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Mechanisms of eukaryotic transcription termination at a glance

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ABSTRACT

Transcription termination is the final step of a transcription cycle, which induces the release of the transcript at the termination site and allows the recycling of the polymerase for the next round of transcription. Timely transcription termination is critical for avoiding interferences between neighbouring transcription units as well as conflicts between transcribing RNA polymerases (RNAPs) and other DNA-associated processes, such as replication or DNA repair. Understanding the mechanisms by which the very stable transcription elongation complex is dismantled is essential for appreciating how physiological gene expression is maintained and also how concurrent processes that occur synchronously on the DNA are coordinated. Although the strategies employed by the different classes of eukaryotic RNAPs are traditionally considered to be different, novel findings point to interesting commonalities. In this Cell Science at a Glance and the accompanying poster, we review the current understanding about the mechanisms of transcription termination by the three eukaryotic RNAPs.

KEY WORDS: Eukaryotic transcription, Gene expression, RNA polymerase, Transcription termination

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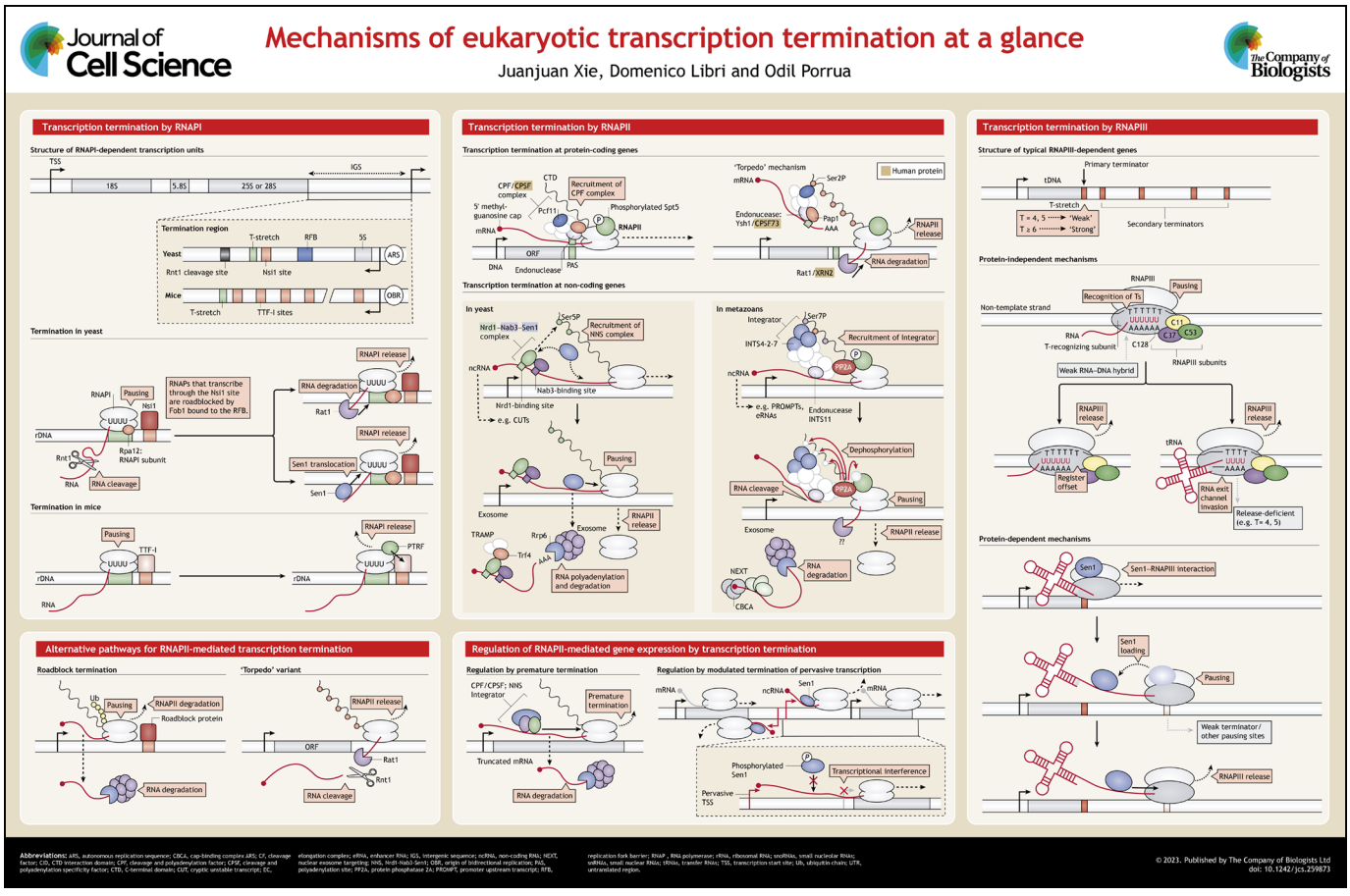
Introduction

