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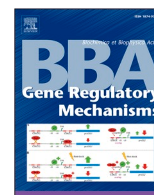

Global control of RNA polymerase II ☆

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RNA polymerase II (Pol II) is the multi-protein complex responsible for transcribing all protein-coding messenger RNA (mRNA). Most research on gene regulation is focused on the mechanisms controlling *which genes* are transcribed *when*, or on the mechanics of transcription. How global Pol II activity is determined receives comparatively less attention. Here, we follow the life of a Pol II molecule from 'assembly of the complex' to nuclear import, enzymatic activity, and degradation. We focus on how Pol II spends its time in the nucleus, and on the two-way relationship between Pol II abundance and activity in the context of homeostasis and global transcriptional changes.

1. Introduction

RNA polymerases are highly conserved enzymes responsible for transcribing RNA from DNA. In eukaryotes, RNA polymerase II (Pol II) is the enzyme responsible for synthesizing messenger RNAs (mRNA), small nucleolar RNAs and long noncoding RNAs. This large, multi-subunit protein complex is assembled in the cytoplasm and imported into the nucleus to carry out its function. Given the essential nature of Pol II for cellular life, many years of research have uncovered the series of complex systems which govern its synthesis, transport, regulated function, and degradation.

mRNA abundance is a primary determinant of the cellular abundance of a protein [1], making the production and degradation of mRNA a critical parameter in the control of cell phenotypes and in cellular homeostasis. Specific mRNAs must be produced for the cell to respond to stimuli, and this must be done within a context where global mRNA concentrations are maintained. Recent work, including our own, has revealed that global Pol II levels are variable between cell states, and respond dynamically to perturbations [2,3]. Therefore, changes in global Pol II abundance should be considered possible, even likely, in contexts where global RNA synthesis rates change.

As a question of the global regulation of a critical enzyme, several important aspects of Pol II function remain unknown. For instance, how is the total level of Pol II controlled? What is the lifetime of a Pol II complex in the cell? Does Pol II abundance determine its global activity or vice versa?

Here, we summarise our current knowledge by following a Pol II molecule through its life cycle – highlighting quantitative studies wherever possible. We then discuss examples of global changes in Pol II activity and abundance that give us insights into its regulation, and demonstrate the importance of its cellular control in diverse biological processes.

2. Building and maintaining a multi-subunit polymerase

Pol II contains 12 subunits, which are named sequentially by size – in humans, POLR2A through POLR2L (Table 1) [4]. The cellular abundances of Pol II subunits are proportional to cell size [3,5]. Estimates of the absolute Pol II numbers per cell range from 30,000 in yeast (volume ≈ 50 fL) [6], 12,500–25,000 (per genome) in *Drosophila* embryos [7], and 12,000–60,000 in human cells (volume ≈ 1–3 pL) [8–10]. In human cells, Pol II abundance is approximately 5 times higher than either RNA Polymerase I or III [10].

A general model of the biogenesis of Pol II via two distinct subassemblies has been proposed [4]. In human cells, the largest subunit, POLR2A associates initially with a subset of other subunits and HSP90 via cofactors, while POLR2B assembles separately together with POLR2C, and GPN1/GPN2/GPN3 [11]. GPN1 and GPN3, small GTPases, have been separately observed to associate with Pol II, with overexpression of dominant negative GPN1 preventing nuclear transport of POLR2A [12]. The pathway appears similar in yeast, with POLR2A and POLR2B subassemblies being brought together via the actions of Rtr1

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Table 1
Nomenclature of Pol II subunits.

Human	Mouse	S cerevisiae	Notes
POLR2A	RPB1	Rpb1/ RPO21	Largest subunit. Contains multiple repeats of conserved YSPTSPS heptad – the site of well characterised post-translational modifications.
POLR2B	RPB2	Rpb2	
POLR2C	RPB3	Rpb3	
POLR2D	RPB4	Rpb4	POLR2D/POLR2G subcomplex (maintained at substoichiometric levels [10,120]). Not essential for viability in *S cerevisiae*.
POLR2E	RPB5	Rpb5	Shared with Pol I and Pol III. Part of Rpb1 subassemby.
POLR2F	RPB6	Rpb6/ RPO26	Shared with Pol I and Pol III. Part of Rpb1 subassemby.
POLR2G	RPB7	Rpb7	POLR2D/POLR2G subcomplex (maintained at substoichiometric levels [10,120]).
POLR2H	RPB8	Rpb8	Shared with Pol I and Pol III. Part of Rpb1 subassemby.
POLR2I	RPB9	Rpb9	Not essential for viability in *S cerevisiae*.
POLR2J	RPB10	Rpb10	Shared with Pol I and Pol III.
POLR2K	RPB11	Rpb11	
POLR2L	RPB12	Rpb12	Shared with Pol I and Pol III.

and Gpn3 [13,14].

