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The transition from transcriptional initiation to elongation

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Transcription is the first step in gene expression, and its regulation underlies multicellular development and the response to environmental changes. Most studies of transcriptional regulation have focused on the recruitment of RNA polymerase to promoters. However, recent work has shown that, for many promoters, post-recruitment steps in transcriptional initiation are likely to be rate limiting. The rate at which RNA polymerase transitions from transcriptional initiation to elongation varies dramatically between promoters and between organisms and is the target of multiple regulatory proteins that can function to both repress and activate transcription.

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Introduction

Transcription is the first step in gene expression and is the major target of regulation. Transcription can be divided into three distinct phases: (i) initiation, (ii) elongation, and (iii) termination. It has been widely assumed that recruitment of RNA polymerase (RNAP) during transcriptional initiation is usually the rate-limiting step in transcription. Indeed, artificial recruitment of the transcriptional machinery is often sufficient for productive transcription in *E. coli*, yeast, and human cells [1–3]. However, recent genome-wide studies indicate that, for many promoters in both prokaryotes and eukaryotes, the rate-limiting step in transcription initiation is likely to occur after recruitment of RNAP. Furthermore, the transition from initiation to elongation is an important target of regulation in both prokaryotes and eukaryotes. In this review we discuss the mechanisms of transition from transcriptional initiation to elongation, how this transition varies between promoters and between species, and how it is regulated by proteins and small molecules.

The transition from transcriptional initiation to elongation in bacteria

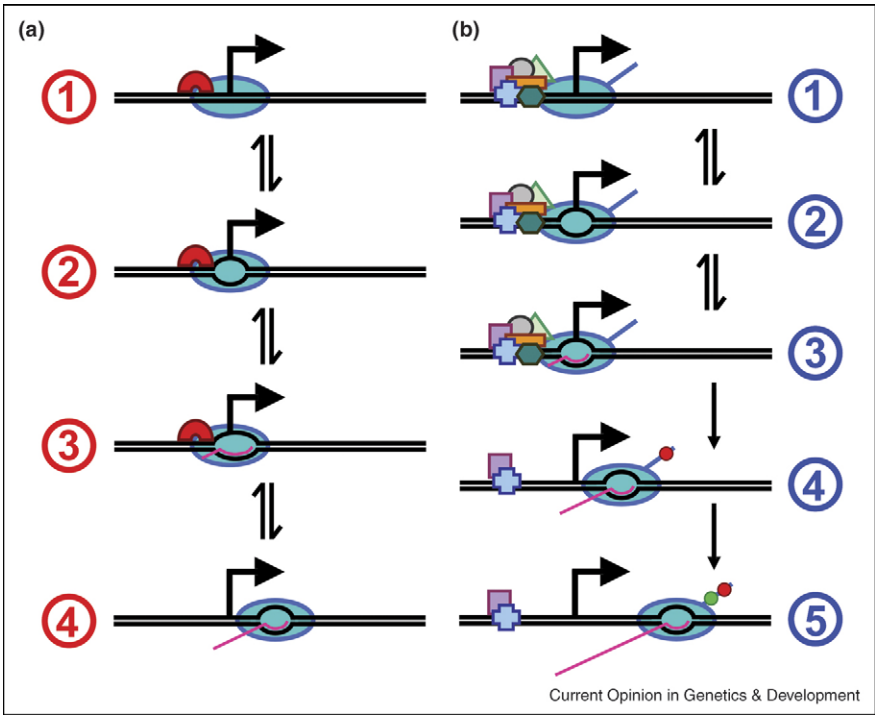
In eubacterial species, transcription of all genes is mediated by a core RNAP complex, typically a 5-subunit ($\alpha_2\beta\beta'\omega$) enzyme. However, in order to recognize promoter DNA sequences, this core enzyme must associate with a σ factor to form RNAP holoenzyme [4]. Initiation occurs at a site that is a fixed distance from the σ recognition sequences. Eubacterial species typically contain multiple σ factors that form distinct classes of RNAP holoenzymes that recognize different promoter sequences and regulate distinct classes of genes [4]. σ does not usually associate with elongating RNAP *in vivo*, although this can occur at a minority of genes under certain environmental conditions [5,6••].

