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CDK9 keeps RNA polymerase II on track

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Abstract

Cyclin-dependent kinase 9 (CDK9), the kinase component of positive transcription elongation factor b (P-TEFb), is essential for transcription of most protein-coding genes by RNA polymerase II (RNAPII). By releasing promoter-proximally paused RNAPII into gene bodies, CDK9 controls the entry of RNAPII into productive elongation and is, therefore, critical for efficient synthesis of full-length messenger (m)RNAs. In recent years, new players involved in P-TEFb-dependent processes have been identified and an important function of CDK9 in coordinating elongation with transcription initiation and termination has been unveiled. As the regulatory functions of CDK9 in gene expression continue to expand, a number of human pathologies, including cancers, have been associated with aberrant CDK9 activity, underscoring the need to properly regulate CDK9. Here, I provide an overview of CDK9 function and regulation, with an emphasis on CDK9 dysregulation in human diseases.

Keywords Cyclin T1 · RNA polymerase II CTD · Transcriptional checkpoint · 7SK RNA · Promoter-proximal pausing · HIV

Introduction

Cyclin dependent kinases (CDK) are serine/threonine kinases that are involved in either cell division (by promoting progression through the cell cycle), transcription by RNAPII, or both [1, 2]. Both ‘cycling CDKs’ and ‘transcriptional CDKs’ associate with a cyclin regulatory subunit that is essential for enzymatic activity. Contrary to cycling CDKs whose activity varies during the cell cycle, due to regulated cyclin degradation (this is the case for CDK1, 2, 3, 4 and 6), transcriptional CDKs keep generally constant levels of expression and activity [3]. CDK9 is the most extensively studied transcriptional CDK. Together with a cyclin T subunit, CDK9 forms the positive transcription elongation factor b (P-TEFb) that is required for efficient transcription of most RNAPII-transcribed genes. P-TEFb stimulates productive elongation through releasing promoter-proximal paused RNAPII into gene bodies [4, 5]. RNAPII pausing and release are decisive steps in the transcription cycle of the vast majority of actively transcribed protein-coding genes in both

basal- and stimulus-regulated conditions, and are thought to enable a synchronous response to developmental cues and external stimuli [6]. The impact of CDK9 on RNAPII transcription extends beyond transcriptional elongation, as it also influences transcription initiation and termination, and helps RNAPII compartmentalization into phase-separated biological condensates [7]. Accurate regulation of CDK9 activity is, therefore, critical to maintain the right transcriptional output, and various pathologies have been associated with dysregulation of P-TEFb activity [8]. Here, I review the current knowledge of CDK9 function and regulation, and its involvement in human diseases.

The general transcription factor P-TEFb

CDK9 is a nuclear protein that is expressed in all human tissues, with higher levels in terminally differentiated cells. The N-terminal region of CDK9 contains a modified PSTAIRE motif (PITALRE) which is characteristic of the CDK family kinases [2]. Two isoforms of CDK9 are expressed from the same gene and termed CDK9₍₄₂₎ and CDK9₍₅₅₎ based on their respective molecular weight. The 55-kDa isoform results from the use of an alternative promoter that generates a 5’-expanded first exon encoding a 117 N-terminal amino acids extension [9]. While the CDK9₍₄₂₎ isoform is generally

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the most highly expressed, the relative abundance of the two proteins varies among cell lines and tissues, with CDK9₍₅₅₎ more highly expressed in brain and liver. The ratio between the two isoforms can also change in response to intra- and extra-cellular signals [9–12]. CDK9₍₄₂₎ and CDK9₍₅₅₎ display the same reactivity and specificity towards substrates and seem to behave similarly [9, 11]. However, specific roles for CDK9₍₅₅₎ in muscle regeneration, DNA repair and apoptosis have been proposed [10, 11, 13].

Both CDK9 isoforms can form heterodimeric complexes with Cyclin T1, T2a or T2b [14] but Cyclin T1 is the predominant CDK9-associated cyclin. Cyclin T2a and T2b arise from alternatively spliced transcripts generated from the same gene. They are usually present at lower levels than Cyclin T1, and are considered as minor CDK9 partners. However, Cyclin T2 may have specific functions in muscle differentiation [15–18] and is essential for mouse embryogenesis [19]. All Cyclin Ts have a characteristic cyclin box at their N-Termini, and a histidine-rich domain implicated in substrate recognition in their C-terminal regions [20, 21]. Since T2-type cyclins often display the same biochemical properties as Cyclin T1, I will consider here the Cdk9₍₄₂₎/CycT1 heterodimer as the positive transcription elongation factor b (P-TEFb).

To be active, CDK9 needs not only to be associated with a Cyclin T, but also to be phosphorylated on the threonine 186 residue (Thr186) located in a conserved region known as the T-loop [2]. Phosphorylation of Thr186 is primarily accomplished by CDK7, another transcriptional CDK, and maybe also by self-phosphorylation [22–24]. The resolution of CDK9/Cyclin T1 crystallographic structure suggests that phosphorylation of Thr186 positions the T-loop correctly for substrate recognition and generates the specificity for the Ser/Thr-Pro motif found in major P-TEFb substrates [23].

Functions of P-TEFb in RNAPII transcription

Transcription by RNAPII can be subdivided into three main stages: initiation, elongation and termination. During initiation, transcription factors (TFs) cooperate with coactivators, such as Mediator, to recruit the general transcription factors and RNAPII to a gene promoter to assemble the pre-initiation complex (PIC). Initiation is a very intricate process that comprises several sequential steps leading to unwinding of the DNA double helix. The transition to elongation occurs when RNAPII begins the synthesis of the RNA molecule and escapes the promoter. During elongation, either RNAPII ‘walks’ along DNA or the DNA is spooled through the polymerase [25]. However, after initiation and promoter escape, movement into the gene is generally blocked 30–60 bp downstream from the transcription start site (TSS) at an early elongation checkpoint (EEC) [6]. A failure of the

RNAPII transcription complex to transition to productive elongation at this stage leads to abortive transcription. If paused RNAPII is successfully converted into elongation-competent polymerase, RNA synthesis resumes and a full-length pre-mRNA can be made. Finally, for genes encoding polyadenylated mRNA, termination occurs after transcription of the polyadenylation site (pA site) when cleavage of the RNA and changes to the RNAPII elongation complex cause termination downstream [26].

P-TEFb activity is critical for release of RNAPII that is stalled at the EEC. CDK9-mediated RNAPII pause release has recently been shown to influence the frequency of transcription initiation [27, 28]. In addition to this well-established function in early elongation control, CDK9 activity is also required for co-transcriptional processing of nascent transcripts, and for the dynamics of RNAPII close to poly(A) sites [29–31]. Thus, P-TEFb potentially regulates, directly or indirectly, all stages of the RNAPII transcription cycle.

Regulation of RNAPII pausing during early elongation

Although other factors have been implicated in pause establishment and release, promoter-proximal pausing primarily relies on the combined action of negative factors that impede RNAPII progression and positive factors that instead stimulate the transition to productive elongation [6] (Fig. 1). Shortly after initiation, RNAPII falls under the influence of two negative factors, DRB-sensitivity inducing factor (DSIF) and negative elongation factor (NELF) [32, 33]. DSIF, a heterodimer composed of Spt4 and Spt5, binds the transcription complex after RNAPII has escaped the promoter when the Spt5-binding site becomes accessible [34, 35]. Association of DSIF to the transcription machinery is also enhanced by the interaction of Spt5 with the emerging nascent RNA [36]. The four-subunit NELF complex (composed of NELF-A-B-C/D and -E) then joins the early elongation complex as it transcribes the promoter-proximal region through recognition of the RNAPII/Spt5 interface [32, 35, 37, 38]. The NELF-E subunit establishes additional contacts with the nascent RNA, where it recognizes a short consensus RNA element enriched at transcriptionally paused genes [39–41]. Whether this interaction assists in RNAPII pausing is not clear. NELF rather stabilizes paused complexes through blocking access to TFIIIS [35], the activity of which is needed to rescue backtracked polymerase to allow resumption of elongation [42, 43]. In addition, NELF binding restrains RNAPII mobility and maintains the active site of paused RNAPII in a tilted conformation that is incompatible with RNA elongation [35]. Thus, DSIF/NELF-associated RNAPII is more likely to experience stable pausing, especially near the TSS, where it encounters the first nucleosome in the transcribed region [44–48]. Apart from DSIF

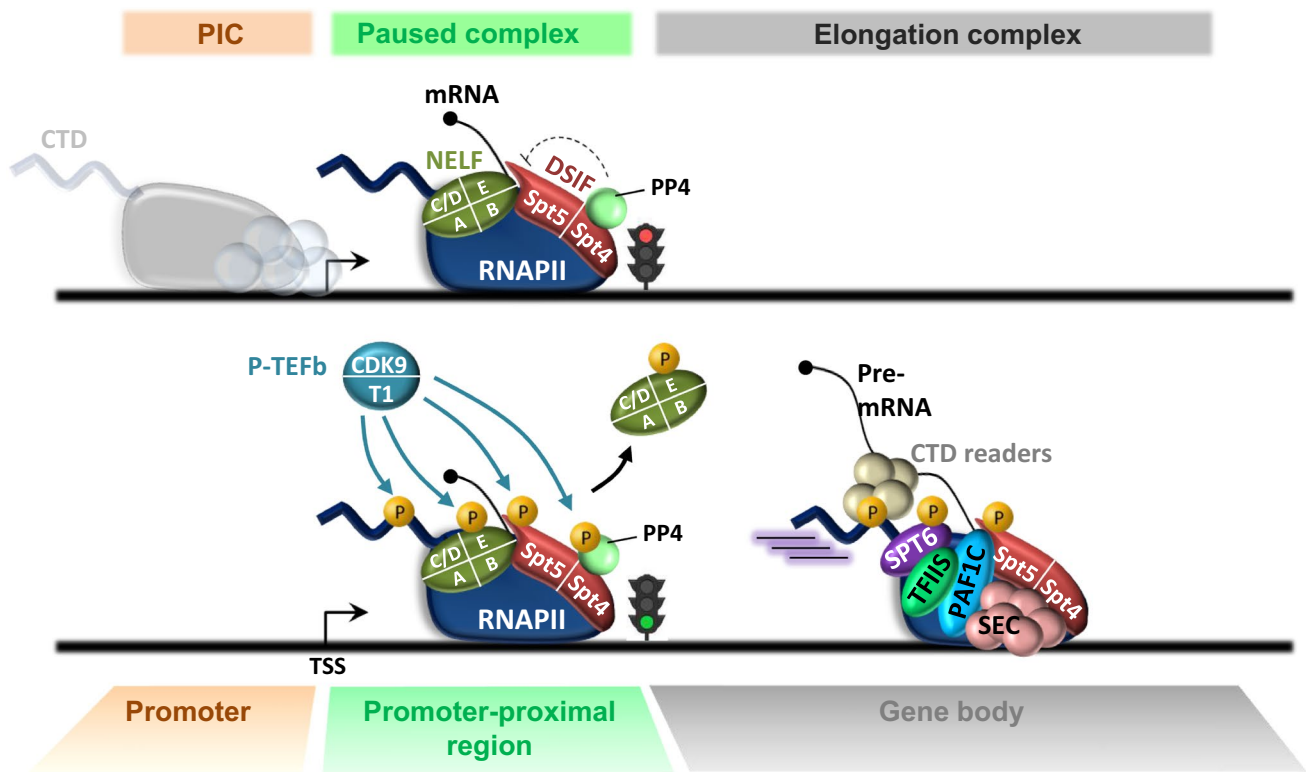


Fig. 1 P-TEFb promotes the release of RNAPII from pause sites. Shortly after initiation, progression of RNAPII along the gene is halted by the coordinated action of NELF and DSIF, while protein phosphatase 4 (PP4) helps maintain the Spt5 subunit of DSIF in an unphosphorylated state. Once P-TEFb is recruited, it phosphorylates DSIF, NELF and the RNAPII CTD. Phosphorylation of Spt5 converts DSIF in a positive elongation factor, and NELF is evicted from the RNAPII complex. Phosphorylation of Spt5 is further reinforced

by inhibitory phosphorylation of PP4. Upon pause release, accessory proteins join the elongation complex to increase RNAPII processivity and to increase the elongation rate. Phosphorylation of the CTD facilitates the recruitment of CTD reader proteins required for pre-mRNA processing. PIC: pre-initiation complex. CTD: carboxyl-terminal domain. NELF: negative elongation factor. DSIF: DRB-sensitivity inhibitory factor. TSS: transcription start site. Pre-mRNA: pre-messenger RNA

and NELF, other factors such as SIRT6 [49], Gdown1 [50, 51], the PAF1 complex (PAF1C) [52, 53], and FACT [54] may also contribute to locking the poised RNAPII, but their direct involvement in pausing is still subject of debate.

As escape of RNAPII from pause sites requires the kinase activity of P-TEFb (Fig. 1), treatment of cells with commonly used CDK9 inhibitors such as DRB (5,6-dichloro-l- β -D-ribofuranosyl benzimidazole) or Flavopiridol (FP) results in an accumulation of RNAPII in the promoter-proximal region of most protein-coding genes [45, 55–58]. Mechanistically, three main CDK9 substrates have been identified within the early elongation complex: the two negative elongation factors, DSIF and NELF, and the carboxy-terminal domain (CTD) of RPB1, the largest RNAPII subunit. Probably the most important target of CDK9 in the pause release process is the Spt5 subunit of DSIF, the phosphorylation of which triggers dissociation of NELF from RNAPII [37, 59]. Phosphorylation of NELF itself, as well as its modification by PARP-1, may also help relieve the DSIF/NELF-mediated blockage of early elongation [60–62]. P-TEFb-mediated

dissociation of NELF from the transcription complex should theoretically allow TFIIS to rescue backtracked RNAPII [35, 43] while converting Spt5 into a positive factor that remains associated with RNAPII across the gene body [58, 63]. Phosphorylation of Spt5 occurs in its repetitive C-terminal repeat region (CTR) that, like the RNAPII CTD, harbors the typical Ser/Thr-Pro motif found in most CDK substrates [59]. CDK9 strengthens SPT5 phosphorylation by also inhibiting protein phosphatase 4 (PP4), a phosphatase that stabilizes promoter-proximal pausing by keeping SPT5 unphosphorylated in the 5' region of genes [64] (Fig. 1). After RNAPII is released, Spt5 enhances RNAPII processivity during transcriptional elongation [59, 65–69] and promotes co-transcriptional recruitment of the PAF1C, Pin1 and Tat-SF1 elongation factors, as well as RNA 3' end processing factors [70–74]. Located near the exiting RNA in the elongation complex [65], the phosphorylated Spt5 CTR could thus behave as a recruitment platform for a number of factors that facilitate elongation and co-transcriptional RNA processing. The P-TEFb-dependent recruitment of PAF1C,

through Spt5, is of particular importance. PAF1C has been recently implicated in regulation of RNAPII pausing [52, 75] and also exerts critical functions in transcription elongation beyond the pause release step [75–78]. Structural studies indicate that NELF and PAF1C bind to RNAPII in a mutually exclusive manner [61]. P-TEFb-mediated phosphorylation of NELF and SPT5 influences the competition between PAF1C and NELF for binding of RNAPII, favoring NELF ejection and PAF1C recruitment. The prolonged presence of PAF1C within the elongation complex could prevent later reassociation of NELF, explaining why RNAPII is less prone to long-lived pausing during productive elongation. PAF1C also favours the later recruitment of CDK12 [75], another transcriptional CDK that targets the RNAPII CTD and reinforce Ser2P in the body of the gene [79].

In addition to DSIF and NELF, the carboxyl-terminal domain (CTD) of the catalytic subunit RPB1 of RNAPII is also a major target of P-TEFb. CDK9 is commonly considered as a kinase targeting serine 2 residues (Ser2) in the consensus heptapeptide Tyr¹-Ser²-Pro³-Thr⁴-Ser⁵-Pro⁶-Ser⁷ (YSPTSPS) that comprises the RNAPII CTD, but it may also phosphorylate Ser5 and Ser7 [80, 81]. Targeted post-translational modifications of residues within the CTD basic unit, which is repeated 52 times in the human enzyme, govern the association of factors with RNAPII during the transcription cycle and coordinate transcription with mRNA processing [82, 83] [84]. Although phosphorylation of Tyr1, Thr4 and Ser7 also occurs, mass spectrometry has revealed that phosphorylation of Ser2 and Ser5 are the most abundant CTD modifications in both yeast and humans [85, 86]. The CTD is hypophosphorylated when RNAPII is recruited within the PIC, and is first phosphorylated on both Ser5 and Ser7 by CDK7, as part as the TFIIF complex, during initiation. Ser5P recruits the capping enzyme and stimulates rapid and efficient capping of the nascent RNA as soon as it emerges from RNAPII, while Ser7P, helped by Tyr1P, primes the CTD for subsequent Ser2 phosphorylation by P-TEFb [80, 81]. Recruitment of P-TEFb to the transcriptional machinery also relies on prior citrullination of a specific arginine residue (R1810) located on repeat 31 of the mammalian RNAPII CTD [87]. Thus, phosphorylation by CDK9 can only take place after prior decoration of the CTD, perhaps to ensure effective initiation and mRNA capping. Ser2P is considered as a hallmark of productive elongation and is markedly enriched across gene bodies, with a peak at the 3' end of genes, over terminator regions. Such high level of Ser2P likely reflects the cooperativity of CDK9 and CDK12 at the 3' end of genes (see below). Phosphorylation of the CTD of elongating RNAPII enables recruitment of additional CTD-associating proteins and positions them near the site of transcription. Factors that recognize the Ser2P mark mainly encompass chromatin-remodeling enzymes and RNA processing complexes [88–95]. Accordingly, CDK9 activity

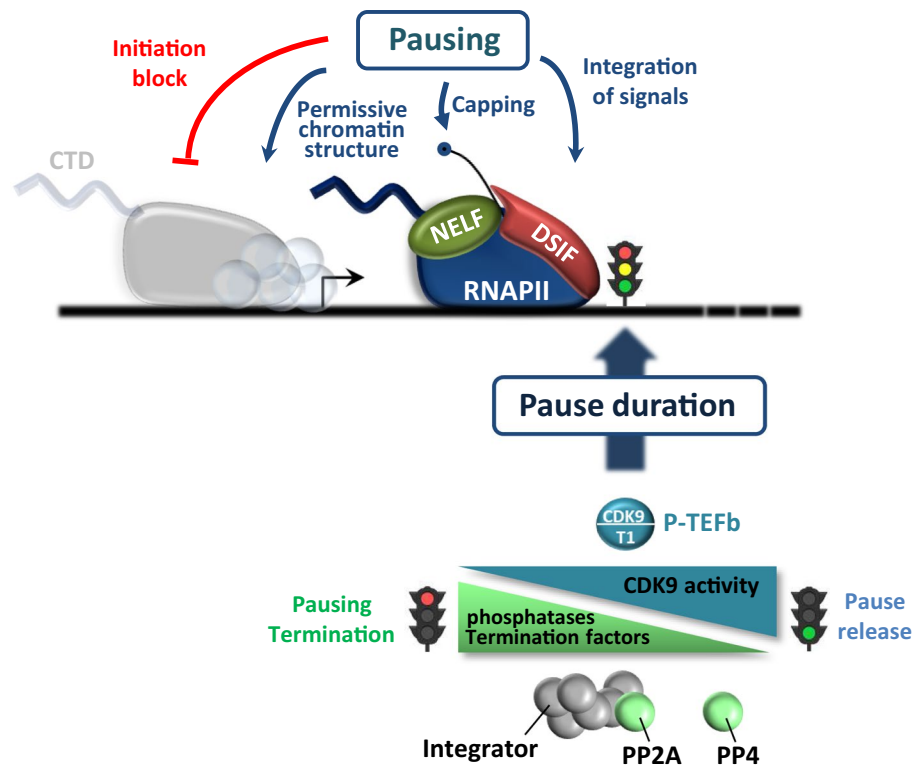
is critical for efficient splicing, and for the recruitment of polyadenylation factors at the 3' ends of RNAPII-transcribed genes [88, 89, 91]. Recent data indicate that P-TEFb also phosphorylates the RNAPII CTD linker region that connects the catalytic core of the enzyme to the CTD during the transition to productive elongation and by doing so, promotes recruitment of the SPT6 histone chaperone to the elongation complex [61, 96, 97]. Spt6 supports efficient transcription elongation through stimulating disassembly and reassembly of nucleosomes during RNAPII progression [98–101]. Finally, P-TEFb is often incorporated into large complexes, called Super Elongation Complexes (SECs), which comprise multiple transcription elongation factors [102]. When recruited to genes, the SEC simultaneously provides several activities that increase RNAPII processivity and stimulate RNA synthesis [103–105].

