

RNA polymerase II speed: a key player in controlling and adapting transcriptome composition

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Abstract

RNA polymerase II (RNA Pol II) speed or elongation rate, i.e., the number of nucleotides synthesized per unit of time, is a major determinant of transcriptome composition. It controls co-transcriptional processes such as splicing, polyadenylation, and transcription termination, thus regulating the production of alternative splice variants, circular RNAs, alternatively polyadenylated transcripts, or read-through transcripts. RNA Pol II speed itself is regulated in response to intra- and extra-cellular stimuli and can in turn affect the transcriptome composition in response to these stimuli. Evidence points to a potentially important role of transcriptome composition modification through RNA Pol II speed regulation for adaptation of cells to a changing environment, thus pointing to a function of RNA Pol II speed regulation in cellular physiology. Analyzing RNA Pol II speed dynamics may therefore be central to fully understand the regulation of physiological processes, such as the development of multicellular organisms. Recent findings also raise the possibility that RNA Pol II speed deregulation can be detrimental and participate in disease progression. Here, we review initial and current approaches to measure RNA Pol II speed, as well as providing an overview of the factors controlling speed and the co-transcriptional processes which are affected. Finally, we discuss the role of RNA Pol II speed regulation in cell physiology.

Keywords co-transcriptional processes; RNA polymerase II; transcription speed

Subject Category Chromatin, Transcription & Genomics

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Introduction

A cell's response to changes in the environment or upon commitment to a specific developmental program is associated with major changes in genome expression. These changes involve quantitative alterations of messenger RNA (mRNA) or non-coding RNA (ncRNA)

expression, but also qualitative changes through differential RNA processing, such as alternative splicing. The correct regulation of genome expression is essential at all steps of an organism's life. Among all the processes involved in genome expression control, transcription by RNA polymerase (pol) II is the first step and one of the most regulated. It has been extensively studied since it controls the production of all mRNAs in eukaryotic cells.

Transcription begins at the so-called "Transcription Start Site" (TSS) immediately downstream of the promoter that contains specific DNA sequences providing stable binding sites for the RNA polymerase and transcription factors. RNA Pol II reaches the polyadenylation (poly(A)) site after transcribing through the whole transcription unit, which can span hundreds of kilobases. When transcribing a protein-coding gene, RNA Pol II produces a pre-mRNA that needs to undergo a maturation process before giving rise to a functional mRNA. This process involves capping of the 5' end, removal of introns by splicing, and cleavage and polyadenylation at the 3' end. All these steps stabilize mRNA and play important roles for its nuclear export and translation. In addition, these different pre-mRNA processing steps are functionally coupled to transcription. Indeed, all these steps are initiated co-transcriptionally, thereby allowing the connection of transcription with mRNA maturation in time and space (Bentley, 2014). Tight regulation of these various processing events allows cells to increase the diversity of the transcriptome, through alternative TSS selection, alternative splicing, back-splicing, or alternative polyadenylation.

The various steps of transcription

RNA Pol II is a multiprotein complex composed of 12 subunits. RNA Pol II on its own cannot initiate transcription or transcribe long DNA sequences, but rather requires many additional protein complexes to perform these tasks. For example, topoisomerases which create transient DNA breaks to relieve DNA supercoiling play multiple roles during the transcription cycle (Pommier *et al.*, 2016; Chen *et al.*, 2018), including reduction of torsional stress to promote efficient transcription elongation (Baranello *et al.*, 2016). Overall, transcription can be divided into four main steps: initiation, promoter-proximal pausing, elongation, and termination (Fig 1) (for excellent reviews on these different steps, see (Porrua & Libri, 2015; Proudfoot, 2016; Core & Adelman, 2019; Schier & Taatjes, 2020)).

The transitions between these steps often involve post-translational modifications of RNA Pol II or associated factors.

The first step of RNA Pol II transcription consists of the assembly of the pre-initiation complex (PIC) on the promoter. PIC assembly often involves the recruitment of histone acetyltransferases and chromatin remodeling complexes that open chromatin to allow accessibility to promoters. The PIC is composed of many factors, including, but not restricted to, the general transcription factors TFIIA, TFIIB, TFIID, TFIIE, TFIIIF, TFIIH, RNA Pol II, and Mediator (Schier & Taatjes, 2020). Once assembled on the promoter, the PIC opens the DNA double strand allowing transcription initiation (Dienemann *et al*, 2019). RNA Pol II thus starts transcribing and a short nascent RNA is produced.

Shortly after transcription initiation, RNA Pol II enters a paused state known as promoter-proximal pausing. In mammals and many other metazoans, promoter-proximal pausing occurs 25–50 base pairs downstream from the TSS and is a major rate-limiting step of

the transcription cycle (Jonkers *et al*, 2014; Mayer *et al*, 2015). It involves a conformational change in the RNA:DNA hybrid within the polymerase active site. This conformational change leads to the absence of a free DNA template base in the active site, which is required for the addition of incoming NTPs by canonical base pairing. As a consequence, the progression of RNA Pol II is prevented (Vos *et al*, 2018b). The negative elongation factors NELF (Negative elongation factor) and DSIF (DRB-sensitivity inducing factor) stabilize and extend the lifetime of the paused complex. RNA Pol II is released from this pausing and allowed to resume transcription by the Cdk9 kinase component of the positive transcription elongation factor b (P-TEFb) complex, which phosphorylates the Spt5 subunit of DSIF, NELF, and serines 2, 5, and 7 (Ser2, Ser5, and Ser7) of the RNA Pol II carboxy terminal domain (CTD) (Czudnochowski *et al*, 2012; Eick & Geyer, 2013; Core & Adelman, 2019). P-TEFb is recruited to promoters through interactions with transcription factors, Mediator, and coactivators (Li *et al*, 2018). The P-TEFb-mediated

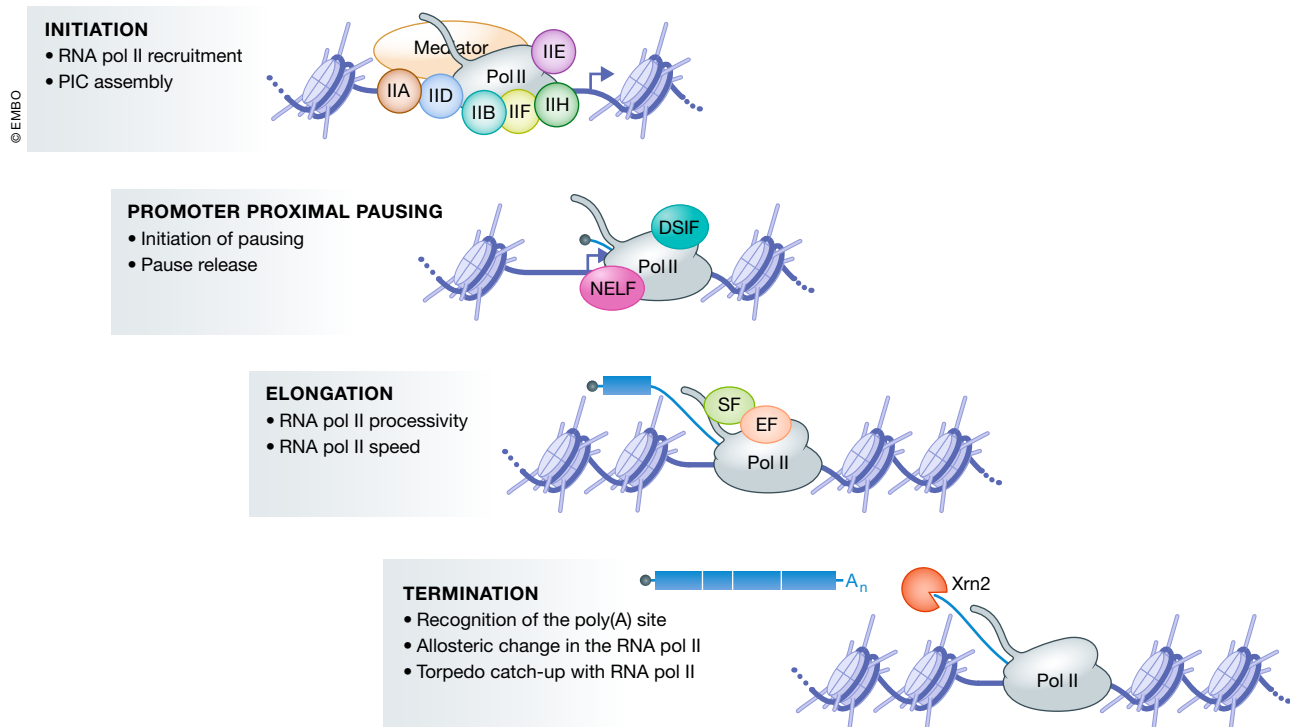


Figure 1. Main regulated steps of the RNA Pol II transcription cycle.

The RNA Pol II transcription cycle can be divided into four main regulated steps. First, transcription initiation starts with recruitment of RNA Pol II to the promoter and assembly of the pre-initiation complex (PIC) which is composed of many factors including, but not restricted to, the general transcription factors TFIIA (IIA), TFIIB (IIB), TFIID (IID), TFIIE (IIE), TFIIIF (IIF), TFIIH (IIH), RNA Pol II, and Mediator. The PIC opens the DNA and RNA Pol II starts transcription. Shortly after transcription initiation, RNA Pol II enters a paused state known as promoter-proximal pausing. Promoter-proximal pausing involves a conformational change in the RNA:DNA hybrid in the polymerase active site that prevents the addition of incoming NTPs by canonical base pairing. In many metazoans, the negative elongation factors NELF (Negative elongation factor) and DSIF (DRB-sensitivity inducing factor) stabilize and extend the lifetime of the paused complex. After its release from promoter-proximal pausing, RNA Pol II enters into productive elongation. During productive transcription elongation, many factors travel with RNA Pol II and help coordinate pre-mRNA processing with transcription. These factors include elongation factors (EF, represented by an orange oval) and splicing factors (SF, represented by a green oval). The term transcription elongation includes at least two regulated processes: RNA Pol II processivity, i.e., the ability of RNA Pol II to travel the entire length of the gene and transcription speed, defined as the number of nucleotides synthesized per unit of time. Finally, transcription termination involves poly(A) site recognition by the cleavage and polyadenylation complex which cleaves the nascent pre-mRNA that is then polyadenylated (A_n). Transcription of the poly(A) site induces an elongation slow-down which promotes transcription termination upon catch-up of RNA Pol II by the exonuclease Xrn2, which degrades the RNA synthesized beyond the poly(A) site from its 3' end.

phosphorylation events lead to both the dissociation of NELF from the RNA Pol II and the switch of Spt5 from a repressor to an activator of transcription (Ivanov *et al*, 2000; Yamada *et al*, 2006). Spt5 remains associated with RNA Pol II and serves as a recruitment platform for additional factors involved in RNA Pol II elongation and RNA processing.

