

# The mechanism of tryptophan induction of tryptophanase operon expression: Tryptophan inhibits release factor-mediated cleavage of TnaC-peptidyl-tRNA<sup>Pro</sup>

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**Expression of the tryptophanase (*tna*) operon of *Escherichia coli* is regulated by catabolite repression and tryptophan-induced transcription antitermination. In a previous study, we reproduced the regulatory features of this operon observed *in vivo* by using an *in vitro* S-30 system. We also found that, under inducing conditions, the leader peptidyl-tRNA (TnaC-peptidyl-tRNA<sup>Pro</sup>) is not cleaved; it accumulates in the S-30 reaction mixture. In this paper, we examine the requirements for TnaC-peptidyl-tRNA<sup>Pro</sup> accumulation and cleavage, *in vitro*. We show that this peptidyl-tRNA remains bound to the translating ribosome. Removal of free tryptophan and addition of release factor 1 or 2 leads to hydrolysis of TnaC-peptidyl-tRNA<sup>Pro</sup> and release of TnaC from the ribosome-mRNA complex. Release factor-mediated cleavage is prevented by the addition of tryptophan. TnaC of the ribosome-bound TnaC-peptidyl-tRNA<sup>Pro</sup> was transferable to puromycin. This transfer was also blocked by tryptophan. Tests with various tryptophan analogs as substitutes for tryptophan revealed the existence of strict structural requirements for tryptophan action. Our findings demonstrate that the addition of tryptophan to ribosomes bearing nascent TnaC-peptidyl-tRNA<sup>Pro</sup> inhibits both TnaC peptidyl-tRNA<sup>Pro</sup> hydrolysis and TnaC peptidyl transfer. The associated translating ribosome therefore remains attached to the leader transcript where it blocks Rho factor binding and subsequent transcription termination.**

**E***scherichia coli* and many other Gram-negative bacteria use the enzyme tryptophanase to degrade L-tryptophan to indole, pyruvate, and ammonia (1). This ability allows tryptophan to be used as a source of carbon, nitrogen, and energy (2). The tryptophanase (*tna*) operon of *E. coli* consists of two major structural genes, *tnaA*, encoding tryptophanase, and *tnaB*, encoding a low affinity, high capacity tryptophan permease (3, 4). This operon also contains a 319-bp transcribed leader regulatory region, preceding *tnaA*. Initiation of transcription of the operon is regulated by catabolite repression. Once initiated, continuation of transcription into the *tnaA-tnaB* structural genes is regulated by a transcription antitermination mechanism that is activated by the inducer, tryptophan. Induction prevents Rho factor from terminating transcription in the leader region of the operon (5–9). The transcript of the *tna* operon leader region contains a coding region, *tnaC*, specifying a 24-residue leader peptide, TnaC (3). There is appreciable evidence demonstrating that translation of *tnaC* is essential for tryptophan induction, and that induction prevents Rho factor-dependent transcription termination in the leader region of the operon. Thus, (i) replacing the *tnaC* start codon by a stop codon eliminates induction (10, 11); (ii) replacing the crucial Trp codon at position 12 of *tnaC* by codons for other amino acids, also eliminates induction (8, 11); (iii) a minimal *E. coli tna* operon introduced into two bacterial species that lack tryptophanase, *Enterobacter aerogenes* and *Salmonella typhimurium*, is tryptophan inducible (12); this

finding suggests that no unique regulatory protein other than TnaC is required for tryptophan regulation; (iv) addition of bicyclomycin, an inhibitor of Rho action, results in constitutive expression of the operon, implicating Rho as a factor required for termination (13); (v) BoxA and *rut* sites located immediately adjacent to the *tnaC* UGA stop codon are essential for Rho-dependent termination (8); and (vi) inactivation of peptide release factor 3 (RF3) increases basal expression of the *tna* operon, suggesting that regulation of ribosome release can influence operon expression (14). These observations support the following working model of the mechanism of induction: In the presence of elevated levels of tryptophan, the TnaC peptide acts in cis on the translating ribosome to inhibit its release at the *tnaC* stop codon. This stalled ribosome then blocks Rho's access to the *rut* site on the *tna* transcript, thereby preventing transcription termination.

Recently, we have reproduced the features of *tna* operon regulation *in vitro* by using an S-30 system (9). We showed that tryptophan addition results in an unusual event, accumulation of TnaC peptidyl-tRNA<sup>Pro</sup> (Pro is the C-terminal amino acid of TnaC; ref. 9). In this report, we examine the requirements for TnaC-peptidyl-tRNA<sup>Pro</sup> accumulation and cleavage, *in vitro*.