No individual subunit of Pol II contains a nuclear localisation sequence and knockdown of individual subunits can lead to cytoplasmic accumulation of other subunits in both human cells and in *S cerevisiae* [11,15], indicating that the assembled core of the Pol II complex is required for correct nuclear localisation. In *S cerevisiae*, Iwr1 directs the import of the assembled Pol II complex, by serving as an adapter to importins [16], however functional conservation in humans remains untested. Moreover, the roles played by many identified accessory subunits in this process remain to be determined [17].

How the abundances of Pol II subunits affect one another was recently addressed in mouse embryonic stem cells (mESCs) by individually tagging each of the twelve subunits with a degron tag [18]. In each case, this allowed inducible depletion of a single Pol II subunit over 1-3 h, while the remaining subunits were monitored by immunoblot and chromatin immunoprecipitation (ChIP). This revealed a complex interplay between subunits: depletion of RPB1, RPB2, RPB5, RPB6, RPB7, and RPB8 led to loss of most other subunits, while depletion of RPB4 and RPB12 did not. Interestingly, the timescales of loss of the other subunits were generally longer (~12 h half-life) compared to the forced removal of the degron-tagged subunit, indicating that individual Pol II subunits are somewhat stable in the absence of the full complex.

Whether individual Pol II subunits have functions outside of the Pol II complex has not been extensively studied, and is an emerging area of interest (reviewed in [19]). Intriguingly, the aforementioned degron study in mESCs found that different Pol II subunits do not occupy the genome uniformly and contribute differently to the transcription process [18]. Moreover, the sub-stoichiometric yeast Rpb4/Rpb7 'subcomplex' associates with the other subunits less stably and has been variously proposed to function in transcription, export, translation, or decay of mRNA (reviewed in [20]).

3. How does a Pol II molecule spend its time?

3.1. Chromatin-binding and phosphorylation of Pol II

Once assembled and localised to the nucleus, the simplest question is what fraction of Pol II becomes active in transcribing RNA, and what fraction is inactive? Pol II must be bound to DNA to carry out its transcriptional function - but what additional steps exist that regulate Pol II transcriptional activity?

Two distinct forms of Pol II can be separated by gel electrophoresis: hypophosphorylated (IIA) and hyperphosphorylated (IIO). Hyperphosphorylation occurs within the YSPTSPS-heptad repeats of the C-terminal domain (CTD) of POLR2A – a sequence feature conserved across domains of life [21]. Transcribing Pol II is of the hyperphosphorylated, IIO form [22]. Biochemical fractionation of chromatin and nucleoplasm indicates that the majority of Pol II in human HeLa cells is bound to chromatin, with ~40 % hypophosphorylated in the free fraction, and the remaining 60 % bound to chromatin in a mix of hyperphosphorylated and hypophosphorylated forms [23]. So, while phosphorylation implies DNA-binding in unperturbed cells, the converse is not necessarily true.

In live cells, the fraction of Pol II bound to chromatin has been examined by fluorescence recovery after photobleaching (FRAP). Early experiments using FRAP of overexpressed GFP-tagged POLR2A in Chinese hamster ovary (CHO) cells observed two major Pol II populations (bound and unbound) but only 20–25 % of Pol II were bound [24,25] (Table 2). In human U2OS cells, using a swap-over system in which tagged POLR2A was overexpressed while the endogenous protein was degraded, FRAP indicated that 40 % of Pol II is chromatin-bound [26]. However, these studies have the confound that total level of Pol II may differ from the wild-type condition. Indeed, using a human MRC5 cell line in which all endogenous POLR2A is GFP-tagged, it was estimated via FRAP that a higher fraction, 60 %, of Pol II was stably chromatin-bound [8], which agrees with experiments in *S cerevisiae* where tagged Pol II was expressed from the endogenous locus [27]. More recently, single particle tracking (SPT) has been used to observe the diffusion dynamics of single molecules directly over time. Using SPT in the swap-over system again led to estimates of ~30–40 % bound [28–30] while with fluorescently tagged endogenous Pol II in *S cerevisiae*, values were again 50–60 % chromatin-bound [5,31]. There is therefore broad agreement across methodologies and systems that neither the bound nor unbound Pol II fraction is overly dominant. The variability in estimates appears to stem from the system used to express a fluorescently tagged Pol II subunit, rather than by technical approach or by the cells examined (Table 2). Best current estimates of the fraction of Pol II which is stably bound are therefore in the range of 50 %, although more extensive studies are needed of endogenously tagged Pol II, particularly in human cells. Thorough recent work in mouse embryonic stem cells, using a combination of FRAP, SPT, and fluorescence correlation spectroscopy (FCS) to examine diffusion of endogenously tagged Pol II, similarly found a bound fraction in the range of 40–50 % for both Halo-Rpb1 and Halo-Rpb3 [32].

