Release of Transcript and Template during Transcription Termination at the trp Operon Attenuator*

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We studied release of trp leader RNA and trp template DNA from RNA polymerase during transcription termination at the attenuator of the trp operon of Escherichia coli. Preliminary evidence had suggested that a stable ternary complex was formed at the trp attenuator. We observed that the complexes between RNA polymerase and trp leader RNA and the DNA template produced during transcription were labile at high salt concentrations and were undetectable when transcription was performed in the presence of heparin. These characteristics are atypical of the stable transcription termination complexes described by others (Richardson, J. P., and Conaway, R. (1980) Biochemistry 19, 4293-4299; Shigesada, K., and Wu, C. (1980) Nucleic Acids Res. 8, 3355-3369). We successfully reconstituted polymerase-trp leader RNA complexes in simple mixing experiments; these and other studies indicated that it is core polymerase that binds the leader transcript and the DNA template. In agreement with this conclusion, it was observed that a factor inhibited binding of RNA polymerase to the trp leader transcript and the DNA template and displaced leader RNA from RNA polymerase during transcription. It seems likely that small amounts of core polymerase present in the holoenzyme preparation, or generated during transcription, are responsible for the nonspecific binding of RNA transcript and DNA template. Our findings, therefore, suggest that the transcription termination event at the trp attenuator normally involves spontaneous dissociation of polymerase, template, and RNA transcript.

The trp operon of Escherichia coli contains an attenuator preceding the structural genes of the operon. The attenuator is an optional transcription termination site that is used in the regulation of transcription of the operon. Studies on attenuation have elucidated many of the molecular events involved in transcription termination and its control (1, 2). However, it was not known whether termination at the attenuator generates a stable termination complex that requires protein factors to dissociate or whether the termination release event is spontaneous. In this report we present an analysis of the transcription termination event at the trp attenuator.

In an in vitro transcription system consisting of RNA polymerase, nucleoside triphosphates, and a DNA restriction fragment containing the trp leader region of E. coli, 96% of the transcribing polymerase molecules terminate transcription at the attenuator, producing a transcript 140 nucleotides in length (3). Polymerase molecules that transcribe through the attenuator continue to the end of the fragment and synthesize a read-through transcript (Fig. 1). Previous studies suggested that the leader transcript and DNA template remained stably associated with RNA polymerase in a ternary termination complex that formed at the attenuator (4). To examine this conclusion, transcription complexes produced in vitro were analyzed by measuring retention of the leader transcript and DNA template on nitrocellulose filters. Our findings demonstrate that the complexes formed in vitro during transcription are not stable. Rather, core polymerase generated during transcription elongation or present in the holoenzyme preparation appears to bind nonspecifically and weakly to the leader transcript and DNA template resulting in their retention on filters. Addition of heparin or purified a factor to the in vitro reaction mixture prevents this nonspecific interaction, suggesting that RNA release and dissociation of RNA polymerase from the DNA template occur spontaneously during transcription termination at the attenuator.

MATERIALS AND METHODS

Preparation of Restriction Fragments Containing the trp Operon Regulatory Region—Plasmids containing the promoter/operator and leader regions of the trp operon (trpP0L) were prepared as described previously (5) and were purified by banding once to equilibrium in cesium chloride-ethidium bromide gradients. The Sau3A-490 restriction fragment used contains the trpP0L region of E. coli; this fragment was isolated from pBN60. Restriction fragments were prepared by cleavage of plasmid DNA with the restriction endonuclease Sau3A (Bethesda Research Laboratories) and electrophoresis of the DNA digest through a 7% acrylamide gel (acylamide-to-bis ratio of 30 to 2.8) and elution of the fragments from the gel as described by Manax and Gilbert (7). The DNA concentration was determined by the rapid estimation technique of Davis et al. (8). Where indicated in figure legends, restriction fragments were labeled at their 3'-end using 15 pCi of [α-32P]dATP or [α-32P]dCTP (specific activity of 2000-3000 Ci/mmol) (Amersham Corp.) by extension of the 3'-recessed ends with the Klengen fragment of DNA polymerase (New England Biolabs).

