JOURNAL OF BACTERIOLOGY, July 2000, p. 3981–3988 Vol. 182, No. 14
0021-9193/00/$04.00+0

Rho-Dependent Transcription Termination in the *tna* Operon of *Escherichia coli*: Roles of the *boxA* Sequence and the *rut* Site

KOUACOU VINCENT KONAN† AND CHARLES YANOFSKY*

Department of Biological Sciences, Stanford University, Stanford, California 94305-5020

Received 23 February 2000/Accepted 28 April 2000

Expression of the tryptophanase (*tna*) operon of *Escherichia coli* is regulated by catabolite repression and by tryptophan-induced transcription antitermination. Tryptophan induction prevents Rho-dependent transcription termination in the leader region of the operon. Induction requires translation of a 24-residue leader peptide-coding region, *tnaC*, containing a single, crucial Trp codon. Studies with a *lacZ* reporter construct lacking the *tnaC-tnaA* spacer region suggest that, in the presence of excess tryptophan, the TnaC leader peptide acts in *cis* on the ribosome translating *tnaC* to inhibit its release. The stalled ribosome is thought to block Rho's access to the transcript. In this paper we examine the roles of the *boxA* sequence and the *rut* site in Rho-dependent termination. Deleting six nucleotides (CGC CCT) of *boxA* or introducing specific point mutations in *boxA* results in high-level constitutive expression. Some constitutive changes introduced in *boxA* do not change the TnaC peptide sequence. We confirm that deletion of the *rut* site results in constitutive expression. We also demonstrate that, in each constitutive construct, replacement of the *tnaC* start codon by a UAG stop codon reduces expression significantly, suggesting that constitutive expression requires translation of the *tnaC* coding sequence. Addition of bicyclomycin, an inhibitor of Rho, to these UAG constructs increases expression, demonstrating that reduced expression is due to Rho action. Combining a *boxA* point mutation with *rut* site deletion results in constitutive expression comparable to that of a maximally induced operon. These results support the hypothesis that in the presence of tryptophan the ribosome translating *tnaC* blocks Rho's access to the *boxA* and *rut* sites, thereby preventing transcription termination.

The enzyme tryptophanase catalyzes the degradation of L-tryptophan to indole, pyruvate, and ammonia (20, 39). Bacterial species that produce this enzyme can utilize tryptophan as a source of carbon, nitrogen, and energy (16). Tryptophanase can also catalyze the reverse reaction and synthesize L-tryptophan from indole and L-serine (or L-cysteine) or from pyruvate and ammonia (29, 47).

The tryptophanase (*tna*) operon from several bacterial species has been cloned and sequenced (7, 15, 17, 19, 24). In *Escherichia coli*, this operon contains two major structural genes, a promoter proximal gene, *tnaA*, encoding tryptophanase and a distal gene, *tnaB*, encoding a low-affinity tryptophan permease (7, 8). Preceding *tnaA* in the *tna* operon is a 319-nucleotide (nt) transcribed regulatory region that contains the coding region for 24-residue leader peptide TnaC. The 220-nt spacer region that separates *tnaC* from *tnaA* contains several transcription pause sites. Studies in vivo and in vitro have shown that these pause sites serve as regulated sites of Rho-dependent transcription termination (40, 41). In the presence of the inducer tryptophan, a transcription antitermination mechanism that increases transcription readthrough into the *tnaA-tnaB* structural gene region of the operon 10- to 100-fold is activated (41). Induction also requires the translation of *tnaC*. Thus, replacing the *tnaC* start codon by a stop codon or replacing the single, crucial Trp codon at position 12 by codons for other amino acids prevents induction (13; M. Eshoo and C. Yanofsky, unpublished results). In contrast, initiation of transcription of the *tna* operon is independent of tryptophan recognition and requires the binding of the cyclic AMP catabolite activator protein (CAP) (3, 4) at a CAP site located just upstream of the *tna* promoter (41).

Additional evidence supporting the essential role of the Rho factor in regulating *tna* operon expression comes from an analysis of Rho mutants (41), examination of Rho-inhibiting drugs (48), and the isolation of *cis*-acting mutants that express the *tna* operon constitutively (41). Mutations in *rho* that reduce Rho factor activity increase basal expression of the *tna* operon significantly (41, 48). Similarly, the drug bicyclomycin, an inhibitor of Rho action, increases expression of the *tna* operon (48, 52). *cis*-acting constitutive mutants, altered near the distal end of *tnaC*, also increase basal-level expression (41). The associated mutations are in a 9-nt sequence (CGC CCT TGA) that is homologous to the *boxA* sequences of the bacteriophage λ early region (9) and of rRNA operons (23). The *boxA* of these operons is necessary for antitermination or prevention of Rho-dependent termination and does not appear to be required for Rho-dependent termination. Several host factors called Nus factors are also involved in antitermination at sites of Rho-dependent termination in the above operons. In particular, in vitro studies with the rRNA operon have shown that a heterodimeric complex of NusB and NusE binds to *boxA* (26, 30). Mutations in *boxA* that prevent rRNA antitermination also interfere with the ability of *boxA* to bind to NusB and NusE (30). In addition, mutations altering either NusB or NusE factor prevent binding of the complex to *boxA* and are associated with reduced antitermination (37). Two other Nus factors, NusA and NusG, are believed to play an indirect role in antitermination by interacting with the transcribing polymerase (25, 30). Unlike findings with the rRNA operon, in vitro studies with the *tna* operon have shown that Rho-dependent transcription termination in the *tna* leader region is enhanced by the NusA factor (40).

Immediately following the *boxA* sequence and *tnaC* stop codon in the *tna* operon transcript, there is a sequence of

* Corresponding author. Mailing address: Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020. Phone: (650) 725-1835. Fax: (650) 725-8221. E-mail: yanofsky@cmgm.stanford.edu.
† Present address: Department of Microbiology/Immunology, Stanford University School of Medicine, Stanford, CA 94305-5124.

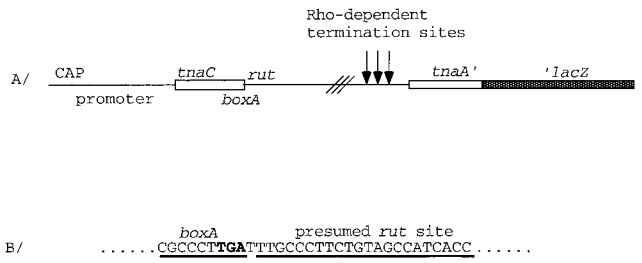

FIG. 1. (A) Schematic diagram of the *tnaA'-'lacZ* fusion used in this study. This construct contains the *tnaC* leader region, the 220-nt spacer region, and the first 20 codons of *tnaA* joined to the ninth codon of *lacZ*. The mutagenized fragments in this study did not include the *lacZ* coding region. (B) Nucleotide sequence of *boxA* and the *rut* site (underlined). The TGA stop codon (boldface) is part of the *boxA* region. CAP, catabolite activator protein binding site.

approximately 22 nt that is rich in cytidylate residues. Comparable sequences, called Rho utilization or *rut* sites (1), are necessary for Rho binding and action in the phage λ early region (32). Interestingly, in phage λ the *boxA* sequence is embedded in part of the *rut* site (*rutA*) of the tR1 terminator (5). Deletion of the *rut* site in the spacer region of the *tna* operon results in semiconstitutive expression of the operon, suggesting that the *rut* site is required for efficient Rho-dependent termination (13).

