Reproducing tna Operon Regulation in Vitro in an S-30 System

TRYPTOPHAN INDUCTION INHIBITS CLEAVAGE OF TnaC PEPTIDYL-tRNA

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Expression of the tryptophanase (tna) operon of Escherichia coli is regulated by catabolite repression and tryptophan-induced transcription antitermination. Catabolite repression regulates transcription initiation, whereas excess tryptophan induces antitermination at Rho factor-dependent termination sites in the leader region of the operon. Synthesis of the leader peptide, TnaC, is essential for antitermination. BoxA and rut sites in the immediate vicinity of the tnaC stop codon are required for termination. In this paper we use an in vitro S-30 cell-free system to analyze the features of tna operon regulation. We show that transcription initiation is cyclic AMP (cAMP)-dependent and is not influenced by tryptophan. Continuation of transcription beyond the leader region requires the presence of inducible levels of tryptophan and synthesis of the TnaC leader peptide. Using a tnaA’-trpE fusion, we demonstrate that induction results in a 15-20-fold increase in synthesis of the tryptophan-free TnaA-TrpE fusion protein. Replacing Trp codon 12 of tnaC by an Arg codon, or changing the tnaC start codon to a stop codon, eliminates induction. Addition of bicyclomycin, a specific inhibitor of Rho factor action, substantially increases basal level expression. Analyses of tna mRNA synthesis in vitro demonstrate that, in the absence of inducer transcription is terminated and the terminated transcripts are degraded. In the presence of inducer, antitermination increases the synthesis of the read-through transcript. TnaC synthesis is observed in the cell-free system. However, in the presence of tryptophan, a peptidyl-tRNA also appears, TnaC-tRNAPro. Our findings suggest that inducer acts by preventing cleavage of TnaC peptidyl-tRNA. The ribosome associated with this newly synthesized peptidyl-tRNA presumably stalls at the tnaC stop codon, blocking Rho’s access to the BoxA and rut sites, thereby preventing termination. 1-Methyltryptophan also is an effective inducer in vitro. This tryptophan analog is not incorporated into TnaC.

The enzyme tryptophanase catalyzes the degradation of L-tryptophan to indole, pyruvate, and ammonia. Degradation allows organisms to utilize tryptophan as a source of carbon, nitrogen, and energy (1, 2). The tryptophanase reaction is reversible; thus, L-tryptophan can be formed from indole and L-serine, L-cysteine, or pyruvate and ammonia (3, 4). The tna operon of Escherichia coli contains two major structural genes, tnaA, encoding tryptophanase, and tnaB, specifying a low affinity tryptophan permease (5, 6). The tna operon promoter is separated from the tnaA structural gene by a 319-nucleotide leader region. This region encodes a 24-residue peptide, TnaC, that is essential for induction. Transcription of the tna operon is regulated by the combined action of catabolite repression and tryptophan-induced transcription antitermination. Regulation by catabolite repression requires the catabolite gene activator protein plus cyclic AMP, and is tryptophan-independent (5, 7, 8). Transcription termination/antitermination in the leader region is regulated in response to high levels of tryptophan. Studies in vivo and in vitro have shown that in the absence of tryptophan, transcription is subject to Rho-dependent termination at several transcription pause sites located between tnaC and tnaA (9, 10). In the presence of inducing levels of tryptophan, termination at these sites is prevented (10). Translation of tnaC is essential for tryptophan induction (11). Specifically, inactivating the tnaC start codon, replacing the single Trp residue of TnaC with a different amino acid, or introducing several other amino acid changes in TnaC, prevents induction (13). BoxA and rut sites located immediately adjacent to the tnaC stop codon are essential for Rho-dependent termination; alteration of either of these sites reduces termination (14). On the basis of these and other findings, it has been proposed that, in the presence of inducer, TnaC acts in cis on its associated translating ribosome to inhibit its release at the tnaC stop codon. Inhibition of ribosome release could block Rho’s access to the BoxA or rut sites of the transcript and thereby prevent transcription termination (15–17).

To define the roles of TnaC, tryptophan, and Rho factor in regulation of tna operon expression, we developed a cell-free S-30 system in which regulation of tna operon could be studied. In this paper we show that the major regulatory features of tna operon observed in vivo can be duplicated in vitro. We examined transcription under inducing and noninducing conditions and demonstrate that in the absence of inducer transcription terminates at several sites in the leader region, and the terminated transcripts are then degraded. In the presence of tryptophan transcription pausing is observed, but the paused transcripts subsequently are elongated. We also detected and analyzed the synthesis and fate of the TnaC peptide. We show that tryptophan induction leads to the accumulation of TnaC-tRNAPro. This finding implicates tryptophan-induced inhibition of cleavage of TnaC-peptidyl-tRNAPro as the event crucial to induction.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—A derivative of the E. coli A19 Rnasel strain containing trpBΔlacZ ΔtrpEΔ2 tnaAβgl::Tn10, was constructed and used as the source of cell-free S-30 extracts. E. coli DH5

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plasmid pPGF4, pGF14 and pGF71 were constructed by replacing the ATG start codon by TAG. Plasmid pGF925 (constructed by P. Gollnick) contains a tnaC-lacz translational fusion. pGF24 was constructed by cloning the PstI-BamHI fragment containing a HindIII-I BamHI fragment containing a tnaA-trpE translational fusion from plasmid pK93 (constructed by Kirt Gish) into pBR322. pGF924 was constructed by ligation a PCR fragment from plasmid pPGD14 and pPGD17 (constructed by P. Gollnick) respectively. In pPGD14, tnaC Trp codon 12 is replaced by an Arg codon, CGG. In pGF71, the tnaC start codon, ATG, was replaced by the TAG stop codon.

