Coupling between Transcription Termination and RNA Polymerase Inchworming

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Summary

Advancement of RNA polymerase of E. coli occurs in alternating laps of monotonic and inchworm-like movement. Cycles of inchworming are encoded in DNA and involve straining and relaxation of the ternary complex accompanied by characteristic leaping of DNA and RNA footprints. We demonstrate that the oligo(T) tract that constitutes a normal part of transcription terminators acts as an inchworming signal so that the leap coincides with the termination event. Prevention of leaping with a roadblock of cleavage-defective EcoRI protein results in suppression of RNA chain release at a termination site. The results indicate that straining and relaxation of RNA polymerase are steps in the termination mechanism.

Introduction

Termination of transcription is a process whereby ternary elongation complex dissociates into RNA transcript, DNA template, and RNA polymerase (RNAP). In bacteria, intrinsic terminator signals in DNA are usually composed of an inverted repeat followed by an oligo(T) sequence, so that in RNA they appear as a GC-rich hairpin and a run of several uridine residues at the 3′ terminus (Platt, 1986; Yager and von Hippel, 1987; Friedman et al., 1987). The hairpin formation is essential to the termination process (Lee and Yanofsky, 1977; Yang and Gardner, 1989; Daube et al., 1995). In the course of elongation, the advancing complex occasionally undergoes structural rearrangements resembling the movements of an inchworm (Krummel and Chamberlin, 1992; Nudler et al., 1994; Chamberlin, 1995; Chan and Landick, 1994; Das, 1993). Inchworming is induced by specific signals in DNA whose nature is unknown. The inchworming signals are at least partly recognized by the front-end domain of RNAP ahead of the growing end of the transcript (Nudler et al., 1994). It was the goal of this work to determine whether inchworming plays any role in termination.

Inchworming is visualized as cyclic changes of the distance (measured in nucleotides) between the RNA 3′ terminus (C) and the front edge of the enzyme (F) mapped by exonuclease III (exo III) footprinting (Nudler et al., 1994) (Figure 1A). During monotonic movement, $C \sim F = 18$. In the course of an inchworming cycle, $C \sim F$ becomes progressively less than 18, and the complex is hypothesized to accumulate internal strain that may lead to collapse into nonproductive dead-end conformation. The cycle is completed in a forward leap of the front edge over several base pairs, which restores $C \sim F = 18$ and relaxes the internal strain. As the complex goes through the cycle, its sensitivity to the transcript cleavage factor GreB displays concomitant cyclic fluctuations. The relaxed monotonic complex is resistant to moderate doses of GreB, while the strained complex is highly sensitive. Cleavage of RNA with GreB restores $C \sim F = 18$ by removing a 3′ terminal increment of RNA, relaxes the strain, and in effect returns the complex to the starting point of the inchworming cycle. Thus, the response to GreB, the predisposition to dead-end formation, and the $C \sim F$ value are three linked diagnostic parameters to follow inchworming in the advancing ternary complex. To study the role of inchworming in termination, we monitored the three inchworming parameters in the ternary complex advancing through a transcription terminator signal.

Results

Inchworming on a Terminator

To study inchworming, we employ RNAP carrying six histidine residues fused to the COOH-terminus of the β′ subunit (Kashlev et al., 1993). Through the His tag, the enzyme adsorbs to Ni^{2+}-chelating agarose beads so that assays can be performed in solid phase. The His-tagged RNAP can be "walked" along the template in controlled steps. The only limitation to obtaining complexes stopped at consecutive single-nucleotide intervals is in cases of repetition of the same nucleotide in the sequence, such as U tracks in terminators.