Transcription termination occurs when the RNA polymerase (RNAP) and the nascent transcript are released from the chromatin. Efficient transcription termination is crucial not only for the correct synthesis of cellular RNAs but also for the regulation of possible conflicts between the different DNA-associated machineries, which can potentially compromise genome stability (reviewed in Porrua and Libri, 2015). Moreover, several reports over the past years have underscored the importance of transcription termination in the regulation of gene expression. For instance,



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termination can occur precociously, leading to the truncation of the functional message and, thus, gene expression downregulation (reviewed in Kamieniarz-Gdula and Proudfoot, 2019). Therefore, timely transcription termination is essential for genome expression and integrity.

The transcription elongation complex (EC), composed of the RNAP, the template DNA and the nascent RNA is a macromolecular assembly characterized by a complex network of protein–protein and protein–nucleic acid interactions. Among these, the association between the RNAP and the RNA–DNA hybrid plays a major role in EC stability (Kireeva et al., 2000). Transcription termination typically involves RNAP pausing and an alteration of the EC interaction network to promote EC destabilization and subsequent dismantling (reviewed in Porrua et al., 2016). Compared to other stages of the transcription cycle, the step of termination remains less well understood. However, in the past few years, technical revolutions in the fields of structural biology and genomics, such as cryo-electron microscopy and high-resolution genomics, have prompted major advances in the comprehension of this important process.

In eukaryotes, transcription of the nuclear DNA is carried out by three types of RNAPs, which are specialized for transcription of different kinds of genes and have opted for seemingly distinct mechanisms to achieve termination (reviewed in Porrua et al., 2016). RNAPII has long been believed to employ multiple protein factors and cis-acting sequences to terminate transcription, whereas termination by RNAPIII is traditionally considered to depend solely on a short DNA sequence (reviewed in Porrua et al., 2016). Nevertheless, recent data have challenged this classical view by unveiling not only common principles, but also a universal termination pathway relying on the conserved helicase Sen1 that operates at all eukaryotic polymerases (Porrua and Libri, 2013; Xie et al., 2022). In this Cell Science at a Glance and accompanying poster, we summarize the strategies adopted by each of the three eukaryotic RNAPs to terminate transcription. We will focus mainly on the factors and mechanisms uncovered in yeast and mammalian models.

Transcription termination by RNAPI

RNAPI synthesizes all ribosomal RNAs (rRNAs), except for 5S. The rDNA locus is located in the nucleolus and is composed of multiple tandem repeats (150–200 copies in yeast and 400 copies in humans) of a transcription unit that contains the sequence coding for the 35S pre-rRNA, which is processed into the three largest rRNAs (18S, 5.8S and 25S in yeast or 28S in mammals), and harbours the 5S gene in antisense. Transcription termination takes place at the intergenic sequence (IGS), separating these repeats. The IGS contains one or several recognition sequences for a protein possessing a Myb-like DNA-binding domain. These sites are often preceded by stretches of thymidine (T) of variable lengths, which partake in RNAPI transcription termination (see poster). Transcribing RNAPIs pause upon encountering a DNA-bound Myb-like protein, which acts as a roadblock, and subsequently additional factors induce the release of the polymerases and nascent transcripts from the chromatin (reviewed in Porrua et al., 2016).

