Transcription Termination at the thr Attenuator

EVIDENCE THAT THE ADENINE RESIDUES UPSTREAM OF THE STEM AND LOOP STRUCTURE ARE NOT REQUIRED FOR TERMINATION*

(Ming-Te Yang, Harlan B. Scott III, and Jeffrey F. Gardner)

From the Department of Microbiology, University of Illinois, Urbana, Illinois 61801

The Escherichia coli thr operon attenuator has a structure similar to other Rho-independent terminators. The DNA sequence immediately upstream of the termina-
sion site is dG+dC-rich and contains a region of dyad symmetry that, when transcribed into RNA, encodes a hairpin structure in the transcript. It also contains a stretch of 9 consecutive dA-dT residues immediately distal to the region of dyad symmetry which encode uridine residues at the 3' end of the terminated transcript. In addition, the thr attenuator has a stretch of 6 dA-dT residues immediately upstream of the region of dyad symmetry which encode 6 adenines. These adenines could potentially pair with the distal uridines to form a hairpin structure extended by as much as 6 A-U base pairs. In this report we have examined the role of the upstream adenines in transcription termination. We used templates that specify mismatches or create new base pairs in the potential A-U secondary structure of the transcript as well as templates that delete segments of the A residues upstream of the hairpin. We conclude that A-U pairing is not required for efficient transcription termination at the thr attenuator. This conclusion is likely to apply to other Rho-independent terminators that contain hairpin-proximal dA-dT residues.

The control of gene expression in bacteria often occurs at the level of transcription termination. Transcription terminators are found upstream of operons, between genes in an operon, and at the ends of operons (for reviews, see Refs. 1–3). In general, transcription terminators have been divided into two classes: Rho-dependent or Rho-independent, depending upon their requirement for Rho factor in vitro (2). Rho-independent terminators terminate transcription in vitro in the absence of Rho protein or other factors, and have two common structural characteristics (2). The first is a dG+dC-rich region of dyad symmetry that encodes a stem-loop or hairpin structure in the nascent mRNA. The second feature is a dA+dT-rich region of 4–9 base pairs immediately distal to the region of dyad symmetry within which the transcript terminates. Thus, when RNA polymerase transcribes through a Rho-independent terminator, the transcript forms a hairpin followed by 3' uridine residues. Evidence from several laboratories has shown that both of these features are required for efficient termination (2, 3).

The thr attenuator is a Rho-independent terminator that has a dG+dC-rich region of dyad symmetry that encodes a hairpin containing a stem of 8 base pairs and a loop of 6 bases. The hairpin is followed by a tract of 9 consecutive dA-dT residues and termination predominately occurs at the 7th or 8th uridine in the transcript (4). The importance of the uridine residues was established by Lynn et al. (5), who made deletions that varied the length of the dA-dT tract. They found that the deletion of 1 or 3 dA-dT residues had no effect on transcription termination in vivo or in vitro. The deletion of 4, 5, or 6 dA-dT residues showed a linear decrease in termination efficiency. When 7 or 8 dA-dT residues were deleted, termination was abolished.

The thr attenuator also has a dA-dT tract of 6 bases immediately upstream of the region of dyad symmetry (4). The dA-dT tract encodes 6 A residues in the transcript that could potentially pair with 6 of the hairpin-distal U residues to extend the length and stability of the RNA hairpin. Approximately one-third to one-half of the Rho-independent terminators characterized to date also contain hairpin-proximal dA-dT residues as part of an AANAA sequence (6), which suggests that pairing of A and U residues could play a functional role in transcription termination. In this report, we describe a systematic analysis of the role of the hairpin-proximal A residues in transcription termination at the thr attenuator.