In the case of tR2 terminator of phage λ (template 1), the run of U's between positions 98 and 106 is interrupted by a single A at 103 (Figure 2). Thus, it was possible to walk the elongation complex (EC) to the beginning (EC⁹⁷), the middle (EC¹⁰²), and the end (EC¹⁰⁶) of the U track. In Figure 2A, each complex, represented by four adjacent lanes on the autoradiogram, was tested for stability (the ability to retain RNA after "washing" of the beads), the sensitivity to GreB, and the ability to resume elongation upon addition of NTPs (chase). In EC⁹⁷, the transcript is stable (lane 2), resistant to GreB (lane 3), and can be chased into the terminated transcripts 104 and 105 and the readthrough product 110 (lane 4). In EC¹⁰², the transcript is stable (lane 6) and is highly sensitive to GreB, which converts it to the 97 nt RNA (lane 7). The chase of EC¹⁰² yields the same products as in the case of EC⁹⁷ (lane 8). The pattern of transcripts observed when the complex is walked to position 106 reflects natural termination at positions 104 and 105 as well as the readthrough to 106 (lane 9). Washing removes nearly all of this material (lane 10), indicating that RNA is indeed released from the complexes. A small fraction of EC¹⁰⁴ and EC¹⁰⁶ that withstands washing (lane 10) is highly sensitive to GreB (lane 11) and
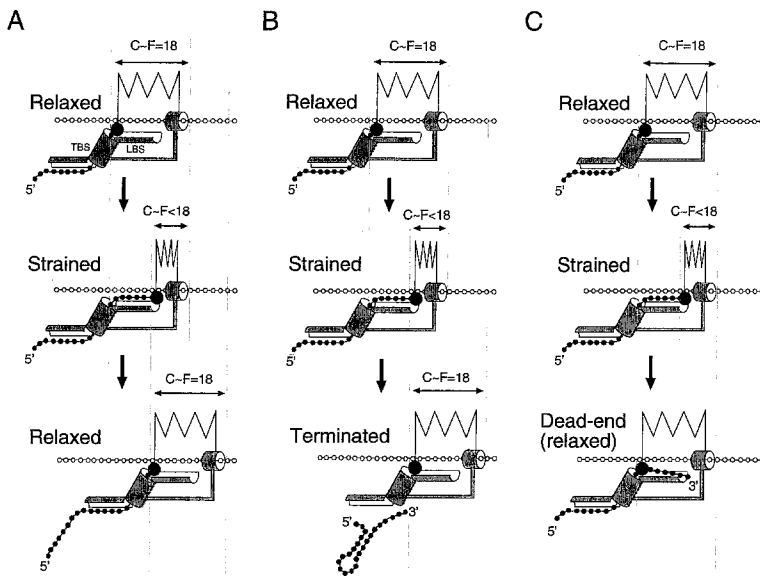
A   B   C

Figure 1. The Inchworming Cycle and Its Role in Transcription Termination

(A) Inchworming on a nonterminator sequence. White and black beads symbolize the template strand of DNA and RNA, respectively. The large ball stands for the active center, and the cylinder around DNA, for the front-end domain. The definition and experimental justification of the loose and tight product-binding sites (LBS and TBS) has been described (Nudler et al., 1994). During straining, LBS is filled with RNA and $C \sim F$ is compressed. Relaxation involves threading of RNA through TBS and leaping of the front end along DNA.

(B) Inchworming on a terminator. The model summarizes the results of this work. During leaping on a terminator, RNA emerging from TBS folds up into a hairpin and the complex is irreversibly committed to termination.

(C) Dead-end formation on a terminator. As explained in the Discussion, this is a side pathway leading from a strained termination complex to RNAP irreversibly locked in a nonproductive stable ternary complex. It is suggested that in the dead end, the catalytic center has disengaged from the 3' terminus (Borukhov et al., 1993a; Chamberlin, 1994) and shifted backward to the site of GreB cleavage between TBS and LBS (Rudd et al., 1994).

cannot be chased (lane 12), reflecting the formation of a dead end. Finally, the transcript in EC^{110} is stable (lane 14), resistant to GreB (lane 15), and fully chaseable (lane 15). Thus, of the ternary complexes studied, EC^{97} and EC^{110} are relaxed, EC^{102} is strained, while EC^{104}, EC^{105}, and EC^{106} apparently pass through a strained stage, but could not be analyzed in real time due to rapid dissociation.

These conclusions were confirmed by the mapping of RNAP front edge with exo III (Figure 2B, top panel). In EC^{95}, the front edge maps at position 113, so that $C \sim F$ = 18. As the transcript grows by 2 nt to EC^{97}, most of the front edge of the complex advances to 115, so that $C \sim F$ = 18 is maintained. In EC^{102}, however, the front edge retreats to 113, resulting in $C \sim F$ = 11. When the complex is walked to position 106, nearly no complex is detected by exo III, reflecting its dissociation at the termination site. The bottom panel of Figure 2B shows radiolabeled RNA in the complexes after treatment with exo III, with or without subsequent chase with NTP. This control demonstrates that exo III treatment does not disrupt the complexes. Note that the 97 nt transcript migrates in the gel ahead of the 95 nt transcript due to the effect of the hairpin. From these results, we conclude that RNAP advancing through the termination site undergoes inchworming. The point of entry into the inchworming cycle is defined by the position of RNA cleavage in strained complexes. As is evident from Figure 2A (lanes 7 and 11), RNA in EC^{102} and EC^{106} is cleaved to yield the 97 nt 5' product. Thus, the entry into inchworming coincides with the beginning of the oligo(T) sequence at position 97, while the leaping of the complex occurs between 103 and 110.