In yeast (*Saccharomyces cerevisiae*), the roadblocking protein is Nsi1 (for NTS1 silencing protein 1), which promotes the termination of ∼90% of transcription events (Merkl et al., 2014; Prescott et al., 2004; Reiter et al., 2012). Nsi1 interacts with Fob1 (Ha et al., 2012), a protein that binds a downstream region called replication fork barrier (RFB). Fob1 binding to the RFB blocks the progression of replication forks coming from a neighbour replication origin (autonomous replication sequence or ARS) to prevent replication-transcription conflicts and also functions as a roadblock for RNAPIs that escape termination at the Nsi1-binding site (El Hage et al., 2008; Ha et al., 2012).

Two independent groups have put forward a model for the release of paused RNAPIs that parallels the so-called ‘torpedo’ model, originally proposed for termination by RNAPII (see below). The 35S pre-RNA is cleaved upstream of the Nsi1-binding site by the RNase III-like endonuclease Rnt1 (Kufel et al., 1999), and the new 5′ end of the nascent transcript thus generated is then targeted by the 5′ to 3′ exonuclease Rat1, which degrades the RNA until it encounters the roadblocked RNAPI and elicits its release (El Hage et al., 2008; Kawauchi et al., 2008). It has also been proposed that the helicase Sen1, a termination factor for RNAPII and RNAPIII (see below), assists Rat1 by removing eventual secondary structures that might hamper the access of Rat1 to the nascent RNA (Kawauchi et al., 2008). However, a recent study has shown that Sen1 associates with RNAPI *in vivo* and can induce the release of paused RNAPIs *in vitro*, strongly suggesting that Sen1 instead provides an alternative route for termination (Xie et al., 2022) (see poster).

Termination of RNAPI transcription in yeast also strongly depends on the non-essential RNAPI subunit Rpa12 (Prescott et al., 2004). Rpa12 is homologous to the C11 subunit of RNAPIII, which plays an important role in RNAPIII transcription termination (see below). Both Rpa12 and C11 have a C-terminal domain with homology to the RNAPII elongation factor TFIIS. This region 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 (Engel et al., 2013). Similar to what has been proposed for C11 (Girbig et al., 2022, see below), it is possible that the C-terminal region of Rpa12, by binding to the catalytic centre of RNAPI, induces conformational changes that contribute to the destabilization and subsequent release of the polymerase mediated by additional factors.

In mammals, the Myb-like protein that pauses RNAPI is transcription termination factor for RNAPI (TTF-I; also known as TTF1), which has several tandem recognition sites within the IGS (Längst et al., 1998). As in yeast, the release of RNAPI from the template depends on an additional factor that in mice is called polymerase I and transcript release factor (PTRF; also known as CAVIN1) and that interacts with both RNAPI and TTF-I (Jansa et al., 1998). PTRF is sufficient to provoke the release of RNAPI paused by TTF-I *in vitro*, but requires the presence of a T-rich sequence upstream of the TTF-I-binding site (Jansa et al., 1998), possibly because the weakness of the resulting RNA–DNA hybrid upon transcription of the T-rich sequence favours the destabilization of the EC. Unlike the yeast RNAPI-releasing factors, PTRF has not been assigned any nucleolytic or ATP-dependent activity. However, murine PTRF can provoke the release of roadblocked yeast RNAPIs from the DNA *in vitro* (Mason et al., 1997), suggesting that yeast and mouse termination factors might induce similar conformational changes in paused polymerases, resulting in their release.

Transcription termination by RNAPII

RNAPII transcribes all protein-coding genes and several classes of non-coding genes, among which are those that produce well-characterized functional RNAs, such as small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs), as well as other highly unstable RNAs resulting from cryptic pervasive transcription. In both yeast and mammals, there are different transcription termination pathways that are specialized for particular RNA classes, as discussed below.

