P-TEFb stimulates transcription elongation and pre-mRNA splicing through multilateral mechanisms

Tina Lenasi & Matjaz Barboric

To cite this article: Tina Lenasi & Matjaz Barboric (2010) P-TEFb stimulates transcription elongation and pre-mRNA splicing through multilateral mechanisms, RNA Biology, 7:2, 145-150, DOI: 10.4161/rna.7.2.11057

To link to this article: http://dx.doi.org/10.4161/rna.7.2.11057

Copyright © 2010 Landes Bioscience

Published online: 01 Mar 2010.

Submit your article to this journal

Article views: 92

View related articles

Citing articles: 20 View citing articles
P-TEFb stimulates transcription elongation and pre-mRNA splicing through multilateral mechanisms

Tina Lenasi and Matjaz Barboric*
Department of Virology; Haartman Institute; University of Helsinki; Helsinki, Finland

Promoter-proximal pausing of RNA polymerase II (RNAPII) across the genome has renewed our attention to the early transcriptional events that control the establishment of pausing and the release of RNAPII into a productive transcription elongation. Here, we review our current understanding of the transcriptional cycle by RNAPII with a particular emphasis on the mechanisms that stimulate transcription elongation and cotranscriptional pre-mRNA splicing through an essential transcriptional kinase, the positive transcription elongation factor b (P-TEFb). We illustrate that by targeting a limited set of transcription elongation factors and paused RNAPII molecule during a promoter-proximal phase of transcription, P-TEFb unleashes an extensive crosstalk between transcription apparatus, RNA processing factors and chromatin for optimal production of mRNA.

Introduction
The control of eukaryotic gene expression programmes is instrumental for the generation and functioning of hundreds of cell types of an organism. Whereas the execution of the programs is enabled by a web of interactions between a myriad of transcription factors and specific regulatory DNA elements of a particular gene, the main executioner is a multi-subunit molecular machine called RNA polymerase II (RNAPII), which transcribes genetic information of all protein-coding genes.1 However, RNAPII is not only the end-point of this complex protein:protein and protein:DNA chatter that positions it to the transcription start site of a gene for the generation of mRNA. Through differential recruitment of RNA processing factors to the C-terminal domain (CTD) of its largest subunit Rpb1, RNAPII also coordinates processing and nuclear export of synthesized transcripts during different steps of transcription and thus effectively links transcription to cotranscriptional maturation of pre-mRNA.2,3 Given that transcript processing reaches back to transcription as well, it is becoming apparent that these processes are intricately intertwined and coupled in a bidirectional manner. Here, we briefly discuss our current view of the RNAPII transcriptional cycle and focus on how the positive transcription elongation factor b (P-TEFb) triggers a series of mechanisms for coordinate stimulation of transcription elongation and splicing of transcripts exiting from RNAPII.

Integration of Transcription and Pre-mRNA Processing via the CTD of RNAPII
Synthesis of mRNA by RNAPII is subjected to a tight control at various steps during the transcription cycle, including the recruitment of transcription preinitiation complex (PIC) to DNA, transcription initiation, promoter clearance, promoter-proximal pausing, elongation, termination, and re initiation.1 Concomitant with the progress of RNAPII through these phases, phosphorylation pattern of the CTD, which consists of multiple tandem heptapeptides (52 in humans, 26 in yeast) with the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser (Y_S P T S P S),
progresses as well.4 The so-called “CTD code” is differentially written by multiple complexes containing cyclin-dependent kinase (Cdk) 7, 8 or 9 and serves as a recognition platform for tethering a variety of transcription and pre-mRNA processing factors, linking transcription to mRNA maturation. Briefly, RNAPII enters the PIC with the unphosphorylated CTD. This step can be antagonized by Cdk8, which phosphorylates the CTD on Ser2 and Ser5 (Ser2P and Ser5P) prior to the PIC assembly such as during mitosis.5 Among the PIC assembly on promoter, Cdk7 of the general transcription factor II H (TFIIH) phosphorylates the CTD on Ser5 and Ser7 (Ser5P and Ser7P), coinciding with transcription initiation and promoter clearance.6,7 Through Ser5P, the CTD is able to recruit and activate the capping enzymes to facilitate cotranscriptional capping of pre-mRNA emerging from the closely positioned RNAPII exit channel.8 Finally, the CTD Ser2P mark, made by the Cdk9 subunit of the P-TEFb kinase, signifies productive transcription elongation.9 In addition, the CTD Ser2P binds splicing factors, including a Ser/Arg-rich (SR) protein SF2/ASF10,11 and Spt6,12,13 and the cleavage/polyadenylation factor Pcf1114 for coordinating transcript elongation with splicing and 3’-end maturation, respectively. Upon transcription termination, the CTD is dephosphorylated by phosphatases such as Fcp1, which enables reassembly of RNAPII with the PIC for re-initiation of transcription.15