## Materials and Methods

**Materials.** [<sup>35</sup>S]methionine (>1,000 Ci/mmol, *in vitro* translation grade) was purchased from NEN (Perkin-Elmer). Puromycin, L-tryptophan, and tryptophan analogs were purchased from Sigma. *S. typhimurium* RF2 (15), *E. coli* RF1 and RF3, ribosome recycling factor (RRF), and elongation factor G (EF-G) were purified after overexpression in *E. coli* (16, 17). Protein concentrations were determined by using the Advanced Protein Assay (Cytoskeleton, Denver).

**Ultracentrifugation.** A Beckman TLA-100 ultracentrifuge equipped with a TLS-55 rotor was used throughout. For 10–30% sucrose density gradient analyses, centrifugation was performed at 4°C for 16 h at 30,000 rpm (about 60,000 × *g*). Fractions were collected from the bottom of the tube. Ribosomes were pelleted by centrifugation at 45,000 rpm (about 150,000 × *g*) for 60 min at 4°C by using the TLS-55 rotor.

**Isolation of Ribosome Complexes.** The conditions used to reproduce *tna* operon regulation in an S-30 system were described in detail (9). A circularized ≈600-bp DNA fragment, bearing the

Abbreviations: RF, release factor; RRF, ribosome recycling factor; EF-G, elongation factor G; uORF, upstream ORF.

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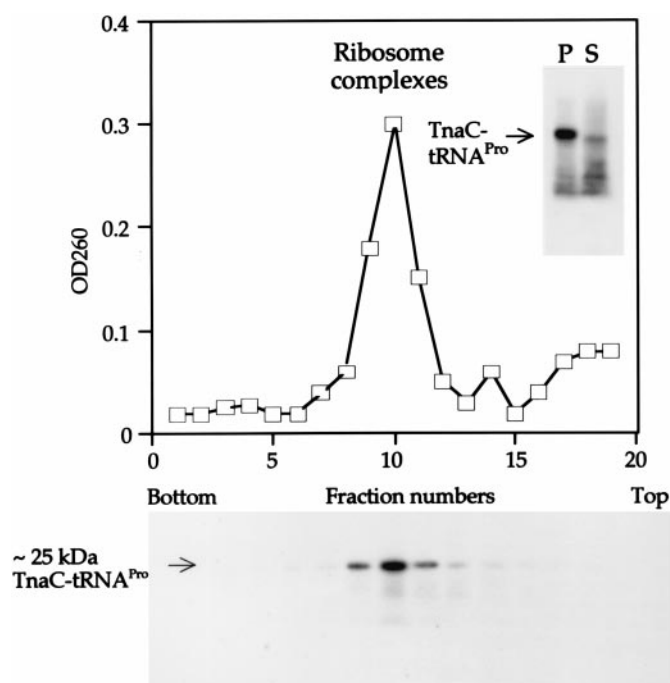
intact *tna* promoter, *tnaC* coding region, and *tna* leader region to bp +306, followed by the *rpoC* terminator sequence, was used as template to direct the S-30 system (9). [<sup>35</sup>S]methionine was used to label newly synthesized TnaC peptide. To isolate ribosome complexes, 200 μl of an S-30 reaction mixture containing 2 mM L-tryptophan was incubated at 37°C for 30 min, layered over an equal volume of a 1.1 M sucrose cushion prepared in TMKN buffer (35 mM Tris acetate, pH 8.0/10 mM magnesium acetate/60 mM potassium acetate/30 mM ammonium acetate/1 mM DTT), and centrifuged as described above. The ribosome pellet was washed twice by resuspension in the same buffer and resedimented by centrifugation. Finally, the ribosome complex pellet was resuspended in 200 μl of TMKN buffer and stored at -80°C.

**In Vitro TnaC-tRNA<sup>Pro</sup> Cleavage.** An amount equal to 10 μl of resuspended ribosome complexes was mixed with the additions indicated in each figure in a final volume of 15 μl, and each mixture was incubated at 37°C for 15 min, unless otherwise indicated. Each reaction was stopped by mixing with 5 μl of 4× Tricine-SDS gel sample buffer and incubation at 80°C for 3 min. The components were separated on 10% Tricine-SDS protein gels (18). Details for each experiment are described in the legends. Gels were dried and analyzed by autoradiography. The levels of <sup>35</sup>S-labeled TnaC peptidyl-tRNA<sup>Pro</sup> were quantified by using a PhosphorImager (Molecular Imager System GS 363, Bio-Rad) and the MOLECULAR ANALYST 2.1 software package.

