Transcription Termination in Vitro by Bacteriophage T7 RNA Polymerase

THE ROLE OF SEQUENCE ELEMENTS WITHIN AND SURROUNDING A ρ-INDEPENDENT TRANSCRIPTION TERMINATOR*

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ρ-Independent transcription terminators in Escherichia coli contain a dG + dC-rich dyad-symmetrical structure that encodes an RNA hairpin structure and an adjacent, downstream dA + dT-rich region which encodes uridines at the 3'-end of the transcript. In the threonyl (thr) attenuator, there are at least six sequence segments in the DNA that might affect termination: the sequence upstream of the attenuator, the deoxythymidine-rich stretch immediately preceding the G + C-rich region, the G + C-rich region itself and its hairpin loop-encoding region, the deoxyadenosine tract following the G + C-rich region, and the following downstream sequence. Our previous studies (Jeng, S.-T., Gardner, J. F., and Gumport, R. I. (1990) J. Biol. Chem. 265, 3823–3830) indicate that both the stability and sequence of the RNA hairpin formed by the G + C-rich region and the length of the uridine tract encoded by the deoxyadenosine stretch influence the termination of T7 RNA polymerase in vitro. In this report, we demonstrate that the template deoxythymidine run upstream of the G + C-rich region, the loop-encoding segment, and the sequences upstream and downstream of the thr attenuator also affect termination. These results indicate that: 1) a deoxythymidine tract is not absolutely required for termination, but increasing the number of deoxythymidines from one to nine base pairs causes T7 RNA polymerase to terminate more efficiently; 2) a template with the natural loop sequence reversed results in a higher termination efficiency than one encoded by the wild-type attenuator; 3) the termination of T7 RNA polymerase is affected by sequences both proximal and distal to the thr attenuator.

Escherichia coli ρ-independent transcription terminators comprise, at a minimum, the following two distinct features: a dG + dC-rich dyad-symmetrical sequence, which encodes an RNA hairpin structure, and a hairpin-distal dA + dT-rich region that encodes a continuous tract of ribouridine residues in the RNA transcript within which RNA polymerase terminates. Studies by several groups have shown that both the dyad symmetrical and the dA + dT-rich regions are required for termination with E. coli RNA polymerase (Farnham and Platt, 1982; Bremer and Trifonov, 1984; Platt, 1986; Gardner, 1982).

Bacteriophage T7 RNA polymerase has been reported to terminate at some ρ-independent terminators, including Tφ, a late terminator found in the T7 genome (McAllister and McCarron, 1977; Carter et al., 1981; Dunn and Studier, 1983) and rrnBT1 of E. coli (Chen and Orozco, 1988; Christiansen, 1988), but it is unable to recognize some other ρ-independent terminators, such as Tε, an early terminator of phage T7 (Millete et al., 1970; Studier, 1972; Kiefer et al., 1977) and the anti-rrnBT1 terminator (Christiansen, 1988).

ρ-Independent terminators are not the only sequence elements that can stop elongating T7 RNA polymerase. A discontinuous deoxyadenosine tract without an apparent, associated region of dyad symmetry efficiently terminates T7 RNA polymerase in vitro (Mead et al., 1986). Furthermore, a DNA template encoding an RNA hairpin with loops of nine adenosines or uridines also abolishes T7 RNA polymerase transcription (Grobe and Uhlenbeck, 1988). Termination by these "unusual" structural or sequence features, which do not fit the standard paradigm for ρ-independent terminators, suggests that the mechanism of transcription termination by T7 RNA polymerase is incompletely understood.

Termination by E. coli RNA polymerase at ρ-independent terminators has been intensively studied. An early model for transcription termination by RNA polymerase was proposed by Farnham and Platt (1980). They postulated that RNA polymerase transcribes the dG + dC-rich region of the terminator and the encoded RNA forms a hairpin in the transcript, causing the polymerase to pause. Unstable interactions between template deoxyadenosines following the dG + dC-rich region and uridines in the transcript were postulated to promote release of the transcript (Martin and Tinoco, 1980). Alternatively, Yager and von Hippel (1991) suggested that a summation of the relative free energies of formation (ΔG) at each nucleotide position of the unwound DNA in the transcription bubble, of the DNA-nascent RNA hybrid in the bubble, and of interactions between RNA polymerase and nearby DNA and RNA could predict the stability of the elongation complex. At certain positions, depending upon the template sequence (usually those sequences containing factor-independent terminators), the complex was relatively unstable and termination was predicted to occur. An extension of this work to consider the kinetics as well as the thermodynamics of the complexes allowed estimations of the efficiencies of termination (Yager and von Hippel, 1991; von Hippel and Yager, 1991, 1992).