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Transcription termination of protein-coding genes

Transcription termination of mRNA-coding genes is coupled to the processing of pre-mRNAs 3′ ends and relies on proteins that are generally highly conserved from yeast to humans (reviewed in Kuehner et al., 2011; Porrua and Libri, 2015). Initially, a multi-subunit complex composed of the cleavage and polyadenylation factor (CPF) and cleavage factors (CF) IA and IB (hereafter referred to as CPF) in yeast, and the cleavage and polyadenylation specificity factor (CPSF) and CFI and CFII (hereafter referred to as the CPSF complex) in mammals, is recruited to the nascent pre-mRNA via the recognition of a relatively loose consensus sequence of the poly(A) signal (PAS) and associated nearby sequence elements in the 3′ untranslated region (UTR) (see poster). The interaction of the complex component Pcf11 with the C-terminal domain (CTD) of the largest subunit of RNAPII possibly contributes to CPF/CPSF recruitment (Lunde et al., 2010). The CTD is composed of tandem repeats of the heptapeptide Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7, which are dynamically phosphorylated at most positions during the transcription cycle and is recognized by multiple factors involved in transcription or transcription-coupled processes (Harlen and Churchman, 2017). Pcf11 preferentially binds the CTD form where the Ser2 residue is phosphorylated (Ser2P), which is a mark that accumulates during transcription elongation, but, at least in yeast, this requires the dephosphorylation of Tyr1P, which occurs at the 3′ end of genes (Mayer et al., 2012). The elongation factor Spt5 is also phosphorylated and dephosphorylated during transcription, which plays an important role in termination by modulating RNAPII elongation rate and the recruitment of termination factors (Cortazar et al., 2019; Kecman et al., 2018; Parua et al., 2018, 2020). Upon recognition of the PAS and auxiliary sequences by CPF/CPSF components, the pre-mRNA is cleaved by a conserved endonuclease (Ysh1 in yeast and CPSF73, also known as CPSF3, in mammals) and a poly(A) tail is added to the pre-mRNA 3′ end by the conserved poly(A)-polymerase Pap1 (PAP, also known as PAPOLA, in mammals), which promotes subsequent export and translation of the mRNA in the cytoplasm (Kumar et al., 2019). The 5′ end of the downstream portion of the cleaved RNA is then targeted by the exonuclease Rat1/XRN2, which degrades the RNA until it encounters RNAPII and, according to the ‘torpedo’ model this collision induces the release of RNAPII from the chromatin (Kim et al., 2004; Park et al., 2015; West et al., 2004) (see poster). Both in yeast and mammals, there is evidence that slowing down or pausing of RNAPII at the 3′ end of genes is critical for efficient transcription termination (Collin et al., 2019; Cortazar et al., 2019; Fong et al., 2015).

Transcription termination of non-coding genes

Two analogous protein complexes play a major role in transcription termination of short non-coding genes in budding yeast and metazoans – the Nrd1–Nab3–Sen1 (NNS) complex and the Integrator complex, respectively.

The NNS complex is the main player in termination of pervasive transcription and in transcription termination of snoRNA genes (reviewed in Porrua and Libri, 2015) (see poster). Initially, the Nrd1 and Nab3 components of the complex are recruited to target RNAs via the recognition of specific motifs that are particularly enriched in non-coding RNAs (ncRNAs) (Carroll et al., 2004; Creamer et al., 2011; Porrua et al., 2012; Schulz et al., 2013). Fully efficient recruitment also requires the interaction of Nrd1 with the Ser5P-containing RNAPII CTD, which is a mark of early elongation (Tudek et al., 2014; Vasiljeva et al., 2008). The same domain of Nrd1 (i.e. the CTD-interaction domain or CID) then interacts with a region of the helicase Sen1 that mimics the RNAPII CTD and promotes Sen1 recruitment to the nascent transcript (Han et al., 2020). After loading onto the RNA, Sen1 uses the energy from ATP hydrolysis to translocate towards RNAPII and induce its release from the DNA. As is the case for termination at the end of protein-coding genes, various studies indicate that polymerase pausing is an important requirement for NNS-dependent termination (Collin et al., 2019; Hazelbaker et al., 2012; Porrua and Libri, 2013). After termination, the released transcript is polyadenylated by the alternative poly(A)-polymerase Trf4, within the TRAMP complex (Wyers et al., 2005), a process that is favoured by the association of the Nrd1 CID with a second CTD mimic in Trf4 (Tudek et al., 2014; Vasiljeva and Buratowski, 2006). Finally, the ncRNA is targeted by the nuclear form of the exosome, containing the exonuclease Rrp6, which mediates full degradation of pervasive transcripts (i.e. cryptic unstable transcripts or CUTs) (see Box 1) or maturation of snoRNAs (LaCava et al., 2005; Wyers et al., 2005). The exosome is a conserved multi-subunit complex with ribonuclease activity that plays an essential role in RNA processing and quality control (reviewed in Zinder and Lima, 2017).