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Plasmid pGF4 was constructed by inserting a HindIII-BamHI fragment containing a tnaA-trpE translational fusion from plasmid pK93 (constructed by Kirt Gish) into pBR322. pGF14 and pGF71 were constructed by replacing the HindIII-NotI fragment of pGF containing the tna promoter through the ATG start codon of tna in a homologous fragment from pPGD14 and pPGD71 (constructed by P. Gollnick), respectively. In pPGD14, tnaC Trp codon 12 is replaced by an Arg codon, CGG. In pGF71, the tnaC ATG start codon, ATG, was replaced by the TAG stop codon.

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Cyclic AMP-dependent Expression of a tnaC-‘lacZ Fusion in an S-30 System—In previous studies it was shown that the expression of the tna operon is regulated by catabolite repression (8). A presumed CAMP-CAP complex-binding site is located just upstream of the -35 region of the tna promoter (5, 10). Plasmid pPDG52 contains a tnaC-‘lacZ translational fusion (Fig. 1A). This plasmid DNA was used as a reporter to examine catabolite repression-dependent expression of the tna operon in vitro. In this construct most of the tna leader region had been removed; thus, expression from the promoter could be tested independently of the effects of the tna operon downstream region and attenuation. The data in Table I show that, in the absence of cAMP and presence of added tryptophan, β-galactosidase activity was almost undetectable. By contrast, in the presence of cAMP and absence of added tryptophan, high levels of β-galactosidase were produced. Addition of tryptophan increased β-galactosidase production less than 2-fold. These results suggest that expression of tnaC-‘lacZ construct in this cell-free system is cAMP-dependent, and that tryptophan produced by protein turnover provides most of the tryptophan required for β-galactosidase synthesis. The less than 2-fold increase observed upon addition of tryptophan is probably not regulatory; it presumably is due to increased completion of synthesis of the 1024-residue β-galactosidase monomer, which contains 39 Trp residues.

Basal and Induced Expression with the tnaA-‘trpE Construct—To study tryptophan induction of tna operon expression in vitro, we prepared a special construct, pGF4, containing the intact tna promoter-leader region followed by a translational fusion that would be tryptophan-free; thus, in vitro synthesis could be examined in the absence as well as the presence of tryptophan. The strain used to prepare the S-30 extract carried a trpR mutation and a trpE deletion; this extract has high levels of Trp-R but no Trp-enzyme activity. As expected, expression of the tnaA-‘trpE fusion was cAMP-dependent; without cAMP, very low levels of TnaA-TrpE enzyme activity were detected either in the absence or presence of tryptophan (Table I). In the presence of cAMP, addition of 300 μM tryptophan led to a 17-fold increase in synthesis of the TnaA-TrpE protein. Addition of a tryptophan analog, 1MT, also led to a 20-fold increase (Table I). We believe that the increase in tryptophan-free TnaA-TrpE protein synthesis in the presence of tryptophan, or 1MT, is due to their action as inducers of antitermination in the leader region.

We also examined the effects of tryptophan addition on TnaA-TrpE synthesis by measuring [35S]methionine incorporation into the TnaA-TrpE protein, following SDS-polyacrylamide gel electrophoresis (Fig. 2). Template plasmid pGF4 contains a β-lactamase gene, which is also expressed in vitro; β-lactamase has four tryptophan residues, and therefore serves as an excellent internal reference to monitor the availability of charged tRNA<sub>Trp</sub> for protein synthesis. In Fig. 2 it can be seen that there was no significant effect of tryptophan addition on β-lactamase production. This suggests that there are appropriate levels of charged tRNA<sub>Trp</sub> (tryptophan presumably generated by protein turnover) for synthesis of proteins like β-lactamase, even in the absence of added tryptophan. A 25-fold increase in TnaA-TrpE synthesis was observed in the presence of 300 μM tryptophan; synthesis was assayed by counting the Cerenkov radiation of 35S-labeled TnaA-TrpE bands (Fig. 2, lane 5 versus lane 2) on the SDS-PAGE gels (data not shown). This increase agrees with the values in Table I, and suggests that the ASase assay results accurately reflect the levels of TnaA-TrpE protein produced in this S-30 system.

Changing the tnaC Start Codon to TAG, or Replacing the tnaC<sup>B</sup>-Trp Codon 12 by an Arg Codon, Prevents Tryptophan Induction—It was shown previously, in vitro, that synthesis of the intact TnaC peptide containing its crucial Trp residue is essential for tryptophan induction of tna operon expression (13). To verify the role of tnaC translation in induction of the tna operon in the S-30 system, we prepared the tnaC<sup>B</sup>-Trp and tnaC<sup>TAG</sup>-Trp constructs, and synthesized the TnaC protein (Table II). Replacing the tnaC start codon by a stop codon (pGF71, Fig. 1B) should eliminate leader peptide synthesis. Basal level expression by this plasmid was decreased 5-fold relative to that of the wild type plasmid pGF4, and induction by tryptophan was abolished (Table II). Replacing Trp codon 12 by an Arg codon (pGF14, Fig. 1B) should allow peptide synthesis to proceed to the normal TGA stop codon of tnaC, generating an altered peptide. TnaA-TrpE production directed by pGF14 was decreased 3–4-fold compared with that of the wild type tnaA-‘trpE fusion plasmid pGF4, and was not inducible by trypto-

### Table I

| Plasmid (5 μM) | Gene fusion | cAMP (1 mM) | Enzymatic activities |
|---------------|-------------|-------------|---------------------|
| pPDG52        | tnaC-‘lacZ  | -           | <1                  |
| pPDG52        | tnaC-‘lacZ  | +           | <1                  |
| pBR322        | tnaC-‘lacZ  | +           | 25                  |
| pGF4          | tnaA-‘trpE  | -           | 4                   |
| pGF4          | tnaA-‘trpE  | +           | 40                  |

* The induction ratios observed upon addition of 300 μM 1-tryptryphan (Trp) or 1L-1-methytryptophan (1MT) are shown in parentheses.

