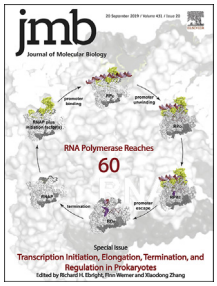

Mechanisms of Bacterial Transcription Termination

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https://doi.org/10.1016/j.jmb.2019.04.003
Edited by R. Ebright

Abstract

Bacterial transcription termination, described mostly for *Escherichia coli*, occurs in three recognized ways: intrinsic termination, an activity only of the core RNAP enzyme and transcript sequences that encode an RNA hairpin and terminal uridine-rich segment; termination by the enzyme Rho, an ATP-dependent RNA translocase that releases RNA by forcing uncharacterized structural changes in the elongating complex; and Mfd-dependent termination, the activity of an ATP-dependent DNA translocase that is thought to dissociate the elongation complex by exerting torque on a stalled RNAP. Intrinsic termination can be described in terms of the nucleic acid movements in the process, whereas the enzymatic mechanisms have been illuminated importantly by definitive structural and biochemical analysis of their activity.

Introduction

Transcription termination is essential both to focus regulated gene expression to discrete segments of DNA and to recycle RNA polymerase (RNAP). Furthermore, active or stalled RNAP complexes that obstruct DNA replication must be removed. There are recent extensive and excellent reviews of bacterial transcription termination [1–3], which will be briefly summarized but not duplicated here; instead, some less known experimental approaches and implications will be emphasized.

There are three known modes of termination of bacterial RNAP, derived principally from the *Escherichia coli* model (Fig. 1): Intrinsic (or Rho-independent) termination that depends solely upon the interaction of nucleic acid elements with RNAP (although other factors like NusA may modulate termination efficiency) [2]; Rho-dependent termination, an activity of the RNA translocase Rho and ATP [3,4]; and Mfd-dependent termination, an activity of the DNA translocase Mfd and ATP. Most of the present discussion will involve intrinsic termination, for which some interesting mechanistic understanding exists. The enzymatic pathways will be discussed largely to consider common elements.

Intrinsic Termination

Background

The intrinsic terminator is well defined in terms of sequence elements and critical dimensions: It encodes an RNA hairpin that forms as the sequences emerge from the elongation complex, followed by a segment that encodes purely or predominantly uridine (U) nucleotides at the released RNA end (Fig. 1) [2]. Invariably, the base of the RNA hairpin is 8–9 nucleotides from the RNA end.

The important function of the hairpin is embodied in the stem, not the loop. This is implied by the fact that transcript release also is induced by an oligonucleotide (originally DNA, although it can be RNA) complementary to a transcript segment where the downstream portion of the stem would be [5]. The structural requirement is quite precisely the same as the natural terminator stem, particularly in the position of the base of the stem relative to the terminated end. In a static reaction, oligonucleotide-mediated release (OMR) does not require the U-rich segment; any arbitrary site tested could be targeted for OMR [5], although detailed study no doubt would reveal different rates and