Integrator is a multi-subunit complex from metazoans that mediates transcription termination at loci producing short unstable ncRNAs, such as promoter upstream transcripts (PROMPTS) and enhancer RNAs (eRNAs), as well as at snRNA genes and some protein-coding genes (Dasilva et al. 2021; Lai et al., 2015; Lykke-Andersen et al., 2021; Skaar et al., 2015) (see poster). Similar to the other termination complexes described above, Integrator recognizes specific phospho-marks of the CTD, in particular Ser2P and Ser7P (Egloff et al., 2010) via its INTS4-2-7 module (Fianu et al., 2021), and also here, the integrity of Tyr1 appears to be critical for its recruitment to the transcribing RNAPII (Shah et al., 2018). Integrator binds preferentially RNAs that are structured (e.g. the so-called 3′ box of snRNA precursors) and catalyses transcript cleavage via its CPSF73 homologue subunit INTS11 (Baillat et al., 2005). Transcription termination by Integrator critically depends on the cleavage of the nascent RNA (Elrod et al., 2019;

Box 1. Regulation of gene expression by modulated termination of non-coding pervasive transcription

Pervasive transcription is a universal phenomenon whereby RNAPII synthetizes a multitude of ncRNAs from regions of the genome; these are different from RNAs encoding proteins or functional RNAs (reviewed in Jensen et al., 2013 and; Villa and Porrua, 2022). These transcription events originate from bi-directional promoters or from cryptic initiation sites when these are not efficiently suppressed by the structure of chromatin (see poster). Although most pervasive transcripts are rapidly degraded and, therefore, lack any obvious function, some of these non-coding transcription events have been shown to have a role in the regulation of gene expression by transcriptional interference (see examples in Martens et al., 2005; Shah et al., 2014; van Werven et al., 2012). Indeed, non-coding transcription that invades the promoter of neighbouring genes can promote the formation of repressive chromatin structures and, thus, induce gene downregulation (Castelnuovo and Stutz, 2015). In budding yeast, it has recently been shown that some non-coding genes that are normally terminated by the NNS pathway and do not regulate the expression of cognate protein-coding genes under standard growth conditions can become repressor genes when the activity of the helicase Sen1 is negatively modulated by phosphorylation (Haidara et al., 2022) (see poster). These findings open up the possibility that a much larger fraction of cryptic transcription events than previously anticipated might serve regulatory purposes through the modulation of their termination efficiency.

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Lykke-Andersen and Jensen, 2007; Nojima et al., 2018; O'Reilly et al., 2014) but also on the activity of its associated protein phosphatase 2A, which catalyses the dephosphorylation of the CTD of RNAPII and SPT5, thus favouring RNAPII pausing and termination (Huang et al., 2020; Vervoort et al., 2021; Zheng et al., 2020). Nevertheless, the precise mechanisms by which RNAPII and the associated transcript are released remain obscure. Finally, the cleaved RNAs are subjected to degradation by the nuclear exosome (Lykke-Andersen et al., 2021), which in the case of PROMPTS depends also on the nuclear exosome targeting (NEXT) and cap-binding complex ARS2 (CBCA) complexes (Andersen et al., 2013).