Both models focused on nucleic acid interactions within or
very near the transcription bubble and the RNA hairpin of the terminator. These models are complicated by the finding that DNA sequences near the promoter and prior to and following the p-independent terminator also affect transcription termination by E. coli RNA polymerase (Reynolds and Chamberlin, 1992; Telesnitsky and Chamberlin, 1989a, 1989b; Goliger et al., 1989). Furthermore, the observation that some terminators can have significantly different termination efficiencies with different RNA polymerases indicates that the enzyme is an important contributor to the process (Reynolds and Chamberlin, 1992; Reynolds et al., 1992; Jeng et al., 1990). Sousa et al. (1992) have proposed a termination model for T7 RNA polymerase that emphasizes interactions of the enzyme with the growing transcript.

We have studied the transcription termination of T7 RNA polymerase with the thr attenuator, a p-independent terminator (Jeng et al., 1990) that has been studied extensively with E. coli RNA polymerase (Lynn et al., 1985, 1987, 1988; Gardner, 1982; Yang and Gardner, 1989). Our previous studies with T7 RNA polymerase indicated that the orientation of the RNA hairpin, and the length of the template deoxyadenosine tract (i.e. the number of potential U residues in the RNA) in the thr attenuator each affect the termination efficiency. With respect to E. coli RNA polymerase, T7 RNA polymerase requires a more perfect RNA hairpin structure and a longer deoxyadenosine tract to terminate with maximum efficiency in vitro (Jeng et al., 1990).

In this report, we describe the effects of sequence variants with deletions in the template deoxythymidine tract upstream and adjacent to the dyad-symmetrical structure, a variant encoding a reversed orientation of the loop within the RNA hairpin, and variants of the sequences immediately surrounding the thr terminator. Our results indicate that (1) although the deoxythymidine tract is not absolutely required for termination, the presence of this sequence feature increases T7 RNA termination efficiency; (2) a template with a reversed loop orientation terminates with higher efficiency; and (3) sequences both proximal and distal to those usually thought to define thr attenuator affect the termination efficiency.

**Experimental Procedures**

**Materials**—CTP labeled with 32P in the α position at 410 Ci/mmol was purchased from Amersham Corp. Unlabeled ribonucleoside triphosphates were obtained from Sigma. T7 RNA polymerase, restriction enzymes, and T4 DNA ligase were obtained from Bethesda Research Laboratories.

**Plasmid Constructions**—Plasmids pTZ18u and pTZ19u (Mead et al., 1986) were obtained from Dr. D. Mead. They contain a T7 promoter and downstream polylinker sites from pUC18 and pUC19, respectively. Plasmids pTZ19t1 and the pTZ19t1 to pTZ19t8 series of plasmids were described previously (Jeng et al., 1990). The difference between pTZ19t1 and the pTZ19t1 series of plasmids is that pTZ19t1 contains five extra base pairs (CCAGCT) that are absent in the pTZ19t1 plasmids (Table I).

The pTZ18thrA series of plasmids was constructed by cloning HindIII-XbaI fragments from pTZ19t1 to the pTZ19t8 series (Table I, Jeng et al., 1990) into HindIII-XbaI-digested pTZ18u and pUC19 (Mead et al., 1986). The pTZ18thrA series contain the thr attenuator in the opposite orientation relative to the T7 promoter (anti-thr orientation) so that successive deletions of the polydeoxythymidine residues are followed by the dyad-symmetrical structure and a deoxyadenosine tract of six base pairs (Table I). Plasmid pTZ18thrA9 contains an extra five base pairs (AGTGC) that are absent in the pTZ18thrA8 to pTZ18thrA1 series (Table I).

Plasmids pTZ18thrA8(W), pTZ18thrA8(W), pTZ18thrA9(W), pTZ19t6(W), and pTZ18thrA9(M) were constructed by cloning synthetic duplex oligodeoxynucleotides into the HinII and XbaI sites of pUC19 and pUC18. One construct, pUC19A6T6(II) contains the thr attenuator with a reversed sequence of the loop-encoding portion of the template and a second construct, pUC19A6T6, contains the wild-type loop-encoding sequence. HindIII-EcoRI fragments from each construct were cloned into the HindIII and EcoRI sites of pTZ18u and pTZ18u to form pTZ19t1W(W), pTZ18A8(W), pTZ19t6(W), and pTZ18A9(M). The designations "W" and "M" indicate that the template encodes a wild-type or reversed loop respectively (Fig. 1a and b).