Isolation of trp Leader RNA—trp leader [α-32P]RNA was isolated from standard in vitro transcription reactions. 25-30 reactions contained either 40 μg/ml of pBN60 or 4 μg/ml of the Sau3A-490 restriction fragment, 22 μg/ml of RNA polymerase, 20 μM GTP, 30 μC of [α-32P]dATP or [α-32P]dCTP (specific activity of 2000-3000 Ci/mmol) (Amersham Corp.) by extension of the 3'-recessed ends with the Klengen fragment of DNA polymerase (New England Biolabs).

Transcription Reactions—Reaction mixtures contained transcription buffer (20 mM Tris-HCl, pH 7.5, 0.1 mM Na2 EDTA, 0.1 mM dithiothreitol, 4 mM MgCl2, 150 mM KCl, 5% glycerol, 20 μg/ml of bovine serum albumin), 150 μM each ATP, CTP, and UTP. Preparative reactions were incubated for 30 min, stopped by the addition of an equal volume of stop buffer (9), and loaded directly on a 7% acrylamide gel containing 7 M urea. RNA was detected by autoradiography and eluted from gel slices as described above for the elution of DNA fragments.

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1 F. Lee, M. Winkler, and C. Yanofsky, unpublished results.
2 B. Nichols, unpublished results.
and [α-32P]GTP. The concentrations of RNA polymerase and DNA used in reactions are indicated in figure legends. Multiple round transcription reactions were initiated by adding RNA polymerase to the reaction mixture at 0 °C and transferring the reaction mixtures to 30 °C. Reaction mixtures were incubated for various periods at 30 °C and were stopped by the addition of rifampicin. This ensured that RNA polymerase molecules engaged in RNA chain elongation could complete a transcription cycle. Aliquots were removed from the reaction at various times and filtered through nitrocellulose filters. Single-round transcription reactions were performed as described previously (10).

The core and holoenzyme RNA polymerase preparations employed were purified from E. coli MR6600 as described (11). σ factor was purified from holoenzyme by the method of Lowe et al. (12). Holoenzyme, core RNA polymerase, and σ factor were generously provided by Robert Fisher (Stanford University). The dilution buffer for σ factor contained 10 mM Tris-HCl, pH 7.9, 0.1 mM Na2EDTA, 0.1 mM dithiothreitol, 0.5 M NaCl, and 50% glycerol.

Various quantities added to transcription reactions included heparin (Invenex), tRNA (Sigma), and purified trp leader RNA. Prior to use, tRNA was phenol extracted, ethanol precipitated, and redissolved in 0.1 mM Na2EDTA and 10 mM Tris-HCl, pH 7.9.

**Reconstitution of RNA Polymerase-trp Leader RNA Complexes**—Mixing experiments performed to reconstitute complexes between RNA polymerase and trp leader RNA were conducted under the standard transcription reaction conditions described above. In reactions containing DNA, ATP, GTP, and TTP were omitted to preclude formation of RNA polymerase complexes containing DNA, ATP, and GTP. The filters were pretreated in 0.4 M KC1 for 20 min at 22 °C, washed, and stored at 4 °C. Immediately before use filters were warmed to 30 °C.

The filter-binding protocol was a modification of the procedure described by Hinkle and Chamberlin (13). 5-μl aliquots from transcription or mixing reactions were added to an equal volume of stop buffer (9) or to 1 ml of filtration buffer (20 mM Tris, pH 7.9, 0.1 mM Na2EDTA, 0.1 mM dithiothreitol, 4 mM MgCl2, 20 μg/ml of bovine serum albumin, and 150 mM KC1, unless indicated otherwise) prewarmed over the course of the experiment. Retention was not due to nuclease action. When the in vitro transcription reaction was heated to 90 °C for 2 min and then filtered, neither RNA nor DNA was retained (Fig. 2, lane 7), suggesting that transcript and template are bound to RNA polymerase in its native conformation.