By counteracting the effects of negative elongation factors, decorating the RNAPII CTD, and supplying RNAPII with key elongation factors, P-TEFb not only releases the DSIF/NELF-mediated elongation barrier but also equips the polymerase to ensure the efficiency of downstream transcriptional and co-transcriptional events. Modulating pause release by P-TEFb is, therefore, an elegant way to fine-tune gene expression to produce the right transcriptional output.

Functional significance of RNAPII pausing

In all metazoans, RNAPII distribution along genes shows an increased density just downstream of the TSS [106, 107]. As this profile primarily results from an equilibrium between RNAPII recruitment, initiation, and pause release into either productive elongation or termination (see below), it suggests that the rate of pause release is generally lower than the rate of initiation. While the kinetics of these two regulatory stages were long considered to be independent of each other, recent studies have highlighted important connections between pausing and the frequency of transcription initiation. In both *Drosophila* and human cells, an inverse correlation between the levels of promoter-proximally paused RNAPII and of newly initiating RNAPII has been observed, with highly paused genes displaying lower initiation rates [27, 28]. Accordingly, increasing RNAPII pausing through inhibition of CDK9-mediated pause release generally results in a concomitant decrease of transcription initiation (Fig. 2). The mechanisms underlying this relationship are not clearly defined, but the presence of RNAPII at pause sites may sterically impede subsequent initiation by another polymerase and limit the frequency of re-initiation [28]. However, RNAPII pausing itself also have a positive effect on transcription initiation as accumulation of RNAPII in promoter-proximal regions creates a permissive chromatin structure that allows factors to access the promoter [108–110]. Indeed, RNA interference-mediated depletion

Fig. 2 Functions of promoter-proximal pausing and regulation of pause duration. Promoter-proximal pausing limits the rate of new initiation by RNAPII, facilitates capping of the nascent pre-mRNA and provides a window of time for integration of external signals or stimuli. Pause duration is tightly regulated and can vary from one gene to another. The residence time of RNAPII at pause sites is driven by the balance between P-TEFb recruitment, phosphatase activities and recruitment of termination factors such as Integrator, which can promote premature termination. CTD: carboxyl-terminal domain. NELF: negative elongation factor. DSIF: DRB-sensitivity inhibitory factor. PP: protein phosphatase



of NELF reduces RNAPII pausing, increases nucleosome occupancy at many promoters and decreases transcription initiation [109, 111] (Fig. 2). Thus, the emerging view is that CDK9-mediated release of pausing stimulates both productive elongation (by promoting progression of RNAPII into gene bodies) and initiation (by reducing RNAPII occupancy at pause sites), which could have synergistic effects on the amount of transcription.

Promoter-proximal pausing was also proposed to provide a window of time for co-transcriptional capping of the nascent RNA to occur [112]. As addition of a 5' methyl-7-Guanosine (m7G) cap protects the 5' end of nascent mRNA from degradation [113], it is important that this happens as soon as possible after the RNA 5' end emerges from RNAPII. Phosphorylation of Ser5 residues by CDK7 early in the transcription cycle turns the RNAPII CTD into a landing pad for the capping machinery [114–117]. The capping enzyme can also access the nascent RNA through interaction with the Spt5 subunit of DSIF, that also stimulates capping [118–120]. Capping appears to start when the transcript is only 20–25 nucleotides long [121] and may occur progressively as RNAPII moves towards the pause region, raising the possibility that nascent RNAs are already capped when NELF-mediated pausing occurs. NELF has recently been shown to recruit the cap-binding complex (CBC) to the transcription machinery [122, 123] to interact with the cap to protect the capped RNA from decapping [124]. The CBC may in turn help to recruit P-TEFb [91].

Thus, DSIF/NELF-mediated RNAPII pausing might function as a quality-control process ensuring that mRNA are properly protected at their 5' end before RNAPII enters productive elongation (Fig. 2).

On some genes, RNAPII is poised for activation and function as scaffold for integration of environmental and developmental signals [125] (Fig. 2). Promoter-proximal pausing could provide storage of transcriptionally engaged, 'ready to go' polymerases, awaiting activation stimuli for rapid and synchronous release of RNAPII into productive elongation [126, 127]. Several key biological processes may rely on poised RNAPII for gene activation, including the heat shock response [128, 129], early embryonic development [126, 130, 131], inflammatory responses [132], the DNA damage response [133, 134] and hormone-regulated signaling [131, 135]. Keeping the polymerase in a state of readiness could thus allow transcription of biologically relevant genes to rapidly respond to external/developmental cues [132, 136]. The early elongation checkpoint thus adds a further possibility to regulate transcription before RNAPII has gone very far [6] and allows synergic or antagonists signals to be integrated during the early steps of transcription.

On the majority of genes, paused RNAPII appears to be quite stable, with a residence time between 5 and 10 min [28, 45, 137]. However, RNAPII can sometimes remain engaged on a gene for 30–60 min while awaiting signals for pause release [28, 56, 137]. It is likely that poor recruitment of P-TEFb to these genes is responsible for increased paused

RNAPII stability. Since paused RNAPII blocks new initiation, prolonged pausing may make a gene refractory to activation [138]. In contrast, a significant proportion of genes harbors very unstable promoter-proximally paused RNAPII that is quickly dissociated from the DNA template through premature termination [110, 139, 140]. The rapid turnover of RNAPII at these genes prevents the polymerase from entering productive elongation, thus repressing gene activity. The instability of this paused RNAPII is due to the recruitment of the integrator complex, whose endonucleolytic activity triggers cleavage of nascent mRNA transcripts and promoter-proximal termination [139]. Recent findings showed that two protein phosphatases, PP2A and PP4, can keep Spt5 unphosphorylated in the promoter-proximal region, thus promoting pausing by counteracting CDK9-mediated phosphorylation [64, 141, 142]. A non-canonical PP2A enzyme, associated with the Integrator complex, may also dephosphorylate the RNAPII CTD [142, 143]. Thus, competitive recruitment of P-TEFb, phosphatases and termination factors during early elongation likely determines the stability and the fate of paused RNAPII (Fig. 2).

CDK9 regulates transcription beyond pausing

Genome-wide nascent transcription analysis techniques such as global run-on sequencing (GRO-seq) confirm that

RNAPII stalls close to the TSS of the majority of genes after addition of CDK9 inhibitors [29, 45, 84, 144]. As visualized on long genes, RNAPII that has already passed the EEC can continue to transcribe for long distances even if CDK9 activity is inhibited, demonstrating that sustained CDK9 activity is not mandatory for transcription through gene bodies [29, 45, 145]. However, when RNAPII reaches the end of the transcribed region, it terminates transcription prematurely at the end of the last exon, near the poly(A) site, at what has been termed a poly(A)-associated elongation checkpoint [29]. Phosphorylation of Spt5 by CDK9 may be critical to negotiate this checkpoint, as Spt5 association with the poly(A) region is drastically reduced by CDK9 inhibition. Indeed, CDK9-mediated phosphorylation of Spt5 is normally erased by protein phosphatase 1 (PP1) when RNAPII passes the poly(A) site [30, 146]. This drop in Spt5 phosphorylation is followed by RNAPII slowing down downstream of the pA site and becomes heavily phosphorylated on Ser2, thus facilitating the recruitment of factors involved in mRNA 3' end processing and termination [79, 147, 148] (Fig. 3). CDK9 activity is required to maintain the phosphorylation status of Spt5 upstream of the poly(A) site, most likely by preventing Spt5 dephosphorylation by PP1. Indeed, PP1 is a target of CDK9 in both yeast and human [30, 31] and this phosphorylation is inhibitory [30, 64]. Thus, CDK9

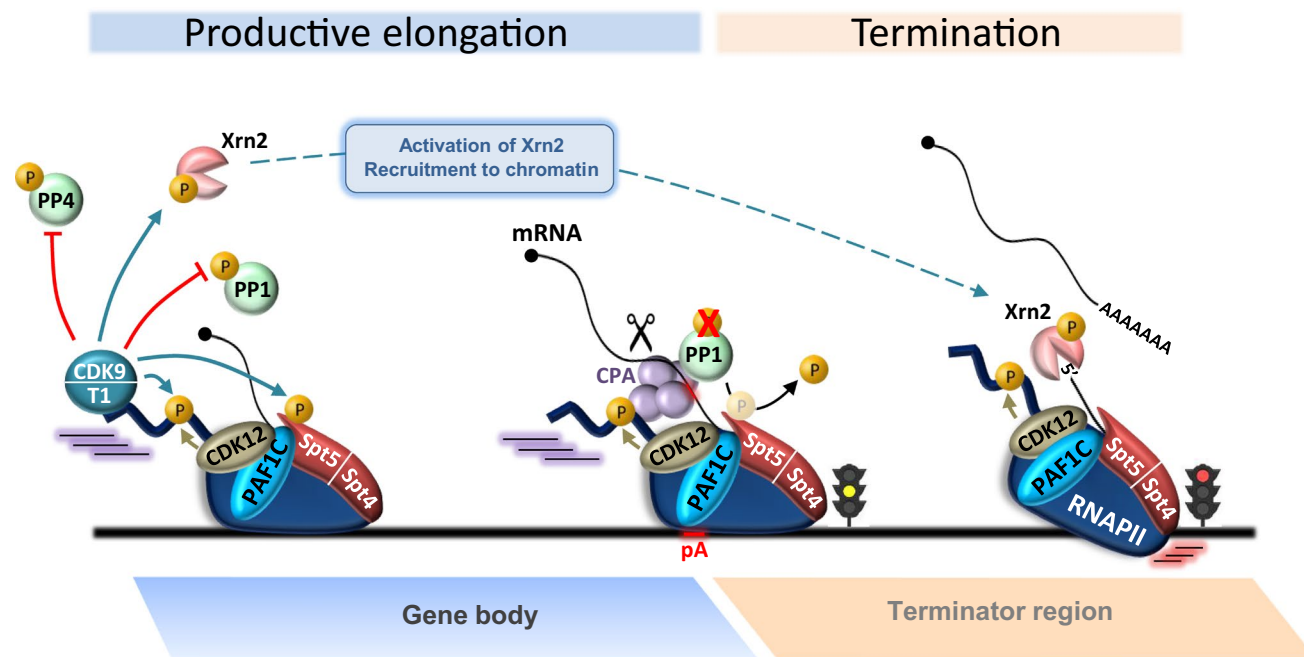


Fig. 3 CDK9 regulates transcription across the polyA site. During elongation, CDK9 phosphorylation inactivates PP4 and PP1 phosphatase activities, thus maintaining high levels of Spt5 phosphorylation. Once RNAPII transcribes through the polyA (pA) site, Ser2P recruits the cleavage and polyadenylation machinery (CPA), which is

critical for proper termination. Concomitantly, CDK9 activity drops and PP1 becomes active, leading to dephosphorylation of SPT5. Unphosphorylated Spt5 acts as a brake for RNAPII that becomes a preferential substrate for CDK9-activated Xrn2 exonuclease. PP: protein phosphatase. CPA: cleavage and polyadenylation factors

neutralizes PP1 activity during elongation, thereby preserving Spt5 phosphorylation until RNAPII reaches the poly(A) site. In turn, Spt5 phosphorylation prevents premature termination by maintaining rapid RNAPII elongation, and perhaps by delaying the action of termination factors until poly(A) site selection has occurred [147, 149].

CDK9 also directly regulates termination by promoting recruitment and activation of the 5' to 3' "torpedo" exonuclease Xrn2 via an RNAPII CTD-independent mechanism [31]. Phosphorylation of Xrn2 by CDK9 appears critical for chromatin localization of Xrn2 and efficient termination in human cells [31]. In the torpedo termination model, the pre-mRNA is cleaved after RNAPII transcribes the poly(A) site, and Xrn2 degrades the downstream RNA fragment co-transcriptionally until it collides with RNAPII and dissociates it from the DNA template [26, 150]. As the torpedo model is based on the ability of Xrn2 to degrade nascent RNA faster than RNAPII synthesizes it, polymerases slowly elongating downstream of the poly(A) site are easily caught by the activated Xrn2, facilitating RNAPII dissociation and transcription termination (Fig. 3).

CDK9, therefore, plays an important role in regulating the elongation to termination switch, a step that is more strictly regulated than initially thought. The addition and removal of phosphate by the CDK9/PP1 pair is critical for Spt5-dependent regulation of RNAPII dynamics during its entry to the terminator region. The mechanism by which PP1 becomes active once RNAPII has transcribed through the poly(A) remains unknown, but it could result from targeted dephosphorylation of PP1 and/or a drop in CDK9 activity. In yeast, the CDK9 homolog dissociates from the elongation complex upstream of the poly(A) site [151], which could provide an explanation for PP1 activation. Recognition of the poly(A) site is also required for the change in Spt5 phosphorylation, suggesting that assembly of a 3' processing complex on the poly(A) site and/or cleavage of the nascent RNA is involved in PP1 activation [146] (Fig. 3). Interestingly, the yeast cleavage and polyadenylation factor (CPF) contains a PP1 holoenzyme [152, 153], and could target PP1 to the transcription machinery at the 3' end of genes.

Additional targets of P-TEFb include the AFF1 and AFF4 subunits of the SEC [154, 155], the androgen receptor [156], pih2 [157], pRB [158] and p53 [159, 160]. P-TEFb also phosphorylates the chromatin regulators histone H1, UBE2A, BRG1 and Tip60 [161–164]. A recent in vitro study aimed at identifying the full repertoire of CDK9 targets revealed numerous additional factors (more than 100), half of which are implicated in transcription and/or RNA metabolism [31]. Decker et al. (2019) used a CDK9 analog-sensitive human cell line to identify 120 phosphosites which were quantitatively decreased upon CDK9 inhibition and confirmed that the major CDK9 substrates were transcription- and RNA processing-associated factors [165]. Potential

new P-TEFb targets include splicing factors, chromatin modifiers, pause release factors and proteins implicated in RNA quality control. One should, therefore, expect future studies to assign additional function(s) to CDK9 and further expand the mechanisms by which it regulates RNAPII transcription.

CDK9 and CDK12: distinct CDKs with the same CTD target

It is important to note that CDK9 is not the only Ser2P kinase, as two other 'transcriptional' CDKs, CDK12 and CDK13, also contribute to this critical CTD modification [79, 166–170]. While both CDK12 and CDK13 are bound to cyclin K and target the CTD in vivo, most studies have focused on CDK12's function, mainly because it has emerged as an important player in human cancers [171, 172]. Depletion or inactivation of CDK12 leads to altered CTD phosphorylation in both *Drosophila* and human cells [166–168, 173–175]. Its genomic distribution suggests that CDK12 functions on elongating RNAPII, downstream of CDK9, and contributes to the increased Ser2P signals towards the 3' end of genes [79, 176] (Fig. 3). However, a number of studies have also identified Ser5 of the CTD as a target of CDK12 [168, 173, 175, 177]. CDK9 and CDK12 are close collaborators, as recruitment of CDK12 depends on CDK9 activity [75]. It makes the relative contribution of each kinase to Ser2P difficult to appraise. Although the CTD appeared to be the main substrate of CDK12 and CDK13, the exact function of these CDKs has long remained unclear [171]. Despite its localization on actively transcribed genes genome-wide [178], CDK12 was shown to specifically promote expression of subsets of human genes, i.e., DNA damage/DNA repair genes [167, 174, 178, 179] and DNA replication genes [173]. However, genome-wide nascent transcription analyses indicate that CDK12 is a global regulator of RNAPII processivity, required for efficient elongation of transcription of the vast majority of RNAPII-transcribed genes [168, 169]. CDK12 is required for stable association of PAF1C and Spt6, two critical elongation factors, with the elongating RNAPII machinery [168]. Maintenance of high RNAPII elongation rates by CDK12 may be critical to prevent premature transcription termination, particularly on long genes [173, 179–181]. Mechanistically, CDK12 activity suppresses cleavage at intronic polyadenylation sites, restricting production of truncated mRNAs. Since DNA damage response genes tend to harbor a higher density of intronic polyadenylation sites than other genes [179], loss of CDK12 primarily affects expression of this class of transcripts. CDK13 displays substantial redundancy with CDK12 for boosting RNAPII processivity [169], but is also specifically required for proper expression of small non-coding RNA genes, by activating RNA processing rather than transcription [174]. Identification of CDK12-associated

proteins and CDK12 targets supports a broad function for this kinase in transcription, and in co-transcriptional pre-mRNA processing [174, 179, 182, 183]. Accordingly, loss of CDK12 has been shown to impede 3' end processing of *C-FOS* and *C-MYC* genes [79, 184], likely reflecting defective Ser2P-mediated recruitment of RNA processing factors. Thus, the primary function of CDK12 (and CDK13) may be to maintain a sustained level of Ser2 phosphorylation on the elongating RNAPII (Fig. 3). By doing so, it helps RNAPII to reach maximum elongation rates to suppress usage of cryptic polyadenylation, which is particularly important on long and polyA site-rich genes [173, 179], but it also stimulates efficient co-transcriptional RNA processing.

CDK9, CDK12 and CDK13, therefore, all cooperate to ensure efficient elongation, as they all target Ser2 residues within the CTD and contribute to Ser2P [169]. Both CDK9 and CDK12 have been proposed to be key players in RNA processing, but it is difficult to disentangle the precise function of each CDK. As blocking CDK9 activity will affect subsequent CDK12 recruitment to the transcription machinery [75], the resulting effects could reflect the loss of both CDK activities. The use of pharmacological inhibitors has proven effective to block CDKs activity and study their functions. Although Flavopiridol and DRB inhibit CDK9 at lower concentrations than CDK12/CDK13 [177], complete inhibition of CDK9 could still significantly affect CDK12 and CDK13 activities, and potentially other kinases as well [182]. The recent development of more selective CDK9 inhibitors [185] and CRISPR/Cas9-engineered cell lines, which allow selective inactivation of CDK9, CDK12 and CDK13 by ATP analogs [27, 165, 168, 169, 173, 175], will help to decipher the function of each individual CDK in CTD phosphorylation and RNAPII transcription.

P-TEFb promotes formation of compartmentalized 'elongation-associated' foci

Microscopy studies have revealed dynamic foci within the nucleus that are enriched in RNAPII, termed transcription 'factories' or 'condensates' [7, 25, 186, 187]. These membrane-less compartments can form by liquid–liquid phase separation and rely on cooperative interactions between intrinsically disordered protein regions (IDRs) [188]. Although their components can continuously interact with the neighboring environment, the local concentration of factors is high within condensates, favoring intermolecular interactions and efficient biochemical reactions. Some transcription (co)factors use their disordered transactivation region to form such condensates at sites of active transcription [186, 187, 189–192]. They recruit and trap RNAPII through its intrinsically disordered hypophosphorylated CTD, providing a mechanism for efficient recruitment of RNAPII to both enhancers and promoter regions to support high rate of transcription initiation [190,

192, 193]. Importantly, CTD phosphorylation by CDK7 weakens interaction with activators and disrupts phase separation as RNAPII escapes the promoter [193, 194]. Thus, transcriptional condensates could be seen as dynamic hubs that enable efficient regulation of RNAPII transcription.

