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Review

Transcription Termination: Variations on Common Themes

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Transcription initiates pervasively in all organisms, which challenges the notion that the information to be expressed is selected mainly based on mechanisms defining where and when transcription is started. Together with post-transcriptional events, termination of transcription is essential for sorting out the functional RNAs from a plethora of transcriptional products that seemingly have no use in the cell. But terminating transcription is not that easy, given the high robustness of the elongation process. We review here many of the strategies that prokaryotic and eukaryotic cells have adopted to dismantle the elongation complex in a timely and efficient manner. We highlight similarities and diversity, underlying the existence of common principles in a diverse set of functionally convergent solutions.

Transcription Termination: on the Importance of Brakes

The transcription elongation complex [TEC, composed of RNA polymerase (RNAP), DNA template, and nascent RNA transcript] is a stable macromolecular assembly able to operate against large opposing forces [1]. Although most studies on the biophysical properties of the TEC have been performed with bacterial RNAPs, many of the general principles uncovered also apply to the eukaryotic enzymes, RNAPI (dedicated to the production of ribosomal RNA), RNAPII (producing mRNAs and non-protein coding RNAs), and RNAPIII (mostly dedicated to tRNAs, rRNA and some non-protein coding RNAs). TEC stability is required for the robust full-length synthesis of transcripts some of which can be long [up to 10^8 nucleotides (nt) in eukaryotes]. However, termination at the end of each transcription unit is required for its regulation independently of surrounding genes and to prevent overlapping transcription events that can interfere with each other. The relatively recent notion that transcription initiation occurs in a rather pervasive manner in virtually all organisms has additionally directed much interest to post-initiation events, including termination, for defining the composition of the functional transcriptome [2].

Because of its stability, disassembling a TEC is an energy-requiring process that depends on the timely and precise action of machineries that can be as complex as the ones involved in its assembly. TEC components are held together by an intricate network of intermolecular interactions that include a key 8–10-bp duplex formed within the RNAP main channel between the DNA template strand and the nascent RNA (Figure 1A). Remodeling of this interaction network as the RNAP moves along DNA (and the RNA chain is extended) can affect the rate and processivity of transcript elongation, but also lead to pausing of the transcriptional machinery on the DNA template and eventually backtracking in a stable or metastable state. Under specific circumstances, the TEC can be driven into an unstable, high-energy state prone to irreversible dissociation (transcription termination). The strategies

Trends

Transcription termination is essential for many aspects of gene expression: often defining the limits of transcription units, dictating the fate of the RNA produced and limiting the extent of noncoding and pervasive transcription.

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Mechanisms of transcription termination are generally specific for every RNA polymerase, but many similarities exist between these mechanisms that sometimes transcend evolutionary distances and reflect common thermodynamic principles underlying the process.

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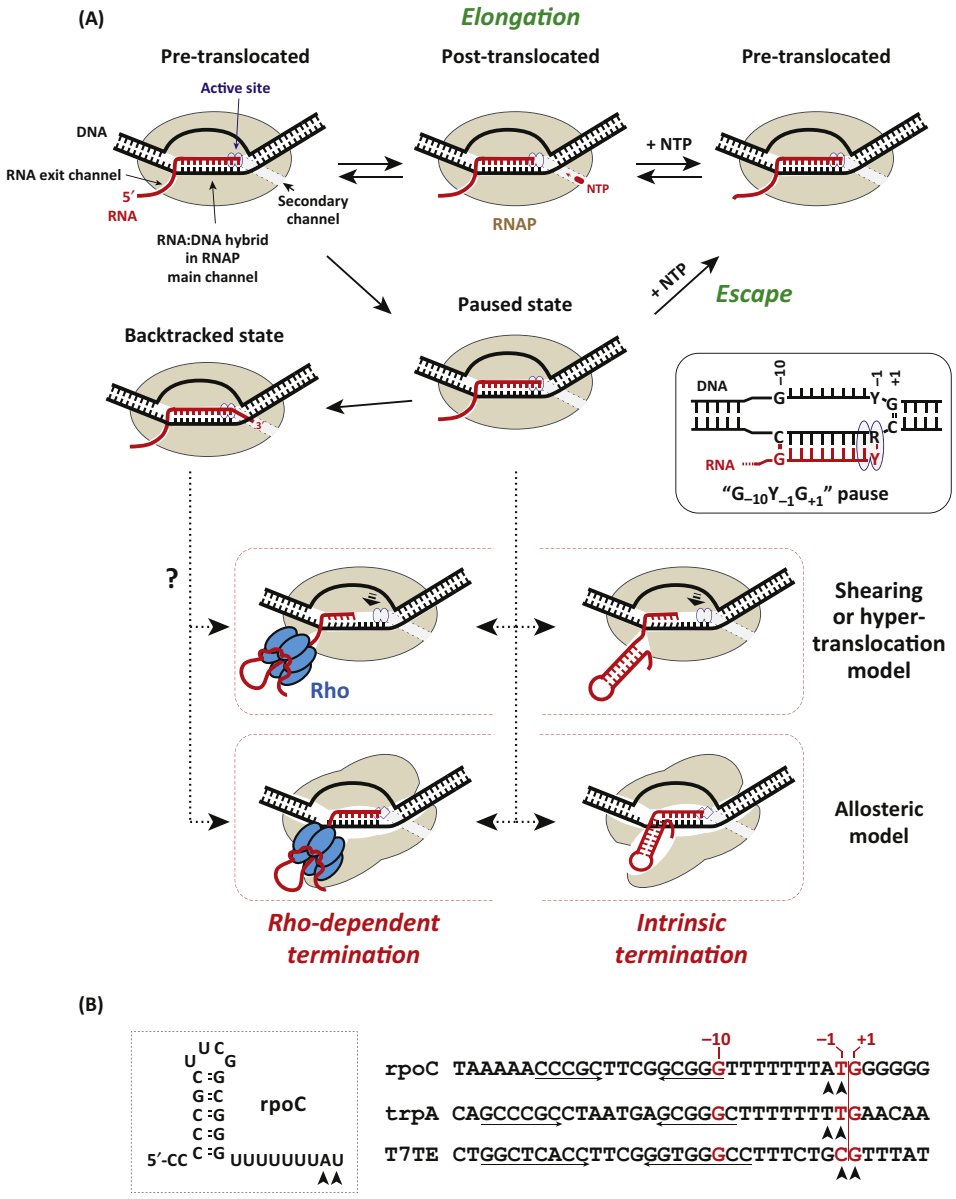