## Results

**Newly Synthesized TnaC Peptidyl-tRNA<sup>Pro</sup> Is Associated with Its Translating Ribosome.** The basic *in vivo* features of *tna* operon regulation, e.g., tryptophan-induced transcription anti-termination and cAMP-dependent transcription initiation, were reproduced *in vitro*, by using an *E. coli* cell-free S-30 system (19). It was shown that tryptophan induction leads to the accumulation of TnaC peptidyl-tRNA<sup>Pro</sup>, presumably in association with the ribosome translating the *tnaC* coding region (9). To establish that TnaC peptidyl-tRNA<sup>Pro</sup> is associated with the translating ribosome, an appropriate template was prepared by self-ligating a DNA fragment bearing the *tna* promoter, the *tna* leader region to bp +306, followed by the *rpoC* terminator. This template was used to direct the synthesis of TnaC peptidyl-tRNA<sup>Pro</sup> in the presence of [<sup>35</sup>S]methionine and high levels of tryptophan. The sole transcription start site observed by using this circular DNA template was the *tna* promoter (9). Ribosome complexes formed in the S-30 system were isolated by centrifugation. [<sup>35</sup>S]TnaC-tRNA<sup>Pro</sup> was detected predominantly in the ribosome pellet, rather than in the supernatant (P and S lanes, Fig. 1 *Inset*). To establish association of the labeled peptidyl-tRNA with ribosomes, a ribosome pellet was resuspended and layered on top of a 10–30% sucrose gradient, the sample centrifuged, and fractions collected. Fig. 1 shows the location of the sedimented ribosomes (UV-absorbing material) and the distribution of [<sup>35</sup>S]Met-labeled TnaC peptidyl-tRNA<sup>Pro</sup> in the various fractions. The majority of the [<sup>35</sup>S]Met-labeled TnaC peptidyl-tRNA<sup>Pro</sup> was in fractions 9–11, the fractions containing the ribosomes. This result demonstrates that the TnaC peptidyl-tRNA<sup>Pro</sup> accumulated under inducing conditions is associated with the ribosome fraction.

If hydrolysis of TnaC peptidyl-tRNA<sup>Pro</sup> is blocked by induction, and this blockage results in stalling of the translating ribosome at the *tnaC* stop codon, it is likely that the *tnaC* transcript would remain bound to the translating ribosome. This expectation was verified by labeling the *tna* transcript species by [<sup>α-<sup>33</sup>P]UTP incorporation in an S-30 reaction under inducing conditions. Ribosome complexes were isolated by centrifugation. As expected, most of the labeled *tna* transcript species were found in the ribosomal pellet (data not shown). This finding</sup>