The exact mechanism by which tryptophan induces antitermination in the *tna* operon is not known. Studies with a *lacZ* reporter construct lacking the spacer region between *tnaC* and *tnaA* suggest that, in the presence of inducer, the nascent TnaC peptide acts in *cis* on the ribosome translating *tnaC* to inhibit its release at the *tnaC* stop codon (21). The stalling of the translating ribosome at this stop codon presumably interferes with Rho binding or action. It has also been shown that a deletion in the *tna* operon leader region of *Proteus vulgaris*, one that places the start codon for TnaA near the *tnaC* stop codon, allows inducer inhibition of ribosome initiation at this start codon (18). The stalled ribosome apparently blocks translation initiation at *tnaA*.

The role of the *tnaC* stop codon in *tna* operon regulation in *E. coli* has been examined by replacing the natural stop codon, UGA, by UAG or UAA. These changes reduced both basal and induced expression of the *tna* operon (22), consistent with other evidence indicating that, in *E. coli*, peptide release factor 1 (RF1; recognizing UAG and UAA stop codons) terminates translation more efficiently than RF2 (recognizing UGA and UAA) (6, 44). It was shown that a mutation that alters RF1 increased basal-level expression of the *tna* operon in strains with UAG or UAA as the *tnaC* stop codon, but not in strains with UGA as the stop codon (22). Additionally, inactivation of the structural gene for RF3 increased basal-level expression of the *tna* operon at least threefold (21, 49), consistent with the role of RF3 in enhancing translation termination at UGA, UAG, and UAA stop codons (14, 27). These results support the hypothesis that, in the presence of tryptophan, the nascent TnaC peptide inhibits ribosome release at the *tnaC* stop codon, thereby preventing Rho-dependent termination.

Although mutations in the *boxA*-like sequence (Fig. 1) result in constitutive expression (at least a threefold increase in basal expression) of the *tna* operon, it was not known whether *tnaC* translation influences the ability of *boxA* mutations to reduce transcription termination. It also was not known whether changes in the nucleotide sequence of *boxA* or the corresponding amino acid sequence or both are responsible for constitutive expression. In the present study we use combinations of point mutations and deletions to answer these questions and to further define the roles of *boxA* and the *rut* site (Fig. 1) in mediating Rho-dependent transcription termination. We show that some nucleotide changes that alter the *boxA* sequence but do not change the sequence of the TnaC peptide also result in constitutive expression of the *tna* operon. We also show that the constitutive expression exhibited by constructs with *boxA* or *rut* mutations is dependent on translation of the *tnaC* coding region. Combining a *boxA* mutation with a *rut* site deletion resulted in elevated *tna* operon expression comparable to that of the induced operon. These results are consistent with the participation of both the *boxA* nucleotide sequence and the *rut* site in Rho-dependent transcription termination in the intact *tna* operon and reveal the importance of *tnaC* translation to the regulatory mechanism controlling *tna* operon expression.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strains and the plasmids used in this study are listed in Table 1. Strains VK1300 to VK3100 are all single lysogens carrying λRS45 (21, 22, 38) with various inserts. To prepare these strains, the respective fusion constructs from pRS552 (Table 1) were independently crossed into phage λRS45 (38) and the recombinant phage genome was inserted into the chromosome of CY15076 (Table 1). Plasmids were introduced into various strains by transformation (34), with selection for the appropriate antibiotic resistance marker.

Media and enzyme assay. Vogel and Bonner minimal medium (45) was used throughout. For β-galactosidase (β-Gal) assays (28), cultures were generally grown with shaking at 37°C in minimal medium plus 0.2% glycerol and 0.05% acid-hydrolyzed casein, with or without L-tryptophan (100 μg/ml). When appropriate, media were supplemented with 30 μg of kanamycin per ml, 15 μg of tetracycline per ml, or 20 μg of bicyclomycin per ml (a noninhibitory concentration). β-Gal assays were performed as described by Miller (28); β-Gal activity is reported in Miller units (28).

Site-directed mutagenesis. The megaprimer PCR method (36) was used throughout to introduce mutations in the *tna* operon. First, the LACZ-RT primer, 5′-GCG ATT AAG TTG GGT AAC GCC AGG-3′, or the VK1 primer, 5′-CGG AAT TCA GCT TCT GTA TTG GTA AG-3′, was used along with the respective mutagenic primer to amplify a PCR product containing the chosen mutation. This product was then purified using a PCR purification kit (Qiagen Inc., Chatsworth, Calif.) and combined in the second PCR with the VK1 or LACZ-RT primer to synthesize the final PCR product. The final product was flanked with an *Eco*RI site at its 5′ end and a *Bam*HI site at its 3′ end; it contained the mutant *tnaC* leader sequence followed by all or part of the *tna* spacer region and the coding sequence for the first 20 amino acids of TnaA (Fig. 1). This product was cloned into the pCRII vector (Invitrogen Co., San Diego, Calif.), and the sequence was confirmed (35). The resulting insert was cleaved with *Eco*RI and *Bam*HI, purified using the GENECLEAN II kit (BIO 101 Inc., La Jolla, Calif.), and subcloned into the *Eco*RI- and *Bam*HI-cleaved pRS552 vector (38).