Although RNA Pol II promoter-proximal pausing also occurs in nematodes, fission yeast, and plants, NELF is absent in these species, suggesting that alternative modes of RNA Pol II promoter-proximal pausing exist. Indeed, pausing at the +1 nucleosome, which does not involve DSIF or NELF, has been identified in fission yeast (Booth *et al*, 2016) and plants (Kindgren *et al*, 2020) but also in mammals (Chiu *et al*, 2018). The mechanisms of this +1 nucleosome pausing are not yet fully understood, but regulation by the MYC transcription factor and sensitivity to flavopiridol (an inhibitor of Cdk9 and Cdk12) have been reported (Chiu *et al*, 2018). In nematodes, although promoter-proximal pausing has been identified at stress response genes during starvation, its mechanism remains largely unknown (Kruesi *et al*, 2013; Maxwell *et al*, 2014). In contrast to these alternative mechanisms to pause RNA Pol II in promoter-proximal regions in nematode, fission yeast, and plants, RNA Pol II promoter-proximal pausing has, to date, not been reported in budding yeast (Booth *et al*, 2016).

After its release from this pause, RNA Pol II enters productive transcription elongation. The term transcription elongation refers to at least two regulated aspects of the transcript elongation process: RNA Pol II processivity and speed. RNA Pol II processivity is the ability of RNA Pol II to travel the entire length of the gene and thus controls the production of full-length transcripts. Premature transcription termination reduces RNA Pol II processivity and can lead to the production of transcripts that depending on the context are either rapidly degraded or stabilized. Stabilized prematurely terminated transcripts can give rise to non-coding RNAs or to proteins with different properties compared to those synthesized from full-length transcripts (Kamieniarz-Gdula & Proudfoot, 2019). The other transcription elongation parameter, transcription speed (or velocity), also referred to as the elongation rate, is defined as the number of nucleotides synthesized per unit of time and is the topic of this review. Note, however, that the term elongation rate is also sometimes used to describe other steps of the transcription cycle, such as RNA Pol II release from promoter-proximal pausing or RNA Pol II processivity.

Finally, when RNA Pol II reaches the end of a gene, it terminates transcription. At the end of almost all protein-coding genes (except replication-dependent histone genes), the termination process involves poly(A) site recognition. The poly(A) site is recognized by the cleavage and polyadenylation complex composed of multi-subunit factors including the cleavage and polyadenylation specificity factor (CPSF), the cleavage stimulatory factor (CstF), and the cleavage factors (CF) I and II. Upon recognition of the poly(A) site by the CPSF30 and WDR33 components of CPSF, the nascent pre-mRNA is cleaved by the CPSF73 endonuclease. The cleaved 3' end of the pre-mRNA is then polyadenylated, while the unprotected 5' end of the nascent RNA provides an entry site for the Xrn2 5'–3' exonuclease, which co-transcriptionally degrades the RNA synthesized beyond the poly(A) site (Eaton & West, 2020). In addition, transcription of the poly(A) site induces allosteric changes in the elongation complex (Logan *et al*, 1987) mediated by dephosphorylation of Spt5

by the PP1 phosphatase, which slow down elongation by at least two- to threefold (Cortazar *et al*, 2019). This reduction of elongation speed facilitates the catch-up of RNA Pol II by the Xrn2 exonuclease, which dislodges RNA Pol II from the DNA template leading to transcription termination (Cortazar *et al*, 2019; Eaton *et al*, 2020).

Regulation of RNA Pol II speed

Initially, studies on transcriptional regulation focused on transcription initiation, demonstrating an essential role for this stage in the regulation of gene expression. However, over the years, it has become increasingly clear that other stages of transcription are critical to control gene expression. For example, RNA Pol II promoter-proximal pausing, which has received increasing attention in the transcription field since the late 90s, was shown to be an early regulatory step that controls expression of most metazoan genes (for excellent reviews on this step, see (Jonkers & Lis, 2015; Chen *et al*, 2018)). Meanwhile, it has become clear that virtually all steps of the transcription cycle can be regulated by RNA Pol II-associated factors and the epigenetic context of the locus.

Until recently however, transcription elongation by RNA Pol II was not considered as a major regulatory step in gene expression. However, studies published over the last 20 years have shown that transcription elongation speed is highly dynamic and extremely regulated within individual genes, as well as between genes, which is the topic of the first section of this review. The use of RNA Pol II mutants displaying accelerated or decelerated transcription rates has also allowed major advances in our understanding of the function of RNA Pol II speed, demonstrating an essential role in many co-transcriptional processes and therefore in the repertoire of RNAs produced from a given locus as discussed in the second section of this review. We will also review the growing list of factors involved in the modulation of RNA Pol II speed highlighting that transcription velocity is a key regulatory step in the transcription cycle. Importantly, the introduction of a point mutation in RNA Pol II, which slows down transcription, was found to be embryonic lethal in mice (Maslon *et al*, 2019) and non-viable in plants (Leng *et al*, 2020). These data demonstrate that correct RNA Pol II speed is essential for development (Maslon *et al*, 2019; Leng *et al*, 2020). Moreover, we and others have shown that transcription speed can be locally adapted in response to intrinsic or extrinsic stimuli, affecting the identity of RNAs produced in the cell (Schor *et al*, 2009; Schor *et al*, 2013; Sharma *et al*, 2014; Muniz *et al*, 2017).

In this review, we will therefore discuss the different techniques used to measure RNA Pol II velocity and the evidences indicating that transcription speed is not static, but highly variable between genes, as well as within individual genes. We will then describe the influence of RNA Pol II velocity on co-transcriptional processes and thus on the identity of RNAs produced from a given gene, as well as the factors that regulate transcription speed. Finally, we will cover the dynamics of RNA Pol II speed regulation and the roles it may play in the response to environmental changes.

Measuring RNA Pol II speed

At which speed does RNA Pol II synthesize RNA? This a long-standing question which has been investigated for over 40 years. Many different methods have been developed to estimate RNA Pol

II speed on endogenous or integrated reporter genes in distinct species (see (Jonkers & Lis, 2015) for an excellent review describing some of these methods). These methods measure the distance traveled by RNA Pol II as a function of time. Importantly, we can only measure an average RNA Pol II speed on any given gene or part of gene, since it depends on both the instantaneous speed at which nucleotides are added by the polymerase to the nascent RNA, as well as polymerase pausing within the gene body. Indeed, transcription elongation is frequently interrupted by pauses induced by roadblocks such as nucleosomes, DNA-binding proteins, or misincorporated nucleotides (Mayer *et al*, 2017), which can thus affect the average speed of transcription. Most of the described methods developed to estimate RNA Pol II speed rely on inducible gene expression or treatments with small drugs globally inhibiting transcription either at the initiation step (with triptolide, for example) or, more commonly, at the promoter-proximal pause release step with reversible P-TEFb inhibitors (e.g., flavopiridol or DRB). Inhibition of P-TEFb and thus repression of the release of RNA Pol II from promoter-proximal pausing has been shown to block transcription of almost all genes (Henriques *et al*, 2013; Jonkers *et al*, 2014). In this scenario, RNA Pol II elongation complexes which are already in the productive elongation phase of the transcription cycle continue transcribing, while no new RNA Pol II enters productive elongation. As such, methods relying on the reversible P-TEFb inhibitors theoretically allow measurement of RNA Pol II speed at most active genes. To calculate transcription speed, the disappearance or appearance of the RNA produced by transcription or RNA Pol II itself is then followed at various locations downstream of gene promoters in kinetics experiments. The distance traveled by RNA Pol II after transcription inhibition (also called the RNA Pol II “retreating wave”) can be followed in response to transcriptional switch-off of inducible genes or after treatment with general transcription inhibitors. Conversely, the distance traveled by RNA Pol II after transcription induction (also called the RNA Pol II “emerging wave”) can be followed in response to transcriptional activation by different stimuli or after globally switching on transcription by removing a reversible inhibitor (Jonkers & Lis, 2015).

RNA Pol II speed measurements by biochemical approaches

Pioneering works have estimated average RNA Pol II speed only on a few genes. By monitoring the appearance of RNA by *in situ* hybridization, nuclear run-on, slot blot, or RT-PCR after expression activation, RNA Pol II speed was estimated between 1.1 kb/min and 4.8 kb/min (Ucker & Yamamoto, 1984; Thummel *et al*, 1990; Sherman & O’Farrell, 1991; O’Brien & Lis, 1993; Tennyson *et al*, 1995; Femino *et al*, 1998; Femino *et al*, 2003; Hanisch *et al*, 2013). Other studies have estimated average RNA Pol II speed between 2 and 6 kb/min by analyzing the sedimentation profile of RNA after [³H] uridine incorporation (Sehgal *et al*, 1976) or RNA Pol II levels by ChIP PCR at several locations in the gene (Mason & Struhl, 2005) after transcriptional repression.

By using quantitative methods to detect RNAs, these measures of RNA Pol II speed were then refined. For instance, the RNA Pol II “emerging wave” has been followed by monitoring the appearance of nascent RNAs by qRT-PCR on exon–intron junctions spanning the genes. This has been performed on a few long genes at different time points after stimulation by tumor necrosis factor- α (TNF α) (Wada *et al*, 2009), interferon β , or DRB release (Singh &

Padgett, 2009). In these studies, the average transcription speed was estimated between 3.1 kb/min and 3.8 kb/min with little variation between individual genes (Singh & Padgett, 2009; Wada *et al*, 2009).

In the late 2000s, many teams began developing methodologies to measure transcription speed genome wide (Table 1). A major improvement in the race for global measurement of RNA Pol II speed came from the demonstration that RNA Pol II “emerging” and “retreating” waves could be followed genome wide by global run-on sequencing (GRO-seq) (Hah *et al*, 2011). This technique has been applied in various human cell lines to follow the “emerging wave” of RNA Pol II after transcriptional activation by different cellular signaling pathways (Danko *et al*, 2013) or by DRB release (Saponaro *et al*, 2014). Other methods have been developed to specifically enrich nascent RNAs and have been applied to follow the RNA Pol II “emerging wave” after DRB or flavopiridol release. These include sequencing of nascent RNAs labeled with 4-thiouridine (Fuchs *et al*, 2014b; Fuchs *et al*, 2015; Liang *et al*, 2018; Baluapuri *et al*, 2019; Gregersen *et al*, 2020) or bromouridine (Veloso *et al*, 2014). As for the RNA Pol II “retreating wave”, it has been followed genome wide by GRO-seq after flavopiridol treatment (Jonkers *et al*, 2014). All these techniques showed in various human cell lines that the average RNA Pol II speed ranges between 1.25 kb/min and 3.5 kb/min (Danko *et al*, 2013; Fuchs *et al*, 2014b; Jonkers *et al*, 2014; Saponaro *et al*, 2014; Veloso *et al*, 2014; Liang *et al*, 2018; Baluapuri *et al*, 2019; Gregersen *et al*, 2020). Contrary to what had been initially shown for a few genes (Singh & Padgett, 2009; Wada *et al*, 2009), the measured transcription speed was found to be extremely variable between genes, with RNA Pol II velocity varying from 0.37 kb/min to 3.57 kb/min for individual genes (Danko *et al*, 2013). RNA Pol II speed was furthermore also shown to be highly variable within individual genes, as RNA Pol II accelerates while traveling throughout the gene body, starting at 0.5 kb/min in the first 10–15 kb and accelerating up to 2–4 kb/min downstream (Danko *et al*, 2013; Fuchs *et al*, 2014b; Jonkers *et al*, 2014; Saponaro *et al*, 2014). In addition, comparison of transcription speed between different cell lines showed that transcription speed on individual genes is overall well conserved between cell lines (Singh & Padgett, 2009; Veloso *et al*, 2014). However, Danko *et al* (2013) found that RNA Pol II velocity can differ by as much as 25–40% on individual genes in different cell lines and in response to diverse signaling pathways. Moreover, we and others have shown that RNA Pol II speed can vary on individual genes or downstream of genes in response to different stimuli (Schor *et al*, 2009; Schor *et al*, 2013; Sharma *et al*, 2014; Muniz *et al*, 2017). Thus, RNA pol II speed is highly regulated between as well as within genes and can change in response to different stimuli.