Within the pool of 'bound' Pol II, the identification of subpopulations with different dynamics may reflect that not all chromatin-bound Pol II is actively transcribing. Indeed, not all Pol II initiation events at genes proceed to elongation into gene bodies (see below). Both FRAP and SPT studies have observed distinct subpopulations of Pol II [8,26,30] and a combination of orthologous technical approaches, such as FCS and SPT will further improve assessment of Pol II dynamics [30,33].

Studies of Pol II dynamics under varied conditions have been largely limited to drastic interventions such as transcription inhibition (Table 2). The effect of a wider variety of perturbations such as those facilitated by protein degron systems on global Pol II dynamics would be of interest. Of note, this combination has been applied already to examine Pol II binding following the degradation of general transcription factor component TATA-binding protein (TBP) [34]. Here it was found that the bound Pol II component was substantially reduced by the loss of TBP, alongside effects on Pol II residence time observed by SPT. This study provides an example of a methodological approach to examine how Pol II dynamic subpopulations may be modulated, to unpick how global Pol II activity is controlled.

3.2. Control of RNA polymerase activity

Regulating the activity of Pol II is a complex process, involving hundreds of gene-specific and general factors. Here, we present a brief overview of the many intersecting Pol II global regulatory systems and

Table 2
Estimates of the fraction of Pol II stably bound in live cells via tagging with a fluorescent protein. Note that studies using overexpression, swap-over models tend to have lower stably bound Pol II fractions (20–40 %) than studies using endogenous expression models (45–60 %).

Study	Biological system	Model	Technique	Pol II stably bound (~%)	Perturbations analysed
Kimura et al. (2002) [25]	Chinese hamster ovary (CHO) cells	Temperature-sensitive swap over EGFP-POLR2A	FRAP/FLIP	20–25 %	Transcription inhibition (DRB)
Hieda et al. (2005) [24]	Chinese hamster ovary (CHO) cells	Temperature-sensitive swap over EGFP-POLR2A	FRAP/FLIP	25 %	Transcription inhibition (DRB) Heat shock
Darzacq et al. (2007) [26]	USO2 human cells	α-Amanitin-resistant swap-over YFP-POLR2A	FRAP	40 %	Transcription inhibition (DRB, actinomycin D)
Fromaget & Cook (2007) [82]	Chinese hamster ovary (CHO) cells	Temperature-sensitive swap over EGFP-POLR2A	FLIP	n/a	Transcription inhibition (DRB, roscovitine, actinomycin D) Proteasome inhibition (Mg132)
Sprouse et al. (2008) [27]	*S. cerevisiae* yeast	Homologous recombination GFP-Rpb1, GFP-Rbp4, GFP-Rbp11	FRAP	62 % (Rbp1) 73 % (Rbp4) 80 % (Rbp11)	n/a
Steurer et al. (2018) [8]	MRC-5 human cells	CRISPR/Cas9 homology directed repair biallelic EGFP-POLR2A	FRAP	60 %	Transcription inhibition (THZ1, flavopiridol, cordycepin, triptolide, α-amanitin, actinomycin D) Proteasome inhibition (Mg132)
Boehning et al. (2018) [29]	USO2 human cells	α-Amanitin-resistant swap-over Halo-POLR2A	SPT	29 %	POLR2A CTD mutation
Teves et al. (2018) [34]	JM8.N4 mouse embryonic stem cells	CRISPR/Cas9 homology directed repair monoallelic Halo-POLR2A	FRAP and SPT	30 % (SPT), ~20 % (FRAP)	Transcription inhibition (triptolide, flavopiridol), TBP degron
McSwiggen et al. (2019) [28]	USO2 human cells	α-Amanitin-resistant swap-over Halo-POLR2A	SPT	35 %	Transcription inhibition (flavopiridol, triptolide)
Collombet et al. (2023) [32]	TX1072 mouse embryonic stem cells	CRISPR/Cas9 homology directed repair biallelic Halo-POLR2A, Halo-POLR2C	SPT, FRAP, and FCS	45–50 % (SPT), 40 % (FRAP)	Transcription inhibition (DRB, flavopiridol), Nucleoplasm versus Xist compartment
Fournier et al. (2023) [30]	USO2 human cells	α-Amanitin-resistant swap-over Halo-POLR2A	SPT	20 %	Transcription inhibition (FCS experiments only)
Nguyen et al. (2021) [31]	*S. cerevisiae* yeast	Homologous recombination Halo-Rbp1	SPT	45 %	PIC disruption (anchor-away system)
Swaffer et al. (2023) [5]	*S. cerevisiae* yeast	Homologous recombination Halo-Rbp1	SPT	50 %	Cell volume

highlight how studies of this system have been facilitated by new tools such as more selective inhibitors. We largely focus on the specifics of the metazoan transcription system.