**RESULTS**

**Nomenclature**—The plasmids used in this study contain the thr attenuator and its variants inserted into the polylinker site of either plasmid pTZ18u or pTZ19u (Mead et al., 1986). The plasmids contain the T7 promoter and polylinker sites from pUC18 and pUC19 to facilitate RNA polymerase assays and further plasmid constructions. When cloned into pTZ19u, the thr attenuator is in its natural orientation with respect to the T7 promoter, and when cloned into pTZ18u, it is in the reversed orientation (anti-thr) (Fig. 1a and b).

**Termination with Variants Containing Successive Deletions in the Polydeoxyadenosine Region of the Anti-thr Attenuator**—The pTZ18thrA and pTZ19T series of plasmids contain nested deletions of the dA + dT tracts preceding and following, respectively, the dG + dC-rich region of dyad symmetry (Table I). The numbers after the pTZ18thrA and pTZ19T series plasmids designate the number of dA or dT residues, respectively, in the non-template strand of the DNA (the strand not complementary to the transcript). The plasmids of the pTZ19T series were constructed as successive deletions in the polydeoxyadenosine region of the thr attenuator (Table I), that occurs downstream of the dyad-symmetrical region. When in the natural orientation and if completely transcribed, these templates would produce a transcript with decreasing numbers of uridines in the terminated transcript. We previously tested these variants in a series of plasmids (pTZ19T1 to 8, Table I) with T7 RNA polymerase in vitro (Jeng et al., 1990). The results showed that T7 RNA polymerase requires an attenuator template with at least 5 continuous deoxyadenosine residues in order to begin termination, and that 8 or 9 are required for maximal termination.

The HindIII-XbaI fragments from pTZ19T series plasmids (Table I) were inserted into HindIII- and XbaI-digested pUC18 to form pTZ18thrA series of plasmids (see "Experimental Procedures"). The pTZ18thrA series plasmids contain the thr attenuator in a reversed orientation (anti-thr) relative to the pTZ19T series plasmids (Table I). In this orientation, these templates encode transcripts with a decreasing number of adenosines preceding the G + C-rich hairpin. The plasmids were linearized by HindIII and subjected to run-off transcription in vitro with T7 RNA polymerase (Fig. 2). As the number of adenosines in the transcript decreases so does the proportion of terminated transcript. The terminated transcripts (thr in Fig. 2) show successively shorter transcript lengths, differing by one base in length, as the number of deoxythymidine residues in the template that precede the dG + dC-rich region of dyad symmetry decreases from 9 to 1.

Effects of Template Sequence on T7 Transcription Termination

Fig. 1. Schematic representation of templates. Each template is shown as a line with the T7 promoter and thr attenuator shown as open and filled thickened segments. Important restriction sites are indicated above the lines. The segments downstream of the T7 promoter are represented for purposes of discussion as composites of discrete regions. The sequence of each region is listed in Table II. Regions 3, 4, and 5 represent the left part of the dyad symmetrical structure, the loop-encoding region, and the right part of the dyad symmetrical structure, respectively. Regions designated with an r indicate a reversed orientation for the corresponding region. Panel a, plasmids pTZ19T6(M) and pTZ19T6 (M) contain the thr attenuator without region 2, which is a 51-bp DNA from phage lambda (Table II). Panel b, plasmids pTZ19thrA6, pTZ19thrA8B, and pTZ19thrA8B contain the thr attenuator without region r-2. Panel c, plasmids pTZ19thr, pTZ19thr, pTZ19thr, and pTZ19thr contain the thr attenuator and region 2. Panel d, plasmids pTZ19thrA6, pTZ19thrA6B, and pTZ19thrA8B contain the anti-thr attenuator with region r-2. Panel e, plasmids pTZ19thr, pTZ19thr, and pTZ19thr contain the thr attenuator and reversed terminator. The termination efficiency ± standard deviation of each construct is indicated to the right of each designation.