MATERIALS AND METHODS

In Vitro Mutagenesis—The procedure described by Kunkel (7, 8) was used for preparation of uracil-containing single-stranded M13mp10-thr DNA templates (9). DNA was isolated from the phage particles by phenol extraction and ethanol precipitation. The "gapped duplex" template formation and primer extension procedure was carried out by the method of Bauer et al. (10). The uracil-containing single-stranded M13mp10-thr derivatives and HisIl-digested M13mp10† RF DNA were mixed in 30 μl of hybridization buffer (40 mM Tris-HCl (pH 7.5), 100 mM NaCl, 20 mM MgCl2, 2 mM β-mercaptoethanol) and boiled for 3 min. The mixture was allowed to cool to 85 °C before addition of 40 pmol of phosphorylated mutagenic primer. The hybridization mixture was slowly cooled to room temperature. Next, 70 μl of primer extension buffer (20 mM Tris-HCl (pH 7.5), 11 mM MgCl2, 1 mM β-mercaptoethanol, 0.83 mM each 2-deoxyribonucleoside triphosphate and 0.4 mM ATP), which contained 0.1 unit of T4 DNA ligase and 0.5 unit of DNA polymerase (Klenow fragment), was added. The reaction mixture was first incubated on ice for 15 min and shifted to room temperature for 5 min. The reaction mixture was then incubated at 30 °C for another 2 h and the reaction stopped by adding 3 μl of 0.5 M EDTA. The mixture was used to transform competent JM105 cells (10) and white plaques on LB plates (tryptone (10 g/liter), yeast extract (5 g/liter), and NaCl (10 g/liter)) containing 40 μg/ml 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal) were screened. Constructs containing the desired mutation were identified by direct DNA sequence analyses.

Construction of thrA-lacZ Protein Fusion Vectors—The construction of the thrA-lacZ protein fusions is shown in Fig. 1A. Restriction fragments containing the Escherichia coli thr operon regulatory region were generated by digesting the appropriate M13mp10-thr derivatives with

* This work was supported by Grant DMB-87-18311 from the National Science Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address: Howard Payne University, Brownwood, TX 76801.

‡ To whom correspondence should be addressed: Dept. of Microbiology, University of Illinois, 407 S. Goodwin Ave., 131 Burrill Hall, Urbana, IL 61801. Tel.: 217-333-7287; Fax: 217-244-6697.
The formation of the 1:2 hairpin structure of the chloro-3-indolyl-
regulatory region and the Hin
electrophoresis on 1% agarose gels. The desired 557-bp DNA frag-
ments containing the whole regulatory region, part of the thrA gene and vector sequences were electroeluted from the agarose gel. These blunt-ended DNA fragments were ligated into the unique
HindIII and EcoRI endonucleases. The HindIII site is upstream of the thr regulatory region and the EcoRI site is in the M13 vector. The 5'-protruding ends of the DNA fragments were removed by treating the DNA with mung bean nuclease. The reaction products were subjected to electrophoresis on 1% agarose gels. The desired 557-bp DNA fragments containing the whole regulatory region were identified by their resistance to ampicillin and their blue color on plates containing X-gal. Construction of thrA-lacZ Fusions with Deletions of Region 1 and 2. The 108-bp BstEII-HpaII fragment from M13mp9-AT45GG containing the sequence from -60 to +107 of the thr leader region was isolated and purified from a 5% polyacrylamide gel. 

1 The abbreviation used are bp, base pair(s); X-gal, 5-bromo-4-
chloro-3-indolyl-β-D-galactopyranoside.

2 H. Scott, unpublished results.

The DNA sequence from +44 to +110 of the thr leader region was replaced by two synthetic oligodeoxyribonucleotides, one 21-mer 5'-CGCAGAGCAAAGCTAGTCTGA-3' and the other 23-mer 5'--CGCATATAACGCTGTTTCTACG-3'. These two oligomers were annealed at 80°C for 1 min and slowly cooled to room temperature. The hybridized oligomers contained HpaII and MluI half-sites at the ends. The HpaII sites of the BstEII-HpaII fragment of the M13mp9-AT45GG and the annealed deoxyribonucleotides were ligated. The resulting 129-bp fragment was purified and ligated with M13mp11-WT thr vector that had been digested with BstEII and MluI endonucleases. The resultant recombinant clone that contained 2 extra base pairs at the ligation junction and deleted the sequences necessary for the formation of the 1:2-hairpin structure of the thr leader region was isolated and named M13mp11-WT thr Δ1:2. The HindIII-EcoRI fragment of M13mp11-WT thr Δ1:2 was then subcloned into the pMC1403 vector to construct pMC1403-WT thr Δ1:2 by following the procedure described above.