Transcriptional initiation by RNAP holoenzyme involves three biochemically defined steps (Figure 1a). RNAP holoenzyme binds to promoter DNA to form the closed 'preinitiation' complex, melts the DNA around the transcription start site to form the open complex, and then transitions from initiation to elongation in a process known as promoter escape. Promoter escape typically includes multiple cycles of abortive initiation where RNAP synthesizes short RNAs of 2–15 nt [7]. Two recent studies have shown that promoter escape involves 'scrunching' of the DNA immediately downstream of the transcription start site [8••,9••]. The upstream face of RNAP remains stationary relative to DNA during abortive initiation while the downstream DNA is drawn into RNAP. This scrunching creates a stressed intermediate state during transcription initiation owing to the unwinding and compaction of DNA. It has been proposed that this stressed intermediate provides the driving force for either abortive initiation or promoter escape [8••,9••].

The transition from transcriptional initiation to elongation in eukaryotes

Eukaryotic cells contain three nuclear RNA polymerases, with RNA polymerase II (Pol II) responsible for transcribing all mRNAs and numerous non-coding RNAs. Pol II, a 12-subunit enzyme with many similarities to bacterial RNAP, does not recognize promoter DNA by itself, but rather as part of the basal Pol II machinery that includes general transcription factors (TFIIA, B, D, E, F, H). Like σ factors, these general transcription factors do not associate with elongating Pol II, and hence rapidly dissociate from Pol II during the transition between initiation and elongation [10]. Numerous factors

Figure 1



Steps in transcription initiation. **(a)** Steps in transcription initiation in eubacteria. 1. Preinitiation closed complex formation at the promoter by RNAP holoenzyme (containing a σ factor). 2. DNA is unwound around the transcription start site to form an open complex. 3. Abortive synthesis of 2–15 nt RNAs requiring DNA 'scrunching'. 4. Promoter escape is typically associated with loss of σ factor. **(b)** Steps in transcription initiation in eukaryotes. 1. Preinitiation complex formation at the promoter with Pol II and general transcription factors. 2. DNA is unwound around the transcription start site to form an open complex. 3. Abortive synthesis of 2–3 nt RNAs. 4. Promoter escape is associated with release of most general transcription factors and with phosphorylation at Serine 5 of the C-terminal domain of the largest Pol II subunit (red circle). In some eukaryotes Pol II pauses after synthesis of 20–50 nt RNAs. 5. Escape from promoter-proximal pauses is associated with phosphorylation at Serine 2 of the C-terminal domain of the largest Pol II subunit (green circle) by pTEFb.

(e.g. FACT, Spt4, Paf1 and TREX complexes, Spt6, Swi/Snf) travel with elongating Pol II throughout the coding region [11]. Importantly, eukaryotic Pol II must contend with nucleosomes that inhibit both initiation and elongation, unlike the *E. coli* RNAP that interacts with a genome that is permissive for binding transcription factors [12].

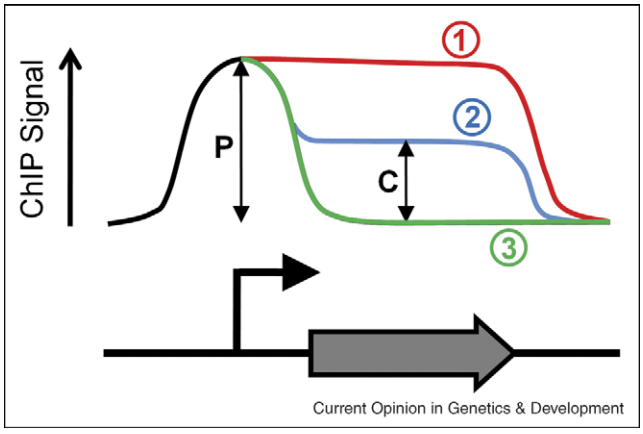
The C-terminal domain (CTD) of the largest Pol II subunit contains multiple copies of a heptad repeat that is phosphorylated at serines 2 and 5 by different kinases [11,13]. After initial association of the unphosphorylated form of Pol II into the preinitiation complex, Serine 5 is phosphorylated by TFIIH at the promoter, and then Serine 2 is phosphorylated by P-TEFb/CTK1 as Pol II elongates through the mRNA coding region. CTD phosphorylation appears to be relatively unimportant for transcriptional initiation or elongation *per se*, but rather plays a crucial role in coupling Pol II elongation to post-transcriptional steps such as mRNA capping, splicing, polyadenylation, export, and chromatin modifications such as histone methylation.

The steps of Pol II association into a closed complex, open complex formation, and promoter escape are analogous to, but mechanistically distinct from, those of bacterial RNAP (Figure 1b). Of particular significance, open complex formation requires the helicase activity of TFIIH, and promoter escape does not coincide with abortive initiation [14]. In addition, unlike most species in which initiation occurs at a fixed position downstream from the TATA element, initiation in *Saccharomyces cerevisiae* occurs at larger and more variable distances, suggesting that there are species-specific differences in how transcription is initiated in eukaryotes. Lastly, as will be discussed in more detail below, there can be an additional Pol II pausing step in the transition between initiation and elongation [15,16].