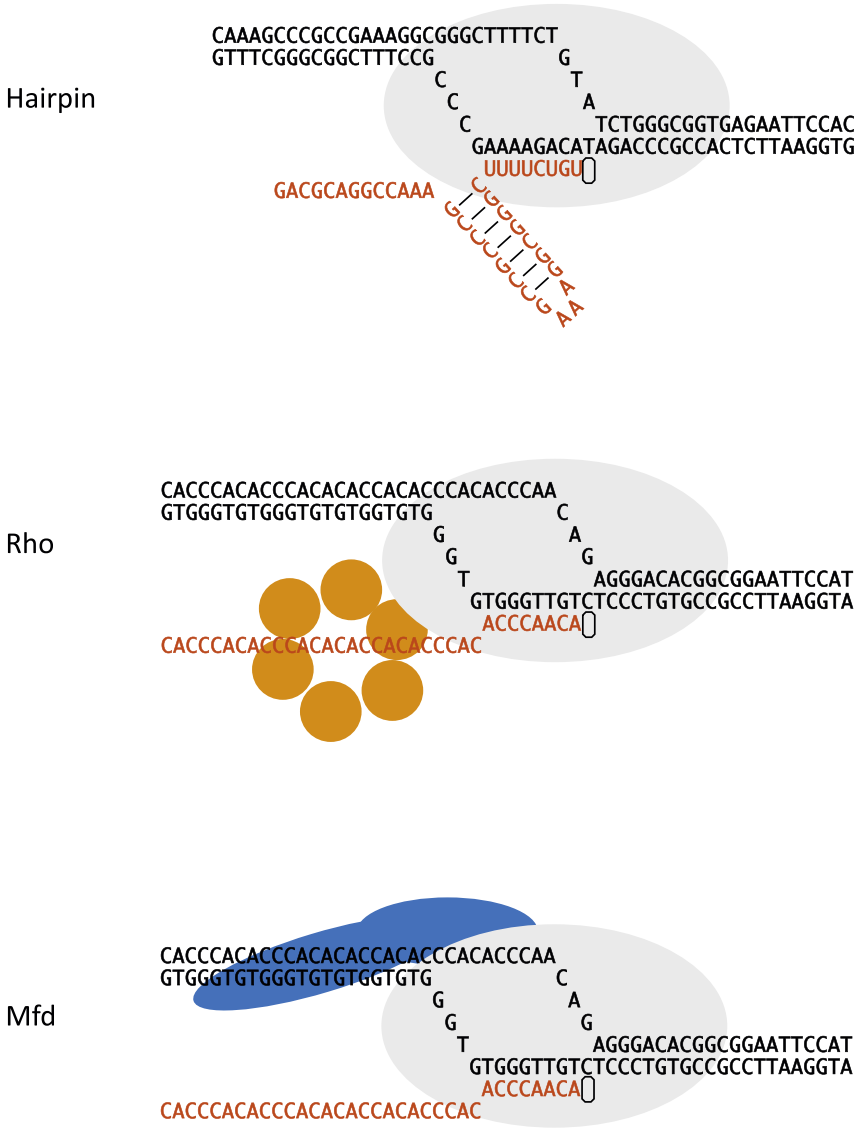

Fig. 1. Three modes of termination in *E. coli.* The intrinsic terminator is illustrated to be initiating termination by forward translocation, although this is only one possible mechanism; see Fig. 2 and the text. The intrinsic terminator illustrated is that described in the text in which the U-rich segment is interrupted by two G/C base pairs. The template illustrated for Rho and Mfd is an artificial template used in Ref. [75] to manipulate these reactions.

efficiencies of release. The activity of oligonucleotides argues that it is the formation of duplex structure in this part of the elongation complex, and not the RNA helix *per se*, that is important to the termination mechanism. And it furthermore implies that the relative instability of the A/U-rich segment likely acts to kinetically favor the dissociation of the RNA rather than being required in some structural sense.

The OMR assay responds in a consistent manner to RNAP mutationally altered to enhance or inhibit termination and, most important, to an antitermination modification; both effects help to authenticate the OMR reaction [5,6]. Modification of RNAP by the single antiterminator polypeptide Q of phage 82, along with the cofactor NusA, strongly inhibits OMR, revealing the Q modification as a structural change that inhibits access to a static RNAP by the termination-inducing polynucleotide sequence. This modification appears to be distinct from the anti-pausing activity of Q and other antiterminators, which is discussed below.