Recent data indicated a function for the histidine-rich domain (HRD) of cyclin T1 in creating another type of transcription condensate that instead of being connected to transcription initiation, is associated with elongation [7, 20] (Fig. 4). The low-complexity HDR of Cyclin T1 is located within a broader IDR in its C-term domain and it was shown to support both interaction with the CTD of RNAPII and phase separation [20, 21]. Consistently, Cyclin T1 lacking the HDR is unable to form droplets, displays a reduced activity towards the CTD as well as a shorter residence time on chromatin. The Cyclin T1 HRD may organize a phase-separated environment leading to local accumulation of RNAPII together with P-TEFb, thereby stimulating efficient hyperphosphorylation of the CTD and elongation. Recent data showed that Cyclin T1 can phase separate in the presence of SEC components [195]. These 'elongation-associated' condensates can be visualized in cells by imaging techniques and correspond to the nuclear speckles [20, 194, 196], which also contain a number of additional elongation and pre-mRNA processing factors [197, 198] (Fig. 4). Incorporation of the CTD within these compartments is enhanced upon pre-phosphorylation by CDK7 [20, 194], suggesting that CTD phosphorylation on Ser5/Ser7 relocates RNAPII from initiation-associated to elongation-associated condensates during early elongation (Fig. 4). Thus, 'elongation-associated' condensates could simultaneously concentrate pause release, elongation and co-transcriptional processing factors around transcriptionally engaged RNAPII complexes to favor robust transcription through chromatin. The fact that hypo and hyper-phosphorylated forms of RNAPII occupy contiguous nuclear locations [199] supports the hypothesis that RNAPII shuttles from 'initiation' to 'elongation' condensates, which would physically separate initiation- and elongation-related factors for increased efficiency. More work is needed to understand the impact of phase separation on each step of the transcription process. Given their reversible nature, future studies should also establish whether the appearance and disappearance of transcription condensates plays a role in regulating transcription programs, such as responses to developmental stimuli, viral infection or external cues.

Regulation of CDK9 activity

Since P-TEFb is a master regulator of RNAPII transcription, controlling its nuclear activity is of particular importance. Two main regulatory mechanisms together govern the rate

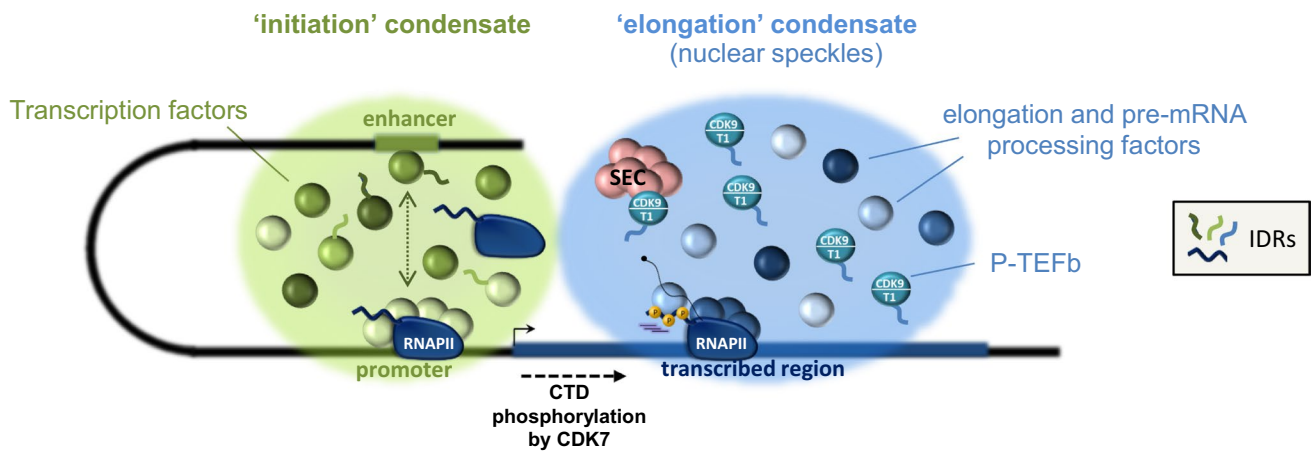


Fig. 4 Cyclin T1 promotes the formation of ‘elongation condensates’ through liquid–liquid phase separation. Intrinsically disordered regions (IDRs) of transcription factors trigger the formation of ‘initiation’ condensates at gene promoters and enhancers and favour recruitment of RNAPII and gene activity. The Cyclin T1 histidine-rich region (HRD) organizes a phase-separated environment that promotes incorporation of P-TEFb into another type of condensate

associated with transcription elongation (or nuclear speckles). These condensates simultaneously concentrate pause release, elongation and processing factors. CTD phosphorylation by CDK7 relocates RNAPII from ‘initiation’ to ‘elongation’ condensates, providing RNAPII with critical activities required for the next stage of the transcription cycle. CTD: carboxy-terminal domain

of CDK9-mediated release of paused RNAPII. First, CDK9 cellular availability can be restrained by the 7SK inhibitory snRNP. Second, P-TEFb has to be efficiently targeted to gene promoters through interaction with transcription factors/complexes.

The 7SK snRNP: a reservoir of CDK9 activity in the nucleoplasm

Binding of cyclin-kinase inhibitors (CKI) is a common way to regulate CDKs activity [200]. CDK9 is, however, the only known CDK that is regulated by an RNA-containing CKI. In human cells, a significant fraction of P-TEFb is found in a large, catalytically inactive complex, associated with the 7SK small nuclear (sn)RNA, MePCE, LARP7 and HEXIM1/2 proteins [5, 201]. Within this ribonucleoparticle (RNP), the 7SK methylphosphate capping enzyme (MePCE) and the La-related Protein 7 (LARP7) stably associate with the 5′ and 3′ ends of the 331 nucleotides-long 7SK snRNA, respectively, and are both essential for 7SK snRNA stability and snRNP integrity [202–204]. Protected by the core snRNP proteins, the 7SK snRNA provides the structural RNA scaffold for the formation of the P-TEFb inhibitory complex. Its 5′ terminal hairpin forms a docking site for a homo- or hetero-dimer of HEXIM1/2 (Hexamethylene Bisacetamide-inducible Protein), binding of which relieves an auto-inhibitory conformation of HEXIM proteins and exposes their otherwise inaccessible P-TEFb binding surface [205–208]. 7SK-dependent binding of HEXIM1 to both CDK9 and Cyclin T1 prevents substrates from accessing the CDK9 catalytic site, thus providing a plausible mechanism

for P-TEFb inhibition [209, 210]. Additional interaction between LARP7 and CDK9 may further reinforce the assembly of 7SK/P-TEFb RNP [211, 212]. Although some structural hints have been provided for binding of MePCE and Larp7 to 7SK [213, 214], the structure of the complete 7SK/P-TEFb snRNP has not yet been resolved. Importantly, only Thr186-phosphorylated CDK9 is successfully incorporated within the inhibitory RNP, ensuring that only activated, ready to use P-TEFb is sequestered into the 7SK/P-TEFb snRNP [215, 216].

The amount of P-TEFb kept ‘in custody’ by the 7SK RNP is dictated by the overall transcriptional need, as well as the proliferative state of the cell [217]. For instance, 50% of P-TEFb is inactivated in actively growing HeLa cells, but 7SK binds up to 90% of P-TEFb in human primary blood lymphocytes [218–221]. While it considerably restricts CDK9 availability under normal conditions, the P-TEFb/7SK RNP rapidly disassembles in response to cellular stress, robustly increasing CDK9 activity [220, 221] (Fig. 5). Thus, the 7SK/P-TEFb snRNP acts as CDK9 storage complex that can release active P-TEFb under stress conditions without requiring new protein synthesis. Stimuli that have been shown to trigger extraction of P-TEFb from its snRNP repressor include transcription blockade, DNA damage, viral infection and activation of various signaling pathways [201, 220–223]. Acetylation of cyclin T1 [224] and ubiquitinylation and phosphorylation of HEXIM1 [225, 226] have all been shown to shift the P-TEFb equilibrium towards the active form at the expense of the inactive form. Erasure of CDK9 T-loop phosphorylation also disassembles the 7SK RNP [227–229], although in this case the released CDK9

**Inactive pool:
7SK/P-TEFb RNP**

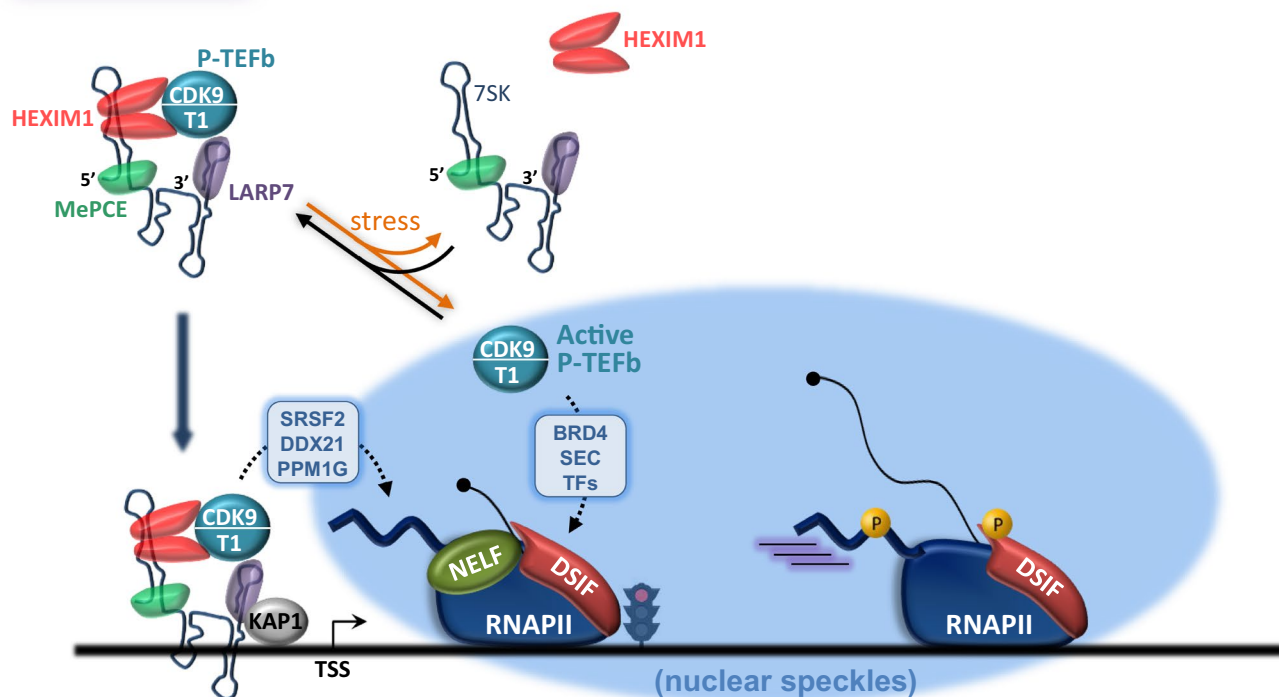


Fig. 5 Regulation of CDK9 availability by the 7SK snRNP. In the nucleoplasm, a fraction of P-TEFb is sequestered into the 7SK/P-TEFb snRNP, where CDK9 activity is inhibited by HEXIM1 in a 7SK-dependent manner. After cellular stress, disassociation of the 7SK/P-TEFb snRNP releases active P-TEFb, which can be loaded onto target genes by BRD4, Super Elongation Complexes (SEC) or gene-specific transcription factors (TF). The 7SK/P-TEFb snRNP is

also directly targeted to gene promoters by KAP1. Factors such as SRSF2, DDX21 or PPM1G can activate P-TEFb from chromatin-anchored 7SK/P-TEFb snRNP in the vicinity of the RNAPII complex for ‘on site’ transcriptional activation. Note that only the active form of P-TEFb can be incorporated into transcription-associated condensates

needs re-phosphorylation on Thr186 to become fully active. Transient rearrangement of 7SK RNA structure [230], destabilization of 7SK RNA [231–233], and binding of various RNA-binding proteins to the 7SK RNP [207, 234–240] may represent alternative/cooperative mechanisms to release P-TEFb. Nuclear speckles could be sites of active assembly/disassembly of inactive P-TEFb complexes [196] and transition from the inactive to the active form may involve phase separation too [195]. Indeed, CyclinT1 is unable to phase separate together with HEXIM1 and the P-TEFb/7SK snRNP is widely dispersed in the nucleus, suggesting that incorporation within the 7SK snRNP prevents CDK9/Cyclin T1 translocation into transcription-associated condensates.

Retention of P-TEFb within the 7SK RNP is a way of separating and storing pre-activated CDK9 in the nucleoplasm, away from the transcription complex. However, recent studies have raised the intriguing possibility that the 7SK/P-TEFb regulatory RNP can also be anchored to chromatin, where it can activate RNAPII transcription locally through ‘on site’ release of active P-TEFb [241] (Fig. 5).

First described as a gene-specific mechanism [228, 242], positioning of the 7SK/P-TEFb RNP on chromatin was next extended to more than 15,000 human genes, where LARP7, CDK9, HEXIM1 and RNAPII co-localize at promoter regions [238, 243]. Widespread 7SK/P-TEFb RNP anchoring to chromatin is mediated by the Kruppel-associated box-interacting protein KAP1 [243], while on site disassembly of the RNP can be driven by PPM1G, SRSF2, DDX21, WDR43 or JMJD6 [228, 232, 233, 236, 238, 244]. However, analyses of 7SK RNA occupancy by chromatin isolation by RNA purification (ChIRP) argue against stable 7SK RNP association with promoter regions, with accumulation of 7SK snRNA being mainly detected on super-enhancers [233, 245], snRNA genes [246] and within the whole transcribed regions of some human protein-coding genes [245, 247]. Microscopy studies also failed to detect 7SK association with a stably integrated active gene [248]. Instead, 7SK RNA transiently associates with the locus upon transcriptional shutdown and was proposed to displace P-TEFb, raising the possibility that 7SK rather serves as a dynamic

P-TEFb carrier ensuring its delivery and/or removal from genes. Thus, it is not clear yet whether the 7SK RNP complex is stably anchored or only loosely associated with promoters, and how widespread this mechanism is.

Targeted delivery of active P-TEFb

The proportion of active and inactive forms of P-TEFb changes according to the proliferative/differentiation state of cells and transcriptional need [217, 249]. Even if the 7SK snRNP is capable of anchoring inactive CDK9 to genomic loci, recruitment of P-TEFb in its active form remains the main CDK9 delivery pathway. The level of P-TEFb brought to the gene will dictate the average pause duration and the fate of poised RNAPII. If active P-TEFb is efficiently recruited by a promoter, the duration of the pause will be shorter and more RNAPII will successfully enter productive elongation. Appropriate P-TEFb recruitment is, therefore, critical to establish specific gene expression programs.

A number of cellular activators can help to target CDK9 to specific DNA or RNA sequences [4]. For example, the bromodomain protein 4 (BRD4) is an important partner of active CDK9 as it directs free P-TEFb to promoters and enhancers through interaction with acetylated histones and/or the Mediator complex [233, 250]. BRD4 directly interacts with Cyclin T1 but the BRD4/P-TEFb interaction also relies on CDK9 phosphorylation on Ser175 [250, 251]. Most of the P-TEFb that is not sequestered in the 7SK/P-TEFb snRNP associates with BRD4 and P-TEFb freshly released from the 7SK snRNP joins the BRD4 pool [240, 252]. Accordingly, RNAi-mediated depletion or chemical inhibition of BRD4 has a profound impact on P-TEFb recruitment, RNAPII transcription elongation and gene expression [240, 250, 253–255]. However, the use of a recently developed BRD4 degron system showed that rapid depletion of BRD4 reduces RNAPII release into gene bodies without affecting P-TEFb occupancy on chromatin [256]. Thus, BRD4 may not be strictly required for targeting P-TEFb to genomic regulatory regions, but may largely function as an activator of chromatin-bound P-TEFb [257]. For instance, BRD4 could collaborate with JMJD6 (Jumanji C-domain-containing protein 6) at enhancer regions to evict P-TEFb from the 7SK inhibitory complex, through demethylation of 7SK cap and/or targeted degradation of MePCE [232, 233]. The resulting ‘on site’ activation of CDK9 would then facilitate pause release and gene activity. However, additional studies will be required to clarify the function of BRD4 in CDK9 recruitment and in P-TEFb-mediated activation of productive elongation.

While association with Brd4 may represent a general way to recruit CDK9 to genes, a number of specific transcription factors (TF) can interact with and deliver active P-TEFb to their respective target genes to facilitate transcriptional activation under basal and/or signal regulated conditions.

TFs targeting P-TEFb to chromatin in a gene-specific manner include NF- κ B [258], c-myc [58], MyoD [16], STAT3 [259] or MEF2 [260]. P-TEFb can also be incorporated into larger Super Elongation Complexes (SEC or SEC-like) together with other elongation factors, such as the eleven-nineteen Lys-rich leukemia (ELL) family members (ELL1/2/3), AF9, ENL, EAF1/2, AFF1 and AFF4 [102]. The SEC stimulates RNAPII processivity very efficiently [104, 105, 124] and helps to recruit CDK9 to active promoters and enhancers through interaction with the Mediator [261], PAF1C [262] or the Integrator complex [263]. Thus, numerous transcription factors and macromolecular complexes orchestrate the coordinated delivery of CDK9 to precise chromatin locations to regulate both basal and activated transcription.

CDK9 and diseases

Given the central importance of P-TEFb in regulating gene expression and maintaining cellular homeostasis, it is perhaps not surprising that CDK9 activity is linked to many pathologic processes. For example, P-TEFb is an essential cellular co-factor required for transcription and replication of the human immunodeficiency virus (HIV), and misregulation of CDK9 activity is associated with cardiac hypertrophy and cancer development [8, 264] (Fig. 6).

P-TEFb is an essential co-factor of HIV

Viruses often subvert the host cell transcriptional machinery for their own needs. P-TEFb is a primary target of HIV, but is also used by other viruses such as human T-lymphotropic virus (HTLV-1), Herpes simplex virus (HSV-1 and HSV-2), human cytomegalovirus (CMV), Epstein-Barr virus (EBV), human adenovirus, Influenza A virus, Dengue virus and Kaposi’s sarcoma-associated virus (KSHV) [264]. Most of the viruses mentioned above hijack P-TEFb via physical interaction with virus-encoded proteins to promote efficient transcription of the viral genome. The discovery that P-TEFb has an essential function in HIV transcription greatly helped to elucidate the molecular mechanisms underlying the replication of the virus. In turn, HIV transcription has become a paradigm for studying P-TEFb function in RNAPII regulation.

The HIV virus integrates into the host genome, and viral gene expression from the Long-Terminal Repeat (LTR) promoter is principally controlled at the early elongation stage by DSIF/NELF-mediated RNAPII pausing [265] (Fig. 6a). Synthesis of full-length HIV transcripts requires expression of a viral transactivator protein, Tat. Activation of elongation primarily relies on Tat-mediated recruitment of P-TEFb to the transactivation response element (TAR) that forms spontaneously at the 5’ end of nascent HIV transcripts [266].