RESULTS

Point mutations and a deletion in the *boxA*-like region of *tnaC* result in semiconstitutive expression of the *tna* operon. There is a 9-nt sequence (CGC CCT TGA) at the end of the *tnaC* leader region (Fig. 1 and Table 2) which has strong homology to those of *boxA* of phage λ (9) and rRNA operons (23). The first 6 nt of this sequence, CGC CCT, encode Arg and Pro residues at positions 23 and 24, respectively, of the TnaC leader peptide. These 6 nt were deleted in construct VK1400 to examine the effects of removal of the presumed *boxA* sequence on Rho-dependent transcription termination. Deleting these 6 nt resulted in an eightfold increase in *tna* operon expression under noninducing conditions (Table 2). The addition of tryptophan did not increase the expression of this deletion construct. As reported previously, replacing the first nucleotide of the CGC CCT sequence, C, by A (Arg-to-Ser change in TnaC) (VK1600) resulted in a fourfold increase in the basal level of expression of the operon; this change allowed a twofold increase in induction (Table 2). Replacing the TnaC Trp residue of this construct by an Arg residue (PDG1181) resulted in higher basal-level expression than that

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genetic characteristics	Reference or source
Strains		
CY15076	W3110 *tnaA2* Δ*lacU169*	22
PDG1114	SVS1100 (λtna_p *tnaC265 tnaA′-′lacZ*)	13
PDG1171	SVS1100 (λtna_p *tnaC275 tnaA′-′lacZ*)	13
PDG1172	SVS1100 (λtna_p *tnaC*-ACU_{24}-*tnaA′-′lacZ*)	This study
PDG1173	SVS1100 (λtna_p *tnaC*-UCU_{24}-*tnaA′-′lacZ*)	This study
PDG1181	SVS1100 (λtna_p *tnaC*-CGG_{12}-AGC_{23}-*tnaA′-′lacZ*)	This study
SVS1100	W3110 *bglR551* Δ(*lac-argF*)*U169*	41
SVS1144	SVS1100 (λtna_p *tnaA′-′lacZ*)	41
VK800	CY15076 (λtna_p *tnaC*-UAG-*tnaA′-′lacZ*)	22
VK1300	CY15076 (λtna_p *tnaC*-UAG_1-CGG_{12}-AGC_{23}-*tnaA′-′lacZ*)	This study
VK1400	CY15076 (λtna_p *tnaC*-Δ[$CGC_{23}CCU_{24}$]-*tnaA′-′lacZ*)	This study
VK1500	CY15076 (λtna_p *tnaC*-UAG_1-Δ[$CGC_{23}CCU_{24}$]-*tnaA′-′lacZ*)	This study
VK1900	CY15076 (λtna_p *tnaC*-UAG_1-Δ[bp 101–123]-*tnaA′-′lacZ*)	This study
VK2000	CY15076 (λtna_p *tnaC*-AGC_{23}-Δ[bp 101–123]-*tnaA′-′lacZ*)	This study
VK2100	CY15076 (λtna_p *tnaC*-UAG_1-AGC_{23}-Δ[bp 101–123]-*tnaA′-′lacZ*)	This study
VK2200	CY15076 (λtna_p *tnaC*-UGA_{11}-AGC_{23}-*tnaA′-′lacZ*)	This study
VK2300	CY15076 (λtna_p *tnaC*-UGA_{18}-AGC_{23}-*tnaA′-′lacZ*)	This study
VK2301	CY15076 (λtna_p *tnaC*-UGA_{18}-*tnaA′-′lacZ*)	This study
VK2400	CY15076 (λtna_p *tnaC*-CGA_{23}-*tnaA′-′lacZ*)	This study
VK2500	CY15076 (λtna_p *tnaC*-CGU_{23}-*tnaA′-′lacZ*)	This study
VK2600	CY15076 (λtna_p *tnaC*-CCA_{24}-*tnaA′-′lacZ*)	This study
VK2700	CY15076 (λtna_p *tnaC*-CCG_{24}-*tnaA′-′lacZ*)	This study
VK2800	CY15076 (λtna_p *tnaC*-UGA_{23}-*tnaA′-′lacZ*	This study
VK2900	CY15076 (λtna_p *tnaC*-UGA_{24}-*tnaA′-′lacZ*)	This study
VK3000	CY15076 (λtna_p *tnaC*-AGA_{23}-*tnaA′-′lacZ*)	This study
VK3100	CY15076 (λtna_p *tnaC*-AGG_{23}-*tnaA′-′lacZ*)	This study
Plasmids		
pRS552	pBR322 derivative, *lac*-based vector	38
pBE621	*trpT su9* (UGA suppressor)	31

of VK1600 but eliminated induction (Table 2). These results confirm that alterations of the nucleotides in the *boxA*-like region of *tnaC* can reduce Rho-dependent transcription termination in the *tna* operon; they also confirm that Trp12 is essential for induction (13).

To determine whether translation of the *tnaC* coding region is required for the semiconstitutive expression observed in constructs with a *boxA* point mutation or deletion, the initiation codon AUG in these constructs was replaced by the stop codon UAG, giving derivatives VK1500 and VK1700. These changes resulted in a 60- to 370-fold decrease in basal-level expression (Table 2) and also eliminated induction. Thus con-

TABLE 2. Alteration of the *boxA*-like sequence in *tnaC* results in constitutive expression of the *tna* operon; constitutive expression depends on *tnaC* translation[a]

Strain	*tnaC* codons	β-Gal activity (Miller units)		Ratio[b]
		−Trp	+Trp	
	boxA			
SVS1144 (wild type)	$AUG_1....UGG_{12}....CGC_{23}CCU_{24}UGA..$	390 ± 17	11,800 ± 1,390	30
SVS1144 + bicyclomycin		4,940 ± 290	18,080 ± 2,080	
VK1400	$\Delta(CGC_{23}CCU_{24})$	3,320 ± 344	3,770 ± 640	
VK1400 + bicyclomycin		4,580 ± 790	5,090 ± 1,275	
VK1600	AGC_{23}	1,870 ± 230	4,190 ± 430	2
VK1600 + bicyclomycin		2,670 ± 325	5,005 ± 1,205	
PDG1114	CGG_{12}	145 ± 9	130 ± 4	
PDG1181	$CGG_{12}...AGC_{23}$	3,210 ± 140	3,005 ± 285	
PDG1171	UAG_1	13 ± 2	19 ± 3	
PDG1171 + bicyclomycin		2,275 ± 270	1,990 ± 220	
VK1500	UAG_1 $\Delta(CGC_{23}CCU_{24})$	55 ± 8	51 ± 10	
VK1700	UAG_1 AGC_{23}	5 ± 0.4	6 ± 0.8	
VK1700 + bicyclomycin		1,230 ± 60	1,300 ± 105	
VK1300	UAG_1 CGG_{12} AGC_{23}	11 ± 1	10 ± 1	
VK2200	UGA_{11} AGC_{23}	150 ± 17	120 ± 10	
VK2300	UGA_{18} AGC_{23}	180 ± 11	190 ± 14	

[a] Cultures were grown at 37°C in minimal medium (45) plus 0.2% glycerol and 0.05% acid-hydrolyzed casein, with (+) or without (−) L-Trp (100 μg/ml). When indicated, 20 μg of bicyclomycin/ml was added to the culture medium. At least five cultures of each type were assayed in each case. Nucleotide changes are underlined.

[b] Ratio of activities for cultures grown with Trp and without Trp.