Initiation of Pol II onto the DNA template has traditionally been thought of as a carefully orchestrated process that takes place at promoters of protein-coding genes. However, transcription is now known to occur much more widely across the genome [35]. Indeed, only 40 % of approximately 20,000 transcription units identified by nascent RNA-sequencing (TT-seq) could be mapped to GENCODE annotations [36], indicating widespread transcription initiation outside of classical gene promoters. A substantial fraction of Pol II which is initiated does not proceed to elongate across the full length of the transcription unit, observed via high resolution genomic localisation of Pol II [37]. We refer to this as premature termination of transcription. Its high prevalence is corroborated by live cell imaging of Pol II dynamics [8]. Moreover, it has been estimated that 95 % of RNA produced by Pol II in mammalian cells never leaves the nucleus, partially reflecting this premature termination, alongside intronic and other RNAs [9].

A series of interacting partners, collectively referred to as the pre-initiation complex, load Pol II onto its DNA template [38]. As part of this initiation, POLR2A is phosphorylated at the Ser5 and Ser7 sites of the CTD via a cyclin-dependent kinase, CDK7, a subunit of transcription factor IIH [39]. Kinase inhibitors selectively targeting CDK7 block Ser5 and Ser7 phosphorylation of Pol II and globally block transcription initiation in mammalian cells [36,40,41].

Following initiation, Pol II localised immediately downstream of the transcription start site can be observed as a peak in ChIP-seq [42]. This 'promoter-proximal' Pol II occurs in 40–70 % of genes and is capable of resuming transcription in in vitro 'run-on' assays [43]. While often referred to as 'paused', this promoter-proximal Pol II may arise from various combinations of kinetic parameters, such as repeated cycles of initiation and premature termination, rather than literal 'pausing' where Pol II becomes immobile at a particular location on the gene before being released into elongation [44]. A recent study combining metabolic labelling with sequencing of transcription start site-associated RNAs, reinforced that a major fraction (~80 %) of promoter-proximal Pol II prematurely terminates and does not progress to productive elongation [45] – in agreement with analyses of Pol II dynamics via FRAP [8,26]. Studies of the rates of Pol II release similarly show that promoter-proximal termination dominates over release to elongation. Monitoring proximal Pol II levels after treating cells with triptolide to inhibit new initiation indicates that Pol II remains associated with promoters for 5–15 min in mammalian cells [46,47].

Which systems maintain Pol II in this cycle of initiation and abortive termination? Pol II is prevented from progressing to active elongation by a combination of negative elongation factor (NELF): a protein complex, and DRB-sensitivity inducing factor (DSIF): a heterodimer of two proteins SPT4 and SPT5 [48]. The specific functions of NELF and DSIF in controlling promoter-proximal Pol II have been recently shown directly using inducible degron approaches [49–52]. Reciprocally, the progression of Pol II into active elongation is positively regulated by the activity of P-TEFb/CDK9, which was initially identified via the activity of transcription inhibitor DRB. P-TEFb phosphorylates the CTD of POLR2A at Ser2 [53]. While DRB is relatively non-selective, the use of more modern inhibitors with greater selectivity for CDK9 over related CDKs [54–56], as well as mutant CDK9 to achieve selective inhibition [57], clearly demonstrate the direct role of CDK9 in releasing Pol II via CTD Ser2 phosphorylation. In addition to acting on the Pol II CTD, P-TEFb phosphorylates both DSIF and NELF [58,59], in order to facilitate progression of Pol II to elongation by a combination of these actions [60]. Pol II is therefore prevented from progressing via negative elongation factors, and P-TEFb/CDK9 mediates its release but what else resolves Pol II which is 'paused' and prevented from progressing?

An important player in antagonising the transition to elongation is Integrator, a multi-subunit complex associating with the CTD of Pol II. This multi-subunit complex has an array of functions, including an endonuclease activity, catalytically cleaving nascent RNA in a function critical to small non-coding RNA and enhancer RNA production [61,62]. Integrator directly associates with NELF and appears to negatively modulate Pol II release, while at the same time being required for correct processivity [63]. Further, the association of Integrator with a protein phosphatase functionally opposes CDK-mediated phosphorylation of the

Pol II CTD [55,64]. By recognising Pol II bound to DSIF and NELF, Integrator facilitates transcription termination at the start of protein coding mRNA genes, attenuating transcription globally [65]. The various subunits of Integrator combine to negatively regulate Pol II progression, and resolve paused Pol II via termination of transcription. Immediately after release from the promoter-proximal region, Pol II progression can also be ceased via premature 3′ end cleavage and polyadenylation (PCPA) – a process that is suppressed through the 'telescripting' function of the U1 small nuclear ribonucleoprotein [66,67].