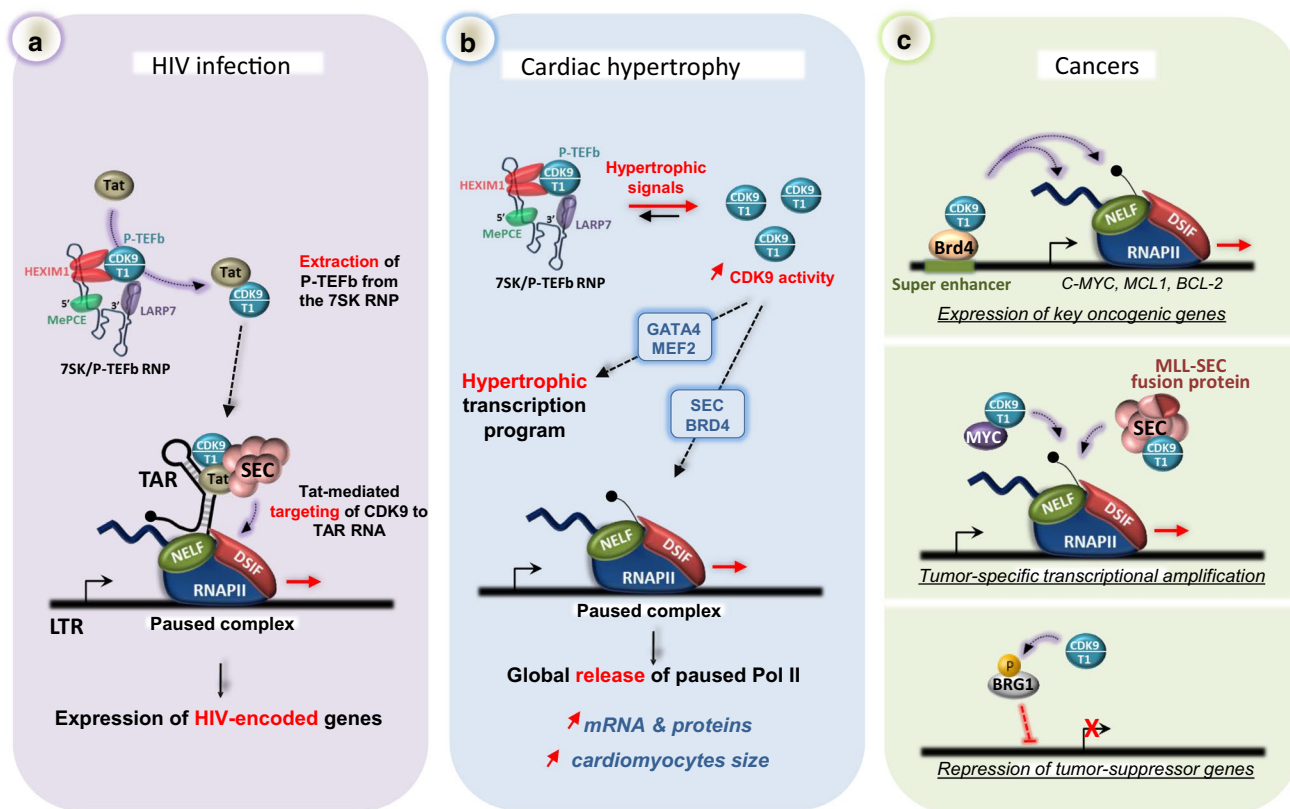


Fig. 6 Involvement of CDK9 in human disease. **a** CDK9 is a primary target of HIV-1. The virus-encoded protein Tat extracts P-TEFb from the 7SK/P-TEFb RNP and increases the level of active P-TEFb in infected cells. P-TEFb is then tethered to the viral genome through interaction with the TAR structure that forms at the 5' end of the viral nascent RNA and serves as a landing pad for the P-TEFb/Tat/SEC complex. Once recruited, P-TEFb releases paused RNAPII in the same way as it does on host protein-coding genes. **b** In cardiomyocytes, hypertrophic signals shift the equilibrium towards the P-TEFb active form. The resulting elevated CDK9 activity leads to the estab-

lishment of a hypertrophic transcription program and a global release of paused RNAPII into gene bodies. Elevated transcription leads to an increased level of mRNA and proteins and enlargement of cardiomyocytes. **c** Misregulation of CDK9 activity can lead to development of cancers. P-TEFb drives the expression of key pro-survival genes such as *C-MYC* and *MCL-1* (top). Mistargeting of P-TEFb by MYC or MLL-SEC fusion proteins promotes tumour-specific transcriptional amplification (middle). P-TEFb represses tumor-suppressor genes through inhibitory phosphorylation of the BRG1 subunit of the BAF chromatin-remodeling complex

The HIV-encoded Tat protein binds to Cyclin T1 and targets P-TEFb to the TAR RNA structure to stimulate transcription elongation in the same way as it acts on cellular genes, i.e., through phosphorylation of DSIF, NELF and the RNAPII CTD. The crystallographic structure of the P-TEFb/Tat complex indicates that binding of Tat induces a conformational change in CDK9 that may alter its substrate specificity [267]. Of note, only Cyclin T1-containing P-TEFb is targeted by Tat, as the interaction relies on a TRM motif that is not found in cyclin T2. Tat and P-TEFb are recruited to TAR within the SEC to stimulate proviral genome transcription [103, 105] and remain associated with the elongating RNAPII machinery throughout transcription. Thus, the Tat/TAR system illustrates another means of recruiting P-TEFb to the transcription machinery through an RNA tethering system. By recognizing the nascent RNA, P-TEFb is directly placed at the vicinity of the transcription complex, close

to its phosphorylation targets, ensuring efficient release of RNAPII pausing.

Interestingly, HIV transcription appears highly sensitive to the level of active CDK9 in the cell [268], making P-TEFb an attractive anti-HIV drug target. Reduced Cyclin T1 levels and hypophosphorylation of CDK9 on Thr186 contribute to HIV latency in primary CD4(+) T cells and these are both substantially up-regulated upon activation [269]. In addition, expression of Tat was shown to significantly increase the cellular pool of available P-TEFb. Tat is capable of disassembling the 7SK RNP by competing with HEXIM1 for binding to both Cyclin T1 and 7SK snRNA [105, 207, 218], and can then capture 7SK-evicted P-TEFb to support efficient viral transactivation (Fig. 6a). In addition, Tat promotes non-degradative ubiquitinylation of HEXIM1 by the UBE2O ubiquitin ligase, which results in cytoplasmic retention of HEXIM1, which may impede reassembly

of the 7SK/P-TEFb RNP [225]. Of note, the HTLV-1 Tax transactivator protein also displaces P-TEFb from the 7SK snRNP, suggesting that 7SK RNP disassembly could be a wide-spread strategy used by viruses to promote viral gene expression [237]. Importantly, Tat and BRD4 form mutually exclusive complexes with P-TEFb as BRD4 interacts with the same region of Cyclin T1 as Tat [240, 250]. Consequently, inhibition of BRD4 favors Tat/P-TEFb interaction, increases HIV transcription and reactivates HIV from latency [270]. Thus, inhibitors of BRD4 such as JQ1 are good candidates to reactivate latent integrated proviruses to facilitate eradication of the viral reservoir. In contrast, CDK9 inhibitors have been shown to be effective in suppressing replication of the virus, but their long-term cytotoxicity remains a serious obstacle to being broadly used in HIV treatment. Targeting the TAR RNA and/or the Tat/P-TEFb interaction surface could be used to inhibit HIV transcription [271]. Additional mechanistic insights into P-TEFb function and regulation may provide the opportunity to develop novel strategies to block RNAPII transcription of the HIV and other viral genomes in the near future.

P-TEFb and cardiac hypertrophy

Cardiac hypertrophy is an adaptive response of the heart to mechanical overload and pressure, frequently seen in case of hypertension and myocardial infarction. It is characterized by an enlargement of the size of cardiac myocytes as a result of an overall increase in RNA and protein content [272]. P-TEFb appears to play a critical role in this process. Known hypertrophic signals, such as stimulation with endothelin-1 or mechanical stresses, lead to disruption of the 7SK/P-TEFb complex and shift the P-TEFb equilibrium towards the active form [273, 274] (Fig. 6b). The molecular mechanisms that lead to P-TEFb de-repression under hypertrophic conditions remain poorly understood, but likely involve activation of calcium-dependent (calcineurin) and Jak/STAT signaling cascades [273, 274]. Disassembly of the 7SK RNP substantially increases the level of active P-TEFb in cells, where CDK9 activity is normally limiting [275], resulting in enhanced RNAPII transcription, increased mRNA/protein synthesis and cell enlargement. The SEC and BRD4 are implicated in targeting P-TEFb to genes to stimulate RNAPII pause release genome-wide [276, 277] (Fig. 6b). Accordingly, a broad BRD4 redistribution occurs during establishment of the cardiomyocyte hypertrophic gene program, with increased chromatin occupancy at promoters and super-enhancers [277, 278]. Consistent with a stimulation of productive elongation, the level of CTD phosphorylation, and in particular that of Ser2, rises under conditions of hypertrophic stress [274]. Besides globally activating RNAPII elongation, stimulation of CDK9 activity is also accompanied by a specific 'hypertrophic' transcriptional

reprogramming (Fig. 6b). For instance, CDK9 is specifically targeted to cardiac hypertrophy-responsive promoters through association with important regulators of cardiomyocytes proliferation such as GATA4 and MEF2 [260, 279]. Over-activation of P-TEFb also represses the promoter activity and subsequent expression of PGC-1 (peroxisome proliferator-activated receptor- γ coactivator-1), a master regulator of mitochondrial function and biogenesis [280]. As a consequence, constitutive activation of CDK9 leads to mitochondrial dysfunction, which engenders myocyte apoptosis and predisposes the patient to heart failure. Importantly, recent data indicate that the heart is refractory to Myc-driven transcription due to the low P-TEFb availability in cardiomyocytes [275]. Thus, abnormal P-TEFb activity in heart cells may profoundly reshape RNAPII transcription by allowing abnormal activation by various transcription factors, including Myc.

Control of P-TEFb availability by the 7SK snRNP appears to be an essential mechanism for cardiomyocyte function and development. Accordingly, manifestation of cardiac hypertrophy can be recapitulated by artificial activation of P-TEFb in mice. For instance, the genetic deletion of the HEXIM1 murine homolog, CLP-1 (cardiac lineage protein 1), results in embryonic lethality and mimics the characteristics of cardiac hypertrophy [281]. Similarly, RNAi-mediated inactivation of 7SK RNA in cardiomyocytes induces CDK9 activation and abnormal cell growth, and mice overexpressing Cyclin T1 also exhibit cardiac hypertrophy [274]. Finally, a recent study showed that 7SK destabilization through LARP7 knock-down also increases Ser2P phosphorylation and cardiomyocyte proliferation in zebrafish [282]. Together, these studies demonstrated that CDK9 is a pivotal regulator of the cardiomyocyte transcriptional program, the perturbation of which can lead to cardiomyopathies. Since CDK9 pharmacological inhibitors dampen hypertrophic signals [274], modulation of CDK9 activity may thus be a relevant therapy for some kinds of heart failure [283]. Reducing CDK9 activity to normal baseline levels could theoretically reduce heart growth, reverse the adverse 'hypertrophic' transcriptional program and restore mitochondrial function. From a scientific point of view, cardiac hypertrophy represents an ideal physiopathological model to interrogate the molecular mechanisms that govern sequestration of P-TEFb by 7SK/HEXIM, and may provide insights into the signaling cascades and post-translational modifications that modulate this critical transcriptional regulatory system.

P-TEFb in cancers

Given that the P-TEFb-dependent transition into productive elongation is an abundantly exploited step in regulating cellular gene expression, it is not surprising that CDK9 contributes to the progression and the maintenance

of many cancer types. Accordingly, P-TEFb hyperactivity promotes malignant transformation of fibroblasts in vitro and is associated with hematological, prostate, ovarian, mammary, hepatic, pancreatic and lung cancers [8]. Due to the broad requirement for P-TEFb during embryonic gene expression, genetic alterations affecting the CDK9 or Cyclin T1 loci are relatively rare. Instead, cancers are often associated with increased CDK9 function resulting from modulation of CDK9 association with regulatory proteins and/or increased targeting to genes (Fig. 6c). Cancers are accompanied by a profound transcriptional reprogramming, and a large proportion of cancer cells display widespread effects on mRNA transcription elongation and processing [284].

Importantly, P-TEFb promotes transcriptional elongation of key signal-responsive genes that are themselves critical for proliferation, development and stress responses (Fig. 6c, top panel). Among genes whose expression is highly dependent on CDK9 activity are those encoding MYC, BCL2 (B cell lymphoma 2) and MCL1 (myeloid cell leukemia 1), which have been found to be over-expressed in a wide range of human malignancies and are thought to drive cell survival [8, 285]. Expression of these pro-survival genes is controlled by super-enhancer-mediated transcription, which can be effectively inhibited by CDK9 inhibitors such as Flavopiridol [286]. Super-enhancers activate key oncogenic drivers in many tumor cells [287]. Interestingly, BRD4, the major CDK9 partner, is heavily loaded onto super-enhancers, including that of *C-MYC*, and could help to recruit P-TEFb to super-enhancer-regulated (onco)genes. Accordingly, BRD4 inhibition impairs CDK9 recruitment to *MYC*, reduces *MYC* transcripts levels, down-regulates Myc-dependent target genes, and has anti-proliferative effects in cancer cells [253, 254, 288]. Thus, CDK9 and BRD4 have emerged as druggable targets for the development of cancer therapies, through suppression of constitutive expression of anti-apoptotic proteins.

Of particular significance is the physical interaction of P-TEFb with MYC [58]. *c-Myc* is a potent oncogene that drives proliferation by globally amplifying gene expression in a P-TEFb-dependent manner [58, 289] (Fig. 6c, middle panel). Mechanistically, Myc-mediated loading of CDK9 to active genes is intensified in cells with high Myc levels, boosting the production of transcripts and resulting in transcriptional amplification. Thus, the interplay between MYC and P-TEFb can contribute to the establishment of tumor-specific gene expression signatures in cancer cells. Incorporation of P-TEFb into the SEC is another source of transcriptional activation in MLL-associated leukemia. As a result of in-frame gene translocations, several components of the SEC, such as AFF1, AFF4, AF9, ELL1 and ENL, form chimeric fusion proteins with the mixed lineage leukemia (MLL) protein [104, 290]. Consequently, SEC can

be mis-targeted to MLL-dependent genes, where P-TEFb can release RNAPII into productive elongation in an uncontrolled manner (Fig. 6c).

Malfunction of the 7SK/P-TEFb regulatory machine that abnormally increases P-TEFb activity can also lead to uncontrolled growth. Low expression levels of 7SK RNP components have been associated with various types of human malignancies, including cervical, thyroid, gastric and breast cancer [291–293]. Accordingly, shifting the P-TEFb functional equilibrium towards the active state causes mammary epithelial transformation [202]. Disruption of the P-TEFb functional equilibrium also promotes an epithelial–mesenchymal transition (EMT), invasion and metastasis of breast cancer cells by favoring the P-TEFb-dependent expression of EMT/metastasis-related genes [293]. Finally, frequent frameshift mutations in the *LARP7* gene are found in gastric cancers, pointing to a tumor suppression function for LARP7 [202, 292]. Together, these observations suggest that shifting back the P-TEFb equilibrium towards its inactive form may reduce proliferation and aggressiveness of cancer cells. In line with this, the anti-cancer activities (inhibition of growth, apoptosis) of BRD4 inhibitors have been shown to rely, at least in part, on transcriptional induction of the *HEXIM1* gene, which allows recapture of P-TEFb into the 7SK inhibitory complex [294, 295].

Like CDKs in general, CDK9 targeting has been an active area of research in oncology, and a number of CDK9 inhibitors have reached preclinical and clinical development [8]. Interestingly, CDK9 inhibition also reactivates, in multiple cancer cells, tumor-suppressive genes that were epigenetically silenced. Reactivation occurs through BRG1-mediated chromatin remodeling at promoters, and it was proposed that P-TEFb-mediated phosphorylation of BRG1 prevent the BAF complex being recruited to chromatin [164] (Fig. 6c, bottom panel). Thus, the anti-tumoral effects of CDK9 inhibition can be attributed to both suppression of constitutive expression of anti-apoptotic proteins (such as MCL1 and/or MYC) and re-expression of tumor suppressor genes.

Concluding remarks

Releasing promoter-proximally paused RNAPII into productive elongation by P-TEFb is now recognized as a key regulatory step in gene expression. In the last decade, CDK9 has emerged as a critical coordinator of RNAPII transcription operating at multiple locations throughout the transcription unit. Mechanisms that dictate the duration of the pause and/or regulate loading of P-TEFb onto selected promoters are currently being intensively dissected, since recruitment of active P-TEFb to genes seems to be the rate-limiting step for pause escape. Through coupling pause release with both initiation and termination, CDK9 has a major impact on

transcriptional outputs. As such, CDK9 represents an attractive target for cancer therapeutics [8], and there are great hopes that inhibition of CDK9 can be used to effectively and selectively restrict cell growth. The list of CDK9 targets and interactors continues to grow, unveiling new avenues of investigation. It is now critical to fully characterize the transcriptional regulatory networks that are organized around CDK9 to understand how they are regulated during development, stress or signaling-induced activation. The most recent advances have uncovered that phosphatases and termination factors can counteract CDK9 activity to regulate pause duration and finely control gene expression. A particularly important objective is now to fully characterize the complex interplay of P-TEFb and the other transcriptional kinases with phosphatase activities throughout the transcription cycle.

