Unravelling the means to an end: RNA polymerase II transcription termination

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Abstract | The pervasiveness of RNA synthesis in eukaryotes is largely the result of RNA polymerase II (Pol II)-mediated transcription, and termination of its activity is necessary to partition the genome and maintain the proper expression of neighbouring genes. Despite its ever-increasing biological significance, transcription termination remains one of the least understood processes in gene expression. However, recent mechanistic studies have revealed a striking convergence among several overlapping models of termination, including the poly(A)- and Sen1-dependent pathways, as well as new insights into the specificity of Pol II termination among its diverse gene targets. Broader knowledge of the role of Pol II carboxy-terminal domain phosphorylation in promoting alternative mechanisms of termination has also been gained.

Cryptic unstable transcripts
Non-coding RNAs (~200–600 nucleotides long) discovered in yeast that are typically transcribed from intergenic regions of the genome (such as promoters) and are rapidly degraded by the exosome.

Stable unannotated transcripts
Non-coding RNAs discovered in yeast that are generally longer and more stable than cryptic unstable transcripts.

Promoter-proximal pausing
Halting of an early RNA polymerase elongation complex that remains competent to eventually resume transcription.

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Regulation of gene expression, which is essential for normal cellular development and homeostasis, often occurs at the level of transcription. In eukaryotes, nuclear RNA polymerase II (Pol II) is responsible for synthesizing all protein-coding RNAs and most non-coding RNAs, including small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), microRNAs (miRNAs), cryptic unstable transcripts (CUTs) and stable unannotated transcripts (SUTs). Pol II is subject to regulatory control at all stages of the transcription cycle, including initiation, elongation and termination (FIG. 1a). During the first stage of initiation, Pol II is recruited to a gene promoter, the DNA is melted to expose the template strand and the first few nucleotides of RNA are synthesized. Pol II escapes from the promoter when RNA reaches a length of ~8–9 nucleotides, which constitutes the full length of the DNA–RNA hybrid that is observed during the elongation stage. Termination occurs when Pol II ceases RNA synthesis and both Pol II and the nascent RNA are released from the DNA template. Perhaps owing to its foremost position in the cycle, initiation is the best-understood process of transcription in terms of both its mechanism and regulation[1]. More recently, the transition from initiation to elongation has been better defined, and the regulation of this transition by promoter-proximal pausing has emerged as an important and widespread phenomenon[2,3]. Much less is known about the mechanism and regulation of Pol II transcription termination.

Transcription termination serves many vital functions in the cell. For example, it prevents Pol II from interfering with downstream DNA elements, such as promoters, and promotes polymerase recycling[4–6]. Contrary to being limited to a constitutive process at the end of a gene, transcription termination is dynamic and can occur upstream, downstream and within open reading frames (ORFs). The idea that termination could be modulated initially came from studies in bacteria, which revealed that premature termination (that is, attenuation) is a sensitive means to regulate clusters of amino acid biosynthesis genes[7,8]. When the amino acid product encoded by these genes is present in excess, an RNA terminator forms within the 5′ untranslated region (UTR) of the mRNA and leads to the release of RNA polymerase before transcription of the protein-coding region. This particular example takes advantage of intrinsic RNA folding and coupled transcription and translation in bacteria, but other variations of attenuation are mediated through RNA-binding proteins and function independently of ribosome activity[8].

The discovery, and ongoing investigation, of attenuation-based regulatory strategies in viruses (for example, HIV) and eukaryotic organisms has substantiated transcription termination as a widespread and versatile target for controlling gene expression[5,9,10]. The importance of termination in genomic partitioning has become all the more compelling with the recent revelation that transcription is not limited to discrete functional units such

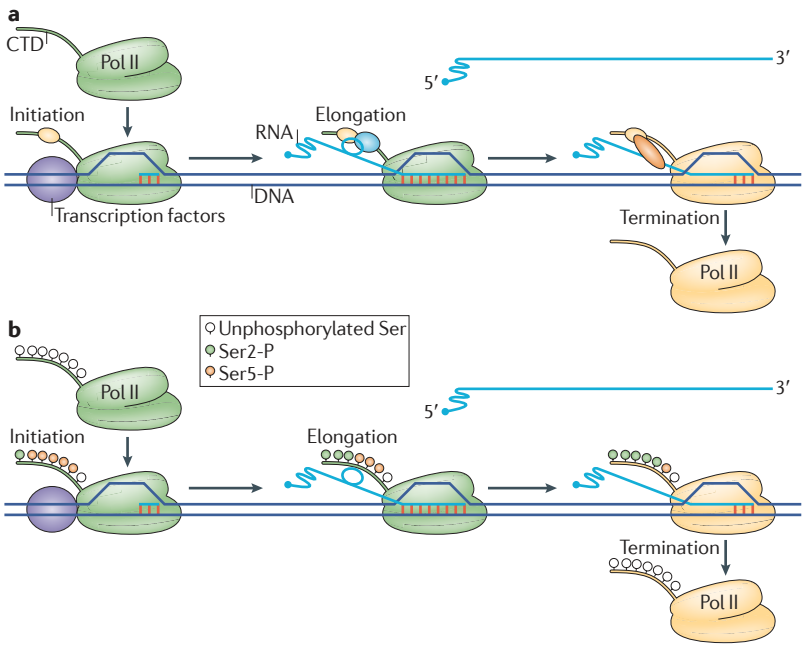


Figure 1 | **Pol II transcription is coordinated with distinct patterns of C-terminal domain phosphorylation. a** | Initiation: RNA polymerase II (Pol II) is recruited to a gene promoter by transcription factors, the DNA is melted to expose the template strand, and the first few nucleotides of RNA are synthesized. Elongation: a full-length RNA–DNA hybrid is formed (~8–9 bp) and Pol II proceeds to extend the transcript. Termination: Pol II ceases RNA synthesis and becomes termination-prone (indicated by its change in colour from green to yellow), and both Pol II and the nascent RNA are released from the template. As shown, destabilization of the RNA–DNA hybrid in the Pol II active site is likely to be a key feature in termination. Protein factors involved in elongation, RNA processing and termination (shown as yellow, blue and orange ovals, respectively) co-transcriptionally associate with the Pol II carboxy-terminal domain (CTD). **b** | The phosphorylation status of the CTD heptad repeats (Tyr_1-Ser_2-Pro_3-Thr_4-Ser_5-Pro_6-Ser_7) changes as Pol II progresses through a gene[28,29]. Hypophosphorylated Pol II is recruited into the pre-initiation complexes and it is phosphorylated on Ser5 by the transcription initiation factor IIH (TFIIH) kinase Kin28 (cyclin-dependent kinase 7 (CDK7) in mammals) during initiation and on Ser2 by CTD kinase subunit 1 (Ctk1; CDK9 in mammals) during elongation. The action of these kinases, combined with their opposing phosphatases (suppressor of Sua7 2 (Ssu72) and regulator of transcription 1 (Rtr1) for Ser5-P and Fcp1 for Ser2-P), sets up a dual gradient of CTD modification, with Ser5-P and Ser2-P being more prevalent when Pol II is towards the 5′ end and the 3′ end of the gene, respectively. Several other kinases and phosphatases contribute to this gradient but are not discussed for simplicity. Each phosphorylation shown on the CTD represents a single heptad repeat (not all repeats are shown) with the general phosphorylation patterns indicated.

as ORFs, but rather that genomes are almost entirely transcribed[11]. The high volume of DNA traffic resulting from genome-wide transcription requires that RNA polymerases terminate efficiently to avoid interfering with neighbouring transcriptional units[12]. Recent studies have also shown that defective termination at the 3′ end of a gene has a greater impact on mRNA synthesis than previously expected. It leads to decreased splicing and increased degradation of the RNA[13], as well as reduced initiation at the gene's promoter, perhaps as a means to shut down transcription when termination fails[14]. As Pol II termination is coupled with RNA 3′-end processing, the timing of Pol II release can also dictate the length of the final RNA product and thus affect the stability, localization and ultimate functionality of nascent transcripts.