TABLE 3. Introducing a UAG stop codon at position 23 or 24 prevents induction[a]

Strain	*tnaC* codons	β-Gal activity (Miller units)		Ratio[b]
		−Trp	+Trp	
SVS1144 (wild type)	AUG..UGG$_{12}$..CGC$_{23}$CCU$_{24}$UGA...	570 ± 15	18,920 ± 365	33
VK800	UAG	140 ± 18	2,100 ± 335	15
VK2900	UAG$_{24}$	240 ± 25	280 ± 30	
VK2900/pMY228	su UAG[c]	145 ± 14	160 ± 19	
VK2800	UAG$_{23}$	930 ± 36	850 ± 60	
VK2800/pMY228	su UAG[c]	540 ± 100	2,350 ± 279	4

[a] Cultures were grown as for Table 2. More than five cultures of each type were assayed in each case. The nucleotide changes introduced are underlined.
[b] Ratio of activities for cultures grown with Trp and without Trp.
[c] su UAG, a tryptophan-inserting UAG suppressor.

stitutive expression by these *boxA* mutants requires *tnaC* translation. The addition of bicyclomycin, an inhibitor of Rho protein function (52), to VK1700, the strain in which the *tnaC* AUG is replaced by UAG and in which there is an altered *boxA* sequence, increased basal expression to a level approaching that of the construct with the start codon AUG (VK1600) (Table 2). These results suggest that, in the absence of translation, the *tnaC* coding sequence serves as a Rho-binding site and that in this context the *boxA* sequence is no longer required for termination. A UGA stop codon was introduced at position 11 or 18 of *tnaC* of the *boxA* mutant constructs (VK2200 and VK2300) to determine whether translation of a portion of *tnaC* is sufficient to allow constitutive *tna* operon expression. These changes increased basal-level expression only slightly, implying that translation of the first 18 codons of *tnaC* is insufficient to permit the semiconstitutive expression typical of the construct with the *boxA* mutation alone. Replacing the *tnaC* start codon by UAG in the construct in which Trp12 was replaced by Arg and in which there was a mutated *boxA* (VK1300) also eliminated the elevated basal-level expression typical of a construct with only the *boxA* change.

Moving the *tnaC* stop codon eliminates induction. It was shown in Table 2 that introducing stop codons at *tnaC* coding positions 11 and 18 eliminated the modest induction observed in VK1600, the construct with the *boxA* point mutation. UAG stop codons were also introduced at positions 23, 24, and 25 to examine their effect on basal-level expression and induction (constructs VK2800, VK2900, and VK800, respectively). These codon changes also alter the natural *boxA* sequence in *tnaC*. It can be seen in Table 3 that introducing UAG stop codons at positions 23, 24, and 25 altered basal-level expression. In addition, introducing stop codons at positions 23 and 24 eliminated induction. To determine if this lack of induction was due to a shortened TnaC peptide, a tryptophan-inserting UAG suppressor was introduced into strains VK2800 and VK2900 to restore translation through codon 24. Suppression of UAG$_{23}$ was observed to restore a low level of induction in comparison to what was found for controls SVS1144 and VK800, whereas suppression of UAG$_{24}$ did not (Table 3). These results suggest that amino acids at positions 23 and 24 of TnaC play a role in setting the basal and induced expression of the *tna* operon. In a previous report, suppression of the UAG stop codon in strain VK800 was shown to increase the basal expression of *tna* but not to affect induced expression (22). These findings do not distinguish whether a correct *tnaC* RNA sequence or TnaC peptide sequence or both are necessary for induction.

Examination of other changes in the *boxA*-like region of *tnaC*, some of which do not change the TnaC amino acid sequence. The finding that some mutational changes in the *boxA* region result in constitutive expression of the operon could indicate that the *boxA* nucleotide sequence must be properly recognized by some factor to obtain efficient Rho-dependent termination. To distinguish between effects of mutations at the DNA or RNA level and those at the protein level, changes that did not change the amino acid specified were introduced at *tnaC* codon 23. Replacing the CGC Arg codon by another Arg codon, either CGG, AGA, or AGG, resulted in a three- to sixfold increase in basal-level expression in comparison to that of the wild-type control (Table 4). These three changes also allow induction, but the basal and induced levels of expression differ from those of the parental control strain, SVS1144. Two additional changes to other Arg codons,

TABLE 4. Some alterations in the nucleotide sequence of *boxA* that do not alter the TnaC amino acid sequence result in constitutive expression of the *tna* operon[a]

Strain	*tnaC* codons	β-Gal activity (Miller units)		Ratio[b]
		−Trp	+Trp	
SVS1144 (wild type)	AUG.....CGC$_{23}$CCU$_{24}$UGA...	520 ± 25	14,830 ± 1,495	29
VK2400	CGA$_{23}$	360 ± 24	5,000 ± 995	14
VK2500	CGU$_{23}$	250 ± 19	4,260 ± 720	17
PDG1178	CGG$_{23}$	1,730 ± 85	17,860 ± 557	10
VK3000	AGA$_{23}$	3,335 ± 260	10,080 ± 1,533	3
VK3100	AGG$_{23}$	1,560 ± 231	8,520 ± 879	5
VK2600	CCA$_{24}$	710 ± 66	5,670 ± 979	8
VK2700	CCG$_{24}$	570 ± 112	5,970 ± 1,035	10
PDG1172	ACU$_{24}$(Thr)	150 ± 19	1,320 ± 245	9
PDG1173	UCU$_{24}$(Ser)	265 ± 25	1,250 ± 214	5

[a] Cultures were grown as for Table 2. More than five cultures of each type were assayed in each case. CGA, CGU, CGG, AGA, and AGG are all Arg codons. CCA and CCG are Pro codons. The nucleotide changes introduced are underlined.
[b] Ratio of activities for cultures grown with Trp and without Trp.

TABLE 5. Combining a deletion of the presumed *rut* site with a point mutation in the *boxA*-like region results in near-maximum expression of the *tna* operon; *tnaC* translation is required for constitutive expression[a]

Strain	*tnaC* codon and spacer region nt		β-Gal activity (Miller units)		Ratio[b]
			−Trp	+Trp	
		(nt101–123)			
SVS1144 (wild type)	$AUG_1...CGC_{23}CCU_{24}UGAU(UU..CC...CC...C..CCA)...$		410 ± 32	12,890 ± 1,268	31
VK1800		Δ(nt 101–123)	3,450 ± 312	4,420 ± 698	
VK1800 + bicyclomycin			3,120 ± 630	3,880 ± 330	
VK2000	$\underline{A}GC_{23}$	Δ(nt 101–123)	8,970 ± 766	9,640 ± 1,027	
VK1900	$\underline{UA}G_1$	Δ(nt 101–123)	480 ± 57	480 ± 72	
VK1900 + bicyclomycin			2,360 ± 65	2,320 ± 44	
VK2100	$\underline{UA}G_1...\underline{A}GC_{23}$	Δ(nt 101–123)	2,365 ± 154	2,320 ± 243	
VK2100 + bicyclomycin			3,075 ± 200	3,100 ± 560	

[a] Cultures were grown as for Table 2. More than five cultures of each type were assayed in each case. The nucleotide changes introduced are underlined.
[b] Ratio of activities for cultures grown with Trp and without Trp.