Besides their roles in controlling pervasive transcription and in the biogenesis of functional ncRNAs, such as sn- and snoRNAs, both the NNS and the Integrator complexes play important roles in gene regulation by promoting the premature transcription termination of protein-coding genes (Arigo et al., 2006; Elrod et al., 2019; Schulz et al., 2013; Tatomer et al., 2019). Such a role has also been shown for the CPSF complex in the case of the *PCF11* gene and possibly several additional genes encoding transcriptional regulators (Kamieniarz-Gdula et al., 2019). A different mechanism of regulation involving transcription termination has recently been reported, whereby the activity of the NNS complex at certain non-coding genes is directly modulated in such a way that it regulates the expression of neighbouring genes (see details in Box 1 and poster).

Finally, in addition to the main termination pathways mentioned above, there are several alternative pathways that operate in a more limited number of cases (see Box 2 and poster).

Transcription termination by RNAPIII

RNAPIII produces short, abundant and highly structured ncRNAs, such as transfer RNAs (tRNAs), the 5S rRNA and the U6 snRNA. Unlike RNAPI and RNAPII, RNAPIII has traditionally been considered capable of terminating efficiently and precisely at a stretch of Ts in the non-template strand (NT) of the DNA without the aid of any additional protein factor (reviewed in Porrua et al., 2016). The length of the canonical terminator directly influences the efficiency of termination (Hamada et al., 2000) and varies among genes and among species. For instance, in budding yeast, stretches of six to seven Ts are the most frequent terminators and shorter ones are considered very weak terminators. In contrast, in humans most genes possess runs of four Ts, which support relatively efficient termination (Braglia et al., 2005).

T-tracts are bipartite transcription termination signals. On one side, the weakness of the rU–dA hybrid that results from transcription of a T-stretch contributes to the destabilization of the EC (Mishra and Maraia, 2019). On the other side, the specific recognition of the unpaired Ts in the NT strand is critical for both RNAPIII pausing and release from the DNA (Arimbasseri and Maraia, 2015). A recent structure–function study in budding yeast has shed light onto the molecular basis of the specific response of RNAPIII to T-stretches (Girbig et al., 2022) (see poster). The Ts in the NT DNA are tightly bound by several conserved residues of the second largest subunit of RNAPIII (C128), and this binding also requires two additional subunits (i.e. the C53–C37 heterodimer), which interact with C128 and appear to pre-position it for the recognition of the Ts. The C11 subunit, which is homologous to the RNAPI subunit Rpa12, is also implicated in transcription termination as it mediates the stable association of C53–C37 with the core polymerase (Landrieux et al., 2006). Moreover, additional data indicate that C11 plays a more direct role in the termination process (Mishra and Maraia, 2019). Similar to its RNAPII-associated homolog TFIIS, C11 restores the elongation capacity of stalled RNAPIIIs by mediating the cleavage of the 3′ end of the nascent RNA (Mishra et al., 2021). Girbig and co-authors have observed that the strong association of C128 with the unpaired Ts induces a register offset between the two DNA strands upon nucleotide addition that might trap RNAPIII in an unproductive state (see poster). They propose that C11 would sense this state and, in an attempt to rescue the stalled EC, it would interact with the active centre of RNAPIII in a way that would promote EC destabilization (Girbig et al., 2022). Another structural study on human RNAPIII reached similar conclusions, suggesting that the molecular determinants of the specific sensitivity of RNAPIII to T-tracts are evolutionarily conserved (Hou et al., 2021).

Several reports indicate that the secondary structures that form on the nascent RNA can also partake in transcription termination. A first *in vitro* study using purified budding yeast RNAPIII proposed that such structures are a strict requirement for the release of RNAPIIIs paused at T-tracts (Nielsen et al., 2013), contrary to the classical model outlined above. These data, however, have been the object of an intense debate (Arimbasseri et al., 2014; Nielsen and Zenkin, 2014). Nevertheless, a more recent report (Xie et al., 2022) showed that RNA structures forming just upstream of T-tracts are instead accessory elements that enhance RNAPIII release at terminators that fall outside of an optimal length range, likely by invading the RNA exit channel of RNAPIII. Furthermore, another study using a reporter system in the human model has provided evidence that RNA secondary structures can stimulate RNAPIII transcription termination *in vivo* (Verosloff et al., 2021).

