A Complementary DNA Oligomer Releases a Transcription Pause Complex*

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The formation of alternative secondary structures in the transcript of the tryptophan (trp) operon leader region regulates expression of the trp operons of Escherichia coli and other bacterial species. During in vitro transcription RNA polymerase pauses near base pair 90 after the first hairpin secondary structure in E. coli trp leader mRNA is formed. The E. coli L-factor enhances transcription pausing at this site (Farnham, P. J., Greenblatt, J., and Platt, T. (1982) Cell 29, 945–951); presumably it does so by facilitating recognition of the RNA hairpin by polymerase. We show that addition of a DNA oligomer complementary to the proximal segment of the RNA hairpin relieves transcription pausing in vitro both in the presence and absence of L-factor. The oligomer apparently interferes with formation of the RNA hairpin which we believe is recognized by polymerase as the pause signal. The oligomer also relieves pausing in L-factor-induced paused complexes, suggesting that the oligomer can disrupt a preformed secondary structure in the transcript.

The mechanism of attenuation in the trp operon of Escherichia coli is best described by a model in which translation of the leader peptide coding region determines which alternative RNA secondary structure forms during transcription of the leader region of the operon. One secondary structure favors transcription termination while the other promotes read-through into the structural genes of the operon (recent reviews, see Refs. 1–4). If attenuation is to function efficiently it is essential that translation of the leader transcript proceed concomitantly with transcription.

RNA polymerase pauses near base pair 90 during in vitro transcription of the trp leader region (5, 6). The 3' end of the pause transcript can form a stable ($\Delta G = -11.2$ kcal/mol) hairpin secondary structure (Fig. 1, structure 1:2). The hairpin secondary structure itself probably elicits the pause by the polymerase (3, 6). How long the polymerase pauses may reflect the stability of the RNA hairpin, the DNA sequence immediately beyond the site of pausing, and the concentrations of available nucleoside triphosphates (3, 5–7). Pausing near base pair 90 may facilitate the coupling of transcription and translation in vivo by providing a transcription delay sufficient to allow a ribosome to begin synthesis of the leader peptide and to disrupt an appropriate leader RNA secondary structure (5). Pausing is influenced by mutational changes in the RNA polymerase $\delta$ subunit (8); lesions which enhance or relieve pausing have been observed (6–8). Pausing in the trp leader region is markedly enhanced in vitro by the presence of L-factor, the nusA gene product (11). L-factor is associated with RNA polymerase during RNA chain elongation (10) and is thought to mediate the interaction of termination and antitermination factors with polymerase (9).

It has been shown that addition of a synthetic oligomer perfectly complementary to segment 1 of the E. coli trp leader transcript promotes transcription read-through at the trp attenuator in vitro (12). Presumably the oligomer interferes with formation of RNA hairpin 1:2, thereby allowing formation of the antiterminator structure, 2:3 (Fig. 1). Here we demonstrate that this same oligomer also relieves transcription pausing; the effect of the oligomer is considerable when pausing is enhanced by the presence of L-factor.

EXPERIMENTAL PROCEDURES

RNA polymerase was prepared by a modification of the procedures of Burgess and Jendrisak (13) and Gonzalez et al. (14) as previously described (15). L-factor was the generous gift of H. Weissbach of the Roche Institute. Heparin was purchased from Invernex. Oligomers were synthesized as previously described (12) and were generously provided by K. Mullis and J. Barnett of Cetus Corporation. The sequence of the complementary oligomer is shown in Fig. 1B. The sequence of the noncomplementary oligomer is d(C-G-C-C-G-C-T-T-T-G-T-G-A-A-C-C). [c$\gamma$-32P]GTP (410 Ci/mmol) was purchased from Amersham Corp. Ribonucleoside triphosphates were purchased from P-L Biochemicals or Sigma. Templates for transcription, prepared as previously described (16), were HpaII restriction fragments which contained the trp promoter, leader, and initial portion of trpE. Synchronized single-round transcription was conducted by preincubating RNA polymerase with template at the indicated temperature in a standard reaction mixture (20 mM Tris-acetate, pH 8.0, 0.1 mM Na$_2$EDTA, 0.1 mM dithiothreitol, 4 mM Mg acetate, 150 mM KCl, 5% glycerol, 20 $\mu$g/ml of bovine serum albumin) containing 150 $\mu$M ATP and 0.4–20 $\mu$M GTP (5). Transcription was initiated by addition of a prewarmed solution containing 150 $\mu$M CTP and UTP and heparin (final concentration = 100 $\mu$g/ml), which prevents subsequent rounds of transcription initiation. Aliquots of the synchronized reaction mixture were removed at the times indicated (after transcription initiation) in individual figures. They were added to an equal volume of stop solution (16) and subjected to electrophoresis on polyacrylamide–TBE$^1$–urea slab gels as previously described (5).