CGA and CGT, reduced the basal level and induced levels two- to threefold. Replacing the CCT Pro codon by two other Pro codons, CCA and CCG, had no significant effect on the basal level but reduced induction about threefold. On the other hand, replacing the CCT Pro codon by ACT (Thr) or TCT (Ser) reduced basal expression by at least 2-fold and allowed 10-fold induction (Table 4). These findings establish that changes in the amino acid sequence of the TnaC peptide are not solely responsible for the altered operon expression observed in the various *tnaC* mutants; changes in the nucleotide sequence of the *boxA* region can also affect both basal-level expression and induced expression.

Deletion of the presumed *rut* site combined with a point mutation in the *boxA* region of *tnaC* results in near-maximal expression of the *tna* operon. It was shown previously, and is confirmed in Table 5, that deletion of 22 nt (nt 101 through 123) from the *tnaC-tnaA* spacer region (VK1800), a presumed *rut* site (Fig. 1), results in an approximately eightfold increase in basal-level expression of the *tna* operon (13). Tryptophan induction of this construct increased expression only slightly (Table 5). Combining the *rut* deletion with the *boxA* mutation at codon 23 in construct VK2000 increased basal-level expression an additional twofold; addition of an inducer had little effect. Combining the *rut* deletion with a mutation replacing the *tnaC* start codon by a stop codon (construct VK1900) reduced basal expression appreciably, and there was no response to an inducer. The addition of bicyclomycin to the strain with this construct elevated expression four- to fivefold, implicating Rho factor in mediating the low level of expression seen in the absence of translation of *tnaC*. Replacing the *tnaC* start codon by a stop codon and combining this change with both the *boxA* change and the *rut* deletion (construct VK2100) allowed moderate expression but no induction. Apparently when both the *boxA* sequence and the *rut* site are altered, the absence of translation of *tnaC* also reduces operon expression, but not to the extent that it does when either the unaltered *boxA* or *rut* site is present. Addition of bicyclomycin to VK2100 increased expression only slightly.

Expression of the *tna* operon does not correlate with changes in the stability of the *tnaC* secondary structure. Nucleotides at positions 52 through 99 of the *tna* leader transcript are predicted to fold and form a relatively stable hairpin structure ($\Delta G = -9.6$ kcal/mol) (40) (Fig. 2 and Table 6). Some of the mutational changes we have examined could exert their effect by altering the stability of this hairpin structure. To explore this possibility, the stabilities of leader mRNA secondary structures for the mRNA segment comprising nt 52 to 99 were predicted using the Zuker MFOLD program (43, 50). In Table 6, it can be seen that many of the mutations that result in noninducibility decrease the stability of this *tnaC* mRNA secondary structure. However, decreased stability also is predicted for transcripts of some mutants that constitutively express the *tna* operon (Table 6). In addition, some changes in the latter group of mutants resulted in a significant increase in the stability of the mRNA secondary structure (Table 6). In the class of mutations that have little or no effect on *tna* expression, there is no significant change in the predicted stability of the leader mRNA structure (Table 6). Considering all of the pre-

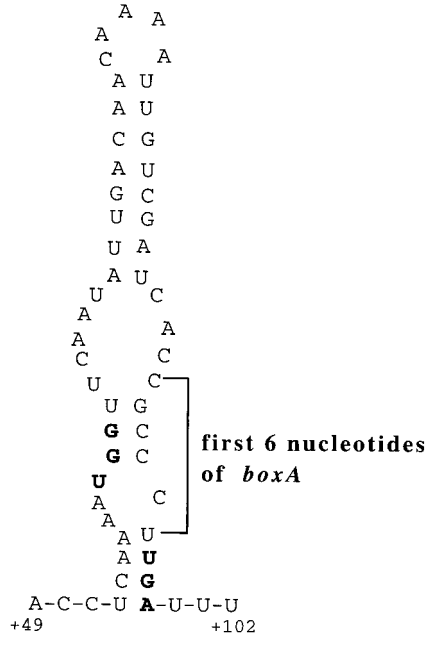

FIG. 2. Predicted RNA secondary structure present at the end of the *tnaC* transcript. The RNA secondary structure was predicted using the MFOLD program of Zuker et al. (50, 51). The free energy of formation of the wild-type structure is −9.6 kcal/mol. Trp codon UGG and the stop codon UGA are in boldface. The UGA stop codon is part of the 9-nt sequence of *boxA* (CGC CCU UGA) in the *tna* operon of *E. coli*.

TABLE 6. Changes in the stability of the *tnaC* secondary structure do not correlate with expression of the *tna* operon

Mutational change	*tna* operon expression	Change in *tnaC* secondary structure stability[a] (kcal/mol)
UUC_{12} (W→F)	Noninducible	+2.9
UGA_{12} (W→stop)	Noninducible	+4.2
UAG_{12} (W→stop)	Noninducible	+3.6
AUG_{12} (W→M)	Noninducible	+3.6
UGC_{13} (F→C)	Noninducible	+3.1
UUU_{15} (I→F)	Noninducible	+0.6
AAU_{15} (I→N)	Noninducible	+2.8
$\Delta(CGC_{23}CCU_{24})$	Constitutive	+1.7
AGC_{23} (R→S)	Constitutive	−0.8
CGG_{23} (R→R)	Constitutive	+0.8
AGA_{23} (R→R)	Constitutive	−1.3
AGG_{23} (R→R)	Constitutive	−0.8
UGG_{13} (F→W)	Constitutive	−6
AUA_{15} (I→I)	Constitutive	+2.6
AUC_{15} (I→I)	Constitutive	−2.1
AUG_{15} (I→M)	Constitutive	+1.7
CAC_{16} (D→H)	Constitutive	+5.5
UUC_{16} (D→F)	Constitutive	+5.7
CGA_{23} (R→R)	Regulated	−1.3
CGU_{23} (R→R)	Regulated	−1.6
CCA_{24} (P→P)	Regulated	+0.1
CCG_{24} (P→P)	Regulated	+0.1
UUU_{13} (F→F)	Regulated	0
GAU_{16} (D→D)	Regulated	+1.9
AAC_{14} (N→N)	Regulated	0
ACU_{24} (P→T)	Regulated	+0.3
UCU_{24} (P→S)	Regulated	+0.3

[a] A positive change indicates reduced stability of the secondary structure. The free energy of formation of the wild-type structure is −9.6 kcal/mol. The results in this table come from this study, from references 11 to 13, 41, and 42, and from unpublished data of M. Eshoo and C. Yanofsky.

dicted changes in this leader mRNA structure, it seems unlikely that changes in its stability are primarily responsible for the phenotypes of the various mutants.