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References

1. Hochegger H, Takeda S, Hunt T (2008) Cyclin-dependent kinases and cell-cycle transitions: does one fit all? *Nat Rev Mol Cell Biol* 9(11):910–916. <https://doi.org/10.1038/nrm2510>
2. Malumbres M (2014) Cyclin-dependent kinases. *Genome Biol* 15(6):122. <https://doi.org/10.1186/gb4184>
3. Garriga J, Bhattacharya S, Calbo J, Marshall RM, Truongcao M, Haines DS, Grana X (2003) CDK9 is constitutively expressed throughout the cell cycle, and its steady-state expression is independent of SKP2. *Mol Cell Biol* 23(15):5165–5173. <https://doi.org/10.1128/mcb.23.15.5165-5173.2003>
4. Peterlin BM, Price DH (2006) Controlling the elongation phase of transcription with P-TEFb. *Mol Cell* 23(3):297–305. <https://doi.org/10.1016/j.molcel.2006.06.014>
5. Bacon CW, D'Orso I (2019) CDK9: a signaling hub for transcriptional control. *Transcription* 10(2):57–75. <https://doi.org/10.1080/21541264.2018.1523668>
6. Core L, Adelman K (2019) Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. *Genes Dev* 33(15–16):960–982. <https://doi.org/10.1101/gad.325142.119>
7. Cramer P (2019) Organization and regulation of gene transcription. *Nature* 573(7772):45–54. <https://doi.org/10.1038/s41586-019-1517-4>
8. Franco LC, Morales F, Boffo S, Giordano A (2018) CDK9: a key player in cancer and other diseases. *J Cell Biochem* 119(2):1273–1284. <https://doi.org/10.1002/jcb.26293>
9. Shore SM, Byers SA, Maury W, Price DH (2003) Identification of a novel isoform of Cdk9. *Gene* 307:175–182. [https://doi.org/10.1016/s0378-1119\(03\)00466-9](https://doi.org/10.1016/s0378-1119(03)00466-9)
10. Giacinti C, Musaro A, De Falco G, Jourdan I, Molinaro M, Bagella L, Simone C, Giordano A (2008) Cdk9-55: a new player in muscle regeneration. *J Cell Physiol* 216(3):576–582. <https://doi.org/10.1002/jcp.21361>
11. Liu H, Herrmann CH (2005) Differential localization and expression of the Cdk9 42k and 55k isoforms. *J Cell Physiol* 203(1):251–260. <https://doi.org/10.1002/jcp.20224>
12. Shore SM, Byers SA, Dent P, Price DH (2005) Characterization of Cdk 9(55) and differential regulation of two Cdk9 isoforms. *Gene* 350(1):51–58. <https://doi.org/10.1016/j.gene.2005.01.015>
13. Liu H, Herrmann CH, Chiang K, Sung TL, Moon SH, Donehower LA, Rice AP (2010) 55K isoform of CDK9 associates with Ku70 and is involved in DNA repair. *Biochem Biophys Res Commun* 397(2):245–250. <https://doi.org/10.1016/j.bbrc.2010.05.092>
14. Peng J, Zhu Y, Milton JT, Price DH (1998) Identification of multiple cyclin subunits of human P-TEFb. *Genes Dev* 12(5):755–762. <https://doi.org/10.1101/gad.12.5.755>
15. Cottone G, Baldi A, Palescandolo E, Manente L, Penta R, Paggi MG, De Luca A (2006) Pkn is a novel partner of cyclin T2a in muscle differentiation. *J Cell Physiol* 207(1):232–237. <https://doi.org/10.1002/jcp.20566>
16. Giacinti C, Bagella L, Puri PL, Giordano A, Simone C (2006) MyoD recruits the cdk9/cyclin T2 complex on myogenic-genes regulatory regions. *J Cell Physiol* 206(3):807–813. <https://doi.org/10.1002/jcp.20523>
17. Marchesi I, Nieddu V, Caracciolo V, Maioli M, Gaspa L, Giordano A, Bagella L (2013) Activation and function of murine Cyclin T2A and Cyclin T2B during skeletal muscle differentiation. *J Cell Biochem* 114(3):728–734. <https://doi.org/10.1002/jcb.24414>
18. Simone C, Stiegler P, Bagella L, Pucci B, Bellan C, De Falco G, De Luca A, Guanti G, Puri PL, Giordano A (2002) Activation of MyoD-dependent transcription by cdk9/cyclin T2. *Oncogene* 21(26):4137–4148. <https://doi.org/10.1038/sj.onc.1205493>
19. Kohoutek J, Li Q, Blazek D, Luo Z, Jiang H, Peterlin BM (2009) Cyclin T2 is essential for mouse embryogenesis. *Mol Cell Biol* 29(12):3280–3285. <https://doi.org/10.1128/MCB.00172-09>
20. Lu H, Yu D, Hansen AS, Ganguly S, Liu R, Heckert A, Darzacq X, Zhou Q (2018) Phase-separation mechanism for

- C-terminal hyperphosphorylation of RNA polymerase II. *Nature* 558(7709):318–323. <https://doi.org/10.1038/s41586-018-0174-3>
21. Taube R, Lin X, Irwin D, Fujinaga K, Peterlin BM (2002) Interaction between P-TEFb and the C-terminal domain of RNA polymerase II activates transcriptional elongation from sites upstream or downstream of target genes. *Mol Cell Biol* 22(1):321–331. <https://doi.org/10.1128/mcb.22.1.321-331.2002>
 22. Larochelle S, Amat R, Glover-Cutter K, Sanso M, Zhang C, Allen JJ, Shokat KM, Bentley DL, Fisher RP (2012) Cyclin-dependent kinase control of the initiation-to-elongation switch of RNA polymerase II. *Nat Struct Mol Biol* 19(11):1108–1115. <https://doi.org/10.1038/nsmb.2399>
 23. Baumli S, Lolli G, Lowe ED, Troiani S, Rusconi L, Bullock AN, Debreczeni JE, Knapp S, Johnson LN (2008) The structure of P-TEFb (CDK9/cyclin T1), its complex with flavopiridol and regulation by phosphorylation. *EMBO J* 27(13):1907–1918. <https://doi.org/10.1038/emboj.2008.121>
 24. Kwiatkowski N, Zhang T, Rahl PB, Abraham BJ, Reddy J, Ficarro SB, Dastur A, Amzallag A, Ramaswamy S, Tesar B, Jenkins CE, Hannett NM, McMillin D, Sanda T, Sim T, Kim ND, Look T, Mitsiades CS, Weng AP, Brown JR, Benes CH, Marto JA, Young RA, Gray NS (2014) Targeting transcription regulation in cancer with a covalent CDK7 inhibitor. *Nature* 511(7511):616–620. <https://doi.org/10.1038/nature13393>
 25. Papantonis A, Cook PR (2013) Transcription factories: genome organization and gene regulation. *Chem Rev* 113(11):8683–8705. <https://doi.org/10.1021/cr300513p>
 26. Proudfoot NJ (2016) Transcriptional termination in mammals: Stopping the RNA polymerase II juggernaut. *Science* 352(6291):aad9926. <https://doi.org/10.1126/science.aad9926>
 27. Gressel S, Schwalb B, Decker TM, Qin W, Leonhardt H, Eick D, Cramer P (2017) CDK9-dependent RNA polymerase II pausing controls transcription initiation. *Elife*. <https://doi.org/10.7554/eLife.29736>
 28. Shao W, Zeitlinger J (2017) Paused RNA polymerase II inhibits new transcriptional initiation. *Nat Genet* 49(7):1045–1051. <https://doi.org/10.1038/ng.3867>
 29. Laitem C, Zaborowska J, Isa NF, Kufs J, Dienstbier M, Murphy S (2015) CDK9 inhibitors define elongation checkpoints at both ends of RNA polymerase II-transcribed genes. *Nat Struct Mol Biol* 22(5):396–403. <https://doi.org/10.1038/nsmb.3000>
 30. Parua PK, Booth GT, Sanso M, Benjamin B, Tanny JC, Lis JT, Fisher RP (2018) A Cdk9-PP1 switch regulates the elongation-termination transition of RNA polymerase II. *Nature* 558(7710):460–464. <https://doi.org/10.1038/s41586-018-0214-z>
 31. Sanso M, Levin RS, Lipp JJ, Wang VY, Greifengberg AK, Quezada EM, Ali A, Ghosh A, Larochelle S, Rana TM, Geyer M, Tong L, Shokat KM, Fisher RP (2016) P-TEFb regulation of transcription termination factor Xrn2 revealed by a chemical genetic screen for Cdk9 substrates. *Genes Dev* 30(1):117–131. <https://doi.org/10.1101/gad.269589.115>
 32. Wada T, Takagi T, Yamaguchi Y, Ferdous A, Imai T, Hirose S, Sugimoto S, Yano K, Hartzog GA, Winston F, Buratowski S, Handa H (1998) DSIF, a novel transcription elongation factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs. *Genes Dev* 12(3):343–356. <https://doi.org/10.1101/gad.12.3.343>
 33. Yamaguchi Y, Takagi T, Wada T, Yano K, Furuya A, Sugimoto S, Hasegawa J, Handa H (1999) NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation. *Cell* 97(1):41–51. [https://doi.org/10.1016/S0092-8674\(00\)80713-8](https://doi.org/10.1016/S0092-8674(00)80713-8)
 34. Schulz S, Gietl A, Smollett K, Tinnefeld P, Werner F, Grohmann D (2016) TFE and Spt4/5 open and close the RNA polymerase clamp during the transcription cycle. *Proc Natl Acad Sci USA* 113(13):E1816–1825. <https://doi.org/10.1073/pnas.1515817113>
 35. Vos SM, Farnung L, Urlaub H, Cramer P (2018) Structure of paused transcription complex Pol II-DSIF-NELF. *Nature* 560(7720):601–606. <https://doi.org/10.1038/s41586-018-0442-2>
 36. Qiu Y, Gilmour DS (2017) Identification of regions in the Spt5 subunit of DRB sensitivity-inducing factor (DSIF) that are involved in promoter-proximal pausing. *J Biol Chem* 292(13):5555–5570. <https://doi.org/10.1074/jbc.M116.760751>
 37. Cheng B, Price DH (2007) Properties of RNA polymerase II elongation complexes before and after the P-TEFb-mediated transition into productive elongation. *J Biol Chem* 282(30):21901–21912. <https://doi.org/10.1074/jbc.M702936200>
 38. Hartzog GA (1829) Fu J (2013) The Spt4-Spt5 complex: a multifaceted regulator of transcription elongation. *Biochem Biophys Acta* 1:105–115. <https://doi.org/10.1016/j.bbarm.2012.08.007>
 39. Pagano JM, Kwak H, Waters CT, Sprouse RO, White BS, Ozer A, Szeto K, Shalloway D, Craighead HG, Lis JT (2014) Defining NELF-E RNA binding in HIV-1 and promoter-proximal pause regions. *PLoS Genet* 10(1):e1004090. <https://doi.org/10.1371/journal.pgen.1004090>
 40. Vos SM, Pollmann D, Caizzi L, Hofmann KB, Rombaut P, Zimniak T, Herzog F, Cramer P (2016) Architecture and RNA binding of the human negative elongation factor. *Elife*. <https://doi.org/10.7554/eLife.14981>
 41. Yamaguchi Y, Inukai N, Narita T, Wada T, Handa H (2002) Evidence that negative elongation factor represses transcription elongation through binding to a DRB sensitivity-inducing factor/RNA polymerase II complex and RNA. *Mol Cell Biol* 22(9):2918–2927. <https://doi.org/10.1128/mcb.22.9.2918-2927.2002>
 42. Palangat M, Renner DB, Price DH, Landick R (2005) A negative elongation factor for human RNA polymerase II inhibits the anti-arrest transcript-cleavage factor TFIIS. *Proc Natl Acad Sci USA* 102(42):15036–15041. <https://doi.org/10.1073/pnas.0409405102>
 43. Sheridan RM, Fong N, D'Alessandro A, Bentley DL (2019) Widespread backtracking by RNA Pol II is a major effector of gene activation, 5' pause release, termination, and transcription elongation rate. *Mol Cell* 73(1):107–118. <https://doi.org/10.1016/j.molcel.2018.10.031>
 44. Egloff S, Al-Rawaf H, O'Reilly D, Murphy S (2009) Chromatin structure is implicated in “late” elongation checkpoints on the U2 snRNA and beta-actin genes. *Mol Cell Biol* 29(14):4002–4013. <https://doi.org/10.1128/MCB.00189-09>
 45. Jonkers I, Kwak H, Lis JT (2014) Genome-wide dynamics of Pol II elongation and its interplay with promoter proximal pausing, chromatin, and exons. *Elife* 3:02407. <https://doi.org/10.7554/eLife.02407>
 46. Kujirai T, Ehara H, Fujino Y, Shirouzu M, Sekine SI, Kurumizaka H (2018) Structural basis of the nucleosome transition during RNA polymerase II passage. *Science* 362(6414):595–598. <https://doi.org/10.1126/science.aau9904>
 47. Teves SS, Weber CM, Henikoff S (2014) Transcribing through the nucleosome. *Trends Biochem Sci* 39(12):577–586. <https://doi.org/10.1016/j.tibs.2014.10.004>
 48. Weber CM, Henikoff S (2014) Histone variants: dynamic punctuation in transcription. *Genes Dev* 28(7):672–682. <https://doi.org/10.1101/gad.238873.114>
 49. Etchegaray JP, Zhong L, Li C, Henriques T, Ablondi E, Nakadai T, Van Rechem C, Ferrer C, Ross KN, Choi JE, Samarakody A, Ji F, Chang A, Sadreyev RI, Ramaswamy S, Nechaev S, Whetstone JR, Roeder RG, Adelman K, Goren A, Mostoslavsky R (2019) The histone deacetylase SIRT6 restrains transcription elongation via promoter-proximal pausing. *Mol Cell* 75(4):683–699. <https://doi.org/10.1016/j.molcel.2019.06.034>
 50. Cheng B, Li T, Rahl PB, Adamson TE, Loudas NB, Guo J, Varzavand K, Cooper JJ, Hu X, Gnatt A, Young RA, Price DH

- (2012) Functional association of Gdown1 with RNA polymerase II poised on human genes. *Mol Cell* 45(1):38–50. <https://doi.org/10.1016/j.molcel.2011.10.022>
51. DeLaney E, Luse DS (2016) Gdown1 associates efficiently with RNA polymerase II after promoter clearance and displaces TFIIF during transcript elongation. *PLoS ONE* 11(10):e0163649. <https://doi.org/10.1371/journal.pone.0163649>
 52. Chen FX, Woodfin AR, Gardini A, Rickels RA, Marshall SA, Smith ER, Shiekhhattar R, Shilatifard A (2015) PAF1, a molecular regulator of promoter-proximal pausing by RNA polymerase II. *Cell* 162(5):1003–1015. <https://doi.org/10.1016/j.cell.2015.07.042>
 53. Chen FX, Xie P, Collings CK, Cao K, Aoi Y, Marshall SA, Rendleman EJ, Ugarenko M, Ozark PA, Zhang A, Shiekhhattar R, Smith ER, Zhang MQ, Shilatifard A (2017) PAF1 regulation of promoter-proximal pause release via enhancer activation. *Science* 357(6357):1294–1298. <https://doi.org/10.1126/science.aan3269>
 54. Tettey TT, Gao X, Shao W, Li H, Story BA, Chitsazan AD, Glaser RL, Goode ZH, Seidel CW, Conaway RC, Zeitlinger J, Blanchette M, Conaway JW (2019) A role for FACT in RNA polymerase II promoter-proximal pausing. *Cell Rep* 27(13):3770–3779. <https://doi.org/10.1016/j.celrep.2019.05.099>
 55. Chao SH, Price DH (2001) Flavopiridol inactivates P-TEFb and blocks most RNA polymerase II transcription in vivo. *J Biol Chem* 276(34):31793–31799. <https://doi.org/10.1074/jbc.M102306200>
 56. Henriques T, Gilchrist DA, Nechaev S, Bern M, Muse GW, Burkholder A, Fargo DC, Adelman K (2013) Stable pausing by RNA polymerase II provides an opportunity to target and integrate regulatory signals. *Mol Cell* 52(4):517–528. <https://doi.org/10.1016/j.molcel.2013.10.001>
 57. Ni Z, Saunders A, Fuda NJ, Yao J, Suarez JR, Webb WW, Lis JT (2008) P-TEFb is critical for the maturation of RNA polymerase II into productive elongation in vivo. *Mol Cell Biol* 28(3):1161–1170. <https://doi.org/10.1128/MCB.01859-07>
 58. Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA (2010) c-Myc regulates transcriptional pause release. *Cell* 141(3):432–445. <https://doi.org/10.1016/j.cell.2010.03.030>
 59. Yamada T, Yamaguchi Y, Inukai N, Okamoto S, Mura T, Handa H (2006) P-TEFb-mediated phosphorylation of hSpt5 C-terminal repeats is critical for processive transcription elongation. *Mol Cell* 21(2):227–237. <https://doi.org/10.1016/j.molcel.2005.11.024>
 60. Fujinaga K, Irwin D, Huang Y, Taube R, Kurosu T, Peterlin BM (2004) Dynamics of human immunodeficiency virus transcription: P-TEFb phosphorylates RD and dissociates negative effectors from the transactivation response element. *Mol Cell Biol* 24(2):787–795. <https://doi.org/10.1128/mcb.24.2.787-795.2004>
 61. Vos SM, Farnung L, Boehning M, Wigge C, Linden A, Urlaub H, Cramer P (2018) Structure of activated transcription complex Pol II-DSIF-PAF-SPT6. *Nature* 560(7720):607–612. <https://doi.org/10.1038/s41586-018-0440-4>
 62. Gibson BA, Zhang Y, Jiang H, Hussey KM, Shrimp JH, Lin H, Schwede F, Yu Y, Kraus WL (2016) Chemical genetic discovery of PARP targets reveals a role for PARP-1 in transcription elongation. *Science* 353(6294):45–50. <https://doi.org/10.1126/science.aaf7865>
 63. Glover-Cutter K, Larochelle S, Erickson B, Zhang C, Shokat K, Fisher RP, Bentley DL (2009) TFIIF-associated Cdk7 kinase functions in phosphorylation of C-terminal domain Ser7 residues, promoter-proximal pausing, and termination by RNA polymerase II. *Mol Cell Biol* 29(20):5455–5464. <https://doi.org/10.1128/MCB.00637-09>
 64. Parua PK, Kalan S, Benjamin B, Sanso M, Fisher RP (2020) Distinct Cdk9-phosphatase switches act at the beginning and end of elongation by RNA polymerase II. *Nat Commun* 11(1):4338. <https://doi.org/10.1038/s41467-020-18173-6>
 65. Bernecky C, Plitzko JM, Cramer P (2017) Structure of a transcribing RNA polymerase II-DSIF complex reveals a multidentate DNA-RNA clamp. *Nat Struct Mol Biol* 24(10):809–815. <https://doi.org/10.1038/nsmb.3465>
 66. Ehara H, Yokoyama T, Shigematsu H, Yokoyama S, Shirouzu M, Sekine SI (2017) Structure of the complete elongation complex of RNA polymerase II with basal factors. *Science* 357(6354):921–924. <https://doi.org/10.1126/science.aan8552>
 67. Fitz J, Neumann T, Pavri R (2018) Regulation of RNA polymerase II processivity by Spt5 is restricted to a narrow window during elongation. *EMBO J*. <https://doi.org/10.15252/embj.201797965>
 68. Henriques T, Scruggs BS, Inouye MO, Muse GW, Williams LH, Burkholder AB, Lavender CA, Fargo DC, Adelman K (2018) Widespread transcriptional pausing and elongation control at enhancers. *Genes Dev* 32(1):26–41. <https://doi.org/10.1101/gad.309351.117>
 69. Shetty A, Kallgren SP, Demel C, Maier KC, Spatt D, Alver BH, Cramer P, Park PJ, Winston F (2017) Spt5 plays vital roles in the control of sense and antisense transcription elongation. *Mol Cell* 66(1):77–88. <https://doi.org/10.1016/j.molcel.2017.02.023>
 70. Chen Y, Yamaguchi Y, Tsugeno Y, Yamamoto J, Yamada T, Nakamura M, Hisatake K, Handa H (2009) DSIF, the Paf1 complex, and Tat-SF1 have nonredundant, cooperative roles in RNA polymerase II elongation. *Genes Dev* 23(23):2765–2777. <https://doi.org/10.1101/gad.1834709>
 71. Lavoie SB, Albert AL, Handa H, Vincent M, Bensaude O (2001) The peptidyl-prolyl isomerase Pin1 interacts with hSpt5 phosphorylated by Cdk9. *J Mol Biol* 312(4):675–685. <https://doi.org/10.1006/jmbi.2001.4991>
 72. Lindstrom DL, Squazzo SL, Muster N, Burckin TA, Wachter KC, Emigh CA, McCleery JA, Yates JR 3rd, Hartzog GA (2003) Dual roles for Spt5 in pre-mRNA processing and transcription elongation revealed by identification of Spt5-associated proteins. *Mol Cell Biol* 23(4):1368–1378. <https://doi.org/10.1128/mcb.23.4.1368-1378.2003>
 73. Mayer A, Schrieck A, Lidschreiber M, Leike K, Martin DE, Cramer P (2012) The spt5 C-terminal region recruits yeast 3' RNA cleavage factor I. *Mol Cell Biol* 32(7):1321–1331. <https://doi.org/10.1128/MCB.06310-11>
 74. Qiu H, Hu C, Gaur NA, Hinnebusch AG (2012) Pol II CTD kinases Bur1 and Kin28 promote Spt5 CTR-independent recruitment of Paf1 complex. *EMBO J* 31(16):3494–3505. <https://doi.org/10.1038/emboj.2012.188>
 75. Yu M, Yang W, Ni T, Tang Z, Nakadai T, Zhu J, Roeder RG (2015) RNA polymerase II-associated factor 1 regulates the release and phosphorylation of paused RNA polymerase II. *Science* 350(6266):1383–1386. <https://doi.org/10.1126/science.aad2338>
 76. Hou L, Wang Y, Liu Y, Zhang N, Shamovsky I, Nudler E, Tian B, Dynlacht BD (2019) Paf1C regulates RNA polymerase II progression by modulating elongation rate. *Proc Natl Acad Sci USA* 116(29):14583–14592. <https://doi.org/10.1073/pnas.1904324116>
 77. Kim J, Guermah M, Roeder RG (2010) The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS. *Cell* 140(4):491–503. <https://doi.org/10.1016/j.cell.2009.12.050>
 78. Van Oss SB, Cucinotta CE, Arndt KM (2017) Emerging insights into the roles of the Paf1 complex in gene regulation. *Trends Biochem Sci* 42(10):788–798. <https://doi.org/10.1016/j.tibs.2017.08.003>
 79. Davidson L, Muniz L, West S (2014) 3' end formation of pre-mRNA and phosphorylation of Ser2 on the RNA polymerase