Figure 1. Transcription Termination in Bacteria. (A) Models of bacterial transcription elongation and termination pathways. A paused intermediate is common to all termination models. In the hybrid-shearing pathway, RNA is pulled (by the action of Rho or hairpin folding) from an otherwise immobile TEC whereas, in the hyper-translocation model, hybrid shortening results from forward movement of RNAP. In the allosteric model, the terminator hairpin (or Rho) invades the RNA exit channel and subsequently the main channel of RNAP, triggering catalytic inactivation and conformational destabilization of the TEC. The configuration of the NA scaffold and RNAP active site (double ovals) at a consensus '$G_{-10}Y_{-1}G_{+1}$' pause site [5] is shown in the inset. (B) Intrinsic terminator sequences encode RNA hairpin motifs followed by U-rich sequences. They also sometimes contain a $G_{-10}Y_{-1}G_{+1}$ motif (red residues) that may contribute to RNAP pausing at the termination sites (black arrowheads). Abbreviations: RNAP, RNA polymerase; TEC, transcription elongation complex.

used to achieve this aim generally differ between prokaryotes and eukaryotes, and sometimes considerably even in the same kingdom. We review here the mechanisms of transcription termination in bacteria and eukaryotes, with a specific focus on the common principles underlying the dismantling of the TEC.

Transcription Termination in Bacteria

Bacterial transcription termination signals are traditionally divided into two classes depending on whether the signals rely solely on the information encoded in DNA (intrinsic terminators) or require the action of a regulatory protein, most often the protein Rho (factor- or Rho-dependent terminators), to destabilize the TEC. The molecular mechanisms underlying intrinsic and Rho-dependent termination have been studied extensively and are detailed in several recent reviews [1,3–5]. Here, we only briefly survey the major aspects and discuss the most recent views. We outline similarities among termination mechanisms that sometimes blur the lines between intrinsic and factor-dependent signals.

Intrinsic Termination

Intrinsic termination is triggered by a nucleic acid signal that alone is sufficient to destabilize the TEC. Characteristic intrinsic termination signals are present in bacterial transcripts in the form of a GC-rich RNA hairpin followed by a run of U residues (Figure 1B). Sequences encoding such hairpin-dependent terminators can be detected by computational approaches [6], and are usually found at the end of bacterial genes and operons or, in some instances, in the 5′ untranslated region of the gene/operon where they can regulate gene expression by inducing early transcription termination (attenuation) [3,7]. RNAP pausing, which occurs early in the termination process, is an important component of intrinsic termination [1]. Pausing is favored by the formation of a poorly stable RNA:DNA hybrid within the main channel, which occurs upon synthesis of the terminator U tract [8]. This provides time for the terminator hairpin to form at a stage where it can invade the RNA exit channel of RNAP (Figure 1A). This event triggers TEC destabilization and dissociation by a mechanism that is still debated [1].

Several hypotheses have been proposed for the role of the hairpin in termination. One model, the hybrid shearing model, proposes that the RNA:DNA hybrid dissociation is initiated at positions most proximal to the nucleating hairpin, and this is facilitated by the presence of uridines [9] (Figure 1A). A second model, the hyper-translocation model, proposes that formation of the terminator hairpin pushes RNAP forward along the DNA, without the addition of nucleotides to the RNA 3′ end, resulting in progressive shortening of the RNA:DNA hybrid [10]. A third model proposes that invasion of the RNA exit channel by the terminator hairpin triggers conformational rearrangements within the RNAP which destabilizes the TEC (allosteric model; Figure 1A [11]. While the two former models focus on the action of the hairpin on the RNA:DNA duplex within the RNAP main channel as the primary determinant of TEC stability, the allosteric model postulates a direct destabilizing action of the hairpin on the RNAP.

Recently, a class of hairpin-dependent terminators that function only in the presence of the protein cofactor, NusA, have been found in *Bacillus subtilis* [12]. These terminators usually contain weak hairpins $\Delta G > -10$ kcal/mol) and hairpin-distal imperfect U tracts. It has been suggested that NusA may slow transcription at these imperfect U tracts and assist the folding of weak hairpins within the RNA exit channel [12], thereby promoting factor-dependent transcription termination through a mechanism analogous to intrinsic termination.

Clear analogies to intrinsic termination in bacteria are found in eukaryotic RNAPIII transcription termination and will be discussed later.

Rho-Dependent Termination

Rho-dependent terminators are characterized by a strict requirement for the bacterial factor Rho and by relaxed sequence determinants [13]. This sequence versatility could allow the presence of Rho-dependent terminators at positions in the genome where strong selective pressure exists on the DNA sequence (e.g., within coding regions).

Rho is a homo-hexameric, ATP-dependent molecular motor protein ring that binds to 85–100 nt long, ribosome-free sections of nascent transcripts at Rho utilization (Rut) sites. Rut sites are traditionally viewed as C-rich, G-poor sequences that contain few stable secondary structures [13]. However, even such a loose definition of Rut sites has exceptions [14,15] and the poor informational content of natural Rut sites can be compensated, at least in part, by the presence of auxiliary sequences that determine terminator usage and/or strength through the recruitment of ancillary factors such as proteins NusA, NusB, or NusG [13].

