Inhibition of Expression of the Tryptophanase Operon in
Escherichia coli by Extrachromosomal Copies
of the tna Leader Region

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Expression of the tryptophanase (tna) operon in Escherichia coli is regulated by catabolite repression and
transcription attenuation. Expression is induced by the presence of elevated levels of tryptophan in a growth
medium devoid of a catabolite-repressing carbon source. Induction requires the translation of a 24-residue
coding region, tnaC, located in the 319-nucleotide transcribed leader region preceding tnaA, the structural gene
for tryptophanase. Multicopy plasmids carrying the tnaC leader region were found to inhibit induction of the
chromosomal tna operon. Mutational studies established that this inhibition was not due to inhibited
transcription initiation, translation initiation, tryptophan transport, or enzyme activity. Rather, multicopy
plasmids inhibited induction by preventing tryptophan-induced transcription antitermination in the
leader region of the tna operon. Translation of the single Trp codon in tnaC of the multicopy plasmids was
shown to be essential for this inhibition. We hypothesize that translation of the Trp codon of the leader peptide
triggers out a trans-acting factor that is essential for tryptophan-induced antitermination in the chromosomal
tna operon. We postulate that this factor is an altered form of tRNA<sub>Trp</sub>.

Tryptophanase (Tnase) is an enzyme that is produced by
many bacterial species and that degrades tryptophan to
indole, pyruvate, and ammonia (28). This β-elimination
reaction is reversible: Tnase can synthesize l-tryptophan
from indole, pyruvate, and ammonia (27) or from indole and
l-serine (18). Tnase allows bacteria to utilize tryptophan as
a sole carbon source (19). The tna operon of Escherichia coli
has been cloned and sequenced (10) and found to contain
two major structural genes, tnaA and tnaB. The promoter-
proximal gene, tnaA, encodes Tnase, while tnaB encodes a
low-affinity, high-capacity tryptophan permease (30). The
tna transcription initiation site lies 319 nucleotides upstream
of the tnaA start codon. This transcribed leader region,
referred to as tna<sub>L</sub>, contains a short coding region, tnaC,
specifying a 24-residue leader peptide (24). Initiation of
transcription at the tnaA promoter is constitutive and
regulated only by the cyclic AMP-catabolite activator protein (CAP)
complex (2, 5) at a characteristic CAP site located immediately
proximal to the tna promoter (24). Transcription of
tna<sub>A</sub> and tna<sub>B</sub> of the tna operon is induced by tryptophan.
Although the precise mechanism of tryptophan induction is
not known, it has been shown that induction occurs by
transcription antitermination at one or more Rho factor-
dependent termination sites located beyond tnaC in the
transcribed leader region (24). cis-acting constitutive mutants
that synthesize large amounts of Tnase in the absence of an
inducer have been isolated (24). Most of these mutants have
single base-pair changes in the distal portion of tnaC, in a
sequence of 9 nucleotides that shows strong homology to the
boxA consensus sequence of bacteriophage λ (11). The boxA
sequence is known to be required for proper antitermination
colour of early lytic gene expression in bacteriophage λ (11).
Other constitutive mutants that have frameshift mutations in
tnaC have been found (24). In these mutants, it is likely that
antitermination is caused by ribosome translation past the
natural tnaC stop codon and into the Rho utilization site (12).
The most striking feature of the regulation of tna operon
expression is the role of the tnaC coding region (12, 25).
Replacing its AUG start codon with AUC decreases expres-
sion more than 60-fold and prevents tryptophan induction.
This start codon mutation is cis acting, a fact suggesting that
the synthesis of the leader peptide is necessary for transcription
antitermination at the adjacent Rho termination site(s). The cis
action of translation of a leader peptide coding region is used
for proper transcription attenuation control in several biosyn-
thetic operons, such as the trp operon of enteric bacteria (29).
Replacing the single tryptophan codon at position 12 of tnaC
with either a UGA or UAG stop codon or a CUG arginine
codon abolishes tryptophan induction (12). Changing the
adjacent phenylalanine codon at position 13 to a tryptophan codon
in a coding region containing an arginine at position 12 (giving
an Arg-12–Trp–13 configuration) does not restore induction
(12). Furthermore, analyses with various suppressor tRNAs
and stop codons at position 12 demonstrate that only when
codon 12 is translated by tRNA<sub>Trp</sub> is there proper tryptophan-
mediated transcription antitermination (12). These studies
demonstrate that induction is dependent on tRNA<sub>Trp</sub> transla-
tion of codon 12 of the leader peptide coding region.