RESULTS

During transcription of the E. coli HpaII-570 restriction fragment containing the trp operon leader region, RNA polymerase pauses at a site near base pair 90 (5, 6). Pausing is readily detected by synchronizing a single round of transcription, as shown in Fig. 2. It is evident that RNA polymerase

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$^1$ The abbreviation used is: TBE, Tris-borate-EDTA.
Fig. 1. Alternative secondary structures in the trp leader transcript. RNA segments which base pair to form intrastrand secondary structures are numbered according to the insert diagram. A, 1:2 and 3:4 (terminator) hairpin structures presumed to signal transcriptional pausing and termination, respectively. B, mutually exclusive alternative 2:3 (antiterminator) secondary structure which signals transcriptional read-through by preventing terminator formation. Base pairing between the complementary oligomer (bold face) and segment 1 of the leader transcript presumably interferes with 1:2 formation and ultimately terminator formation by facilitating antiterminator formation. Bases are numbered from the 5' end of the transcript. The arrow indicates the predominant 3' terminus of the pause transcript.2

Fig. 2. Transcription pausing in the trp leader region. Synchronized single round transcription experiments were performed at 20 °C as described under “Experimental Procedures” using 20 μM [α-32P]GTP (50 μCi) and 150 μM ATP, CTP, and UTP. At the indicated times after initiation of transcription aliquots of the reaction mixture were removed and the reaction stopped. Transcription products were analyzed on a 10% acrylamide TBE-urea denaturing gel as previously described (5). The three transcripts are the 92-nucleotide pause RNA species (P), the 140-nucleotide terminated leader RNA (L), and the 260-nucleotide read-through transcript (RT).

molecules transcribing the trp leader region dwell for a considerable time at the pause site relative to other segments of the leader region, since the only transcript bands detected are those of the pause, attenuated (terminated), and read-through species. Reducing the GTP concentration in the reaction mixture accentuates pausing (Fig. 3), presumably because the GTP concentration is far below the Kₐ for elongation. By lowering the GTP concentration as shown, one can form a relatively stable complex at the pause site; the half-life of paused complexes formed at 37 °C in 0.4 μM GTP is approximately 20-fold greater than in 20 μM GTP (t₁/₂ = 9.5 min and 0.5 min, respectively). When pausing is prolonged by lowering the GTP concentration we can test for effects on pausing that are not obvious at high GTP concentrations. We were particularly interested in determining whether pausing would be affected by the addition of a DNA oligomer that is perfectly complementary to RNA segment 1 of the 1:2 hairpin loop of the E. coli trp leader transcript (Fig. 1). It was inferred from previous studies that this oligomer competes with RNA segment 2 for RNA segment 1 during structure 1:2 formation, allowing the formation of the alternate secondary structure, 2:3 (12). Formation of the antiterminator 2:3 presumably precludes formation of the mutually exclusive 3:4 structure, the terminator. The net effect, therefore, should be an increase in read-through transcription at the attenuator, which is what is observed (12).

We determined the molar ratios of the pause, terminated leader, and read-through transcripts as a measure of relief of pausing. (Conditions which relieve pausing will decrease the amount of pause RNA relative to the other two RNA species.) Addition of the complementary oligomer to a synchronized transcription reaction mixture alleviated pausing by RNA polymerase (Table I, line 1). Addition of a noncomplementary oligomer of approximately the same G+C content as the complementary oligomer (12) did not affect pausing (Table I, line 1). When either oligomer was present during synchronized transcription of a Serratia marcescens trp leader template (the S. marcescens ΔRSA5 transcript contains no sequences complementary to either oligomer (17)), no effect on RNA polymerase pausing was detected (Table I, line 2). The presence of L-factor during in vitro transcription greatly enhanced pausing in the trp leader region (Fig. 4, Ref. 11). As was the case at low GTP concentrations, this enhancement allowed us to test for alterations in pausing. When the complementary oligomer was added to a synchronized transcription reaction mixture containing L-factor, pausing was dramatically relieved (Table I, line 3; Fig. 5). Addition of the noncomplementary oligomer had no effect. The effects of the two conditions, low GTP concentration and presence of L-factor, were not additive, however. At 0.4 μM GTP, pausing was not further enhanced by the presence of L-factor (data not shown). Note that addition of the complementary oligomer not only alleviated pausing, but, as described previously (12), it also promoted read-through beyond the attenuator (Fig. 5). Use of an altered RNA polymerase that enhances both termination and pausing (7) also revealed oligomer effects on pausing. In these experiments as well, only the complementary oligomer relieved transcription pausing (Table I, line 4).