Genomic partitioning
Separation of adjacent DNA functional units from one another by transcription termination and/or DNA-binding proteins (such as chromatin) in order to prevent transcriptional interference.

A comprehensive chronicle of eukaryotic transcription termination by all of the major nuclear RNA polymerases was expertly discussed a short time ago[6] and is not the focus of this Review. Instead, we concentrate on the most recent findings pertaining to Pol II transcription termination, and particularly on the selection, usage and functional outcome of alternative termination pathways in the model eukaryote *Saccharomyces cerevisiae*. The corresponding human termination pathways are discussed where appropriate. We also compare models of Pol II termination with better-characterized examples from bacterial transcription termination, and expound on the possibility of a general and conserved allosteric mechanism for all cellular RNA polymerases.

Pol II termination pathways

Although the release of RNA polymerase and its product are clearly important, these events must be balanced with the needs of a cell to transcribe genes quickly and completely. The remarkable speed of the Pol II elongation complex (~1–4 kb per min (REF. 15)), and its processivity, depends on an intricate network of interactions between polymerase proteins and nucleic acids (DNA and RNA) and base-pairing interactions between single-stranded RNA and DNA. The RNA–DNA hybrid of 8 nucleotides in length that is maintained during the elongation stage has been proposed to be the primary stability determinant of Pol II[16,17], and as such, disruption of this heteroduplex may be the pivotal event that results in termination (FIG. 1). Pol II termination can be elicited through different pathways, depending on the RNA 3′-end processing signals and termination factors that are present at the end of a gene[6,18,19]. Two of the best-studied pathways, the poly(A)-dependent pathway and the Sen1-dependent pathway, are presented below to illustrate common themes and principles involved in termination. An extensive collection of termination factors has been revealed, and mutational analysis has helped tease apart some of the dual roles that these proteins serve in the relevant RNA processing and termination decisions (TABLE 1; also see Supplementary information S1 (table)).

Poly(A)-dependent termination. Pol II termination downstream of most protein-coding genes is functionally coupled with an RNA maturation event in which the 3′ end of the nascent transcript undergoes cleavage and polyadenylation[20,21] (FIG. 2a). This 3′-end processing reaction can be broken down into two steps: first, transcription of a poly(A) site is followed by pausing of Pol II transcription and endoribonucleolytic cleavage of the nascent transcript; and second, the upstream cleavage product is polyadenylated, whereas the downstream cleavage product is degraded. The 3′-end processing reaction is initiated when *cis*-acting elements in the poly(A) site of the nascent transcript are recognized by RNA-binding factors that associate with Pol II, and the efficiency of termination correlates well with the strength of the poly(A) site[22–24]. The 3′-end processing machinery that carries out this seemingly simple reaction is highly complex, comprising more than 20 polypeptides in yeast and over 14 proteins in mammals[25,26]. An exhaustive analysis of

Table 1 | **Factors involved in Pol II termination***

Yeast complex	Yeast protein	Human homologue?	Protein function	Putative role in termination		Refs
				Poly(A)-dependent	Sen1-dependent	
CFIA	Pcf11	PCF11	Binds Pol II Ser2-P CTD, scaffolding protein	Promotes RNA cleavage and Rat1 recruitment, disrupts Pol II hybrid by bridging CTD and RNA	Disrupts Pol II hybrid by bridging CTD and RNA	40,97, 122–124
CPF	Cft1	CPSF160	Binds Pol II Ser5-P and Ser2-P CTD, binds poly(A) site RNA	Promotes Pol II pausing and RNA cleavage for Rat1 entry	N.D.	34,125
CPF	Ysh1	CPSF73	Endoribonuclease, cleaves poly(A) site RNA	Provides entry point for Rat1	Provides entry point for exoribonuclease‡	97,126
CPF	Yth1	CPSF30	Binds poly(A) site RNA and Pol II	Promotes Pol II pausing	N.D.	34
CPF(APT§)	Glc7	PP1	Ser/Thr phosphatase, dephosphorylates Sen1	N.D.	Promotes Sen1 recruitment and/or helicase activity	127,128
CPF (APT)	Pta1	Symplekin	Scaffolding protein, bridges CFIA and CPF	N.D.	Maintains integrity of APT complex	127,129
CPF (APT)	Ssu72	SSU72	Pol II Ser5-P CTD phosphatase	Promotes recruitment of Pcf11 to Pol II‖	Promotes recruitment of Pcf11 to Pol II	99,100, 130,131
Rat1–Rai1–Rtt103	Rat1	XRN2	5′-3′ exoribonuclease, degrades Ysh1 (or CPSF73)-generated downstream cleavage product	Promotes Pcf11 and Rna15 recruitment, collides with Pol II near RNA exit channel	No effect observed	38–40,97
Rat1–Rai1–Rtt103	Rai1	DOM3Z	De-capping endoribonuclease, pyrophosphohydrolase	Promotes Rat1 stability and activity	N.D.	38,71,132
Rat1–Rai1–Rtt103	Rtt103	–	Binds Pol II Ser2-P CTD, bridges Rat1 to Pol II CTD	Recruits Rat1 and Pcf11 to Pol II	N.D.	38,41
–	–	p54NRB and PSF	RNA-binding proteins, bind Pol II CTD	Promotes XRN2 recruitment to Pol II	N.D.	133
Sen1–Nrd1–Nab3	Nrd1	SCAF8 and SCAF4	Binds Pol II Ser5-P CTD, RNA-binding protein	No effect observed	Disrupts Pol II hybrid by bridging CTD and RNA, recruits Sen1 to Pol II	50,94, 97,134
Sen1–Nrd1–Nab3	Nab3	–	RNA-binding protein, bridges Nrd1 and Sen1	N.D.	Recruits Sen1 to Pol II	50,128
Sen1–Nrd1–Nab3	Sen1	Senataxin	Binds Pol II CTD, 5′-3′ RNA–DNA helicase	Promotes Rat1 activity by exposing RNA	Unwinds RNA–DNA hybrid in Pol II	48,51,52, 58,60,97
Pol II	Rpb1	RPB1	CTD serves as docking site for transcription and RNA processing factors	Recruits Pcf11 and Rat1 to Pol II	Recruits Nrd1, Pcf11 and Sen1 to Pol II	38,50, 52,94, 135,136
Pol II	Rpb3	RPB3	Forms heterodimer with Rpb11	N.D.	Transduces termination signal to Pol II	137
Pol II	Rpb11	RPB11	Forms heterodimer with Rpb3	N.D.	Transduces termination signal to Pol II	137
Paf1C	Paf1	PAF1	Associates with Pol II, scaffolding protein	N.D.	Promotes recruitment of Pcf11 and Nrd1 to Pol II	103,138, 139
–	Ess1	PIN1	Prolyl-isomerase; binds Ser5-P and Pro_6 Pol II CTD	Promotes recruitment of Ssu72 to Pol II‖	Promotes recruitment of Ssu72 to Pol II	99,100
–	Ctk1	CDK9	Pol II Ser_2 CTD kinase	Promotes recruitment of CFIA and CPF¶	Promotes recruitment of Pcf11	24,50,89
–	Chd1	CHD1	Chromatin-remodelling factor	Enhances Pol II pausing	N.D.	104