Pol II continues to be modulated during elongation. Multiple studies employing selective inhibitors [68] alongside analog-sensitive CDKs [69,70] have recently defined the role of an additional pair of kinases, CDK12/13, in maintaining the activity of elongating Pol II. CDK12/13 directly phosphorylates Ser2 of the CTD, but appears to be restricted to maintaining this after release from the promoter-proximal zone. Inhibition of these enzymes leads to Pol II redistributing toward the start of genes.

The picture of Pol II activity which emerges here is one of a regulated system, in which multiple often competitive processes control the eventual production of complete mRNA (Fig. 1). At initiation, release to elongation, and even once elongating, Pol II is subject to many distinct regulatory interventions. It bears emphasising that premature termination — initiated Pol II that terminates before completion of a full-length transcript — is common across the genome [36]. As noted previously, P-TEFb/CDK9 inhibition prevents transcription globally, whereas only a fraction of genes display obvious promoter-proximal peaks [43]. This suggests a model where, rather than discrete checkpoints, competing kinetic rates (of pTEFb, NELF/DSIF-binding, Integrator, U1 snRNP, etc.), direct Pol II to elongate or terminate within the proximal-promoter zone, or even further downstream. The interaction of those rates will give rise to various distributions of Pol II across the gene, including apparently paused peaks. This view contrasts somewhat with a 'checkpoint' model, where Pol II must proceed linearly through a series of defined states (e.g. DSIF/NELF-mediated pausing and subsequent release), where orderly assembly of a functional RNA synthesis and processing complex is validated.

The combination of new approaches to examine Pol II activity in living cells, including SPT which discerns multiple sub-populations of bound Pol II [30] with a wide variety of new tools to perturb the Pol II system, including protein degron systems, provide an avenue to thoroughly characterising the activity of the global Pol II pool. Selective inhibitors of various points in the transcription cycle would allow the testing of which regulatory elements define these populations. It is interesting to note that the precise function of these intersecting systems, differing as they do from yeast to metazoans, in regulating transcription globally has not been defined. It has been discussed, for instance, that premature termination may constitute a core aspect of gene regulation, act to buffer transcriptional noise, or facilitate the correct progression of co-transcriptional processes such as splicing [43,47,71]. A greater understanding of which processes act in unperturbed cells on the global Pol II pool may contribute to answering this question.

3.3. Pol II degradation and responsiveness of the Pol II regulatory system to perturbation

Of all the steps in the Pol II lifecycle, degradation of the protein complex is the least well understood, and has mostly been studied in the context of DNA damage (reviewed in [71]). It therefore remains unclear how the total level of Pol II is maintained in the cell, and whether this is coordinated through regulation of its synthesis or its degradation. The protein complexes responsible for Pol II ubiquitination remain poorly characterised: the ubiquitin ligase BRCA1 [72]; von Hippel-Lindau protein [73]; the Elongin A complex [74]; and an ARMC5-containing ubiquitin ligase complex [75] have all been proposed to target Pol II for degradation. What regulates these complexes, and which sites on Pol II are targeted for ubiquitination remain important topics for further investigation.

In human cells, POLR2A is rapidly degraded by the ubiquitin-proteasome system upon transcriptional inhibition. This occurs upon treatment with direct POLR2A inhibitor, α-amanitin [8,76,77]; TFIIH inhibitor, triptolide [3,8,77–79]; CDK7 inhibitor, THZ1 [80]; and CDK9 inhibitors, DRB [79] and AZD4573 [3,81]. Treatment with CDK9 inhibitors DRB [25,82] or flavopiridol [8,28] increases the fraction of free Pol II, consistent with a model where CDK9 is necessary to allow Pol II to remain actively elongating (see [3,8,26]). Acute loss of pausing factor SPT5 also leads to degradation of Pol II [50], suggesting that general loss of transcription activity may lead to loss of Pol II. A generic mechanism for adapting Pol II abundance to Pol II activity was recently proposed, which relies on the non-chromatin-bound fraction being the one typically targeted for degradation [3]. In this model, any perturbation (such as CDK9 inhibition or SPT5 depletion) that leads to unbinding of Pol II would therefore lead to overall reduction in Pol II levels, but conversely less degradation would occur in conditions where more enzyme is loaded onto the DNA template. This model is supported by a live-cell imaging study that found accumulation of non-chromatin-bound POLR2A when triptolide or α-amanitin were combined with proteasome inhibitors [8]. Moreover, DNA intercalation with actinomycin D