Plasmids pMC1403 and pMC1396 are analogous to each other, except that pMC1403 contains a unique EcoRI site (11). To construct the pMC1403 attenuator mutants derivatives with the same deletion in the 1:2 region, the MluI-BamHI fragments from the corresponding M13mp9-AT45GG derivatives (Fig. 1A) were isolated and ligated with the EcoRI-MluI fragment of pMC1403-WT thr Δ1:2. The ligation products were then subcloned between the EcoRI and BamHI sites of the pMC1403 vector (see Fig. 1C). These constructions produced plasmids that are isogenic with pMC1403-WT thr Δ1:2.

Construction of Plasmids Containing Deletions of the dA-dT Tracts—M13mp8 derivatives (5) carrying a nested set of deletions with 1, 3, 5, or 8 dT residues distal to the region of dyad symmetry of the thr attenuator were used as sources of DNA for the constructs. The terminators were isolated as 42-49-base pair RsaI-XbaI fragments.

FIG. 1. Construction of the thrA-lacZ protein fusion plasmids. A, the scheme for the construction of pMC1396 derivatives that contain an in-frame fusion of the thrA gene to the 5'-end of the lacZ coding sequence of the plasmid pMC1396 is shown. The EcoRI (*) site is only present in pMC1396. B and C, the schemes for the construction of thrA-lacZ protein fusion vectors which are deleted for the sequences involved in the formation of the 1:2 hairpin structure of the thr leader region are shown. See "Materials and Methods" for details of the constructions.
Transcription Termination at the thr Attenuator

Fig. 2. Construction of templates containing deletions of the da-dT tract upstream and downstream of the hairpin. A, construction of templates containing deletions of the hairpin-distal da-dT tract. Rsal-Xbal fragments containing varying numbers of da-dT base pairs in the underlined region of the sequence were cloned into the HindIII-Xbal sites of pUC19. The construct shown corresponds to A6-T8(+) (See text and Fig. 5). A6-T6(+), A6-T3(+), and A6-T1(+) contain 6, 3, or 1 da-dT base pairs in the underlined region, respectively. B, construction of templates containing deletions of the da-dT tract upstream of the hairpin. Rsal-Xbal fragments, identical to those above, were inserted into the Xbal-HindIII sites of pUC18. The construct shown corresponds to A8-T6(−) (See text and Fig. 5). A6-T6(−), A3-T6(−), and A1-T6(−) contain 6, 3, or 1 da-dT base pairs in the underlined region, respectively.

Approximately 100 µg of plasmid DNA containing the various da-dT tract deletions (5) were digested with Rsal and Xbal, and the fragments were separated by electrophoresis in a 10% polyacrylamide gel. After staining with ethidium bromide and visualization with ultraviolet light, the appropriate segments of gel containing the bands were excised and resuspended in formamide dyes (80% formamide, 1% TBE buffer, 0.1% xylene cyanol, and 0.1% bromphenol blue). The products of the transcription reactions were analyzed on 8% polyacrylamide, 8 M urea gels. After autoradiography, regions of the gel corresponding to the terminated and read-through transcripts (9) were cut out of the gel and counted in a Bedman LSI801 scintillation counter. The molar ratios of the terminated and read-through transcripts were calculated by correcting for the lengths and base compositions of the transcripts (9).

In Vivo Recombination and Single λ Prophage Selection—In vivo recombination of thrA-lacZ fusions onto ARZ-5 (16) was performed as follows. Strains containing the thrA-lacZ fusion plasmids were grown in LB medium overnight. Cells (0.1 ml) were subcultured into 5 ml of LB medium (tryptone (10 g/liter), yeast extract (5 g/liter), NaCl (10 g/liter), maltose (20 mg/liter), and 10 mM MgSO4) and shaken at 37°C for 2 h. One ml of the cell suspension was infected with 10 µl of ARZ-5 at a multiplicity of infection of 2–5. The cultures were then grown at 37°C until lysis occurred. Chloroform (50 µl) was added, and the cultures were incubated for 15 min. The lysates were collected as the supernatant fraction after centrifugation at 5,000 rpm for 10 min. The phage lysates were diluted 1:100 and 1:1000 into X-gal starter buffer (10 mM Tris-HCl (pH 7.9), 10 mM MgSO4, and 5 mM CaCl2) and 0.1 ml of each dilution was added to an equal volume of overnight MC4100 cells. The mixtures were then spread on T-plates (tryptone (10 g/liter), NaCl (5 g/liter), and Bacto-agar (12.5 g/liter)) containing 25 µg/ml X-gal. The transduced MC4100 strains were identified by their sensitivity to ampicillin and X-gal.