Finally, RNA Pol II speed has also been inferred from existing total RNA-seq data (Ameur *et al*, 2011; Jonkers *et al*, 2014; Kawamura *et al*, 2019). Indeed, intronic reads form a decreasing gradient from the 5’ to the 3’ end of the intron. This gradient depends on the kinetics of RNA Pol II and co-transcriptional splicing and leads to formation of a “saw-tooth” pattern of read coverage over the length of the gene (Ameur *et al*, 2011). RNA Pol II speed can thus be inferred for long introns by calculating the 5’–3’ slope of intron reads coverage. Faster RNA Pol II will take less time to reach the downstream exon where co-transcriptional splicing can take place, thus limiting RNA accumulation at the 5’ end of introns, thereby

Table 1. Average RNA pol II speed estimations on a genome-wide basis.

| RNA pol II speed | Cell line, organism | Number of genes | Measurement method | Study |
|---|---|---|--|-------------------------------|
| 2.1 kb/min 2.8 kb/min | MCF-7, Human AC16, Human | 140 genes 26 genes | GRO-seq (sequencing of nascent RNA extended in the presence of BrUTP in run-on experiments) following transcriptional activation by estrogen signaling or cytokine treatment—follows the “emerging wave” of RNA pol II | Danko <i>et al</i> (2013) |
| 3.5 kb/min | HeLa, Human | 1,577 genes | 4sUDRB-seq (sequencing of nascent RNA labeled with 4-thiouridine following DRB release)—follows the “emerging wave” of RNA pol II | Fuchs <i>et al</i> (2014) |
| 1.25 kb/min 1.75 kb/min 1.25 kb/min 1.25 kb/min 1.75 kb/min | HF1, Human TM, Human CS-B, Human K562, Human MCF-7, Human | 2,702 genes 2,469 genes 1,932 genes 2,270 genes 2,399 genes | BruDRB-seq (sequencing of nascent RNA labeled with bromouridine following DRB release)—follows the “emerging wave” of RNA pol II | Veloso <i>et al</i> (2014) |
| 3.13 kb/min | HEK293, Human | 237 genes | DRB/GRO-seq (sequencing of nascent RNA extended in the presence of BrUTP in run-on experiments following DRB release)—follows the “emerging wave” of RNA pol II | Saponaro <i>et al</i> (2014) |
| 2 kb/min | mESC, Mouse | More than 1,000 genes | GRO-seq (sequencing of nascent RNA extended in the presence of BrUTP in run-on experiments) following flavopiridol treatment to block RNA pol II promoter-proximal pause release—follows the “retreating wave” of RNA pol II | Jonkers <i>et al</i> (2014) |
| 3.27 kb/min | U2OS, Human | 2,163 genes | 4sUDRB-seq (sequencing of nascent RNA labeled with 4-thiouridine following DRB release)—follows the “emerging wave” of RNA pol II | Baluapuri <i>et al</i> (2019) |
| Around 3.2 kb/min | HEK293, Human | 982 genes | 4sUFP-seq (sequencing of nascent RNA labeled with 4-thiouridine following flavopiridol release)—follows the “emerging wave” of RNA pol II | Liang <i>et al</i> (2018) |
| Around 1 kb/min | S2, <i>Drosophila</i> | Not mentioned | 4sUDRB-seq (sequencing of nascent RNA labeled with 4-thiouridine following DRB release)—follows the “emerging wave” of RNA pol II | Akhtar <i>et al</i> (2019) |
| 2.3 kb/min | HEK293, Human | 4,869 genes | DRB/TT _{chem} -seq (sequencing of nascent RNA labeled with 4-thiouridine following DRB release)—follows the “emerging wave” of RNA pol II | Gregersen <i>et al</i> (2020) |

decreasing the 5′–3′ slope of intronic reads coverage. This technique is however far less accurate than the methods designed to follow the emerging or retreating waves of RNA Pol II. It may, nonetheless, give an opportunity to compare RNA Pol II speed in thousands of published datasets from different cell types and in response to many different stimuli.

RNA Pol II speed measurements by imaging technologies

Interestingly, studies performed in live cells came to very similar estimations of RNA Pol II speed. RNA Pol II speed in live cells was estimated to range from 0.6 kb/min to 4.3 kb/min by fluorescence recovery after photobleaching (FRAP) experiments using MS2 fluorescent proteins to label nascent MS2 repeat-containing RNAs (Boireau *et al*, 2007; Darzacq *et al*, 2007; Ben-Ari *et al*, 2010; Yunger *et al*, 2010; Brody *et al*, 2011; Maiuri *et al*, 2011; Muramoto *et al*, 2012; Corrigan *et al*, 2016). By using constructs carrying arrays of MS2 and PP7 repeats in the 5′ and 3′ regions of the same gene, RNA Pol II speed was estimated to range from 0.84 kb/min to 3.66 kb/min (Hocine *et al*, 2013; Fukaya *et al*, 2017). Other imaging techniques such as fluctuation analysis of fluorescently labeled nascent RNA or high-speed 3D fluorescence nanoimaging techniques reported speed in the same range, albeit slightly higher between 0.6 and 12 kb/min (Larson *et al*, 2011; Martin *et al*, 2013; Annibale & Gratton, 2015). Measurement of RNA Pol II velocity on endogenous genes in living cells was also attempted by FRAP experiments using EGFP tagged RNA Pol II. The authors calculated an RNA Pol II speed between 1.1 to 1.5 kb/min when measured on the Hsp70 genes following heat shock in *Drosophila* (Yao *et al*, 2007; Ardehali *et al*, 2009). These studies also found that RNA Pol

II speed was highly variable between cells (Hocine *et al*, 2013), as well as between identical copies of the same gene at different locations in a gene array, measured simultaneously in the same cell (Annibale & Gratton, 2015). Indeed, in this latter study, a fourfold variation in RNA Pol II velocity was measured between identical copies of the same gene and overall, the different RNA Pol II speeds measured in the gene array almost span the range of transcription speeds reported using the MS2 containing constructs, from below 0.6 kb/min to over 12 kb/min (Annibale & Gratton, 2015). Along the same line, Maiuri *et al* (2011) even reported an extremely high RNA Pol II speed (about 80 kb/min) on an HIV-based vector integrated in one copy, whereas when integrated in 35 copies, RNA Pol II velocity was estimated at 1.6 kb/min, similar to earlier estimates from multicopy HIV-derived vector insertions (Boireau *et al*, 2007). Nevertheless, single integration of the CCND1 gene under the control of an endogenous or viral promoter led to an RNA Pol II speed of 0.31–0.78 kb/min (Yunger *et al*, 2010) and the number of gene units in the tandem array had no effect on RNA Pol II speed in another study (Brody *et al*, 2011). In agreement with biochemical measurements of RNA Pol II speed, all these data show that RNA Pol II speed can be variable between genes but also within genes, highlighting the fact that RNA Pol II speed is highly regulated.

RNA Pol II speed measurements in organisms

As mentioned above, over the last 20 years, RNA Pol II speed has been estimated by several different methods. All these studies show that transcription speed is highly dynamic and highly regulated within, as well as between genes, suggesting that it might play an

important role in the transcription cycle. The most accurate and least biased methods to measure RNA Pol II speed are based on activation or inhibition of transcription and following RNA Pol II progression throughout genes over time. This allows estimating the distance traveled by RNA Pol II during a given amount of time. However, most of these studies, in particular those analyzing RNA Pol II speed genome wide, used drugs that inhibit either transcription initiation or promoter-proximal pause release. It is important to consider that these drugs may have important secondary effects, since most of them target kinases involved in cell-cycle control, which may affect the measurements. Another issue with most of these studies is that they were performed using cell lines. Whether the observed features can be generalized or whether they are a peculiarity of the respective analyzed cell lines thus remains an open question. The use of drugs can be overcome by activating different signaling pathways to induce transcription of specific genes, hence limiting secondary effects. Although the use of these induction systems restricts the analysis to only a handful of genes, the method can be scaled up to whole organisms, as shown in a recent study measuring RNA Pol II speed in budding yeast and *A. thaliana* seedlings (Leng *et al*, 2020). Moreover, this study together with a recent study measuring RNA Pol II speed in mouse ESCs (Embryonic Stem Cells) and neurons showed that these methods are readily transferable to more complex systems and still give comparable results (Maslon *et al*, 2019; Leng *et al*, 2020). Clearly, further developing novel technologies allowing accurate measurement of RNA Pol II speed at genome-wide level in physiological situations and in complex organisms will be a major advance in the field.

RNA Pol II speed controls many co-transcriptional processes and thus affects the composition of the transcriptome

In the early 80s, a screen was performed to discover mutations rendering *Drosophila* RNA Pol II resistant to the transcription inhibitor α -amanitin (Greenleaf *et al*, 1979). This screen led to the discovery of mutations affecting transcription speed. These mutations, mostly located in the largest RNA Pol II subunit RPB1 (Greenleaf, 1983), which either slow down or accelerate transcription, have allowed huge progress in the characterization of the role of RNA Pol II speed in the regulation of gene expression. At least two different mutations in human RNA Pol II are known to slow down transcription. The R749H substitution in the funnel domain is homologous to the C4 mutation in *Drosophila* (de la Mata *et al*, 2003) and likely affects translocation of the polymerase such that the forward and backward movement of the mutant elongation complex is slowed-down (Chen *et al*, 1993; Chen *et al*, 1996). The H1108Y substitution is homologous to the H1085Y substitution in the yeast Rpb1 (Kaplan *et al*, 2008) and mutates a contact with the β -phosphate of incoming NTPs (Fong *et al*, 2014) which also slows down transcription. Conversely, the E1126G substitution in human RPB1 (Fong *et al*, 2014) (homologous to the E1103G substitution in the yeast RPB1; Kaplan *et al*, 2008; Kireeva *et al*, 2008) accelerates transcription by stabilizing a triple alpha helix at the base of the trigger loop, concomitantly with a loss of fidelity that is reduced by α -amanitin. Average speed for these RNA Pol II mutants is 0.5 Kb/min or even slower for the slow mutants (R749H and H1108Y,