Binding to a Rut site is the primary determinant of Rho activity [16,17]. Once bound, Rho is thought to load the RNA chain into its central ring channel where RNA–protein contacts allosterically couple RNA translocation to ATP hydrolysis [18–20]. The ATP-fueled Rho motor then translocates towards the transcript 3′-end in order to catch up with and dissociate RNAP at a downstream transcription termination site (TTS) in the DNA template [1,4]. Rho is moderately processive and dissociates from RNA after translocating only 60–80 nt [21,22]. This distance should nonetheless be sufficient in most cases because Rho translocates 2–5 times faster than RNAP and should thus quickly overtake it [21]. It is possible that Rho processivity (and, thus, the window of opportunity for termination along the DNA template) is tuned by accessory factors or physical roadblocks such as proteins bound to RNA [21,23] but this hypothesis has not yet been rigorously tested.

The phenotype of slow- and fast-acting RNAP and Rho mutants supports the notion of a kinetic competition between Rho and RNAP (kinetic coupling model) [24] and is consistent with the findings that TTSs appear to systematically coincide with RNAP pause sites but do not display common sequence features [1,16,17]. An analogous model has been proposed for Sen1-dependent termination in yeast, which will be discussed later.

Rho is a rather strong motor [23] and it is conceivable that, once abutted to RNAP, it can apply sufficient force on the RNA chain to mechanically pull it from the TEC. This variant of the hybrid shearing model adapted to Rho-dependent termination (Figure 1A) [1] has not been tested yet. Rho-dependent termination may also rely on a hyper-translocation mechanism similar to that proposed for intrinsic termination (Figure 1A) [25].

An alternative model, based on *in vitro* experiments, posits the allosteric destabilization of the TEC by Rho [26]. A two-step mechanism has been proposed whereby Rho quickly inactivates the TEC before triggering its slow dissociation. Invasion of the RNA exit channel by Rho would trigger TEC conformational rearrangements analogous to those induced by an intrinsic terminator hairpin (Figure 1A) [26]. In the context of this model, the authors also propose the existence of a persistent, RNA-independent association of Rho with RNAP [26], which is still subject to debate [27,28].

Recent genome-wide analyses [29–31] have shed light on the mechanism and extent of Rho-dependent termination. Although considered an important cofactor [29], the NusG protein only stimulates Rho action at a minority (~20%) of the termination hotspots uncovered by transcriptomics [32]. By contrast, these largely colocalize with binding sites for the histone-like nucleoid-structuring (H-NS) protein [32]. *In vitro*, bridged H-NS filaments facilitate Rho termination at sites that are otherwise poor TTSs and where H-NS also stimulates transcriptional pausing and RNAP backtracking [33]. Whether H-NS simply increases the time window for Rho action at specific pause sites [33] or promotes structural changes in backtracked TECs that facilitate destabilization by Rho remains to be determined.

In *Escherichia coli*, ~20% of the roughly 4500 genes coding for mRNAs, small RNAs, and tRNAs are terminated in a Rho-dependent manner [29,30]. In addition, genome-wide analyses suggest

that a large fraction of the 1300 Rho-dependent terminators specifically play a role in preventing transcription antisense to coding genes [32]. A similar analysis of the *B. subtilis* transcriptome [31] also highlighted repression of antisense transcription as a major function of Rho. The mechanism of Rho action allows additional opportunities for regulation of gene expression. Rho-dependent termination can lead to regulated attenuation upon structural remodeling of a 5′-leader mRNA region containing a conditional Rut site either exposed or sequestered in a secondary structure [15,34,35]. Rho function can be regulated by cellular factors that prevent Rho binding to RNA [e.g., YaeO protein shielding the primary binding site (PBS) of Rho] [36], inhibit the maturation of the Rho:RNA complex into its active isoform (as proposed for the Hfq protein [37] or for the RARE sequence motif found in the mgtC leader of *Salmonella enterica* [38]), or increase TEC processivity [3]. Rho binding is also known to be regulated by translation when located within open reading frames. This situation is specific to bacteria where nascent mRNAs are translated during their synthesis by RNAP and arises when a genetic (e.g., nonsense mutation) or metabolic (e.g., amino acid depletion) condition impairs translation [39].

Finally, Rho has been implicated in genome-wide repression of R loops; RNA:DNA hybrids that form when nascent RNA anneals with template DNA in the wake of transcribing RNAP. This has been proposed to be the major function of Rho in *E. coli* [40], which can dissociate RNA:DNA hybrid structures *in vitro* using its ATPase-powered helicase activity [41]. However, whether these R loops form naturally during transcription or arise from codirectional collisions between the replisome and arrested (backtracked) TECs, is unclear, and Rho might prevent R-loop formation simply by dislodging the arrested TEC [42]. In line with this proposal, impairing Rho function in *E. coli* favors the formation of DNA double-strand breaks that typically occurs upon transcription–replication conflicts [43].

Transcription Termination in Eukaryotes

Mechanism of RNAPI Termination

RNAPI transcribes the ribosomal genes that generate the largest precursor of rRNAs. rDNA is organized in multiple tandem repeats (150–200 copies in yeast and 400 copies in humans) and is transcribed in the nucleolus. Each repeat contains the sequence corresponding to the different mature rRNAs (18S, 5.8S and 25S in yeast or 28S in humans) flanked by external or internal transcribed spacers. The different repeats are separated by an intergenic sequence (IGS) (Figure 2A). Termination of transcription by RNAPI occurs at the IGS, which contains one or several recognition sequences for DNA-binding proteins of the Myb-family, often preceded by T-rich sequences. Most studies agree that transcribing RNAPs are roadblocked by a Myb-like factor bound to the DNA and the subsequent release of the polymerases is promoted by additional factors. RNAPI release is stimulated by the presence of T stretches of different lengths, which, however, are absent from the human main terminator, suggesting that these might not be a strict requirement for termination [44].

In mammals the Myb-like protein that pauses the elongation complex is TTF-I (transcription termination factor for RNAPI) and the release of RNAPI from the template depends on polymerase I and transcript release factor (PTRF), a factor that associates with RNAPI and interacts directly with TTF-I [44]. However, little is known about the precise mechanisms by which PTRF elicits dissociation of the elongation complex. PTRF is sufficient to provoke the release of RNAPI paused by TTF-I in an ATP-independent manner *in vitro* (Figure 2B). This strictly requires the presence of a T-rich sequence upstream of the TTF-I binding site box [45], and could be due to the weakness of the RNA:DNA hybrid at the U-rich sequence, which destabilizes the elongation complex paused by TTF-I. This is reminiscent of intrinsic termination in bacteria and RNAPIII termination (see below). Whether the release of RNAPI involves the simultaneous interaction of PTRF with TTF-I and RNAPI remains to be elucidated.