Fig. 3 shows a comparison of termination efficiencies for pTZ18thrA and pTZ19T plasmids. As the number of template deoxythymidines preceding the hairpin increases from 1 to 9, the termination efficiencies of the pTZ18thrA plasmids increase, from 19 to 47% (Fig. 3). The pTZ18thrA1 plasmid containing only 1 template deoxythymidine terminates with an efficiency of 19%. In contrast, pTZ19T plasmids with up to 4 deoxyadenosines following the hairpin show no detectable termination. These results show that the presence of a deoxythymidine stretch preceding the dG + dC-rich region is not required for T7 RNA polymerase to terminate efficiently in vitro. However, the ratio of termination efficiencies for pTZ18thrA9 to pTZ18thrA1 (0.47:0.19) is approximately 2.5, indicating that the presence of the deoxythymidine residues assists termination. In contrast, the ratio of termination efficiencies of pTZ19t, the wild-type terminator, to pTZ19T (0.35:0.012) is approximately 29, a value which shows that the polydeoxyadenosine residues following the hairpin are more critical for efficient termination.

Interestingly, both pTZ18thrA6 and pTZ19T6 contain 6 deoxythymidine residues before and 6 deoxyadenosine residues after the dyad-symmetrical structure (Table I), but the termination efficiency of pTZ18thrA6 (29%) is nearly twice that of pTZ19T6 (15%). In terms of the structure of the product RNA, the stem regions of the thr hairpin are identical for both pTZ18thrA6 and pTZ19T6, but their loop regions differ (Table I). We can thus conclude that the difference in termination efficiencies likely results from either the region of the template encoding the loop to differences in more distal sequences. The sequences surrounding the attenuator in these constructs were changed as a consequence of the reversed cloning. We therefore constructed templates with these two regions independently varied to understand their relative contributions to termination.

VARIANTS IN THE LOOP-ENCODING TEMPLATE AND SURROUNDING SEQUENCES—Termination of E. coli RNA polymerase at the thr attenuator has been the subject of several studies (Gardner, 1982; Lynn et al., 1985, 1987, 1988; Yang and Gardner, 1988), using numerous variants that had been isolated by genetic or in vitro mutagenetic procedures. We introduced some of these thr attenuator variants into the pTZ plasmids to place them downstream of the T7 promoter in order to study termination by T7 RNA polymerase. The constructs were designed to permit us to analyze the functions of both the sequences surrounding the encoded G + C-rich hairpin and the loop-encoding portions of the template. To study these functions easily, DNA sequences in the poly linker location of pTZ plasmids were divided into different regions, and each region was designated by an Arabic numeral (Table II and Fig. 1). Table II lists the sequences in the wild-type and the reversed orientations of the thr attenuator. Fig. 1 shows schematic representations of templates, the numbers designating these regions, and their termination efficiencies.

The only difference between pTZ19T6(W) (wild-type) and pTZ19T6(M) (reversed) (Fig. 1a) is the orientation of the loop template sequence. Plasmid pTZ19T6(M) with a reversed-loop template (region r-4 in Fig. 1a) terminates with a higher efficiency than does pTZ19T6(W) with the wild-type loop template (region 4 in Fig. 1a) (29 versus 10%). Analogous results occur with pTZ18A6(M) and pTZ18A6(W) (24 versus 15%) (Fig. 1b). These results indicate that the loop sequence itself influences T7 RNA polymerase termination and that the template with the reversed loop is a better terminator. The differences between the sequences of pTZ19thrT6 (Fig. 1c) and pTZ19T6(W) (Fig. 1a) is in region 2 with a 51-bp DNA fragment from phage λ having been inserted in pTZ19thrT6. The termination efficiency of pTZ19thrT6 (53%) is 3-fold higher than that of pTZ19T6(W) (10%) (Fig. 1, a and c). This result indicates that, under some circumstances, some feature of the 51-bp promoter-proximal fragment enhances the termination of T7 RNA polymerase. Plasmids pTZ18A6(M) (Fig. 1b) and pTZ18thrA6 (Fig. 1d) are

The abbreviation used is: bp, base pair(s).
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The sequences of anti-thr attenuator in the pTZ18thrA series plasmids and thr attenuator in the pTZ19T series plasmids

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Sequence*</th>
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<tr>
<td>pTZ18thrA9</td>
<td>CTCATTAGTCGAAAGCAGCCGACCTGCAGTTCTCTAGAG...</td>
</tr>
<tr>
<td>pTZ18thrA8</td>
<td>CTCATTAGAAAGCAGCCGACCTGCAGTTCTCTAGAG...</td>
</tr>
<tr>
<td>pTZ18thrA6</td>
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<td>pTZ18thrA3</td>
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</tr>
<tr>
<td>pTZ18thrA2</td>
<td>CTCATTAGAAAGCAGCCGACCTGCAGTTCTCTAGAG...</td>
</tr>
<tr>
<td>pTZ18thrA1</td>
<td>CTCATTAGAAAGCAGCCGACCTGCAGTTCTCTAGAG...</td>
</tr>
</tbody>
</table>

* The nested deletion regions in pTZ18thrA and pTZ19T series of plasmids are indicated with the open and closed circles, respectively. The anti-thr attenuator already terminates effectively region 5-2. The core dyad symmetrical regions in the thr and anti-thr attenuator are indicated by dashed lines. The five extra bases in pTZ18thrA9 and pTZ19TT are indicated in boldface (see text).