APT, associated with Pta1; CDK9, cyclin-dependent kinase 9; CFIA, cleavage factor IA; Chd1, chromodomain helicase DNA-binding 1; CPF, cleavage and polyadenylation factor; CPSF, cleavage and polyadenylation specificity factor; CTD, carboxy-terminal domain; Ctk1, CTD kinase subunit 1; DOM3Z, DOM-3 homologue Z; N.D., not determined; Nab3, nuclear polyadenylated RNA-binding 3; Paf1C, Paf1 complex; Pcf11, protein 1 of CFI; PIN1, peptidyl-prolyl *cis–trans* isomerase NIMA-interacting 1; Pol II, RNA polymerase II; PP1, protein phosphatase 1; Rai1, Rat1-interacting 1; Rat1, RNA-trafficking protein 1; Rtt103, regulator of Ty1 transposition 103; SCAF, SR-related and CTD-associated factor; Ssu72, suppressor of Sua7 2; XRN2, 5′–3′ exoribonuclease 2; *This is an abbreviated listing owing to space constraints. A more complete table is included in Supplementary information S1 (table). ‡Termination defects are allele-specific. §APT is a subcomplex of yeast CPF. ‖Role in termination seems to be limited to specific mRNAs (for example, short genes and targets of Sen1-dependent attenuation). ¶Role in termination seems to be limited to mRNAs with weak poly(A) sites.

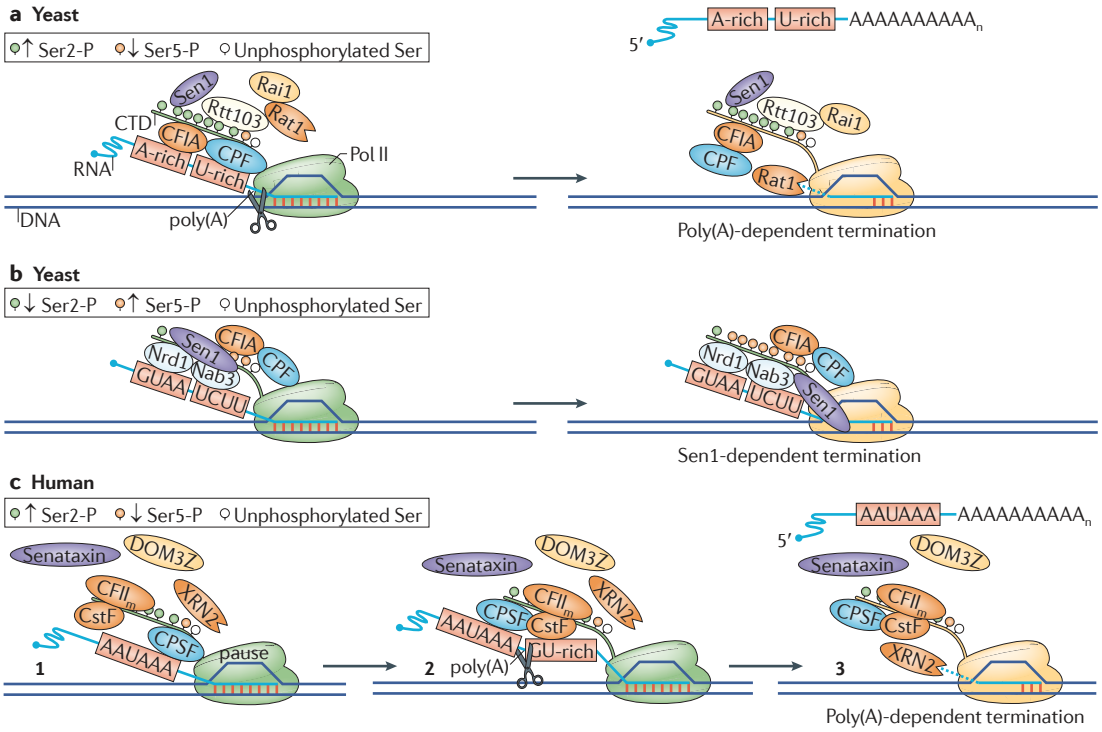


Figure 2 | **Factors involved in poly(A)-dependent and Sen1-dependent termination.** Counterparts of termination factors in yeast and humans are shown in the same colour, and known interactions between RNA, RNA polymerase II (Pol II) and other factors are indicated by direct contacts. Pol II carboxy-terminal domain (CTD) phosphorylation dynamics are indicated as in FIG. 1b, with Ser2-P being higher than Ser5-P in regions of poly(A)-dependent termination, and the reverse pattern being observed in regions of Sen1-dependent termination. **a** | In poly(A)-dependent termination in yeast, the 5′–3′ exoribonuclease RNA-trafficking protein 1 (Rat1; XRN2 in mammals) is recruited to Pol II via proteins that interact with phosphorylated Ser2 in the CTD (such as regulator of Ty1 transposition 103 (Rtt103)) and poly(A) site RNA elements (such as the indicated A-rich and U-rich sequences). In what has been called the 'torpedo' model, Rat1 degrades the downstream RNA (dashed light blue line) that results from the 3′-end processing cleavage event (scissors), which may result in disruption of the Pol II active site hybrid. In addition to contacting the CTD, cleavage and polyadenylation factor (CPF; homologous to human cleavage and polyadenylation specificity factor (CPSF)) may also interact with the body of Pol II through its suppressor of Sua7 2 (Ssu72) subunit. Optimal association of Rat1 with chromatin requires cleavage factor IA (CFIA; homologous to human cleavage stimulatory factor (CstF)), but direct contacts with CFIA have not been reported. **b** | In Sen1-dependent termination in yeast, the mechanism that applies to most non-coding RNAs, Sen1 is recruited to Pol II via proteins that interact with Pol II Ser5-P CTD (such as Nrd1) and specific RNA elements (such as GUAA repeats). Sen1 may unwind the Pol II active site hybrid via its helicase activity. **c** | In poly(A)-dependent termination in humans, pausing of human Pol II is induced when CPSF bound to the body of Pol II recognizes the AAUAAA signal sequence that emerges in the nascent transcript (step **1**). Upon exposure of the GU-rich binding site, CstF dislodges CPSF (step **2**). Following cleavage at the poly(A) site, 5′–3′ exoribonuclease 2 (XRN2) degrades the downstream RNA product, which may displace Pol II as described above for Rat1 (step **3**). $CFII_m$, mammalian CFII (which contains the human homologue of yeast protein 1 of CFI (Pcf11)); DOM3Z, DOM-3 homologue Z; Nab3, nuclear polyadenylated RNA-binding 3; Rai1, Rat1-interacting 1.

the factors associated with the core mammalian 3′-end processing machinery recovered over 80 proteins, many of which mediate crosstalk with other co-transcriptional processes[27].