Mechanism

How do the sequence elements actually destabilize the RNAP elongation complex in intrinsic termination? Three models describe putative movements of nucleic acids or protein during the process: *forward translocation* [5,7], *slippage* [8,9], and "*allosteric*" [10,11], the latter illustrated as "bubble collapse" in order to show a surmised pathway (Fig. 2). In forward translocation (or hypertranslocation), the normal movement of DNA continues, but without extension of the RNA chain, resulting in shortening and destabilizing of the RNA/DNA hybrid and leading to dissociation; the G/C-rich

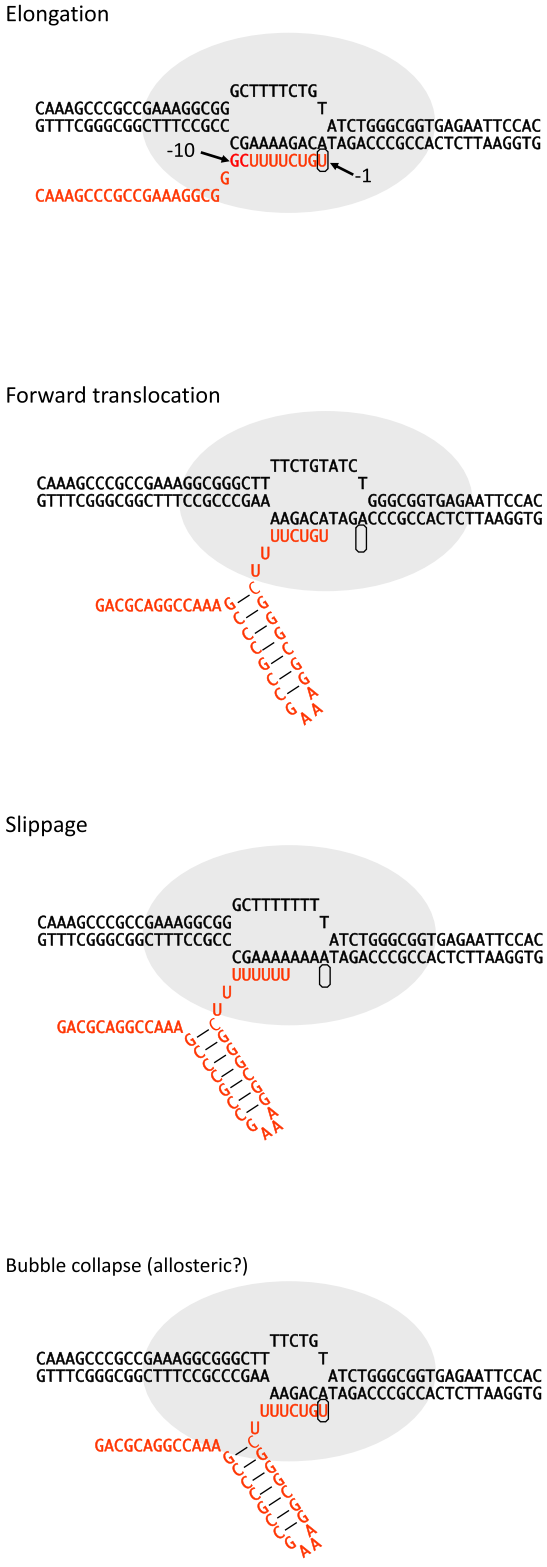

Fig. 2. A current model of the elongation complex and three proposed modes of termination by an intrinsic terminator.

hairpin formation is proposed to compensate for binding energy lost as the hybrid shortens. Slippage (also called "hybrid shearing") is essentially pulling the transcript out of the complex, impelled by hairpin formation, with intermediate stages of annealing stabilized by the homopolymeric sequence. The "allosteric" model is less defined but presumably involves some conformational change responding to hairpin formation that opens the enzyme and leads to bubble collapse, but does not involve the nucleic acid movements required of the other models. Of course bubble collapse ultimately would occur as termination initiated by forward translocation or slippage is completed.