Induction experiments performed with strains bearing mul-
ticopy plasmids containing the tna operon result in only 2- to
3-fold induction, compared with the nearly 100-fold induction
observed with the wild-type strain containing a single chro-
mosomal tna operon. In this report, we analyze this inhibi-
tion. We show that it is translation of tnaC on a multicopy
plasmid that interferes with normal induction of the chromoso-
mal tna operon. We also demonstrate that the translation of
Trp codon 12 of tnaC is required for this inhibition.

MATERIALS AND METHODS

Bacterial strains and plasmids. Strains that contained any
combination of three tna operon configurations were used:
**tna**, designate the "natural" chromosomal tna operon; tna pl designates the plasmid tna promoter leader region; and tnaA' lacz designates the chromosomal gene fusion tna p, tnaC' tnaA' lacz. The E. coli K-12 strains used in this study are listed in Table 1. Plasmids pKG69-00, -14, -16, -59, -62, -74, -75, and -77 were designed to test the inhibitory effects of various tnaC alleles and were constructed by excising the 629-bp BamHI fragments from pSVS14-00, pPDG14, pPDG16, pSVS14-59, pSVS14-62, pPDG74, pPDG75, and pPDG77, respectively (12, 24, 25). These homologous fragments, each containing the tnaA promoter through the first 21 codons of tnaA, were ligated into BamHI-digested pBR322 (4). The specific nucleotide differences between these homologous fragments are indicated in Fig. 1. The orientation in all the pKG69 plasmids was such that transcription from the tna promoter proceeded in the same direction as transcription from the tet promoter within the host vector. Plasmid pKG57 was designed to test the hypothesis that the overproduction of tRNA^TTR_y can overcome the inhibitory effect of pKG69-00. pKG57 was constructed by first inserting the 264-bp EcoRI fragment containing trpT (the structural gene for tRNA^TTR_y) from pMY231 (31) into the EcoRI site of pBluescript SK+. A 710-bp PvuII fragment from the resulting recombinant plasmid (pKG54) was then ligated into Scal-digested pACYC184 (7). The orientation of the inserted fragment was such that transcription from the lac promoter produced sense tRNA^TTR_y and anti-sense mRNA for the tetracycline resistance gene of the host vector. Plasmid pPLT15 (P. Tavormina) contains the nusA gene under the control of the synthetic tac promoter (21) and was used to examine the role of NusA in inhibition by pKG69-00.

**Media.** 3-(N-Morpholino)propanesulfonic acid (MOPS)-based minimal medium (17) was prepared from a modified

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**TABLE 1. Bacterial strains and plasmids**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Relevant genotype</th>
<th>Source or reference</th>
</tr>
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<tr>
<td>W3110</td>
<td>F^- λ^- prototroph</td>
<td>1</td>
</tr>
<tr>
<td>SVS1100</td>
<td>W3110 bgIR551 Δ(lac-argF)U169</td>
<td>24</td>
</tr>
<tr>
<td>SVS1142</td>
<td>SVS1100 (tnaC' lacz)</td>
<td>24</td>
</tr>
<tr>
<td>SVS1144</td>
<td>SVS1100 (tnaA' lacz)</td>
<td>24</td>
</tr>
<tr>
<td>PDG1116</td>
<td>SVS1100 (tnaC278 tnaA' lacz)</td>
<td>12</td>
</tr>
<tr>
<td>CY15202</td>
<td>SVS1100 tnaB271: Tn5 (tnaA' lacz)</td>
<td>30</td>
</tr>
<tr>
<td>SE5000</td>
<td>MC4100 recA56 Δ(lac-argF)U169 thi</td>
<td>22</td>
</tr>
<tr>
<td>KG150</td>
<td>SVS1144/pBR322</td>
<td>This study</td>
</tr>
<tr>
<td>KG151</td>
<td>SVS1144/pKG69-00</td>
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</tr>
<tr>
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</tr>
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<td>SVS1142/pBR322</td>
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</tr>
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</tr>
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<td>SE5000 (tnaC' lacz)</td>
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</tr>
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<td>KG300/pKG69-77</td>
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</tr>
<tr>
<td>KG6957</td>
<td>KG300/pKG69-00/pKG57</td>
<td>This study</td>
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Plasmids

- pACYC184: P15A replicon
- pBluescript SK+: lac,
- pBR322: CoE1 replicon
- pMY231: trpT-
- pPLT15: lac, nusA repE replicon
- pKG54: lac, trpT P15A replicon
- pKG57: lac, trpT P15A replicon
- pKG69-00: tna, tnaC' tnaA'
- pKG69-14: tna, tnaC261 tnaA'
- pKG69-16: tna, tnaC262 tnaA'
- pKG69-49: tna, tnaC259 tnaA'
- pKG69-59: tna, tnaC262 tnaA'
- pKG69-74: tna, tnaC267 tnaA'
- pKG69-75: tna, tnaC266 tnaA'
- pKG69-77: tna, tnaC278 tnaA'

The strains and plasmids listed above were described in this study.
was less of specific activity standard.