**P-TEFb Stimulates the Transition from RNAPII Pausing to Productive Elongation Through Multi-Tiered Mechanisms**

Among the various phases of the RNAPII transcription cycle, mechanisms directing the recruitment of RNAPII to promoters and transcription initiation have received most attention over the past three decades. In turn, it has been commonly thought that the recruitment of RNAPII was a major rate-limiting stage in eukaryotic transcription. However, findings of recently performed global analyses have seriously challenged the sanctum of this prevailing paradigm. Namely, genome-wide studies in *S. cerevisiae*, *D. melanogaster*, mammalian embryonic stem cells, and mammalian differentiated cells have established that a major fraction of genes contains disproportionally high levels of RNAPII at their 5’-ends regardless of their activity.16 Moreover, thousands of these genes contain hallmarks of transcription initiation, such as the presence of initiating transcripts and trimethylation of histone H3 on lysine 4 (H3K4me3), but are devoid of productive transcription elongation.17,18 Given that developmental genes and stress-response genes, such as those essential for DNA-damage and immune-response pathway, are enriched in paused RNAPII,19 it is very likely that this mode of gene regulation enables a rapid transcriptional response to changing extracellular stimuli. Importantly, predicting these global analyses, investigation of a small set of genes, including those of HIV-1, the Drosophila hsp70, and human c-myc and fos, has revealed that stimulating the escape from RNAPII promoter-proximal pausing can be a rate-limiting step in transcription.20 Collectively, these studies indicate that post-recruitment control is another major mechanism controlling eukaryotic gene expression.

Which factors are essential for the generation of promoter-proximal pausing of RNAPII and its release from it? Pioneering studies employing in vitro transcription assays have determined that DBR sensitivity-inducing factor (DSIF/Spt4-Spt5) and a multi-subunit negative elongation factor (NELF) act cooperatively to arrest the RNAPII soon after promoter clearance.20 In support of its important role in the establishment of pausing, depletion of NELF in *Drosophila* S2 cells reduces the fraction of promoter-proximal paused genes by more than half.19 Notably, although many of these NELF-dependent genes are up-regulated, more then two thirds of them display lower expression due to increased nucleosome density at the promoter regions.21 In addition, recent work demonstrates the requirement of the kinase activity of Cdk7 for the promoter-proximal recruitment of NELF,7 further underscoring an interconnected nature of transcriptional mechanisms. The release of RNAPII from the pause can be stimulated by the recruitment of P-TEFb, which is in humans composed of the catalytic subunit Cdk9 and one of the three cyclin (Cyc) regulatory subunits CycT1, CycT2a and Cyc2Tb.7 Diverse mechanisms of recruiting P-TEFb have been described and not surprisingly, all of them share a common theme of tethering P-TEFb to RNAPII soon after promoter clearance. For example, in yeast *S. cerevisiae* and fission yeast *S. pombe*, a yeast P-TEFb orthologue Bur1/Bur2 and P-TEFb complexed with the mRNA capping enzyme, respectively, are recruited to RNAPII after promoter escape by binding the CTD Ser5P.22–23 Perhaps even more elegantly, the HIV-1 transactivator recruits P-TEFb to paused RNAPII by interacting cooperatively with the transactivation response RNA element that is present at 5’ termini of all viral transcripts.24 Finally, P-TEFb can be tethered to paused RNAPII by a number of promoter- or enhancer-bound transcriptional activators3 or via chromatin by the double bromodomain-containing protein Brd4, which reads a histone code generated in a signal-dependent manner.25–27 Despite the fact that numerous transcription units depend on P-TEFb,9 its general requirement for antagonizing promoter-proximal pausing of RNAPIII in metazoans remains an open question.