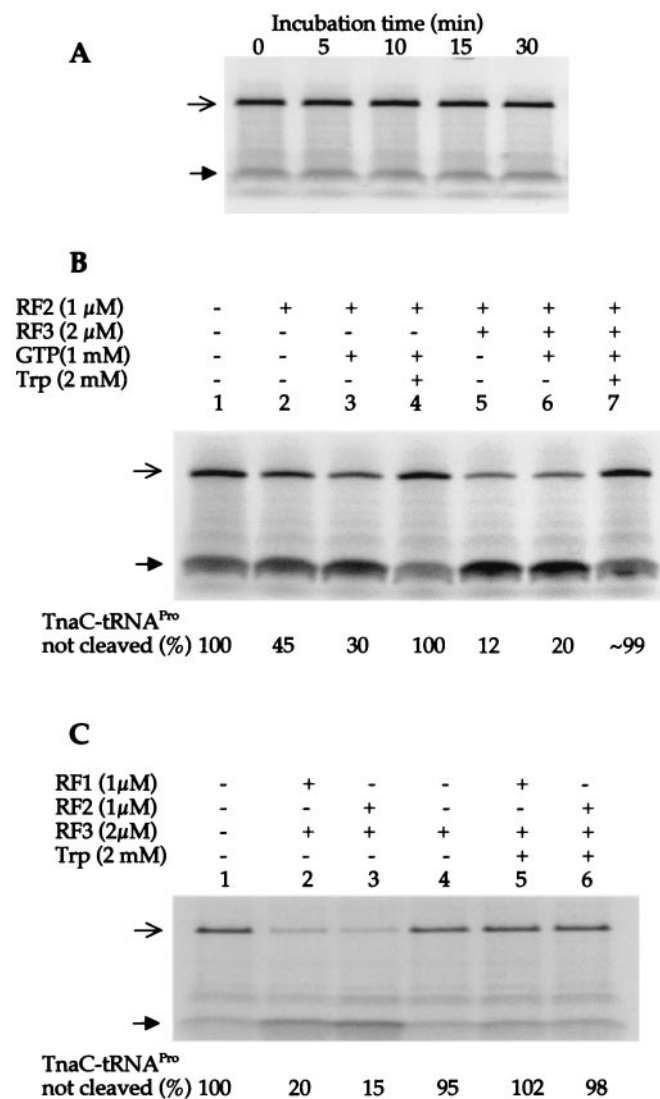


**Fig. 1.** [<sup>35</sup>S]TnaC peptidyl-tRNA<sup>Pro</sup> is ribosome associated under inducing condition. A tryptophan-induced S-30 reaction mixture was centrifuged, and the ribosome pellet was resuspended in TMKN buffer. Portions of the ribosome pellet (P) and supernatant (S) were analyzed electrophoretically (*Upper Inset*). The remainder of the resuspended ribosome pellet was layered on a 10–30% sucrose gradient and centrifuged. Fractions were collected, and absorbance at 260 nm was monitored (*Upper*). Proteins in each fraction were recovered by cold acetone precipitation, dissolved in 20 μl 1× Tricine-SDS gel sample buffer, separated electrophoretically, and analyzed by autoradiography. Prestained protein molecular weight standards were used as markers (not shown); TnaC-tRNA<sup>Pro</sup> was found to be ≈25 kDa (*Lower*).

suggests that the *tna* transcript produced in the S-30 reaction under inducing conditions is in a complex with the translating ribosome and TnaC-tRNA<sup>Pro</sup>. When this presumed TnaC peptidyl-tRNA<sup>Pro</sup>-*tna* mRNA-ribosome complex was toeprinted to determine the location of the stalled ribosome on the *tna* transcript, strong “toeprint” signals were observed ≈15 nt beyond the *tnaC* stop codon, corresponding to a ribosome stalled at the *tnaC* stop codon (data not shown). These findings establish that, in the presence of tryptophan, *tnaC* is translated, and that cleavage of its translated product, TnaC peptidyl-tRNA<sup>Pro</sup>, is inhibited. The translating ribosome bearing TnaC peptidyl-tRNA<sup>Pro</sup> appears to be stalled at the *tnaC* UGA stop codon.

**RF-Mediated TnaC Peptidyl-tRNA<sup>Pro</sup> Cleavage and Its Inhibition by L-Tryptophan.** Spontaneous hydrolysis of TnaC peptidyl-tRNA<sup>Pro</sup> in the purified, washed, ribosome complex (TnaC peptidyl-tRNA<sup>Pro</sup>-*tna* mRNA-ribosome) was first examined (Fig. 2*A*). It is apparent that, despite the absence of added tryptophan, the TnaC peptidyl-tRNA<sup>Pro</sup> present in these washed ribosome complexes is stable; its concentration remained unchanged after 30 min of incubation at 37°C.

During translation, termination RFs decipher the stop codons in the ribosomal A site and activate hydrolysis of peptidyl-tRNA located in the ribosomal P site (16, 20). In *E. coli*, there are two codon-specific RFs that mediate peptidyl-tRNA cleavage, RF1 and RF2. RF1 recognizes UAG and UAA stop codons, and RF2 recognizes UGA and UAA stop codons. Their action is aided by the G-protein, RF3 (21, 22). RF3 has recently been shown to accelerate dissociation of RF1 and RF2 from ribosomes in a GTP-dependent manner (23). To determine whether RF2 can mediate TnaC



**Fig. 2.** Release factor-mediated cleavage of TnaC-tRNA<sup>Pro</sup>. TnaC-tRNA<sup>Pro</sup> (open arrow) and TnaC (filled arrow) bands are indicated. (A). Spontaneous cleavage of TnaC-tRNA<sup>Pro</sup> is not evident on incubation of isolated ribosome complexes at 37°C. Samples were taken at the indicated times and analyzed by electrophoresis and autoradiography. (B) RF-2-mediated TnaC-tRNA<sup>Pro</sup> cleavage and its inhibition by L-tryptophan. Ribosome complexes with the indicated additions were incubated at 37°C for 15 min, and then analyzed electrophoretically. The TnaC-tRNA<sup>Pro</sup> concentration in lane 1 was set at 100%. (C) A comparison of RF1- and RF2-mediated TnaC-tRNA<sup>Pro</sup> cleavage. Incubation was performed as in the middle figure. It was also found (not shown) that there is no significant cleavage when GTP is added with RF3. The TnaC-tRNA<sup>Pro</sup> concentration in lane 1 was set at 100%.

