Use of complementary DNA oligomers to probe *trp* leader transcript secondary structures involved in transcription pausing and termination

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ABSTRACT

DNA oligomers were synthesized that are perfectly complementary to different segments of the tryptophan (*trp*) operon leader transcript. These 15 nucleotide long oligomers were used as probes of the involvement of transcript secondary structures in two processes: transcription pausing at the pause site located near base pair 90 in the leader region, and transcription termination at the attenuator. The 15-mers were complementary to the four segments of the *trp* leader transcript which have been shown to form the alternative secondary structures that are believed to be responsible for pausing, termination, and antitermination. Oligomers complementary to RNA segments 1 and 3 relieved termination while the 15-mer complementary to RNA segment 1 relieved pausing. 15-mers complementary to segment 2 had no effect on pausing and the oligomer complementary to segment 4 had virtually no effect on termination.

INTRODUCTION

Alternative secondary structures in the *trp* operon leader transcript regulate expression of the *trp* operon of *Escherichia coli* and other bacterial species (for recent reviews, see 1-6). This regulation involves control of transcription termination at the attenuator, a site which lies between the promoter and the first major structural gene of the operon. One secondary structure, the terminator (Fig. 1), is believed to signal the transcribing RNA polymerase to terminate transcription at the attenuator (7, 8). An alternative, mutually exclusive transcript secondary structure, the antiterminator (Fig. 1), is thought to prevent formation of the terminator and thereby permit transcriptional read-through beyond the attenuator into the structural genes of the operon. Expression of other bacterial amino acid biosynthetic operons also is regulated by attenuation (9-17).

Translation of a leader peptide coding region (18-20) determines which alternative transcript secondary structure is formed as RNA polymerase transcribes the operon leader region. For attenuation to control operon expression efficiently it is essential that translation of the leader peptide

Fig. 1 Alternative secondary structures in the *E. coli* *trp* leader transcript. RNA segments which base pair to form intrastrand secondary structures are numbered according to the insert schematic diagram. Bases are numbered from the 5' end of the transcript. The bold lines indicate the segments of the *trp* leader transcript to which the different oligomers (designated by the letters adjacent to the bold lines) are perfectly complementary.

Upper left: 1:2 and 3:4 (terminator) hairpin structures presumed to signal transcriptional pausing and termination, respectively. The arrow indicates the predominant 3' terminus of the pause transcript (R. Fisher and C. Yanofsky, unpublished data).

Upper right: mutually exclusive 2:3 hairpin (antiterminator) which promotes transcriptional read-through by preventing formation of the terminator. Lower: The linear sequence of the 3' portion of the terminated *trp* leader transcript. Segments 1, 2, 3 and 4 of the leader transcript that participate in intrastrand base pairing are boxed by heavy lines. The sequence of each of the five complementary DNA oligomers used in this study, each designated by a different letter, is indicated above or below the complementary segment of the linear transcript sequence.

coding region be tightly coupled to transcription of the leader region. This is necessary so that the appropriate RNA secondary structure will form when the translating ribosome stalls at the Trp codons or moves to the stop codon on the transcript. A mechanism which might provide this synchronization, transcription pausing, was suggested by *in vitro* transcription experiments with *trp* operon templates (Figure 1)(21-23). RNA polymerase pauses near base pair 90, just distal to the 1:2 stem of the *trp* leader transcript (21-23). Pausing at this site could promote coupling of transcription and translation *in vivo* by delaying transcription while a ribosome initiates synthesis of the leader peptide, moves on the transcript, and disrupts the 1:2 leader RNA secondary structure as it approaches the paused polymerase.

We have previously shown in *in vitro* transcription experiments that addition of a synthetic oligodeoxynucleotide complementary to segment 1 of the *E. coli trp* leader transcript (oligo-A, Fig. 1) not only promotes transcription read-through at the attenuator, but also relieves transcription pausing at the pause site (23, 24). These effects presumably result from oligo-A's interference with formation of the 1:2 RNA hairpin, the pause signal, thereby promoting formation of the 2:3 antiterminator secondary structure (Fig. 1). Formation of the antiterminator then presumably prevents terminator structure (3:4) formation and thereby promotes read-through at the attenuator (26-28). There was no effect on pausing or read-through when a non-complementary oligomer with approximately the same G+C content as oligo-A was present during transcription (Fig. 1, refs. 23, 24). Here we test synthetic 15-mers complementary to other segments of the *trp* leader transcript which are involved in secondary structure formation and presumably are responsible for transcription pausing and termination.