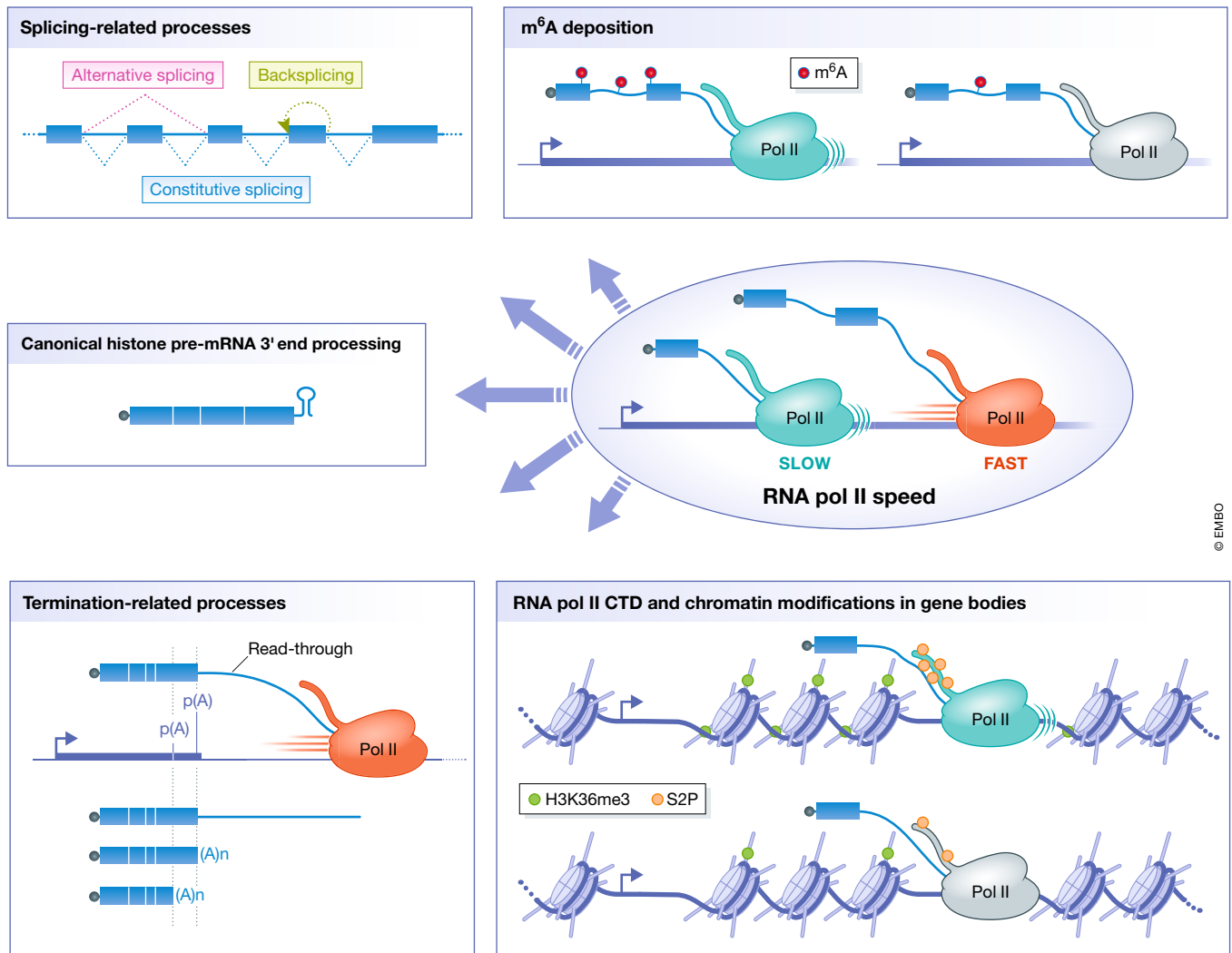
respectively) and 1.9 Kb/min for the fast mutant as compared to 1.7 Kb/min for the human α -amanitin-resistant WT pol II (Fong *et al*, 2014). Nevertheless, prolonged α -amanitin treatment has been shown to induce accelerated degradation of several proteins including the elongation factor DSIF (Tsao *et al*, 2012). Measurements of RNA Pol II speed obtained using α -amanitin-resistant RNA Pol II mutants should thus be taken with care.

By analyzing these different mutants in different systems, RNA Pol II velocity has been shown to control many co-transcriptional processes. Indeed, transcription speed determines the rate at which binding sites for RNA-binding proteins are synthesized within the nascent RNA and is thus in kinetic competition with co-transcriptional processes. Co-transcriptional processes shown to be regulated by RNA Pol II speed include constitutive splicing, alternative splicing, back-splicing, alternative polyadenylation, and transcription termination (Fig 2). All of these co-transcriptional processes contribute to the generation of transcriptome diversity that is critical for proper cell functioning and will be discussed in further detail in the following sections.

Control of splicing-dependent processes

Constitutive splicing is the joining of consecutive exons by removal of intervening introns. The majority of individual introns are constitutively spliced. However, splicing efficiency is not 100% and unspliced RNAs are predominantly retained in the nucleus and degraded through a quality-control mechanism (Palazzo & Lee, 2018). Using microarrays designed to study splicing in budding yeast, slow RNA Pol II mutants were shown to enhance splicing, while fast mutants were shown to reduce constitutive splicing (Braberg *et al*, 2013). This anti-correlation between transcription speed and splicing efficiency has been confirmed in budding yeast by RNA sequencing-based methods (Aslanzadeh *et al*, 2018). Likewise, other studies using sequencing-based methods showed that a slow mutant enhances constitutive splicing in *Drosophila* (Howe *et al*, 2003; Khodor *et al*, 2011; Braberg *et al*, 2013; Aslanzadeh *et al*, 2018), as does a fast mutant in plants (Leng *et al*, 2020). This discrepancy between plants on one hand and *Drosophila* and budding yeast on the other hand might seem surprising; however, both slow and fast RNA Pol II mutants inhibit constitutive splicing in human cells (Fong *et al*, 2014). These results show that while RNA Pol II speed differentially affects constitutive splicing in various species, proper control of intragenic RNA Pol II velocity is essential for proper regulation of constitutive splicing efficiency.

In contrast to constitutive splicing, alternative splicing contributes to the proteome diversity through the biogenesis of several mRNA splice variants from the same pre-mRNA. Indeed, transcripts can undergo one or more alternative splicing events such as inclusion or skipping of an exon, use of alternative 5' or 3' splice sites, intron retention, or splicing of adjacent mutually exclusive exons, thus generating several different transcript isoforms from the same gene. More than 95% of human genes undergo alternative splicing in a developmental, tissue-specific or signal transduction-dependent manner (Nilsen & Graveley, 2010). Alternative splicing regulation is extremely complex, with dynamic alternative splicing networks identified in many different cell types and organs, as well as in different physiological conditions. Many types of RNA-binding proteins can control alternative splicing through binding to *cis* elements (splicing enhancers or silencers), although the full set of factors



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Figure 2. Co-transcriptional processes regulated by RNA Pol II speed.

RNA Pol II speed can influence the following:

- Splicing-related processes such as constitutive splicing, alternative splicing, and back-splicing;
- The RNA methylome by controlling the deposition of N⁶-methyladenosine in RNAs;
- Normal 3' end processing of replication-dependent core histone mRNAs by controlling the folding of nascent RNA at histone genes;
- Termination-related processes such as alternative polyadenylation (represented by two poly(A) sites) and the distance traveled by RNA Pol II beyond the poly(A) site, i.e., read-through transcription;
- Protein modifications on gene bodies: post-translational modifications of RNA Pol II CTD such as serine 2 phosphorylation and chromatin modifications such as trimethylation of lysine 36 of histone H3.

involved is not known. Like constitutive splicing, alternative splicing is also sensitive to RNA Pol II speed. Indeed, the use of gel-based methods in yeast, human, and mouse cells (de la Mata *et al*, 2003; Howe *et al*, 2003; Schor *et al*, 2009; Ip *et al*, 2011; Dujardin *et al*, 2014) or of RNA sequencing-based methods in human cells (Fong *et al*, 2014) showed that slow and fast transcription either increased or decreased inclusion of alternative exons depending on the genes analyzed. Moreover, RNA sequencing analyses showed that slow transcription obtained using an RNA Pol II knock-in mutant in mouse ESCs or fast transcription induced by an RNA Pol II point mutant in plants leads to changes in both exon inclusion and skipping (Maslon *et al*, 2019; Leng *et al*, 2020).

The effects of RNA Pol II velocity on splicing events can be explained by the fact that splicing is in kinetic competition with transcription elongation. According to the “window of opportunity” model, altering transcription speed may regulate splicing by modulating the presence of *cis*-acting sequence elements on the nascent RNA and thus the recruitment of both positive and negative splicing factors. However, slow and fast RNA Pol II mutants often have the same effect on alternative exon inclusion or skipping (Fong *et al*, 2014; Aslanzadeh *et al*, 2018), which is not predicted by the “window of opportunity” model. These observations have thus led to the emergence of another model termed “Goldilocks” model in which an optimal RNA Pol II speed is required to allow proper

nascent RNA folding and factors binding to splice sites, splicing enhancers and silencers (Fong *et al.*, 2014). This model predicts that RNA Pol II speed has evolved under positive selection pressure to allow optimal co-transcriptional splicing. However, it remains to be determined how RNA Pol II speed affects nascent RNA folding and/or binding of factors to influence co-transcriptional splicing.

RNA Pol II speed, through controlling splicing efficiency, has also been implicated in the regulation of circular RNA (circRNA) production. CircRNAs belong to a newly described class of RNAs that also contribute to transcriptome diversity. CircRNAs are produced by back-splicing of pre-mRNAs, which corresponds to the joining of a downstream 5' splice site to an upstream 3' splice site in the reversed orientation, thus producing a circular RNA molecule (for excellent reviews on circRNA biogenesis and function, see (Chen, 2020; Xiao *et al.*, 2020)). Tens of thousands of circRNAs have been identified in metazoans, some of which are evolutionarily conserved. While a majority of these circRNAs are expressed at low levels, some of them accumulate to levels exceeding those of their linear counterparts (Jeck *et al.*, 2013; Salzman *et al.*, 2013). Indeed, although back-splicing is generally much less efficient than canonical splicing, owing to their circular structure, circRNAs are resistant to degradation by exonucleases, which allows them to accumulate to high levels. CircRNAs exhibit tissue and cell type-specific expression patterns with a tendency to accumulate in the brain (Rybak-Wolf *et al.*, 2015). To date, only a small subset of all the identified circRNAs has been functionally studied, leaving a full repertoire of circRNAs of unknown function. Moreover, because of their low expression level, the biological significance of some circRNAs has been questioned; however, recent findings suggest that circRNAs can work as a group to achieve measurable effects. Furthermore, accumulating evidence indicates that circRNAs are tightly regulated transcripts that in some cases play essential biological functions. Indeed, a growing number of circRNAs have been implicated in the regulation of innate immunity, cell proliferation, transformation, and neuronal function (Chen *et al.*, 2019; Liu *et al.*, 2019; Hollensen *et al.*, 2020). Misregulation of some circRNAs has also been implicated in multiple diseases among which cancer, some circRNAs playing important roles in cancer progression (Chen *et al.*, 2019). CircRNAs can regulate transcription, splicing, and chromatin interactions. They can also act as microRNA decoys, function as protein scaffolds, sequester proteins, or be themselves translated (Kristensen *et al.*, 2019). Moreover, because circRNAs are very stable and can be detected in body fluids (blood and urine, for example), they are now considered as promising disease biomarkers (Vo *et al.*, 2019; Wang *et al.*, 2021). Importantly, circRNA biogenesis is tightly regulated, although the mechanisms regulating their levels are not completely understood. Among key elements regulating their production, intronic complementary repeats in introns flanking the circularized exons have been shown to be important for the biogenesis of circRNAs (Liang & Wilusz, 2014). Base pairing between these repeats is required for the biogenesis of these circRNAs, most probably by bringing the splice sites into close proximity. Many proteins are also involved in the production of circRNAs, such as spliceosome components, RNA-binding proteins, and RNA helicases (Patop *et al.*, 2019). In addition, some recent studies point to RNA Pol II speed as another determinant of circRNA production. Indeed, the average RNA Pol II velocity is higher on genes producing circRNAs than on genes not producing circRNAs (Zhang *et al.*, 2016) and

especially on introns of these genes (Ragan *et al.*, 2019). Moreover, the use of slow and fast RNA Pol II mutants has shown that transcription speed directly correlates with the circularization efficiency of circRNAs (Ashwal-Fluss *et al.*, 2014; Zhang *et al.*, 2016).