### RESULTS

**Cyclic AMP-dependent Expression of a tnaC-‘lacZ Fusion in vitro**

In vitro S-30 reaction conditions and enzyme assay procedures are described under “Experimental Procedures.” Each assay was performed in duplicate on at least three separate occasions, and the average is given here.

![Fig. 2. A 10% Tricine-SDS-PAGE analysis of pGF4-directed in vitro translation products.](http://www.jbc.org/)

**TABLE II**

| Enzymatic activities |
|---------------------|
| -Trp                |
| +Trp                |
| +1MT                |

**FIG. 2.** A 10% Tricine-SDS-PAGE analysis of pGF4-directed in vitro translation products. In vitro reactions and Tricine-SDS-PAGE conditions are described under “Experimental Procedures.” Polypeptide bands were labeled with [35S]methionine. The templates used were: no plasmid (lane 1), 5 μM pGF4 (lanes 2-5), 5 μM pBR322 (lane 6), and 15 μM pGF4 (lanes 7 and 8). Protein molecular weight standards (Low Range; Life Technologies, Inc.) were used as markers; their molecular weights are given. The positions of TnaA-TrpE and β-lactamase are marked by arrows. *50 μM Trp was present in this reaction.

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mid concentration was varied, as shown in Fig. 3. When 5 nM B there was no significant increase in TnaA-TrpE activity. Plasmin, whereupon it began to level off. In the absence of inducer, TrpE enzyme activity was observed to increase linearly for 40 course experiment performed in the presence of inducer. TnaA- A concentration of inducer. Fig. 3

ables: reaction time, plasmid pGF4 concentration, and the con- 
To optimize reaction conditions, we examined three vari-

TABLE II
Effect of added tryptophan and bicyclomycin on expression of tnaA’-trpE plasmids

| tnaA’-trpE plasmid (5 nM) | tnaC mutation | ASase-specific activitya | +Trp | +BCb | +Trp and BC |
|--------------------------|---------------|-------------------------|------|------|------------|
| pBB322                   | Wild type     | <0.1                    | <0.1 | <0.1 | <0.1       |
| pGF4                     | Wild type     | 20                      | 360 (18) | 380 | 453        |
| pGF71                    | ATG to TAG    | 4                       | 5 (1.3) | 348 | ND*        |
| pGF14                    | Trp12 to Arg  | 6                       | 5 (0.8) | 360 | ND         |

a The induction ratios observed with 300 μM Trp are shown in parentheses.
b BC, 50 μg/ml bicyclomycin.
* ND, not determined.

Bicyclomycin Increases Basal Level Expression of All tnaA’-trpE Constructs—Bicyclomycin is a specific inhibitor of E. coli Rho factor. This antibiotic inhibits the poly(C)-stimulated ATPase activity of E. coli Rho factor (22, 23). Consistent with this finding, it has been shown that bicyclomycin increases basal level expression of the tna operon in vivo (24). Addition of an appropriate concentration of this antibiotic directly to the S-30 system should therefore inhibit Rho factor activity and increase basal level expression of the tna operon. To examine this possibility, 50 μg/ml bicyclomycin was added in vitro with tnaA’-trpE plasmids that did or did not have tnaC mutations. Bicyclomycin relieved Rho-dependent termination in the tna operon, irrespective of whether an intact TnaC peptide could be synthesized (Table II).

Characterization of tna Operon Regulation in the S-30 System—To optimize reaction conditions, we examined three variables: reaction time, plasmid pGF4 concentration, and the concentration of inducer. Fig. 3A presents the results of a time-course experiment performed in the presence of inducer. TnaA-TrpE enzyme activity was observed to increase linearly for 40 min, whereupon it began to level off. In the absence of inducer, there was no significant increase in TnaA-TrpE activity. Plasmid concentration was varied, as shown in Fig. 3B. When 5 nM pGF4 was present, there was 18-fold induction by 300 μM tryptophan. Increasing the pGF4 plasmid concentration to 10 nM elevated basal level expression appreciably, and there was only 1.2-fold induction by added tryptophan. When the pGF4 plasmid concentration was increased to 15 nM or greater, basal level expression increased further and there was no significant effect of added tryptophan. These results are consistent with the intensities of the TnaA-TrpE bands observed on a SDS-PAGE gel (Fig. 2, compare lanes 7 and 8); when 15 nM pGF4 was used, the [35S]methionine-labeled TnaA-TrpE protein bands in lane 7 (without added tryptophan) and lane 8 (with 300 μM tryptophan) were indistinguishable. These data suggest that the S-30 system employed is limiting for some component required for efficient transcription termination under noninducing conditions.

1MT, an analog of tryptophan, effectively induces tna operon expression in vivo (25). It is thought that 1MT induces tna operon without being charged onto tRNATrp or being incorporated into protein (see below) (13). The effect of varying 1MT concentration on TnaA-TrpE production in our S-30 system is shown in Fig. 3C. A concentration of 0.5–1 mM was sufficient to fully induce tna operon expression; maximum induction pro-

FIG. 3. Characterization of the cell-free S-30 system. In vitro protein synthesis and ASase assays were carried out as described under “Experimental Procedures.” Induction ratios are shown in parentheses. A, a time-course experiment with plasmid pGF4-programmed TnaA-TrpE synthesis. B, effect of plasmid pGF4 concentration on TnaA-TrpE production, both in the presence and absence of added tryptophan. C, effect of DL-1-methyl-tryptophan (DL-1-IMT) concentration on the production of TnaA-TrpE protein.
Addition of purified Rho lowers basal level expression when high concentrations of tnaA-trpE plasmids are used