A key scaffold for the recruitment of Pol II termination factors is the extended unstructured carboxy-terminal domain (CTD) of its Rpb1 subunit. The Pol II CTD consists of tandem heptad repeats (26 in yeast, 52 in human), which are made up primarily of the amino acid consensus sequence Tyr_1-Ser_2-Pro_3-Thr_4-Ser_5-Pro_6-Ser_7. The CTD is targeted by a wide range of post-translational modifications, of which the best-studied is phosphorylation[28,29]. As Pol II progresses through different stages of transcription, changes in the pattern of CTD phosphorylation alter the affinity of CTD-binding proteins, suggesting that a CTD code may regulate Pol II function[30] (FIG. 1b). Binding of 3′-end processing factors to the Pol II CTD and to RNA could transmit enough force to shear the active site RNA–DNA hybrid. However, this association may also make Pol II more susceptible to pausing, which has been observed several-hundred base pairs beyond the poly(A) site on many human genes[31,32]. Maximal recruitment of cleavage and polyadenylation factors coincides with paused Pol II[32]. Elegant *in vitro* studies from the Martinson laboratory[33–35] using cell extracts show that transcription through a poly(A) signal reduces the rate of Pol II elongation and causes pausing downstream of the poly(A) site. These findings support a model in

which the human cleavage and polyadenylation specificity factor (CPSF; homologous to cleavage and polyadenylation factor (CPF) in yeast) is recruited to the elongation complex by interaction with the Pol II body, whereas the cleavage stimulatory factor (CstF; homologous to cleavage factor IA (CFIA) in yeast) interacts with the CTD (FIG. 2c, step 1). Then, when the AAUAAA sequence that CPSF recognizes is transcribed, CPSF binding to this site induces pausing. When CstF binds to the downstream GU-rich processing signal, CPSF binds to CstF, releases its hold on the Pol II body and accompanies CstF to the CTD, leading to CPSF-mediated cleavage and release of the paused Pol II (FIG. 2c, steps 2 and 3).

Thus, pausing appears to be an important intermediate step leading to termination in many mammalian genes. However, pausing of Pol II per se does not lead to termination. For example, Pol II pauses at other positions in a gene, such as just proximal to the promoters of inducible metazoan genes[3] or in terminal exons in yeast genes, as a way to allow sufficient time for splicing[36,37]. Efficient release of Pol II paused downstream of a poly(A) site requires not only 3′-end processing factors, such as CPF and CFIA, but also the 5′–3′ exoribonuclease RNA-trafficking protein 1 (Rat1; XRN2 in mammals) in complex with its activating cofactor Rat1-interacting 1 (Rai1) and its CTD-interacting partner regulator of Ty1 transposition 103 (Rtt103)[38,39] (FIGS 2a,c). In what has been called the 'torpedo' model, Rat1 is recruited to the 3′ end of the gene via interactions of the CTD with 3′-end processing factors and with Rtt103 (REFS 40,41). Creation of an unprotected 5′ end by the 3′-end processing endoribonuclease, or by other cleavage events downstream of the poly(A) site[42–45], allows Rat1 (or XRN2) to rapidly 'chew' its way along the RNA tether to the RNA exit channel on Pol II. Collision of Rat1 (or XRN2) with Pol II would then promote termination[38,39,46] (FIGS 2a,c). In this scenario, the function of the 3′-end processing complex is to help recruit Rat1 to the Pol II CTD, to introduce an entry site for Rat1 and to induce a pause or slowing of Pol II that allows Rat1 to quickly catch up with the polymerase. The pause may also give the processing complex time to assemble and cleave the transcript. Combined with the Rat1 torpedo, the allosteric changes that slow Pol II and perhaps loosen its grip on the DNA template lead to destabilization of Pol II and its release.

Sen1-dependent termination. An alternative Pol II termination pathway for most non-coding RNAs was first discovered in the yeast *S. cerevisiae* (FIG. 2b). Unlike mRNAs, the 3′-ends of yeast snRNAs and snoRNAs are generated by endoribonucleolytic cleavage and/or exoribonucleolytic trimming by the nuclear exosome–TRAMP complex and do not possess a poly(A) tail in their mature form[47]. A distinct set of core factors is required for recognition and transduction of the transcription termination signal, including the RNA-binding proteins Nrd1 and nuclear polyadenylated RNA-binding 3 (Nab3) and the putative RNA and DNA helicase Sen1 (REFS 48–51), which is also needed for 3′-end processing of non-coding RNAs[52,53] and the resolution of R-loops formed during transcription[54] (FIG. 2b). In addition to targeting snRNAs and snoRNAs, the Sen1-dependent pathway is required for termination of CUTs[55,56]. Several mRNA 3′-end processing factors contribute to the transcription termination of non-coding RNAs (TABLE 1). As with the poly(A)-dependent mechanism, the interaction of these factors with the Pol II CTD and with their RNA-binding sites could provoke conformational changes. However, instead of a collision with Rat1 dislodging Pol II, in this pathway Sen1 is proposed to terminate Pol II by unwinding the RNA–DNA hybrid in the active site[49], analogously to the proposed action of bacterial Rho helicase[57]. Sen1, and its human homologue senataxin, also contributes to poly(A)-dependent termination[58–60], and short mRNA-encoding genes are particularly susceptible to loss of Sen1 function[51]. The role of Sen1 in poly(A)-dependent termination is as yet unknown, but it has been speculated to make RNA more accessible to Rat1 by removing proteins or RNA secondary structure[58].

It remains to be seen if a distinct type of termination pathway targets non-coding RNAs in higher eukaryotes. Unlike their homologues in yeast, most of the mammalian snoRNAs are encoded within introns and termination is not needed in this arrangement[61]. However, most metazoan snRNAs are transcribed from independent genes, and a promoter-specific pre-initiation complex helps to recruit the integrator complex that mediates 3′-end cleavage of the primary transcripts[62,63]. Interestingly, the integrator complex contains homologues to the mRNA 3′-end processing factors CPSF73 and CPSF100 (REFS 64,65), suggesting that metazoan snRNA termination is mediated, at least in part, by co-transcriptional cleavage and a 5′–3′ exoribonuclease 2 (XRN2) torpedo mechanism. An alternative, or perhaps additional, mechanism for termination downstream of the mammalian U2 snRNA-encoding gene appears to involve a nucleosomal roadblock, the negative elongation factor (NELF), and the insulator protein CCCTC-binding factor (CTCF)[66]. Unlike for Sen1 in yeast, senataxin does not seem to be needed at snRNA-encoding genes[60]. For the unadenylated miRNA transcripts, the action of XRN2 following Drosha-mediated cleavage of miRNA precursors induces transcriptional termination[67]. Termination mechanisms for the many other types of mammalian non-coding RNAs transcribed by Pol II[68] need to be investigated.

In summary, three mechanisms are thought to cause Pol II termination: conformational changes induced by binding of factors to the elongation complex; the collision of Rat1 or XRN2 with Pol II; and the action of a helicase, such as Sen1. Despite the accumulated evidence supporting the contributions of these different factors, the precise nature of changes occurring in the Pol II elongation complex, and how these are elicited by the factors discussed above, remains unresolved.

For example, one conundrum that has arisen is the role of Rat1. Rat1 degradation of the nascent transcript *in vitro*, and presumably its collision with Pol II, is not sufficient for release[69]; nor is degradation by Xrn1, the cytoplasmic counterpart of Rat1, when Xrn1 is directed to the nucleus[40]. Furthermore, 'rear-ending' of a paused

Cleavage and polyadenylation specificity factor
A mammalian protein complex containing an endoribonuclease that is required for efficient mRNA 3′-end processing and RNA polymerase II (Pol II) transcription termination. Homologous to yeast cleavage and polyadenylation factor, which contains additional subunits required for efficient Pol II termination at small nuclear RNA-encoding and small nucleolar RNA-encoding genes.

Cleavage stimulatory factor
A mammalian protein complex that is required for efficient mRNA 3′-end processing and transcription termination. Homologous to yeast cleavage factor IA, which is also required for efficient RNA polymerase II termination at genes encoding small nuclear RNAs and small nucleolar RNAs.

Exosome
A protein complex that targets various types of RNA for degradation primarily via its 3′–5′ exoribonuclease activity.