A critical single-molecule experiment detected slippage occurring for a terminator with a homopolymeric U-rich segment, but also found evidence that forward translocation occurs for a terminator with a U-rich segment interrupted by two G/C base pairs [8] (Fig. 1). Thus, at least two distinct classes of intrinsic terminator may exist [1]. In biochemical experiments to test the forward translocation model, utilizing the same terminator with two G/C base pairs in its U-rich segment mentioned above, an interstrand crosslink was placed just downstream of the site of termination; if forward translocation were essential to the termination mechanism, the crosslink would prevent it. This crosslink impeded but did not prevent RNA release, indicating that forward translocation is a more efficient pathway, but that release also occurs without translocation [7]. OMR with a truncated version of the same terminator but lacking the upstream hairpin half showed that a crosslink placed from one to four nucleotides downstream of the released end slowed release at least several-fold [7]. Note that this experiment illustrates a valuable application of OMR: having the attacking complementary polynucleotide in solution rather than immediately attached to the downstream segment, which would facilitate a rapid first-order formation of the duplex structure, slows the process into the manual biochemical time realm. Transcript release from the crosslinked template might occur either by bubble collapse, allowing the RNA/DNA hybrid to dissociate (Fig. 2), or possibly by slippage despite the intruding G/C base pairs. *Peters et al*. [1] noted that terminators with an interrupted terminal U-rich segment tended to have weaker base pairs *downstream* of the release site, consistent with facilitated melting of the helix as forward translocation occurs for this class of terminators.

Sequence changes are most detrimental to terminator function in the portion of the U-rich segment adjacent to the hairpin stem [12]. This is consistent with all of the mechanisms envisioned, in which termination is initiated by melting of this hairpin-adjacent segment as the hairpin stem forms up to at least −11 [13]. The RNA segment −11 to −14 is in the channel that normally accommodates single-stranded RNA in the elongation

complex and would be pulled out in either the forward translocation or slippage model as the hairpin forms and hairpin-adjacent base pairs are melted. A pause-strengthening hairpin associated with attenuation regulatory pauses forms in the channel; presumably, the termination hairpin would as well [14,15]. Termination hairpin formation in the channel is expected to trigger conformational changes in RNAP, just as the pause hairpin does [14], and in turn may promote bubble collapse.

For a terminator bearing two G/C base pairs in the U-rich segment, the same terminator for which evidence for the forward translocation mechanism was obtained (Fig. 1), reannealing of DNA strands in the −11 to −7 region of the transcription bubble is essential to efficient termination [16]. Substitutions in the non-template strand that prevent reannealing but do not affect templating of either the RNA hairpin or U-rich segment nonetheless prevent RNA release and allow continued elongation. In fact, the complex stalls at the site of release but over many minutes recovers into elongation, perhaps because an intermediate in termination is stabilized or the complex backtracks [16]. The effect of nontemplate substitutions is most consistent with the forward translocation model and bubble collapse. It is unknown if these results apply to a terminator with a pure U-rich segment.

Pausing and intrinsic termination

Transcription pausing is central both to termination and to a fundamental mode of antitermination, namely, anti-pausing, so that understanding of one illuminates the other. Pausing is believed to be an essential step in the function of an intrinsic terminator. Mutationally altered RNAP selected to increase or decrease the efficiency of an intrinsic terminator correspondingly increases or decreases pausing in *in vitro* transcription [17,18]. A transcription pause site has been shown experimentally to be a component of the downstream portion of the intrinsic terminator; thus, deletion of DNA encoding the upstream hairpin segment leaves a pause-inducing sequence that acts at the site of terminator release [5,10]. It is often surmised that the U-rich encoding segment induces the pause, but it likely results from what has become known as the elemental pause sequence (EPS). The EPS was revealed first by single-molecule RNAP elongation analysis [19] and more completely understood through genomic-scale sequencing of elongating complexes [20,21]. Its predominant elements are a G/C base pair at the site where the RNA/DNA hybrid is attempting to unwind (designated −10; Fig. 1), strongly favoring G in the nontemplate sequence; a pyrimidine base at the RNA 3′ end; and G at the next position (+1) in the nontemplate strand. Not all of these elements are required, and other sequences, especially in the hybrid region, certainly influence the intensity of the pause [22]. The function of the EPS can be understood as predominantly a consequence of base pairing energy; that is, the G at −10 disfavors RNA/DNA hybrid unwinding, and G following the pause site constitutes a strong DNA base pair that inhibits downstream unwinding. The following G also influences pausing through a base-specific interaction with the core RNAP; since this interaction favors the fully post-translocated state, it inhibits pausing [21].