This mixture of phosphate, 100 mM pyridoxal phosphate, 10 mM β-mercaptopethanol, 2 mM EDTA and resuspended in 2 ml of lysis buffer. Cells were sonicated for 30 s on ice (Sonicator W-225R; Heat Systems Ultrasonics), and the cell debris was removed by centrifugation in 1.5-ml tubes in a Sorvall Microspin centrifuge for 2 min at 12,000 × g. Cell extracts were mixed with assay buffer (100 mM potassium phosphate [pH 7.8], 100 μM pyridoxal phosphate, 20 mM β-mercaptopethanol, 1 mg of bovine serum albumin per ml) in a final volume of 500 μl. This mixture was preincubated at 30°C for 5 min, and the reaction was started by the addition of 500 μl of prewarmed 0.66 mM SOPC. The reaction was stopped by the addition of 100 μl of 1 M NaOH, and the optical densities were measured at 470 nm. Enzyme activities were calculated by use of an extinction coefficient of 626 M\(^{-1}\) cm\(^{-1}\) for o-nitrothiophenolate. Tnase was found to be stable in extracts stored overnight at 4°C. SOPC was a generous gift of Robert Phillips, University of Georgia. Protein concentrations were determined in cell extracts by use of the Bio-Rad protein assay reagent (6) and bovine serum albumin as the protein standard. Tnase specific activities were calculated as follows: \((A_{470} \times 1.1\text{-ml reaction volume})/(0.626 \text{ μmol/mg}) \times \text{time (minutes)} \times \text{protein concentration (micrograms per milliliter)} \times \text{cell extract volume (milliliters)})]. One unit of Tnase specific activity = 1 μmol of o-nitrothiophenolate produced per min at 30°C. All extracts were assayed in triplicate, and enzyme activity was found to be linear with amount of extract added. Deviation among triplicate samples was less than 10%.

β-Galactosidase (β-Gal) activity was determined by measuring the conversion of o-nitrophenyl-β-D-galactopyranoside (ONPG) to o-nitrophenol by a slight modification of the method of Miller (16). Cells were grown in 15 ml of medium in 150-ml flasks at 37°C with shaking to the mid-log phase and harvested by centrifugation. The cell pellet was resuspended in an equal volume of Z buffer (100 mM sodium phosphate [pH 7.0], 10 mM KCl, 1 mM MgSO\(_4\), 50 mM β-mercaptoethanol), and cell extracts were prepared by sonication as described above. Extracts were diluted with Z buffer to a final volume of 1 ml, and enzyme activity was determined at 30°C. The reaction was started by the addition of 200 μl of a 4-mg/ml ONPG solution and stopped by the addition of 500 μl of 1 M Na\(_2\)CO\(_3\). Optical densities were measured at 420 nm, and enzyme activities were calculated by use of an extinction coefficient of 4,300 M\(^{-1}\) cm\(^{-1}\) for o-nitrophenol. β-Gal was found to be stable in extracts stored overnight at 4°C. Protein concentrations were determined as described above. All extracts were assayed in triplicate, and enzyme activity was found to be linear with amount of extract added. Deviation among triplicate samples was less than 10%. Although specific activities varied by two- to threefold on different days, the percent differences between like samples varied by less than 10%; therefore, tabular results are presented as relative specific activities.