2 R. Fisher and C. Yanofsky, unpublished experiments.
Table I

| Template                  | RNA polymerase | Additions                                      | Ratio of leader + read-through RNAs to pause RNAs |
|---------------------------|----------------|-----------------------------------------------|--------------------------------------------------|
|                           |                |                                               | Control                                        |
|                           |                |                                               | +Noncomplementary 16-mer | +Complementary 15-mer |
| E. coli wild type         | Wild type      |                                               | 1                                              |
| S. marcescens RSA5        | Wild type      |                                               | 1.1                                            |
| E. coli wild type         | Wild type      | L-factor*                                     | 1                                              |
| E. coli wild type         | Termination* proficient |                                  | 1.5                                            |

* In the presence of L-factor (5 μg/ml), 20 μM GTP was employed.
* The termination proficient RNA polymerase was purified from E. coli rifR strain CY 15028 (8) as previously described (7).

Fig. 4. Effect of L-factor on transcription pausing. Synchronized single round transcription reactions containing 23.5 nM RNA polymerase and 10 nM E. coli HpaII-570 wild type restriction fragment were performed at 37 °C as described in Fig. 2 in the absence and presence of 5 μg/ml of L-factor. Samples were taken at the indicated times, in minutes, after initiation of transcription.

Fig. 5. The complementary oligomer relieves L-factor-enhanced pausing. Synchronized single round transcription reactions containing 23.5 nM RNA polymerase and 5 μg/ml of L-factor were performed at 37 °C as described in the legend to Fig. 2 in the absence (control) or presence of noncomplementary (+NC-16mer) and complementary (+C-15mer) oligomers. The oligomers, at a 500-fold molar excess over template, were present during the synchronizing preincubation period. Samples were taken at the indicated times, in minutes, after initiation of transcription.

The oligomer used in this study did not significantly alter the half-life of the pause species formed at high GTP concentrations in the absence of L-factor. However, L-factor increased the half-life of pause RNA approximately 4-fold (Table II). In the presence of L-factor the complementary oligomer markedly decreased the half-life of the paused complex, whereas the noncomplementary oligomer had no effect (Table II). This is consistent with the view that L-factor prolongs pausing by enhancing the ability of the polymerase to recognize the crucial hairpin structure in the transcript. The complementary oligomer, by inhibiting hairpin 1:2 formation, may effectively eliminate the L-factor-enhanced signal that RNA polymerase recognizes. Alternatively, L-factor may simply stabilize the interaction of RNA polymerase with the RNA hairpin that signals pausing.

To determine if the complementary oligomer could disrupt the presumptive pause signal we added oligomer to paused complexes formed in the presence of L-factor. The oligomer was added to the reaction mixture at the time indicated in Fig. 6. Presence of the oligomer resulted in immediate relief of pausing; it reduced the half-life of the paused complex by a factor of 4, to about 30 s (Fig. 7). Approximately 95% of the paused complexes decayed with this accelerated half-life following addition of the complementary oligomer (Fig. 7), indicating that the oligomer was able to interact with virtually every paused complex. This result suggests that added oligomer can disrupt a preformed RNA hairpin.

In other studies we showed that L-factor-enhanced pausing on the trp leader template of wild type S. marcescens was not relieved by addition of the oligomer that is effective with the E. coli template (data not presented). However, this oligomer is not complementary to either strand of the 1:2 hairpin secondary structure of S. marcescens. This finding supports the view that the oligomer relieves pausing on E. coli trp DNA by pairing with its complementary RNA sequence.

Table II

| L-factor | Oligomer                  | Pause RNA half-life |
|----------|---------------------------|---------------------|
| -        | Noncomplementary 16-mer   | 31                  |
| -        | Complementary 15-mer       | 36                  |
| -        | Complementary 15-mer       | 38                  |
| +        | Noncomplementary 16-mer    | 120                 |
| +        | Complementary 15-mer       | 130                 |
the synchronized single-round reaction mixture were removed and the time at which 5 m), to the pause transcript (0, are obtained by multiplying the values by 4.5. The Cerenkov radiation as previously described (5). The autoradiography, excised from the gels, and quantified by determining the reaction stopped at the indicated times. Reaction products were analyzed on 10% polyacrylamide TBE-urea denaturing gels as described under "Experimental Procedures." RNA bands corresponding to the pause transcript (C, ), the terminated leader transcript (C, ), and the run-off read-through transcript (A, ) were detected by autoradiography, excised from the gels, and quantified by determining Cerenkov radiation as previously described (5). The arrow indicates the time at which 5 m complementary oligomer (C, D) or buffer (C, D) was added to the reaction mixture. Actual molar amounts are obtained by multiplying the values by 4.5 x 10^{-14}.