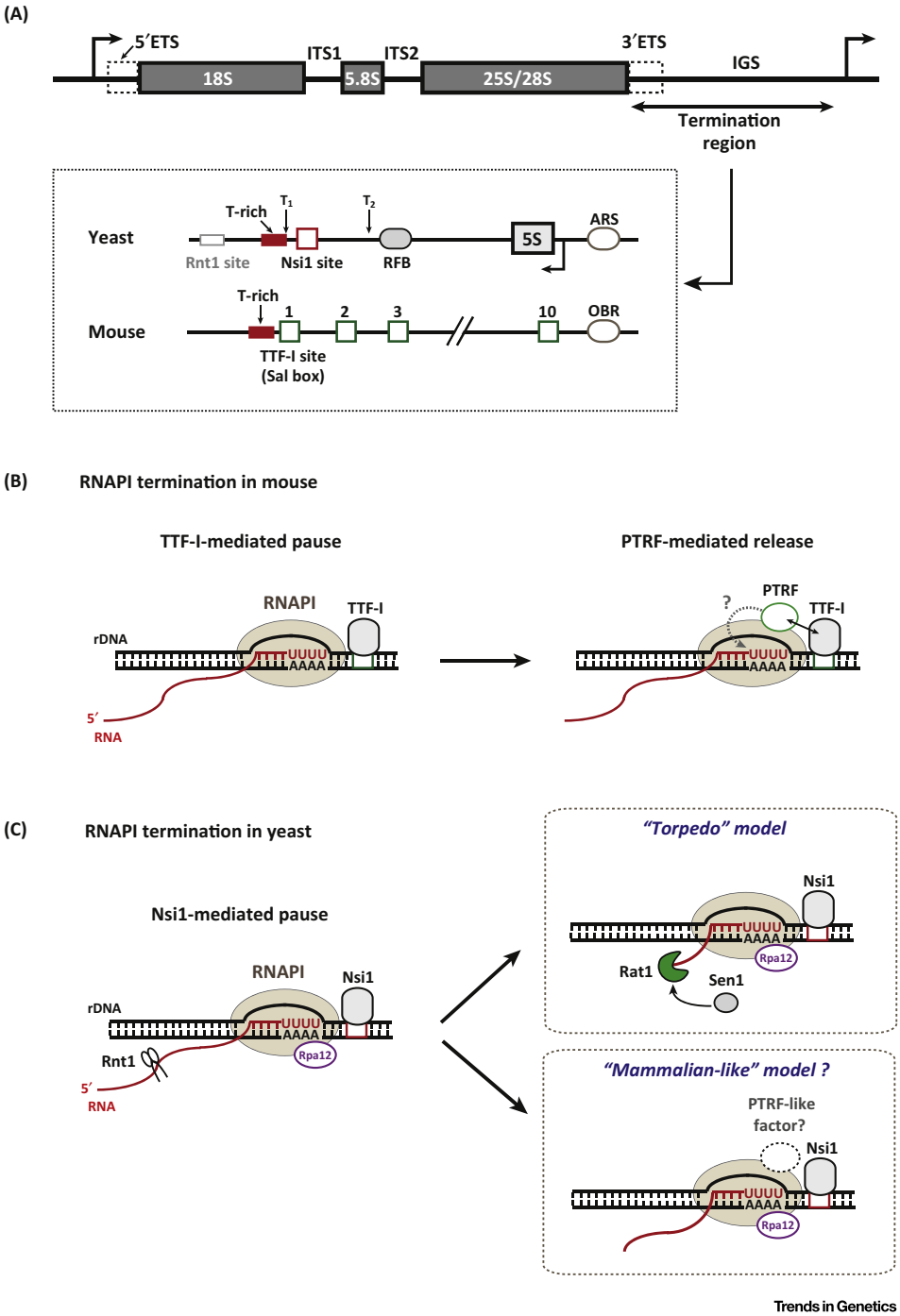


Figure 2. Termination of RNAPI in eucaryotes. (A) Structure of the rDNA locus. The structure of the termination region is shown in detail both for the yeast and the mammalian models. T_1 and T_2 are alternative termination sites at the Nsi1 binding site and at the RFB. Open green boxes for the mammalian termination region indicate alternative and redundant sites of termination. (B) Model for transcription termination in mouse and yeast. More details in the text. Abbreviations: ETS, external transcribed space; IGS, intergenic sequence; ITS, internal transcribed spacer; Nsi1, NTS1 silencing protein 1; PTRF, polymerase I and transcript release factor; RFB, replication fork barrier; TTF-I, transcription termination factor.

The generalities of RNAPI transcription termination are conserved in *Saccharomyces cerevisiae*, although a PTRF homolog is not present in yeast and the identity of the release factor is unknown. Although early work suggested that Reb1 is the roadblocking factor [46,47], later studies have shown that this role is carried out by another protein containing a Myb-like DNA binding domain, Nsi1 (NTS1 silencing protein 1), which recognizes a sequence that is similar to the Reb1 binding site present at the rDNA terminator [48,49]. The specific implication of Nsi1 instead of Reb1 might be due to some subtle differences in DNA binding of the two proteins [48]. Also, Nsi1 interacts with Fob1, a factor that binds a sequence called replication fork barrier (RFB) in the IGS (Figure 2A), which isolates the rDNA locus from a replication origin to prevent replication–transcription interference [50]. It has to be noted, however, that Nsi1 is not essential in yeast, suggesting the existence of redundant mechanisms of termination. Indeed, direct observation of RNAPI transcription in yeast by electron microscopy indicates that ~90% of transcription terminates immediately upstream of the Nsi1 binding site [51], whereas the 10% remaining terminates at either a second site dubbed T2 or at the RFB, possibly because Fob1 can also act as a roadblock [52]. Consistent with this notion, in metazoans the IGS harbors several tandem terminator sequences (up to 10 in mice), containing TTF-I binding sites (Figure 2A) [44]. Strikingly, roadblocking yeast RNAPI by Reb1 *in vitro* is not sufficient for efficient release of the polymerase, which, however, occurs by addition of murine PTRF [53,54], suggesting that a PTRF-like factor, yet unidentified, could be responsible for dismantling the paused TEC in yeast.