TRAMP
A polyadenylation complex that enhances exosome-mediated degradation of aberrant RNAs.

Drosha
A ribonuclease III enzyme that initiates processing of microRNAs.

Pol II by an actively transcribing one does not dislodge the stalled one, but rather promotes its moving beyond the pause site[70], suggesting that sheer force of collision is not enough. What then is needed to pry Pol II off its DNA template? Rat1 possesses an unusual tower domain that protrudes above the exoribonuclease active site and is longer than the equivalent region in Xrn1 (REFS 71,72). Degradation of the RNA may position such domains of Rat1 against the Pol II body in a way that promotes release. This event may also require the full constellation of processing factors, which were not present in the *in vitro* analysis mentioned above[69]. Indeed, recruitment of cleavage and polyadenylation factors has been proposed as an alternative function for Rat1 in termination[40]. If a helicase is the missing ingredient, then Sen1 cannot be the only source of this activity, as loss of Sen1 or senataxin function does not affect termination at all poly(A)-dependent genes[51,59].

Transcription termination in bacteria

The study of transcription termination in bacterial systems has been an active field of exploration for decades and may be a useful model for elucidating the molecular mechanisms that govern termination in eukaryotes. Many of the structural features and biochemical activities of bacterial RNA polymerases are conserved in higher eukaryotic RNA polymerases, and numerous bacterial regulatory factors have eukaryotic homologues or orthologues. Thus, information gleaned from the study of termination in these bacterial systems, which are relatively simpler than those in eukaryotes, may reveal general features of termination and important points of contact between polymerases and termination factors that are common to all cellular RNA polymerases. Some of the recent advances made in the field of bacterial transcription termination are highlighted below.

Rho-dependent termination. A potentially paradigm-shifting report on termination has emphasized the role of allostery in mediating Rho-dependent transcription termination[73]. Rho is an RNA–DNA helicase that serves as a general bacterial transcription termination factor[57]. Rho binds preferentially to unstructured, C-rich nascent RNA and traverses RNA in the 5′–3′ direction via RNA-dependent ATP hydrolysis, all the while threading RNA through its central cavity. A widely accepted model for Rho-dependent termination posits that Rho catches up to the elongation complex by translocating along the nascent transcript and, at certain sites, dissolves the elongation complex by pulling out the transcript. Contrary to this model, Epshtein *et al.*[73] proposed that Rho loads directly onto RNA polymerase early in elongation and traps the elongation complex in an inactivated conformation at Rho-dependent termination sites. The observed dependence of Rho-mediated termination on functional RNA polymerase β′-lid and trigger loop domains led to the 'allosteric' model that Rho pushes against the lid to mediate hybrid melting (FIG. 3a). Pushing against the lid may also transmit an allosteric signal to the trigger loop, to which it is connected, thereby facilitating catalytic inactivation and eventual dissociation of the elongation complex.

Some evidence suggests that this allosteric model of bacterial transcription termination may have bearing on the mechanisms governing Pol II transcription termination. *Escherichia coli* Rho has been shown to dismantle Pol II elongation complexes assembled *in vitro*[74]. Interestingly, neither Pol I nor Pol III demonstrated susceptibility to Rho-mediated termination, indicating that Rho activity was specific to Pol II. Given the structural conservation between bacterial RNA polymerase and eukaryotic Pol II, perhaps the Pol II Rpb1 lid serves as an effector domain contacted by Sen1 or Rat1 to elicit Pol II termination, similar to the way Rho mediates *E. coli* RNA polymerase termination (FIGS 3a,b).

Rho cofactors in termination. In addition to the Rho termination factor, insights have been gained into the structure and function of two Rho cofactors, N utilization substance A (NusA) and NusG (an orthologue of the eukaryotic transcriptional regulator Spt5). NusA enhances intrinsic termination, whereas NusG enhances Rho-dependent termination[75,76]. In a seemingly opposing manner, both Nus factors participate in ribosomal RNA (*rrn*)- and λ-antitermination complexes[77–79]. Recent studies have helped elucidate the mechanisms by which these factors function as terminators in some instances and antiterminators in others. Mutational analysis of NusA and chemically induced cleavage of NusA-bound RNA polymerase have led to proposed sites of interaction between NusA and RNA polymerase[80]. The amino-terminal domain (NTD) of NusA possesses a concave hydrophobic core that is sufficient to stimulate pausing and termination through an interaction with the β-flap, which makes up part of the RNA exit channel of RNA polymerase, and the β′-dock domains (FIG. 3a). In addition to contacting the RNA exit site, the NusA NTD interacts with the nascent transcript and shields positions -17 to -30 from interaction with other macromolecules. The NusA-mediated stabilization of contacts between the β-flap and the emerging transcript is thought to produce a distal termination signal that is allosterically transmitted to the RNA polymerase active site, thereby constraining the catalytic residues and disfavouring elongation[81].

The λQ protein, which forms part of the λ-antitermination complex, interacts with NusA in the vicinity of the β-flap, and this λQ–NusA complex can also effectively shield and restrict access to the emerging transcript, thereby inhibiting Rho-dependent termination[79]. Interaction with λQ may thus mask the pausing- and termination-mediating structural features of NusA, as λQ-mediated antipausing is decidedly dominant over NusA-mediated pausing. Another study suggests that λQ protein bound to the β-flap may prevent the flap from adopting a termination-competent conformation[79]. Given the prominent role of the bacterial RNA polymerase β-flap as a major site of termination and antitermination regulation, the Pol II Rbp2 flap could participate in an analogous role in eukaryotic termination. This is an intriguing possibility as the site of RNA exit on Pol II is located in the vicinity of the last ordered residue of Rpb1, which marks the beginning of the linker to the CTD (FIGS 3b,c). In yeast, the CFIA and CPF processing factors and the Rat1-associated protein

Tower domain
A pronounced and conserved α-helix near the active site of the yeast nuclear 5′–3′ exoribonuclease RNA-trafficking protein 1 (Rat1; XRN2 in mammals) but not its cytoplasmic orthologue 5′–3′ exoribonuclease 1 (Xrn1).