**Effect of Ionic Strength on Complex Stability**—Richardson (14, 15) has shown that ternary transcription elongation and termination complexes that form at certain sites in bacteriophage T7 DNA are, while in 0.5 M KC1, whereas binary complexes between RNA polymerase and RNA or DNA are not. To characterize the nature of the complexes formed between RNA polymerase and the trp leader transcript, we analyzed the effect of ionic strength on their stability. The KC1 concentration of the filtration buffer and the transcription reaction was 150 mM, greater than 70% of nascent leader RNA was retained on filters. However, raising the KC1 concentration of the filtration buffer above 150 mM markedly decreased RNA retention. At 0.5 M KC1 no RNA was filter-bound (Fig. 3). These findings established that RNA polymerase-trp leader RNA complexes are salt-labile.

**The Effect of Heparin on Complex Formation**—Heparin is a potent inhibitor of transcription initiation (16). Current evidence suggests that heparin interacts with RNA polymerase at the RNA product site and acts by disrupting the association of the σ and β subunits of RNA polymerase (17). To analyze the effect of this polyanion on complex formation, heparin was added along with the nucleoside triphosphates when the transcription reaction was started. After heparin addition, only those RNA polymerase molecules prebound to the template could initiate transcription. In the control reaction rifampicin was added in lieu of heparin to restrict transcription to one cycle. Fig. 4 illustrates that heparin completely eliminated the retention of leader and read-through RNAs and the DNA template. The possibility that heparin directly disrupts a termination complex seems unlikely, since it does not dissociate RNA polymerase from T7 transcripts at sites at which ρ factor is required for release (15, 18). Like heparin, tRNA added to a transcription reaction eliminated RNA polymerase binding to nascent leader RNA and the DNA template (data not shown). These findings suggested that heparin and tRNA blocked a site or sites on RNA polymerase that could bind leader RNA and the DNA template.

**Reconstitution of Complexes between RNA Polymerase and the Leader Transcript**—The instability of complexes in high salt and the inhibition of complex formation by heparin suggest that the leader transcript binds nonspecifically to RNA polymerase. To examine this possibility we compared retention of nascent leader RNA in a transcription reaction to that of purified leader RNA added to a transcription reaction

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3 Under the conditions we generally used in in vitro reactions approximately 3 to 10% of the template molecules were transcribed.

4 M. Winkler and C. Yanofsky, unpublished data.
Retention of nascent trp leader and read-through RNA transcripts and trp DNA template on nitrocellulose filters. A 40-µl transcription reaction containing 0.5 µg/ml of 3'-end labeled Sau3A-490 DNA fragment (30,000 cpm), 30 µCi of [α-32P]GTP, and 13.5 µg/ml of RNA polymerase was incubated for 26 min at 30 °C. 10 µg/ml of rifampicin was added to stop initiation. 5-µl aliquots were removed at various times after rifampicin addition and were added either to an equal volume of stop buffer (9) or were filtered through a nitrocellulose filter. Nucleic acid was eluted from filters. Filtered and unfiltered samples were analyzed by gel electrophoresis. The radioactivity in bands corresponding to leader transcript was measured. The amount of leader transcript was eluted from filters. Filtered and unfiltered samples were analyzed by gel electrophoresis. The radioactivity in bands corresponding to leader transcript was measured.

Retention of purified and nascent leader RNAs differed only slightly; approximately 44 and 53% were filter-bound, respectively (results not shown). In a reaction mixture lacking nucleoside triphosphates, complexes formed between RNA polymerase and purified leader RNA were stable for more than 3 h. Omission of DNA from the reaction had no effect on leader RNA filterability (Fig. 5). These results not only demonstrated that complexes can form between RNA polymerase and free leader RNA but also suggested that complex formation and transcription termination are independent events.