In vitro S-30 reaction conditions and the ASase assay procedure are described under "Experimental Procedures." Each assay was performed in duplicate on at least three separate occasions, and the average is given here. Plasmid concentration is shown in parentheses. 300 μM Trp was added, as indicated.

| tnaA-trpE plasmid | tnaC mutation | No added Rho | +Rho (6 μM) |
|-------------------|---------------|--------------|-------------|
|                   |              | −Trp | +Trp | +/- ratio | −Trp | +Trp | +/- ratio |
| pGF4 (5 nM)       | Wild type    | 20   | 360  | 18       | ND   | ND   |          |
| pGF14 (5 nM)      | Trp^{12} to Arg | 6    | 5    | 0.8      | ND   | ND   |          |
| pGF71 (5 nM)      | ATG to TAG   | 4    | 5    | 1.3      | ND   | ND   |          |
| pGF4 (15 nM)      | Wild type    | 425  | 456  | 1.1      | 53   | 328  | 6.0      |
| pGF14 (15 nM)     | Trp^{12} to Arg | 360  | 404  | 1.1      | 37   | 35   | 1.0      |
| pGF71 (15 nM)     | ATG to TAG   | 375  | 380  | 1.0      | 37   | 40   | 1.0      |

ND, not determined.

provided a 23-fold increase in ASase-specific activity over that observed in the absence of added 1MT. Increasing the tryptophan concentration from 50 μM to 300 μM (Fig. 2, lane 4 versus lane 5) also led to a significant increase in TnaA-TrpE production. Since tryptophanyl-tRNA synthase has a relatively high affinity for tryptophan (K_{m} of 5 × 10^{-5} M; Ref. 26), the tRNATrp in our S-30 should be fully charged in the presence of 50 μM added tryptophan. Taken together, these findings suggest that tna induction is not based on the extent of charging of tRNATrp.

Rho Is Limiting in Our Cell-free System—One unexpected finding was that, when the concentration of plasmid pGF4 was increased from 5 to 15 nM, basal level expression of tnaA-trpE increased dramatically (Fig. 3B). This observation suggests that a factor(s) responsible for (or participating in) basal level expression in the S-30 system might be limiting, or titrated out. To determine whether increased translation of tnaC is responsible for this presumed titration effect, constructs with mutations in tnaC that prevent induction were tested. It can be seen in Table III that, with 15 nM pGF71 (or pGF14) (Fig. 1B) as template, the results obtained are similar to those observed with pGF4 (Fig. 3B). These findings indicate that translation of tnaC is not responsible for the apparent titration effect.

A second reasonable explanation is that Rho may become limiting because the additional tna transcripts produced contribute BoxA and rut binding sites that sequester much of the available Rho. If this is the case, addition of purified Rho protein should reverse the titration effect. As expected, when 6 μM Rho was added to the reaction mixture (Table III), both the basal and induced levels of tna operon expression were lowered, and now 6-fold tryptophan induction was observed with wild type tnaC plasmid pGF4. No induction was observed with either pGF14 or pGF71, as expected. These findings support the interpretation that Rho is responsible for setting the basal level of expression of the tna operon.

Measurement of tna mRNA Synthesis—To demonstrate directly that tryptophan has no effect on cAMP-dependent transcription initiation at the tna promoter, a circularized DNA template was constructed that contains the intact tna promoter but lacks the leader region beyond bp +6, CF-tna+6rpoBC"t". This circular template was used to direct the S-30 system (Fig. 4A). The predicted −130-nucleotide transcript would contain only the first 6 nucleotides of the tna leader region, immediately followed by the E. coli rpoBC terminator (rpoBC"t"). In Fig. 4A it can be seen that with this template transcription initiation from the tna promoter was highly efficient and was dependent on the presence of cyclic AMP in the reaction mixture. No effect of added L-tryptophan was evident.

To confirm that the tryptophan induction observed with the tnaA-trpE translational fusion construct was due to relief from Rho-dependent termination, another circularized construct, CF-tna+306rpoBC"t", was examined (Fig. 5A). This construct contains the intact tna promoter, tna leader region to +306, followed by the rpoBC transcription terminator. Transcripts produced from this template that escape Rho-dependent termination should terminate at the rpoBC terminator, yielding a −430-nucleotide read-through transcript (Fig. 5A). No transcripts were observed when cAMP or the template was omitted from the S-30 system (Fig. 5B, −cyclic AMP lane). In the presence of cAMP and added tryptophan, a prominent read-through (RT) transcript band was observed (Fig. 5B, last lane). Only a faint read-through transcript was produced when tryptophan was omitted (Fig. 5B, −Trp lane). Consistent with the translational results described above, no effect of tryptophan was observed when two templates with different tnaC

Fig. 4. Demonstration of the requirement for cAMP for initiation of transcription of the tna operon. A, schematic representation of the circularized fragment (CF-tna+6rpoBC"t") used as template to program the cell-free reactions illustrated in A. The first 6 nucleotides of the tna leader transcript were followed by the E. coli rpoBC terminator (rpoBC"t"). The source of the predicted −130-nucleotide transcript is shown. B, analysis of transcription in an S-30 system. A 6% polyacrylamide, 7 M urea gel was used. Transcription initiation at the tna promoter was cAMP-dependent and tryptophan-independent. The S-30 reactions (50 μl each) were incubated at 37 °C for 10 min in the presence or absence of cyclic AMP and/or L-tryptophan. 20 μCi of α-32PUTP was then added, and after a 5-min incubation, the reactions were stopped by phenol extraction and the contents loaded onto an RNA gel.
Regulation of E. coli tna Operon in Vitro

In the absence of added tryptophan, all templates gave RNA expression patterns similar to those obtained with the wild type template in the absence or presence of tryptophan. 