peptidyl-tRNA<sup>Pro</sup> cleavage at the *tnaC* UGA stop codon, purified RF2 was added to our washed ribosome complexes (Fig. 2B). A control reaction with the washed ribosome complex alone is shown in lane 1. When RF2 or RF2 + GTP was added (Fig. 2B, lanes 2 and 3), 55% and 70% of the TnaC peptidyl-tRNA was cleaved, respectively, and a corresponding amount of free TnaC peptide was observed. When both RF2 and RF3 were added, without or with GTP, 88% and 80% of the TnaC peptidyl-tRNA<sup>Pro</sup>, respectively, were cleaved (lanes 5 and 6). When 2 mM L-tryptophan was added with RF2 + GTP (lane 4), or with RF2, RF3, and GTP (lane 7), the added tryptophan completely inhibited TnaC peptidyl-tRNA<sup>Pro</sup> cleavage. In a separate experiment, RF1 was compared with RF2 in mediating TnaC peptidyl-tRNA<sup>Pro</sup> cleavage (Fig. 2C). A control

reaction containing only the washed ribosome complex is shown in lane 1. When RF1 and RF3 were present (lane 2), 80% of the TnaC peptidyl-tRNA<sup>Pro</sup> was cleaved. When RF2 and RF3 were present (lane 3), 85% of the TnaC peptidyl-tRNA<sup>Pro</sup> was cleaved. When RF3 was added alone, there was no significant cleavage of TnaC peptidyl-tRNA<sup>Pro</sup> (lane 4). When RF3 was tested in the presence of GTP, there also was no cleavage (data not shown). Addition of 2 mM L-tryptophan completely inhibited both RF1- and RF2-promoted cleavage of TnaC peptidyl-tRNA<sup>Pro</sup> (lanes 5 and 6).

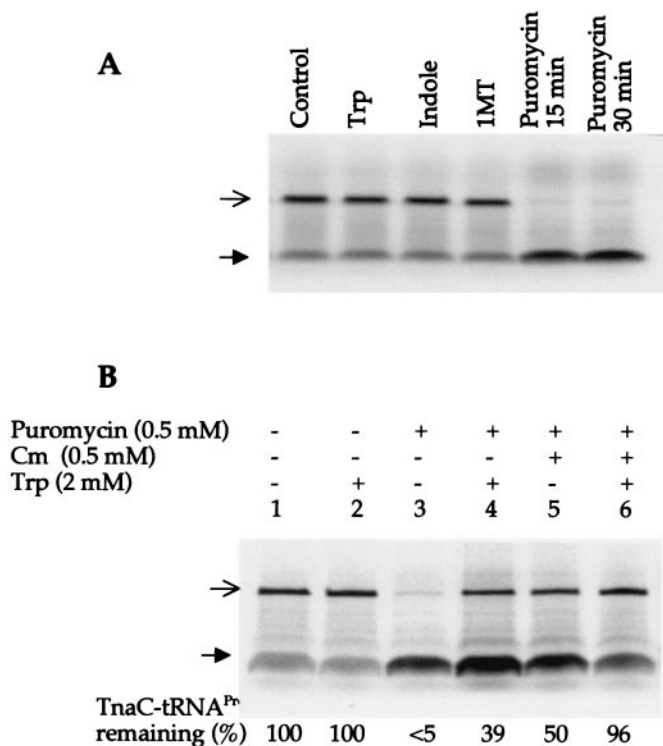
Two other protein factors known to be involved in translation elongation or translation termination, namely, EF-G and RRF, were also incubated with isolated ribosome complexes to examine their effects on the stability of TnaC peptidyl-tRNA<sup>Pro</sup>. EF-G is a GTPase that is involved in the translocation of bacterial ribosomes along messenger RNA during protein biosynthesis (24). RRF is an essential translation termination factor in *E. coli*; it is responsible for the release of the translation terminating ribosome from mRNA (25, 26). EF-G and RRF were tested in both the presence and the absence of GTP. These additions did not lead to cleavage of TnaC-tRNA<sup>Pro</sup> (data not shown).