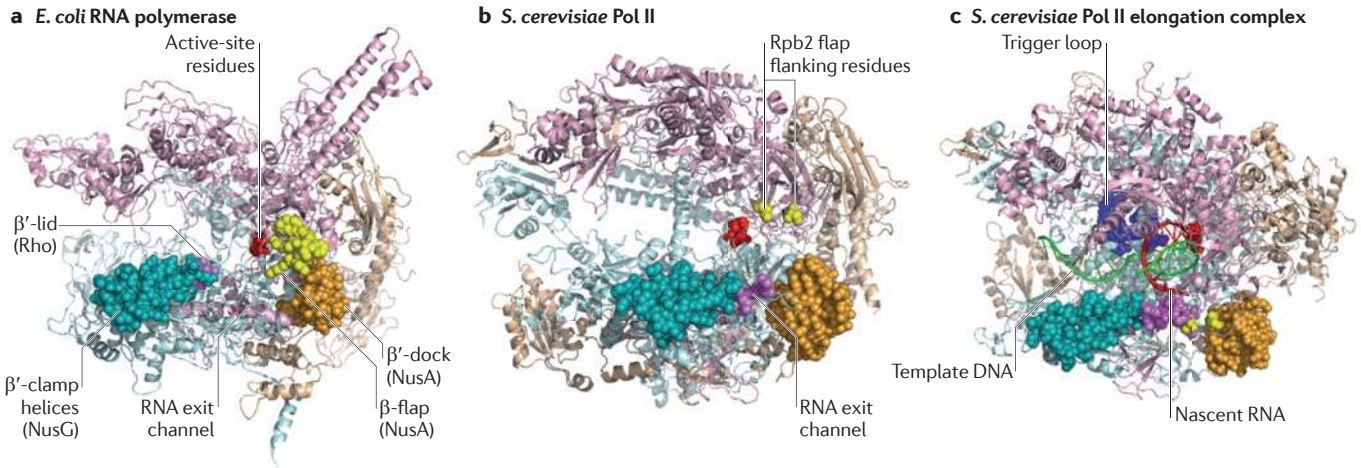


Figure 3 | **Comparison of *E. coli* RNA polymerase and *S. cerevisiae* Pol II structures.** A summary of the putative sites on bacterial RNA polymerase that are contacted by Rho, N utilization substance A (NusA) and NusG, and their corresponding regions in yeast RNA polymerase II (Pol II), are shown. **a**,**b** | A molecular model of *Escherichia coli* RNA polymerase[119] (**a**) and a crystal structure model of *Saccharomyces cerevisiae* Pol II[120] (**b**) are depicted in cartoon rendering (β′-subunit (Rpb1 in *S. cerevisiae*), pale teal; β-subunit (Rpb2 in *S. cerevisiae*), light pink; other subunits, wheat; active site, red). The key sites of interaction on *E. coli* RNA polymerase are highlighted in space-filled rendering (β-flap, yellow; β′-clamp helices, teal; β′-lid, violet; β′-dock, orange), as are the relative sites on Pol II. Note that only the flanking amino-terminal and carboxy-terminal residues of the Pol II Rpb2 flap are shown as the loop is too disordered to be depicted here. **c** | Crystal structure model of the *S. cerevisiae* Pol II elongation complex[121] depicted as detailed for *E. coli* RNA polymerase in (**a**), but rotated to show the trigger loop (highlighted in blue in space-filled rendering). The template DNA (green) and the nascent transcript (red) are also shown, to emphasize the close proximity of the features detailed in (**a**) to the RNA exit path. For simplicity, the Rpb4 and Rpb7 subunits of the Pol II elongation complex are hidden. The structures in this figure were created with PyMOL (Schrödinger) using Protein Data Bank files 3LU0 for RNA polymerase and 1I3Q and 1Y1W for Pol II.

Rtt103 interact directly with the CTD. Therefore, an expansion of the model for Pol II termination described above would be that degradation of the nascent chain by Rat1 brings these factors to the backside of Pol II, where they contact the Rpb2 flap domain and allosterically elicit termination.

Similar to NusA, NusG possesses an NTD that contacts RNA polymerase, as determined by NMR and mutational studies[83]. The concave, hydrophobic surface of the NusG NTD is proposed to bind the complementary convex tip of the β′-clamp helices of RNA polymerase (FIG. 3a). Such a model is supported by an observed genetic interaction between NusG and the RNA polymerase clamp helices[84]. Whereas the NTD is responsible for RNAP binding, the CTD of NusG interacts with Rho and thus stimulates Rho-dependent termination (FIG. 3a). When recruited into λ-antitermination complexes, the NusG CTD may be prevented from binding to Rho, thereby inhibiting Rho-mediated termination. Such masking is reminiscent of the λQ-mediated modification of NusA termination activities. Another antiterminator, RfaH, is an operon-specific virulence factor that reduces Rho-mediated termination by competing with NusG for its binding site on the RNA polymerase β′-clamp helices[85,86]. Thus the clamp helices, much like the flap domain, may be putative binding sites for regulators of transcription termination in eukaryotes. A comparison of the structures of *E. coli* RNA polymerase and *S. cerevisiae* Pol II is provided to highlight the relative positions of the regions contacted by bacterial transcription termination factors (FIG. 3).

Selecting a Pol II termination pathway

The CTD of the Rpb1 subunit is a binding surface for termination factors unique to eukaryotic Pol II and not found in bacterial or other RNA polymerases. Analysis of CTD phosphorylation dynamics across several model yeast genes revealed a dual gradient, with the ratio of Ser5-P to Ser2-P being high when Pol II is at the 5′ end of genes and low at the 3′ end[87] (FIG. 1b). Proteins involved in 'early' transcription events, such as RNA 5′-end capping, preferentially bind Ser5-P CTD[87], whereas proteins involved in 'late' events of Pol II transcription, such as RNA 3′-end processing, preferentially bind Ser2-P CTD[88,89]. Ser7-P has more recently emerged as another major CTD modification and, like Ser5-P, it is enriched on Pol II at the 5′ ends of both protein-coding and non-coding genes[62,90]. Thus far, the only known function for Ser7-P in Pol II transcription is in recruitment of the integrator complex to mammalian snRNA-encoding genes[62].

Role of CTD phosphorylation in Pol II termination. Pol II termination is facilitated when *cis*-acting elements in the nascent transcript are recognized by RNA-binding proteins that co-transcriptionally associate with the CTD (FIG. 2). In yeast, the type of CTD phosphorylation influences the mechanism of termination, and in metazoan cells it may prevent the use of strong poly(A) signals located near the promoter[91]. Based on the distance that Pol II has progressed from a transcription start site (TSS) and the associated change in CTD phosphorylation status, yeast Pol II becomes more