In the presence of added tryptophan (Fig. 6, A), multi-

Figure 5. Requirements for in vitro synthesis of the read-through transcript. A, schematic representation of the circularized DNA fragment, CF-tna +306rpoBC tat, used as template to program the cell-free reactions in A. This fragment contains the intact tna promoter, the tna leader region to bp +306, followed by the rpoBC transcription terminator. The predicted tna transcripts in the absence or presence of tryptophan are also shown. B, analysis of the requirements for read-through transcription. A 6% polyacrylamide, 7 M urea gel was run with transcription/translation assay mixtures. S-30 reaction mixtures with 20 μCi of [α-32P]UTP (50 μl reaction mixture) with the indicated additions were incubated at 37 °C for 20 min. The reactions were then stopped by phenol extraction, and the RNA samples were loaded on RNA gels. An arrow points to the position of the read-through transcript (RT).

mutations (ATG start codon changed to TAG, or Trp codon 12 changed to an Arg codon, in CF-tna +306rpoBC tat) were used (data not shown). Addition of bicyclomycin with cAMP increased basal level expression with all templates (data not shown).

Single-round Transcription Analyses in the S-30 System—In the experiment described in Fig. 5B (-Trp lane), only low levels of terminated transcripts were observed. Why were bands of comparable intensity to those in the +Trp lane not also seen? In an attempt to explain this observation, we analyzed transcription more closely using the "single-round transcription" approach (27). Using this procedure, transcription/translation assays (coupled to translation) were performed in an S-30 system. Circularized fragment CF-tna +306rpoBC tat (see Fig. 5B) was used as template. S-30 reactions (100 μl) without CTP and UTP, in the absence or presence of tryptophan, were incubated at 37 °C for 10 min, then 50 μCi of [α-32P]UTP, 200 μCi CTP, and 200 μg/ml rifampicin were added together to the reaction mixture. Samples (10 μl) were taken at indicated time points, stopped by phenol extraction, and loaded on an RNA gel. The read-through transcript (RT) and the RNA doublet observed only in the presence of tryptophan are marked by arrows. B, effect of addition of the Rho inhibitor bicyclomycin, to a reaction mixture incubated in the absence of tryptophan. Single-round transcription assays (coupled to translation) were performed as described in A. CF-tna +306rpoBC tat was used as template. Control reactions (+ Trp) are shown on the left.

In other experiments, we tested templates analogous to CF-tna +306rpoBC tat but bearing various tnaC mutations (ATG to TAG, or Trp12 → Arg). Both in the presence and absence of tryptophan, all templates gave RNA expression patterns similar to those obtained with the wild type template in the absence of tryptophan (Fig. 6A, -Trp lanes); thus, we do not show these results. We also examined the effects of adding the translation leader region to bp 

Figure 6. Single-round transcription analyses examining the effects of added tryptophan and bicyclomycin. A, single-round transcription analyses (coupled with translation) were performed in an S-30 system. Circularized fragment CF-tna +306rpoBC tat (see Fig. 5B) was used as template. S-30 reactions (100 μl) without CTP and UTP, in the absence or presence of tryptophan, were incubated at 37 °C for 10 min, then 50 μCi of [α-32P]UTP, 200 μCi CTP, and 200 μg/ml rifampicin were added together to the reaction mixture. Samples (10 μl) were taken at indicated time points, stopped by phenol extraction, and loaded on an RNA gel. The read-through transcript (RT) and the RNA doublet observed only in the presence of tryptophan are marked by arrows. B, effect of addition of the Rho inhibitor bicyclomycin, to a reaction mixture incubated in the absence of tryptophan. Single-round transcription assays (coupled to translation) were performed as described in A. CF-tna +306rpoBC tat was used as template. Control reactions (+ Trp) are shown on the left.
Regulation of E. coli tna Operon in Vitro

Previous attempts to synthesize TnaC of E. coli in an S-30 system were unsuccessful (28). When we used the tnaA'–trpE construct pGF4 to program protein synthesis, we also did not observe TnaC peptide production. Synthesis was examined using a Tricine-SDS protein gel (see Fig. 2). To improve our ability to detect TnaC, we decided to eliminate competition for transcription or translation. Therefore, we changed our strategy and used a circularized small PCR fragment, CF-tna +306proBC +4rpoBC, as template. This circularized template contains only the tna promoter, the tna leader region to bp +306, followed by the rpoBC terminator. The only transcripts expected would be those initiated at the tna promoter, and the resulting peptide product would be TnaC. In Fig. 7A it can be seen that, with [35S]methionine as label, two new bands (a ~3-kDa band and a ~25-kDa band) appear in the presence of added tryptophan. The ~3-kDa band (but not the ~25-kDa band) is present in the absence of added tryptophan. Neither of these bands is present when the CF-tna +306proBC +4rpoBC template is omitted from the S-30 reaction (Fig. 7A, lane 1). The ~3-kDa band could be TnaC, and the ~25-kDa band could be TnaC-peptidyl-tRNA. Some nonspecific products were also detected in all the reactions. The 2.9-kDa 24-residue TnaC peptide predicted from the tnaC nucleotide sequence should contain 1 Trp, 4 Ile, 1 Pro, and 25-kDa molecule was separated from the gel and treated with RNase A, proteinase K (Fig. 8A), and DNase. After RNase A treatment, the ~25-kDa molecule disappeared and the labeled product shifted to the TnaC position. The ~25-kDa molecule disappeared after proteinase K digestion (Fig. 8A). DNase treatment had no effect (data not shown). These data suggest that this ~25-kDa molecule contains TnaC linked to a RNA.