RESULTS

Inhibition of Tnase induction. Strain KG300 (SE5000 \([λtna\_tnac\_tnac\_II\_lacZ]) was transformed with pKG69-00, a multicopy plasmid containing the wild-type \(tna\) promoter (\(tna\_p\)), leader region, and first 21 codons of \(tna\_A\) (Fig. 2), or with control plasmid pBR322 (parental vector of pKG69-00) to yield strains KG3200 and KG301, respectively. Plasmid-containing cultures were grown in the presence or absence of tryptophan, and Tnase and β-Gal activities were determined (Table 2). These enzymes are specified by chromosomal copies of the \(tna\_AB\) operon \((tna\_p\_tna\_A\_II\_lacZ)) and an operon containing a \(tna\_A\_II\_lacZ\) translational fusion (\(tna\_A\_II\_lacZ\_chr\)) driven by a second copy of the \(tna\_A\) regulatory region. Cultures either without a plasmid or with the control plasmid (pBR322) showed normal induction (Table 2) (24). However, strain KG3200 containing plasmid pKG69-00 showed a significant reduction (about 70%) in the induction of chromosomal expression of both \(tna\_chr\) and \(tna\_A\_II\_lacZ\_chr\) (Table 2). These findings demonstrate that the promoter leader region of the \(tna\_p\) operon, when present on a multicopy plasmid, inhibits expression of the chromosomal \(tna\_p\) operon, irrespective of
the chromosomal operon configuration or the enzyme assayed. Basal-level expression was unaffected by pKG69-00. Inhibition occurred immediately upon the addition of tryptophan and was unaffected by cell density (Fig. 3).

**Effect of different promoters and gene fusions on inhibition competence.** The experiments described above demonstrated that Tnase activity was severely inhibited in cells harboring multiple copies of a plasmid containing the *tna* leader region. For determination of whether the inhibited event was transcription initiation, experiments in which the promoter of the chromosomal *tnaA'-lacZ* operon was replaced were performed.

**TABLE 2. Inhibition of *tnaA* and *tnaA'-lacZ* induction by extrachromosomal copies of *tnaC***

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Plasmid*</th>
<th><em>tnaC</em></th>
<th>% Relative sp act of:*</th>
<th>Tnase</th>
<th>β-Gal</th>
<th>–Trp +Trp Ratio*</th>
<th>–Trp +Trp Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG300</td>
<td>–</td>
<td>+2.2</td>
<td>100° 45</td>
<td>3.6</td>
<td>100° 28</td>
<td>97</td>
<td>30</td>
</tr>
<tr>
<td>KG301 pBR322</td>
<td>–</td>
<td>2.0 99</td>
<td>50</td>
<td>3.2</td>
<td>97 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KG3200 pKG69-00</td>
<td>+</td>
<td>2.0 28</td>
<td>14</td>
<td>3.1</td>
<td>23</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>KG200</td>
<td>–</td>
<td>2.1 85</td>
<td>40</td>
<td>0.084</td>
<td>3.5</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>KG201 pBR322</td>
<td>–</td>
<td>1.8 100</td>
<td>55</td>
<td>0.071</td>
<td>3.8</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>KG2200 pKG69-00</td>
<td>+</td>
<td>2.0 32</td>
<td>16</td>
<td>0.076</td>
<td>1.1</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

* KG300, KG301, and KG3200 contain λ*tnaB*, *tnaC* and *tnaA'-lacZ*; and KG200, KG201, and KG2200 contain λ*tnaB*, *tnaC* and *tnaA'-lacZ*.

† Plasmid pKG69-00 is a derivative of pBR322 carrying the *tna* leader region.

‡ Induced or not induced with l-tryptophan at 100 μg/ml of growth medium ( –Trp or +Trp, respectively). The induced specific activity in KG300 was set at 100%.

§ Ratio of induced to noninduced specific activities.

Paradoxically, the chromosomal *tnaA* operon was uninduced in strains KG350 and KG351, the integrated λ contains the *tnaC* fusion. The *tnaC* fusion is caused by the insertion of one A in

**FIG. 2. Schematic diagram of the inhibiting *tnaC* plasmid and the chromosomal *tna* operons that are inhibited by this plasmid. A 629-bp BamHI fragment containing the CAP site, *tna* promoter (*tna_p*), leader peptide coding region (*tnaC*), and first 21 codons of *tnaA* (*tnaA’) was inserted into the *tet* gene of plasmid pBR322 to yield pKG69. The 319-nucleotide transcribed leader region is indicated (*tnaL*).**
codon 18 of tnaC (Fig. 1) and was previously shown to result in constitutive expression of the tnaA'.' lacZ fusion (12). Inhibition of chromosomal β-Gal production in strain KG151 with the inhibiting plasmid and in strain KG150 with the control plasmid occurred as in strains KG301 and KG3200 (Tables 2 and 3). Likewise, inhibition of Tnase activity by the inhibiting plasmid in both KG251 and KG351 was the same as seen in KG151, KG200, and KG2200 (Tables 2 and 3); all of these strains carry the wild-type chromosomal tna operon. β-Gal expression in control strains from the tnaC'.' lacZ fusion (KG250) or from the tnaC261 tnaA'.' lacZ fusion (KG350) was constitutive, in agreement with previous reports (12, 24). However, inhibition of β-Gal production did not occur in these strains when the inhibiting plasmid was present (KG251 and KG351) (Table 3). Therefore, these data support the conclusion that inhibition is not due to an effect on transcription initiation (tnaC'.' lacZ fusion data) or tnaA translation initiation (tnaC261 tnaA'.' lacZ fusion data). Rather, inhibition must be due to interference with proper regulation of the chromosomal tna operon.