Stabilization of Termination Complex by a Protein Roadblock

To stop the elongating complex precisely at the major point of chain termination at 104, we employed the road-blocking mutant restriction endonuclease EcoRI(Q111) defective in DNA cleavage (Wright et al., 1989; Pavco and Steege, 1990). The EcoRI restriction site was engineered in the template DNA (template 2) so that bound EcoRI(Q111) blocked transcription exactly at the termination point. As can be seen from Figure 3A, in the absence of EcoRI(Q111), transcription at template 2 terminates at the natural positions 104 and 105 (lane 1). At both these sites, RNA chains are released since they cannot be found in the immobilized complexes after washing (lane 2). Bound EcoRI(Q111) prevents extension of RNA beyond position 104 (lane 3). The crucial observation of this work is that chains stopped at 104 by the EcoRI(Q111) roadblock are not released (lane 4) and remain in the complex even after prolonged incubation. The roadblocked EC^{104} is highly sensitive to RNA cleavage with GreB (lane 5). The EcoRI(Q111) roadblock can be removed from DNA by brief exposure to high ionic strength. This procedure leads to immediate dissociation of most of RNA from the complex (lane 6). The residual material remaining in the complex after salt treatment is readily cleaved by GreB (lane 7), indicating that it represents dead-end complex that accumulates upon incubation of the roadblocked complex. Thus, the roadblocked termination complex is stable and strained.

It is tempting to suggest that the roadblock prevents chain release by suppressing leaping of the strained complex. In other words, in the absence of the roadblock, the
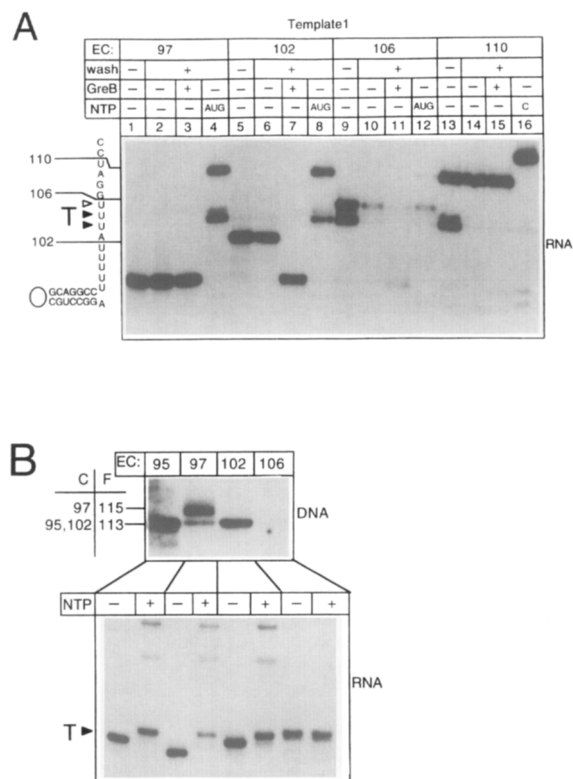
Figure 2. Advancement of the Elongation Complex through the tR2 Terminator

(A) The autoradiogram shows the RNA in four immobilized elongation complexes (EC) before (lanes 1, 5, 9, and 13) and after (lanes 2, 6, 10, and 14) washing. The washed complexes were treated with GreB (lanes 3, 7, 11, and 15) or chased by adding the indicated NTPs (lanes 4, 8, 12, and 16). The RNA sequence of tR2 is shown on the left. Termination points at 104, 105, and 106 are indicated by arrows. GreB was added in the amount of 0.5 pmol.

(B) The top panel shows the protection of terminally ^{32}P-labeled non-template DNA strand from exo III digestion. The table lists the positions of the front end (F) in each complex and the length of the transcript. The bottom panel represents RNA in the complexes after treatment with exo III before or after the chase with the four NTPs. We emphasize the importance of this control aimed at choosing the concentration of exo III that does not disrupt the complexes. At higher exo III doses, the treated complex becomes unchaseable and the footprint is pushed upstream resulting in erroneous estimate of the front edge position.
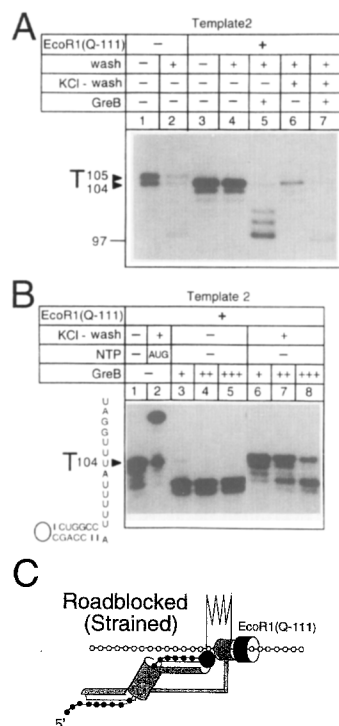

Figure 3. The Effect of EcoRI(Q111) Roadblock on Termination

Template 2 contained EcoRI restriction site at 16 nt downstream from the major termination point.