The most likely identity of the ~25-kDa band is TnaC-peptidyl-tRNA Pro (the C-terminal residue of TnaC is Pro). To examine this possibility, the ~25-kDa molecule was separated from E. coli total tRNA by long distance Tricine-SDS-PAGE, located by autoradiography, excised, and used as template for RT-PCR using primers based on the sequence of tRNA Pro. A S-30 sample that had not been incubated with the circularized DNA template was also loaded on the same gel, and the corresponding band was recovered as a control to test for tRNA contamination. The S-30 control did not yield a product (Fig. 8B, lane 2), and the lane in which read-through was omitted also lacked a product (lane 3), while a 70–80 bp PCR product was observed using the ~25-kDa molecule as template (lane 4). As a positive control, total tRNA from E. coli (Sigma) was used as template. We observed the same 70–80 bp product plus a shorter product (lane 1). These findings indicate that the ~25-kDa molecule observed in the presence of tryptophan is TnaC.
The Effect of Tryptophan Concentration on the Accumulation of TnaC-\(\text{tRNA}^{\text{Pro}}\), and the Read-through Transcript—As shown above, tryptophan, the inducer of the \(tna\) operon, plays a crucial role in the accumulation of peptidyl-\(\text{tRNA}\) and the production of the read-through transcript. To determine the tryptophan concentration dependence of the events leading to peptidyl-\(\text{tRNA}\) and read-through transcript production, we performed experiments with our circularized CF-\(\text{tna}+306\text{ropBC}\) DNA template, and varied the tryptophan concentration (Fig. 9). As the tryptophan concentration in the S-30 reaction was increased, stronger TnaC peptide and TnaC-\(\text{tRNA}^{\text{Pro}}\) signals were detected, with an apparent maximum at 1 mM tryptophan (Fig. 9A). When the read-through transcript level was determined, using \([\alpha^{35}\text{P}]\text{UTP}\) to label transcripts in the presence of increasing concentrations of tryptophan, the maximum level of read-through transcript was observed at the highest tryptophan concentration tested, 0.5 mM (Fig. 9B). These findings indicate that, in the S-30 system, a tryptophan concentration in excess of 0.25 mM is probably required to obtain 50% or greater induction of \(tna\) operon expression.

DISCUSSION

Tryptophan-mediated induction of \(tna\) operon expression proceeds by preventing transcription termination at Rho factor-dependent termination sites in the leader region of the operon (9–11). The simplest model consistent with all of our previous experimental findings is that, in the presence of inducing levels of tryptophan, the nascent TnaC peptide acts in cis on its translating ribosome to inhibit its release at the \(tnaC\) stop codon. The stalled ribosome would presumably block Rho’s access to the BoxA and \(\text{rut}\) site adjacent to the \(tnaC\) stop codon, and thereby prevent Rho-mediated transcription termination (29).

In this study we successfully reproduced all the in vivo features of \(tna\) operon regulation by tryptophan-induced transcription antitermination using a coupled transcription/translation S-30 system from \(E.\ coli\). In addition, we provide the first demonstration of \(E.\ coli\) TnaC peptide synthesis in the S-30 system (in vitro synthesis of \(P.\ vulgaris\) TnaC has been shown (Ref. 28)). Most importantly, we show that the presence of inducing levels of tryptophan leads to the accumulation of TnaC-\(\text{tRNA}^{\text{Pro}}\). We also show that moderately high tryptophan concentrations are required for induction and for peptidyl-\(\text{tRNA}\) accumulation. Our results provide direct support for the hypothesis that, in the presence of inducing levels of tryptophan, TnaC-\(\text{tRNA}^{\text{Pro}}\) is not cleaved. This peptidyl-\(\text{tRNA}\) presumably blocks release of the translating ribosome at the \(tnaC\) stop codon, and prevents Rho action. Our preliminary findings (data not shown) demonstrate that the peptidyl-\(\text{tRNA}^{\text{Pro}}\) is associated with the translating ribosome.

The S-30 Cell-free System—\(E.\ coli\) strain \(\text{RNase I- trpR} \\Delta\text{lacZ }\text{trpE2 trnaA bgl::Tn10}\) was used to prepare our S-30 extracts. This strain was derived from the classic \(\text{RNase I minus A-19}\) strain. \(\text{trpE}\) and \(\text{lacZ}\) deletions were introduced into this strain; consequently, any \(\text{Trp}\) or \(\text{LacZ}\) activity detected in the S-30 extract would result from plasmid-programmed synthesis. This strain is \(\text{trp}\) repressor minus; thus, S-30 extracts from this strain have elevated levels of the \(\text{TrpG-D protein (30)}\) that are sufficient to fully activate all the TnaA-\(\text{trp}\) protein synthesized during the course of an experiment. This conclusion has been confirmed by measuring TnaA-\(\text{Trp}\) activity in the \(\text{NH}_{3}\) dependent anthranilate synthase reaction that does not require \(\text{TrpG-D protein (data not shown)}\).

The Level of Tryptophanyl-\(\text{tRNA}^{\text{Pro}}\)—How added tryptophan serves as the signal that leads to inhibition of Rho-dependent termination is a basic unanswered question. Translation of \(t\) \(c\) \(d\) \(o\) \(n\) 12 of \(\text{tnaC} \) by \(\text{tRNA}^{\text{Pro}}\) is believed to be essential for tryptophan-induced antitermination with the wild type \(t\) \(n\) \(a\) \(n\) operon (13). It has been suggested that the TnaC peptide might be modified in some manner when excess tryptophan is present (15). Since full induction of the \(t\) \(n\) operon in the S-30 system is also observed with 1 mM 1M as inducer, and since unlabeled 1M does not appear to reduce incorporation of labeled tryptophan into TnaC or TnaC-\(\text{tRNA}^{\text{Pro}}\) (Fig. 7B), it now seems unlikely that the Trp residue at position 12 is modified. Simi-
1982