Whether complexes form between RNA polymerase and purified leader RNA at RNA polymerase/RNA ratios characteristic of the in vitro transcription reaction was also examined. In the in vitro transcription reactions, approximately one leader transcript is synthesized for every 50 to 100 RNA polymerase molecules in the reaction. Under these conditions 40 to 60% of the nascent leader transcripts bind to RNA polymerase. These values correlate with the retention of purified leader RNA. At RNA polymerase/RNA ratios between 50 and 100, 39 to 46% of the purified leader transcripts added to reactions lacking DNA were filter-bound (Fig. 6). At high RNA polymerase/RNA ratios (≥100), the amount of nascent and purified leader RNA retained on filters varied between 60 to 90% and 50 to 80%, respectively.

The retention of leader transcripts at high RNA polymerase/RNA ratios suggests that contaminating core polymerase in the holoenzyme preparation may be responsible for the formation of nonfilterable complexes. Although our enzyme preparation contained stoichiometric amounts of α according to conventional assays, these assays do not detect modest quantities of contaminating core polymerase.

Fig. 2. Retention of nascent trp leader and read-through RNA transcripts and trp DNA template on nitrocellulose filters. A 40-µl transcription reaction containing 0.5 µg/ml of 3'-end labeled Sau3A-490 DNA fragment (30,000 cpm), 30 µCi of [α-32P]GTP, and 13.5 µg/ml of RNA polymerase was incubated for 26 min at 30 °C. 10 µg/ml of rifampicin was added to stop initiation. 5-µl aliquots were removed at various times after rifampicin addition and were added either to an equal volume of stop buffer (9) or were filtered through a nitrocellulose filter. Nucleic acid was eluted from filters. Filtered and unfiltered samples were analyzed by gel electrophoresis. The radioactivity in bands corresponding to leader transcript was measured. From the specific radioactivities of [α-32P]GTP and the [32P]-labeled RNA fragment (Sau3A-490) was measured. From the specific radioactivities of [α-32P]GTP and the [32P]-labeled RNA fragment (Sau3A-490) was measured. From the specific radioactivities of [α-32P]GTP and the [32P]-labeled RNA fragment (Sau3A-490) was measured. From the specific radioactivities of [α-32P]GTP and the [32P]-labeled RNA fragment (Sau3A-490) was measured. From the specific radioactivities of [α-32P]GTP and the [32P]-labeled RNA fragment (Sau3A-490) was measured. From the specific radioactivities of [α-32P]GTP and the [32P]-labeled RNA fragment (Sau3A-

Fig. 3. Effect of KCl concentration on trp leader RNA-RNA polymerase complexes. 25-µl transcription reactions containing 0.4 µg/ml of unlabeled Sau3A-490 DNA fragment, 15 µCi of [α-32P]GTP, and 13.5 µg/ml of RNA polymerase were incubated and stopped as described in Fig. 2. 5-µl aliquots were removed from reactions 20 min after rifampicin addition and were added directly to an equal volume of stop buffer (9) or to filtration buffer containing various concentrations of KCl. The latter were filtered through nitrocellulose. RNA was eluted from filters. Filtered and unfiltered samples were analyzed by gel electrophoresis. The radioactivity in bands corresponding to the leader transcript was measured. The amount of leader RNA was calculated as a percentage of the total amount of leader RNA synthesized in the sample. The x axis refers to the KCl concentration of the filtration buffer.

25-µl transcription reactions containing 0.4 µg/ml of unlabeled Sau3A-490 DNA fragment, 15 µCi of [α-32P]GTP, and 13.5 µg/ml of RNA polymerase were incubated and stopped as described in Fig. 2. 5-µl aliquots were removed from reactions 20 min after rifampicin addition and were added directly to an equal volume of stop buffer (9) or to filtration buffer containing various concentrations of KCl. The latter were filtered through nitrocellulose. RNA was eluted from filters. Filtered and unfiltered samples were analyzed by gel electrophoresis. The radioactivity in bands corresponding to the leader transcript was measured. The amount of leader RNA was calculated as a percentage of the total amount of leader RNA synthesized in the sample. The x axis refers to the KCl concentration of the filtration buffer.