The nested deletion regions in pTZ18thrA and pTZ19T series of plasmids are underlined, and the core dyad symmetrical regions in the thr and anti-thr attenuator are indicated by dashed lines. The five extra bases in pTZ18thrA9 and pTZ19TT are indicated in boldface (see text).

The sequences

The nested deletion regions in pTZ18thrA and pTZ19T series of plasmids are underlined, and the core dyad symmetrical regions in the thr and anti-thr attenuator are indicated by dashed lines. The five extra bases in pTZ18thrA9 and pTZ19TT are indicated in boldface (see text).

Fig. 2. Transcription from templates with deoxythymidine deletions. Transcription reactions used the pTZ18thrA derivatives (Table I) linearized with HindIII as templates for T7 transcription. The positions of the terminated transcripts of the terminated (thr) and the readthrough transcript (RT) are indicated. The lengths of terminated transcripts differ from each other and there are 81, 75, 73, 72, 71, 70, 69, and 68 bases for pTZ18thrA9, -A8, -A6, -A5, -A4, -A3, -A2, and -A1, respectively. Lane a, pTZ18thrA9; lane b, pTZ18thrA8; lane c, pTZ18thrA6; lane d, pTZ18thrA5; lane e, pTZ18thrA4; lane f, pTZ18thrA3; lane g, pTZ18thrA2; lane h, pTZ18thrA1.

The difference between pTZ19T6 (Fig. 1e) and pTZ19thrT6 (Fig. 1c) is in the sequence downstream of the thr attenuator. Plasmid pTZ19T6 contains the rrnC terminator (region 6) and pTZ19thrT6 contains part of the polylinker region (region 7). The termination efficiency of pTZ19T6 (15%) is approximately half that of pTZ19thrT6 (33%). These results indicate that the presence of the rrnC terminator segment decreases the termination at the upstream thr attenuator. The same conclusion also can be drawn from a comparison of the termination efficiencies of pTZ19tt (35%) (Fig. 1e) versus pTZ19thr (43%) (Fig. 1c) and pTZ19T8 (32%) (Fig. 1e) versus pTZthrT8 (46%) (Fig. 1c). The downstream rrnC terminator (region 6) shows the greatest effect in templates that contain 6 deoxyadenosine template residues. A comparison of the termination efficiencies of templates with 6 deoxyadenosine residues, pTZ19T6 (15%) and pTZ19thrT6 (33%), shows a 2-fold effect while templates with 9 deoxythymidine residues, pTZ19tt (35%) and pTZ19thr (43%), show a smaller relative difference. These findings imply that the termination enhancement caused by region 7 is small, and when the thr attenuator already terminates effectively region 7 cannot further enhance it.

Region 5–2 has five more bases (CGACT) than region 5–3 (Fig. 1c and Table II) as a result of the construction of the continuous deletion variants (“Experimental Procedures”). The termination efficiencies of pTZ19thrT8 with region 5–3
TABLE II
DNA sequences of the various regions in the pTZ plasmids used for this study

<table>
<thead>
<tr>
<th>Region*</th>
<th>Sequence³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AAAGTTGCAAGCTGTCAGGTC</td>
</tr>
<tr>
<td>2</td>
<td>GACGGATCTCCACTGCCAGTCGACGTCGAAGCTCGGGATCCCTCTAG</td>
</tr>
<tr>
<td>3</td>
<td>TACAGGAAACACAGAAAAGCCCGCAC</td>
</tr>
<tr>
<td>4</td>
<td>CGTCA</td>
</tr>
<tr>
<td>5-1</td>
<td>GTGCCGGTCTTTTT</td>
</tr>
<tr>
<td>5-2</td>
<td>GTGCCGGGCTTTTTTCGACT</td>
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<tr>
<td>5-3</td>
<td>GTGCCGGCTTTTTTTTTTTTT</td>
</tr>
<tr>
<td>5-4</td>
<td>GTGCCGGCTTTTTTTTTTCGACT</td>
</tr>
<tr>
<td>6</td>
<td>AGAGAAGATCCGGCAACGGGATGCCGTCGAGTTTGCAGGTGCGGGTTTTTGTCAG</td>
</tr>
<tr>
<td>7</td>
<td>ATCCCATACCTTCAAGCGAAGCTAAGGATTTTTCACGGCCTGGAGTTGGTCAGGAGCT</td>
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<tr>
<td>r-7</td>
<td>GAATTGACCTGTTCGTCACTGGGATCCCTTCTAG</td>
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<td>r-5-1</td>
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<td>r-5-2</td>
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<td>r-5-3</td>
<td>AAAAAAGCGCGAC</td>
</tr>
<tr>
<td>r-4</td>
<td>TGTCAAG</td>
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<tr>
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<td>r-2</td>
<td>CGCGTGCCTGTTCCTCCGGCTCGAGGGACTTTCGACAGTGGATTGGATCGTC</td>
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<tr>
<td>r-1</td>
<td>GACCTGAGCATGCAAGGTC</td>
</tr>
</tbody>
</table>