(A) Roadblock on a natural termination site. RNA products are shown that were synthesized on template 2 in the absence or in the presence of roadblocking EcoRI(Q111) protein. The starting complex carrying 32 nt RNA was chased by adding the four NTPs followed by standard wash where indicated. The washed complexes were treated with GreB and briefly exposed to high salt as indicated.

(B) Roadblock on a terminator with the hairpin destabilized by inosine. The complex was walked to position 104 with inosine (I) incorporated at the indicated positions of the hairpin. Control in lane 2 presents chasing of the complex after brief exposure to high salt. The effect of the increasing dose of GreB on the complex before or after high salt challenge is shown in lanes 3–8. GreB was added in the amounts of 0.5 pmol (+), 5 pmol (++), or 50 pmol (+++).

(C) The scheme of the roadblocked complex (see Figure 1 for symbols).

EC[104] complex should be expected to leap, relax, and release RNA. To test this prediction, the roadblock experiment was repeated when the hairpin was destabilized by incorporation of inosine in place of guanine, so that RNA release is suppressed and EC[104] could be studied in real time (Figure 3B). Under these conditions, the roadblocked complex EC[104] (lane 1) is stable, and most of it can be chased if the roadblock is removed by brief exposure to high ionic strength (lane 2). The roadblocked complex is highly sensitive to GreB (lanes 3–5). However, brief exposure to high ionic strength makes the complex resistant to GreB (lanes 6–8), indicating that the removal of the roadblock leads to relaxation (leaping) of the complex.

In the experiments on the salt effect on the roadblocked complex, it was necessary to rule out that salt does not directly induce release of RNA, but acts through the removal of the roadblocking EcoRI. To this end, a control experiment showed that the kinetics of dissociation of the inosine-containing complex after the removal of the roadblock was nearly the same at 150 mM and 700 mM KCl (data not shown). We also note that the efficiency of in vitro termination at tR2 is essentially unchanged in the range of KCl concentration between 150 mM and 1 M (Reynolds et al., 1992), testifying against the direct effect of salt on chain release in this system. Thus, we conclude that the leaping of RNAP at the terminator precisely coincides with the termination point. Suppression of the leap prevents RNA chain release.
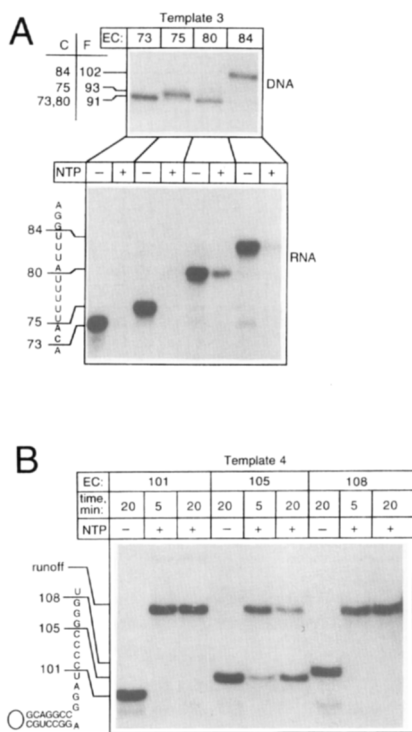
Figure 4. Dissection of tR2 Terminator into Elements

(A) Transcription through U track alone. Template 3 was derived from template 1 by deletion from position 75–97 to remove the hairpin. The top panel shows the protection of DNA from exo III digestion. The bottom panel presents the control showing RNA after treatment with exo III before or after the chase with the four NTPs.

(B) Transcription through the hairpin alone. Template 4 was derived from template 1 by deleting the U track to generate the GC-rich sequence from position 97–106. Complexes stalled at 101, 105, and 108 were incubated under standard conditions for 5 or 20 min prior to chase.