**DISCUSSION**

Sites at which RNA polymerase molecules pause during transcription are either G + C-rich or are adjacent to or within DNA regions which specify transcript segments that can form stable secondary structures (5, 6, 18-27). The transcription pause site in the trp operon leader region displays both of these features (5). A G + C-rich secondary structure (ΔG = -11.2 kcal) is formed in the transcript and this structure immediately precedes the 5' end of the pause transcript (5). Thus secondary structure is thought to be the signal that the transcribing RNA polymerase recognizes and responds to by pausing. The similarity of the pause RNA secondary structure to secondary structures that promote transcription termination suggests that the same domain of the RNA polymerase molecule recognizes both structures. In agreement with this conclusion we have found that mutationally altered RNA polymerase molecules that respond abnormally to RNA termination signals also behave aberrantly when they encounter pause signals (7).

Pausing in the trp leader region is exaggerated when the GTP concentration in the transcription reaction mixture is reduced, when L-factor is added, or when a mutationally altered polymerase is employed. Each of these conditions also reduces the rate of RNA chain elongation. A longer polymerase is employed. Each of these conditions also reduces the rate of RNA chain elongation. A longer polymerase half-life about 4-fold, from 120 s to about 30 s. Actual molar amounts are obtained by multiplying the values by 4.5 x 10^{-14}.

**FIG. 6.** Addition of the complementary oligomer to L-factor-induced paused complexes relieves pausing. Synchronized single-round transcription reactions containing 23.5 nM RNA polymerase holoenzyme, 10 nM E. coli HpaII-570 wild type restriction fragment, and 5 μg/ml L-factor were performed at 37 °C using 20 μM [α-32P]GTP (50 μCi) and 150 μM ATP, CTP, and UTP. Aliquots of the synchronized single-round reaction mixture were removed and the reaction stopped at the indicated times. Reaction products were analyzed on 10% polyacrylamide TBE-urea denaturing gels as described under "Experimental Procedures." RNA hands corresponding to induced paused complexes relieves pausing. The similarity of the pause RNA secondary structure immediately precedes the formation of structure 1:2 as RNA segment 1 we believe that the presence of the complementary oligomer decreased the pause RNA half-life about 4-fold, from 120 s to about 30 s. Actual molar amounts are obtained by multiplying the values by 4.5 x 10^{-14}.

We used conditions that prolong pausing to test the assumption that pausing is dependent upon the formation and/or stability of a particular RNA secondary structure, hairpin 1:2 of the trp leader transcript. We showed that addition of a DNA oligomer complementary to RNA segment 1 of the transcript relieved pausing. Since the oligomer employed is perfectly complementary to RNA segment 1 we believe that it acts by base-pairing with this segment and interferes with the formation of structure 1:2 as RNA segment 2 is being synthesized (12). Addition of the oligomer also appears to disrupt preformed 1:2 paused complexes (Fig. 6). Thus the half-life of the paused complex was reduced appreciably when the oligomer was added to complexes preformed in the presence of L-factor (Fig. 7). The complementary DNA oligomer apparently can displace segment 1 from structure 1:2 and thereby destroy the transcription pause signal.

Analysis of the kinetics of pausing of preformed paused complexes indicates that the oligomer interacts with virtually every complex to eliminate pausing. The same paused complex half-life value is obtained when the oligomer is added to the reaction mixture in the presence or absence of L-factor (Table II). Thus L-factor enhancement of pausing is totally overcome by disruption of RNA secondary structure by the oligomer.

**FIG. 7.** Addition of the complementary oligomer to preformed paused complexes alters the half-life of the paused complex. The data for the pausing experiment in Fig. 6 were replotted to determine the exponential rate of decay of the paused complexes after addition of the complementary oligomer ( ) or buffer ( ). The presence of the complementary oligomer decreased the pause RNA half-life about 4-fold, from 120 s to about 30 s. Actual molar amounts are obtained by multiplying the values by 4.5 x 10^{-14}.

Disruption of structure 1:2 by the added oligomer would be expected to enhance formation of structure 2:3 and hence reduce formation of the terminator structure, 3:4. As illustrated in Fig. 6, addition of the complementary oligomer...
increased read-through from 1 to 15%. Although the oligomer appears to interact with virtually every paused complex, termination relief at the attenuator is far from complete. In this context it is important to note that the oligomer is not complementary to all of segment 1 of trp leader RNA (Fig. 1). The portion of segment 1 which is not complementary to the oligomer could still base pair to its complementary region in segment 2. Such base pairing could prevent antiterminator formation in that region of 2:3 which precludes terminator formation. Our findings bolster the view that RNA polymerase recognition of RNA secondary structure is fundamental to both pausing and termination events.

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