- II CTD are reciprocally coupled in human cells. *Genes Dev* 28(4):342–356. <https://doi.org/10.1101/gad.231274.113>
80. Czudnochowski N, Bosken CA, Geyer M (2012) Serine-7 but not serine-5 phosphorylation primes RNA polymerase II CTD for P-TEFb recognition. *Nat Commun* 3:842. <https://doi.org/10.1038/ncomms1846>
 81. Mayfield JE, Irani S, Escobar EE, Zhang Z, Burkholder NT, Robinson MR, Mehaffey MR, Sipe SN, Yang W, Prescott NA, Kathuria KR, Liu Z, Brodbelt JS, Zhang Y (2019) Tyr1 phosphorylation promotes phosphorylation of Ser2 on the C-terminal domain of eukaryotic RNA polymerase II by P-TEFb. *Elife*. <https://doi.org/10.7554/eLife.48725>
 82. Buratowski S (2009) Progression through the RNA polymerase II CTD cycle. *Mol Cell* 36(4):541–546. <https://doi.org/10.1016/j.molcel.2009.10.019>
 83. Egloff S, Dienstbier M, Murphy S (2012) Updating the RNA polymerase CTD code: adding gene-specific layers. *TIG* 28(7):333–341. <https://doi.org/10.1016/j.tig.2012.03.007>
 84. Zaborowska J, Egloff S, Murphy S (2016) The pol II CTD: new twists in the tail. *Nat Struct Mol Biol* 23(9):771–777. <https://doi.org/10.1038/nsmb.3285>
 85. Schuller R, Forne I, Straub T, Schreieck A, Texier Y, Shah N, Decker TM, Cramer P, Imhof A, Eick D (2016) Heptad-specific phosphorylation of RNA polymerase II CTD. *Mol Cell* 61(2):305–314. <https://doi.org/10.1016/j.molcel.2015.12.003>
 86. Suh H, Ficarro SB, Kang UB, Chun Y, Marto JA, Buratowski S (2016) Direct analysis of phosphorylation sites on the Rpb1 C-terminal domain of RNA polymerase II. *Mol Cell* 61(2):297–304. <https://doi.org/10.1016/j.molcel.2015.12.021>
 87. Sharma P, Lioutas A, Fernandez-Fuentes N, Quilez J, Carbonell-Caballero J, Wright RHG, Di Vona C, Le Dily F, Schuller R, Eick D, Oliva B, Beato M (2019) Arginine citrullination at the C-terminal domain controls RNA polymerase II transcription. *Mol Cell* 73(1):84–96. <https://doi.org/10.1016/j.molcel.2018.10.016>
 88. Ahn SH, Kim M, Buratowski S (2004) Phosphorylation of serine 2 within the RNA polymerase II C-terminal domain couples transcription and 3' end processing. *Mol Cell* 13(1):67–76. [https://doi.org/10.1016/s1097-2765\(03\)00492-1](https://doi.org/10.1016/s1097-2765(03)00492-1)
 89. David CJ, Boyne AR, Millhouse SR, Manley JL (2011) The RNA polymerase II C-terminal domain promotes splicing activation through recruitment of a U2AF65-Prp19 complex. *Genes Dev* 25(9):972–983. <https://doi.org/10.1101/gad.2038011>
 90. Lenasi T, Barboric M (2010) P-TEFb stimulates transcription elongation and pre-mRNA splicing through multilateral mechanisms. *RNA Biol* 7(2):145–150. <https://doi.org/10.4161/rna.7.2.11057>
 91. Lenasi T, Peterlin BM, Barboric M (2011) Cap-binding protein complex links pre-mRNA capping to transcription elongation and alternative splicing through positive transcription elongation factor b (P-TEFb). *J Biol Chem* 286(26):22758–22768. <https://doi.org/10.1074/jbc.M111.235077>
 92. Liu Y, Warfield L, Zhang C, Luo J, Allen J, Lang WH, Ranish J, Shokat KM, Hahn S (2009) Phosphorylation of the transcription elongation factor Spt5 by yeast Bur1 kinase stimulates recruitment of the PAF complex. *Mol Cell Biol* 29(17):4852–4863. <https://doi.org/10.1128/MCB.00609-09>
 93. Lunde BM, Reichow SL, Kim M, Suh H, Leeper TC, Yang F, Mutschler H, Buratowski S, Meinhart A, Varani G (2010) Cooperative interaction of transcription termination factors with the RNA polymerase II C-terminal domain. *Nat Struct Mol Biol* 17(10):1195–1201. <https://doi.org/10.1038/nsmb.1893>
 94. Yoh SM, Cho H, Pickle L, Evans RM, Jones KA (2007) The Spt6 SH2 domain binds Ser2-P RNAPII to direct Iws1-dependent mRNA splicing and export. *Genes Dev* 21(2):160–174. <https://doi.org/10.1101/gad.1503107>
 95. Bentley DL (2014) Coupling mRNA processing with transcription in time and space. *Nat Rev Genet* 15(3):163–175. <https://doi.org/10.1038/nrg3662>
 96. Chun Y, Joo YJ, Suh H, Batot G, Hill CP, Formosa T, Buratowski S (2019) Selective kinase inhibition shows that Bur1 (Cdk9) phosphorylates the Rpb1 linker in vivo. *Mol Cell Biol*. <https://doi.org/10.1128/MCB.00602-18>
 97. Sdano MA, Fulcher JM, Palani S, Chandrasekharan MB, Parnell TJ, Whitby FG, Formosa T, Hill CP (2017) A novel SH2 recognition mechanism recruits Spt6 to the doubly phosphorylated RNA polymerase II linker at sites of transcription. *Elife*. <https://doi.org/10.7554/eLife.28723>
 98. Ardehali MB, Yao J, Adelman K, Fuda NJ, Petesch SJ, Webb WW, Lis JT (2009) Spt6 enhances the elongation rate of RNA polymerase II in vivo. *EMBO J* 28(8):1067–1077. <https://doi.org/10.1038/emboj.2009.56>
 99. Endoh M, Zhu W, Hasegawa J, Watanabe H, Kim DK, Aida M, Inukai N, Narita T, Yamada T, Furuya A, Sato H, Yamaguchi Y, Mandal SS, Reinberg D, Wada T, Handa H (2004) Human Spt6 stimulates transcription elongation by RNA polymerase II in vitro. *Mol Cell Biol* 24(8):3324–3336. <https://doi.org/10.1128/mcb.24.8.3324-3336.2004>
 100. Kaplan CD, Laprade L, Winston F (2003) Transcription elongation factors repress transcription initiation from cryptic sites. *Science* 301(5636):1096–1099. <https://doi.org/10.1126/science.1087374>
 101. Nojima T, Tellier M, Foxwell J, Ribeiro de Almeida C, Tan-Wong SM, Dhir S, Dujardin G, Dhir A, Murphy S, Proudfoot NJ (2018) Deregulated expression of mammalian lncRNA through loss of SPT6 Induces R-loop formation, replication stress, and cellular senescence. *Mol Cell* 72(6):970–984. <https://doi.org/10.1016/j.molcel.2018.10.011>
 102. Luo Z, Lin C, Shilatifard A (2012) The super elongation complex (SEC) family in transcriptional control. *Nat Rev Mol Cell Biol* 13(9):543–547. <https://doi.org/10.1038/nrm3417>
 103. He N, Liu M, Hsu J, Xue Y, Chou S, Burlingame A, Krogan NJ, Alber T, Zhou Q (2010) HIV-1 Tat and host AFF4 recruit two transcription elongation factors into a bifunctional complex for coordinated activation of HIV-1 transcription. *Mol Cell* 38(3):428–438. <https://doi.org/10.1016/j.molcel.2010.04.013>
 104. Lin C, Smith ER, Takahashi H, Lai KC, Martin-Brown S, Florens L, Washburn MP, Conaway JW, Conaway RC, Shilatifard A (2010) AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. *Mol Cell* 37(3):429–437. <https://doi.org/10.1016/j.molcel.2010.01.026>
 105. Sobhian B, Laguette N, Yatim A, Nakamura M, Levy Y, Kiernan R, Benkirane M (2010) HIV-1 Tat assembles a multifunctional transcription elongation complex and stably associates with the 7SK snRNP. *Mol Cell* 38(3):439–451. <https://doi.org/10.1016/j.molcel.2010.04.012>
 106. Guenther MG, Levine SS, Boyer LA, Jaenisch R, Young RA (2007) A chromatin landmark and transcription initiation at most promoters in human cells. *Cell* 130(1):77–88. <https://doi.org/10.1016/j.cell.2007.05.042>
 107. Kim TH, Barrera LO, Zheng M, Qu C, Singer MA, Richmond TA, Wu Y, Green RD, Ren B (2005) A high-resolution map of active promoters in the human genome. *Nature* 436(7052):876–880. <https://doi.org/10.1038/nature03877>
 108. Gilchrist DA, Dos Santos G, Fargo DC, Xie B, Gao Y, Li L, Adelman K (2010) Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation. *Cell* 143(4):540–551. <https://doi.org/10.1016/j.cell.2010.10.004>
 109. Gilchrist DA, Nechaev S, Lee C, Ghosh SK, Collins JB, Li L, Gilmour DS, Adelman K (2008) NELF-mediated stalling of Pol

- II can enhance gene expression by blocking promoter-proximal nucleosome assembly. *Genes Dev* 22(14):1921–1933. <https://doi.org/10.1101/gad.1643208>
110. Krebs AR, Imanci D, Hoerner L, Gaidatzis D, Burger L, Schubeler D (2017) Genome-wide single-molecule footprinting reveals high RNA polymerase II turnover at paused promoters. *Mol Cell* 67(3):411–422. <https://doi.org/10.1016/j.molcel.2017.06.027>
 111. Core LJ, Martins AL, Danko CG, Waters CT, Siepel A, Lis JT (2014) Analysis of nascent RNA identifies a unified architecture of initiation regions at mammalian promoters and enhancers. *Nat Genet* 46(12):1311–1320. <https://doi.org/10.1038/ng.3142>
 112. Sims RJ 3rd, Belotserkovskaya R, Reinberg D (2004) Elongation by RNA polymerase II: the short and long of it. *Genes Dev* 18(20):2437–2468. <https://doi.org/10.1101/gad.1235904>
 113. Ramanathan A, Robb GB, Chan SH (2016) mRNA capping: biological functions and applications. *Nucleic Acids Res* 44(16):7511–7526. <https://doi.org/10.1093/nar/gkw551>
 114. Fabrega C, Shen V, Shuman S, Lima CD (2003) Structure of an mRNA capping enzyme bound to the phosphorylated carboxy-terminal domain of RNA polymerase II. *Mol Cell* 11(6):1549–1561. [https://doi.org/10.1016/s1097-2765\(03\)00187-4](https://doi.org/10.1016/s1097-2765(03)00187-4)
 115. Ghosh A, Shuman S, Lima CD (2011) Structural insights to how mammalian capping enzyme reads the CTD code. *Mol Cell* 43(2):299–310. <https://doi.org/10.1016/j.molcel.2011.06.001>
 116. Ho CK, Shuman S (1999) Distinct roles for CTD Ser-2 and Ser-5 phosphorylation in the recruitment and allosteric activation of mammalian mRNA capping enzyme. *Mol Cell* 3(3):405–411. [https://doi.org/10.1016/s1097-2765\(00\)80468-2](https://doi.org/10.1016/s1097-2765(00)80468-2)
 117. Schwer B, Shuman S (2011) Deciphering the RNA polymerase II CTD code in fission yeast. *Mol Cell* 43(2):311–318. <https://doi.org/10.1016/j.molcel.2011.05.024>
 118. Mandal SS, Chu C, Wada T, Handa H, Shatkin AJ, Reinberg D (2004) Functional interactions of RNA-capping enzyme with factors that positively and negatively regulate promoter escape by RNA polymerase II. *Proc Natl Acad Sci USA* 101(20):7572–7577. <https://doi.org/10.1073/pnas.0401493101>
 119. Pei Y, Shuman S (2002) Interactions between fission yeast mRNA capping enzymes and elongation factor Spt5. *J Biol Chem* 277(22):19639–19648. <https://doi.org/10.1074/jbc.M200015200>
 120. Wen Y, Shatkin AJ (1999) Transcription elongation factor hSPT5 stimulates mRNA capping. *Genes Dev* 13(14):1774–1779. <https://doi.org/10.1101/gad.13.14.1774>
 121. Martinez-Rucobo FW, Kohler R, van de Waterbeemd M, Heck AJ, Hemann M, Herzog F, Stark H, Cramer P (2015) Molecular basis of transcription-coupled pre-mRNA capping. *Mol Cell* 58(6):1079–1089. <https://doi.org/10.1016/j.molcel.2015.04.004>
 122. Aoi Y, Smith ER, Shah AP, Rendleman EJ, Marshall SA, Woodfin AR, Chen FX, Shiekhattar R, Shilatifard A (2020) NELF regulates a promoter-proximal step distinct from RNA Pol II pause-release. *Mol Cell* 78(2):261–274. <https://doi.org/10.1016/j.molcel.2020.02.014>
 123. Narita T, Yung TM, Yamamoto J, Tsuboi Y, Tanabe H, Tanaka K, Yamaguchi Y, Handa H (2007) NELF interacts with CBC and participates in 3' end processing of replication-dependent histone mRNAs. *Mol Cell* 26(3):349–365. <https://doi.org/10.1016/j.molcel.2007.04.011>
 124. Gonatopoulos-Pournatzis T, Cowling VH (2014) Cap-binding complex (CBC). *Biochem J* 457(2):231–242. <https://doi.org/10.1042/BJ20131214>
 125. Nechaev S (1809) Adelman K (2011) Pol II waiting in the starting gates: regulating the transition from transcription initiation into productive elongation. *Biochem Biophys Acta* 1:34–45. <https://doi.org/10.1016/j.bbarm.2010.11.001>
 126. Boettiger AN, Levine M (2009) Synchronous and stochastic patterns of gene activation in the *Drosophila* embryo. *Science* 325(5939):471–473. <https://doi.org/10.1126/science.1173976>
 127. Lin C, Garrett AS, De Kumar B, Smith ER, Gogol M, Seidel C, Krumlauf R, Shilatifard A (2011) Dynamic transcriptional events in embryonic stem cells mediated by the super elongation complex (SEC). *Genes Dev* 25(14):1486–1498. <https://doi.org/10.1101/gad.2059211>
 128. Buckley MS, Kwak H, Zipfel WR, Lis JT (2014) Kinetics of promoter Pol II on Hsp70 reveal stable pausing and key insights into its regulation. *Genes Dev* 28(1):14–19. <https://doi.org/10.1101/gad.231886.113>
 129. Wu CH, Yamaguchi Y, Benjamin LR, Horvat-Gordon M, Washinsky J, Enerly E, Larsson J, Lambertsson A, Handa H, Gilmour D (2003) NELF and DSIF cause promoter proximal pausing on the hsp70 promoter in *Drosophila*. *Genes Dev* 17(11):1402–1414. <https://doi.org/10.1101/gad.1091403>
 130. Lagha M, Bothma JP, Esposito E, Ng S, Stefanik L, Tsui C, Johnston J, Chen K, Gilmour DS, Zeitlinger J, Levine MS (2013) Paused Pol II coordinates tissue morphogenesis in the *Drosophila* embryo. *Cell* 153(5):976–987. <https://doi.org/10.1016/j.cell.2013.04.045>
 131. Williams LH, Fromm G, Gokey NG, Henriques T, Muse GW, Burkholder A, Fargo DC, Hu G, Adelman K (2015) Pausing of RNA polymerase II regulates mammalian developmental potential through control of signaling networks. *Mol Cell* 58(2):311–322. <https://doi.org/10.1016/j.molcel.2015.02.003>
 132. Adelman K, Kennedy MA, Nechaev S, Gilchrist DA, Muse GW, Chinenov Y, Rogatsky I (2009) Immediate mediators of the inflammatory response are poised for gene activation through RNA polymerase II stalling. *Proc Natl Acad Sci USA* 106(43):18207–18212. <https://doi.org/10.1073/pnas.0910177106>
 133. Lavigne MD, Konstantopoulos D, Ntakou-Zamplara KZ, Liakos A, Fousteri M (2017) Global unleashing of transcription elongation waves in response to genotoxic stress restricts somatic mutation rate. *Nat Commun* 8(1):2076. <https://doi.org/10.1038/s41467-017-02145-4>
 134. Williamson L, Saponaro M, Boeing S, East P, Mitter R, Kantidakis T, Kelly GP, Lobley A, Walker J, Spencer-Dene B, Howell M, Stewart A, Svejstrup JQ (2017) UV irradiation induces a non-coding RNA that functionally opposes the protein encoded by the same gene. *Cell* 168(5):843–855. <https://doi.org/10.1016/j.cell.2017.01.019>
 135. Aiyar SE, Sun JL, Blair AL, Moskaluk CA, Lu YZ, Ye QN, Yamaguchi Y, Mukherjee A, Ren DM, Handa H, Li R (2004) Attenuation of estrogen receptor alpha-mediated transcription through estrogen-stimulated recruitment of a negative elongation factor. *Genes Dev* 18(17):2134–2146. <https://doi.org/10.1101/gad.1214104>
 136. Gilchrist DA, Fromm G, dos Santos G, Pham LN, McDaniel IE, Burkholder A, Fargo DC, Adelman K (2012) Regulating the regulators: the pervasive effects of Pol II pausing on stimulus-responsive gene networks. *Genes Dev* 26(9):933–944. <https://doi.org/10.1101/gad.187781.112>
 137. Chen F, Gao X, Shilatifard A (2015) Stably paused genes revealed through inhibition of transcription initiation by the TFIIF inhibitor triptolide. *Genes Dev* 29(1):39–47. <https://doi.org/10.1101/gad.246173.114>
 138. Suter DM, Molina N, Naef F, Schibler U (2011) Origins and consequences of transcriptional discontinuity. *Curr Opin Cell Biol* 23(6):657–662. <https://doi.org/10.1016/j.ceb.2011.09.004>
 139. Elrod ND, Henriques T, Huang KL, Tatomer DC, Wilusz JE, Wagner EJ, Adelman K (2019) The integrator complex attenuates promoter-proximal transcription at protein-coding genes. *Mol Cell* 76(5):738–752. <https://doi.org/10.1016/j.molcel.2019.10.034>