**Inhibition is not due to reduced activity of tnaB-dependent tryptophan transport.** The tnaB gene encodes a low-affinity, high-capacity tryptophan permease (30). aroP and mtr also encode permeases capable of transporting tryptophan (8, 13, 20). To demonstrate that the inhibition of tna induction is not due to the reduced activity of tryptophan transport, we transformed the tnaB strain CY15202 (30) with the control plasmid, pBR322, to yield strain KG450, or with the inhibiting plasmid, KG69-00, to yield strain KG451 and examined expression (Table 4). Full induction of the tna operon of tnaB+ strains occurs with 100 μg of tryptophan or 10 μg of 1-methyltryptophan (1-MT) per ml (30). When tnaB strain KG450 harboring control plasmid pBR322 was induced with tryptophan, only modest induction was observed, in agreement with previous reports (30). When this tnaB strain was induced with 1-MT, full induction was observed. However, when tnaB strain KG451 harboring inhibiting plasmid KG69-00 was induced with 1-MT, full induction was not observed (Table 4); inhibition was comparable to that seen in tnaB+ strains carrying the inhibiting plasmid (Tables 2 and 3). These results demonstrate that there is no requirement for the presence of a functional TnaB tryptophan permease

**TABLE 3. Inhibition of constitutively expressed tnaA'.' lacZ and tnaC'.' lacZ**

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Plasmid*</th>
<th>tnaC*</th>
<th>% Relative sp act (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG150</td>
<td>pBR322</td>
<td>–</td>
<td>100*</td>
</tr>
<tr>
<td>KG151</td>
<td>pKG69-00</td>
<td>+</td>
<td>2.3</td>
</tr>
<tr>
<td>KG250</td>
<td>pBR322</td>
<td>–</td>
<td>2.1</td>
</tr>
<tr>
<td>KG251</td>
<td>pBR322</td>
<td>–</td>
<td>2.1</td>
</tr>
<tr>
<td>KG350</td>
<td>pBR322</td>
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</tr>
<tr>
<td>KG351</td>
<td>pKG69-00</td>
<td>+</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* KG150 and KG151 contain λtnaC* tnaA'.' lacZ; KG250 and KG251 contain λtnaC'.' lacZ; and KG350 and KG351 contain λtnaC261 tnaA'.' lacZ.
* Plasmid KG69-00 is a derivative of pBR322 carrying the tnaA leader region.
* Induced or not induced with 1-tryptophan at 100 μg/ml of growth medium (–Trp or +Trp, respectively). The induced specific activity in KG150 was set at 100%.
* Ratio of induced to noninduced specific activities.
* 8.7 μmol of o-nitrothiophenolate formed per min per mg of protein.
* 55 μmol of o-nitrophenol formed per min per mg of protein.
for the inhibition of chromosomal tnaA··lacZ expression by the tnaC-containing plasmid.

Inhibition capability of various tnaC alleles. Although the above-described experiments suggested that the presence of multiple copies of the tna operon leader region was responsible for the inhibition of tryptophan-induced transcription antitermination, the mediator of the inhibition was unclear. For determination of whether inhibition by the multicopy plasmid containing the tna operon leader region was due to a particular DNA segment, the segment’s transcript, the coupled translation of the segment, or the leader peptide itself, we introduced various tnaC alleles onto multicopy plasmid pBR322 and examined the ability of the resulting plasmids to inhibit chromosomal tnaA··lacZ induction (Table 5). When the introduced plasmid contained a mutation replacing the start codon of tnaC, with AUG (strain KG3262), the plasmid no longer inhibited the induction of chromosomal tnaA··lacZ. This result suggested that either the synthesis of the leader peptide or the TnaC peptide itself was responsible for the inhibition of chromosomal tnaA··lacZ expression.