Control of transcription termination-related processes

RNA Pol II speed is also in competition with the rate of processes linked to transcription termination such as poly(A) site usage and transcription termination itself.

A gene can possess multiple poly(A) sites, and differential usage of these sites leads to the formation of distinct mRNA isoforms, a phenomenon termed alternative polyadenylation. Alternative polyadenylation is very common in eukaryotes, and around 70% of mammalian protein-coding genes are subjected to alternative polyadenylation (Derti *et al.*, 2012; Hoque *et al.*, 2013). When an alternative poly(A) site is present in the coding region, the use of this alternative polyadenylation site can produce mRNA isoforms that differ in their coding capacity, resulting in different final protein products with specific cellular functions. In contrast, and most frequently, the alternative poly(A) sites are located downstream of the coding region and their differential usage generates mRNAs that share the same coding sequence, but have different 3' untranslated region (UTR) lengths. The alternative 3' UTR sequence may contain regulatory elements that interact with RNA-binding proteins or miRNAs, modulating mRNA stability, translation, transport, and/or localization (Tian & Manley, 2017). On several genes possessing alternative poly(A) sites, a slow RNA Pol II mutant shows a switch to the use of upstream poly(A) sites (Pinto *et al.*, 2011; Liu *et al.*, 2017; Geisberg *et al.*, 2020; Yague-Sanz *et al.*, 2020), while a fast RNA Pol II mutant shows a slight downstream switch in poly(A) site usage (Geisberg *et al.*, 2020). In addition, a slow RNA Pol II mutant shows a tissue-specific effect on alternative polyadenylation as it affects poly(A) site usage differentially in the *Drosophila* head or body (Liu *et al.*, 2017).

Transcription termination is an essential step of the transcription cycle. It requires disassembly of the stable complex formed between the nascent RNA, RNA Pol II, and the DNA template. Transcription termination is important to recycle RNA Pol II and to prevent interference between adjacent transcription units. For most genes, transcription termination is triggered by recognition of the poly(A) site, but it does not occur at a conserved site or distance from the 3' end of genes (Hofer & Darnell, 1981; Weintraub *et al.*, 1981; Hagenbüchle *et al.*, 1984). It rather occurs at diffuse sites, at variable distances, up to several kilobases, downstream of the poly(A) site (Fong *et al.*, 2015). Transcription termination has been shown to be shifted closer to the poly(A) site by a slow RNA Pol II mutant (Fong *et al.*, 2015), whereas a fast RNA Pol II mutant shifted transcription termination further downstream from the poly(A) site (Fong *et al.*, 2015; Leng *et al.*, 2020). Consistent with the results obtained with slow RNA Pol II mutants, slowing-down transcription using mutants of the elongation factor TFIIS also shifts termination upstream (Sheridan *et al.*, 2019; Zatreanu *et al.*, 2019). RNA Pol II velocity can thus control the distance traveled by RNA Pol II downstream of genes, in other words, the extent of transcriptional read-through. These results suggest that a kinetic competition between the elongating RNA Pol II and the degradation of the nascent RNA by Xrn2 is controlling termination (Fong *et al.*, 2015). Interestingly, we recently showed that transcription speed increases downstream of

two genes in response to activation of an oncogene and that this increase correlates with an increase in transcriptional read-through downstream of these genes (Muniz *et al*, 2017). This implies that transcriptional read-through might be regulated by RNA Pol II speed in response to intra-cellular stimuli.

Control of mRNA modifications

N6-methyladenosine (m⁶A) is the most abundant modification in mRNA, detected in thousands of human transcripts (about 25% of mRNAs contain at least one m⁶A) (Dominissini *et al*, 2012; Meyer *et al*, 2012). More than 99% of total m⁶A in polyadenylated mRNA is deposited by a heterodimeric complex composed of METTL3-METTL14 in a highly specific manner (Shi *et al*, 2019; Zaccara *et al*, 2019). m⁶A influences splicing, degradation, and translation of mRNA (Knuckles & Bühler, 2018; He & He, 2021). A slow RNA Pol II mutant induces an increase in the level of m⁶A deposition on mRNAs, and this has been linked to a reduced efficiency of mRNA translation (Slobodin *et al*, 2017). Thus, transcription speed, by affecting m⁶A deposition on mRNAs, may influence mRNA stability as well as translation efficiency and therefore proteome composition.

Control of histone pre-mRNA processing through alteration of nascent RNA structure

mRNAs encoding replication-dependent core histones (H2A, H2B, H3, and H4) produced during S-phase lack the normal poly(A) tail and instead present a 3' stem-loop (Marzluff & Koreski, 2017). They undergo endonucleolytic cleavage at the 3' side of this stem-loop, and correct folding of this structure is absolutely essential for normal 3' processing of histone mRNAs (Williams *et al*, 1994; Battle & Doudna, 2001; Dominski *et al*, 2003). Slow RNA Pol II mutants reduce histone pre-mRNA processing (Saldi *et al*, 2018), the stem-loop failing to properly fold in transcripts synthesized by these mutants. This is likely due to a competition mechanism between the rate of RNA synthesis and the rate of nascent RNA folding, thus explaining the failure to process 3' ends. These results show that regulation of transcription speed can also control pre-mRNA processing by changing nascent RNA structure.

Control of protein modifications on gene bodies

The CTD of RNA Pol II, which consists of tandem repeats of the heptapeptide YSPTSPS, plays a central role in the coordination of co-transcriptional processing through its association with a large number of enzymes and protein/RNA-binding factors. The CTD is the target of many post-translational modifications, yielding specific patterns (referred to as “the CTD code”), which are recognized by factors playing essential roles throughout the transcription cycle (Harlen & Churchman, 2017). Importantly, these RNA Pol II CTD modifications are dynamic, allowing spatial and temporal regulation of processing factors binding. Eukaryotic transcription units are, for instance, characterized by conserved 5' to 3' profiles of specific pol II CTD phosphorylation events deposited co-transcriptionally. Phosphorylation on Ser5 is normally high at the 5' ends of genes where it facilitates mRNA capping, whereas Ser2 phosphorylation is high at 3' ends where it is important for mRNA 3' end formation (Buratowski, 2009; Heidemann *et al*, 2013; Bentley, 2014). Interestingly, *in vitro* transcription analyses have shown that the RNA Pol II CTD phosphorylation patterns and the factors recognizing them change as a function of post-initiation time rather than distance elongated

(Joo *et al*, 2019). Moreover, slow RNA Pol II mutants shift RNA Pol II Ser2 hyperphosphorylation to the 5' end of genes (Fong *et al*, 2017). These results suggest that the dwell time spent by RNA Pol II within the “target zone” where it associates with a modifier can modulate the deposition or removal of post-translational modifications (Fong *et al*, 2017). Altogether, these studies indicate that CTD phosphorylation is controlled by RNA Pol II speed.

Eukaryotic transcription units are also characterized by conserved 5' to 3' profiles of specific histone modifications deposited co-transcriptionally. These histone modifications profiles are sensitive to RNA Pol II speed. Indeed, fast transcription correlates with distal localization of H3K36me3 (Jonkers *et al*, 2014). Moreover, a fast RNA Pol II mutant shifts H3K4me2/me3 in the 3' direction (Soares *et al*, 2017), while a slow RNA Pol II mutant shifts these marks toward the 5' end of genes (Fong *et al*, 2017; Soares *et al*, 2017). In addition, H2Bub1 levels are highly correlated with RNA Pol II velocity and inhibition of transcription elongation strongly affects global H2Bub1 levels (Fuchs *et al*, 2014a). These results suggest that transcription speed can modulate co-transcriptional chromatin modification patterns.

Control of TSS selection

Most eukaryotic promoters are known to utilize several different TSSs; nevertheless, how the TSSs are chosen remains an open question. Transcripts with different start sites can differ in their stability or translational efficiency (Rojas-Duran & Gilbert, 2012) and give rise to transcriptome variations during development and cell differentiation (Hu *et al*, 2020). In budding yeast, RNA Pol II speed controls the use of differential TSSs, with fast RNA Pol II mutants shifting to upstream TSSs, while slow RNA Pol II mutants shift to downstream TSSs (Kaplan *et al*, 2012; Braberg *et al*, 2013; Qiu *et al*, 2020).

Control of pausing

Following transcription initiation and promoter clearance, RNA Pol II stalls and accumulates at high levels in the promoter-proximal region. Promoter-proximal pausing is a key rate-limiting step of the transcription cycle that RNA Pol II experiences at almost all genes in metazoans (Henriques *et al*, 2013; Jonkers *et al*, 2014), at around 20% of genes in plants (Kindgren *et al*, 2020) and at about 30% of genes in fission yeast (Booth *et al*, 2016). In *Drosophila*, a slow RNA Pol II mutant has been shown to shift pausing, which is stabilized by NELF in this species, closer to the TSS (Li *et al*, 2013), while in plants, a fast RNA Pol II mutant decreases the extent of +1 nucleosome promoter-proximal pausing (Leng *et al*, 2020). As such, RNA Pol II velocity can control both the location relative to the TSS and the extent of promoter-proximal pausing.

Control of gene expression

Finally, RNA Pol II speed has been linked to regulation of gene expression levels in several studies. Indeed, genes with the highest measured RNA Pol II velocity have been shown to generate more mRNAs (Danko *et al*, 2013; Jonkers *et al*, 2014; Cohen *et al*, 2018). Moreover, transcription by a fast RNA Pol II mutant in *A. thaliana* increased production of nascent RNAs (Leng *et al*, 2020), suggesting that transcription speed can, to some extent, affect gene expression levels. However, other studies have found no relationship between RNA Pol II speed and expression levels (Singh & Padgett, 2009;

Fukaya *et al*, 2017). Nevertheless, RNA Pol II velocity has also been suggested to affect the timing of mRNA accumulation and could thus play a role in the timing of some developmental regulatory processes (Thummel *et al*, 1990; Fukaya *et al*, 2017; Maslon *et al*, 2019). Indeed, early stages of embryonic development display great variations in cell-cycle duration with some extremely fast cell divisions (Ciemerych & Sicinski, 2005; Ferree *et al*, 2016). It is thus possible that during these short cell divisions, RNA Pol II speed plays a crucial role in maintaining the required level of some essential mRNAs that need to be expressed at given developmental stages.