EXPERIMENTAL PROCEDURES

In vitro transcription

The *E. coli* Hpa II₅₇₀ restriction fragment which contains the *trp* promoter, leader region and initial portion of *trpE* was used as a template for transcription. This fragment, prepared as previously described (25), contains the sites at which RNA polymerase pauses and terminates transcription. Synchronized single-round transcription experiments were conducted by preincubating RNA polymerase and template in the presence or absence of a 500-fold molar excess of 15-mer over the Hpa II restriction fragment template. The standard transcription reaction mixture (23) contained 20mM Tris-acetate, pH 8.0, 0.1 mM Na₂EDTA, 0.1 mM dithiothreitol,

4 mM Mg acetate, 150 mM KCl, 5% (v/v) glycerol, 20 μ g/ml bovine serum albumin, 150 μ M ATP, 0.4 or 20 μ M GTP, 23.5 nM RNA polymerase holoenzyme and 10 nM template. Incubation was at 37°C. Transcription was initiated by the addition of a prewarmed solution containing 150 μ M CTP and UTP, and heparin (final concentration = 100 μ g/ml), which prevents subsequent rounds of transcription initiation. Aliquots of the synchronized reaction mixture were removed after transcription initiation. They were added to an equal volume of stop solution (25), and subjected to electrophoresis on 10% polyacrylamide- TBE-urea slab gels as previously described (25). Following location by autoradiography the RNA bands were excised from the gels and radioactivity was measured by Cerenkov counting as previously described (26). Pause RNA half-lives were estimated by plotting the exponential decrease in cpm in the pause transcript.

For multiple rounds of transcription the reaction mixture contained all four ribonucleoside triphosphates and was incubated at 37°C in the presence or absence of a 500-fold molar excess of the indicated 15-mer over the restriction fragment. After 15-20 min an equal volume of stop solution was added to the reaction mixture and the products were subjected to electrophoresis as described above. Following autoradiography and Cerenkov counting, the percentage of read-through transcription was calculated from the molar amounts of terminated leader and read-through transcripts.

Oligomer Synthesis

Both the DNA 15-mer complementary to segment 1 of the trp leader transcript and the non-complementary oligomer were synthesized as previously described (24, 29) and were generously provided by K. Mullis and J. Barnett of Cetus Corporation. The other DNA 15-mers (Figure 1) were synthesized using standard phosphotriester block coupling solid phase methods (30, 31) with a Bachem manual DNA synthesizer and Bachem reagents. The sequence of each synthetic oligonucleotide was verified by sequencing using the Maxam and Gilbert sequencing reactions (32).

RESULTS

RNA secondary structure involvement in transcription termination.

Transcript secondary structures that form as RNA polymerase transcribes the trp leader region signal the polymerase to pause near base pair 90 and subsequently either terminate transcription at the attenuator or read-through into the structural genes of the operon (23, 27, 28). As probes of the involvement of leader transcript secondary structures in these polymerase

Table 1

Effect of oligomers complementary to segments of the *trp* leader transcript on transcription pausing^a and transcription read-through.

Oligomer ^b	Pause RNA Half-life (sec)	Molar % Read-through
none	130	4.5
A	38	31.5
B	115	4.4
C	122	6.5
D	144	15.7
E	n.t. ^c	5.1
non-complementary	120	4.4

^a Pause RNA half-life values were determined in the presence of L-factor, the *nusA* gene product (5 µg/ml).

^b The oligomers used are described in Fig. 1.

^c n.t. = not tested.

activities, we used synthetic DNA 15-mers that are perfectly complementary to segments of the *trp* leader transcript which form the various RNA secondary structures. We synthesized two overlapping 15-mers (B and C, Fig. 1) to probe the interaction of RNA segment 2 with segments 1 and 3 of the *trp* leader transcript. Oligo-B is perfectly complementary to the entire loop between segments 1 and 2 as well as to the promoter-proximal half of segment 2 (nucleotides 68-82); oligo-C is complementary to segment 2 predominantly (nucleotides 73-87)(Fig. 1). These 15-mers had virtually no effect on termination at the attenuator when they were included in the *in vitro* transcription reaction mixture (compare Table 1, line 1 with lines 3 and 4).