Dissection of a Terminator into Functional Elements

What is the role of the hairpin and the oligo(T) sequence in the inchworming at the terminator? To address this question, we dissected the terminator signal into the two elements by engineering transcription templates lacking either of them. When RNAP was walked through the tR2 terminator from which the hairpin has been deleted (template 3), an inchworming cycle was observed on the oligo(T) sequence (Figure 4A) equivalent to that observed on the intact terminator (template 1, see Figure 2A). On template 3, the extension of RNA from 75–80 (equivalent to $97 \rightarrow 102$ on template 1) is accompanied by a 2 nt retreat of the front edge so that in EC^{80} $C \sim F = 11$. In EC^{84} (equivalent to EC^{106} in template 1), the $F \sim C = 18$ is restored reflecting the leap, while a small fraction of EC^{80} has collapsed into the dead end (Figure 4A, bottom panel). These observations are corroborated by the high sensitivity of EC^{80}, and the resistance of EC^{75} and EC^{84}, to GreB (data not shown). Thus, the T track of tR2 is sufficient to cause the inchworming cycle in the absence of the hairpin. Tracks of T's also cause inchworming in other sequence contexts (data not shown).

When the T track is removed from the tR2 terminator (template 4), no inchworming is observed. The elongation complexes in the segment immediately downstream from the hairpin ($97 \rightarrow 108$) maintain $F \sim C = 18$ and are resistant to GreB (data not shown). We found, however, that the hairpin alone causes substantial destabilization of elongation complex stopped at position 105, i.e., 8 nt downstream from the hairpin base. This is illustrated by Figure 4B, in which EC^{101}, EC^{105}, and EC^{108} on template 4 were challenged with the mixture of NTPs (chase) after 5 or 20 min incubation. It is clear that an increasing fraction of EC^{105} is unchaseable. Control experiments confirmed that this is due to chain release rather than dead-end formation. Thus, the hairpin plays no role in inchworming, but destabilizes relaxed elongation complexes at the downstream site where termination normally occurs. This result is consistent with the report of complex-destabilizing effect of random hairpins (Arndt and Chamberlin, 1990).

Discussion

Discontinuous Mechanism of Transcription Elongation (Inchworming)

The results of this study are interpreted below in terms of a hypothetical model based on the original idea of Chamberlin (1994) that envisages RNA polymerase as a molecular machine composed of several flexibly connected parts (Figure 4). The key elements of the model are the loose and the tight product-binding sites (LBS and TBS, respectively), which are operationally defined by the outcome of internal cleavage of RNA in ternary complexes. LBS is defined as the locality from which the 3' terminal fragment falls out; TBS is defined as the site where the 5' terminal fragment is held (Borukhov et al., 1993a). The concept of LBS and TBS is reinforced by the phenomenon of upward "slippage" of tetranucleotide transcripts that leads to dramatic stabilization of certain promoter complexes (Borukhov et al., 1993b; Severinov and Goldfarb, 1994). The notion of "filling" of LBS with RNA is supported by the observation that the priming nucleotide secured in the 5' NTP-binding pocket can be extended by 8 nt before the transcription stops (Mustaev et al., 1993). It should be emphasized that LBS and TBS are conceptual notions inferred from functional analysis; their structural basis remains to be established.

In the framework of the above model, inchworming is viewed as two synchronized cycles of movement: the leap-like advancement of RNAP front edge along DNA (probed by exo III) and the filling and emptying of LBS (probed by GreB). The leap of the front edge coincides with the loss of GreB sensitivity, interpreted as the translocation of RNA from LBS to TBS. Again, the two-stroke mechanism of inchworming (filling–leaping) is a speculative notion inferred from the exo III and GreB footprints; its physical relevance has to be tested by direct approaches.

In addition to the RNAP front edge and the GreB cleavage site, two other parameters of the ternary complex display inchworming: these are the trailing edge of RNAP (probed by DNase I; Krummel and Chamberlin, 1992) and the boundaries of the transcription bubble (probed by a

variety of DNA reagents; Zaychikov et al., 1995). The data reported for a limited number of complexes appear to fit the inchworming cycles established by the exo III and GreB analysis (Nudler et al., 1994; unpublished data). However, a systematic mapping of the trailing edge and the bubble will have to be performed before the synchronicity of all four parameters in the inchworming cycle can be firmly established.

An essential element of the working model of inchworming is the concept of physical strain present in the complexes with filled LBS. This assumption is based on the observation that only GreB-sensitive complexes (i.e., complexes undergoing inchworming) are prone to collapse into the nonproductive dead-end state. The rate of dead-end formation increases with the extent of LBS filling (i.e., the length of the 3' cleavage product). The leap of the front edge and concomitant emptying of LBS are accompanied by the cessation of the collapse-prone state. We define this transition as the "relaxation" of the ternary complex (Nudler et al., 1994; unpublished data).