**Transfer of TnaC Peptidyl-tRNA<sup>Pro</sup> to Puromycin and Its Inhibition by L-Tryptophan.** Puromycin is an antibiotic that mimics A-site-bound aminoacyl-tRNA. Peptidyl-tRNA in the ribosomal P site is attacked by puromycin, resulting in covalent attachment of the carboxyl terminus of the peptidyl moiety to puromycin. The results presented above suggest that TnaC peptidyl-tRNA<sup>Pro</sup> is located in the ribosomal P site and is accessible to RF-mediated hydrolysis. When puromycin is added to ribosomes bearing TnaC peptidyl-tRNA<sup>Pro</sup>, the final product should be TnaC with puromycin covalently attached at its C terminus. Addition of puromycin did lead to TnaC peptidyl transfer, and the formation of TnaC-puromycin; greater than 95% of the TnaC peptidyl-tRNA<sup>Pro</sup> initially present was cleaved during 15 min of incubation with 1 mM puromycin (Fig. 3A). Incubation of the partially purified ribosome complex with tryptophan, indole, or 1-methyl-tryptophan had no effect on the stability of TnaC peptidyl-tRNA (Fig. 3A). Addition of tryptophan with puromycin inhibited cleavage of the TnaC peptidyl-tRNA and peptidyl transfer to puromycin;  $\approx$ 40% of the TnaC peptidyl-tRNA<sup>Pro</sup> remained intact (Fig. 3B, lane 4). When both puromycin and chloramphenicol, a known peptidyl transferase inhibitor, were present, 50% of the TnaC peptidyl-tRNA<sup>Pro</sup> present was uncleaved (lane 5). At the concentrations used, the inhibitory effects of chloramphenicol and tryptophan were additive (compare lane 6 with lanes 4 and 5).

**Examination of the Structural Features of L-Tryptophan Required for Inhibition of Puromycin Action.** Analogs of L-tryptophan were examined for their ability to mimic L-tryptophan and inhibit TnaC peptidyl transfer to puromycin. Of the analogs tested, appreciable inhibition was observed only with 1-methyl-L-tryptophan, 5-methyl-L-tryptophan, 5-fluoro-L-tryptophan, 5-hydroxy-L-tryptophan, and glycyl-L-tryptophan (Fig. 4A and B). 1-methyl-L-tryptophan and 5-methyl-L-tryptophan are known to be effective inducers of *tna* operon expression *in vivo*. 5-Fluoro-L-tryptophan, 5-hydroxy-L-tryptophan, and glycyl-L-tryptophan were found to be effective inducers in the S-30 system (data not shown).

We also determined whether indole, indole-3-propionic acid, tryptamine, or D-tryptophan could compete with L-tryptophan in inhibiting puromycin action. The L-tryptophan concentration used was 0.2 mM, and the analog concentration used was 2 mM. L-tryptophan inhibited cleavage by 30% (Fig. 4C). Of the analogs tested, only tryptamine effectively competed with L-tryptophan (Fig. 4C). These findings suggest that a stereospecific tryptophan binding site may exist or be created in the TnaC peptidyl-tRNA<sup>Pro</sup> ribosome complex.