DISCUSSION

In the present report, we extend our analysis of the role of the *boxA*-like sequence and the *rut* site in mediating transcription termination in the *tna* operon leader region. We show that deleting 6 nt of the *boxA* sequence or substituting a Ser for Arg codon 23 results in elevated constitutive (at least threefold increase in the basal-level) expression of the *tna* operon (Table 2). In further analyses with these constitutive *boxA* mutant constructs, we observed that eliminating translation of all or part of the *tnaC* coding sequence reversed the elevated expression of the *tna* operon associated with the *boxA* change and reduced expression well below that of the wild-type parental construct (Table 2). Addition of bicyclomycin, an inhibitor of Rho activity (48, 52), increased expression from these constructs, confirming that the reduced basal expression observed in the absence of translation is due to Rho action (Table 2). Interestingly, bicyclomycin addition had only a slight stimulatory effect on the expression of *boxA* deletion or missense mutant constructs which exhibit moderately high basal-level expression, whereas it had a significant effect on the wild-type construct and on constructs with very low basal levels of expression (Tables 2 and 5). In Table 3, introduction of a stop codon at position 23 or 24 of *tnaC* drastically changed the *boxA* sequence. These changes resulted in alterations in the basal level and loss of tryptophan induction. In addition, suppression of UAG_{23} slightly increased (fourfold) induced expression whereas suppression of UAG_{24} had no effect on basal or induced expression. These results suggest that the nature of the amino acids at positions 23 and 24 of *tnaC* and perhaps the nucleotide sequence in *boxA* contribute to the basal level of *tna* expression observed in wild-type cultures.

We examined the specific role of the *boxA* RNA sequence by introducing mutational changes in Arg codon 23, some of which did not alter the amino acid specified at this position. Three such mutations, which introduced the Arg codons CGG, AGA, and AGG at codon position 23 of *tnaC*, resulted in elevated basal-level expression of the *tna* operon (Table 4). This result establishes that the RNA sequence itself, specifically the sequence of the *boxA*-like region of *tnaC*, plays a role in determining the extent of Rho-mediated termination in the *tna* operon leader region. The *boxA* sequence at the end of the *tnaC* leader region does not behave like a typical *boxA* sequence. Indeed, the *boxA* sequence in the *tna* operon overlaps the *tnaC* stop codon, UGA. Furthermore, the *boxA* sequences of phage λ (9, 10) and rRNA operons (2, 23) are required for transcription antitermination, not termination.

A sequence resembling a Rho utilization (*rut*) site is located immediately downstream of the *boxA* sequence (41). We confirm the report that deleting this *rut* site elevates expression of the *tna* operon in the absence of inducer (Table 5) (13). Interestingly, the *rut* site responsible for Rho-dependent transcription termination at the λtR1 terminator contains *boxA* in one of its two discrete *rutA* sites (5). We analyzed the relative contribution of *boxA* and the *rut* site in Rho-dependent termination with a construct containing the *rut* deletion combined with a substitution of Ser codon AGC for Arg codon CGC in *boxA*. The combined mutations resulted in near-maximal expression of the *tna* operon with or without tryptophan; thus their effects are additive (Table 5). These findings suggest that, in the absence of tryptophan, both *boxA* and the *rut* site contribute to efficient Rho-dependent transcription termination in the *tna* operon. Rho may bind directly to *boxA* and the *rut* site, or the binding of Rho at the *rut* site may be enhanced by interactions with a cellular factor(s) bound at *boxA*. Interaction of *boxA* with NusA, NusB, or NusE factor alone appears to have been ruled out. Indeed, previous studies with a *nusA1* (41) mutant strain have shown no defect in *tna* operon regulation. In addition, *nusA1*, *nusB100*, and *nusE100* mutant strains (33, 46) were examined for *tna* operon expression by measuring tryptophanase (7) levels in cultures grown with or without inducing levels of tryptophan; enzyme levels in these mutants were indistinguishable from that of the wild-type control (data not shown). NusA, NusB, and NusE could bind to *boxA* as a complex; in this case, any particular mutation in any one of these factors might have a negligible effect on tryptophanase regulation. In any event, since protein factors do bind at lambda's *boxA*, it is likely that they could influence Rho's activity through interaction. This possibility is under continuing examination.

A summary of the nucleotide and amino acid changes that have been introduced to date in *tnaC* and its peptide product is presented in Fig. 3. In the first group of mutations (Fig. 3A), substitution of a stop codon for a sense codon at codon position 1, 11, 12, 18, 23, or 24 prevents tryptophan induction, consistent with the importance of synthesis of the TnaC peptide in relieving Rho-dependent transcription termination (Fig. 3A). Tryptophan inducibility also is lost when some *tnaC* codons conserved between *E. coli*, *P. vulgaris*, and *Enterobacter aerogenes* (12) are replaced by codons specifying different amino acids (Fig. 3A). Most importantly, replacing Trp12 by a codon specifying a different amino acid eliminates induction.

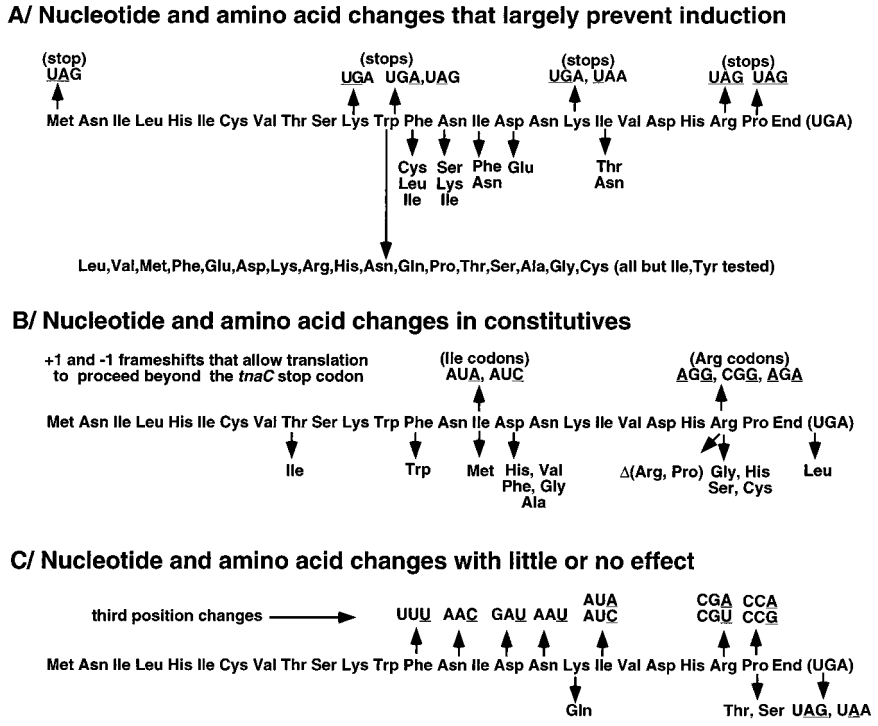

FIG. 3. Nucleotide and amino acid changes in *tnaC* and the TnaC peptide that do or do not affect *tna* operon expression. Nucleotide changes that do not lead to amino acid changes are underlined and are above the TnaC leader peptide sequence. The results come from this study, from references 11 to 13, 41, and 42, and from unpublished data of M. Eshoo and C. Yanofsky.