In fact, the requisite intrinsic terminator sequence elements enrich for the EPS. The G/C base pair is frequently present at −10 (and a G/C-rich region invariably is), perhaps camouflaged through its more evident role to encode the G-rich segment of the base of the downstream hairpin stem. The G/C-rich stem of intrinsic terminators is strongly biased toward G being the base in the downstream stem segment, placing the G's appropriately to be the critical G at the −10 position at the pause site of an EPS. The frequent terminal U satisfies the pyrimidine preference, and the requirement for a terminal U at one terminator has been demonstrated explicitly by mutation [10].

Why is there a transcription pause at the site of termination? Likely it simply allows time for structural rearrangements to liberate the transcript before elongation can proceed. Consistently, there is a competition between termination and elongation at the site of transcript release in *in vitro* transcription [23,24]. Particularly for weaker terminators and for a mutationally altered RNAP deficient in termination, a lower concentration of the nucleoside triphosphate (NTP) for the nucleotide following the release site favors termination, whereas a higher concentration favors readthrough [23,24]. The result is consistent with the existence of a critical pause, the length of which can be adjusted by NTP concentration *in vitro*, and which is necessary to allow the terminator to work. For several intrinsic terminators tested at different NTP concentrations, a span of release sites occurs, perhaps corresponding to the span of nontemplate strand G's that constitute the critical −10 position of the EPS. For one terminator examined, the sites match in detail: a U nucleotide interrupting a span of G's around −10 causes a gap in the set of release sites exactly 10 nucleotides downstream [23].

Pausing, antipausing, and antitermination

Two-well characterized antiterminators, products of bacteriophage lambda (and related phage) genes *Q* and *N*, modulate the elongation behavior of RNAP in a way that likely reflects a direct antagonism of the EPS activity. A strong argument derives from the activity of the bacteriophage lambda Q protein antiterminator [25]. Q modifies purified *E. coli* RNAP so that it overcomes both intrinsic and Rho-dependent terminators. Q initially binds and acts at a promoter-proximal early engagement site

which is also a pause, located 15–25 bp from the initiating nucleotide among different Q variants [26]. Importantly, a consequence of Q binding to the RNAP complex at the pause is to attenuate the pause, releasing RNAP into downstream elongation, during which it is modified to the antitermination state [26]. In fact, the Q-modified RNAP shows less pausing throughout transcription of hundreds of nucleotides downstream [27], a property also of the lambda gene *N* antiterminator [24]. The promoter-proximal pause requires the σ70 initiation factor, which remains complexed with RNAP after promoter escape to bind a partial but active repeat of the −10 hexamer promoter binding sequence; this repeat was originally identified as the pause-inducing sequence [28]. However, this pause also requires an EPS that determines the site of the pause. The steps in establishing the pause involve initial binding of σ70 to anchor the enzyme, followed by DNA "scrunching" (as in initiation) as transcription proceeds to an EPS pause site [29,30].

The telling activity of the Q modification is the nature of the escape from the pause: Q of phage lambda initially *extends* the scrunching beyond the EPS site [31], from +16 to +25. Since scrunching continues beyond +16, the σ70 anchor must initially remain in place, so that the continued transcription that induces further scrunching must reflect overcoming the EPS pause. Thus, Q activity directly targets the EPS. Since an EPS is an essential component of the intrinsic terminator, this activity explains the anti-pausing related antitermination activity of Q. It is noteworthy that the Q-dependent extended scrunching itself frequently stalls at +25 due to an apparent EPS sequence [31], from which some complexes backtrack if Gre protein is not present. Thus, the antipausing effect is quantitative and not absolute: with the further tension provided by extended scrunching, an EPS can still stop elongation. The lambda gene *N* antiterminator also has antipausing activity and might act in a similar way [24].