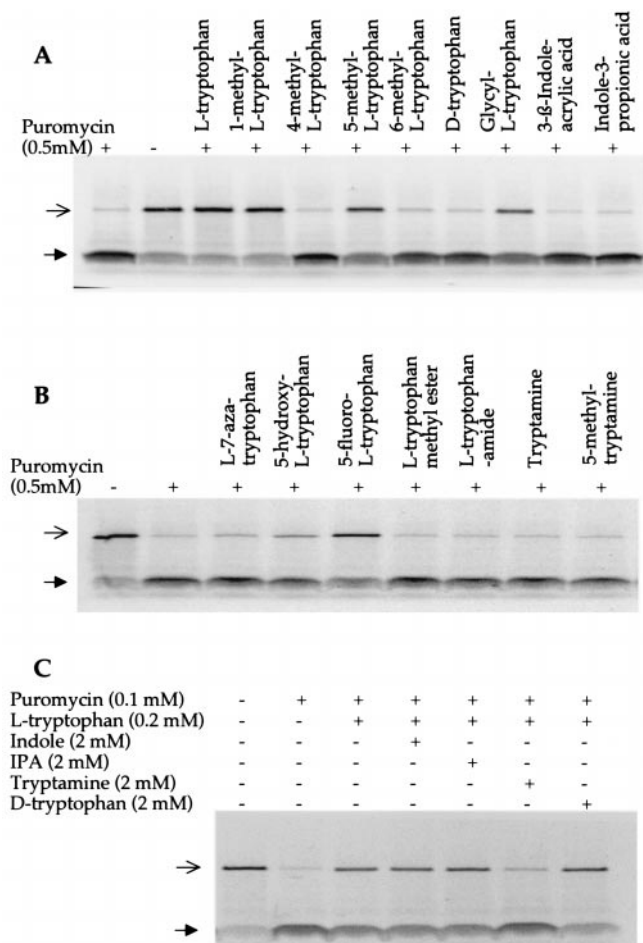


**Fig. 3.** Transfer of TnaC from isolated TnaC peptidyl-tRNA<sup>Pro</sup>-*tnaC* mRNA-ribosome complexes to puromycin. TnaC, TnaC-puromycin (filled arrow), and TnaC-tRNA<sup>Pro</sup> (open arrow) bands are marked. (A) Puromycin-mediated TnaC peptidyl transfer. Ribosome complexes were mixed with 1 mM L-tryptophan, indole, 1-methyl-L-tryptophan, or puromycin, respectively, and incubated at 37°C for 15 min. A sample containing puromycin was also incubated for 30 min. (B) Chloramphenicol (Cm) or tryptophan inhibits puromycin-mediated TnaC peptidyl transfer. Experimental details are described in *Materials and Methods*. The TnaC peptidyl-tRNA<sup>Pro</sup> concentration was not changed when Cm alone was present (not shown). The TnaC-tRNA<sup>Pro</sup> concentration in lane 1 was set at 100%. It is unclear why TnaC and TnaC-puromycin signals in lane 4 are stronger than those in lane 3 on this gel, but this discrepancy was not generally observed. No significant differences in the stability of TnaC and TnaC-puromycin were detected.

## Discussion

**On the Mechanism of *tna* Operon Induction.** We have used a *tna* operon template in an *in vitro* S-30 system to demonstrate that tryptophan induction of *tna* operon expression is a consequence of tryptophan inhibition of cleavage of newly synthesized TnaC peptidyl-tRNA<sup>Pro</sup>, at the *tnaC* stop codon. This peptidyl-tRNA remains in a complex with the stalled translating ribosome and its associated *tna* transcript. We purified these stalled ribosome complexes and demonstrated that the uncleaved TnaC-tRNA<sup>Pro</sup> could be cleaved on incubation with either RF1 or RF2. Most importantly, tryptophan addition prevented release factor-mediated TnaC peptidyl-tRNA<sup>Pro</sup> cleavage. These findings establish that unique properties of the translating ribosome bearing TnaC peptidyl-tRNA<sup>Pro</sup>, and L-tryptophan, prevent release factor from promoting cleavage of TnaC-tRNA<sup>Pro</sup> at the *tnaC* stop codon. In other studies, we have shown that crude S-30 preparations or an S-100 soluble fraction can promote cleavage of TnaC-tRNA<sup>Pro</sup>; cleavage by these preparations also is prevented by added tryptophan (unpublished observations). We also showed that replacing Trp12 of TnaC by Arg eliminates the ability of tryptophan to inhibit cleavage of this altered peptidyl-tRNA (unpublished observations). Our findings establish the basis of inducer inhibition of Rho action.

**How and Where Does Tryptophan Act?** Our findings indicate that the presence of TnaC peptidyl-tRNA<sup>Pro</sup> and tryptophan in the



**Fig. 4.** Analysis of the structural features of L-tryptophan required for inhibition of puromycin-mediated TnaC peptidyl transfer. TnaC (filled arrow) and TnaC-tRNA<sup>Pro</sup> (open arrow) bands are indicated. Ribosome complexes with the indicated additions were incubated at 37°C for 15 min, and then analyzed electrophoretically. (A and B) Test of the ability of tryptophan analogs (2 mM) to inhibit puromycin-mediated TnaC peptidyl transfer. (C) Tryptamine prevents L-tryptophan inhibition of puromycin-mediated TnaC transfer. IPA, indole-3-propionic acid.

translating ribosome inhibits both peptidyl transfer and peptidyl-tRNA hydrolysis. The precise mechanisms of release factor-mediated peptidyl-tRNA hydrolysis and stop codon recognition remain unknown (27). There is substantial evidence demonstrating that peptidyl transferase is responsible for hydrolysis of peptidyl-tRNA during translation termination (20, 28–30). In other studies, we have shown that a stop codon must follow the *tnaC* coding region for tryptophan induction to efficiently stall the translating ribosome (unpublished observations). It seems likely that it is the unique properties of TnaC peptidyl-tRNA<sup>Pro</sup> and tryptophan, both retained within the translating ribosome, that prevent peptidyl-tRNA hydrolysis. The finding that RF1, as well as RF2, mediates TnaC-tRNA<sup>Pro</sup> cleavage at the *tnaC* UGA stop codon suggests that recognition of the stop codon may not be essential for release factor to activate peptidyl-tRNA cleavage in this isolated specific ribosome complex. The significance of this finding is being investigated.