All the lysogens were verified by their sensitivity to λirx and resistance to λi. 

RESULTS AND DISCUSSION

Transcription Termination with Templates Containing Substitution Mutations—Like other Rho-independent terminators, the λ ir enhancer has a dG+dC-rich region of dyad symmetry that encodes an RNA hairpin followed by 9 consecutive da-dT residues that encode 7 or 8 uridine residues at the 3' end of the transcript. In addition, there are da-dT residues that encode adenosines in the transcript immediately preceded by leader sequence. The resultant DNA was sequenced by the dye detection method of Sanger et al. (15) with a modified T7 DNA polymerase, Sequenase™ from U. S. Biochemical Corp. Plasmid DNA from the constructs containing the deletions was sequenced using a Taq Tag Kit from Promega.

In Vitro Transcription—In vitro transcription reactions were performed with purified RNA polymerase essentially as described by Yang and Gardner (9). The final concentrations of the components of the transcription reaction mixtures were the following: 20 mM Tris acetate (pH 7.9), 0.1 mM Na2 EDTA, 0.1 mM dithiothreitol, 150 mM KCl, 150 µM GTP, 150 µM ATP, 150 µM UTP, 50–80 µM CTP, and 10–20 µCi of [α-32P]CTP, 1–2 pmol of DNA template, and 1–2 pmol of E. coli RNA polymerase. In some experiments, GTP was replaced by ITP at a concentration of 150 µM. Templates carrying the da-dT tract deletions contain Plac. Reactions using these templates contained 1.25 µM CAMP and 0.34 mg of CRP protein.

Transcription reactions (50 µl) were terminated after 10–20 min at 37°C by the addition of an equal volume of phenol, and carrier tRNA was added to a final concentration of 0.5 mg/ml. After the phenol extraction, the aqueous phase was adjusted to 0.3 mM sodium acetate and the samples were ethanol-precipitated, desalted, dried in vacuo, and resuspended in formamide dyes (80% formamide, 1% TBE buffer, 0.1% xylene cyanol, and 0.1% bromphenol blue). The products of the transcription reactions were analyzed on 8% polyacrylamide, 8 M urea gels. After autoradiography, regions of the gel corresponding to the terminated and read-through transcripts (9) were cut out of the gel and counted in a Bedman LSI801 scintillation counter. The molar ratios of the terminated and read-through transcripts were calculated by correcting for the lengths and base compositions of the transcripts (9).
ing the region of dyad symmetry. The 3' uridine residues are complementary to the stretch of adenosine residues, and the stability and length of the helix would be enhanced by extending the base pairs from the G+C-rich hairpin to include the 6 A-U base pairs. Such base pairing could be important in promoting transcription termination at terminators that contain this feature by a variety of mechanisms. For example, the increased stability of the hairpin provided by the A-U base pairs could be required for termination or the extended helix could be required as a structural component of the termination signal by interacting with the enzyme. In templates that contain deletions of the upstream DA-dT residues, the stability and length of the hairpin would be reduced resulting in less efficient transcription termination. Alternatively, the formation of the extended helix in the transcript could compete for uridine residues that are paired with the template and disrupt U-dA interactions at the 3' end of the transcript to promote termination and dissociation of the enzyme. Thus, when the length of the A-U helix is less than 6 base pairs, the length of the RNA-DNA duplex would increase and possibly reduce transcription termination.

Several other Rho-independent terminators including the phe (19), leu (20), and ilvB (21) attenuators also contain upstream adenosines as part of a conserved AANAA sequence that can also potentially form base pairs with the uridines. In addition, the bidirectional tonB/P14 terminator and the o1 operon terminator, o1,2, contain stretches of adenosines upstream of the hairpin (22, 23). If A-U base pairing is important for efficient transcription termination at these terminators, a simple prediction is that disruption of the base pairs, by introducing substitutions upstream of the hairpin or in the hairpin-distal region of the template, should reduce the efficiency of transcription termination. In addition, compensating mutations that restore base pairing should direct efficient transcription termination.