Factors controlling RNA Pol II speed

As described above, dynamic regulation of transcription speed within gene bodies is essential for controlling co-transcriptional processes, which determine the composition of the transcriptome. Factors regulating RNA Pol II velocity therefore play an essential role in cells and are the topic of several recent studies using genome-wide RNA Pol II speed measurement methods (Table 2) (Saponaro *et al*, 2014; Liang *et al*, 2018; Baluapuri *et al*, 2019; Cortazar *et al*, 2019; Gregersen *et al*, 2019; Hou *et al*, 2019; Sheridan *et al*, 2019; Fan *et al*, 2020). RNA Pol II speed can be controlled by different factors including, but not restricted to, DNA sequence, gene structure, histone modifications, chromatin remodelers, and RNA Pol II-associated factors and modifications.

DNA sequence and nascent RNA folding

During transcription elongation, RNA Pol II encounters roadblocks that can cause it to enter a paused state and backtrack, sliding backwards on the DNA template. Backtracking thus causes a misalignment of the nascent RNA 3' end with its active site, which needs to be resolved before transcription can restart. RNA Pol II pausing and backtracking lead to an overall decrease in RNA Pol II average speed. The DNA sequence in the transcription bubble and the secondary structure adopted by the nascent RNA can influence RNA Pol II speed through controlling pausing and backtracking. Indeed, *in vitro* transcription assays showed that RNA Pol II pauses are shorter and less frequent on GC-rich templates. This is probably due to co-transcriptional folding of nascent RNA which, at GC-rich sequences, may impose an energy barrier to pausing by impeding backtracking along the DNA (Zamft *et al*, 2012). Along the same line, *in vivo* experiments in budding yeast showed that fast transcription elongation by RNA Pol II is associated with strong nascent RNA structures which would prevent RNA Pol II from backtracking thus pushing it forward (Turowski *et al*, 2020). Short DNA subsequences have also been shown to influence the frequency and duration of pauses in budding yeast. However, here, no correlation has been found between the GC content of these short sequences and RNA Pol II speed (Cohen *et al*, 2018). Moreover, the GC content within gene bodies has been shown to correlate negatively with RNA Pol II speed (Jonkers *et al*, 2014; Veloso *et al*, 2014). As GC rich templates have been shown to increase RNA Pol II speed *in vitro* (Zamft *et al*, 2012), the role played by the GC content in RNA Pol II speed *in cellulo* might be more complex than anticipated. For example, while high GC content in the transcription bubble slows down RNA polymerase I transcription, strong nascent RNA

structures formed close to the polymerase promote forward movement (Turowski *et al*, 2020). Finally, in budding yeast, RNA Pol II pauses were found to correlate with the stability of the RNA:DNA hybrid, rather than the stability of the DNA:DNA duplex (Lukačičin *et al*, 2017).

Gene structure

Interestingly, the gene structure itself, and in particular its intron and exon composition, can also play a role in regulating RNA Pol II velocity. Indeed, a positive correlation has been identified between intron 1 size and RNA Pol II speed (Jonkers *et al*, 2014) and the presence of introns increases transcription speed (Fukaya *et al*, 2017). Conversely, exon density correlates negatively with RNA Pol II velocity (Jonkers *et al*, 2014; Veloso *et al*, 2014), which may be the result of several contributing factors.

First, increased nucleosome occupancy occurs on exons with respect to introns (Andersson *et al*, 2009; Nahkuri *et al*, 2009; Schwartz *et al*, 2009; Spies *et al*, 2009; Tilgner *et al*, 2009; Chodavarapu *et al*, 2010). Nucleosomes can induce RNA Pol II pausing *in vitro* and *in vivo* and may even be the major source of pausing (Churchman & Weissman, 2011; Bintu *et al*, 2012). Consistent with the idea that nucleosomes represent a transcriptional barrier, histone depletion from human cells increases transcription speed (Jimeno-González *et al*, 2015). Although no general correlation has been found between nucleosome occupancy and RNA Pol II speed (Jonkers *et al*, 2014), nucleosomes bound within exons could nonetheless slow down RNA Pol II transcription.

Second, pre-mRNA splicing may be involved in the reduction of RNA Pol II velocity over exons, which could, in turn, affect co-transcriptional splicing. Indeed, RNA Pol II ChIP on chip and ChIP-seq data showed that RNA Pol II accumulates on exons in yeast and human, suggesting that RNA Pol II pauses over exons (Brodsky *et al*, 2005; Schwartz *et al*, 2009; Alexander *et al*, 2010; Carrillo Oesterreich *et al*, 2010; Kwak *et al*, 2013; Mayer *et al*, 2015; Nojima *et al*, 2015; Harlen *et al*, 2016). Furthermore, in budding yeast, RNA Pol II ChIP qPCR allowed observation of RNA Pol II pausing at the 3' splice site (Alexander *et al*, 2010). This transcriptional pausing over the 3' splice site is splicing-dependent, since it was lost upon introduction of a mutation in the 5' or 3' splice sites (Alexander *et al*, 2010; Chathoth *et al*, 2014). A mutation in the branch site of the intron also inhibited this RNA Pol II pausing, which was restored by introducing a complementary mutation in the U2 snRNA (Alexander *et al*, 2010). Finally, this pausing is enforced by ubiquitylation of the catalytic subunit of RNA Pol II (Milligan *et al*, 2017).

More recently, PRO-seq (precision run-on sequencing) and NET-seq (native elongating transcript sequencing), methods offering global and strand-specific mapping of RNA Pol II density at nucleotide resolution, were used to investigate RNA Pol II pausing throughout genes. These methods confirmed the existence of a pause at different positions over exons and intron-exon boundaries in several different species. These methods also confirmed the existence of a pause at the 3' splice site in budding yeast (Harlen *et al*, 2016) and *Drosophila* (Kwak *et al*, 2013), at the 3' and 5' splice sites in human cells (Mayer *et al*, 2015; Nojima *et al*, 2015) and at the 5' splice site in plants (Kindgren *et al*, 2020). Moreover, higher RNA Pol II accumulation has been reported at spliced exons, as compared to skipped exons (Kwak *et al*, 2013; Mayer *et al*, 2015; Nojima *et al*, 2015). All these studies show that exons and intron-exon

Table 2. List of factors controlling transcription speed

| Factor | Study | Effect on RNA pol II speed | Organism | Number of genes analyzed and measurement method |
|---|-------------------------------|--|------------------------------|--|
| RNA pol II-associated factors and modifications | | | | |
| Ccr4-Not | Kruk <i>et al</i> (2011) | Increases | Yeast | Measured on 1 gene (GAL1-YLR454W) by RNA pol II ChIP qPCR at +2 Kb into the gene body at several time points after transcription inhibition by switching from galactose to dextrose containing growth medium |
| CDK12 and CDK13 | Fan <i>et al</i> (2020) | Increases | Human | Measured on 368 genes longer than 100 Kb by PRO-seq at several time points following DRB release |
| DBIRD | Close <i>et al</i> (2012) | Increases locally at AT-rich exon-intron junctions | Human | Measured on 2 genes by RNA pol II ChIP qPCR and nascent RNA analysis at several locations into the gene |
| MYC | Liang <i>et al</i> (2018) | Increases | Human | Measured on 1,021 genes by 4sU-seq 15 min after flavopiridol release |
| | Baluapuri <i>et al</i> (2019) | Increases | Human | Measured on 2,163 genes by 4sU-seq 10 min after DRB release |
| | Caggiano <i>et al</i> (2019) | Increases | Human | Measured on 1 gene (Sam68) by RT-qPCR with primers designed in two regions of the gene and by calculating the abundance of distal versus proximal pre-mRNA 20 min following DRB release |
| Paf1C | Hou <i>et al</i> (2019) | Increases | Mouse | Measured on 1,708 genes longer than 70 Kb by PRO-seq at several time points following DRB treatment |
| | Mason and Struhl (2005) | No effect | Yeast | Measured on 1 gene (GAL1-YLR454W) by RNA pol II ChIP PCR at several locations into the gene 4 min after transcription inhibition by switching from galactose to glucose containing growth medium |
| Rtf1 subunit | Vos <i>et al</i> (2020) | Increases | <i>Sus scrofa</i> RNA pol II | Measured <i>in vitro</i> using reconstituted elongation complexes |
| PNUTS-PP1 | Cortazar <i>et al</i> (2019) | Decreases | Human | Measured on 4,324 non-overlapping genes of more than 60 Kb by RNA pol II ChIP-seq at several time points following DRB release |
| RECQL5 | Saponaro <i>et al</i> (2014) | Decreases | Human | Measured on 237 long genes by GRO-seq at several time points following DRB release |
| SCAF8 | Gregersen <i>et al</i> (2019) | Increases | Human | Measured on 4,869 genes between 60 and 300 Kb by DRB/TT _{chem} -seq at several time points following DRB release |
| Super elongation complex | Liang <i>et al</i> (2018) | Increases | Human | Measured on 982 genes by 4sU-seq 15 min after flavopiridol release |
| Spt5 | Quan and Hartzog (2010) | Increases | Yeast | Measured on 1 gene (GAL1-YLR454W) by RNA pol II ChIP qPCR at several locations into the gene 5 min after transcription inhibition by switching from galactose to glucose containing growth medium |
| | Fitz <i>et al</i> (2018) | No effect | Mouse | Measured on 8 long and 2 short genes by RT-qPCR on exon-intron junctions at several time points following flavopiridol release |
| Spt6 | Ardehali <i>et al</i> (2009) | Increases | <i>Drosophila</i> | Measured on 1 gene (Hsp70) by RNA pol II ChIP qPCR at several locations into the gene and at several time points following activation by heat shock |
| Sub1 | Garcia <i>et al</i> (2012) | Increases | Yeast | Measured on 1 gene (GAL1-YLR454W) by RNA pol II ChIP PCR at several locations into the gene 4 min after transcription inhibition by switching from galactose to glucose containing growth medium |
| TFIIS | Mason and Struhl (2005) | No effect | Yeast | Measured on 1 gene (GAL1-YLR454W) by RNA pol II ChIP PCR at several locations into the gene 4 min after transcription inhibition by switching from galactose to glucose containing growth medium |
| | Sheridan <i>et al</i> (2019) | Increases | Human | Measured on 600–1,200 genes of more than 75 Kb by RNA pol II ChIP-seq or Bru-seq at several time points following DRB release |
| | Zatreanu <i>et al</i> (2019) | Increases | Human | Measured on 1 long gene by RT-qPCR at several locations on the nascent RNA (labeled with 4SU) 40 min following DRB release |
| Factors affecting the chromatin landscape | | | | |
| Brm (Swi-Snf subunit) | Batsché <i>et al</i> (2006) | Decreases locally at alternative exon junctions | Human | Measured on 1 gene (CD44) by RNA pol II ChIP qPCR at several locations into the gene |

Table 2 (continued)

| Factor | Study | Effect on RNA pol II speed | Organism | Number of genes analyzed and measurement method |
|----------------------|---------------------------------|----------------------------|----------|---|
| H3K9me2 and H3K27me3 | Schor <i>et al</i> (2013) | Decreases | Mouse | Measured on 1 gene (NCAM) by RT-qPCR at several locations into the gene and at several time points following DRB release |
| Histone acetylation | Schor <i>et al</i> (2009) | Increases | Mouse | Measured on 1 gene (NCAM) by RT-qPCR with primers designed in different introns and by calculating the abundance of distal versus proximal introns in a given region |
| | Sharma <i>et al</i> (2014) | Increases | Mouse | Measured on 3 genes by RT-qPCR on the nascent RNA (labeled with BrU) with primers located in different exons spanning the entire genes at several time points following DRB release |
| HP1 proteins | Saint-André <i>et al</i> (2011) | Decreases | Human | Measured on 1 gene (CD44) by RNA pol II ChIP qPCR at several locations into the gene |
| | Allo <i>et al</i> (2009) | Decreases | Human | Measured on 1 reporter gene by RT-qPCR with primers designed in different regions of the gene and by calculating the abundance of distal versus proximal pre-mRNA in a given region |

boundaries are regions of RNA Pol II transcription slow-down and this slow-down seems to be induced by the splicing process itself and could, in turn, affect co-transcriptional splicing.