Two independent groups (Figure 2C) [52,55] have proposed a different mechanism for RNAPI termination, which would occur by a 'torpedo' mechanism, analogous to that proposed for RNAPII termination (see below). The 35S pre-RNA is cleaved at the 3′ end of the mature 25S and upstream of the Nsi1-binding site by the RNase III-like endonuclease Rnt1 [56], which likely occurs before the end of transcription [57]. The 5′ end of the RNA cleavage product thus generated would be phosphorylated by the kinase Gcr3p [58] to provide an entry point for the 5′ to 3′ exonuclease Rat1. The latter is proposed to degrade the nascent transcript until it encounters RNAPI roadblocked at the Nsi1 site and elicits its release by an unknown mechanism. It has also been proposed that the helicase Sen1, a termination factor for RNAPII (see below), cooperates with Rat1 by removing possible RNA structures that might prevent the access of Rat1 to the nascent RNA [55]. Because no Rnt1 cleavage has been identified in mammalian rRNA precursors, this mechanism might not apply to metazoan RNAPI termination, or might require cleavage by some other endonucleases.

Termination of RNAPI transcription is also strongly dependent on the nonessential Rpa12 (or AC12.2) subunit [51]. Rpa12 is homologous to the C11 subunit from RNAPIII (see below) and both subunits share homology to Rpb9 from RNAPII in their N-terminal region and to the RNAPII transcription elongation factor TFIIS in their C-terminal region (Table 1). The region of homology with TFIIS contains a C-terminal ribbon, also present in C11, that reaches the active site of the polymerase and stimulates the RNA cleavage activity important for proofreading [59]. Because this C-terminal ribbon occupies the binding site for backtracked RNA, it is possible that forced backtracking by a roadblock like TTF-I or Nsi1 provokes a conformational change involving Rpa12 that favors termination.

Mechanisms of RNAPII Termination

Termination of transcription by RNAPII has been the subject of a few recent reviews [60–62]. We therefore only discuss the generalities of the different pathways and highlight some of the common principles and differences with the other known mechanisms.

CPF Pathway

Two main pathways of termination exist in yeast, only one of which is conserved in metazoans. The CPF pathway terminates transcription of mRNAs (Figure 3A). A large, multisubunit complex

Table 1. Subunit Composition of Eukaryotic RNA Polymerases

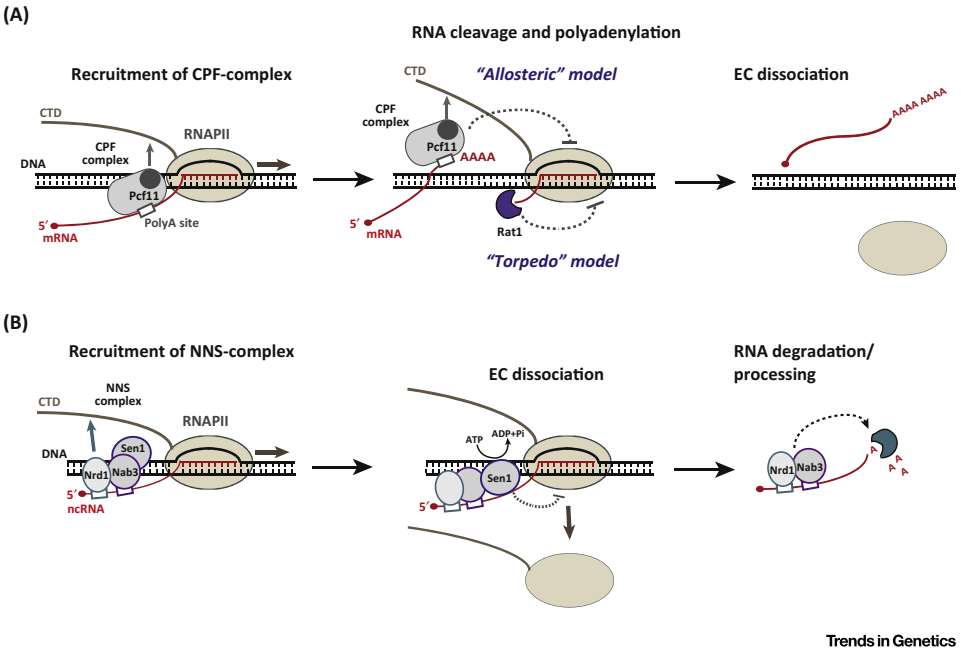
Module/function	RNAPI	RNAPII	RNAPIII
Core subunits	A190	Rpb1	C160
	A135	Rpb2	C128
	AC40	Rpb3	AC40
	Rpb5	Rpb5	Rpb5
	Rpb6	Rpb6	Rpb6
	Rpb8	Rpb8	Rpb8
	A12.2[a]	Rpb9	C11[a]
	Rpb10	Rpb10	Rpb10
	AC19	Rpb11	AC19
	Rpb12	Rpb12	Rpb12
Stalk subcomplex[b]	A14	Rpb4	C17
	A43	Rpb7	C25
Initiation and termination	A49		C37[c]
	A34.5		C53[c]
Initiation			C31
			C34[d]
			C82[d]

[a]C-terminal domain corresponds to a zinc-ribbon domain of the elongation factor for RNAPII TFIIS responsible for stimulation of transcript cleavage.
[b]Conserved module with diverse functions in transcription *in vivo*, among which the interaction with transcription factors [100].
[c]TFIIF-like subcomplex.
[d]TFIIE-like subunits.