The strict structural requirements needed for a tryptophan analog to inhibit TnaC peptidyl transfer to puromycin, and the reversal of tryptophan inhibition by tryptamine (Fig. 4), suggest that a specific tryptophan binding site may exist or be created in the stalled ribosome complex. The recently described structure

of the 50S ribosomal subunit indicates that all nascent polypeptides must pass through a 100-Å exit tunnel in this subunit before emerging from the ribosome (31). TnaC residue Trp12, as well as the residues surrounding it, namely KWFNID, has been shown to be important for induction (12). This TnaC segment could interact with a portion of the inner surface of the tunnel and help create a transient tryptophan binding site. Bound tryptophan could prevent peptidyl transferase and peptidyl hydrolytic center action. Alternatively, tryptophan bound in the ribosomal A site could exploit some feature of TnaC-tRNA<sup>Pro</sup>, or induced by TnaC-tRNA<sup>Pro</sup>, that prevents peptidyl-tRNA cleavage. A third possibility is that tryptophan alters contact between TnaC-tRNA<sup>Pro</sup> and the surface of the peptide exit tunnel, and improperly orients the peptidyl-tRNA in the ribosomal P site. Improper orientation might prevent RF action.

The ribosome tunnel surface is mostly formed by domains I through V of 23S rRNA. However, proteins L4, L22, and L39e make significant contributions (31). It is interesting to note that, when TnaC-tRNA<sup>Pro</sup> is located in the P site, TnaC segment KWFNID would likely be at or very close to the narrowest segment of the tunnel formed by proteins L22 and L4. It has been suggested that this location might be where the nature of the nascent chain is sensed (31). It will be of interest to learn whether a specific part of the polypeptide exit tunnel surface must interact with the KWFNID segment of TnaC-tRNA<sup>Pro</sup> for cleavage to be prevented.

**Other Instances of Inhibition of Peptidyl-tRNA Cleavage and Ribosome Stalling.** Expression of several bacterial genes that confer antibiotic resistance has been shown to be antibiotic inducible (32). Expression is due to stalling of a translating ribosome at a unique site in an upstream ORF (uORF). In these examples, stalling depends on both the sequence of the encoded peptide and the structure of the antibiotic effector. Ribosome stalling generally alters the adjacent secondary structure of the transcript, permitting translation to proceed in a downstream coding region (32). These examples suggest that the nascent peptide, in the presence of its coregulatory antibiotic, inhibits peptidyl trans-

ferase activity irreversibly, resulting in ribosomal stalling (32). Another example concerns *E. coli* SecM, the product of gene *secM*, located upstream of *secA*. SecM is responsible for secretion-responsive control of SecA translation. The nascent SecM peptide has been reported to undergo self-translational arrest; this arrest alters the secondary structure of the *secM-secA* transcript and enhances *secA* translation (33).

In several eukaryotic systems, translation of an upstream ORF (uORF) has also been shown to affect translation of a downstream coding region. The mechanisms used also appear to depend on the sequences of the encoded uORF peptides (34, 35). Among these are fungal uORFs encoding the arginine attenuator peptides (AAPs; refs. 36 and 37), uORF2 of the human cytomegalovirus gpUL4, and the uORF preceding the coding region for mammalian *S*-adenosyldecarboxylase (AdoMetDC; ref. 35). Nascent AAPs, in the presence of high levels of arginine, cause regulated stalling of ribosomes involved in both elongation and termination. In contrast, the nascent peptides encoded by the 22-codon gpUL4 uORF and the 6-codon AdoMetDC uORF act on terminating not elongating ribosomes. Similar to the results reported in this paper, the gpUL4 uORF-encoded nascent peptide remains covalently attached to the tRNA<sup>Pro</sup> responsible for decoding the final sense codon of the uORF. This finding suggests that this nascent peptide interferes with cleavage of the peptidyl-tRNA at the translation stop codon (38). These examples reveal that exploitation of the basic features of translation allow ribosomes to sense and respond to specific signals inherent in the nascent peptide, and provided by outside signals. The features of the ribosomal peptide exit tunnel may play an important role in these instances by contributing to nascent peptide recognition.

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