RNA Pol II-associated factors and modifications

Many transcription factors play a role in transcription elongation; however, few of them have been shown to directly influence the speed of transcription by RNA Pol II (Table 2). Backtracked RNA Pol II can be rescued by the elongation factor TFIIS which stimulates RNA Pol II intrinsic cleavage activity (Cheung & Cramer, 2011). RNA Pol II cleaves the nascent RNA 3' end, releasing an RNA fragment of 2–14 bases long (Izban & Luse, 1993) and producing a new 3' end perfectly aligned with its active site. RNA Pol II can then resume transcription. TFIIS thus reduces the duration of pausing and has been shown to accelerate RNA Pol II transcription *in vitro* (Ishibashi *et al*, 2014; Schweikhard *et al*, 2014). Accordingly, TFIIS increases RNA Pol II speed in human cells (Sheridan *et al*, 2019; Zatreanu *et al*, 2019), while its deletion had no effect on RNA Pol II velocity in budding yeast (Mason & Struhl, 2005). Another factor which can accelerate transcription through reactivation of backtracked RNA Pol II is the Ccr4-Not complex (Kruk *et al*, 2011), which is considered a master regulator of eukaryotic gene expression. Reactivation of backtracked RNA Pol II by the Ccr4-Not complex is different from reactivation by TFIIS. *In vitro* transcription assays showed that in contrast to TFIIS, this complex is unable to reactivate RNA Pol II which has backtracked after incorporation of the chain terminator O-me-GTP, indicating that its activity does not involve stimulation of the intrinsic RNA Pol II cleavage activity (Kruk *et al*, 2011). The Ccr4-Not complex is instead thought to push RNA Pol II forward through binding to the nascent RNA until realignment of the 3' end of the nascent transcript in the active site (Kruk *et al*, 2011). Finally, interplays between these two complexes have been described: While Ccr4-Not can rescue backtracked RNA Pol II in the absence of TFIIS, further *in vitro* characterization showed that Ccr4-Not also interacts with TFIIS and recruits it to the arrested elongation complex resulting in enhanced transcript cleavage (Dutta *et al*, 2015).

Besides factors stimulating the progression of backtracked RNA Pol II, other general transcription factors are involved in controlling transcription speed by mechanisms that are for the most unclear.

These factors, which we introduce in the following section, are mostly proteins or complexes associated with RNA Pol II which are also involved in other transcription steps. Sub1, which is a functional component of the pre-initiation complex playing a role in TSS selection is also involved in transcription elongation and has been shown to increase RNA Pol II velocity in budding yeast (García *et al*, 2012). Another factor reported to increase RNA Pol II speed is the transcription elongation factor Spt6, which physically interacts with histone H3 (Bortvin & Winston, 1996; Winkler *et al*, 2000) and can facilitate nucleosome reassembly (Adkins & Tyler, 2006). Spt6 binds to the phosphorylated RNA Pol II CTD and facilitates RNA Pol II promoter-proximal pause release (Vos *et al*, 2018a). In *Drosophila*, Spt6 has also been shown to increase RNA Pol II speed (Ardehali *et al*, 2009).

The super elongation complex (SEC) is a large protein complex with compositional and functional diversity and is one of the most active P-TEFb containing complexes (Luo *et al*, 2012). In addition to its role in promoter-proximal pause release and elongation processivity, the SEC facilitates RNA Pol II transcription, as disruption of this complex slows down RNA Pol II (Liang *et al*, 2018). RNA Pol II CTD heptapeptide residues Tyr1, Ser2, Thr4, Ser5, and Ser7 are dynamically phosphorylated and dephosphorylated by different CTD kinases and phosphatases throughout the transcription cycle (Harlen & Churchman, 2017). CDK12 and CDK13 are multitask RNA Pol II CTD kinases playing important roles for proper gene expression and genome stability (Greenleaf, 2019). Dual inhibition of both kinases significantly decreases RNA Pol II CTD Ser2 and Thr4 phosphorylation, as well as RNA Pol II processivity and speed (Fan *et al*, 2020).

Spt5 (suppressor of Ty 5) is a highly conserved RNA Pol II-associated factor, which, in eukaryotes, is part of the DSIF complex together with Spt4. In budding yeast, Spt5 increases transcription speed (Quan & Hartzog, 2010). However, in mouse embryonic fibroblasts it controls RNA Pol II processivity, but not RNA Pol II velocity (Fitz *et al*, 2018). Nevertheless, consistent with a role of Spt5 in the regulation of RNA Pol II speed, the PP1 phosphatase together with its nuclear targeting subunit (PNUTS) can dephosphorylate Spt5, which slows down RNA Pol II transcription in human cells (Cortazar *et al*, 2019). Moreover, the transcription factor MYC, which directly binds to Spt5 and recruits it to promoters, allowing

Spt5 transfer onto RNA Pol II (Baluapuri *et al*, 2019), has been shown to be required for fast transcription elongation (Liang *et al*, 2018; Baluapuri *et al*, 2019; Caggiano *et al*, 2019).

Furthermore, involved in regulating RNA Pol II speed is the conserved polymerase-associated factor 1 complex (Paf1C), which plays multiple roles in chromatin transcription and genomic regulation (Van Oss *et al*, 2017). Paf1C specifically increases RNA Pol II velocity within the first part of gene bodies in mice (Hou *et al*, 2019), although no effect of the loss of this factor could be measured in budding yeast (Mason & Struhl, 2005). On the other hand, consistent with a role of the Paf1C complex in RNA Pol II speed regulation, recent data show that the dissociable Paf1C subunit Rtf1 increases RNA Pol II speed in *in vitro* transcription assays (Vos *et al*, 2020).

Two more components affecting RNA Pol II speed are SCAF8 and the DBIRD complex. SCAF8, an RNA Pol II CTD-interacting protein, which plays a role in suppressing the use of proximal poly(A) sites, is a positive RNA Pol II elongation factor that increases RNA Pol II speed (Gregersen *et al*, 2019). Depletion of DBIRD, a protein complex formed by DBC1 and ZNF326, induces an accumulation of RNA Pol II on A/T-rich alternatively spliced internal exons in human cells, suggesting a role for DBIRD in the control of RNA Pol II speed over exons (Close *et al*, 2012).

Finally, RECQL5, a member of the conserved family of RECQ DNA helicases, decreases RNA Pol II speed and suppresses genome instability (Saponaro *et al*, 2014). The hypothesis behind these two observations is that decreasing RNA Pol II speed smoothens transcription elongation and thus renders RNA Pol II less prone to pausing and arrest which can induce transcription stress (Saponaro *et al*, 2014).

As exemplified above, some factors such as Paf1C, TFIIS, or Spt5 have been reported to be important for transcription speed in certain species, but not in others. This may represent a real biological difference between species or could be due to the fact that these factors are involved only for some genes or under particular conditions. However, this difference could also be caused by technical issues: In yeast, genes are quite short (around 1.5–2 kb long on average, although the gene YLR454W, used for most of the experiments measuring RNA Pol II speed in budding yeast, is exceptionally large with a size of around 8 kb (Table 2) (Mason & Struhl, 2005; Quan & Hartzog, 2010; Kruk *et al*, 2011; García *et al*, 2012)). It might thus be difficult to assess the effect of a factor on transcription speed because the methods used to measure RNA Pol II speed offer poor resolution on short genes. Indeed, in mammalian cells most studies only consider very long genes to accurately decipher an elongation speed (Saponaro *et al*, 2014; Cortazar *et al*, 2019; Gregersen *et al*, 2019; Hou *et al*, 2019; Sheridan *et al*, 2019; Fan *et al*, 2020).

Chromatin landscape

Chromatin modifications can also regulate RNA Pol II velocity by facilitating or hindering RNA Pol II progression through nucleosomes (Table 2). Genome-wide studies have shown that H3K79me2, H2B mono-ubiquitylation, and H4K20me1 positively correlate with RNA Pol II speed in the gene body (Fuchs *et al*, 2014a; Jonkers *et al*, 2014; Veloso *et al*, 2014). Besides these global correlations, other histone modifications locally influence RNA Pol II velocity: modifications triggering chromatin relaxation increase RNA Pol II speed, while modifications triggering chromatin compaction slow down RNA Pol II. For instance, a reduction in the level of

class II HDACs on specific genes and increased H3 and H4 acetylation correlate with faster RNA Pol II transcription on these genes (Sharma *et al*, 2014). Similarly, H3K9 hyperacetylation correlates with a change in the ratio of distal versus proximal pre-mRNA levels suggesting a local increase in RNA Pol II speed (Schor *et al*, 2009), whereas an increase in H3K9 di-methylation and H3K27 trimethylation correlates with a slow-down of RNA Pol II transcription (Schor *et al*, 2013).

The evolutionary conserved family of non-histone chromosomal proteins HP1 (heterochromatin protein 1) consists of three isoforms in human. HP1 proteins bind to di- and trimethylated H3K9, as well as to other proteins involved in various epigenetic functions, such as histone methyltransferases (HMTs) (Zeng *et al*, 2010). HP1 α and HP1 β isoforms are mainly heterochromatic, while HP1 γ is present in both heterochromatin and euchromatin (Zeng *et al*, 2010). Through RNA Pol II ChIP measurements, HP1 γ has been suggested to locally increase RNA Pol II pausing on alternatively spliced exons enriched in H3K9me3 marks (Saint-André *et al*, 2011). The mechanism is thought to involve the RNAi machinery (Saint-André *et al*, 2011; Ameyar-Zazoua *et al*, 2012). Similarly, HP1 α recruitment to local H3K9me2 enrichment induced by siRNA targeting correlates with a change in the ratio of distal versus proximal pre-mRNA levels suggesting a decreased local RNA Pol II speed (Alló *et al*, 2009).