Upon recruitment, the kinase activity of P-TEFb is essential for stimulating transcription elongation through various mechanisms, which include alleviation of negative elongation factors and promotion of activity or recruitment of positive elongation factors that directly affect RNAPII elongation or its passage through chromatin (Fig. 1). P-TEFb achieves these tasks by phosphorylating CTD on Ser2, as well as the C-terminal region of the Spt5 subunit of DSIF.20,27 These events release NELF from RNAPII and convert DSIF into a positive elongation factor. In turn, DSIF cooperates with the elongation factors Tat-SF1 and the Paf1 complex (Paf1C) to directly stimulate the elongation of RNAPII by a yet to be defined mechanism.27 In addition, this trimeric module seems to be required for monoubiquitination of histone H2B at lysine 120 (H2BK120ub1),27 and at least in yeast, the P-TEFb orthologue Bur1/Bur2 stimulates the Rad6/Brel E3 ubiquitin ligase activity (RNF20/40 and UbcH6 in humans) essential for the analogous
H2BK123ub1 histone mark. In cooperation with H2BK120ub1, FACT (for facilitates chromatin transcription) helps RNAPII navigate through chromatin by displacing the H2A/H2B dimer from the nucleosome barrier and mediating nucleosome reassembly after the passage of RNAPII. Likewise, the elongation and splicing factor Spt6 that can function as a H3/H4 chaperone, binds the CTD Ser2P and may further assists FACT in nucleosome dynamics during RNAPII elongation. Finally, a reciprocal crosstalk between P-TEFb-dependent transcription elongation and splicing (see below) results in additional reinforcement of transcription elongation by RNAPII.

**7SK snRNP Represses the Kinase Activity of P-TEFb**

Given its key role in stimulating the release of RNAPII from promoter-proximal pausing, P-TEFb is subjected to a tight control in cells. Whereas transcriptionally active P-TEFb binds Brd4 and transcriptional activators such as NF-κB, a major fraction of P-TEFb is sequestered in a transcriptionally inactive complex [referred to herein as 7SK small nuclear ribonucleoprotein (7SK snRNP)], where its kinase activity is repressed. In addition to P-TEFb, 7SK snRNP contains the non-coding 7SK small nuclear RNA (7SK), hexamethylene bisacetamide-induced protein (HEXIM) 1 and 2, the La related protein 7 (LARP7), and a 7SK γ methylphosphate capping enzyme (MePCE), also called BCDIN3, which caps 7SK at its 5’-end. Therein, 7SK serves as a molecular scaffold, whose stability is ensured through its interaction with LARP7 and MePCE/BCDIN3, which form a trimeric, cell stress-resistant core 7SK snRNP. On the other hand, 7SK turns HEXIMs into P-TEFb inhibitors by causing their conformational change, which enables them to bind the cyclin subunits of P-TEFb and subsequently repress the kinase activity of Cdk9 through a yet to be defined mechanism. Supporting the biological relevance of the negative control of P-TEFb by 7SK snRNP, derepression of P-TEFb in the wake of 7SK snRNP disintegration has been linked to widespread defects of embryonic development and the onset of hypertrophic and hyperproliferative states such as cardiac hypertrophy and tumorigenesis. Considering that upon the release of P-TEFb, core 7SK snRNP serves as a platform for sequestering inhibitory splicing factors of a heterogeneous nuclear
ribonucleoprotein (hnRNP) protein family such as hnRNP A1 and A2/B1.\(^{37,38}\) 7SK snRNP may function as rheostat that controls a global and/or local supply of positive and negative regulators affecting cotranscriptional pre-mRNA splicing.