(called here for simplicity the CPF, cleavage and polyadenylation factor; for a more detailed description, see [60]) and several additional factors are required for efficient dismantling of the elongation complex at the end of target genes. Release of RNAPII is associated and generally preceded by 3′-end processing of the nascent transcript, which includes cleavage and polyadenylation [61,62].

3′-end processing and termination are triggered by multipartite signals on the nascent RNA, which are recognized by components of the CPF complex that also directly interacts with the polymerase. It is generally accepted that cleavage of the nascent transcripts occurs before release of the enzyme from the DNA template, which occurs further downstream. The biochemical details of the termination reaction are still unclear, mainly because it has not been possible to fully recapitulate the whole reaction *in vitro* with purified components. Two, non-mutually exclusive lines of thought predominate (Figure 3A). According to the first, upon encountering termination signals the polymerase undergoes a conformational change that commits the enzyme to termination (allosteric model) [63–66]. The second posits that cleavage of the nascent transcript provides an entry point for a 5′–3′ exonuclease (Rat1 in yeast and XRN2 in humans) that degrades the nascent RNA still attached to RNAPII and elicits termination (torpedo model) [67–71]. Kinetic competition has been proposed to occur *in vivo* between the elongating polymerase and the pursuing exonuclease [65,69]. Combinations of the allosteric and torpedo models have also been proposed [65,69,72].

In spite of intense investigation and supporting evidence for both models, many mechanistic issues are still unresolved [66]. For instance the nature of the conformational change in the



Trends in Genetics

Figure 3. Major Pathways of RNAPII Termination. (A) Transcription termination by the CPF pathway. The two main models for release of the polymerase are shown above (allosteric model) or below (torpedo model) of the scheme. Action of Rat1 depends on previous cleavage of the nascent RNA by the CPF complex. According to the allosteric model, the elongation complex that encounters termination signals is committed to termination because of a conformational change, possibly due to the loss of elongation factors. (B) Termination of noncoding RNA by the NNS complex in yeast. Nrd1 and Nab3 interact with the nascent RNA and recruit Sen1 for dismantling the elongation complex with a mechanism that requires interaction with the nascent RNA and ATP hydrolysis. Abbreviations: CPF, cleavage and polyadenylation factor; EC, elongation complex; NNS, Nrd1-Nab3-Sen1; RNAPII, RNA polymerase II.

elongation complex remains elusive: it has been shown that elongation factors are lost *in vivo* at this stage [63] and that one of the CPF components, Pcf11, can dismantle the elongation complex *in vitro* by simultaneously binding to the nascent RNA and RNAPII [64], but whether these observations are relevant for the termination step remains unclear. Similarly, the molecular event that would trigger RNAPII release once the nascent RNA has been degraded by the Rat1 exonuclease are unknown and conflicting evidence exists as to whether this can elicit termination *in vitro* [70,71,73]. It could be speculated that backtracking of RNAPII when the nascent RNA has been cleaved and degraded by Rat1 might rapidly lead to loss of the DNA:RNA hybrid in the catalytic center of the polymerase, but there is no supportive evidence for this hypothesis.

NNS Pathway

The other main pathway of termination (Figure 3B), the NNS (Nrd1-Nab3-Sen1) pathway, is dedicated to the production of functional and stable noncoding RNAs, such as the small nuclear and small nucleolar RNAs, involved in splicing and the modification of rRNA respectively, and to the termination of transcription of a class of unstable RNAs produced by pervasive transcription called CUTs (cryptic unstable transcripts) [62]. In this pathway, two RNA-binding proteins, Nrd1 and Nab3, recognize short sequence elements on the nascent RNA, which is thought to be the main determinant of specificity [74–77]. Nrd1 also interacts directly with RNAPII [78–80]. Subsequently, an RNA helicase, Sen1, interacts with the nascent RNA and dislodges the transcribing polymerase [81], a step that presumably implies a kinetic competition between Sen1 translocation on the RNA and RNAPII elongation [82]. This model posits that Nab3 and Nrd1 function as adaptors to position Sen1 for timely and specific termination, but this does not exclude their additional roles in termination.

Box 1. Alternative Pathways for RNAPII Termination

RNAPII transcription can also be terminated by a roadblock mechanism, which has been studied recently for the transcription factor Reb1, and possibly extends to other DNA-binding proteins [101]. With some analogy to termination for RNAPI, Reb1 induces pausing of the polymerase, which *per se* is not sufficient to induce release of the polymerase from the template. Rather, the paused enzyme is ubiquitylated and likely degraded by the proteasome, similar to the fate it faces when encountering DNA damage [102]. This mode of termination is disruptive, in that both the polymerase and the released RNA are destroyed. Because of this, roadblock termination is considered to be a backup mechanism to neutralize termination leakage events that might occlude nearby promoters [101]. A similar mechanism is also used in *E. coli*, whereby elongation complexes stalled by a roadblock or by a DNA lesion are removed by the transcription–repair coupling factor Mfd, an ATPase motor that translocates on double stranded DNA [103–105].

Another backup mechanism is proposed to depend on cleavage of the nascent transcript by the RNaseIII like enzyme Rnt1 [106,107]. Similar to what was discussed for RNAPI termination, Rnt1 cleavage would provide an entry point for Rat1 and promote degradation of the nascent RNA thus provoking termination.

Finally, of mechanistic interest is a variant of the torpedo model proposed for fail-safe termination of mRNAs in *Schizosaccharomyces pombe* [108]. In this model, a fraction of elongation complexes reading through CPF terminators would be terminated in an exosome-dependent manner. The RNA exosome is a multicomponent complex that degrades the RNA from 3′ to 5′; both in the cytoplasm and the nucleus. In a paused and backtracked elongation complex, the 3′ end of the nascent RNA would be accessible from the secondary channel of the polymerase for degradation by exosome, which would prompt release of the polymerase. Whether the elongation complex backtracks sufficiently for the 3′ end of the nascent RNA to be accessible and how its degradation by the exosome provokes termination remain, however, unclear.

