of tryptophanase synthesis to about 5 to 10% of the control value. In contrast, glycerol or pyruvate had little effect on β-galactosidase synthesis.

Tryptophanase production in various mutants. Strains carrying lesions in *trpR* or *trpS* showed essentially wild-type tryptophanase levels with or without induction (Table 2). We believe that the slightly elevated uninduced tryptophanase level in the *trpR* strain simply reflected overproduction of tryptophan in this mutant. These findings suggest that *tna* regulation is distinct from that of the *trp* operon.

Other studies (see below) indicated that ρ-dependent transcription termination may be involved in *tna* regulation. Indeed, several *rho* mutants were partially constitutive for tryptophanase production (Table 2). The *nusA1* allele reduces *N* gene product-mediated antitermination control in bacteriophage λ (24), but it had little effect on *tna* expression (Table 2).

Comparision of *tna* induction by tryptophan and 5-MT. Newton and Snell (45) showed that 5MT is an efficient tryptophanase inducer. We extended their results, using a *tnaA'-lacZ*$^+$ gene fusion to monitor the induced rate of tryptophanase production. We grew parallel cultures of strain SVS1500 in MOPS-AHC medium supplemented with 0.05 mM tryptophan. At cell densities of about 3 × 10^8 CFU/ml, we added L-tryptophan to a final conentration of 0.5 mM or 5-methyl-DL-tryptophan to a final concentration of 1 mM. We removed samples at 5-min intervals and assayed β-galactosidase activity directly in permeabilized cells (40). The rate of hybrid β-galactosidase synthesis was similar with either tryptophan or 5MT as inducer, approximately 40 U of activity per min per 10^8 CFU.

Comparison of *tnaA* and *tnaC* expression. The *tna* leader region (*tnaL*) contains a coding sequence (*tnaC*) for a 24-residue peptide (Fig. 2). Strains carrying a *tnaC'-lacZ*$^+$ gene fusion produced active β-galactosidase, indicating that this coding region was translated in vivo (see below). Thus, the transcript of *tnaL* contains a translated region, *tnaC*, and a long untranslated region, from +100 to +319 (Fig. 2).

The *tnaC'-lacZ*$^+$ fusion deleted the nontranslated portion of the leader region (Fig. 1). Comparing the relative expression of *tnaC'-lacZ*$^+$ and *tnaA'-lacZ*$^+$ suggested a regulatory role for this region. β-Galactosidase synthesis from *tnaC'-*