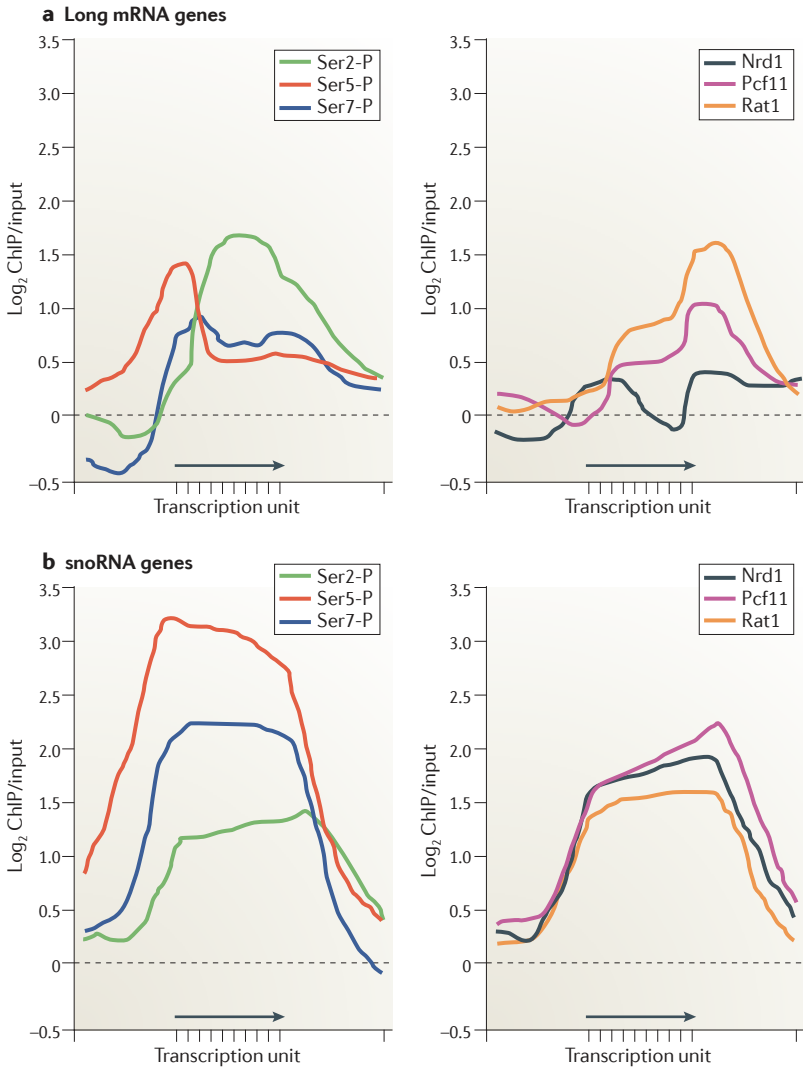


Figure 4 | **Genome-wide localization of yeast Pol II CTD phosphorylations and termination factors.** The localization of RNA polymerase II (Pol II) phospho-carboxy-terminal domain (CTD) isoforms (left) and protein 1 of CFI (Pcf11), RNA-trafficking protein 1 (Rat1) and Nrd1 (right) were mapped across the entire yeast genome by ChIP–chip. Average distributions for highly expressed long mRNA-encoding genes (>2,000 bases, 128 genes) (**a**) and short small nucleolar RNA (snoRNA)-encoding genes (<700 bases, 31 genes) (**b**) are shown. Transcription units for mRNA-encoding genes (transcription start site (TSS) to poly(A) site) and snoRNA-encoding genes (TSS to mature 3′ end), indicated by arrows, are divided into 10 equal intervals and flanked with 1 kb of 5′ and 3′ sequence. Images are modified, with permission, from REF. 24 © (2010) Macmillan Publishers Ltd. All rights reserved.

prone to use either the Sen1-dependent or the poly(A)-dependent termination pathway[92]. Generally speaking, Sen1-dependent termination predominates at distances <1 kb from a TSS (typical for genes encoding snRNAs and snoRNAs), whereas poly(A)-dependent termination predominates at distances >1 kb from a TSS (typical for mRNA-encoding genes)[51,92,93]. Nrd1, protein 1 of CFI (Pcf11; a component of the CFIA complex), and the Rat1-associated Rtt103 protein serve as 'readers' of the CTD code, with Nrd1 preferentially binding to Ser5-P[94] and Pcf11 and Rtt103 to Ser2-P CTD[38,88]. In addition to the temporal recruitment of termination factors by the CTD, nascent RNA elements recruit some termination factors independently of CTD phosphorylation status[92]. Therefore, the decision to terminate early or late during transcription is likely to be determined by kinetic competition between phosphorylation-specific CTD-binding proteins and sequence-specific RNA-binding proteins that associate with Pol II-elongation complexes[29].

A revised Pol II CTD code. The dual gradient model of CTD phosphorylation was initially formulated using a small collection of model yeast genes, but until recently it was unknown whether the model applied generally across all Pol II-transcribed genes. Several recent genome-wide studies analysing the association of Pol II and termination factors with chromatin have provided a wealth of information that expands our understanding of the timing and specificity of these events[24,95,96]. In general, the average global CTD phosphorylation pattern of most well-isolated and highly expressed long mRNAs is consistent with earlier results, namely that Ser5-P and Ser7-P are enriched on Pol II at the 5′ end of genes and Ser2-P is enriched on Pol II at the 3′ end (FIG. 4a). However, CTD phosphorylation patterns do not follow these canonical profiles at all genes. For example, a significant fraction of genes exhibit Pol II CTD Ser2-P enrichment at the 5′ end and/or Ser5-P enrichment at the 3′ end, perhaps owing to the presence of CUTs and SUTs[96]. The current model for the CTD code will probably need to be revised as the functional consequence of these alternative CTD phosphorylation patterns is investigated.

In addition to the canonical and noncanonical patterns of CTD phosphorylation observed when Pol II is at the beginning and end of genes, genome-wide studies have also provided a clearer picture of changes across the middle of genes. Ser7-P and Ser5-P overlap to some degree, but overall the Ser7-P profile is more variable than Ser5-P[24]. Whereas Ser5-P exhibits a dramatic drop as Pol II progresses beyond the 5′ end of a gene, Ser7-P remains present at higher levels and is retained all the way until transcription termination[24,96] (FIG. 4a). The widespread occurrence of Ser7-P across coding regions suggests that it may contribute to efficient Pol II elongation, particularly in light of its enrichment in Pol II that is at highly transcribed genes involved in ribosome biogenesis[96]. In contrast to mRNA-encoding genes, the trend of Ser7-P and Ser5-P patterns overlaps more closely across non-coding RNA genes, and Ser2-P is under-represented even when gene size is taken into consideration[96] (FIG. 4b). An unexpected finding from the global analysis, given what had been deduced from the model genes, is that the ratio of Ser2-P to Ser5-P is not scaled to gene length and only increases within the first 500 bases of a transcription unit. Thus, the dual gradient model applies best to short genes[24]. After the 500-base transition point, the ratio of Ser2-P to Ser5-P does not change substantially and thus would not provide a clear metric for Pol II position. Taken together, these data suggest that a combination of Ser7-P and Ser5-P enrichment and low Ser2-P serves as a signal for early Pol II transcription events, such as Sen1-dependent termination and the processing of short non-coding RNAs[96] (FIG. 2b). At later events during transcription, such

ChIP–chip
A technique that combines chromatin immunoprecipitation (ChIP) with microarray technology (chip) to investigate genome-wide protein–DNA interactions.

as poly(A)-dependent termination and the processing of long mRNAs, Pol II position may be directed by a combination of other, unidentified CTD modifications and/or Pol II transit through termination signals.

Global localization of Pol II termination factors. Genome-wide association studies of Pol II termination factors have confirmed some previously observed patterns from individual genes[24]. Consistent with their functions in Sen1-dependent termination and poly(A)-dependent termination, respectively, Nrd1 is generally enriched at non-coding RNA genes (for example, snRNAs and snoRNAs, CUTs and SUTs) (FIG. 4b) and Pcf11 and Rat1 are enriched at mRNA 3′ ends (FIG. 4a). Pcf11 also exhibits widespread recruitment to snoRNA-encoding genes along with Rat1 (FIG. 4b), which is somewhat surprising given that Rat1 exoribonuclease activity is not required for termination at several of the snoRNA-encoding genes tested[97]. Overall, this pattern of recruitment supports the idea that the machinery for both Sen1-dependent and poly(A)-dependent termination is available to target Pol II during the elongation of all genes[97], and in fact may provide a way to ensure fail-safe termination[43,44].

The global genomic studies have also revealed new patterns of termination factor recruitment corresponding with distinct forms of CTD phosphorylation. The association of Nrd1 at non-coding RNA genes (for example snRNAs and snoRNAs, CUTs and SUTs) strongly colocalizes with Ser7-P[24] (FIG. 4b). This localization pattern suggests that, in addition to its previously demonstrated interaction with Ser5-P, Nrd1 may interact with Ser7-P. Precedence for recognition of a dual CTD mark comes from studies of proteins that bind Ser5-P and Ser2-P, such as the histone methyltransferase SET domain-containing 2 (Set2) that functions during elongation[28], and from protein complexes that bind Ser7-P and Ser2-P, such as the integrator complex[98]. Pcf11 colocalizes with Nrd1 at regulatory non-coding RNAs located at the 5′-ends of some mRNA-encoding genes (for example, *HRP1*, IMP dehydrogenase 2 (*IMD2*), *NRD1* and *URA2*)[24]. Pcf11 also colocalizes with Nrd1 across many snoRNA-encoding genes (FIG. 4b), which is somewhat surprising given that Pcf11 binds Ser2-P *in vitro*[41,88] and snoRNA-encoding genes exhibit relatively meagre levels of Ser2-P CTD[96] (FIG. 4b). However, this localization is entirely consistent with the requirement of Pcf11 for both poly(A)-dependent and Sen1-dependent termination[97].