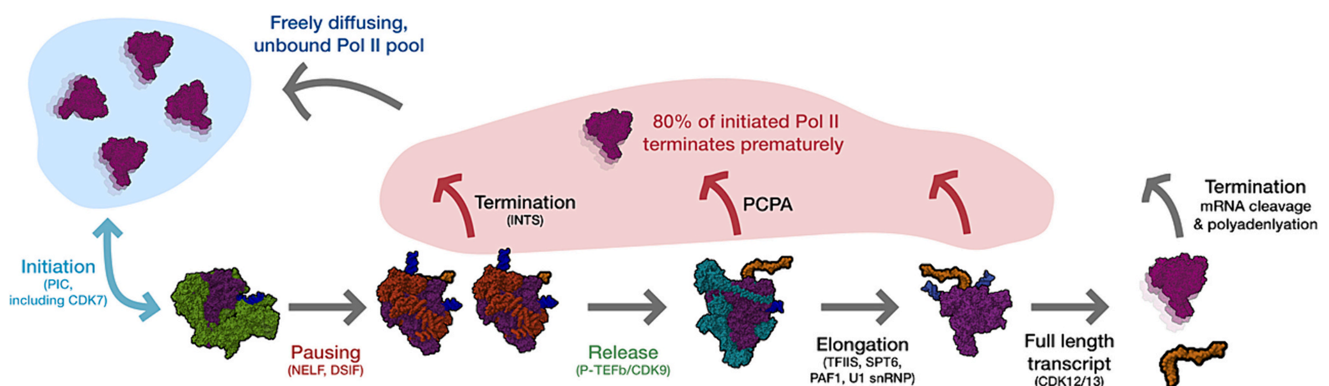

Fig. 1. Global activity of Pol II in the live cell. The global Pol II pool is split between a freely diffusing component, and a remaining fraction bound to chromatin. Initiation occurs via the pre-initiation complex, after which Pol II progression is paused. Pol II can be either released from the paused state, or prematurely terminated at either this or later stages in transcription. A major fraction of Pol II does not proceed to elongation of a complete mRNA but aborts before this point. However, due to the comparatively long time it takes to produce a full-length transcript, more than half of all Pol II molecules are stably bound to chromatin at any given time. Many distinct and competing processes regulate Pol II activity on DNA globally. Rendering of Pol II complex structures for this figure taken from the Protein Data Bank, PDB IDs 8A40, 8CEN, 6GML, and 7XN7 [8,26,45–47].

decreased the relative fraction of free Pol II [8,25,82,83], and also reduced α-amanitin-induced degradation [76]. Such a model would also be capable of coordinating Pol II abundance with transcriptional activity not just in the context of perturbations but also in homeostasis and potentially in situations of dynamic changes in RNA metabolism. We now discuss what is known about global Pol II control in these processes.

4. RNA polymerase II in mRNA homeostasis

Cellular mRNA concentration is under homeostatic control. This involves coordination of mRNA abundance with cell volume, and also coordination of mRNA synthesis and decay rates with one another (reviewed in [84]). For example, genetic perturbation of factors involved in RNA production leads (directly) to global changes in RNA synthesis rates. However, such perturbations typically also lead (indirectly) to corresponding changes in RNA degradation rates. The converse is also true: perturbation of decay factors results in changes in RNA synthesis rates. This phenomenon has been termed mRNA buffering (reviewed in [85]) and although it has been observed in yeast and mammalian model systems, it remains poorly understood. Recent acute perturbation experiments have shown that this mechanism is rapid. For example, degradation of nuclear RNA exosome subunit DIS3 in human cells, led to a rapid reduction in transcription, on the same time-scale as mRNA began to accumulate in the nucleus [3] (DIS3-targets are predominantly produced by Pol II [86]). Similarly, targeting Xrn1 (the predominant cytoplasmic 5′-3’ RNA exonuclease) in *S cerevisiae* led to rapid accumulation of mRNA within one hour, followed by complete reversion to wild-type levels over the following three hours [87]. This homeostatic adaptation of mRNA levels was underpinned by transcription-rate adaptations following Xrn1 depletion.

A recent genome-wide screen in human cells for factors regulating RNA synthesis rates revealed that perturbations associated with reduced or increased global RNA synthesis rates typically have corresponding reductions or increases in the nuclear concentration of Pol II (specifically POLR2A was measured, by either immunofluorescence or GFP-tagging) [3]. Interestingly, many of the perturbations that disrupted RNA synthesis targeted components downstream of transcription, suggesting that Pol II levels are modulated as part of the mechanism of transcriptional adaptation. In support of this, it was observed that transcript production is reduced *before* loss of RNA Pol II in response to nuclear RNA exosome depletion – mirroring Pol II loss seen upon chemical inhibition of transcription (see above). This suggests some feedback mechanism coordinates Pol II activity, which then modulates Pol II levels, although the mechanism of this feedback has not been determined (reviewed in [84]). Intriguingly, the global regulator of transcription P-TEFb is itself under a complex control mechanism, which sequesters and releases the activating enzyme CDK9 in response to stimuli such as transcription inhibition [88,89].