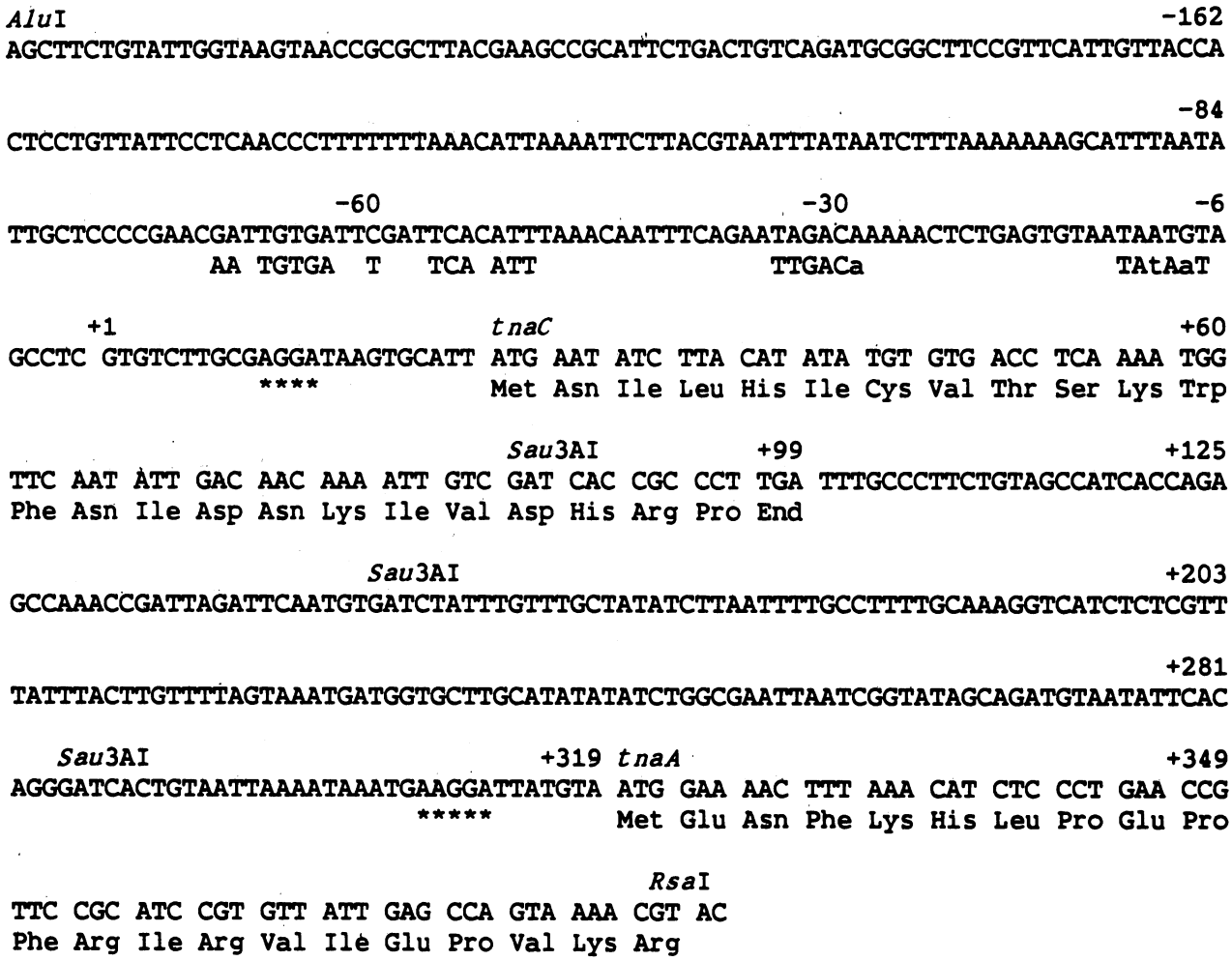

FIG. 2. Nucleotide sequence of restriction fragment *Alu*I-*Rsa*I619. The sequence is numbered in relation to the first transcription start site, +1; the second start is at +3 (18). The consensus promoter sequence is shown directly below the −10 and −30 regions (33). The cAMP-CRP binding site is at −60; the consensus sequence is shown directly below (16). Shine-Dalgarno regions are marked with asterisks (28). The *Sau*3AI site at +85 was used to construct the *tnaC'-lacZ*$^+$ gene fusion (Fig. 1). The sequence shown differs from that previously reported (17) at three positions: an additional A at +138; A instead of T at +150; and C instead of T at +358.

lacZ$^+$ was insensitive to the presence of tryptophan and was repressed about 10-fold by glucose (Table 3). This indicates that transcription initiation from the *tna* promoter is subject to catabolite repression but not to tryptophan control. In contrast, β-galactosidase synthesis from *tnaA'-lacZ*$^+$ was induced about 20-fold by tryptophan; the induced level was repressed over 100-fold by glucose (Table 3).

We pulse-labeled the same cultures with [^{3}H]uridine and measured the rate of specific mRNA synthesis. In the absence of tryptophan, *lacZ* mRNA production from *tnaC'-lacZ* was high, whereas that from *tnaA'-lacZ*$^+$ was low (Table 3). In the presence of tryptophan, *lacZ* mRNA production from both fusions was high. This suggests that the region between *tnaC* and *tnaA* contains a tryptophan-responsive transcription terminator and that this terminator is used to regulate *tna* expression.

We also measured tryptophanase enzyme activity and *tnaA* mRNA levels in the same experiments. The relative levels of *tnaA* induction paralleled those of *tnaA'-lacZ*$^+$ (Table 3), suggesting that the *tnaA'-lacZ*$^+$ gene fusion provided a legitimate indicator of *tnaA* expression.

Under inducing conditions, the hybrid β-galactosidase specific activity from *tnaC'-lacZ*$^+$ was 20-fold lower than that from *tnaA'-lacZ*$^+$, even though *tnaC'-lacZ*$^+$ produced nearly twice as much *lacZ* mRNA (Table 3). This difference may have been due at least in part to a lower rate of translation initiation at *tnaC*, although the specific activities or stabilities of the two hybrid enzymes may also have differed. The spacing between the Shine-Dalgarno region and the AUG initiation codon of *tnaC* is 10 base pairs (Fig. 2), which may result in relatively poor translation of the *tnaC* coding region (28).