T Tracks as Inchworming Signals

When inchworming was first discovered (Krummel and Chamberlin, 1992), the initial suggestion was that discontinuous RNAP advancement is in fact the essence of the elongation mechanism. However, in our previous work, it was shown that inchworming is not intrinsic to elongation but rather constitutes a response to specific DNA sites. Between such sites, the movement of the elongation complex is monotonic. The nature of the inchworming signal in DNA as well as physiological significance of inchworming remained unknown. In this work, we show that oligo(T) tracks in rho-independent terminators act as inchworming signals so that the leaping of the complex is precisely phased with the termination event. It should be noted that the oligo(T) track is not the only type of inchworming signals. Previously, we have observed inchworming on sites without many T's (Nudler et al., 1994). It would be interesting to determine whether the unusual intrinsic terminators that do not have T sequences (Studier and Rosenberg, 1981; Telesnitsky and Chamberlin, 1989) would still display inchworming.

A Model of Intrinsic Termination

We demonstrate that termination at tR2 involves occurrence of two events: the leaping of the strained complex at the end of the T track and the formation of destabilizing hairpin ~ 8 nt upstream. The same is true for at least two other rho-independent terminators (data not shown). Based on these observations, we propose a model explaining rho-independent termination in terms of the conceptual view of RNAP outlined above. The maximally strained complex at the end of the T track is envisaged as the principal termination intermediate. The leaping–relaxation of this complex plays a dual role. First, the leap brings the hairpin in the nascent RNA into a presumed destabilization site whose topology within the RNAP molecule is defined by the 8 nt distance between the hairpin base and the 3' terminus in the relaxed complex. Second, we suggest that threading of RNA through RNAP during

the leap is associated with transient disruption of RNA–protein interactions in the tight product-binding site (TBS, see Figure 1B). This effect amplifies the destabilizing effect of the hairpin, thus leading to irreversible termination. Without the hairpin, the transient loosening of the "grip" of TBS on RNA would not be sufficient to cause termination. In other words, the model proposes the existence of an additional RNA-holding site upstream from TBS that serves as a backup during the leap. The appropriately phased hairpin somehow disrupts RNA holding in the backup site. This model finds support in recent studies on direct RNA binding to RNAP (Johnson and Chamberlin, 1994). The authors report that there are multiple sites for RNA binding that are single-strand specific. Formation of stem-loop hairpins greatly reduce RNA interaction with these sites.

The notion that leaping transiently destabilizes the complex and leads to termination, although speculative, is attractive because it explains how an inchworming signal in DNA leads to accumulation of intramolecular strain in the advancing enzyme, which is then used to disrupt forces holding together the remarkably stable ternary elongation complex. The two-site model involving TBS and an upstream backup site is consistent with the original suggestion by Chamberlin (1995) that elongation is accomplished through alternation of a pair of protein–nucleic acid clamps.

In a classical thermodynamic model of rho-independent termination, the diminished stability of oligo(dA:rU) hybrid was assigned the key role in dissociation of the ternary complex (Yager and von Hippel, 1987, 1991). Our results are inconsistent with this view and point to an alternative mechanism in which protein–nucleic acid interactions play a crucial role in the maintenance of the elongation complex, while termination involves breakage of these interactions through a conformational transition in RNAP (see Chamberlin, 1994).

Dead-End Complexes as a Side Pathway in Termination

Earlier, we have shown that strained ternary complexes at the end of inchworming cycles are prone to collapsing into the dead-end configuration (Nudler et al., 1994). Here, we demonstrate that a fraction of ternary complexes at the termination site result in a dead end. This fraction varies at different terminators and may be as high as 20% in the case the trpA attenuator (data not shown). Thus, dead-end formation is a feature of the termination process and apparently represents a side pathway whereby the normal act of relaxation goes awry (Figure 1C).

The formation of a dead end during termination offers a possible biological role to the phenomenon of transcript cleavage induced by GreA and GreB proteins (Borukhov et al., 1992, 1993a). The in vivo consequences of a termination-dependent dead end is likely to be fatal because it will lock RNAP on the template. The relief of dead ends through cleavage and restart must therefore be advantageous. These considerations are in agreement with the observation that oligo(T) tracks cause elongations arrest in the case of eukaryotic RNA polymerase II, which is re-

lieved by elongation factor TFIIS via cleavage and restart pathway (Reines et al., 1989; Kerppola and Kane, 1991; Izban and Luse, 1992, 1993).