Rate-limiting steps in transcription initiation vary among genes and organisms

The transition between initiation and elongation *in vivo* can be investigated by using chromatin immunoprecipitation (ChIP) to determine RNAP association at promoters and transcribed regions. Recently, this transition has

Figure 2



Possible profiles of ChIP signal for RNAP. The graph shows three possible ChIP profiles for RNAP across a gene. In all cases RNAP associates with promoter DNA sequences at an equivalent level. In case (1) the ChIP signal is constant throughout the promoter and coding sequence, indicating rapid transition of RNAP from initiation to elongation. In case (2) the ChIP signal is reduced within the coding sequence as compared with the promoter, although ChIP signal in the coding sequence is above background. This indicates that some or all RNAP complexes transition slowly from initiation to elongation. As ChIP measures a population of cells it is impossible to determine whether all RNAP complexes transition at the same rate. In case (3) the ChIP signal is only present at the promoter. This indicates 'poised' RNAP at the promoter, that is, RNAP that is unable to make the transition from initiation to elongation. If the peak of RNAP ChIP signal is downstream of the transcription start site this indicates that RNAP is paused in early elongation. In all cases a Traveling Ratio (TR) can be calculated as the ratio of coding sequence signal (C) to promoter signal (P). Hence, TR can be used as a measure of the rate of transition from initiation to elongation at a given promoter.

been analyzed on a genome-wide scale in several organisms using tiled, high-density microarrays (ChIP-chip). If the transition from initiation to elongation is rapid, the level of RNAP at a promoter will be roughly equivalent to the level in the corresponding coding sequence. If the transition is slow, RNAP association will be much greater at the promoter than in the corresponding coding sequence (Figure 2). Thus, the ratio of promoter association to coding sequence association is a simple measure of the rate of transition from initiation to elongation. We refer to this ratio as the 'Traveling Ratio' (TR) [6••].

In rapidly growing *E. coli*, the TR values for different transcribed regions are highly variable, ranging from 0 to 1 [6••]. In most cases, TR values are <1, and the median value is 0.43, suggesting (i) that the transition from initiation to elongation is limiting at most transcribed regions and (ii) that RNAP spends about 1–3 s at a promoter, which is ~50-fold more time than at a given position within the coding region. Strikingly, for 23% of transcribed regions where RNAP association is observed, there is no detectable transcript, indicating that RNAP at these promoters is unable to transition from initiation to elongation and hence is 'poised'. It should be noted this genome-wide pattern of RNAP reflects the σ^{70} containing form that is responsible for transcription of the vast majority of *E. coli* genes. By contrast, σ^{54} containing RNAP is typically poised at the promoter, and the transcription requires an activator protein and ATP hydrolysis [17].

In the budding yeast *S. cerevisiae*, genome-wide analyses [18,19•,20] indicate that the level of Pol II association at promoters is roughly equivalent to that within the corresponding coding sequence in almost all cases; average TR value = ~0.9 [6••,18]. This observation indicates a rapid transition from initiation to elongation, which is consistent with the very strong correlation between the level of promoter-bound TBP (a general transcription factor) and the level of transcription [21,22]. There are, however, isolated examples of transcriptionally inactive genes whose promoters bind the transcriptional machinery, probably with Pol II in a closed complex [21,23]. In addition, Pol II associates with many transcriptionally inactive promoters during stationary phase [19•], particularly for genes whose transcription is induced within 3 min of exiting stationary phase, suggesting that Pol II is poised for rapid activation. Intriguingly, the Rpb1 CTD is hypophosphorylated in yeast cells during stationary phase, suggesting that global regulation of transcription could be brought about by controlling CTD phosphorylation [19•].

The distribution of Pol II in human and *Drosophila* cells is very different from that in growing yeast cells, and in fact is more similar to the RNAP pattern in *E. coli*. For most transcribed regions, the level of Pol II association at the promoter is substantially higher than that in the corresponding coding sequence [24–26,27••,28,29••,30••], indicating that post-recruitment steps in transcription initiation are generally slow. Furthermore, 20–50% of Pol II-bound promoters correspond to transcriptionally inactive genes [27••,29••,30••,31]. Several lines of evidence suggest that Pol II at most of these transcriptionally inactive genes is 'paused' in early elongation 20–50 bp beyond the initiation site, in a manner similar to that described two decades ago for *Drosophila* heat shock genes [14]. First, this class of genes contains chromatin with tri-methylated H3-K4, a histone mark that is generated after transcriptional initiation [27••]. Second, the average position of Pol II at these genes is 50 bp downstream from the initiation site [30••]. Third, in all cases tested, RNA can be detected at the extreme 5′ end of the gene, but not further downstream [27••]. Fourth, in all cases tested, permanganate mapping reveals an open transcription bubble around the pause site [29••,30••]. As is the case for *E. coli* promoters with 'poised' RNAP containing σ^{70}, TFIID and presumably other general transcription factors are associated with promoters containing paused Pol II [14,25,28,31]. However, the