The induction ratios of the *tnaA'-lacZ*$^+$ enzyme and of tryptophanase were approximately 20 and 100, respectively. An analogous observation was made in comparing *trpE* and *trp-lac* expression (64). Others have noted that the induced rate of tryptophanase synthesis is lower when the *tnaA* gene is located at λ *att*, on a specialized transducing prophage (59). Thus, we believe that the difference in induction ratios may have been due to position effects rather than to artifacts associated with the gene fusions.

Constitutive mutants. We isolated constitutive tryptophanase mutants after mutagenesis with ethyl methanesulfonate or 2-aminopurine, or as spontaneous mutants.

TABLE 2. Tryptophanase expression in wild-type and mutant strains

Strain	Relevant genotype	Relative sp act[a] of tryptophanase after growth in tryptophan (mM)			
		0	0.05	0.5	0.5 plus glucose
SVS100	Wild type	1.3	15	100[b]	<0.5
SVS120	*trpR*	4.6	—[c]	118	—
SVS121	*trpS*[d]	—	18	118	—
SVS124	*nusA1*	1.3	—	77	<0.5
SVS7	*rho-15*	49	—	98	2.0
SVS8	*rho-101*	9.5	—	102	—
SVS9	*rho-102*	10	—	100	—
SVS10	*rho-103*	9.3	—	94	<0.5

[a] The induced specific activity in SVS100 is set at 100%.
[b] 0.43 μmol of *o*-nitrothiophenolate formed per min per mg.
[c] —, Not determined.
[d] *trpS* strains are tryptophan auxotrophs (19).

The majority of the lesions (over 98%) were linked to *bgl-552*::Tn*10* and thus were linked to the *tna* operon. A few mutants had constitutive alterations which were unlinked to *bgl*. Most of these strains grew very poorly and are not considered further in this report. DNA sequence analysis (see below) established that all of the *bgl*-linked constitutive mutants we studied had lesions in the *tnaC* region; we designate these genetic alterations as *tnaC* (TnaC$^-$ phenotype).

We chose 12 mutants for detailed analysis and backcrossed each to strain SVS300. Six of the alleles were isolated in strain SVS4 (*tnaC250* to *tnaC255*), and six were isolated in strain SVS300 (*tnaC256* to *tnaC261*). These mutants represented the range of phenotypes we observed, from relatively poor to excellent growth on indole medium lacking 5MT. We mapped three alleles by reciprocal three-point crosses and found the order *tnaC-tnaA2-bglR* (data not shown).

TABLE 3. Comparison of tryptophanase and *tna-lac* hybrid β-galactosidase expression with mRNA expression

Strain	Assay[a]	Relative levels[b]					
		Enzyme sp act			mRNA[c]		
		−Trp	+Trp	+Trp, +Glc	−Trp	+Trp	+Trp, +Glc
SVS1144 *tnaA'-lacZ+*	Tna	0.9	100[f]	—[e]	15	100[c]	—
SVS1144 *tnaA'-lacZ+*	Lac	5.3	100[g]	0.46	16	100[h]	<0.1
SVS1142 *tnaC'-lacZ+*	Lac	4.6	4.8	0.48	177	179	12

[a] Tna, Tryptophanase enzyme or mRNA; Lac, β-galactosidase enzyme or mRNA.
[b] The induced specific activity in SVS1144 is set at 100%. −Trp, Uninduced (no tryptophan in growth medium); +Trp, induced (20 mM tryptophan in growth medium); +Trp, +Glc, catabolite repressed (tryptophan plus glucose in growth medium).
[c] *tna* mRNA was measured by hybridization to pMD6, and *lac* mRNA was measured by hybridization to pMC931.
[d] 0.6 μmol of *o*-nitrothiophenolate formed per min per mg.
[e] —, Not determined.
[f] 0.38% of total labeled RNA.
[g] 20.5 μmol of *o*-nitrophenol formed per min per mg.
[h] 0.54% of total labeled RNA.