140. Erickson B, Sheridan RM, Cortazar M, Bentley DL (2018) Dynamic turnover of paused Pol II complexes at human promoters. *Genes Dev* 32(17–18):1215–1225. <https://doi.org/10.1101/gad.316810.118>
141. Huang KL, Jee D, Stein CB, Elrod ND, Henriques T, Mascibroda LG, Baillat D, Russell WK, Adelman K, Wagner EJ (2020) Integrator recruits protein phosphatase 2A to prevent pause release and facilitate transcription termination. *Mol Cell*. <https://doi.org/10.1016/j.molcel.2020.08.016>
142. Vervoort SJ, Welsh SA, Devlin JR, Barbieri E, Knight DA, Offley S, Bjelosevic S, Costacurta M, Todorovski I, Kearney CJ, Sandow JJ, Fan Z, Blyth B, McLeod V, Vissers JHA, Pavic K, Martin BP, Gregory G, Demosthenous E, Zethoven M, Kong IY, Hawkins ED, Hogg SJ, Kelly MJ, Newbold A, Simpson KJ, Kauko O, Harvey KF, Ohlmeyer M, Westermarck J, Gray N, Gardini A, Johnstone RW (2021) The PP2A-Integrator-CDK9 axis fine-tunes transcription and can be targeted therapeutically in cancer. *Cell*. <https://doi.org/10.1016/j.cell.2021.04.022>
143. Zheng H, Qi Y, Hu S, Cao X, Xu C, Yin Z, Chen X, Li Y, Liu W, Li J, Wang J, Wei G, Liang K, Chen FX, Xu Y (2020) Identification of Integrator-PP2A complex (INTAC), an RNA polymerase II phosphatase. *Science*. <https://doi.org/10.1126/science.abb5872>
144. Saponaro M, Kantidakis T, Mitter R, Kelly GP, Heron M, Williams H, Soding J, Stewart A, Svestrup JQ (2014) RECQL5 controls transcript elongation and suppresses genome instability associated with transcription stress. *Cell* 157(5):1037–1049. <https://doi.org/10.1016/j.cell.2014.03.048>
145. Booth GT, Parua PK, Sanso M, Fisher RP, Lis JT (2018) Cdk9 regulates a promoter-proximal checkpoint to modulate RNA polymerase II elongation rate in fission yeast. *Nat Commun* 9(1):543. <https://doi.org/10.1038/s41467-018-03006-4>
146. Cortazar MA, Sheridan RM, Erickson B, Fong N, Glover-Cutter K, Brannan K, Bentley DL (2019) Control of RNA Pol II speed by PNUITS-PP1 and Spt5 dephosphorylation facilitates termination by a “Sitting Duck Torpedo” mechanism. *Mol Cell* 76(6):896–908. <https://doi.org/10.1016/j.molcel.2019.09.031>
147. Kecman T, Kus K, Heo DH, Duckett K, Birot A, Liberatori S, Mohammed S, Geis-Asteggiane L, Robinson CV, Vasiljeva L (2018) Elongation/termination factor exchange mediated by PP1 phosphatase orchestrates transcription termination. *Cell Rep* 25(1):259–269. <https://doi.org/10.1016/j.celrep.2018.09.007>
148. Fong N, Saldi T, Sheridan RM, Cortazar MA, Bentley DL (2017) RNA Pol II dynamics modulate co-transcriptional chromatin modification, CTD phosphorylation, and transcriptional direction. *Mol Cell* 66(4):546–557. <https://doi.org/10.1016/j.molcel.2017.04.016>
149. Tellier M, Ferrer-Vicens I, Murphy S (2016) The point of no return: the poly(A)-associated elongation checkpoint. *RNA Biol* 13(3):265–271. <https://doi.org/10.1080/15476286.2016.1142037>
150. West S, Gromak N, Proudfoot NJ (2004) Human 5' → 3' exonuclease Xrn2 promotes transcription termination at co-transcriptional cleavage sites. *Nature* 432(7016):522–525. <https://doi.org/10.1038/nature03035>
151. Mayer A, Lidschreiber M, Siebert M, Leike K, Soding J, Cramer P (2010) Uniform transitions of the general RNA polymerase II transcription complex. *Nat Struct Mol Biol* 17(10):1272–1278. <https://doi.org/10.1038/nsmb.1903>
152. Schrieck A, Easter AD, Etzold S, Wiederhold K, Lidschreiber M, Cramer P, Passmore LA (2014) RNA polymerase II termination involves C-terminal-domain tyrosine dephosphorylation by CPF subunit Glc7. *Nat Struct Mol Biol* 21(2):175–179. <https://doi.org/10.1038/nsmb.2753>
153. Vanoosthuysse V, Legros P, van der Sar SJ, Yvert G, Toda K, Le Bihan T, Watanabe Y, Hardwick K, Bernard P (2014) CPF-associated phosphatase activity opposes condensin-mediated chromosome condensation. *PLoS Genet* 10(6):e1004415. <https://doi.org/10.1371/journal.pgen.1004415>
154. Bitoun E, Oliver PL, Davies KE (2007) The mixed-lineage leukemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. *Hum Mol Genet* 16(1):92–106. <https://doi.org/10.1093/hmg/ddl444>
155. Kuzmina A, Krasnopolsky S, Taube R (2017) Super elongation complex promotes early HIV transcription and its function is modulated by P-TEFb. *Transcription* 8(3):133–149. <https://doi.org/10.1080/21541264.2017.1295831>
156. Gordon V, Bhadel S, Wunderlich W, Zhang J, Ficarro SB, Mollah SA, Shabanowitz J, Hunt DF, Xenarios I, Hahn WC, Conaway M, Carey MF, Gioeli D (2010) CDK9 regulates AR promoter selectivity and cell growth through serine 81 phosphorylation. *Mol Endocrinol* 24(12):2267–2280. <https://doi.org/10.1210/me.2010-0238>
157. Bagashev A, Fan S, Mukerjee R, Claudio PP, Chabrashvili T, Leng RP, Benchimol S, Sawaya BE (2013) Cdk9 phosphorylates Pirh2 protein and prevents degradation of p53 protein. *Cell Cycle* 12(10):1569–1577. <https://doi.org/10.4161/cc.24733>
158. De Luca A, Esposito V, Baldi A, Claudio PP, Fu Y, Caputi M, Pisano MM, Baldi F, Giordano A (1997) CDC2-related kinase PITALRE phosphorylates pRb exclusively on serine and is widely expressed in human tissues. *J Cell Physiol* 172(2):265–273. [https://doi.org/10.1002/\(SICI\)1097-4652\(199708\)172:2%3c265::AID-JCP13%3e3.0.CO;2-8](https://doi.org/10.1002/(SICI)1097-4652(199708)172:2%3c265::AID-JCP13%3e3.0.CO;2-8)
159. Claudio PP, Cui J, Ghafouri M, Mariano C, White MK, Safak M, Sheffield JB, Giordano A, Khalili K, Amini S, Sawaya BE (2006) Cdk9 phosphorylates p53 on serine 392 independently of CKII. *J Cell Physiol* 208(3):602–612. <https://doi.org/10.1002/jcp.20698>
160. Radhakrishnan SK, Gartel AL (2006) CDK9 phosphorylates p53 on serine residues 33, 315 and 392. *Cell Cycle* 5(5):519–521. <https://doi.org/10.4161/cc.5.5.2514>
161. Brauns-Schubert P, Schubert F, Wissler M, Weiss M, Schlicher L, Bessler S, Safavi M, Miething C, Borner C, Brummer T, Maurer U (2018) CDK9-mediated phosphorylation controls the interaction of TIP60 with the transcriptional machinery. *EMBO Rep* 19(2):244–256. <https://doi.org/10.15252/embr.201744311>
162. O'Brien SK, Cao H, Nathans R, Ali A, Rana TM (2010) P-TEFb kinase complex phosphorylates histone H1 to regulate expression of cellular and HIV-1 genes. *J Biol Chem* 285(39):29713–29720. <https://doi.org/10.1074/jbc.M110.125997>
163. Shchebet A, Karpiuk O, Kremmer E, Eick D, Johnsen SA (2012) Phosphorylation by cyclin-dependent kinase-9 controls ubiquitin-conjugating enzyme-2A function. *Cell Cycle* 11(11):2122–2127. <https://doi.org/10.4161/cc.20548>
164. Zhang H, Pandey S, Travers M, Sun H, Morton G, Madzo J, Chung W, Khowsathit J, Perez-Leal O, Barrero CA, Merali C, Okamoto Y, Sato T, Pan J, Garriga J, Bhanu NV, Simithy J, Patel B, Huang J, Raynal NJ, Garcia BA, Jacobson MA, Kadoch C, Merali S, Zhang Y, Childers W, Abou-Gharbia M, Karanicolos J, Baylin SB, Zahnow CA, Jelinek J, Grana X, Issa JJ (2018) Targeting CDK9 reactivates epigenetically silenced genes in cancer. *Cell* 175(5):1244–1258. <https://doi.org/10.1016/j.cell.2018.09.051>
165. Decker TM, Forne I, Straub T, Elsaman H, Ma G, Shah N, Imhof A, Eick D (2019) Analog-sensitive cell line identifies cellular substrates of CDK9. *Oncotarget* 10(65):6934–6943. <https://doi.org/10.18632/oncotarget.27334>
166. Bartkowiak B, Liu P, Phatnani HP, Fuda NJ, Cooper JJ, Price DH, Adelman K, Lis JT, Greenleaf AL (2010) CDK12 is a transcription elongation-associated CTD kinase, the metazoan ortholog of yeast Ctk1. *Genes Dev* 24(20):2303–2316. <https://doi.org/10.1101/gad.1968210>

167. Blazek D, Kohoutek J, Bartholomeeusen K, Johansen E, Hulinkova P, Luo Z, Cimermancic P, Ule J, Peterlin BM (2011) The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. *Genes Dev* 25(20):2158–2172. <https://doi.org/10.1101/gad.16962311>
168. Tellier M, Zaborowska J, Caizzi L, Mohammad E, Velychko T, Schwalb B, Ferrer-Vicens I, Blears D, Nojima T, Cramer P, Murphy S (2020) CDK12 globally stimulates RNA polymerase II transcription elongation and carboxyl-terminal domain phosphorylation. *Nucleic Acids Res* 48(14):7712–7727. <https://doi.org/10.1093/nar/gkaa514>
169. Fan Z, Devlin JR, Hogg SJ, Doyle MA, Harrison PF, Todorovski I, Cluse LA, Knight DA, Sandow JJ, Gregory G, Fox A, Beilharz TH, Kwiatkowski N, Scott NE, Vidakovic AT, Kelly GP, Svejstrup JQ, Geyer M, Gray NS, Vervoort SJ, Johnstone RW (2020) CDK13 cooperates with CDK12 to control global RNA polymerase II processivity. *Sci Adv* 6(18):aaz5041. <https://doi.org/10.1126/sciadv.aaz5041>
170. Greifenberg AK, Honig D, Pilarova K, Duster R, Bartholomeeusen K, Bosken CA, Anand K, Blazek D, Geyer M (2016) Structural and functional analysis of the Cdk13/Cyclin K complex. *Cell Rep* 14(2):320–331. <https://doi.org/10.1016/j.celrep.2015.12.025>
171. Greenleaf AL (2019) Human CDK12 and CDK13, multi-tasking CTD kinases for the new millenium. *Transcription* 10(2):91–110. <https://doi.org/10.1080/102541264.2018.1535211>
172. Lui GYL, Grandori C, Kemp CJ (2018) CDK12: an emerging therapeutic target for cancer. *J Clin Pathol* 71(11):957–962. <https://doi.org/10.1136/jclinpath-2018-205356>
173. Chirackal Manavalan AP, Pilarova K, Kluge M, Bartholomeeusen K, Rajecy M, Oppelt J, Khirsariya P, Paruch K, Krejci L, Friedel CC, Blazek D (2019) CDK12 controls G1/S progression by regulating RNAPII processivity at core DNA replication genes. *EMBO Rep* 20(9):e47592. <https://doi.org/10.15252/embr.201847592>
174. Liang K, Gao X, Gilmore JM, Florens L, Washburn MP, Smith E, Shilatifard A (2015) Characterization of human cyclin-dependent kinase 12 (CDK12) and CDK13 complexes in C-terminal domain phosphorylation, gene transcription, and RNA processing. *Mol Cell Biol* 35(6):928–938. <https://doi.org/10.1128/MCB.01426-14>
175. Bartkowiak B, Yan C (1849) Greenleaf AL (2015) Engineering an analog-sensitive CDK12 cell line using CRISPR/Cas. *Biochem Biophys Acta* 9:1179–1187. <https://doi.org/10.1016/j.bbagr.2015.07.010>
176. Heidemann M, Hintermair C, Voss K (1829) Eick D (2013) Dynamic phosphorylation patterns of RNA polymerase II CTD during transcription. *Biochem Biophys Acta* 1:55–62. <https://doi.org/10.1016/j.bbagr.2012.08.013>
177. Bosken CA, Farnung L, Hintermair C, Merzel Schachter M, Vogel-Bachmayr K, Blazek D, Anand K, Fisher RP, Eick D, Geyer M (2014) The structure and substrate specificity of human Cdk12/Cyclin K. *Nat Commun* 5:3505. <https://doi.org/10.1038/ncomms4505>
178. Zhang T, Kwiatkowski N, Olson CM, Dixon-Clarke SE, Abraham BJ, Greifenberg AK, Ficarro SB, Elkins JM, Liang Y, Hannett NM, Manz T, Hao M, Bartkowiak B, Greenleaf AL, Marto JA, Geyer M, Bullock AN, Young RA, Gray NS (2016) Covalent targeting of remote cysteine residues to develop CDK12 and CDK13 inhibitors. *Nat Chem Biol* 12(10):876–884. <https://doi.org/10.1038/nchembio.2166>
179. Krajewska M, Dries R, Grasseti AV, Dust S, Gao Y, Huang H, Sharma B, Day DS, Kwiatkowski N, Pomaville M, Dodd O, Chipumuro E, Zhang T, Greenleaf AL, Yuan GC, Gray NS, Young RA, Geyer M, Gerber SA, George RE (2019) CDK12 loss in cancer cells affects DNA damage response genes through premature cleavage and polyadenylation. *Nat Commun* 10(1):1757. <https://doi.org/10.1038/s41467-019-09703-y>
180. Dubbury SJ, Boutz PL, Sharp PA (2018) CDK12 regulates DNA repair genes by suppressing intronic polyadenylation. *Nature* 564(7734):141–145. <https://doi.org/10.1038/s41586-018-0758-y>
181. Quereda V, Bayle S, Vena F, Frydman SM, Monastyrskyi A, Roush WR, Duckett DR (2019) Therapeutic targeting of CDK12/CDK13 in triple-negative breast cancer. *Cancer Cell* 36(5):545–558. <https://doi.org/10.1016/j.ccell.2019.09.004>
182. Bartkowiak B, Greenleaf AL (2015) Expression, purification, and identification of associated proteins of the full-length hCDK12/CyclinK complex. *J Biol Chem* 290(3):1786–1795. <https://doi.org/10.1074/jbc.M114.612226>
183. Bartkowiak B, Yan CM, Soderblom EJ, Greenleaf AL (2019) CDK12 activity-dependent phosphorylation events in human cells. *Biomolecules*. <https://doi.org/10.3390/biom9100634>
184. Eifler TT, Shao W, Bartholomeeusen K, Fujinaga K, Jager S, Johnson JR, Luo Z, Krogan NJ, Peterlin BM (2015) Cyclin-dependent kinase 12 increases 3' end processing of growth factor-induced c-FOS transcripts. *Mol Cell Biol* 35(2):468–478. <https://doi.org/10.1128/MCB.01157-14>
185. Olson CM, Jiang B, Erb MA, Liang Y, Doctor ZM, Zhang Z, Zhang T, Kwiatkowski N, Boukhali M, Green JL, Haas W, Nomanbhoy T, Fischer ES, Young RA, Bradner JE, Winter GE, Gray NS (2018) Pharmacological perturbation of CDK9 using selective CDK9 inhibition or degradation. *Nat Chem Biol* 14(2):163–170. <https://doi.org/10.1038/nchembio.2538>
186. Cho WK, Spille JH, Hecht M, Lee C, Li C, Grube V, Cisse II (2018) Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. *Science* 361(6400):412–415. <https://doi.org/10.1126/science.aar4199>
187. Chong S, Dugast-Darzacq C, Liu Z, Dong P, Dailey GM, Catglio C, Heckert A, Banala S, Lavis L, Darzacq X, Tjian R (2018) Imaging dynamic and selective low-complexity domain interactions that control gene transcription. *Science*. <https://doi.org/10.1126/science.aar2555>
188. Hnisz D, Shrinivas K, Young RA, Chakraborty AK, Sharp PA (2017) A phase separation model for transcriptional control. *Cell* 169(1):13–23. <https://doi.org/10.1016/j.cell.2017.02.007>
189. Bojja A, Klein IA, Sabari BR, Dall'Agnese A, Coffey EL, Zamudio AV, Li CH, Shrinivas K, Manteiga JC, Hannett NM, Abraham BJ, Afeyan LK, Guo YE, Rimel JK, Fant CB, Schuijers J, Lee TI, Taatjes DJ, Young RA (2018) Transcription factors activate genes through the phase-separation capacity of their activation domains. *Cell* 175(7):1842–1855. <https://doi.org/10.1016/j.cell.2018.10.042>
190. Kwon I, Kato M, Xiang S, Wu L, Theodoropoulos P, Mirzaei H, Han T, Xie S, Corden JL, McKnight SL (2013) Phosphorylation-regulated binding of RNA polymerase II to fibrous polymers of low-complexity domains. *Cell* 155(5):1049–1060. <https://doi.org/10.1016/j.cell.2013.10.033>
191. Lu Y, Wu T, Gutman O, Lu H, Zhou Q, Henis YI, Luo K (2020) Phase separation of TAZ compartmentalizes the transcription machinery to promote gene expression. *Nat Cell Biol* 22(4):453–464. <https://doi.org/10.1038/s41556-020-0485-0>
192. Sabari BR, Dall'Agnese A, Bojja A, Klein IA, Coffey EL, Shrinivas K, Abraham BJ, Hannett NM, Zamudio AV, Manteiga JC, Li CH, Guo YE, Day DS, Schuijers J, Vasile E, Malik S, Hnisz D, Lee TI, Cisse II, Roeder RG, Sharp PA, Chakraborty AK, Young RA (2018) Coactivator condensation at super-enhancers links phase separation and gene control. *Science*. <https://doi.org/10.1126/science.aar3958>
193. Boehning M, Dugast-Darzacq C, Rankovic M, Hansen AS, Yu T, Marie-Nelly H, McSwiggen DT, Kokic G, Dailey GM, Cramer P, Darzacq X, Zweckstetter M (2018) RNA polymerase II clustering through carboxy-terminal domain phase separation.