paused Pol II observed at human and *Drosophila* promoters is transcriptionally engaged [14], and in this respect may differ from 'poised' *E. coli* RNAP at promoters of inactive genes.

The prevalence of paused Pol II indicates that this is a major rate-limiting step in transcription in flies, human, and presumably most eukaryotic species. Strikingly, paused Pol II in *Drosophila* appears to be preferentially localized at genes involved in development, suggesting that Pol II pausing may have evolved to allow regulation of specific cellular processes [29••,30••].

Why does Pol II pause at many eukaryotic genes *in vivo*?

Three potential mechanisms, not mutually exclusive, might be considered for paused Pol II *in vivo*, given that such pauses do not occur *in vitro* with the minimal core Pol II machinery [14]. First, negative elongation factors (e.g. NELF and DSIF) associating with the preinitiation or early initiation complex might block the transition to full elongation. Second, positive factors might be required to dissociate Pol II from general initiation factors that are localized to the promoter, thereby limiting the distance Pol II can travel downstream from the initiation site. Third, nucleosomes near the initiation site might inhibit elongation, in which case variability in the position of paused Pol II among different genes might be explained by variations in position of the promoter-proximal nucleosome with respect to the initiation site. For all of these mechanisms, elongation beyond the pause requires the recruitment of positive elongation factors that remove the negative factors and/or mobilize or alter nucleosomes. Examples of such positive elongation factors are P-TEFb, which phosphorylates Serine 2 of the CTD, and chromatin-modifying factors such as JIL-1 kinase (phosphorylates histone H3 at Serine 10) [32•], FACT (an H2A-H2B chaperone), Paf1 complex (required for H3 methylation at lysines 4, 36, and 79), and Spt6 (an H3-H4 chaperone). TFIIS, the transcript cleavage factor, also plays a role in releasing paused Pol II, and DSIF remains associated with elongating Pol II after release from the pause and may act as a positive elongation factor [14].

Why does paused Pol II not occur in *S. cerevisiae*, especially considering that almost all the relevant factors are present in this organism? Possible explanations include (1) the absence of NELF, (2) differences in histone variants and possibly stability or positioning of nucleosomes, or (3) the absence of activators that (directly or indirectly) recruit the preinitiation complex but not elongation factors. An intriguing possibility is that lack of paused Pol II is linked to the longer and more variable distance between the TATA element and initiation site in *S. cerevisiae* than in most eukaryotic organisms [33]. This difference in start-site selection occurs *in vitro* and is primarily due to TFIIB and Pol II [34]. As the stereochemistry of TFIIB with respect to initiation site is conserved, we speculate that *S. cerevisiae* Pol II rapidly dissociates from the preinitiation complex owing to a weak interaction with TFIIB, whereupon it travels down the gene for a variable distance before initiating transcription at a site defined by the initiator element. An inherently unstable preinitiation complex in yeast cells is supported by an unusually large open complex between the TATA element and initiation site [35] and the lack of Mediator at core promoters [36].

Controlling the rate of transition by DNA sequence

Gene-specific variation in the rate of transition from initiation to elongation can be due to differences in promoter DNA sequence. In *E. coli*, such differences can affect both the affinity of RNAP for the promoter and the rate of post-recruitment steps [37], such as the level of abortive initiation and promoter escape [38,39], the formation of unproductive 'moribund' RNAP complexes [40,41], and σ-dependent pausing of RNAP in the initial transcribed region [42,43]. As initial transcription occurs through scrunching, an intriguing possibility is that the presence of promoter-proximal σ-dependent pause sites may cause RNAP to favor forward translocation and hence promoter escape, rather than repeated cycles of abortive initiation. In addition, relative differences in DNA melting temperature around promoters may influence the rate of transition from initiation to elongation [6••].

In eukaryotes, sequence-dependent effects on the transition between initiation and elongation have yet to be described, although some of the above mechanisms may be involved. Sequence-dependent effects might also arise from the fact that core promoters have a great deal of structural and functional diversity, particularly with respect to the TATA, initiator, and downstream promoter elements [44]. In addition, Pol II initiation and elongation is strongly inhibited by nucleosomes, and DNA sequences in the vicinity of the promoter may differ significantly with respect to nucleosome positioning and stability.