TABLE 4. Complementation analysis of *tnaC* mutants

Strain	Allele	Relative sp act[a]					
		Tryptophanase			β-Galactosidase		
		−Trp	+Trp	Ratio[b]	−Trp	+Trp	Ratio[b]
SVS1600	*tnaC*$^+$	1.1	100[c]	91	3.9	100[d]	25
SVS1650	*tnaC250*	24	74	3.1	4.7	120	25
SVS1651	*tnaC251*	24	72	3.0	4.7	124	26
SVS1652	*tnaC252*	47	104	2.2	4.2	119	28
SVS1653	*tnaC253*	39	175	4.5	4.6	124	27
SVS1654	*tnaC254*	24	94	3.9	4.6	134	29
SVS1655	*tnaC255*	20	74	3.7	3.9	126	32
SVS1656	*tnaC256*	25	110	4.4	4.7	121	26
SVS1657	*tnaC257*	39	153	3.9	4.7	133	28
SVS1658	*tnaC258*	55	163	3.0	8.7	123	15
SVS1659	*tnaC259*	74	182	2.5	9.4	147	16
SVS1660	*tnaC260*	74	168	2.3	8.6	136	16
SVS1661	*tnaC261*	76	86	1.1	7.8	146	19

[a] The induced specific activity in SVS1600 is set at 100%. −Trp, Uninduced (no tryptophan in growth medium); +Trp, induced (0.5 mM tryptophan in growth medium).
[b] Ratio of induced to uninduced specific activities (induction ratio).
[c] 0.51 μmol of *o*-nitrothiophenolate formed per min per mg.
[d] 13.5 μmol of *o*-nitrophenol formed per min per mg.

Expression and complementation analysis. The *tnaC* mutants were partially constitutive, expressing *tnaA* at uninduced levels approximately 20 to 80% of the wild-type induced level. Most responded to inducer, and many were hyperinducible, with induced levels approximately 150 to 180% of the wild-type induced level (Table 4). We also examined glycerol- and glucose-mediated catabolite repression in these mutants, but none showed more than a slight relief of catabolite repression on either carbon source (data not shown). Thus, the phenotypes of these strains were very similar to those of previously reported constitutive mutants, termed *tnaR* (18, 21, 26).

All *tna* constitutive mutants previously tested are *cis*-dominant (18, 21, 27). We used a *tnaA'-lacZ*$^+$ gene fusion for complementation tests, to determine the *cis-trans* behavior of our *tnaC* mutants. We constructed *trp*$^+$ Δ*lac recA* derivatives of each of the 12 *tnaC* mutants, lysogenized each derivative with a *tnaA'-lacZ*$^+$ specialized transducing phage, and measured both tryptophanase and β-galactosidase specific activities after growth under noninducing or inducing conditions. Each mutant exhibited constitutive tryptophanase production but retained wild-type-like regulation of the hybrid β-galactosidase product (Table 4). Thus, the *tnaC* lesions were *cis*-dominant, as defined by this test.

We constructed a *tnaA'-lacZ*$^+$ gene fusion carrying the *tnaC255* allele to confirm the validity of *tnaA'-lacZ*$^+$ as an indicator for the complementation analyses described above. We measured both tryptophanase and β-galactosidase specific activities in a set of four strains (SVS1744, 1746, 1844, 1846) carrying all possible combinations of *tnaC*$^+$ or *tnaC255* linked to either *tnaA*$^+$ or *tnaA'-lacZ*$^+$. The *tnaC255* allele conferred a strictly *cis*-acting phenotype, regardless of its chromosomal location (data not shown).

DNA sequence analysis. The above experiment demonstrated that the *tnaC255* lesion was contained within the *Alu*I-*Rsa*I619 restriction fragment. This fragment contains the *tna* promoter and leader region, as well as the first 20 codons of *tnaA*. We determined the DNA sequence, on both strands, for both the wild-type and the *tnaC255 Alu*I-*Rsa*I619 fragments. The DNA sequences differed at a single position, withn *tnaC*. We then sequenced the corresponding

Allele	Mutagen	Sequence
		+99
		AAA ATT GTC GAT CAC CGC CCT TGA TTT GCC CTT CTG TAG
		CGC TCT TtA
tnaC250	EMS	G
tnaC251	EMS	G
tnaC252	EMS	G
tnaC253	EMS	A
tnaC254	2AP	G
tnaC255	2AP	G
tnaC256	EMS	T
tnaC257	EMS	A
tnaC258	none	A
tnaC259	none	A
tnaC260	none	A
tnaC261	none	+A