One of the previously characterized constitutive mutants, tnaC259 (24), has a single base change in codon 23 of tnaC (Fig. 1). This change is in a nonamer sequence homologous to boxA of phage λ. The boxA sequence is presumably a site recognized by a NusB-S10 heterodimer (9, 15) and is required for proper antitermination control of early lytic gene expression (11). This mutation reduces homology to the consensus boxA sequence, changes the sequence of TnaC, and results in constitutive expression. With tnaC259 on a multicopy plasmid in strain KG3259, the induction of chromosomal tnaA··lacZ expression was reduced compared to that observed with a plasmid containing wild-type tnaC (KG3200) (Table 5). Since inhibition still occurred, it is not likely that the tna boxA sequence of the multicopy plasmid was responsible for the observed inhibition.

It was shown previously that mutations in tnaCeh are strictly cis acting and that wild-type TnaC produced in trans cannot overcome these defects (25). However, it is conceivable that the high level of TnaC peptide presumably produced by the multicopy pKG69 plasmids inhibits chromosomal tna induction. To explore this possibility, we examined two different multicopy plasmids carrying frameshift mutations in tnaC, tnaC276, and tnaC261 (Fig. 1), for their ability to inhibit chromosomal tnaA··lacZ expression.

In the plasmid carrying the tnaC276 frameshift allele (strain KG3278), the nucleotides of codon 12 of tnaC are read in a different reading frame and translation presumably terminates at a new in-phase stop codon, codon 16. Introducing this allele onto a multicopy plasmid did not lead to the inhibition of chromosomal tnaA··lacZ expression (Table 5). The tnaC261 mutation introduces a frameshift in tnaC codon 16. This mutation presumably allows translation to proceed in the +1 reading phase to a new in-phase stop codon, 25 codons past the normal UGA stop codon, thereby doubling the size of the translated peptide. A plasmid carrying the tnaC261 allele was fully capable of inhibiting chromosomal tnaA··lacZ expression when present in strain KG3261 (Table 5). These data suggest that translation past Trp codon 12 of the leader peptide, in the natural reading frame, and not the TnaC peptide itself, is required for plasmid inhibition of the induction of the chromosomal tna operon.

Role of tRNA<sup>Trp</sup> in inhibition. Translation of codon 12 (Trp) of tnaC by tRNA<sup>Trp</sup> is believed to be essential for tryptophan-induced transcription antitermination (12). We examined the role of codon 12 and of tRNA<sup>Trp</sup> in the inhibition conferred by the multicopy tnaC plasmid in strain KG3265, which carries a plasmid in which Trp codon 12 is replaced by Arg codon CGG (tnaC265) (Fig. 1). Inhibition was abolished by this change (Table 5). In a plasmid construct in which this Arg codon was present at codon 12 and a Trp codon was present at codon 13 (tnaC266), inhibition of chromosomal tna expression also was abolished. Further-

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**TABLE 4. tnaC inhibition in a tryptophan permease (tnaB) mutant**

<table>
<thead>
<tr>
<th>Strain</th>
<th>tnaC allele on plasmid</th>
<th>% Relative β-Gal activity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG450</td>
<td>tnaC+</td>
<td>4.7 18 100&lt;sup&gt;c&lt;/sup&gt; 3.8 21</td>
</tr>
<tr>
<td>KG451</td>
<td>tnaC+</td>
<td>4.7 16  16 3.4 3.4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Relevant genotype: tnaB271··tna5 containing tnaA··lacZ.
<sup>b</sup> −Trp and +Trp, without and with 100 µg of t-l-tryptophan per ml of growth medium, respectively. The induced specific activity in strain KG450 was set at 100%.
<sup>c</sup> Ratio of Trp-induced to noninduced specific activities.