Structural correlates of termination and pausing

The three termination models emphasize movements of nucleic acids during termination, and they are thought to occur or at least initiate within the normal RNAP elongation structure. However, there certainly are important structural changes in the enzyme that accompany termination. Several molecular structures of the RNAP/DNA/RNA elongation complex exist during stages of pausing and thus, presumably, the initial step of termination [14,15,32]. There are extensive biochemical investigations of structural changes that accompany pausing [11,33]. Numerous variant structures have been found, including different opening and closing states of the "clamp" that constitutes the major catalytic cleft, as well as different states of some central molecular elements of the active center, such as the trigger loop/helices and bridge helix. Importantly, the hairpin of a hairpin-stabilized pause element has been shown to penetrate the RNA exit channel, requiring some structural relaxation [15]. It is worth noting that the various structures can be obtained only if they are stable intermediates (or even off-pathway states), from which a continuum of structural movements is then surmised.

The most persuasive of these is a partially translocated structure [14,15,34]. There were previous images at the molecular level of the pre-translocated and post-translocated states, but several examples now exist of a putative paused complex in which the RNA has moved to the translocated position, but the template DNA strand has not moved; despite this apparent disjunction, RNA/DNA base pairing is maintained, resulting in "slanted" base pairs in the hybrid. This structure had been observed previously in elongation complexes of yeast RNA polymerase II [35]. Importantly, the base pair immediately after the base templating the RNA end is still intact; this is the site of the nontemplate G important to the EPS, and this base pair is thought to be one of the constraining elements of the EPS. Clearly, the unavailability of the template nucleotide for the next step of synthesis would prevent elongation and is consistent with the paused state. In addition, the RNA/DNA base pair at position −10 is still present. Thus, the structure embodies some of the central elements of the EPS and may be a visualization of it. Certainly, an additional interesting question is whether this semi-translocated structure is a normal intermediate of the translocation process, here frozen by the nucleic acid interactions that impede DNA/RNA or DNA duplex melting.

Rho-Dependent Termination

Background

Rho is an ATP-dependent RNA translocase [3,4] that terminates transcription at certain specific genomic sites, especially in lambda bacteriophage [36–38] and in a well-characterized riboswitch [39], but more widely at genetically undefined sites that are revealed by the condition of transcription, for example, transcription without translation by accompanying ribosomes [40–42]. Rho proteins are widely represented in different bacterial sequences, although not universal [43]. Rho is responsible for genetic polarity in *E. coli*, the restriction of expression of downstream genes in an operon after a nonsense codon that interrupts translation [44,45]. More generally, Rho inhibits non-productive transcription, particularly in the antisense direction from promoters [42]. Rho has a particular role in diminishing transcription from certain prophage

DNAs that represent intrusive horizontally obtained sequences [46]. One consequence of non-productive transcription is believed to be the accumulation of free RNA that invades negatively supercoiled DNA, creating R-loops [47]; these structures may be particularly deleterious by obstructing movement of the replication fork. In fact, inhibition of Rho activity gives rise to double-strand breaks in DNA [48].