| Time (min) | L  | RT | DNA |
|-----------|----|----|-----|
| 10        | 9.0| 0.9| 12.5|
| 15        | 8.9| 0.6| 12.7|
| 21        | 10.0| 0.8| 11.7|
| 33        | 9.1| 0.9| 12.9|
| 45        | 11.0| 0.9| 12.9|
| average   | 9.6| 0.8| 12.5|

RNA or DNA Bound
pmoles (x10^-5)

Time (min)
L  RT  DNA

Average values
9.6  0.8  12.5

Effect of KCl concentration on trp leader RNA-RNA polymerase complexes. 25-µl transcription reactions containing 0.4 µg/ml of unlabeled Sau3A-490 DNA fragment, 15 µCi of [α-32P]GTP, and 13.5 µg/ml of RNA polymerase were incubated and stopped as described in Fig. 2. 5-µl aliquots were removed from reactions 20 min after rifampicin addition and were added directly to an equal volume of stop buffer (9) or to filtration buffer containing various concentrations of KCl. The latter were filtered through nitrocellulose. RNA was eluted from filters. Filtered and unfiltered samples were analyzed by gel electrophoresis. The radioactivity in bands corresponding to the leader transcript was measured. The amount of leader RNA was calculated as a percentage of the total amount of leader RNA synthesized in the sample. The x axis refers to the KCl concentration of the filtration buffer.
were eluted from filters. Filtered and unfiltered samples were analyzed.

Both reactions were incubated at 30 °C. Thereafter, 5-μl aliquots were removed from reactions and were either added to an equal volume of other reaction along with UTP and CTP to initiate transcription.

One reaction and 0.4 mg/ml of heparin (1.25 units) was added to the DNA template (Sau3A-490) are indicated. RNA and DNA from reactions lacking heparin were alternately removed from reactions and were filtered in 3-min intervals beginning 12 min after the start of transcription. Lanes containing DNA were applied directly to Whatman 3MM filters. Aliquots were filtered through nitrocellulose. Aliquots were removed alternately from reactions and were filtered in 3-min intervals beginning 12 min after the start of transcription. Lanes were added to stop buffer 10, 35, 11, and 36 min after the start of transcription. Lanes of purified RNA polymerase-trp leader RNA (12,000 cpm); RNA polymerase/RNA ratio was 50. 5 μg/ml of the Sau3A-490 DNA fragment was added to one reaction; DNA dilution buffer (1 mM Tris, pH 7.9, 0.1 mM NaCl, EDTA) was added to the control reaction. ATP and GTP were omitted. Reactions were started by the addition of RNA polymerase and transfer of the reaction mixture from 0 to 30 °C. Thereafter, 5-μl portions were removed from reactions and were analyzed as described in the legend to Fig. 5. Each point is an average of 3 determinations.

The Effect of σ Factor on Complex Formation—To determine whether leader RNA binds to residual amounts of core polymerase in the holoenzyme preparation, the effect of added σ factor on complex formation was examined. Various amounts of σ were added to reactions containing core polymerase or holoenzyme. In reactions containing either enzyme preparation, the proportion of leader RNA retained on filters decreased directly as a function of the σ/polymerase ratio. However, inhibition of complex formation required higher amounts of σ/RNA polymerase ratios in reactions containing core polymerase than in reactions containing holoenzyme. The difference between the curves in Fig. 7 indicated that the molar amount of added σ required to inhibit complex formation depended on the fraction of RNA polymerase molecules that were unretained by filters.
Polymerase complexes during transcription at various u/RNA ratios. To one reaction, 12 μg/ml of σ factor was added. To the other reaction σ dilution buffer was added. To start the reactions, UTP, CTP, and 10 μg/ml of rifampicin were added. Reactions were incubated for 20 min at 30 °C. 5-μl aliquots were removed from reactions and were either added to an equal volume of stop buffer (9) or were filtered through nitrocellulose. Aliquots were removed alternately from reactions and were filtered at 3-min intervals beginning 18 min after the addition of rifampicin. RNA and DNA were eluted from filters. Filtered and unfiltered samples were analyzed by gel electrophoresis. Lanes 1, 5, 6, 10, RNA and DNA in unfiltered aliquots added directly to stop buffer 14, 34, 15 and 35 min, respectively, after the start of transcription. Lanes 2-4 and 7-9, RNA and DNA from reactions lacking or containing σ, respectively, and retained on nitrocellulose filters. Leader (L) and read-through (RT) transcripts and the DNA template (Sau3A-490) are indicated. RNA and DNA from filtered and unfiltered samples lacking σ (−σ) or containing σ (+σ).