However, when a stop codon at position 12 is suppressed by a tRNA that inserts tryptophan, induction is restored (12, 13).

The second group of mutations results in partial constitutive (at least a threefold increase in basal-level) expression of the operon; many of these mutations remain inducible in the presence of tryptophan (Fig. 3B). Some of these changes alter the TnaC amino acid sequence, while others, such as some of the Arg codon replacements described in this report, do not. This class also includes frameshift mutations and a mutation (replacement of UAA with UGA) that allows translation to proceed beyond the natural *tnaC* stop codon. Interestingly, some mutations that change residues in the conserved amino acid sequence near Trp residue 12 also result in constitutive expression (12). These findings establish that the nucleotide sequence of the *tnaC* coding region must play a role in establishing the low Rho-dependent basal-level expression of the operon. Nucleotide changes that alter amino acid coding specificity could act either at the nucleotide sequence level or by altering the amino acid sequence of the TnaC peptide.

The third group of mutations (Fig. 3C) introduces changes in *tnaC* or its product that have little or no effect on basal or induced expression of the *tna* operon (Fig. 3C). Some of these changes (e.g., in the Arg23 or Pro24 codon) appear to increase Rho-dependent termination, but tryptophan-mediated induction is retained (Table 4). Other changes (e.g., replacement of UAG or UAA with UGA) are known to increase the efficiency of ribosome release at the *tnaC* stop codon.

An analysis of the mutational changes made in this and other studies (12, 13, 21, 41, 42) shows a lack of correlation between the stability of the *tnaC* mRNA secondary structure and expression of the *tna* operon. These observations suggest that changes in the stability of the *tnaC* leader mRNA cannot alone account for the phenotypes of the respective *tna* mutants.

In conclusion, it is apparent that both the TnaC peptide and the sequence of its encoding transcript play a role in Rho-dependent transcription termination and tryptophan-induced antitermination in the *tna* operon of *E. coli*. The studies described define some of the features of the *boxA* sequence and the *rut* site in Rho-dependent transcription termination and their relationship to translation of the leader peptide coding region.

ACKNOWLEDGMENTS

We thank the Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan, for providing samples of bicyclomycin. We are indebted to Virginia Horn for assistance with *E. coli* strain construction. We are also grateful to Kurt Gish, Ajith Kamath, and Peter Margolis for their helpful comments.

These studies were supported by grant GM09738 to C.Y. from the United States Public Health Service. K.V.K. was a Postdoctoral Fellow.

REFERENCES

1. **Bektesh, S. L., and J. P. Richardson.** 1980. A rho-recognition site on phage lambda cro gene mRNA. Nature **283:**102–104.
2. **Berg, K. L., C. Squires, and C. L. Squires.** 1989. Ribosomal RNA operon anti-termination. Function of leader and spacer region box B-box A sequences and their conservation in diverse microorganisms. J. Mol. Biol. **209:**345–358.
3. **Bilezikian, J., R. Kaempfer, and B. Magasanik.** 1967. Mechanism of tryptophanase induction in *Escherichia coli*. J. Mol. Biol. **27:**495–506.
4. **Botsford, J. L., and R. D. DeMoss.** 1971. Catabolite repression of tryptophanase in *Escherichia coli*. J. Bacteriol. **105:**303–312.
5. **Chen, C. Y., and J. P. Richardson.** 1987. Sequence elements essential for rho-dependent transcription termination at lambda tR1. J. Biol. Chem. **262:** 11292–11299.
6. **Craigen, W. J., and C. T. Caskey.** 1986. Expression of peptide chain release factor 2 requires high-efficiency frameshift. Nature **322:**273–275.
7. **Deeley, M. C., and C. Yanofsky.** 1981. Nucleotide sequence of the structural