Structure and mechanism

Structural analysis of the bacterial termination factor Rho, a homohexameric translocase (or helicase) [49], has been greatly revealing of its mode of function [50,51]. Early biochemical analysis identified two sorts of nucleic acid binding sites: *primary* sites that mediate recognition of available RNA sequences, which are characterized particularly by a lack of secondary structure and a predominance of C residues, and *secondary* sites on a distinct surface of the enzyme that stimulate the ATPase activity [52]. Initial binding of RNA occurs to the primary sites of the six subunits, followed by its interaction with secondary sites that constitute the translocase motor. Structural analysis shows that Rho is a broken hexameric ring (a "lockwasher" structure) [51], which provides a passageway for RNA to enter and bind the secondary site, following which the structure closes into the active form that mediates termination [50,53,54]. In its enzymatic function, Rho is a translocase, moving along RNA using the energy of ATP. There is evidence that Rho engages in "tethered tracking," consistent with an early proposal [55,56], remaining bound through its primary sites to an initial RNA binding sequence while RNA tracks through the secondary sites toward RNAP. How Rho interacts with RNAP and exactly how it terminates transcription are still quite obscure. It could be significant that Rho can terminate transcription by eukaryotic RNA polymerase II [57] and archaeal RNAP [58], which are unlikely to have conserved binding sites [57], although this might reflect simply the powerful translocase activity of Rho acting against an obstacle. The translocase activity likely pulls the transcript through Rho in the 5′–3′ direction, meeting and then pushing against RNAP—exerting a force that would tend to pull the transcript out of the complex, as discussed further below. There likely are specific interaction sites between Rho and its natural RNAP, although no well-defined locus of interaction has been found.

There is no consensus Rho termination site beyond the preference for cytidine nucleotides in the segment that binds the primary site of the enzyme, and a preference for Y residues in the secondary binding site [54] The well-studied set of release sites for Rho in bacteriophage lambda also is pause sites [59], as is the riboswitch terminator [39], although the nature of these pauses is unknown. Not all pause sites are release sites, presumably because the upstream segment is not favorable (i.e., C-rich and relatively unstructured). And since Rho must act at a wide variety of sites in suppressing futile transcription, it is unlikely that specific pause sequences are involved. The activity of Rho also is modulated by transcription rate, which introduces a kinetic element in the selection of sites [60].

Importantly, Rho function is stimulated by the transcription elongation factor NusG, a homolog of the eukaryotic factor Spt5 [61]. Structural analysis shows that NusG and Spt5 bind equivalent regions of the bacterial [61], eukaryotic [62] and archaeal [63] core enzymes, and are thus likely have at least in part equivalent functions. *nusG* was Identified originally through its role in the bacteriophage lambda gene *N* antitermination system [64], as well as its ability to bind Rho [65]. NusG stimulates termination by binding a specific site of Rho and favoring transition from the inactive open ("lock washer") to active closed-ring conformation [50]. NusG activity also lessens the requirement for C residues in primary site binding, expanding potential termination sites [50]. NusG is involved intimately in the coupling of translation and transcription, because the site where Rho binds NusG alternatively engages the ribosomal protein S10 [50,66]. Thus, a ribosome binds NusG on RNAP if translation occurs, and prevents Rho interaction; but if translation fails, NusG is freed to bind Rho, thus stimulating termination [67].

Mfd-Dependent Termination

Background

Mfd is predominantly a DNA repair protein that mediates the process of transcription-coupled repair [68–70], and an important component of its activity is to act as a release factor that removes RNAP from DNA. Mfd recognizes a stalled RNAP and removes it from DNA, and simultaneously recruits the excision repair machinery by binding UvrA protein [71]. Unlike the other transcription termination modes that involve RNA, Mfd is an ATP-dependent *DNA* translocase, binding simultaneously to DNA and RNAP, and using the energy of ATP to remove RNAP. The Mfd DNA translocase activity is invoked by binding RNAP, which induces conformational rearrangements in the multidomain protein [72]. However, recent single-molecule analysis of Mfd function shows that Mfd also can bind and translocate along DNA without first binding RNAP [73]. In this mode, Mfd patrols the DNA, moving more slowly than RNAP: if RNAP moves normally, Mfd does not catch up, but a stalled RNAP is overtaken and the release/recruitment activity of Mfd ensues. There is evidence that after transcript release, RNAP remains bound to Mfd and the complex continues movement along the DNA [71]; however, RNAP presumably

would be a passive passenger not engaged in transcription.