Regulation of E. coli tna Operon in Vitro

Fig. 9. Tryptophan dependence of TnaC, TnaC-tRNA<sup>Pro</sup>, and read-through transcript production. A, a 10% Tricine-SDS protein gel of S-30 reactions performed at increasing tryptophan concentrations. S-30 reactions (50 μl each) were performed with CF-tna + 306proBC<sup>T</sup> in the presence of the indicated concentrations of L-tryptophan. Incubation was at 37°C for 10 min, then 40 μCi of [35S]methionine was added for 10 min, and the reactions were stopped by acetone precipitation, boiled in 1× Tricine-SDS sample buffer for 3 min, and then loaded onto a 10% Tricine-SDS gel. Levels of TnaC peptide and TnaC-tRNA<sup>Pro</sup> were quantified using a PhosphorImager. The levels of TnaC and TnaC-tRNA<sup>Pro</sup> in the 1 ms tryptophan lane were set at 100%, respectively. B, a 6% polyacrylamide, 7 M urea gel of a transcription/translation reaction examining the effect of increasing tryptophan concentration on the production of read-through (RT) transcript. CF-tna + 306proBC<sup>T</sup> was used as template. The S-30 reaction mixtures (50 μl each), in the presence of the indicated concentrations of L-tryptophan, were incubated at 37°C for 10 min, then 40 μCi of [35S]UTP and 200 μg/ml rifampicin were added. After 1, 5, or 15 min of incubation, samples were taken and reactions were stopped by phenol extraction and loaded on an RNA gel.

Fig. 10. Model depicting the mechanism of tryptophan induction of tna operon expression. When a ribosome reaches the tnaC termination codon, UGA, in the presence of tryptophan, the 24-residue TnaC peptide (chain of circles) remains covalently joined to tRNA<sup>Pro</sup>. Consequently the ribosome associated with the peptidyl-tRNA stalls at the tnaC stop codon. This blocks Rho’s access to the BoxA and rut sites, and thereby prevents transcription termination. The positions at which amino acid residue changes have been shown to largely prevent induction are represented by black circles; positions where changes can result in constitutive expression are white circles; positions where changes result in mixed effects are indicated by white circles with a diagonal line; positions for which there are insufficient data are indicated as gray circles (14). The crucial Trp codon 12 is shown as an enlarged black circle. PTC, peptidyltransferase center.

conclusion. The antibiotic bicyclomycin is known to interact with Rho and inhibit its action (22–24). Addition of bicyclomycin to our in vitro system led to a 15-fold increase in basal level expression (Table II). In a recent study, it has been shown that a BoxA site and a rut site (both are adjacent to the tnaC stop codon) are crucial for Rho action in vivo (14).

TnaC-tRNA<sup>Pro</sup> Synthesis, Ribosome Stalling at the tnaC Stop Codon, and the RNA Doublet—The observation that TnaC-tRNA<sup>Pro</sup> accumulates in the presence of inducing levels of tryptophan suggests that the translating ribosome may be stalled at the tnaC stop codon. If this is correct, the associated transcript should not be available for subsequent rounds of translation. In the absence of added tryptophan, TnaC would presumably be synthesized and released. Thus, one might expect to detect a higher level of TnaC peptide in the absence versus the presence of added tryptophan. However, in the presence of tryptophan, TnaC would exist as two species, free and as peptidyl-tRNA. Unfortunately, the TnaC peptide is labile in the S-30 system (data not shown); therefore, we could not reliably measure the level of TnaC that is synthesized.

Appearance of the RNA doublet only in the presence of tryptophan provides additional support for the interpretation that induction results in ribosome stalling at the tnaC stop codon. It is very likely that the RNA doublet arises from RNA processing. When we employ a template with a 5-bp deletion at the 5′-end of the tna leader region instead of the wild type template, a corresponding decrease is observed in the length of RNA doublet (data not shown). This establishes that the 3′ end of the doublet is identical using either template. The normal length RNA doublet was also observed when a template was used that has the rut region deleted (from bp +101 to +123, just beyond the stop codon of tnaC), suggesting that the RNA secondary structure that forms just beyond the tnaC stop codon (9) is not responsible for formation of the doublet (data not shown). Strephtolydigin was added to an incubation mixture to inhibit all ongoing transcription, RNA doublet accumulation was still observed, consistent with doublet formation resulting from RNA processing.

Our combined results show that tna operon regulation can be
reconstituted in an S-30-coupled transcription/translation system, with expression dependent on catabolite repression. Rho-dependent transcription termination, and tryptophan-induced antitermination. We also conclude that, in the presence of inducer, the newly synthesized TnaC-peptidyl-tRNAPro is resistant to cleavage. We assume that TnaC-peptidyl-tRNAPro is in the P site of the translating ribosome, and that the tnaC UGA stop codon is in the ribosomal A site. We previously reported that inactivation or overproduction of release factor 3 affects both basal level expression and induction of the tna operon (31). These findings imply that the release factor 3-mediated event in normal ribosome release can influence the unusual events associated with induction. A schematic representation of the hypothetical stalled translation termination complex that forms in the presence of tryptophan is shown in Fig. 10. In this representation the peptidyl portion of TnaC-peptidyl-tRNAPro is placed in the polypeptide exit tunnel of the 50 S ribosomal subunit; however, the presumed active segment of this TnaC-peptidyl-tRNAPro could interact with a surface of the peptide tunnel or with a region of the ribosomal A site (32, 33). Wherever TnaC-peptidyl-tRNAPro is located, its presence, plus inducing levels of tryptophan, appear to prevent release factor 2 from mediating cleavage of TnaC-peptidyl-tRNAPro. Similar findings have been described by Cao and Geballe (34–36) in studies on translational regulation of gene expression in the cytomegalovirus. They have shown that translation of an upstream open reading frame, uORF2, regulates translation of a downstream coding region, corresponding to gene ULA. The uORF2 polypeptide, 22 residues in length, like TnaC, accumulates as a peptidyl-tRNAPro, at the stop codon of the uORF coding region. This peptidyl-tRNA is also resistant to cleavage, resulting in ribosome stalling and blockage of translation of the downstream coding region for ULA (35). The features of this example also suggest that certain amino acid sequences in a nascent peptide can interfere with peptidyl-tRNA cleavage when the translating ribosome is reading a stop codon. Addition of puromycin does not result in cleavage of this peptidyl-tRNA, and its release from the ribosome (37). By contrast, TnaC-peptidyl-tRNAPro can be released and cleaved in response to puromycin. Somewhat related examples in bacteria concern antibiotic inhibition of peptide chain elongation, leading to ribosome stalling. This influences the availability of a downstream nucleotide sequence needed for translation initiation. Chloramphenicol action during translational attenuation in the CAT operon is perhaps the best understood example of this type (12).