Fig. 8. The effect of σ factor on the formation of trp leader RNA and read-through RNA-RNA polymerase complexes during transcription. Two 25-μl single-round transcription reactions contained 8 μg/ml of the 3′-end labeled Sau3A-490 DNA fragment (20,000 cpm), 15 μCi of [α-32P]GTP, and 22 μg/ml of RNA polymerase. To one reaction, 12 μg/ml of σ factor was added. To the other reaction σ dilution buffer was added. To start the reactions, UTP, CTP, and 10 μg/ml of rifampicin were added. Reactions were incubated for 20 min at 30 °C. 5-μl aliquots were removed from reactions and were either added to an equal volume of stop buffer (9) or were filtered through nitrocellulose. Aliquots were removed alternately from reactions and were filtered at 3-min intervals beginning 18 min after the addition of rifampicin. RNA and DNA were eluted from filters. Filtered and unfiltered samples were analyzed by gel electrophoresis. Lanes 1, 5, 6, 10, RNA and DNA in unfiltered aliquots added directly to stop buffer 14, 34, 15, and 35 min, respectively, after the start of transcription. Lanes 2-4 and 7-9, RNA and DNA from reactions lacking or containing σ, respectively, and retained on nitrocellulose filters. Leader (L) and read-through (RT) transcripts and the DNA template (Sau3A-490) are indicated. RNA and DNA from filtered and unfiltered samples lacking σ (−σ) or containing σ (+σ).

Fig. 9. Formation of trp leader RNA- and trp DNA-RNA polymerase complexes during transcription at various σ/RNA polymerase ratios. 25-μl single-round transcription reactions contained 8 μg/ml of the 3′-end labeled Sau3A-490 DNA fragment (25,000 cpm), 5 μCi of [α-32P]GTP, 22 μg/ml of RNA polymerase, and various concentrations of σ factor. Reactions were started by the addition of UTP, CTP, and 10 μg/ml of rifampicin and were incubated for 20 min at 30 °C. 5-μl aliquots were removed from reactions and were added to an equal volume of stop buffer (9) or were filtered through nitrocellulose. RNA and DNA from filtered and unfiltered aliquots were analyzed by gel electrophoresis. The radioactivity in bands corresponding to the leader transcript and DNA template was measured to determine the molar amounts of DNA and RNA retained on filters. Each point is an average of 2 determinations. RNA polymerase-trp leader RNA (●) and –trp DNA (●) complexes.

In view of the above results, σ factor was also added to preformed complexes between RNA polymerase and leader RNA. In a reaction in which 83% of the leader transcripts were complexed with RNA polymerase and retained on filters, addition following complex formation reduced RNA retention 71% (results not shown). When σ was added during a transcription reaction, binding of RNA polymerase to the nascent leader and read-through transcripts was also inhibited. At an added σ/RNA polymerase ratio of 3, only traces of either RNA transcript were filter-bound (Fig. 8). The radioactivity in the Sau3A-490 fragment in this experiment (Fig. 8) was too low to analyze the effect of σ on DNA retention. However, an effect of σ factor on DNA retention was apparent when the specific radioactivity of the DNA template added to the transcription reaction was increased more than 10-fold. As Fig. 3 illustrates, increasing the σ/RNA polymerase ratio from 0 to 3.2 coordinately reduced the retention of leader RNA and the DNA template.