Experimental Procedures

Transcription Templates

Template 1 was a PCR-amplified 324 bp DNA fragment carrying the T7A1 promoter and tR2 phage λ terminator (Nudler et al., 1994). Its transcribed sequence is ATCGAGAGGG ACACGGCGAA TAGC-CATCCC AATCGACACC GGGGTCCGGG ATCTGGATCT GGATCG-CTAA TAACA**GGCCT GCTGGTAATC GCAGGCC**TTT TTATTTGGAT CCCCGGGTAC CGAGCTCGAA... (the elements of the terminator are highlighted). Other templates were prepared by PCR-mediated mutagenesis from template 1. Template 2 carried an EcoRI site downstream from the major termination point to yield the sequence (starting with the T track with EcoRI site underlined): TTTTTA*TTT*GGATCCCCGGG-TGAATTCACT.... The position of EcoRI site was based on the data of Pavco and Steege (1990). Template 2 also carried a 27 nt deletion fusing positions 44 and 71 of the transcription unit of template 1 in order to simplify walking to terminator. Template 3 carried a 22 nt deletion precisely removing the hairpin sequence between positions 75 and and 97 of template 1. Template 4 carried a 9 nt deletion precisely removing the T track of template 1.

Transcription Reactions

His-tagged RNA polymerase was purified and immobilized on Ni–NTA–agarose as described (Kashlev et al., 1993). The walking reactions, washing of the complexes, chase, GreB cleavage, and exo III footprinting were performed as described previously (Nudler et al., 1994). In brief, preformed promoter complexes were allowed to initiate transcription using the tetranucleotide primer CpApUpC (50 μM, Oligos, Inc.) in the presence of ATP and GTP (25 μM each). This resulted in a stable ternary complex stalled at position +11. The complex was then labeled by incorporation of [α-^{32}P]CTP (3000 Ci/mmol) followed by extension to the position +20 with unlabeled ATP, GTP, and CTP (5 μM each). This complex was the starting material for the walking or chase reactions. Each reaction contained ~0.2 pmol of defined ternary complex. GreB was added in the amounts indicated in figure legends. The dose of exo III and GreB was established by titration in order to avoid artifacts due to "pushing back" of the complex by the footprinting agent. In the case of exo III, excessive exposure resulted in unchaseable dead-end complexes even when the front edge was pushed back by only one nucleotide. In the case of GreB, excessive doses resulted in more than one cleavage product reflecting processive RNA degradation (see Nudler et al., 1994).

Roadblocking Experiment

The EcoRI(Q111) mutant protein was provided by G. Runyon and D. G. Bear (University of New Mexico School of Medicine). It was diluted immediately prior to use with ice-cold 20 mM KPO₄ (pH 7.4), 0.2 M KCl, 0.2 mM DTT, 1 mM EDTA, and 10% (v/v) glycerol. Each sample (~0.2 pmol DNA) was incubated with 1.6 pmol of EcoRI(Q111) for 20 min at 25°C. The DNA–EcoRI(Q111) complex was 100% stable during walking reaction. To remove EcoRI(Q111), the complexes were washed once with 700 mM KCl (~30 s) followed by a standard wash with transcription buffer (Kashlev et al., 1993). The completeness of EcoRI(Q111) removal was verified by a control demonstrating 100% readthrough from an upstream position.

References

Arndt, K. M., and Chamberlin, M. J. (1990). RNA chain elongation by Escherichia coli RNA polymerase: factors affecting the stability of elongating ternary complex. J. Mol. Biol. 213, 79–108.

Borukhov, S., Polyakov, A., Nikiforov, V., and Goldfarb, A. (1992). GreA protein: a transcription elongation factor from Escherichia coli. Proc. Natl. Acad. Sci. USA 89, 8899–8902.

Borukhov, S., Sagitov, V., and Goldfarb, A. (1993a). Transcript cleavage factors from E. coli. Cell 72, 459–466.

Borukhov, S., Sagitov, V., Josaitis, C. A., Gourse, R. L., and Goldfarb, A. (1993b). Two modes of transcription initiation in vitro at the rrnB P1 promoter of Escherichia coli. J. Biol. Chem. 268, 23477–23482.

Chamberlin, M. (1995). New models for the mechanism of transcription elongation and its regulation. Harvey Lect. 88, 1–21.

Chan, C. L., and Landick, R. (1994). New perspectives on RNA chain elongation and termination by E. coli RNA polymerase. In Transcription: Mechanisms and Regulation, R. C. Conavay and J. W. Conaway, eds. (New York: Raven Press), pp. 297–321.

Das, A. (1993). Control of transcription termination by RNA-binding proteins. Annu. Rev. Biochem. 62, 893–930.

Daube, S. S., Hart, C. R., and von Hippel, P. H. (1995). Coupling of RNA displacement and intrinsic termination during transcription from synthetic RNA–DNA bubble duplexes. Proc. Natl. Acad. Sci. USA, in press.

Friedman, D. I., Imperiale, M. J., and Adhya, S. L. (1987). RNA 3' end formation in the control of gene expression. Annu. Rev. Genet. 21, 453–488.