Pol II levels have also been measured in the context of cell-size changes in *S cerevisiae*. Here it was found that abundance of all Pol II subunits scale linearly with cell size (like most proteins), however their enrichment on chromatin scales sublinearly (as measured by spike-in normalised ChIP) [5]. It was also observed that rapid 50 % depletion of Rpb1 from the nucleus led (within 40 min) to 50 % reduction of Rpb1 occupancy on chromatin. This, together with data showing that over-expression of Pol II led to increased Pol II on chromatin suggests that Rpb1 levels in *S cerevisiae* are normally maintained at a level that is limiting for transcription. These findings lend support to the ‘limiting-factor model’ of cell-size scaling (reviewed in [84]), in which cellular Pol II levels dictate transcriptional activity. Reconciling these observations with the complex and dynamic feedback mechanisms that impinge on global Pol II activity and abundance [84] remains a topic for future work. An interesting outstanding question in this field remains, though, in the quantitative relationship between Pol II occupancy (as measured by ChIP or imaging) and transcriptional activity. To what extent does Pol II binding to DNA directly relate to transcriptional output, and does the former linearly predict the latter?

5. Changes to global RNA polymerase activity

In situations of cellular homeostasis, one may expect that robust regulatory feedbacks would be advantageous for precisely controlling the levels of cellular transcription and Pol II abundance. However, there are biological situations where transcription must be globally altered. We now discuss several of these, focusing in each on what is known mechanistically about Pol II activity changes.

5.1. Mitosis

In human cells, transcription is globally repressed before nuclear envelope breakdown and Pol II is almost entirely excluded from highly compacted mitotic chromosomes [90–92], along with many sequence-specific transcription factors (reviewed in [93]). Mitotic transcriptional shutdown involves inhibition of new initiation by phosphorylation of general transcription factors, including TFIIB, TFIID and TFIIH [94,95] as well as clearance of already paused Pol II by CDK9-mediated release into elongation [92]. Despite these mechanisms, low levels of Pol II activity can still be detected on mitotic chromatin [96], particularly at kinetochores [97]. Pol II is re-imported into the nucleus during late telophase [91], but Ser2-phosphorylated POLR2A can be detected only later, in early G1 [98]. Post-mitotic transcription is re-established in an orchestrated manner, with cell structure and growth genes expressed before cell-type-specific genes [96].

5.2. Zygotic genome activation

In animals, the fertilised oocyte is transcriptionally silent, with development initially dependent on maternal supplies of protein and RNA. Zygotic genome activation (ZGA) represents a process of carefully orchestrated global increase in transcriptional activity. In the non-mammalian model organisms *Xenopus*, zebrafish and *Drosophila*, more than ten rounds of rapid cell division take place before a characteristic lengthening of cell cycle duration called the mid-blastula transition (MBT). This coincides with the onset of widespread transcription of the zygotic genome (reviewed in [99]). In these organisms, MBT/ZGA timing appears to be controlled by titration of an inhibitory factor against the exponentially increasing amounts of DNA in the developing embryo (reviewed in [100]). In mammals, ZGA occurs much earlier (in terms of cell numbers): at the two-cell stage in mouse [101] and at the 4–8-cell stage in human [102]. Pol II regulation seems to differ in the different systems studied.

In *Xenopus*, nuclear import appears to be a key regulatory step in ZGA for many chromatin-associated proteins, with the timing of a protein's import immediately preceding its activity [103]. Pol II is no exception. It accumulates before ZGA and is constant during ZGA [104], with nuclear import occurring only around the onset of transcription. This is similar in zebrafish, however the appearance of phosphorylated Pol II in the nucleus is preceded by nuclear import of developmental transcription factors and histone acetylation [105,106]. Moreover, supplying excess quantities of an exogenous ‘writer’ (P300) and ‘reader’ (BRD4) of histone acetylation (together) results in premature ZGA [107]. This suggests that Pol II is not limiting for transcriptional activation, but is dependent on an appropriately configured chromatin template.

In *Drosophila*, the timing of Pol II import differs, with hypophosphorylated Pol II detectable in the nucleus several cell cycles before genome activation [108] – again indicating that Pol II nuclear localisation is insufficient to activate transcription at early stages. During ZGA, phosphorylated Pol II appears heterogeneously distributed in the nucleus, localising to large histone locus bodies as well as small foci that depend on the Zelda (Zinc-finger early *Drosophila* activator) transcription factor [109]. Interestingly, in *Zelda* mutant embryos (which lack small Pol II foci), nuclear Pol II levels still increase normally over

developmental time, with excess Pol II accumulating at histone locus bodies instead of Zelda targets [109]. This suggests that global levels of phosphorylated Pol II in the nucleus may be independently regulated and decoupled from activation at specific genes.