It is noteworthy that a molar excess of σ factor decreased the overall efficiency of the in vitro transcription reaction 50% (Fig. 8). The effect of σ on transcription efficiency depended on the σ concentration in the reaction. At a σ/RNA polymerase ratio of 3, decreasing the σ concentration from 12 μg/ml to 6 μg/ml reduced the inhibition of transcription by σ (results not shown).

Discussion

Preliminary in vitro studies suggested that when RNA polymerase terminates transcription at the trp operon attenuator, the RNA transcript, DNA template, and RNA polymerase form a ternary complex. However, attempts to determine the DNA segment protected by RNA polymerase molecules that terminated transcription at the attenuator, using standard “footprinting” (20) techniques and DNA protection studies, were unsuccessful. Subsequent studies, reported herein, demonstrate that complexes formed between RNA...
polymerase and the leader transcript and DNA template differ from some other ternary termination complexes in several respects. Whereas some transcription elongation and termination complexes have been reported to be stable in 0.5 M KCl (14, 15), trp leader RNA-RNA polymerase complexes dissociate at KCl concentrations above 150 mM. Furthermore, although stable ternary complexes that form at certain termination sites in bacteriophage T7 are resistant to the action of heparin (15, 18), complexes formed between RNA polymerase and trp leader RNA and the trp DNA template are not.

In reconstitution experiments RNA polymerase formed nonfilterable complexes with purified leader RNA at RNA polymerase/RNA ratios characteristic of the in vitro transcription reaction. $\sigma$ factor and tRNA inhibited RNA polymerase from binding to both nascent and purified leader RNA. By these criteria, reconstituted RNA polymerase-leader RNA complexes are analogous to those that form during transcription suggesting that complex formation does not depend on the event of transcription termination. During transcription termination RNA polymerase does not protect any specific segment of DNA in the attenuator region from endonuclease digestion providing additional evidence that a specific termination complex does not form at the attenuator.

The effect of $\sigma$ on complex formation supports the interpretation that core polymerase is responsible for the nonfilterability of leader RNA and the DNA template. $\sigma$ addition can prevent complex formation as well as displace leader RNA from RNA polymerase. These findings suggest that $\sigma$ may directly or indirectly block a site or sites on core RNA polymerase that can bind RNA or DNA. Why a molar excess of $\sigma$ is required to inhibit RNA polymerase from binding to the leader transcript and DNA template is unclear. One possible explanation is that binding of $\sigma$ to core polymerase is not optimal in the in vitro transcription reaction.

Based on these results, we propose that RNA polymerase terminates transcription at the trp operon and releases the leader transcript and DNA template. No accessory factors appear to be required for this release. $\rho$ and L factor (also called nusA protein), two proteins that facilitate transcription termination at other sites on E. coli DNA (21, 22), have no effect on the formation or stability of "complexes" between RNA polymerase and the leader transcript and DNA template.9 These findings conflict with the interpretation of experiments in which $\rho$ factor stimulates the synthesis of trp leader RNA in vitro (4). We believe that the explanation for this observation may be that $\rho$ competes with RNA polymerase for RNA binding and, in so doing, it frees some polymerase molecules for additional rounds of transcription.

In vivo in E. coli the molar amount of $\sigma$ varies between 30 to 60% that of core polymerase and correlates with the estimated molar amount of unbound RNA polymerase (23). Thus, there appears to be sufficient $\sigma$ to saturate all free polymerase molecules. If these estimates are correct, one important in vivo function of $\sigma$ may be to prevent core polymerase from binding nonspecifically to DNA and nascent RNA. It is also possible, however, that other factors present in vivo function in this capacity. For example, basic proteins may bind to RNA and DNA and decrease their affinity for RNA polymerase. By inhibiting the formation of nonspecific RNA polymerase-nucleic acid complexes, $\sigma$ and other regulatory proteins may increase the proportion of RNA polymerase molecules available for transcription.

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