* The specific features of these regions are shown in Fig. 1.
³The sequences shown here indicate the template strand of the DNA.

and pTZ19thr78B with region 5-2 are the same (46%) (Fig. 1c), and the differences in termination efficiencies between pTZ18thrA8 with region r-5-3 and pTZ18thrA8B with region r-5-2 are small (38 versus 32%) (Fig. 1d). These results indicate that five extra bases of the given sequence in this location do not affect termination in either orientation. Taken together, these results suggest that the loop-encoding template and some sequences within regions 2 and 6 affect termination. However, other sequences, e.g. the additional CGACT in region 5-2, do not do so.

DISCUSSION

At least six sequence segments in or near the thr attenuator could affect termination by T7 RNA polymerase: remote sequences upstream of the thr attenuator, the template deoxythymidine stretch immediately preceding the dG + dC-rich region, the dG + dC-rich region itself, the region encoding the loop of the hairpin, the template deoxyadenosine tract immediately adjacent to the dG + dC-rich region, and sequences more distal to the termination site. Our previous studies (Jeng et al., 1990) indicated that both the integrity and sequence of the RNA hairpin formed by the dG + dC-rich region and the length of the uridine tract encoded by the distal deoxyadenosine stretch influenced termination of T7 RNA polymerase in vitro. Similar results concerning the RNA hairpin sequence dependence were recently reported for E. coli RNA polymerase in vitro (Cheng et al., 1991). Here, we find that the length of the deoxythymidine-rich stretch, upstream of the region of dyad symmetry, the template encoding the loop, and sequences both upstream and downstream of the thr attenuator also modulate T7 termination.

In the wild-type orientation, the length of the deoxyadenosine tract that encodes the uridine residues at the 3'-end of the transcript influences the frequency of termination (Jeng et al., 1990). Termination failed to occur when the length of the deoxyadenosine tract was from 1 to 4 residues in length. Upon the addition of more deoxyadenosine residues, the efficiency increased linearly as the length increased from 5 to 9 residues (Jeng et al., 1990) (Fig. 2). Interestingly, the anti-thr attenuator terminates with higher efficiency than the wild-type attenuator. The anti-thr attenuator has but 6 deoxyadenosines following the hairpin-encoding template in contrast to the wild-type thr attenuator with 9. Therefore, the number of deoxyadenosines in this region cannot itself solely determine the T7 RNA polymerase termination efficiency. In addition, Christiansen (1988) obtained similar results with T7 RNA polymerase and the rrnB1 terminator and indicated that sequences other than the template deoxyadenosines were involved in the termination process. The rrnB1 and anti-rrnB1 terminators encode G + C-rich hairpins that are identical except the anti-rrnB1 hairpin contains a C-A mismatch at a position where the rrnB1 hairpin contains a G-U pair. Both encode 4 hairpin-proximal adenines and hairpin-distal interrupted uridine tracts containing 7 uridines (5'-UUUCGUUUU-3'). T7 RNA polymerase terminates well at the rrnB1 terminator but is unable to abort at the anti-rrnB1 terminator (Christiansen, 1988). This result, along with those discussed in the introduction, shows that sequences other than those usually considered to constitute a classical terminator make important contributions to T7 RNA polymerase termination. Christiansen (1988) also suggested that T7 RNA polymerase termination might be sensitive to the hairpin loop sequence or to additional, unidentified RNA secondary structures.