The genome-wide occupancy of Pcf11 measured across mRNA-encoding genes does not correlate with Ser2-P CTD[24,95] (FIG. 4a). This apparent discrepancy could be explained by Ser2-P CTD being masked across genes *in vivo* or CTD-independent recruitment of Pcf11 by other 3′-end processing factors[95]. Even though CTD Ser2-P levels are lower at snoRNA-encoding genes relative to mRNA-encoding genes, their pattern at snoRNA-encoding genes correlates better with Pcf11 association[24] (FIG. 4b), suggesting that Ser2-P may be more important for Pcf11 recruitment at Sen1-dependent terminators than at poly(A)-dependent terminators. This finding is consistent with the fact that mutations in the Pcf11 CTD-interaction domain (CID) disrupt termination at snoRNA-encoding genes but not mRNA-encoding genes[97]. It's also in line with the observation that loss of the major Ser2-P kinase, CTD kinase subunit 1 (Ctk1; cyclin-dependent kinase 9 (CDK9) in mammals), has no effect on the termination of most yeast mRNA-encoding genes[24,89]. It is important to note that the localization patterns of CTD phosphorylation and termination factors might also be influenced by other factors. Examples of such factors could be the cooperative binding of two different proteins to neighbouring CTD repeats[41], the action of prolyl isomerases (for example, Ess1 and peptidyl-prolyl *cis–trans* isomerase NIMA-interacting 1 (PIN1)) that alter the presentation of phosphorylated Ser residues[28,99,100], and non-uniform phosphorylation patterns on the CTD, an idea that remains to be tested experimentally. Nevertheless, the global localization of Pol II CTD phosphorylation and termination factors reveals wider overlap than previously appreciated.

Conclusions and future directions

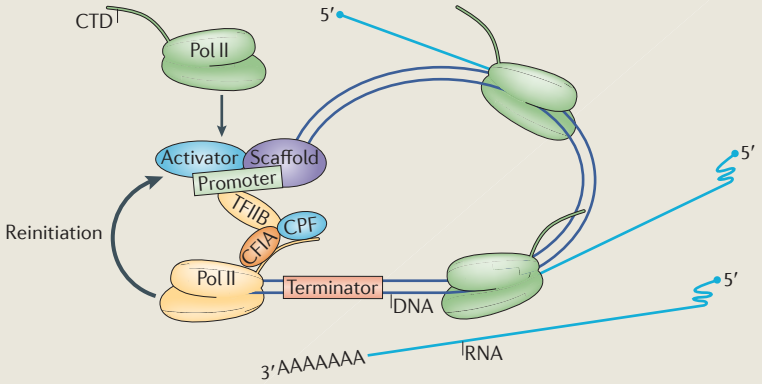
A strong argument can be made that the further Pol II advances through the transcription cycle, the less we understand about its underlying mechanism and regulation. At least part of this gap in knowledge is inherent to the fact that transcription becomes progressively more interconnected with other cellular processes as Pol II enters the later stages of RNA synthesis. Furthermore, the diversity of transcripts that Pol II synthesizes, and the importance of buffering its widespread genomic activity, has probably placed significant pressure on the cell to evolve alternative and redundant termination mechanisms. Over the past 25 years, significant progress has been made towards unravelling the intricate details of this fundamental process. Two dominant models for the molecular mechanism of termination at protein-coding genes have waxed and waned in the spotlight, eventually converging into a unified model. A distinct but overlapping pathway has emerged for yeast genes encoding non-coding RNA, and this is strongly reminiscent of the Rho-mediated transcription termination of bacterial RNA polymerase. Most recently, studies of individual model genes have been expanded to the genome-wide level, confirming some ideas and challenging others, as well as raising new important questions in the field.

Numerous elongation factors contribute to how well Pol II traverses a gene[101] and in this Review we have not addressed how recruitment and/or loss of these elongation factors influence the likelihood of Pol II termination. Yeast elongation factors exit via a two-step 3′ transition, during which some factors (for example, Paf1 and Bur1) are released upstream of the poly(A) site and others (for example, Spt4 and Spt5) are released downstream[95,102]. Paf1 has been implicated in mRNA poly(A) site selection and Sen1-dependent termination, perhaps through the recruitment of Pcf11 and Nrd1 (REF. 103). However, the specific contribution of Paf1 and other such elongation factors to Pol II termination remains unclear. Recent work also supports a close physical juxtaposition of the 5′ and 3′ ends of genes, called gene-looping (BOX 1), and the implications of this linkage on termination will surely be the focus

Prolyl isomerases
Enzymes that catalyse the interconversion of *cis* and *trans* isomers of peptide bonds with the amino acid Pro.

Box 1 | **The end is the beginning is the end**

Perhaps owing to their seemingly distal position from one another, the transcriptional events that take place at the beginning and end of a gene are often studied independently from one another. However, RNA polymerase II (Pol II) initiation factors and the 3′-end processing and termination machinery are more intertwined than one might expect[110], and several recent papers provide new insight into the biological significance of this linkage.



Transcription initiation factor IIB (TFIIB) is a general transcription factor that directs assembly of the pre-initiation complex scaffold at the promoter[111]. In yeast, TFIIB also interacts with the cleavage and polyadenylation factor (CPF) 3′-end processing complex, which contributes to the juxtaposition of promoter and terminator DNA through a process known as gene looping[112] (see the figure). New evidence reveals that gene looping in yeast also requires the interaction of TFIIB with the cleavage factor IA (CFIA) 3′-end processing factor, a linkage that is dependent on a transcriptional activator[113]. This interaction is conserved in the mammalian system, in which it is regulated by TFIIB phosphorylation and necessary for productive elongation[114], and this is consistent with a model in which gene loops promote Pol II reinitiation[112,115,116].

TFIIH is a general transcription factor that facilitates promoter clearance, at least in part, through the action of its Kin28 subunit (cyclin-dependent kinase 7 (CDK7) in mammals), a kinase that phosphorylates the Pol II carboxy-terminal domain (CTD) at Ser_5 and Ser_7 (REF. 111). TFIIH activity was thought to be restricted to the promoter, but a recent report reveals that CDK7 and the core subunit p62 also localize to coding regions and the 3′ ends of genes[117]. Furthermore, inhibition of CDK7 causes delays in both promoter-proximal pausing and transcription termination.

The recruitment of RNA-trafficking protein 1 (Rat1; XRN2 in mammals), the exoribonuclease that helps trigger Pol II termination[38,39], peaks at the 3′ end of genes. However, Rat1 can also be detected at gene promoters and coding regions. A new report reveals a function for Rat1 at these more upstream positions, namely to terminate Pol II molecules engaged in the production of uncapped RNA[118]. Overall, the connections highlighted above reveal that template DNA and its associated transcription factors are more versatile than previously appreciated, and this flexibility provides additional avenues for control of both Pol II activity and RNA quality.

of future studies. Other areas meriting further investigation are the roles of chromatin modifications, nucleosome positioning and epigenetic modifications, which have all been implicated in poly(A) site selection and stalling of Pol II[104–108]. Finally, the use of alternative poly(A) sites during cancer progression and in normal cell development is widespread and functionally important[109] but poorly understood mechanistically. How much of this is dictated by selection of the poly(A) site by the processing machinery, and how much by the decision of where to terminate? Hopefully, future work will lead to a more integrated model for termination that takes into account not only changes in Pol II but also its recent past and its immediate surroundings.

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Competing interests statement
The authors declare no competing financial interests.

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