We constructed a series of mutant templates that contained single or double mutations in the thr attenuator and measured their transcription termination efficiencies in vitro and in vivo. The mutants specified potential base pair mismatches or new base pairs in the stretch of A and U residues of the transcript (Fig. 3B). Most of these variants, each containing single or double base changes, were made by oligonucleotide-directed mutagenesis. L131G is a substitution mutant with an A to G change in the run of A residues immediately 5' to the attenuator. Mutants L153C, L160A, and L160G disrupt the U tract by substituting C, A, or G for U at positions +158 and +160, respectively. Mutants L153A (24) and L160C3 were isolated by a genetic selection that involved isolating mutants that decrease transcription termination at the thr attenuator in vivo. Variants L131U/L160A and L131G/L160C contain different base substitutions at the same positions: A to U or G at position +131 and U to A or C at position +160, respectively. These two variants disrupt the sequences of both the A and U stretches, but maintain the potential complementarity at the base of the stem. Variant L131A/L160C, which contains two single base changes at positions +153 and +160, has disruptions in both the G+C-rich region and the U tract of the thr attenuator.

The BstEII-SstI restriction fragments bearing the thr operon leader region and different attenuator mutants were isolated from the M13mp10-thr constructs and used for in vitro transcription studies (Fig. 3A). The transcribed RNA was subjected to electrophoresis on 8% polyacrylamide, 8 M urea gels and subjected to autoradiography. The terminated (164 bases) and read-through (300 bases) transcripts were isolated, and the amount of radioactivity in each gel species was measured. The in vitro transcription termination efficiencies of the attenuator variants are presented in Fig. 4 and Table I. The results showed that variant L153A/L160C, which contains C-A mismatches in both the dG+dC-rich region and the U stretch of the attenuator, significantly decreased the termination frequency. Variants with substitutions at position +160 only (L160C, L160G, and L160A) showed termination frequencies that were only slightly lower than wild type. All of the remaining variants bearing base substitution(s) that disrupted the runs of A or U residues terminated efficiently.

It has been shown previously (9) that substitution of ITP for GTP caused a decrease in termination frequencies for several other thr attenuator variants. Inclusion of ITP decreased termination at the mutant terminators dramatically but had only a slight effect on termination at the wild type site (9). If ITP caused a similar decrease in the termination frequencies of the mutants constructed in this study, it would be possible to determine the effects of base changes that either disrupted or restored the putative complementarity in the runs of A and U residues of the thr attenuator.

The termination frequencies obtained from ITP-substituted

\[ \text{Fig. 3. In vitro transcription template and the RNA secondary structure of the thr attenuator and its variants. A, a BstEII-SstI fragment carrying the thr regulatory region was used for in vitro transcription experiments shown in Fig. 4. The terminated and read-through transcripts are 164 and 300 bases, respectively. B, the secondary structure of the thr attenuator RNA is presented in the conformation that maximizes base pairing interactions. The positions of the nucleotides in the transcript are numbered starting from the transcription initiation site of the thr leader RNA, which is designated as +1. The mutational changes in the thr attenuator that were generated by in vitro mutagenesis are indicated as bold letters. Variant L160C was originally isolated by a genetic method that showed relief of transcription termination at the thr attenuator in vivo.} \]
in vitro transcription experiments are shown in Fig. 4 and Table I. As observed previously, incorporation of ITP in the transcripts showed only a slight effect when the wild-type template was used with a termination value of 65% (9). Variants L160C, L160G, and L160A, which contain disruptions in the U tract by substituting C, G, or A, respectively for U at position +160, showed drastically decreased termination frequencies. Since these three variants have the same G+C-rich sequences and are expected to form hairpin structures identical to the wild type with one potential mismatch in the A-U region, it is likely that the decrease in the termination values for these variants is caused by the effects of the substituted bases at the U stretch, possibly by affecting RNA-DNA template interactions or by direct effects of the sequence changes themselves. This interpretation was further supported by comparing the results from two attenuator variants, L131G/L160C and L131U/L160A. These two variants were specifically constructed so that the putative base pairing at the base of the stem of the thr attenuator was restored; i.e., I-C and U-A base pairs in L131G/L160C and L131U/L160A, respectively. If the effect exhibited by the single mutants (L160C or L160A) arose from disruption of the A-U base pairings at the base of the thr attenuator, the two variants would be expected to terminate as efficiently as the wild type. The results show that both of the variants retained termination frequencies similar to the variants with the same change in the U stretch only (L160C or L160A). Taken together, the in vitro transcription studies with ITP suggested that single base changes in the run of A residues have no effect on termination.