ATP-dependent chromatin remodeling complexes regulate the access of different factors to genomic DNA during transcription, replication, and repair by altering the contacts between histones and DNA within nucleosomes. The SWI/SNF family of ATP-dependent chromatin remodeling complexes controls the expression of many genes by sliding or evicting nucleosomes positioned on binding sites for transcription activators or repressors (Clapier *et al*, 2017). SWI/SNF complexes contain more than 10 protein subunits, including a single ATPase, either SMARCA4 (BRG1) or SMARCA2 (BRM), that utilizes the energy of ATP hydrolysis to alter nucleosome positioning (Euskirchen *et al*, 2012). The SWI/SNF complex subunit BRM has been suggested to locally decrease RNA Pol II speed in response to activation of the MAP kinase pathway. Indeed, BRM knockdown has been shown to decrease RNA Pol II accumulation on alternatively spliced exons as measured by RNA Pol II ChIP (Batsché *et al*, 2006).

Adapting RNA Pol II speed to the cellular environment

Over the last 20 years, the use of kinetic RNA Pol II mutants has shown that RNA Pol II speed regulation plays an essential role in many co-transcriptional processes thus controlling the composition of the transcriptome. The use of such mutants has also shown that RNA Pol II speed regulation is essential for development (Maslon *et al*, 2019; Leng *et al*, 2020). However, RNA Pol II mutants globally affect RNA Pol II velocity and thus may have side effects on the expression of transcription factors involved in these co-transcriptional events or in physiological processes such as development. These studies thus left the question of the physiological relevance of transcription speed regulation open. Recent studies have however given a hint of an answer to this question by revealing that RNA Pol II velocity can be adapted to respond to intra- and extra-cellular stimuli (Fig 3). First, RNA Pol II velocity differs by as much as 25–40% on individual genes stimulated by diverse signaling pathways in different cell lines

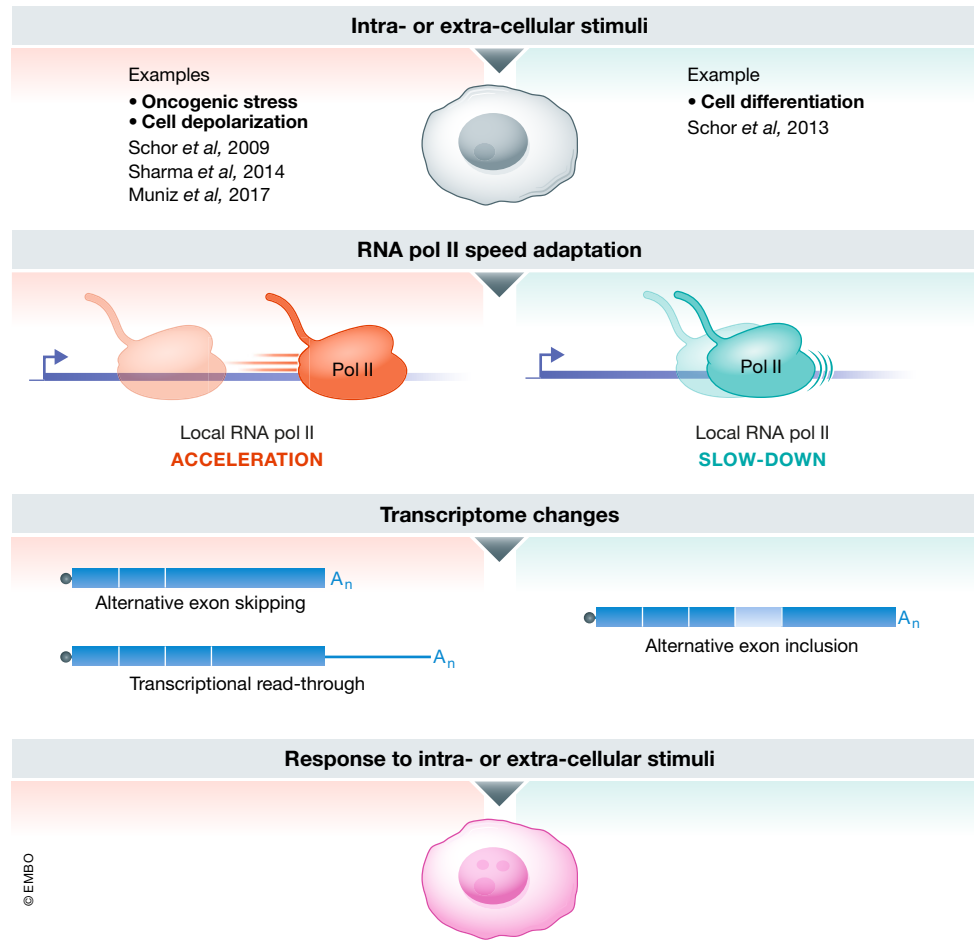


Figure 3. RNA Pol II speed is regulated in order to adapt the transcriptome composition in response to intra- or extra-cellular stimuli.

In response to intra- or extra-cellular stimuli such as oncogenic stress, cell depolarization, or cell differentiation, RNA Pol II can either accelerate or slow down locally, inducing a change in alternative splicing or the extent of read-through which could play a role in the response to stimuli.

(Danko *et al*, 2013). Second, RNA Pol II velocity changes around alternatively spliced exons in specific genes, in response to cell depolarization and cellular differentiation (Schor *et al*, 2009; Schor *et al*, 2013; Sharma *et al*, 2014). Along the same lines, RNA Pol II accumulates on alternatively spliced exons of specific genes in response to activation of the protein kinase C or MAP kinase pathways, suggesting a decrease in RNA Pol II speed (Batsché *et al*, 2006; Saint-André *et al*, 2011; Ameyar-Zazoua *et al*, 2012). Third, transcription speed increases downstream of some protein-coding genes in response to an oncogenic signal (Muniz *et al*, 2017). Finally, UV-induced DNA damage has been proposed to induce a reduction in RNA Pol II speed (Muñoz *et al*, 2009; Lavigne *et al*, 2017; Williamson *et al*, 2017). Although this last result can be explained by transcription-blocking DNA lesions accumulating along gene bodies, it remains plausible that DNA damage signaling also directly triggers RNA Pol II transcription slow-down.

These variations in transcription speed in response to changes in the cellular environment may have important consequences on transcriptome composition. Indeed, changes in RNA Pol II velocity measured in response to cell depolarization and cell differentiation

regulate alternative splicing and thus RNA identity (Schor *et al*, 2009; Schor *et al*, 2013; Sharma *et al*, 2014). Moreover, we have shown that the increase in RNA Pol II velocity downstream of genes is accompanied by an increase in transcriptional read-through which interferes with and thus decreases the expression of downstream convergent protein-coding genes (Muniz *et al*, 2017). Increased transcriptional read-through is known to interfere with the expression of downstream genes also in other contexts such as cancer or HSV-1 infection in human cells (Grosso *et al*, 2015; Rutkowski *et al*, 2015; Hennig *et al*, 2018), as well as cold acclimation in plants (Kindgren *et al*, 2018). Transcriptional read-through can also give rise to a chimeric transcript which can be spliced and translated into an aberrant fusion protein, if it invades the downstream gene in a pair of tandem genes (Grosso *et al*, 2015; Hennig *et al*, 2018). Therefore, by controlling the extent of transcriptional read-through, RNA Pol II speed can have dramatic consequences on the expression of surrounding genes.

Importantly, transcriptome modifications induced by RNA Pol II speed changes can be extremely fast, since they do not require new transcription initiation events. Furthermore, these transcriptome

modifications could participate in the response to intra- or extra-cellular stimuli. Indeed, we have shown that those convergent genes, antisense to the transcriptional read-through induced in response to oncogene-induced senescence, are enriched in pro-proliferative genes (Muniz *et al*, 2017). Thus, by repressing those genes, the transcriptional read-through may participate in the control of the genetic program of senescence. Moreover, transcriptional read-through is induced downstream of thousands of genes in response to different kinds of stresses (Rutkowski *et al*, 2015; Vilborg *et al*, 2015; Vilborg *et al*, 2017; Cardiello *et al*, 2018; Hennig *et al*, 2018) and has been proposed to play an important role in the stress response by reinforcing the nuclear scaffold integrity after nuclear stress (Vilborg *et al*, 2015). Finally, read-through transcription can also affect the genome 3D structure: In read-through regions, elongating RNA Pol II displaces cohesin from CTCF sites thus eliminating chromatin loops and locally decompacting chromatin (Heinz *et al*, 2018). Therefore, by controlling the extent of transcriptional read-through, RNA Pol II speed could affect both gene expression and genome 3D organization. More generally speaking, regulation of RNA Pol II speed might thus be important for the cell response to intra- and extra-cellular stimuli by affecting the whole transcriptome, from splicing-dependent processes to gene expression and chromatin organization.

Conclusion and perspectives

More and more studies point to the importance of RNA Pol II speed in determining the identity of RNAs produced from a specific locus. Indeed, changes in transcription speed induce changes in the splicing repertoire, with modifications of alternative splicing and of the production of circular RNAs by back-splicing (see section on co-transcriptional processes regulated by RNA Pol II speed). These changes also affect transcription termination-related processes such as poly(A) site usage and the extent of transcriptional read-through. In summary, changes in RNA Pol II speed modify the whole spectrum of coding and non-coding RNAs produced from the genome. By changing the spectrum of RNAs produced by transcribed genes, RNA Pol II speed can thus provide another layer of complexity in genome expression control.

An important issue is to which extent this speed can be regulated upon changes in the cellular environment. As exemplified above, a number of factors are known to participate in controlling transcription speed. Some of them are general transcription factors probably affecting all transcribing RNA polymerases. Thus, any modification of the function of these factors presumably affects RNA Pol II velocity genome wide, leading to major changes in the repertoire of coding and non-coding RNAs. However, some features of the chromatin landscape, such as histone modifications or chromatin bound factors, can locally control RNA Pol II speed. Chromatin is a highly dynamic structure, which is known to harbor local changes depending on the cellular environment. Such local changes within a transcribed region are thus susceptible to lead to local changes in RNA Pol II velocity and thus to modifications in the identity of the RNAs produced by this locus. We recently exemplified such a local modification of chromatin and RNA Pol II speed in cells undergoing oncogene-induced senescence downstream of two genes, leading to extended transcriptional read-through (Muniz *et al*, 2017). Along

the same lines, local changes of histone modifications such as acetylation or methylation and RNA Pol II speed occur on alternative spliced exons during cell response to depolarization and differentiation (Schor *et al*, 2009; Schor *et al*, 2013; Sharma *et al*, 2014). Whether such observations can be generalized to various environmental changes is clearly an open issue which merits further investigation. Deciphering the mechanisms underlying such changes, as well as the signaling pathways involved, will also clearly be a major step toward our understanding of the genome response to environmental changes. Similarly, the local regulation of RNA Pol II velocity upon activation of specific signaling pathways may be essential during the development of multicellular organisms and therefore represents a challenging line of research in the field of developmental biology.

It would also be important to investigate whether deficient regulations of RNA Pol II speed participate in disease progression, again through the resulting consequences on local or global genome expression. Cancer cells have probably exploited such mechanisms given the variety of circular and read-through RNAs whose expression is affected during cancer progression (Grosso *et al*, 2015; Smid *et al*, 2019). Most importantly, recent results point to a causative role of circular RNAs on cancer progression (Chen *et al*, 2019). Investigating the developmental-programmed and cancer-induced modifications in RNA Pol II velocity and the complexity of the RNAs affected thus clearly opens a new avenue of research in the fields of physiology and physiopathology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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