Izban, M. G., and Luse, D. S. (1992). The RNA polymerase II ternary complex cleaves the nascent transcript in a 3'→5' direction in the presence of elongation factor IIS. Genes Dev. 6, 1342–1356.

Izban, M. G., and Luse, D. S. (1993). The increment of SII facilitated transcript cleavage varies dramatically between elongation competent and incompetent RNA polymerase II ternary complexes. J. Biol. Chem. 268, 12874–12885.

Johnson, T. L., and Chamberlin, M. J. (1994). Complexes of yeast RNA polymerase II and RNA are substrates for TFIIS-induced RNA cleavage. Cell 77, 217–224.

Kashlev, M., Martin, E., Polyakov, A., Severinov, K., Nikiforov, V., and Goldfarb, A. (1993). Histidine-tagged RNA polymerase: dissection of the transcription cycle using immobilized enzyme. Gene 130, 9–14.

Kerppola, T. K., and Kane, C. M. (1991) RNA polymerase: regulation of transcript elongation and termination. FASEB J. 5, 2833–2842.

Krummel, B., and Chamberlin, M. J. (1992). Structural analysis of ternary complexes of Escherichia coli RNA polymerase: individual complexes halted along different transcription units have distinct and unexpected biochemical properties. J. Mol. Biol. 225, 221–234.

Lee, F., and Yanofsky, C. (1977). Transcription termination at the trp operon attenuator of Escherichia coli and Salmonella typhimurium: RNA secondary structure and regulation of termination. Proc. Natl. Acad. Sci. USA 74, 4365–4369.

Mustaev, A., Kashlev, M., Zaychikov, E., Grachev, M., and Goldfarb, A. (1993) Active center rearrangement in RNA polymerase initiation complex. J. Biol. Chem. 268, 19185–19187.

Nudler, E., Goldfarb, A., and Kashlev, M. (1994). Discontinuous mechanism of transcription elongation. Science 265, 793–796.

Pavco, P. A., and Steege, D. A. (1990). Elongation by Escherichia coli RNA polymerase is blocked in vitro by a site-specific DNA binding protein. J. Biol. Chem. 265, 9960–9969.

Platt, T. (1986). Transcription termination and the regulation of gene expression. Annu. Rev. Biochem. 55, 339–372.

Reines, D., Chamberlin, M. J., and Kane, C. M. (1989). Transcription elongation factor IIS (TFIIS) enables RNA polymerase II to elongate through a block to transcription in a human gene in vitro. J. Biol. Chem. 264, 10799–10809.

Reynolds, R., Bermudez, C. R. M., and Chamberlin, M. J. (1992). Parameters affecting transcription termination by Escherichia coli RNA

polymerase. J. Mol. Biol. *224*, 31–51.

Rudd, M. D., Izban, M. G., and Luse, D. S. (1994). The active site of RNA polymerase II participate in transcript cleavage within arrested ternary complexes. Proc. Natl. Acad. Sci. USA *91*, 8057–8061.

Severinov, K., and Goldfarb, A. (1994). Topology of the product binding site in RNA polymerase revealed by transcript slippage at the phage λ P$_L$ promoter. J. Biol. Chem. *269*, 31701–31705.

Studier, F. W., and Rosenberg, A. H. (1981). Genetic and physical mapping of the late region of bacteriophage T7 DNA by use of cloned fragments of T7 DNA. J. Mol. Biol. *153*, 503–525.

Telesnitsky, A., and Chamberlin, M. J. (1989). Terminator-distal sequences determine the *in vitro* efficiency of the early terminators of bacteriophage T3 and T7. Biochemistry *28*, 5210–5218.

Wright, D. J., King, K., and Modrich, P. (1989). The negative charge of Glu-111 is required to activate the cleavage center of EcoRI endonuclease. J. Biol. Chem. *264*, 11816–11821.

Yager, T. D., and von Hippel, P. H. (1987). Transcript elongation and termination in *Escherichia coli*. In *Escherichia coli* and *Salmonella typhimurium*, F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger, eds. (Washington, D. C.: American Society for Microbiology), pp. 1241–1275.

Yager, T. D., and von Hippel, P. H. (1991). A thermodynamic analysis of RNA transcript elongation and termination in *E. coli*. Biochemistry *30*, 1097–1118.

Yang, M.-T., and Gardner, J. F. (1989). Transcription termination directed by heteroduplex *thr* attenuator templates. J. Biol. Chem. *264*, 2634–2639.

Zaychikov, E., Denissova, L., and Heumann, H. (1995). Translocation of the *Escherichia coli* transcription complex observed in the registers 11 to 20: "jumping" of RNA polymerase and asymmetic expansion and contraction of the "transcription bubble". Proc. Natl. Acad. Sci. USA *92*, 1739–1743.