**TABLE 5. Inhibition of tnaA··lacZ induction by multicopy plasmids harboring various tnaC alleles**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid tnaC allele</th>
<th>Description of allele</th>
<th>% Relative β-Gal activity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>KG300</td>
<td>tnaC+</td>
<td>Wild type</td>
<td>5.9 100&lt;sup&gt;d&lt;/sup&gt; 17</td>
</tr>
<tr>
<td>KG301</td>
<td>tnaC+</td>
<td>AUG start codon changed to AUC</td>
<td>4.9 91 18</td>
</tr>
<tr>
<td>KG3200</td>
<td>tnaC+</td>
<td>boxA constitutive</td>
<td>4.9 19 19</td>
</tr>
<tr>
<td>KG3262</td>
<td>tnaC262</td>
<td>AUG start codon changed to AUC</td>
<td>4.5 81 18</td>
</tr>
<tr>
<td>KG3259</td>
<td>tnaC259</td>
<td>boxA constitutive</td>
<td>4.9 15 3.1</td>
</tr>
<tr>
<td>KG3278</td>
<td>tnaC278</td>
<td>+1 frameshift in Lys-11</td>
<td>4.5 83 18</td>
</tr>
<tr>
<td>KG3261</td>
<td>tnaC261</td>
<td>+1 frameshift in Lys-18</td>
<td>5.9 5.0 17</td>
</tr>
<tr>
<td>KG3265</td>
<td>tnaC265</td>
<td>Trp-12 to Arg</td>
<td>4.5 79 17</td>
</tr>
<tr>
<td>KG3266</td>
<td>tnaC266</td>
<td>Trp-12 to Arg, Phe-13 to Trp</td>
<td>4.5 118 26</td>
</tr>
<tr>
<td>KG3267</td>
<td>tnaC267</td>
<td>Phe-13 to Trp</td>
<td>4.2 41 9.8</td>
</tr>
</tbody>
</table>

<sup>a</sup> See Fig. 1 for locations of mutational changes.
<sup>b</sup> −Trp and +Trp, without and with 100 µg of t-l-tryptophan per ml of growth medium, respectively. The induced specific activity in strain KG300 was set at 100%.
<sup>c</sup> Ratio of Trp-induced to noninduced specific activities.
<sup>d</sup> 28.8 µmol of o-nitrophenol formed per min per mg of protein.
more, when an additional Trp codon was introduced at codon 13 of the plasmid to yield a Trp-12–Trp-13 configuration (strain KG3267), the induction ratio was reduced, but less than twofold, compared with a nearly fivefold reduction when the wild-type tnaC allele was present on the plasmid.

The wild-type trpT gene, encoding tRNA<sup>Trp</sup>, was introduced onto a separate, compatible plasmid, pKG57, and this plasmid was transformed into strain KG3200 harboring inhibiting plasmid pKG69-00. In the resulting strain, KG6957, inhibition of chromosomal expression was not affected (data not shown). Plasmid pKG57 was tested for its ability to direct tRNA<sup>Trp</sup> synthesis in vivo and was found to complement a temperature-sensitive trpT mutant strain (data not shown). These results suggest that tnaC plasmid inhibition is not simply a consequence depletion of the intracellular concentration of tRNA<sup>Trp</sup>.

**DISCUSSION**

The precise mechanism of tryptophan-mediated induction of tna operon expression is not known. Induction occurs by prevention of transcription termination at one or more Rho factor-dependent termination sites in the leader region of the operon (23, 24). We are currently considering two models to explain how this antitermination is accomplished. The first model proposes that tryptophan induces frameshifting during translation of the tnaC leader peptide coding region. This would allow the translating ribosome to proceed in the +1 frame an additional 75 nucleotides along the mRNA before termination and dissociation, thereby interfering with the binding of Rho factor to the nascent message. This model is supported by two observations: (i) a deletion of the 24 nucleotides immediately following the tnaC stop codon results in constitutive expression of the operon, presumably by removing the Rho utilization site (12), and (ii) frameshift mutants that allow translation to proceed past the normal tnaC stop codon exhibit constitutive expression (12). However, attempts to date to establish the existence of frameshifting in tnaC have been unsuccessful (unpublished results). The second model proposes that upon tryptophan induction, an antitermination complex that renders RNA polymerase immune to the action of Rho is formed on the tna transcript. Formation of this antitermination complex would occur when the intracellular tryptophan concentration is high. This model further assumes that translation of the leader peptide coding region up to codon 12 is essential for complex formation. Support for this model comes primarily from the findings that most cis-acting constitutive mutations map to a boxA-like region at the end of tnaC (24) and that translation of Trp codon 12 of tnaC is essential for induction (25).

The experiments described in this report address the question of whether there are cell components that are limiting for tryptophan-mediated induction of the tna operon. A key observation that bears directly on this question is the finding that multicopy plasmids containing tnaC inhibited chromosomal tna operon induction. This inhibition also was observed for a chromosomal tnaC<sup>−</sup> lacZ operon fusion and when the wild-type promoter in the chromosomal fusion was replaced by the tet promoter (Table 2). Thus, plasmid inhibition is independent of the promoter directing the expression of the chromosomal tna operon.