The function of the deoxythymidine stretch preceding the dG + dC-rich region had not been previously examined with T7 RNA polymerase. Such a deoxythymidine-rich stretch is present in over one-third of the RNA independent terminators characterized (Brendel et al., 1986) suggesting that it may play a role in termination. The results of our study indicate that the deoxythymidine stretch is not as critical for termination by T7 RNA polymerase as is the downstream deoxyadenosine region in the thr attenuator. The presence of the template deoxythymidine stretch increases the termination efficiency of T7 RNA polymerase from 19% (1 deoxythymidine) to 45% (9 deoxythymidines) (Fig. 3). The competition occurring between the formation of the RNA hairpin and the RNA-DNA heteroduplex may offer an explanation for these results. During transcription elongation, a RNA-DNA heteroduplex occurs as RNA polymerase transcribes along the DNA template. The formation of the terminator RNA hairpin and the unstable dA-U stretch following it increases the probability of transcript release from the template (Parnham and Platt, 1980; Yager and von Hippel, 1987). The transcript
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adensine stretch, which is encoded by the template deoxythymidine stretch preceding the G + C-rich region has the potential to form base pairs with the uridine stretch encoded by the region containing the template deoxycytidines distal to the G + C-rich region. Such base pairing would thereby extend the length and stability of the stem of the G + C-rich RNA hairpin. Because these adenosine residues may compete for 3'-terminal uridine residues that are paired with dA residues in the DNA template, an increased potential for the formation of A-U base pairs could decrease the strength or potential for forming of base pairs between dA and U, i.e., between the transcript and template, which could consequently facilitate transcript release from the DNA template in accord with the model of Yager and von Hippel (1987). Our observations may also be rationalized to fit another model for termination that involves shorter transcript-DNA heteroduplexes and a greater role for enzyme-nucleic acid interactions (Surratt et al., 1991) by postulating that the potentially longer hairpin affects either of the two elements of the model to enhance cessation of synthesis.

Wright et al. (1992) reported that the deoxythymidine stretch preceding the dG + dC-rich region of a terminator also affects the termination efficiency of E. coli RNA polymerase. They deleted the DNA template d(T): stretch preceding the dG + dC-rich region of the a-operon terminator, t517, and the mutant terminator caused 6-fold more readthrough than the wild-type sequence with E. coli RNA polymerase in vivo (Wright et al., 1992). Therefore, they suggested that the deoxythymidine stretch encodes adenosines that associate with complementary RNA uridines to help unzip the 3'-end of the RNA from its template DNA. Their explanation, which was based on the results from E. coli RNA polymerase, is consistent with our results with T7 RNA polymerase. In contrast, Yang et al.1 reported that the template deoxythymidine stretch does not significantly modulate termination at the thr attenuator with E. coli RNA polymerase. A consideration of the potential contribution of the extension of the hairpins by the formation of A-U base pairs may account for the opposite behaviors of the E. coli enzyme at the two terminators. The t517 terminator falls in the short stem class of ρ-independent structures described by d'Aubenton Carafa et al. (1990), whereas the thr attenuator is in the long stem class; the G + C-rich stems of the two terminators contain five and eight base pairs, respectively. The addition of seven A-U base pairs to the t517 stem to form a total of 12 base pairs could contribute a greater differential termination capability than the increment of six A-U base pairs to the eight base pair core of the thr attenuator. Finally, the difference in the results with the bacteriophage and bacterial enzyme at the thr attenuator indicate the importance of the interaction between a specific RNA polymerase and a given terminator in setting termination efficiency.

The requirement for an RNA hairpin in the termination of ρ-independent terminators has been dogma for years (Farnham and Platt, 1980; Yager and von Hippel, 1987). However, the function of the loop in the hairpin had not yet been widely studied. Here, by comparing the termination efficiencies of pTZ19T6(W) with pTZ19T6(M) and pTZ18T6(W) with pTZ18T6(M) (Fig. 1, a and b), we find that thr attenuators in which the loop orientation reversed terminate more efficiently than those with the wild-type loop orientation. Qualitatively similar results also were obtained from transcription termination experiments with E. coli RNA polymerase.1 Two possibilities may explain why both RNA polymerases favor one loop orientation over another.

First, the loop sequence may act indirectly by altering the stability of the G + C-rich hairpin. Tuerk et al. (1998) showed that RNA hairpins containing a loop with the sequence UUGC is more stable than a loop with the sequence UUGG. Also, an RNA hairpin with a UUGC loop forms an unusually stable structure (Antao et al., 1991). The reversed-loop sequence of the thr attenuator is UGUCAG (Fig. 1, a and b), and thus contains a disrupted UUGC sequence. Whether or not this interrupted sequence similarity is the cause, the thr hairpin with the reversed loop may be more stable than the one with the wild-type loop, and thus the increased stability of the hairpin may contribute to the higher termination efficiencies of the T7 and E. coli RNA polymerases. Grebe and Uhlenbeck (1988) have demonstrated that the loop size affects the stability of an RNA hairpin when tested with varying length homopolymeric loops. Because the size of both the wild-type and reversed loops in our study is identical, the contribution of size as opposed to sequence on the stability of the RNA hairpin should be similar. Hence, a direct interaction between RNA polymerase and the template encoding the loop or the RNA loop itself may provide a second possible explanation for the effect of a given loop orientation.