To determine the effects of the variants in vivo, the same mutants were subcloned into the vector pMC1403 to construct in-frame thrA-lacZ protein fusions (Fig. 1, B and C). These constructs lack the leader region that encodes the upstream secondary RNA structures that are involved in regulating the formation of the thr terminator structure encoded by the attenuator. These plasmids were constructed to avoid possible complications introduced by the upstream sequences. The fusions were then crossed onto iRZ5 by homologous recombination and single-copy lysogens were constructed. The level of β-galactosidase expression should only reflect the termination efficiencies of these attenuator variants in vivo.

Table II shows the results of β-galactosidase assays performed on cells grown in minimal medium. The results show that variant L131G exhibited the same level of β-galactosidase activity as wild type. This result could be explained by the formation of a G-U base pair in the helix. However, variants which only disrupted the U tract showed 3.7-4.0-fold (L160C and L160G) and 7.5-7.9-fold (L160A and L160G) higher β-galactosidase activities than that of wild type. Furthermore, the β-galactosidase activities of the two variants bearing base changes that could potentially form base pairs in the transcript at both the runs of A and U were 3.1 times (L131G/L160C) and 5.9 times (L131U/L160A) the wild-type value. The results again suggested that the restoration of complementarity at the base of stem cannot compensate for the effects of a single substitution mutation in the U stretch. In vivo β-galactosidase assays of the variants that contain an intact thr leader region also gave similar results (data not shown).

In Vitro Transcription with Templates Containing Deletions of the Hairpin-proximal and Hairpin-distal da-dT Tracts—A second prediction of the model proposing that A-U base pairing is important for transcription termination is that deletions of the adenosine residues upstream of the hairpin would decrease the length of the helix and, consequently, decrease the efficiency of transcription termination. We constructed templates that encoded varying numbers of adenosine residues without

![Fig. 4. Transcription termination in vitro.](image-url)
changing the sequence of the hairpin and downstream uridine residues. RsaI-XbaI fragments (42–49 bp) were isolated from several deletion mutants previously constructed by Lynn et al. (5) that contained a series of successive deletions of the hairpin-distal dA-dT tract. These fragments contain the threonine attenuation plus approximately 15 bp upstream of it. The fragments were cloned into pUC18 and pUC19 at the HindIII and XbaI sites (Fig. 2). The attenuator was in the wild-type orientation, with respect to the direction of transcription from the lac promoter in pUC18 and in the inverse orientation in pUC19. The number of dA-dT residues distal to the region of dyad symmetry varied in the pUC18 clones and the number of dA-dT residues upstream of the region of dyad symmetry varied in the pUC18 clones (Fig. 2). Since transcription termination is efficient even on a template that contains a deletion that removes 5 of the 6 adenosine residues upstream of the hairpin, these results also indicate that A-U base pairing is not important for efficient transcription termination.

In summary, we have constructed variants of the thr attenuator to determine if the tract of 6 dA-dT residues upstream of the hairpin is essential for transcription termination. Both the in vitro and in vivo results indicate that an intact dA-dT tract is not essential for efficient transcription termination. These results argue against a model that proposes that pairing between the adenosine residues upstream of the hairpin and downstream uridine residues is necessary for transcription termination. In addition, the dA-dT tract upstream of the hairpin does not contribute essential structural or sequence information because deletion of 5 of the 6 A residues from the transcript does not affect the efficiency of termination. The results are compatible with current models that propose that termination is a multi-step process involving active participation of the RNA polymerase (3, 25, 26) or that termination is controlled by the relative stabilities of DNA-DNA, RNA hairpin, and DNA-RNA interactions at the termination site in the transcription bubble (2, 27).