Structure and mechanism

The Mfd polypeptide has seven domains that mediate RNAP beta subunit binding, UvrA binding, and DNA translocase activity [74]. The translocase domains have strong homology to those of the recombination helicase RecG, giving insight into how they are arrayed on DNA [75]. Although the natural target of Mfd is RNAP stalled by a DNA lesion such as a pyrimidine dimer, a sensitive experimental substrate is RNAP blocked by nucleotide substrate deprivation at an engineered site, or by a blocking protein such as a repressor or the Gln111 variant of EcoRI endonuclease, which binds DNA but cannot cut [76]. Mfd removes a stalled RNAP, measured by freeing of the transcript from complex bound to magnetic beads, in an ATP- or dATP-dependent reaction [77]. Mfd binds the upstream surface of RNAP, placing the translocase domains on DNA that emerges from the elongating complex and requiring about 25 bp of free upstream DNA to function (Fig. 1) [77].

A strong insight into its mechanism is the demonstration that Mfd induces forward translocation of a backtracked complex [77]. Using an irreversibly backtracked RNAP complex in an experimental system, it was shown that Mfd rescues this complex into productive elongation. Essentially all backtracked complexes could be rescued, meaning that all were propelled forward until the RNA 3′ end was available to prime continued elongation in the enzyme active site, and that complexes were not released in the backtracked state. In the same reaction, omission of NTP substrates resulted in release of all transcripts. These results indicate strongly that the translocase activity that pushes RNAP forward is also the activity that collapses the complex and releases the transcript when elongation cannot occur. We consider models for this activity below.

Common Features of the Termination Pathways

A noteworthy feature of the three termination pathways is that each displays a detectable tendency to induce forward translocation of the stalled complex. The hairpin of an intrinsic terminator, tested in the absence of the U-rich segment required for transcript release, enables RNAP to push through an EcoR1 Gln111 block with higher efficiency than without the hairpin; in one example, readthrough was increased from 12% to 48% [7]. The OMR reaction demonstrates the same effect.

The ATP-dependent enzymes Rho and Mfd do not utilize a U-rich segment to favor release, and given their promiscuous function, there is unlikely to be any particular sequence requirement of the transcription bubble at the release site; thus, both translocases presumably transmit enough force to induce release at an arbitrary site. The forward translocation tendency of both Mfd and Rho is seen in the pattern of transcripts of RNAP stopped by EcoR1Gln111 [78]. If no NTPs are present to allow further elongation, release occurs at the site where elongation stops. However, when a low concentration of substrate for the next two positions is present, the release site predominantly moves downstream one or two nucleotides, and release is still efficient at these sites. This is interpreted to mean that the encounter between RNAP and EcoR1Gln111 is somewhat elastic, allowing added force to translocate RNAP further against the block [78].

The primary mechanism of release of both Mfd and Rho thus may be a forward translocation mechanism, corresponding to that illustrated for the intrinsic terminator in Fig. 2; this was suggested particularly because arbitrary sites will have G/C base pairs, as does the type of intrinsic terminator that is thought to release by forward translocation [2]. For RNAP blocked by EcoR1GlnIII, or some other strong barrier, as for the crosslinked intrinsic terminator, a different pathway of release by Mfd and Rho must be followed, that is, slippage or the bubble collapse pathway of Fig. 2.

Finally, there may exist undiscovered or uncharacterized termination mechanisms of bacterial RNAP. One interesting candidate would be some component of the DNA replication fork, which very efficiently removes potentially disruptive transcription complexes, particularly those codirectional with replication fork movement [79]. The ability of the DNA translocase Mfd to push RNAP off DNA could provide a model for such an activity.

Acknowledgment

I thank Eric Strobel for advice on the manuscript and for helpful discussion.

Received 2 March 2019;
Received in revised form 28 March 2019;
Accepted 2 April 2019
Available online 9 April 2019

Keywords:
RNA hairpin;
transcription pause;
EPS (elementary pause sequence);
Rho factor;
Mfd

Abbreviations used:
RNAP, RNA polymerase; OMR, oligonucleotide-mediated release; EPS, elemental pause sequence; NTP, nucleoside triphosphate.

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