FIG. 3. Nucleotide sequence changes in *tnaC* alleles. Only the relevant portion of the wild-type leader region nucleotide sequence around position +99 is shown (Fig. 2). A consensus *boxA* sequence is shown directly below the *tnaC* sequence at positions 90 to 99 (24). The nucleotide change resulting from each mutational event is shown below the sequence. Mutagens: EMS, ethyl methanesulfonate; 2AP, 2-aminopurine.

region in each of the recombinant pMD4 plasmids which carried the remaining *tnaC* alleles. In each mutant, we found a single base change in *tnaC* (Fig. 3). The 12 independent mutants defined six different changes from the wild-type sequence. Some of these changes were represented by only a single example, so other *tnaC* lesions may exist.

Attempt to mimic the TnaC$^-$ phenotype. One class of *tnaC* lesion changed the *tnaC* opal termination codon (UGA) to a tryptophan codon (UGG). This alteration would allow translation to proceed to the amber codon (UGA) located five codons downstream from the *tnaC* opal termination codon (Fig. 3). We attempted to examine the contribution of this additional translation to the TnaC$^-$ phenotype by introducing an efficient opal suppressor, Su7-UGA, into an otherwise wild-type strain. Su7-UGA probably inserts tryptophan and is expressed from a *lac* promoter in plasmid pSWC101 (51). The level of tryptophanase was indistinguishable between strains with plasmids carrying either Su^0 (strain SVS1910) or Su7-UGA (SVS1911) and was indifferent to the *lac* inducer, isopropyl-β-D-thiogalactoside (data not shown). Control experiments established that SVS1911 expressed the opal suppressor. This result suggests that translational readthrough was not responsible for the constitutive phenotype in this class of *tnaC* mutant, although it is also possible that the Su7-UGA suppressor is inefficient in this particular context.

DISCUSSION

The evidence presented in this paper has led us to draw several conclusions regarding *tna* operon expression. We will consider each in turn and then propose a working hypothesis for *tna* regulation based on these conclusions.

***tna* operon regulation is distinct from *trp* operon regulation.** The tryptophan (*trp*) biosynthetic operon is regulated by two mechanisms: tryptophan-mediated repression and tryptophanyl-tRNA^{Trp}-mediated attenuation (64). The tryptophan aporepressor lesion *trpR2* had no significant effect on tryptophanase induction (Table 2). We conclude that the *trpR* product plays no direct role in *tna* expression. Attenuation in the *trp* operon responds to the level of tryptophanyl-tRNA^{Trp} and is relieved in certain mutants altered in *trpS*, the structural gene for tryptophanyl-tRNA^{Trp} synthetase (19, 63). The *trpS10343* lesion had no effect on *tna* expression (Table 2). Further, tryptophanase synthesis was efficiently induced by 5MT (45; this work), which is very poorly charged onto tRNA^{Trp} (19). Thus, we conclude that *tna* expression is not influenced by the level of tryptophanyl-tRNA^{Trp}. This is consistent with the in vivo role of an amino-acid catabolizing enzyme, which should only be synthesized when its substrate is well in excess of the level required for tRNA charging and protein synthesis.

***tna* operon regulation involves ρ-dependent transcription termination.** All four *rho* alleles we tested rendered *tna* expression partially constitutive. Tryptophanase gene expression in the *rho-15* strain was striking, with an uninduced enzyme level roughly one-half that of the induced value (Table 2). The only known function of ρ protein is as a factor required for transcription termination at certain termination sites (52, 58). These results suggest that in wild-type cells, ρ-dependent transcription termination prevents *tna* operon expression under noninducing conditions.

Further support for this conclusion comes from our analysis of transcription termination in the *tna* leader region (V. Stewart, R. Landick, and C. Yanofsky, submitted for publication). ρ factor promoted efficient transcription termination within the leader region in an in vitro transcription system. We have also detected mRNA species in vivo which appear to correspond to the in vitro-terminated tna leader transcripts.

Indeed, the nucleotide sequence of the *tna* leader region has many characteristic features of ρ-dependent transcription termination sites (38, 41). It encodes a long (over 200 base pairs) stretch of untranslated RNA, and it contains relatively few G residues (Fig. 2).