It is interesting to note that a study by Wright et al. (23) with the tL17 terminator has shown that a deletion of the tract of 7 adenosine residues upstream of the dG+dC-rich element increases the frequency of read-through at the terminator by a factor of 10 in vivo. They concluded that pairing between the adenosines in the A-tract and the uridines in the distal U tract is important for termination. We have no obvious explanation for the differences observed between the thr and tL17 terminators. As discussed by Wright et al. (23) it is possible that, as a consequence of the deletion of the A tract, the formation of an alternative stem-and-loop structure could occur. It would contain 4 instead of 5 G-C base pairs in the stem, and the loop would be 6 rather than 4 bases. It is also possible that the length of the helix in the stem of the RNA could be important in determining the requirement for pairing between the A tract and the distal uridines. The thr and tL17 stems are 8 and 9 base pairs in length, respectively. Perhaps the tL17 terminator requires A-U base pairs in addition to the G-C base pairs to extend the length of the stem helix in order to act as an efficient terminator. Additional systematic studies will be required to determine if either of these explanations is correct.

Acknowledgments—We thank R. Gumport, D. Friedman, L. Hales and M. MacWilliams for comments on the manuscript, and S.-T. Jeng for helpful discussions. We also thank E. Morgan and J. Harmon for their gifts of RNA polymerase and CRP protein and S. Henson for preparing the manuscript.

REFERENCES
1. Bauer, C. E., Carey, J., Kasper, L. M., Lynn, S. P., Waechter, D. A., and Gardner, J. F. (1983) in Gene Function in Prokaryotes (Beckwith, J., Davies, J., and Gallant, J. A., eds) pp. 73–81, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
2. Yager, T. D., and von Hippel, P. H. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham,
Transcription Termination at the thr Attenuator

J. L. Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) Vol. 2, pp. 1241–1275, American Society for Microbiology, Washington, DC
3. Chamberlin, M. (1994) Harvey Lect. 88, 1–21
4. Gardner, J. F. (1982) J. Biol. Chem. 257, 3896–3904
5. Lynn, S. P., Kasper, L. M., and Gardner, J. F. (1988) J. Biol. Chem. 263, 472–479
6. Brendel, V., Hamm, G. H., and Trifonov, E. N. (1986) J. Biomol. Struct. Dyn. 3, 705–723
7. Kunkel, T. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 488–492
8. Kunkel, T. A., Roberts, J. D., and Zakour, R. A. (1987) Methods Enzymol. 154, 367–382
9. Yang, M.-T., and Gardner, J. F. (1989) J. Biol. Chem. 264, 2634–2639
10. Bauer, C. E., Hesse, S. D., Waechter-Brulla, D. A., Lynn, S. P., Gumport, R. I., and Gardner, J. F. (1985) Gene (Amst.) 37, 73–81
11. Casadaban, M. J., Chou, J., and Cohen, S. N. (1980) J. Bacteriol. 143, 971–980
12. Zagursky, R., and Berman, M. L. (1984) Gene (Amst.) 27, 183–191
13. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) in Molecular Cloning: A Laboratory Manual pp. 249–253, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
14. Kraft, R., Tardiff, J., Krauter, K. S., and Leinwand, L. A. (1988) BioTechniques 6, 544–547
15. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
16. Silhavy, T. J., Berman, M. L., and Enquist, L. W. (1984) Experiments With Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Miller, J. H. (1972) Experiments in Molecular Genetics, pp. 352–355, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
18. Sly, W. S., and Rabideau, K. (1969) J. Mol. Biol. 42, 385–400
19. Zurawski, G., Brown, K., Killingly, D., and Yanofsky, C. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 4271–4275
20. Keller, E. B., and Calvo, J. M. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 6189–6190
21. Friden, P., Newman, T., and Freundlich, M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 6156–6160
22. Postle, K., and Good, R. F. (1985) Cell 41, 577–585
23. Wright, J. J., Kumar, A., and Hayward, R. S. (1992) EMBO J. 11, 1957–1964
24. Lynn, S. P., Bauer, C. E., Chapman, K., and Gardner, J. F. (1985) J. Mol. Biol. 183, 529–541
25. Reynolds, R., Bermúdez-Cruz, R. M., and Chamberlin, M. J. (1992) J. Mol. Biol. 224, 31–51
26. Nudler, E., Kashlev, M., Nikiforov, V., and Goldfarb, A. (1995) Cél 81, 351–357
27. Yager, T. D., and von Hippel, P. H. (1991) Biochemistry 30, 1097–1118