Oligo-D is complementary to segment 3 of the terminator, while oligo-E is complementary to the entire loop between 3 and 4 and segment 4 of the terminator (Fig. 1). When oligo-D was included in an *in vitro* transcription reaction mixture, read-through at the attenuator was 350% that observed in the absence of the 15-mer (Table 1, compare lines 1 and 5). This presumably results from the 15-mer directly interfering with formation of the 3:4 terminator. When oligo-E (Fig. 1) was included in an *in vitro* transcription reaction, there was virtually no effect on termination at the attenuator (Table 1, lines 1 and 6).

RNA secondary structure involvement in transcription pausing.

The 1:2 RNA hairpin is thought to signal RNA polymerase to pause near base pair 90 as it transcribes the *trp* leader region (23). Since we previously observed that disruption of the 1:2 hairpin by addition of oligo-A (Fig. 1) relieved pausing, we tested oligos B and C, which are complementary to other regions of the 1:2 hairpin. We observed that neither 15-mer had a

significant effect on the pause RNA half-life (Table 1, compare lines 1, 3 and 4).

We also determined if either of these 15-mers would behave like oligo-A and disrupt a preformed pause complex (23); neither reduced the pause RNA half-life (data not shown). Oligo-D, as expected, had no effect on pausing during transcription (Table 1, line 1 and 5) and did not reduce the half-life of preformed pause complexes (data not shown).

DISCUSSION

Oligomers that are complementary to different segments of the trp leader transcript affect transcription pausing at the leader pause site and alter termination efficiency at the attenuator. We showed previously that an oligomer complementary to RNA segment 1 reduced pausing and relieved termination, presumably by promoting formation of the antiterminator and thereby indirectly preventing formation of the terminator (23). In the present study we show that a second oligomer, D, complementary to RNA segment 3, increases read-through at the attenuator, presumably by interfering directly with formation of the RNA terminator. The findings with these two oligomers therefore provide support for the view that specific secondary structures in the trp leader transcript are responsible for pausing and termination. Oligo-A gives greater relief of termination than does oligo-D. This difference is probably due to the greater time that the 1:2 hairpin is available for an effective interaction than the 3:4 hairpin. This extended time would provide additional opportunities for oligo-A to promote structure 2:3 formation and the accompanying relief of termination.

Whereas oligos A and D relieve attenuation 7-fold and 3.5-fold, respectively, through interference with terminator formation, point mutations in 3:4 which affect terminator stability increase read-through 5 to 15-fold (33, 34). In addition, replacing GTP by its analog ITP during transcription, resulting in the formation of the weaker I-C base pairs in the terminator, leads to virtually complete read-through at the attenuator (35). These observations illustrate the importance of secondary structure stability and recognition to the termination process (26).

Unexpectedly, oligomers complementary to RNA segment 2 had no effect on pausing or termination, and the oligomer complementary to RNA segment 4 did not reduce termination. These oligomers theoretically could interfere with formation of structures 1:2 and 3:4 respectively, and therefore could influence pausing and termination comparably to the oligomers complementary

to RNA segments 1 and 3. There are other relevant factors that should be considered however, which may provide a basis for reasonable explanations for our unanticipated findings. During the formation of the transcription pause and termination complexes, RNA segments 2 and 4 may not be as readily accessible for pairing with added oligomers as RNA segments 1 and 3. Thus the transcribing RNA polymerase molecule may sequester portions of RNA segments 2 and 4 by direct interaction and thereby prevent them from pairing with complementary sequences to which they are not covalently joined. Similarly, secondary structures 1:2 and 3:4 may have to "breathe" for an added oligomer to contact and pair with its complement. If this "breathing" were always initiated at the 5' end of the 1:2 and 3:4 structures, and if breathing rarely extended to the loop end of these structures, then oligomers complementary to segments 1 and 3 could initiate pairing at the 5' end and effectively displace segments 2 and 4 whereas oligomers complementary to segments 2 and 4 would not have this opportunity, i.e., the fact that the chain growing point is at the 3' end of structures 1:2 and 3:4 could be responsible for restricting accessibility to the 5' end of a complementary oligomer. Additionally, when RNA segments 1 and 3 are first synthesized in the presence of a complementary oligomer, segments 2 and 4 are not yet synthesized and therefore oligomers complementary to segments 1 and 3 have an opportunity to pair before there can be competition by segments 2 and 4. Another possible explanation is that the inactive oligomers complementary to RNA segments 2 and 4 form base paired structures with these segments which are recognized as the 1:2 and 3:4 secondary structures that are pause and termination signals; i.e., an oligo B or C: segment 2 base-paired structure and an oligo E: segment 4 base paired structure may be equivalent to the 1:2 and 3:4 structures. Which of these alternative explanations is correct, if any, is not known at this time.

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