Regulating the rate of transition by proteins and small molecules

In bacteria, the transition from initiation to elongation is regulated by a wide variety of proteins and mechanisms. Some DNA-binding repressors stabilize initiating RNAP:promoter DNA complexes, thereby trapping RNAP at the promoter [45]. The nucleoid-associated protein, H-NS, can trap RNAP at promoters by forming looping interactions between binding sites upstream and downstream of the promoter [46]. Intriguingly, loop formation, and hence repression, occur when RNAP is bound to σ^{70} but not to the alternative σ factor, σ^{38} [46]. The transition from initiation to elongation can also

be accelerated by certain activator proteins that bind directly to both promoter DNA and RNAP and stimulate transcription at a post-recruitment step [47–50]. The transcription elongation factor GreA, a homolog of eukaryotic TFIIS, can also increase the rate of transition from initiation to elongation [51,52], presumably through its RNA cleavage activity that can rescue unproductive RNAP complexes [40,41].

The transition from initiation to elongation can also be regulated by small molecules. For example, RNAP is poised at the *osmY* promoter under conditions of low glutamate but is released when the glutamate concentration increases, indicating a direct role for glutamate in controlling the conformation of RNAP [53]. Small molecules can function through transcription factors. ArgP either represses or activates transcription at the *argO* promoter in the presence of lysine or arginine, respectively. In both cases RNAP binds promoter DNA and forms open complex but in the presence of lysine RNAP is unable to complete promoter escape [54•]. ppGpp, a small molecule produced during nutrient starvation, binds directly to RNAP and downregulates transcription at promoters that have intrinsically unstable open complexes [55]. ppGpp can also upregulate transcription of certain genes, and DksA has been implicated in modulating ppGpp function [55]. Promoters with unstable open complexes are also regulated by the concentration of the initiating nucleotide, which increases the half-life of open complexes by mass action [56].

In eukaryotes, the widespread existence of paused Pol II (except in *S. cerevisiae*) strongly suggests that this a major step at which the transition between initiation and elongation is regulated [14]. The classic example of such regulation is the induction of heat shock genes in *Drosophila*, in which the release of paused Pol II is mediated by HSF, a DNA-binding transcriptional activator protein. It is presumed that HSF recruits factors that release paused Pol II and permit it to traverse the gene, although it is unclear which factors are direct targets and exactly how Pol II release occurs. P-TEFb plays an important role, because artificial recruitment bypasses the pause [57], and chemical inhibition blocks release from the pause [58]. It is likely that other activators (e.g. the HIV Tat protein) will function in a manner analogous to HSF, although the precise details may differ [59]. Conversely, some repressors (e.g. PIE-1) [60] might function by inhibiting CTD-Serine 2 phosphorylation and/or recruitment of elongation factors. P-TEFb is negatively regulated through its association with a complex containing 7SK RNA, HEXIM, and other proteins [59].

The transition between initiation and elongation can be regulated in other ways. For example, the interaction between phosphorylated ELK1 and the Med23 subunit of Mediator is important not only for recruitment of Mediator to the Egr1 promoter, but also for a step after preinitiation complex assembly that permits Pol II to escape the promoter [61]. However, the salt-sensitivity of Pol II in the uninduced state indicates that regulation does not involved paused Pol II. It is also worth noting that transcription of ribosomal RNA by Pol I is regulated after recruitment to the promoter, with UBF1 and nuclear actin being implicated in promoter clearance in human cells [62,63].

In vivo, the basic Pol II machinery is unable to access the chromatin template unless it is recruited (directly or indirectly) by DNA-binding activator proteins [64]. As a consequence, and given the existence of paused Pol II, there must be a class of 'activators' that can recruit the core machinery to the promoter but is unable to recruit elongation factors and hence stimulate Pol II transcription. On the basis of the *Drosophila* heat shock genes, the GAGA factor is likely to be such an 'activator'. Such 'activators' are analogous to, although mechanistically distinct from, bacterial activators that require regulatory signals to stimulate post-recruitment steps. It is possible that *S. cerevisiae* lacks activator proteins of this type.

Conclusions

Post-recruitment steps in transcription initiation are rate limiting at many promoters in species ranging from *E. coli* to humans. There is great variability within the rate of these steps both within and between species, and under different growth conditions. Since most studies of transcriptional regulation have focused on recruitment of RNAP to promoters there is still much to learn about the transition from initiation to elongation, in particular with regard to the proteins and small molecules that regulate this process.

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