Induction does not act at the *tna* promoter. The presumptive *tna* promoter has been identified by in vitro transcription experiments (18). Expression of the *tnaC'-lacZ*$^+$ gene fusion was indifferent to tryptophan, but it was decreased by

glucose (Table 3). This indicates that the *tna* promoter is not regulated by tryptophan but that it is sensitive to catabolite repression, presumably mediated through cAMP-CRP (18).

Induction involves suppression of transcription termination in the *tna* leader region. mRNA from the *tnaC'-lacZ*$^+$ gene fusion was synthesized at a high constitutive level, while mRNA synthesis from the *tnaA'-lacZ*$^+$ gene fusion was induced by tryptophan (Table 3). These results suggest that induction acts to relieve transcription termination in the region between *tnaC* and *tnaA*. The *tnaC'-lacZ*$^+$ gene fusion deletes the distal, nontranslated portion of the leader region, which contains the sites for ρ-dependent transcription termination (Fig. 1; see above).

Most constitutive alterations define a *boxA*-like sequence. A sequence termed *boxA*, near the bacteriophage λ early rightward terminator (λ tR1), is thought to provide a recognition site for a transcription control factor (23, 24, 48, 49). The *boxA5* allele changes this sequence from 5'-CGCTCTTA-3' to 5'-CTCTCTTA-3' and significantly reduces *N* gene product-mediated antitermination at λ tR1 (49). Most of the *tnaC* alleles define a sequence, 5'-CGCCCTTGA-3', that is similar to *boxA* (Fig. 3). Thus, alterations in the *boxA*-like sequence in the *tna* leader region may impair the action of a transcription factor and thus reduce the efficiency of transcription termination under noninducing conditions.

The *tnaC* and *boxA5* alterations have opposite phenotypes; the former apparently reduced transcription termination, while the latter reduces transcription readthrough. One reason for this difference may involve the position of the sequence relative to a translated region. The *boxA* sequence near λ tR1 is located eight base pairs distal to the translation termination codon of the *cro* gene. Transcription readthrough at λ tR1 still occurs when *cro* translation is terminated early or abolished. However, readthrough is greatly reduced when *cro* translation is extended by as little as four base pairs (48, 49, 60). In contrast, the *boxA*-like sequence in the *tna* leader actually overlaps the *tnaC* coding region (Fig. 3).

The *nusA1* allele reduces antitermination in bacteriophage λ lytic gene expression (reviewed in references 23 and 24), but it had little or no effect on *tna* expression (Table 2). Friedman and co-workers have proposed that *boxA* provides a recognition site for the *nusA* protein, which is required for proper antitermination in bacteriophage λ lytic gene expression (23, 24, 48, 49). Our experiments did not address the hypothesis that the *boxA*-like sequence in the *tna* leader region serves as a recognition site for the *nusA* protein.

***tna* operon regulation may involve antitermination control.** In view of these results, we propose a hypothesis for antitermination control of *tna* operon expression. This hypothesis closely parallels that for bacteriophage λ lytic gene expression (reviewed in reference 23). In our model, transcription initiation at the *tna* promoter occurs efficiently, regulated only by cAMP-CRP-mediated transcription activation. In the absence of tryptophan, ρ-dependent transcription termination occurs in the *tna* leader region. We hypothesize that *E. coli* produces a tryptophan-responsive antitermination protein analogous to the N or Q proteins of λ. With inducing levels of tryptophan, this hypothetical protein would act to reduce transcription termination in the leader region, thus allowing RNA polymerase to continue into the major structural genes of the *tna* operon.

We have not yet examined the role of the presumed *tnaC* peptide in *tna* operon regulation. The strictly *cis*-acting nature of *tnaC* mutants suggests that the peptide is not a diffusible regulatory element (see below). Further, the efficient induction by 5MT indicates that *tnaC* translation does not play a role analogous to that of the *trp* leader peptide (63) (see above). A simple working hypothesis is that *tnaC* translation serves to couple translation termination with ρ-dependent transcription termination.

***tna* operon regulation does not involve repression control.** A previous report described constitutive alterations that were thought to define a gene for a *tna* repressor protein (18). We were unable to confirm that conclusion using those mutant strains (V. Stewart, unpublished observations).