gene for tryptophanase of *Escherichia coli* K-12. J. Bacteriol. **147:**787–796.
8. **Edwards, R. M., and M. D. Yudkin.** 1982. Location of the gene for the low-affinity tryptophan-specific permease of *Escherichia coli*. Biochem. J. **204:**617–619.
9. **Friedman, D. I., and E. R. Olson.** 1983. Evidence that a nucleotide sequence, "boxA," is involved in the action of the NusA protein. Cell **34:**143–149.
10. **Friedman, D. I., E. R. Olson, L. L. Johnson, D. Alessi, and M. G. Craven.** 1990. Transcription-dependent competition for a host factor: the function and optimal sequence of the phage λ*boxA* transcription antitermination signal. Genes Dev. **4:**2210–2222.
11. **Gish, K., and C. Yanofsky.** 1993. Inhibition of expression of the tryptophanase operon in *Escherichia coli* by extrachromosomal copies of the *tna* leader region. J. Bacteriol. **175:**3380–3387.
12. **Gish, K., and C. Yanofsky.** 1995. Evidence suggesting *cis* action by the *tnaC* leader peptide in regulating transcription attenuation in the tryptophanase operon of *Escherichia coli*. J. Bacteriol. **177:**7245–7254.
13. **Gollnick, P., and C. Yanofsky.** 1990. $tRNA^{TRP}$ translation of leader peptide codon 12 and other factors that regulate expression of the tryptophanase operon. J. Bacteriol. **172:**3100–3107.
14. **Grentzmann, G., D. Brechemier-Baey, V. Heurgue, L. Mora, and R. Buckingham.** 1994. Localization and characterization of the gene encoding release factor RF3 in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:**5848–5852.
15. **Hirahara, T., S. Suzuki, S. Horinouchi, and T. Beppu.** 1992. Cloning, nucleotide sequences, and overexpression in *Escherichia coli* of tandem copies of a tryptophanase gene in an obligately symbiotic thermophile, *Symbiobacterium thermopilum*. Appl. Environ. Microbiol. **58:**2633–2642.
16. **Hopkins, F. G., and S. W. Cole.** 1903. A contribution to the chemistry of proteid. Part II. The constitution of tryptophanase, and the action of bacteria upon it. J. Physiol. (London) **29:**451–466.
17. **Kamath, A. V., and C. Yanofsky.** 1992. Characterization of the tryptophanase operon of *Proteus vulgaris*. J. Biol. Chem. **267:**19978–19985.
18. **Kamath, A. V., and C. Yanofsky.** 1997. Roles of the *tnaC-tnaA* spacer region and Rho factor in regulating expression of the tryptophanase operon of *Proteus vulgaris*. J. Bacteriol. **179:**1780–1786.
19. **Kawasaki, K., A. Yokota, S. Oita, C. Kobayashi, S. Yoshikawa, S. Kawamoto, S. Takao, and F. Tomita.** 1993. Cloning and characterization of a tryptophanase gene from *Enterobacter aerogenes* SM-18. J. Gen. Microbiol. **139:**3275–3281.
20. **Kazarinoff, M. N., and E. E. Snell.** 1977. Essential arginine residues in tryptophanase from *Escherichia coli*. J. Biol. Chem. **252:**7598–7602.
21. **Konan, K. V., and C. Yanofsky.** 1997. Regulation of the *Escherichia coli tna* operon: nascent leader peptide control at the *tnaC* stop codon. J. Bacteriol. **179:**1774–1779.
22. **Konan, K. V., and C. Yanofsky.** 1999. Role of ribosome release in regulation of *tna* operon expression in *Escherichia coli*. J. Bacteriol. **181:**1530–1536.
23. **Li, S. C., C. L. Squires, and C. Squires.** 1984. Antitermination of E. coli rRNA transcription is caused by a control region segment containing lambda nut-like sequences. Cell **38:**851–860.
24. **Martin, K., G. Morlin, A. Smith, A. Nordike, A. Eisenstark, and M. Golomb.** 1998. The tryptophanase gene cluster of *Haemophilus influenzae* type b: evidence for horizontal gene transfer. J. Bacteriol. **180:**107–118.
25. **Mason, S. W., and J. Greenblatt.** 1991. Assembly of transcription elongation complexes containing the N protein of phage lambda and the Escherichia coli elongation factors NusA, NusB, NusG, and S10. Genes Dev. **5:**1504–1512.
26. **Mason, S. W., J. Li, and J. Greenblatt.** 1992. Direct interaction between two Escherichia coli transcription antitermination factors, NusB and ribosomal protein S10. J. Mol. Biol. **223:**55–66.
27. **Mikuni, O., K. Ito, J. Moffat, K. Matsumura, K. McCaughan, T. Nobukuni, W. Tate, and Y. Nakamura.** 1994. Identification of the *prfC* gene, which encodes peptide-chain-release factor 3 of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:**5798–5802.
28. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Newton, W. A., and E. E. Snell.** 1964. Catalytic properties of tryptophanase, a multifunctional pyridoxal phosphate enzyme. Proc. Natl. Acad. Sci. USA **51:**382–389.
30. **Nodwell, J. R., and J. Greenblatt.** 1993. Recognition of boxA antiterminator RNA by the E. coli antitermination factors NusB and ribosomal protein S10. Cell **72:**261–268.
31. **Raftery, L. A., J. B. Egan, S. W. Cline, and M. Yarus.** 1984. Defined set of cloned termination suppressors: in vivo activity of isogenic UAG, UAA, and UGA suppressor tRNAs. J. Bacteriol. **158:**849–859.
32. **Richardson, L. V., and J. P. Richardson.** 1996. Rho-dependent termination of transcription is governed primarily by the upstream rho utilization (rut) sequences of a terminator. J. Biol. Chem. **271:**21597–21603.
33. **Robledo, R., B. L. Atkinson, and M. E. Gottesman.** 1991. *Escherichia coli* mutations that block transcription termination by phage HK022 Nun protein. J. Mol. Biol. **220:**613–619.
34. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
35. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
36. **Sarkar, G., and S. S. Sommer.** 1990. The "megaprimer" method of site-directed mutagenesis. BioTechniques **8:**404–407.
37. **Sharrock, R. A., R. L. Gourse, and M. Nomura.** 1985. Defective antitermination of rRNA transcription and derepression of rRNA and tRNA synthesis in the nusB5 mutant of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82:**5275–5279.
38. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53:**85–96.
39. **Snell, E. E.** 1975. Tryptophanase: structure, catalytic activities, and mechanism of action. Adv. Enzymol. **42:**389–446.
40. **Stewart, V., R. Landick, and C. Yanofsky.** 1986. Rho-dependent transcription termination in the tryptophanase operon leader region of *Escherichia coli* K-12. J. Bacteriol. **166:**217–223.
41. **Stewart, V., and C. Yanofsky.** 1985. Evidence for transcription antitermination control of tryptophanase operon expression in *Escherichia coli* K-12. J. Bacteriol. **164:**731–740.
42. **Stewart, V., and C. Yanofsky.** 1986. Role of leader peptide synthesis in tryptophanase operon expression in *Escherichia coli* K-12. J. Bacteriol. **167:**383–386.
43. **Stiegler, P., P. Carbon, M. Zuker, J. P. Ebel, and C. Ehresmann.** 1981. Structural organization of the 16S ribosomal RNA from E. coli. Topography and secondary structure. Nucleic Acids Res. **9:**2153–2172.
44. **Uno, M., K. Ito, and Y. Nakamura.** 1996. Functional specificity of amino acid at position 246 in the tRNA mimicry domain of bacterial release factor 2. Biochimie **78:**935–943.
45. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218:**97–106.
46. **Ward, D. F., A. DeLong, and M. E. Gottesman.** 1983. *Escherichia coli nusB* mutations that suppress nusA1 exhibit lambda N specificity. J. Mol. Biol. **168:**73–85.
47. **Watanabe, T., and E. E. Snell.** 1972. Reversibility of the tryptophanase reaction: synthesis of tryptophan from indole, pyruvate and ammonia. Proc. Natl. Acad. Sci. USA **69:**1086–1090.
48. **Yanofsky, C., and V. Horn.** 1995. Bicyclomycin sensitivity and resistance affect Rho factor-mediated transcription termination the *tna* operon of *Escherichia coli*. J. Bacteriol. **177:**4451–4456.
49. **Yanofsky, C., V. Horn, and Y. Nakamura.** 1996. Loss or overproduction of polypeptide release factor 3 influences expression of the tryptophanase operon of *Escherichia coli*. J. Bacteriol. **178:**3755–3762.
50. **Zuker, M.** 1989. Computer prediction of RNA structure. Methods Enzymol. **180:**262–288.
51. **Zuker, M., J. A. Jaeger, and D. H. Turner.** 1991. A comparison of optimal and suboptimal RNA secondary structures predicted by free energy minimization with structures determined by phylogenetic comparison. Nucleic Acids Res. **19:**2707–2714.
52. **Zwiefka, A., H. Kohn, and W. R. Widger.** 1993. Transcription termination factor rho: the site of bicyclomycin inhibition in Escherichia coli. Biochemistry **32:**3564–3570.