Sequences linked to prokaryotic promoters affect the efficiency of downstream termination by E. coli RNA polymerase (Telesnitsky and Chamberlin, 1989a; Goliger et al., 1988; Reynolds and Chamberlin, 1992). Telesnitsky and Chamberlin (1989a) suggested that the conformation of E. coli RNA polymerase changes during transcription depending upon the sequence of the DNA template transcribed and that the conformationally altered polymerases may terminate differently at a downstream terminator. A 51-bp fragment (region 2 in Table II, Fig. 1, c and e) from bacteriophage λ was used as a linker between BamHI and MluI sites that were used during the construction of the plasmid pTZ19tt (Jeng et al., 1990), and its presence increased the termination of T7 RNA polymerase unexpectedly (pTZ19T6(W) (10%) (Fig. 1a) versus pTZ19thrT6 (33%) (Fig. 1c)). As hypothesized for E. coli RNA polymerase, the conformation of the phage polymerase may be altered when it reads through this 51-bp region, and its termination potential at the downstream thr terminator may be affected similarly. Alternatively, some feature of the DNA of this 51-bp fragment or its RNA transcript may interact with the enzyme during the termination event to alter its efficiency.

T7Te and T3Te are natural terminators in their respective bacteriophages and terminate transcription by E. coli RNA polymerase with efficiencies of 74 and 9%, respectively (Telesnitsky and Chamberlin, 1989b). Transcription with hybrid terminator templates, in which the downstream sequence of T7Te was changed to that of T3Te, revealed that the termination efficiency of hybrid T7Te by E. coli RNA polymerase was increased from 74 to 7%. Since the structures of the G + C-rich region of dyad symmetry and the template polyanucleotide tract were unchanged in the hybrid terminator, the difference in termination efficiencies could only have been due to the downstream sequences (Telesnitsky and Chamberlin, 1987b). Lee et al. (1990) also suggested that not-yet-transcribed DNA templates affect the pausing in upstream DNA sequences with E. coli RNA polymerase. In our study, constructs containing the rrrC terminator in region 6 (Table II) decreased the termination efficiency of the upstream thr terminator (compare pTZ19tt to pTZ19thr, pTZ19T8 to pTZ19thrT8, and pTZ19T6 to pTZ19thrT6) (Fig. 1, c and e). Therefore, explanations of ρ-independent terminator efficiency in vitro with T7 RNA polymerase must also consider the template sequence downstream of the terminator. Such observations with either enzyme are puzzling for this region...
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of the template has not been, nor will it ever be, transcribed in the terminated transcripts. Effects of these downstream sequences upon the unwinding of the upstream template strands to expose them for transcription or interactions of the enzyme with those regions may play roles in this effect. With another terminator Lee et al. (1990) argue against unwinding as an explanation for the downstream effects seen with E. coli RNA polymerase.

A five-base sequence, CGACT, immediately downstream from the template polydeoxyadenosine tract of the thr terminator is present in region 5-2 but not in region 5-3 (Table I and Fig. 1c). These five bases have no effect on the termination of T7 RNA polymerase at the thr terminator, and each of these regions also affects the termination efficiency of E. coli RNA polymerase. Each of these regions also affects the termination by the T7 enzyme.

In this and our previous report (Jeng et al., 1990), we have demonstrated that all six segments of the thr terminator, i.e. the promoter-linked sequence, the proximal deoxynucleotide region, the G + C-rich dyad-symmetrical region, the template encoding the loop, the distal deoxyadenosine stretch, and yet another distant deoxycytidine stretch, affect termination by T7 RNA polymerase. Each of these regions also affects the termination efficiency of E. coli RNA polymerase at the thr terminator and other ρ-independent terminators (Telesnitsky and Chamberlin, 1989a, 1989b; Goliger et al., 1989). It is difficult to understand how some of these template changes can have direct effects on the RNA structure encoded by the attenuator or upon DNA-RNA interactions, and thereby offer an explanation for the altered termination efficiencies.

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