Although RNAPIII was traditionally believed to terminate transcription very efficiently, high-resolution genome-wide studies of RNAPIII-mediated transcription have observed a substantial fraction of polymerases reading through the first T-tract (i.e. the primary terminator) after tRNA genes (tDNAs), which constitute the majority of RNAPIII-dependent genes (Turowski et al., 2016; Xie et al., 2022). Additional canonical termination sequences (i.e. secondary terminators) can be found downstream of primary terminators and likely contribute to termination of read-through polymerases. However, secondary terminators are most often short, and thus weak, and a different pathway, which relies on the helicase Sen1, appears to play a more prominent role in

Box 2. Alternative pathways for RNAPII transcription termination

As for RNAPI, it has been shown that transcriptional regulators containing a Myb-type DNA-binding domain, such as Reb1 and Rap1, can also act as roadblocks for RNAPII, at least in budding yeast (Candelli et al., 2018; Colin et al., 2014). In this case, clearing of paused polymerases is not mediated by specialized termination factors but rather involves RNAPII ubiquitylation followed by proteasomal degradation. This mechanism plays a role in preventing cryptic transcription from invading promoter regions in some instances and also provides an additional fail-safe pathway for RNAPIIs that do not terminate at canonical CPF-dependent termination sites.

In addition to this alternative pathway for RNAPII transcription termination, a variant of the 'torpedo' mechanism has been described in yeast where the entry site for the Rat1 exonuclease is provided by Rnt1-mediated RNA cleavage, as in the case of RNAPI-dependent transcription units (Ghazal et al., 2009) (see poster).

termination of RNAPIIIs that fail to cease transcription at the primary site (see poster). Indeed, combined biochemical and genomic data indicate that Sen1 interacts with RNAPIII and induces the release of RNAPIIIs that pause at weak termination signals or at other unrelated pausing sites. One of the two Sen1 homologues in *Schizosaccharomyces pombe* has also been shown to be involved in RNAPIII transcription termination (Rivosecchi et al., 2019) indicating that this alternative termination pathway is conserved in distant organisms. Therefore, not only nucleic acid-dependent but also protein-dependent mechanisms operate to ensure fully efficient transcription termination by RNAPIII.

Concluding remarks

Because of the essential nature of the process of transcription termination, eukaryotic cells have evolved multiple complementary, and often partially redundant, mechanisms to prevent the unscheduled persistence of transcribing RNAPs in the genome. Transcription termination pathways come in different flavours, but common patterns can be observed. First of all, transcriptional pausing or slowing down is a universal requisite for termination by all three classes of RNAPs. Polymerase pausing can be provoked by physical barriers, such as road-blocking proteins bound to the DNA, by specific DNA sequences such as T-tracts, as in the case of RNAPIII, or by dephosphorylation of the transcription machinery and associated factors, in the case of RNAPII. The weakness of the RNA–DNA hybrid might also generally contribute to destabilization of the EC. Indeed, although the importance of this parameter has been clearly established for transcription termination by RNAPI and RNAPIII, recent data have also pointed to a possible involvement of weak AU-rich hybrids in termination at RNAPII-dependent snRNA genes (Davidson et al., 2020).

Finally, from a mechanistic point of view, the step of RNAP release remains a ‘black box’. Although recent structure–function analyses have shed light onto the protein-independent mechanism of termination by RNAPIII and a plausible molecular model is starting to emerge, protein-dependent pathways for termination are far less understood. However, the fact that the helicase Sen1 is capable of dislodging the three polymerases without the aid of any additional factor strongly suggests that similar structural transitions underlie the release of eukaryotic polymerases during termination. Indeed, Sen1-mediated termination, which relies solely on transcriptional pausing and the action of Sen1 through the nascent RNA emerges as a universal termination pathway, thus implying the existence of more commonalities between the different types of RNAPs than previously appreciated.

Deciphering all the key molecular events leading to dissociation of an EC remains doubtlessly a challenging task. Nevertheless, the extraordinary progress of cryo-electron microscopy and its capacity to generate snapshots of complex biological processes promises major advances in this respect in the near future.

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Competing interests

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Cell science at a glance

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