The NNS pathway is analogous to Rho dependent termination in bacteria in many aspects. Despite the marked structural differences between Rho and Sen1, both interact with the nascent transcript to dismantle the elongation complex in an ATP-dependent manner. In both cases the reaction can be fully recapitulated *in vitro* with purified components, demonstrating that a simple system containing only the helicase and the elongation complex is sufficient for transcription termination. Sen1 (like Rho) is an active helicase [83,84] and has been implicated in unwinding DNA:RNA hybrids (R loops) [85], but whether termination requires translocation on the nascent RNA and/or unwinding of nucleic acids duplexes is unclear. Deciphering whether Sen-1 dependent termination involves hybrid shearing, hyper-translocation or an allosteric change, as proposed for bacterial termination, remains a challenge for future studies.

Several alternative pathways that often function as fail-safe pathways of termination have also been described recently (Box 1).

Mechanism of RNAPIII Termination

RNAPIII-dependent transcripts are short (generally <400 nt long), structured, and abundant. The high expression of RNAPIII genes is due in part to highly processive elongation and very efficient termination, but also to rapid reloading of RNAPIII on the same gene to undertake multiple transcription cycles, a process called facilitated reinitiation [86].

RNAPIII is traditionally considered to be able to terminate transcription efficiently and precisely upon encountering a T stretch of variable length on the non-template strand without a specific need for accessory factors [87]. In yeast, most RNAPIII terminators contain six or seven Ts, whereas in mammals, terminators longer than four or five Ts are rare and often even shorter than four Ts [88]. The flanking sequences can significantly influence the efficiency of termination both in yeast and in metazoans, especially in those terminators that contain short T stretches [89,90], but there is no conserved sequence systematically found either upstream or downstream of the T tract. RNAPIII pauses upon transcribing the T tract so that the rU:dA hybrid occupies the main channel of the polymerase [91,92]. The poor stability of the rU:dA hybrid [93] is believed to be a critical requirement for RNAPIII termination [87]. Recent structural studies have shown that the RNA:DNA duplex is significantly less tightly bound by the active site in the RNAPIII elongation

complex than in the case of RNAPII, which might explain the particularly high sensitivity of RNAPIII to the weak rU:dA hybrid [94].

Recent work [92] has shed some light on the mechanism by which T stretches as short as 5 nt can induce termination. Using an *in vitro* experimental setup, Arimbasseri et al. have shown that upon transcription of the first four Us, the elongation complex pauses and enters a metastable state, which can result in either further elongation or if a fifth U is added, in termination. Importantly, entering this state requires both the presence of T_3 and T_4 in the non-template strand and the integrity of the C53–C37 heterodimer, two RNAPIII subunits homologous to the RNAPII TFIIF transcription factor that are involved both in initiation and termination [92,95]. Early release of the transcript upon incorporation of U_5 also requires the presence of T_5 in the non-template strand and the C53–C37 heterodimer. Residues 226–230 of C37 are critical for this mode of termination [92] and crosslinking and structural studies suggest that they contact the unpaired non-template DNA [94,96]. In the absence of such a facilitated release at short T stretches, termination can

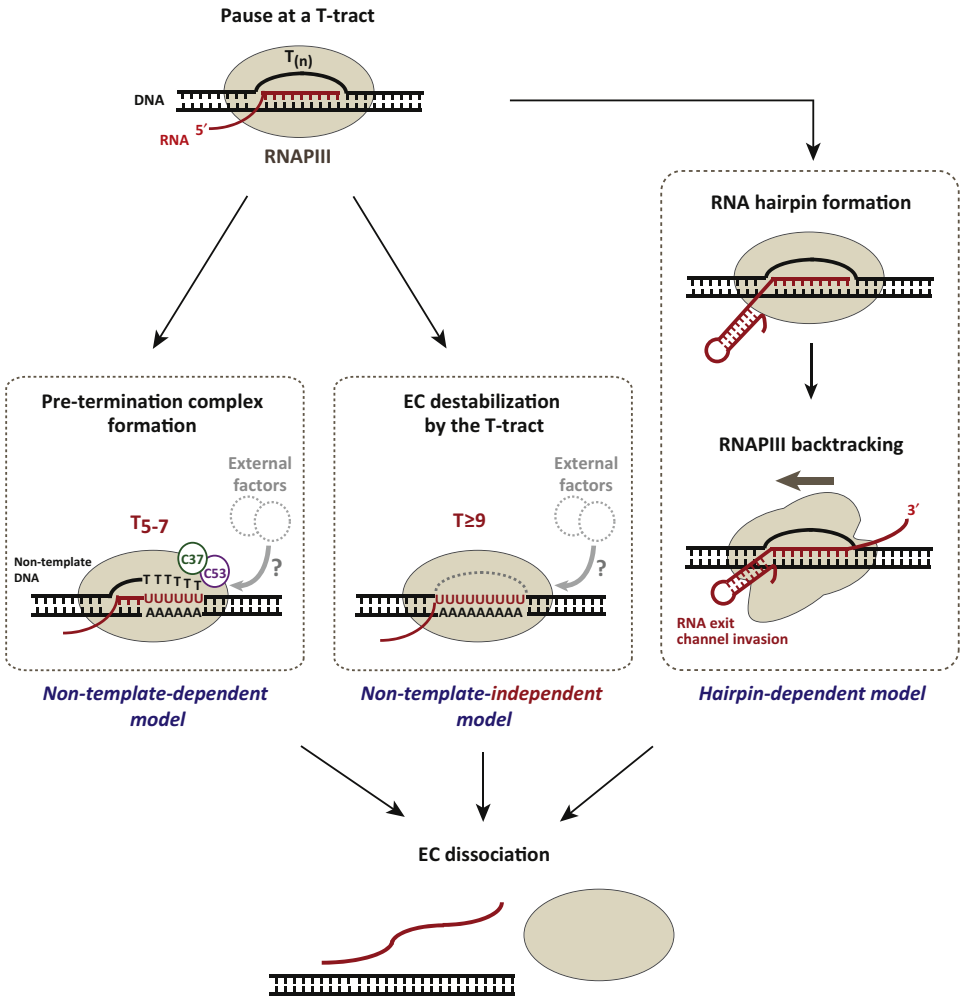


Figure 4. Models of Transcription Termination by RNAPIII. Termination occurs after pausing upon synthesis of a U-rich tract. The elongation complex is destabilized depending on contacts with the C53-C37 subunits of RNAPIII, by the synthesis of a longer U-rich tract or by invasion by a structured region of the newly synthesized RNA. See the text for more details. Abbreviations: EC, elongation complex; RNAPIII, RNA polymerase III.

occur distally in a non-template-strand- and C53–C37-heterodimer-independent manner, presumably depending only on the poor stability of the rU:dA hybrid in the RNAPIII main channel (Figure 4). Whether this latter mechanism is physiologically relevant, for instance, in the case of long but imperfect U-rich terminators, is unclear.