In mouse, transcription is silenced in the final stages of oocyte maturation, when Pol II dissociates from chromatin [110,111]. After fertilisation, Pol II is imported into both the maternal and paternal pronuclei during the one-cell stage [110], where it is bound to chromatin at accessible regions and CG-rich promoters. Toward the end of the one-cell stage Pol II relocates to gene targets before their activation [110] with Ser2-phosphorylated Pol II detected by the two-cell stage [112]. The nuclear RNA/DNA-binding protein TDP-43 is imported at the same stage and appears important for POLR2A to localise correctly on the genome [112].

It has not been investigated if the machinery used for Pol II nuclear import in interphase cells is also used during ZGA. It appears, however, that nuclear Pol II is necessary but not sufficient for widespread transcription. Dilution of inhibitory factors, expression/import of specific transcription factors, and chromatin modification are some of the many key steps regulating cellular transcription rates at this stage. This contrasts with the homeostatic control mechanisms discussed above, where Pol II levels are closely linked with activity.

5.3. Immune cell activation

Activation of immune cells such as lymphocytes and monocytes is associated with major cell morphology and metabolic changes. For example, activated B-cells become larger and accumulate more RNA and protein [113], including an estimated 17-fold increase in RNA Pol I and Pol III and 8-fold increase in Pol II [113]. However, considering the interdependence of cell size, RNA abundance, and transcriptional activity; cause and consequence are not well understood in this process. Moreover, because cell volume is often not measured, or measured imprecisely, changes in the *concentrations* of rRNA, mRNA, and Pol II remain uncertain. Recent research, predominantly in mouse, has centred on the role of the transcription factor c-Myc (Myc) in modulating transcription during immune-cell activation. Myc is induced early in immune-cell activation and is essential for many of the cell-state changes, including cell growth and accumulation of RNA [114]. Its direct targets include rDNA and genes encoding ribosomal proteins [115,116]. Increases in bulk RNA synthesis rates are also Myc-dependent, however, it remains disputed whether Myc ‘amplifies’ transcription at all expressed genes [117,118], or whether its activities are limited to a more restricted subset of genes [115,116]. It is generally unclear how this important process mechanistically interacts with (or overcomes) homeostatic regulation of Pol II and mRNA concentration.

5.4. DNA damage response

Control of Pol II levels are also central to the DNA damage response. For example, UV exposure results in DNA lesions and rapid inhibition of transcription elongation, followed by global transcriptional shutdown – mediated by ubiquitin-dependent proteasomal degradation of POLR2A (reviewed in [71]). Blocking this Pol II decay pathway (by mutation of the lysine residue on POLR2A that is normally ubiquitinated) leads to preferential production of mRNAs from shorter transcription units upon DNA damage, presumably as they are less likely to have barriers to transcriptional elongation [2]. The global reduction in transcription driven by global Pol II reduction is therefore thought to allow cell survival in the DNA damage response by avoiding an unbalanced (gene-length-dependent) transcriptome [2]. Whether RNA stabilities are also modulated to maintain constant RNA concentrations during the DNA damage response has not been tested.

6. Conclusions and future directions

Control of gene expression at the transcriptional level is a major determinant of variability between cell types and of differences in protein levels between single cells [1]. Furthermore, the examples of cell-size scaling, mitosis, ZGA, immune-cell activation, and DNA-damage response highlighted in this review all represent different, evolved strategies for global Pol II control. It is therefore unsurprising that the number of regulatory steps that govern Pol II activity is enormous. While some progress has been made in understanding global Pol II control, simple aspects of this system such as the control of total Pol II activity in response to perturbation, and how the total level of the Pol II complex is maintained remain outstanding questions.

To gain a more complete understanding of global Pol II behaviour, imaging studies (which directly measure global Pol II dynamics at the level of single molecules or populations of molecules (Table 2)) must be more deeply integrated with genomic methodologies (in particular, metabolic labelling [36,45], which can provide kinetic and genomic resolution in snapshot-measurements). Linking both viewpoints has the potential to reveal the global abundances, binding kinetics and phosphorylation states of Pol II, the timescales of transitions between these states, as well as how these rates vary across genes. We believe that integration of these diverse data sources is most naturally achieved through mathematical modelling. Modelling has recently been successfully applied to understand coordination of transcription with cell size [3,5,119], as well as to infer changes in transcription kinetics from snapshot genomics data [2,44,69] and live-cell imaging [8,26]. Unifying these models to capture both the genomic distributions and dynamics of the entire (transcribing and non-transcribing) pool of Pol II molecules will be a major goal of the field over the next decade. This will provide a detailed picture of the precise control points used by the multiple biological processes that regulate global Pol II activity and abundance, and may lead to an increased ability to precisely control gene expression in vivo.

CRediT authorship contribution statement

Alexander Gillis: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Scott Berry:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Scott Berry reports financial support was provided by Australian Research Council. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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