A selection for constitutive mutants should yield two classes of mutants if the structural genes in question are subject to repression control. First, *cis*-acting constitutive lesions in such an operon should define the repressor-binding site (operator). In fact, the *tnaC* changes do not define a classical-type operator region; the sequence in this region does not make up part of a dyad symmetry, and it is located over 90 base pairs away from the promoter. Second, null (i.e., chain terminating or insertion) constitutive alterations would define the structural gene for the aporepressor and would act in *trans*. We have exhaustively searched for such lesions, and we believe that they do not exist (see Materials and Methods). Constitutive expression of the *tnaC'-lacZ*$^+$ fusion (see above) also argues against repression control of *tna* operon expression.

We have no direct evidence for the hypothetical *tna* antitermination protein. If it is a positive regulator, then null alterations in its structural gene would confer an uninducible phenotype rather than a constitutive phenotype. We are developing methods to select for such mutants.

Most constitutive alterations do not act by affecting translation. The *tnaC* changes were strictly *cis*-dominant, indicating that the presumed *tnaC* peptide does not act as a diffusible regulatory element (see above). In addition, if *tnaC255* acted by allowing readthrough of the *tnaC* peptide, then we believe that the high-efficiency, tryptophan-inserting suppressor Su7-UGA would have produced a constitutive phenotype in a wild-type strain (Fig. 3). These findings suggest that most of the *tnaC* alterations did not act by changing the amino acid sequence of the *tnaC* peptide. We favor the alternate explanation: the lesions changed a *boxA*-like site within the *tna* leader region that is important for proper transcription termination (see above). We do not know if *tnaC* translation palys a role in regulating wild-type *tna* expression.

One allele, *tnaC261*, resulted from an insertion rather than a change of a single base pair. This lesion was outside of the *boxA*-like sequence defined by the other alleles (Fig. 3), and it was also unusual in that *tna* was expressed at a high level irrespective of inducer (Table 4). The *tnaC261* allele should have a frameshift effect and extend the *tnaC* coding region 25 codons past the normal termination codon to an ochre codon at position +171 (Fig. 2). Current models postulate that ρ-mediated transcription termination requires a large segment (over 60 base pairs) of untranslated RNA (38, 41, 58). We suggest that extended translation in the *tnaC261* mutant simply masked RNA regions required for ρ binding or action.

Possible role for the *tna* leader in mediating catabolite repression. *tna* expression is subject to an unusually severe and complex catabolite repression (10, 11, and references therein). Ullmann and colleagues (30) have proposed a role for ρ in mediating catabolite repression of some genes. Several *rho* alleles had no detectable effect on glucose-mediated catabolite repression of *tna* expression (30; this work). We also failed to observe relief of glycerol-mediated

catabolite repression in the *rho* mutants (data not shown). However, glucose depressed mRNA expression from the *tnaC'-lacZ*$^+$ fusion only about 15-fold, while it depressed expression from the *tnaA'-lacZ*$^+$ fusion well over 100-fold (Table 3). This suggests that a catabolite repression-sensitive regulatory site may exist in the leader region, in addition to the cAMP-CRP-sensitive site at the promoter.

Pastan and Perlman (50) had initially suggested that cAMP affects tryptophanase expression at a posttranscriptional step, but this conclusion has not been pursued (e.g., see reference 16). Our results suggest that both induction and glucose-mediated catabolite repression regulate the levels of *tnaA* mRNA production.

Other examples of antitermination control. Attenuation control of amino acid biosynthetic operons involves regulated transcription termination as a mechanism for controlling gene expression (63). Attenuation is *cis*-acting in that no operon-specific diffusible factors are required for regulation. Transcription antitermination control of gene expression in lambdoid bacteriophages requires *trans*-acting regulatory proteins (23, 24, 29, 52). However, few examples of antitermination have been thoroughly documented in *E. coli*. A bacterial antitermination factor may be involved in *rrn* operon expression (1). In addition, the bacterial genes *sfrA* and *sfrB* may mediate antitermination control of the F factor *tra* operon expression (5, 25). If *tna* operon expression is subject to antitermination control, it represents the first example of this type of regulation of a dispensable, catabolic operon. Perhaps this unusual mechanism of genetic regulation is more common than currently appreciated.

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