Recent work has challenged two pillars of the previous model, that is, that a T tract alone is sufficient for RNAPIII termination and that release of the polymerase is prompted by the weakness of the rU:dA hybrid [91]. Using a different protocol for RNAPIII preparation, the authors have shown that RNAPIII pauses efficiently at T tracts but cannot dissociate from the DNA template unless a double-strand region can be formed on the upstream RNA (Figure 4). In much the same way as intrinsic termination in bacteria, the hairpin would invade the RNA exit channel provoking the destabilization and subsequent release of RNAPIII. RNAPIII backtracking after pausing at the T stretch would allow some flexibility in the position of the hairpin relative to the T tract and, importantly, provoke release of the enzyme at positions where the internal RNA:DNA hybrid is not necessarily a weak rU:dA tract. The positional flexibility of the hairpin and the natural abundance of structured regions in RNAPIII substrates would explain why such elements of the termination signal were not previously identified. This work has prompted a debate on the *in vivo* relevance of the two models [97,98]. Most differences appear to reside in the purity of the RNAPIII preparation and it is possible that a loosely bound factor, lost in the study by Nielsen *et al*. [91], favors termination even in the absence of a destabilizing hairpin. Consistent with this notion, several factors have been proposed to be involved in RNAPIII termination [87], including the La protein that binds the terminal U tract of RNAPIII transcripts and interacts with the transcription factor TFIIIC [99]. Alternatively, it is possible that, as for RNAPII (and likely RNAPI), alternative termination pathways coexist and are adopted depending on the particular context of each transcription unit.

Concluding Remarks

Whether the considerable variations on the theme of termination reflects a genuine diversity of solutions or simply blurs the quest for common principles remains unclear. However, clear commonalities emerge. The first is the general requirement for pausing of RNAP: this can be induced by the presence of a roadblock or by a signal on the nascent RNA or the DNA, but also by mechanisms that remain to be elucidated as for the more complex NNS- or CPF-dependent termination in eukaryotes. Whether the binding of a protein complex to the nascent RNA, eventually accompanied by the simultaneous interaction with the RNAP as for Nrd1 or for the CPF component Pcf11, is sufficient to slow down elongation and induce pausing is unclear. Termination factors might also simply prevent TEC escape from a paused or backtracked state formed as a consequence of the normal transcription process.

A relatively unstable DNA:RNA hybrid in the TEC is also an important termination determinant that might, however, be restricted to situations depending more heavily on the inherent stability of the TEC. This could be more prominent when a specific machinery for dismantling a stable TEC is absent as for intrinsic termination in bacteria and RNAPIII (and to some extent RNAPI) in eukaryotes, but less so for RNAPII (neither the CPF- nor the NNS-dependent pathways) or for Rho-dependent termination in bacteria. Whether the composition of the hybrid also impacts the position of termination in these cases remains, however, an open and interesting question that must await the precise identification of termination sites.

As for children's games, chasing and touching the RNAP for stopping it is a recurrent theme. Beyond the apparent similarities, however, degradation of the nascent RNA by the chasing Rat1/XRN2 exonuclease, and translocation on the nascent RNA of a helicase (Rho or Sen1) might not translate into the same molecular transitions for releasing the RNAP. Although the molecular mechanism of the reaction is still under debate even for the well-studied

Outstanding Questions

What are the mechanisms that induce pausing during or before termination? How do factors that bind the nascent RNA affect elongation?

What is the role of the RNA:DNA hybrid in systems that use complex machineries for termination?

How can helicases or nucleases that chase the polymerase induce termination?

What technical improvements are required to decrypt the mechanisms of termination in component-controlled systems?

How extensive is redundancy in transcription termination? What is the importance of roadblock termination and other fail-safe mechanisms?

What is the role of chromatin (or nucleoid organization) in termination?

Rho-dependent termination, plausible models based on the enzymatic function of helicases exist. These, however do not seem to easily fit the analogous role of Rat1/XRN2.

With the exception of roadblock termination for RNAPI and RNAPII, the role of the DNA is generally considered to be a minor one. However, the recent discovery that the sequence of the non-template strand is recognized by specific subunits of RNAPIII [92] might reopen the debate: are similar contacts also present in the context of other terminating TEC intermediates? The subunits involved in RNAPIII termination are present in other transcription systems, but in the form of initiation factors [95]; whether other proteins fill this gap is not clear.

In spite of the many *in vivo* studies, understanding the mechanism of eukaryotic termination still requires *in vitro* analyses, which, however, need to face the hurdles of the higher complexity of the systems. Plausible models derived from these analyses then have to accommodate the *in vivo* complexity where many additional factors weigh directly and indirectly on the processivity and stability of the TEC.

Finally, the marked redundancy that characterizes transcription termination in many instances is also a common principle. This testifies to the absolute necessity of preventing the persistent occupancy of the DNA by nonfunctional elongation complexes, again highlighting the essential nature of termination. Redundancy appears to be most important where the compactness of the genome imposes a strong selective pressure on restricting the access of RNAPs to sensitive regions as promoters/nucleosome-free regions, but also applies to larger genomes.

Understanding how transcription ends remains a major future challenge for appreciating how the cell partitions activities that require reading information from contiguous and often overlapping sources (see Outstanding Questions).

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