Four important questions remain unanswered regarding tna operon regulation: how is tryptophan recognized, what is the role of TnaC, what is the role of Trp at position 12, and, under inducing conditions, how is cleavage of TnaC-tRNAPro prevented?

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REFERENCES

1. Kazarinoff, M. N., and Snell, E. E. (1977) J. Biol. Chem. 252, 7598–7602
2. Hopkins, P. G., and Cole, S. W. (1983) J. Physiol. 289, 451–466
3. Newton, W. A., and Snell, E. E. (1984) Proc. Natl. Acad. Sci. U. S. A. 51, 382–389
4. Watanabe, T., and Snell, E. E. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 1086–1090
5. Deeley, M. C., and Yanofsky, C. (1981) J. Bacteriol. 147, 787–796
6. Edwards, R. M., and Yudkin, M. D. (1982) Biochem. J. 204, 617–619
7. Bilenkian, J., Kaempfer, R., and Magasanik, B. (1967) J. Mol. Biol. 27, 495–506
8. Botsford, J. L., and DeMoss, R. D. (1971) J. Bacteriol. 105, 303–312
9. Stewart, V., Landick, R., and Yanofsky, C. (1986) J. Bacteriol. 166, 217–223
10. Stewart, V., and Yanofsky, C. (1985) J. Bacteriol. 164, 731–740
11. Stewart, V., and Yanofsky, C. (1986) J. Bacteriol. 167, 383–386
12. Lovett, P. S., and Rogers, E. J. (1996) Microbiol. Rev. 60, 366–385
13. Gish, K., and Yanofsky, C. (1995) J. Bacteriol. 177, 2399–2405
14. Konan, K. V., and Yanofsky, C. (2000) J. Bacteriol. 182, 3981–3988
15. Konan, K. V., and Yanofsky, C. (1999) J. Bacteriol. 181, 1530–1536
16. Konan, K. V., and Yanofsky, C. (1997) J. Bacteriol. 179, 1774–1779
17. Gish, K., and Yanofsky, C. (1995) J. Bacteriol. 177, 7245–7254
18. Zubay, G. (1973) Annu. Rev. Genet. 7, 267–287
19. Schagger, H., and von Jagow, G. (1987) Anal. Biochem. 166, 368–379
20. Lesley, S. A. (1995) in Methods in Molecular Biology: In Vitro Transcription and Translation Protocols (Tymms, M. J., ed) Vol. 37, pp. 265–278, Humana Press Inc., Totowa, NJ
21. Yanofsky, C., and Horn, C. (1994) J. Bacteriol. 176, 6245–6254
22. Park, H. G., Zhang, X., Moon, H. S., Zwiefka, A., Cox, K., Gaskell, S. J., Widger, W. R., and Kohn, H. (1995) Arch. Biochem. Biophys. 323, 447–454
23. Zwiefka, A., Kohn, H., and Widger, W. R. (1993) Biochemistry 32, 3564–3570
24. Yanofsky, C., and Horn, C. (1995) J. Bacteriol. 177, 4451–4456
25. Yanofsky, C., Horn, V., and Gollnick, P. (1991) J. Bacteriol. 173, 6009–6017
26. Squires, C. L., Rose, J. K., Yanofsky, C., Yang, H. L., and Zubay, G. (1973) Nat. New Biol. 245, 131–133
27. Winkler, M. E., and Yanofsky, C. (1981) Biochemistry 20, 3738–3744
28. Kamath, A. V., and Yanofsky, C. (1992) J. Biol. Chem. 267, 19978–19985
29. Yanofsky, C., Konan, K. V., and Sarotero, J. P. (1996) Biochimie 78, 1017–1024
30. Yanofsky, C., Miles, E., Bauerle, R., and Kirschner, K. (1999) in Encyclopedia of Molecular Biology (Creighton, T. E., ed) pp. 2676–2689, John Wiley & Sons, Inc., New York
31. Yanofsky, C., Horn, V., and Nakamura, Y. (1996) J. Bacteriol. 178, 3755–3762
32. Ban, N., Nissen, P., Hansen, J., Moore, P. B., and Steitz, T. A. (2000) Science 289, 985–990
33. Nissen, P., Hansen, J., Ban, N., Moore, P. B., and Steitz, T. A. (2000) Science 289, 990–993
34. Cao, J., and Geballe, A. P. (1995) J. Virol. 69, 1030–1036
35. Cao, J., and Geballe, A. P. (1996) Mol. Cell. Biol. 16, 7109–7114
36. Cao, J., and Geballe, A. P. (1998) Mol. Cell. Biol. 16, 603–608
37. Cao, J., and Geballe, A. P. (1998) RNA 4, 181–188

3 F. Gong and C. Yanofsky, unpublished results.
Reproducing tna Operon Regulation in Vitro in an S-30 System: TRYPOTOPHAN INDUCTION INHIBITS CLEAVAGE OF TnaC PEPTIDYL-tRNA

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