**P-TEFB Triggers Distinct Mechanisms to Link Transcription Elongation with Pre-mRNA Splicing**

Several recent studies have contributed fresh insights into a long-standing quest of what may be the nature of coupling between transcription elongation and cotranscriptional pre-mRNA splicing. Given that the phosphorylated CTD enhances all pre-mRNA maturation processes in the course of transcription in vitro and that CTD deletion impairs these cotranscriptional reactions in vivo,\(^2,3\) it may not come as a surprise that several coupling mechanisms involve an interplay between transcription elongation factors, splicing regulators, and the CTD of RNAPII (Fig. 1B,C). Perhaps the most direct demonstration of how a transcription elongation factor can impact splicing has been provided by circumventing the negative control of P-TEFB, which has revealed that derepression of P-TEFB stimulates not only elongation, but also alternative splicing of nascent pre-mRNA.\(^{11}\) Specifically, upon disintegration of 7SK snRNP via depletion of LARP7 or MePCE/BCDIN3, P-TEFB promotes inclusion of an alternative exon containing an exonic splicing enhancer (ESE) element and a sub-optimal 3’ splice site through its increased occupancy at the promoter and exonic region of the gene.

As a consequence, this event results in elevated levels of the CTD Ser2P, which in turn facilitates the recruitment of the SR splicing factor SF2/ASF and possibly other SR proteins to RNAPII. Importantly, a significant change in splicing patterns of two developmentally-regulated genes has also been observed in response to disassembly of 7SK snRNP in zebrafish embryos. According to a current model of P-TEFB-mediated modulation of alternative splicing, SR splicing factors such as SF2/ASF facilitate splice site recognition, leading to stimulation of the early steps of spliceosome formation.\(^{19}\) In this scenario, the CTD Ser2P-bound SF2/ASF interacts with the spliceosomal U1 and U2 snRNPs and the U2 auxiliary factor (U2AF) heterodimer to tether them to the elongating RNAPII. In turn, these regulators have immediate access to their cognate RNA elements present on the nascent mRNA precursors. Specifically, by binding the ESE element, SF2/ASF recruits the U1 snRNP to the 5’ splice site and the U2AF complex and U2 snRNP to the 3’ splice site. Overall, these protein:protein and protein:RNA interactions nucleate assembly of the pre-spliceosome, which is completed by the addition of the U4/U6-U5 tri-snRNP. Upon an extensive restructuring, including the release of the U1 and U4 snRNPs, the active spliceosome is formed, resulting in the inclusion of an alternative exon. Of note, knockdown of SF2/ASF and a related SR protein SC35 reduce the recruitment of P-TEFB and levels of the CTD Ser2P at the promoter and exonic regions of genes.\(^{11,40}\) Moreover, depletion of SC35 induces accumulation of RNAPII on the body of select genes, leading to attenuated transcription elongation.\(^{40}\) Collectively, these studies establish an important role of 7SK snRNP and P-TEFB in alternative splicing and illustrate further that transcription elongation and cotranscriptional splicing can influence each other in a reciprocal manner.

Another exciting aspect related to a control of nascent transcript maturation by P-TEFB comes from studies of an inducible inflammatory gene expression programme in macrophages triggered by Toll-like receptor signalling.\(^{44}\) In the absence of the external trigger, many of the GC-rich primary response genes have preloaded RNAPII containing the CTD Ser5P but lacking considerable levels of the CTD Ser2P mark. Surprisingly, RNAPII elongation still occurs, perhaps due to the basal CTD Ser2P levels, but is effectively divorced from cotranscriptional processes as the synthesized transcripts contain splicing and most likely also 3’-end maturation defects. In contrast, signal-dependent recruitment of P-TEFB by Brd4, which detects inducibly acquired histone H4 acetylation on lysines 5, 8, and 12, leads to robust levels of the CTD Ser2P and subsequent generation of high amounts of mature, spliced transcripts of the primary response genes.