Plasmid-mediated inhibition appears to act through interference with proper transcription antitermination during transcription of the chromosomal tna operon. This conclusion is supported by the finding that plasmid inhibition does not affect either tnaA translation initiation (KG351; Table 3) or tna transcription initiation (KG251; Table 3). Furthermore, inhibition occurs almost immediately upon the addition of tryptophan (Fig. 3), a result indicating that time-dependent synthesis of an additional cellular factor is not necessary for this effect.

Analysis of the nature of this plasmid inhibition led to several conclusions. First, since plasmids containing a start codon mutation in the tnaC leader peptide coding region (tnaC262) did not inhibit expression (Table 5), the synthesis of TnaC appeared to be essential for inhibition. Second, plasmids containing a frameshift mutation before Trp codon 12 (tnaC278) or containing the Trp-12–Arg mutation (tnaC265) did not inhibit tnaA′-lacZ expression (Table 5). However, when a frameshift was introduced after Trp-12 of tnaC<sub>pr</sub>, inhibition still occurred. These findings indicate that translation past Trp codon 12 of tnaC is required for plasmid-mediated inhibition of tna operon expression. The copy numbers of the inhibiting plasmid (carrying the tnaC<sup>−</sup> allele) and an analogous plasmid unable to cause inhibition (carrying tnaC265) were found to be indistinguishable (data not shown).

Additional analysis of the tnaC<sub>pr</sub> coding region indicated that the context of the codons near Trp codon 12 was critical for conferring inhibition competence to multicopy plasmids containing tnaC. When codons Trp-12 and Phe-13 of tnaC<sub>pr</sub> were replaced by Arg and Trp codons, respectively, to yield an Arg-12–Trp-13 codon sequence (tnaC266), inhibition was abolished (Table 5). Furthermore, replacement of Phe codon 13 with Trp to yield the Trp-12–Trp-13 configuration (tnaC267) reduced inhibition significantly. In this regard, it is noteworthy that the 6 contiguous codons surrounding Trp codon 12 are conserved, while the others have diverged, when the 24-residue tnaC coding region of E. coli is compared with the 34-residue tnaC coding region of *Proteus vulgaris* (14).

The above-described results not only establish that the codon context of tnaC is essential for inhibition but also reduce the possibility that inhibition is due simply to depletion of normal charged tRNA<sup>Trp</sup>. Translation of codon Trp-13 of tnaC266 presumably requires the same amount of charged tRNA<sup>Trp</sup> as translation of codon Trp-12 of tnaC<sup>+</sup>, and translation of the Trp-12–Trp-13 codons of tnaC267 presumably requires twice the amount of charged tRNA<sup>Trp</sup>. However, neither of these alleles, when carried on a multicopy plasmid, inhibit chromosomal tna expression as well as wild-type tnaC. Additionally, cultures containing extra tRNA<sup>Trp</sup>, supplied by additional copies of the trp<sup>T</sup> gene, are fully inhibited by plasmid-borne tnaC (data not shown).

The findings described above indicate that the mechanism of inhibition of induction involves depletion of some cellular component that is necessary for transcription antitermination-mediated induction of the chromosomal tna operon. NusA is an *E. coli* protein known to be involved in the proper regulation of *N* gene-mediated antitermination (9). However, when NusA was overexpressed from a nusA-containing plasmid (pPLT15), inhibition by plasmid pKG69-00 was not overcome (data not shown). Previous mutational analyses demonstrated that the 9-nucleotide boxA-like sequence at the end of tnaC<sub>pr</sub> (24) and tRNA<sup>Trp</sup> translation of tnaC<sub>pr</sub> codon 12 (12) are required for proper regulation of the tna operon. Here we show that although Trp codon 12 is necessary for tnaC plasmid inhibition, an intact boxA-like sequence is not. Thus, in the tnaC259 allele (Fig. 1), homology to boxA is reduced, yet a plasmid with this change inhibited induction normally (Table 5). This
result suggests that while inhibition is most likely due to titration of some factor necessary for tna induction, it is not due to titration of a factor that specifically acts at boxA.

Tryptophanyl-tRNA is modified by the miaA gene product, and induction of tna operon expression in a miaA mutant was previously shown to be defective (12). Since the translation of tnaC Trp codon 12 in its particular context appears to be critical for the inhibition of chromosomal tna expression, we suggest that the limiting trans-acting component responsible for inhibition acts in conjunction with translation of codon 12 and therefore may be an altered form of tRNA^Trp.

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