In addition to the above-mentioned stimulation of cotranscriptional splicing by P-TEFB through the tethering of the spliceosomal components to RNAPII via the CTD Ser2P-associated SR proteins, a variety of distinct mechanisms may operate alone or in cooperation to link transcription elongation with pre-mRNA splicing (Fig. 1B,C). An intriguing mechanism that couples P-TEFB-mediated phosphorylation of CTD on Ser2 to splicing of nascent transcripts is operating through Spt6, which binds a series of CTD Ser2P residues within the N-terminal stretch of 15 tandem consensus repeats of the CTD.\(^{41}\) In fact, abrogation of this interaction or depletion of the Spt6-binding protein Iws1 (for interacts with Spt6) leads to splicing defects of HIV-1 and c-myc transcripts.\(^{12}\) Although the underlying mechanism remains to be defined, the CTD Ser2P-bound Spt6/Iws1 heterodimer may connect transcription elongation to splicing via strengthening the recruitment of the histone H3 lysine 36 (H3K36) methyltransferase HYPB/Sed5 to the doubly phosphorylated CTD on Ser2 and Ser5 for trimethylation of histone H3 on lysine 36 (H3K36me3).\(^{13}\) The H3K36me3 mark, which is found in the body of genes and is a hallmark of productive transcription elongation, seems to be specifically enriched on exons\(^{40}\) and could thus serve as a recognition platform of a yet to be identified protein complex that might stimulate splicing reaction in the course of transcription. Finally, several lines of evidence suggest that P-TEFB promotes splicing upon conversion of DSIF into a positive transcription elongation factor. For example, transcription elongation factor Tat-SF1 associates with multiple spliceosomal U snRNPs\(^{42}\) and its yeast counterpart, Cus2p, interacts genetically and physically with the splicing machinery.\(^{43}\) Moreover, accumulation of unspliced transcripts, particularly of highly transcribed genes with long introns, occurs in S. cerevisiae.\(^{44,45}\) Importantly, P-TEFB and Pat1C are instrumental for monoubiquitination of H2BK120, which could be analogously to the scenario in S. cerevisiae a prerequisite for the H3K4me3 mark.\(^{25}\) This chromatin modification
indicative of productive transcription at 5’-ends of genes is subsequently recognized by an ATP-dependent chromatin remodeling protein and histone chaperone CHD1, which recruits post-initiation factors including U2 snRNP to 5’ ends of genes. Taken together, it is thus becoming apparent that upon phosphorylation of the Spt5 subunit of DSIF by P-TEFb, the subsequently assembled trimeric DSIF/Paf1C/Tat-SF1 complex not only stimulates RNAPII elongation, but also increases a local supply of splicingosomale U snRNPs for promoting cotranscriptional pre-mRNA splicing.

Perspectives

Since the initial identification of P-TEFb and negative transcription elongation factors by using in vitro transcription reconstitution assays, we have come a long way in our understanding of a transcriptional checkpoint operating at the transition from RNAPII promoter-proximal pausing to productive transcription elongation. Further use of purified transcription systems will likely yield novel players controlling transcription elongation. On the other hand, as the wall between transcription and cotranscriptional processes continues to fall, additional unexpected roles of transcription elongation factors in pre-mRNA processing and RNA processing factors in transcription are awaiting to be revealed. Moreover, in the light of recent evidence indicating a preferential nucleosome positioning in exons, particularly in those flanked by suboptimal splice sites, a Brd4-mediated deployment of P-TEFb from chromatin could be an elegant way to regulate alternative splicing. Also, given that in the case of divergent transcription, a common characteristic of active promoters, productive transcription elongation occurs only in the direction of genes, it would be interesting to determine to what extent could this transcriptional directionality be ascribed to the reciprocal coupling of RNAPII elongation to pre-mRNA maturation. Finally, P-TEFb is assuming an important role in embryonic development and its deregulation has been associated with the onset of fatal diseases such as cardiac hypertrophy and cancer. Considering that alternative pre-mRNA processing events operate during transcription of most genes and have been linked to a variety of unwanted cellular processes including tumorigenesis, identification of mature transcriptome critical for enabling the unrestricted cell growth in response to overly active P-TEFb remains a pressing challenge for the future.

Acknowledgments

We would like to thank Kalle Saksela and B. Matija Peterlin for support. This work was funded by the Academy of Finland FiDiPro programme (grant number 1127313). We would like to apologize to many colleagues for not being able to cite all relevant work due to space restrictions.

References