- Nat Struct Mol Biol 25(9):833–840. <https://doi.org/10.1038/s41594-018-0112-y>
194. Guo YE, Manteiga JC, Henninger JE, Sabari BR, Dall'Agnese A, Hannett NM, Spille JH, Afeyan LK, Zamudio AV, Shrinivas K, Abraham BJ, Boija A, Decker TM, Rimel JK, Fant CB, Lee TI, Cisse II, Sharp PA, Taatjes DJ, Young RA (2019) Pol II phosphorylation regulates a switch between transcriptional and splicing condensates. *Nature* 572(7770):543–548. <https://doi.org/10.1038/s41586-019-1464-0>
 195. Guo C, Che Z, Yue J, Xie P, Hao S, Xie W, Luo Z, Lin C (2020) ENL initiates multivalent phase separation of the super elongation complex (SEC) in controlling rapid transcriptional activation. *Sci Adv* 6(14):eaay4858. <https://doi.org/10.1126/sciadv.aay4858>
 196. Dow EC, Liu H, Rice AP (2010) T-loop phosphorylated Cdk9 localizes to nuclear speckle domains which may serve as sites of active P-TEFb function and exchange between the Brd4 and 7SK/HEXIM1 regulatory complexes. *J Cell Physiol* 224(1):84–93. <https://doi.org/10.1002/jcp.22096>
 197. Galganski L, Urbanek MO, Krzyzosiak WJ (2017) Nuclear speckles: molecular organization, biological function and role in disease. *Nucleic Acids Res* 45(18):10350–10368. <https://doi.org/10.1093/nar/gkx759>
 198. Herzel L, Ottoz DSM, Alpert T, Neugebauer KM (2017) Splicing and transcription touch base: co-transcriptional spliceosome assembly and function. *Nat Rev Mol Cell Biol* 18(10):637–650. <https://doi.org/10.1038/nrm.2017.63>
 199. Ghamari A, van de Corput MP, Thongjuea S, van Cappellen WA, van Ijcken W, van Haren J, Soler E, Eick D, Lenhard B, Grosveld FG (2013) In vivo live imaging of RNA polymerase II transcription factories in primary cells. *Genes Dev* 27(7):767–777. <https://doi.org/10.1101/gad.216200.113>
 200. Lim S, Kaldis P (2013) Cdks, cyclins and CKIs: roles beyond cell cycle regulation. *Development* 140(15):3079–3093. <https://doi.org/10.1242/dev.091744>
 201. Quaresma AJC, Bugai A, Barboric M (2016) Cracking the control of RNA polymerase II elongation by 7SK snRNP and P-TEFb. *Nucleic Acids Res* 44(16):7527–7539. <https://doi.org/10.1093/nar/gkw585>
 202. He N, Jahchan NS, Hong E, Li Q, Bayfield MA, Marais RJ, Luo K, Zhou Q (2008) A La-related protein modulates 7SK snRNP integrity to suppress P-TEFb-dependent transcriptional elongation and tumorigenesis. *Mol Cell* 29(5):588–599. <https://doi.org/10.1016/j.molcel.2008.01.003>
 203. Jeronimo C, Forget D, Bouchard A, Li Q, Chua G, Poitras C, Therien C, Bergeron D, Bourassa S, Greenblatt J, Chabot B, Poirier GG, Hughes TR, Blanchette M, Price DH, Coulombe B (2007) Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme. *Mol Cell* 27(2):262–274. <https://doi.org/10.1016/j.molcel.2007.06.027>
 204. Krueger BJ, Jeronimo C, Roy BB, Bouchard A, Barrandon C, Byers SA, Searcey CE, Cooper JJ, Bensaude O, Cohen EA, Coulombe B, Price DH (2008) LARP7 is a stable component of the 7SK snRNP while P-TEFb, HEXIM1 and hnRNP A1 are reversibly associated. *Nucleic Acids Res* 36(7):2219–2229. <https://doi.org/10.1093/nar/gkn061>
 205. Egloff S, Van Herreweghe E, Kiss T (2006) Regulation of polymerase II transcription by 7SK snRNA: two distinct RNA elements direct P-TEFb and HEXIM1 binding. *Mol Cell Biol* 26(2):630–642. <https://doi.org/10.1128/MCB.26.2.630-642.2006>
 206. Michels AA, Fraldi A, Li Q, Adamson TE, Bonnet F, Nguyen VT, Sedore SC, Price JP, Price DH, Lania L, Bensaude O (2004) Binding of the 7SK snRNA turns the HEXIM1 protein into a P-TEFb (CDK9/cyclin T) inhibitor. *EMBO J* 23(13):2608–2619. <https://doi.org/10.1038/sj.emboj.7600275>
 207. Muniz L, Egloff S, Ughy B, Jady BE, Kiss T (2010) Controlling cellular P-TEFb activity by the HIV-1 transcriptional transactivator Tat. *PLoS Pathog* 6(10):e1001152. <https://doi.org/10.1371/journal.ppat.1001152>
 208. Yik JH, Chen R, Nishimura R, Jennings JL, Link AJ, Zhou Q (2003) Inhibition of P-TEFb (CDK9/Cyclin T) kinase and RNA polymerase II transcription by the coordinated actions of HEXIM1 and 7SK snRNA. *Mol Cell* 12(4):971–982. [https://doi.org/10.1016/s1097-2765\(03\)00388-5](https://doi.org/10.1016/s1097-2765(03)00388-5)
 209. Kobbi L, Demey-Thomas E, Braye F, Proux F, Kolesnikova O, Vinh J, Poterszman A, Bensaude O (2016) An evolutionary conserved Hexim1 peptide binds to the Cdk9 catalytic site to inhibit P-TEFb. *Proc Natl Acad Sci USA* 113(45):12721–12726. <https://doi.org/10.1073/pnas.1612331113>
 210. Schulte A, Czudnochowski N, Barboric M, Schonichen A, Blazek D, Peterlin BM, Geyer M (2005) Identification of a cyclin T-binding domain in Hexim1 and biochemical analysis of its binding competition with HIV-1 Tat. *J Biol Chem* 280(26):24968–24977. <https://doi.org/10.1074/jbc.M501431200>
 211. Barboric M, Lenasi T, Chen H, Johansen EB, Guo S, Peterlin BM (2009) 7SK snRNP/P-TEFb couples transcription elongation with alternative splicing and is essential for vertebrate development. *Proc Natl Acad Sci USA* 106(19):7798–7803. <https://doi.org/10.1073/pnas.0903188106>
 212. Muniz L, Egloff S, Kiss T (2013) RNA elements directing in vivo assembly of the 7SK/MePCE/Larp7 transcriptional regulatory snRNP. *Nucleic Acids Res* 41(8):4686–4698. <https://doi.org/10.1093/nar/gkt159>
 213. Eichhorn CD, Yang Y, Repeta L, Feigon J (2018) Structural basis for recognition of human 7SK long noncoding RNA by the La-related protein Larp7. *Proc Natl Acad Sci USA* 115(28):E6457–E6466. <https://doi.org/10.1073/pnas.1806276115>
 214. Yang Y, Eichhorn CD, Wang Y, Cascio D, Feigon J (2019) Structural basis of 7SK RNA 5'-gamma-phosphate methylation and retention by MePCE. *Nat Chem Biol* 15(2):132–140. <https://doi.org/10.1038/s41589-018-0188-z>
 215. Chen R, Yang Z, Zhou Q (2004) Phosphorylated positive transcription elongation factor b (P-TEFb) is tagged for inhibition through association with 7SK snRNA. *J Biol Chem* 279(6):4153–4160. <https://doi.org/10.1074/jbc.M310044200>
 216. Li Q, Price JP, Byers SA, Cheng D, Peng J, Price DH (2005) Analysis of the large inactive P-TEFb complex indicates that it contains one 7SK molecule, a dimer of HEXIM1 or HEXIM2, and two P-TEFb molecules containing Cdk9 phosphorylated at threonine 186. *J Biol Chem* 280(31):28819–28826. <https://doi.org/10.1074/jbc.M502712200>
 217. Zhou Q, Yik JH (2006) The Yin and Yang of P-TEFb regulation: implications for human immunodeficiency virus gene expression and global control of cell growth and differentiation. *Microbiol Mol Biol Rev* 70(3):646–659. <https://doi.org/10.1128/MMBR.00011-06>
 218. Barboric M, Yik JH, Czudnochowski N, Yang Z, Chen R, Contreras X, Geyer M, Matija Peterlin B, Zhou Q (2007) Tat competes with HEXIM1 to increase the active pool of P-TEFb for HIV-1 transcription. *Nucleic Acids Res* 35(6):2003–2012. <https://doi.org/10.1093/nar/gkm063>
 219. Kim YK, Mbonye U, Hokello J, Karn J (2011) T-cell receptor signaling enhances transcriptional elongation from latent HIV proviruses by activating P-TEFb through an ERK-dependent pathway. *J Mol Biol* 410(5):896–916. <https://doi.org/10.1016/j.jmb.2011.03.054>
 220. Nguyen VT, Kiss T, Michels AA, Bensaude O (2001) 7SK small nuclear RNA binds to and inhibits the activity of CDK9/cyclin T complexes. *Nature* 414(6861):322–325. <https://doi.org/10.1038/35104581>

221. Yang Z, Zhu Q, Luo K, Zhou Q (2001) The 7SK small nuclear RNA inhibits the CDK9/cyclin T1 kinase to control transcription. *Nature* 414(6861):317–322. <https://doi.org/10.1038/35104575>
222. Napolitano G, Amente S, Lavadera ML, Di Palo G, Ambrosio S, Lania L, Dellino GI, Pelicci PG, Majello B (2013) Sequence-specific double strand breaks trigger P-TEFb-dependent Rpb1-CTD hyperphosphorylation. *Mutat Res* 749(1–2):21–27. <https://doi.org/10.1016/j.mrfmmm.2013.07.005>
223. Sun Y, Liu Z, Cao X, Lu Y, Mi Z, He C, Liu J, Zheng Z, Li MJ, Li T, Xu D, Wu M, Cao Y, Li Y, Yang B, Mei C, Zhang L, Chen Y (2019) Activation of P-TEFb by cAMP-PKA signaling in autosomal dominant polycystic kidney disease. *Sci Adv* 5(6):3593. <https://doi.org/10.1126/sciadv.aaw3593>
224. Cho S, Schroeder S, Kaehlecke K, Kwon HS, Pedal A, Herker E, Schnoelzer M, Ott M (2009) Acetylation of cyclin T1 regulates the equilibrium between active and inactive P-TEFb in cells. *EMBO J* 28(10):1407–1417. <https://doi.org/10.1038/emboj.2009.99>
225. Faust TB, Li Y, Bacon CW, Jang GM, Weiss A, Jayaraman B, Newton BW, Krogan NJ, D'Orso I, Frankel AD (2018) The HIV-1 Tat protein recruits a ubiquitin ligase to reorganize the 7SK snRNP for transcriptional activation. *Elife*. <https://doi.org/10.7554/eLife.31879>
226. Mbonye UR, Wang B, Gokulrangan G, Chance MR, Karn J (2015) Phosphorylation of HEXIM1 at Tyr271 and Tyr274 promotes release of P-TEFb from the 7SK snRNP complex and enhances proviral HIV gene expression. *Proteomics* 15(12):2078–2086. <https://doi.org/10.1002/pmic.201500038>
227. Chen R, Liu M, Li H, Xue Y, Ramey WN, He N, Ai N, Luo H, Zhu Y, Zhou N, Zhou Q (2008) PP2B and PP1alpha cooperatively disrupt 7SK snRNP to release P-TEFb for transcription in response to Ca²⁺ signaling. *Genes Dev* 22(10):1356–1368. <https://doi.org/10.1101/gad.1636008>
228. McNamara RP, McCann JL, Gudipaty SA, D'Orso I (2013) Transcription factors mediate the enzymatic disassembly of promoter-bound 7SK snRNP to locally recruit P-TEFb for transcription elongation. *Cell Rep* 5(5):1256–1268. <https://doi.org/10.1016/j.celrep.2013.11.003>
229. Wang Y, Dow EC, Liang YY, Ramakrishnan R, Liu H, Sung TL, Lin X, Rice AP (2008) Phosphatase PPM1A regulates phosphorylation of Thr-186 in the Cdk9 T-loop. *J Biol Chem* 283(48):33578–33584. <https://doi.org/10.1074/jbc.M807495200>
230. Krueger BJ, Varzavand K, Cooper JJ, Price DH (2010) The mechanism of release of P-TEFb and HEXIM1 from the 7SK snRNP by viral and cellular activators includes a conformational change in 7SK. *PLoS ONE* 5(8):e12335. <https://doi.org/10.1371/journal.pone.0012335>
231. Elagib KE, Rubinstein JD, Delehanty LL, Ngoh VS, Greer PA, Li S, Lee JK, Li Z, Orkin SH, Mihaylov IS, Goldfarb AN (2013) Calpain 2 activation of P-TEFb drives megakaryocyte morphogenesis and is disrupted by leukemogenic GATA1 mutation. *Dev Cell* 27(6):607–620. <https://doi.org/10.1016/j.devcel.2013.11.013>
232. Lee S, Liu H, Hill R, Chen C, Hong X, Crawford F, Kingsley M, Zhang Q, Liu X, Chen Z, Lengeling A, Bernt KM, Marrack P, Kappler J, Zhou Q, Li CY, Xue Y, Hansen K, Zhang G (2020) JMJD6 cleaves MePCE to release positive transcription elongation factor b (P-TEFb) in higher eukaryotes. *Elife*. <https://doi.org/10.7554/eLife.53930>
233. Liu W, Ma Q, Wong K, Li W, Ohgi K, Zhang J, Aggarwal A, Rosenfeld MG (2013) Brd4 and JMJD6-associated anti-pause enhancers in regulation of transcriptional pause release. *Cell* 155(7):1581–1595. <https://doi.org/10.1016/j.cell.2013.10.056>
234. Barrandon C, Bonnet F, Nguyen VT, Labas V, Bensaude O (2007) The transcription-dependent dissociation of P-TEFb-HEXIM1-7SK RNA relies upon formation of hnRNP-7SK RNA complexes. *Mol Cell Biol* 27(20):6996–7006. <https://doi.org/10.1128/MCB.00975-07>
235. Bugai A, Quresma AJC, Friedel CC, Lenasi T, Duster R, Sibley CR, Fujinaga K, Kukanja P, Hennig T, Blasius M, Geyer M, Ule J, Dolken L, Barboric M (2019) P-TEFb activation by RBM7 shapes a pro-survival transcriptional response to genotoxic stress. *Mol Cell* 74(2):254–267. <https://doi.org/10.1016/j.molcel.2019.01.033>
236. Calo E, Flynn RA, Martin L, Spitale RC, Chang HY, Wysocka J (2015) RNA helicase DDX21 coordinates transcription and ribosomal RNA processing. *Nature* 518(7538):249–253. <https://doi.org/10.1038/nature13923>
237. Cho WK, Zhou M, Jang MK, Huang K, Jeong SJ, Ozato K, Brady JN (2007) Modulation of the Brd4/P-TEFb interaction by the human T-lymphotropic virus type 1 tax protein. *J Virol* 81(20):11179–11186. <https://doi.org/10.1128/JVI.00408-07>
238. Ji X, Zhou Y, Pandit S, Huang J, Li H, Lin CY, Xiao R, Burge CB, Fu XD (2013) SR proteins collaborate with 7SK and promoter-associated nascent RNA to release paused polymerase. *Cell* 153(4):855–868. <https://doi.org/10.1016/j.cell.2013.04.028>
239. Van Herreweghe E, Egloff S, Goiffon I, Jady BE, Froment C, Monsarrat B, Kiss T (2007) Dynamic remodelling of human 7SK snRNP controls the nuclear level of active P-TEFb. *EMBO J* 26(15):3570–3580. <https://doi.org/10.1038/sj.emboj.7601783>
240. Yang Z, Yik JH, Chen R, He N, Jang MK, Ozato K, Zhou Q (2005) Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. *Mol Cell* 19(4):535–545. <https://doi.org/10.1016/j.molcel.2005.06.029>
241. D'Orso I (2016) 7SKing on chromatin: move globally, act locally. *RNA Biol* 13(6):545–553. <https://doi.org/10.1080/15476286.2016.1181254>
242. D'Orso I, Frankel AD (2010) RNA-mediated displacement of an inhibitory snRNP complex activates transcription elongation. *Nat Struct Mol Biol* 17(7):815–821. <https://doi.org/10.1038/nsmb.1827>
243. McNamara RP, Reeder JE, McMillan EA, Bacon CW, McCann JL, D'Orso I (2016) KAP1 Recruitment of the 7SK snRNP complex to promoters enables transcription elongation by RNA polymerase II. *Mol Cell* 61(1):39–53. <https://doi.org/10.1016/j.molcel.2015.11.004>
244. Bi X, Xu Y, Li T, Li X, Li W, Shao W, Wang K, Zhan G, Wu Z, Liu W, Lu JY, Wang L, Zhao J, Wu J, Na J, Li G, Li P, Shen X (2019) RNA targets ribogenesis factor WDR43 to chromatin for transcription and pluripotency control. *Mol Cell* 75(1):102–116. <https://doi.org/10.1016/j.molcel.2019.05.007>
245. Flynn RA, Do BT, Rubin AJ, Calo E, Lee B, Kuchelmeister H, Rale M, Chu C, Kool ET, Wysocka J, Khavari PA, Chang HY (2016) 7SK-BAF axis controls pervasive transcription at enhancers. *Nat Struct Mol Biol* 23(3):231–238. <https://doi.org/10.1038/nsmb.3176>
246. Egloff S, Vitali P, Tellier M, Raffel R, Murphy S, Kiss T (2017) The 7SK snRNP associates with the little elongation complex to promote snRNA gene expression. *EMBO J* 36(7):934–948. <https://doi.org/10.15252/emboj.201695740>
247. Mumbach MR, Granja JM, Flynn RA, Roake CM, Satpathy AT, Rubin AJ, Qi Y, Jiang Z, Shams S, Louie BH, Guo JK, Gennert DG, Corces MR, Khavari PA, Atianand MK, Artandi SE, Fitzgerald KA, Greenleaf WJ, Chang HY (2019) HiChIRP reveals RNA-associated chromosome conformation. *Nat Methods* 16(6):489–492. <https://doi.org/10.1038/s41592-019-0407-x>
248. Prasanth KV, Camiolo M, Chan G, Tripathi V, Denis L, Nakamura T, Hubner MR, Spector DL (2010) Nuclear organization and dynamics of 7SK RNA in regulating gene expression. *Mol*

- Biol Cell 21(23):4184–4196. <https://doi.org/10.1091/mbc.E10-02-0105>
249. He N, Pezda AC, Zhou Q (2006) Modulation of a P-TEFb functional equilibrium for the global control of cell growth and differentiation. *Mol Cell Biol* 26(19):7068–7076. <https://doi.org/10.1128/MCB.00778-06>
 250. Jang MK, Mochizuki K, Zhou M, Jeong HS, Brady JN, Ozato K (2005) The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. *Mol Cell* 19(4):523–534. <https://doi.org/10.1016/j.molcel.2005.06.027>
 251. Mbonye UR, Gokulrangan G, Datt M, Dobrowolski C, Cooper M, Chance MR, Karn J (2013) Phosphorylation of CDK9 at Ser175 enhances HIV transcription and is a marker of activated P-TEFb in CD4(+) T lymphocytes. *PLoS Pathog* 9(5):e1003338. <https://doi.org/10.1371/journal.ppat.1003338>
 252. Schroder S, Cho S, Zeng L, Zhang Q, Kaehlcke K, Mak L, Lau J, Bisgrove D, Schnolzer M, Verdin E, Zhou MM, Ott M (2012) Two-pronged binding with bromodomain-containing protein 4 liberates positive transcription elongation factor b from inactive ribonucleoprotein complexes. *J Biol Chem* 287(2):1090–1099. <https://doi.org/10.1074/jbc.M111.282855>
 253. Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, Robson SC, Chung CW, Hopf C, Savitski MM, Huthmacher C, Gudgin E, Lugo D, Beinke S, Chapman TD, Roberts EJ, Soden PE, Auger KR, Mirgouet O, Doehner K, Delwel R, Burnett AK, Jeffrey P, Drewes G, Lee K, Huntly BJ, Kouzarides T (2011) Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. *Nature* 478(7370):529–533. <https://doi.org/10.1038/nature10509>
 254. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastriitis E, Gilpatrick T, Paranani RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel PL, Ghobrial IM, Richardson PG, Young RA, Hahn WC, Anderson KC, Kung AL, Bradner JE, Mitsiades CS (2011) BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* 146(6):904–917. <https://doi.org/10.1016/j.cell.2011.08.017>
 255. Kanno T, Kanno Y, LeRoy G, Campos E, Sun HW, Brooks SR, Vahedi G, Heightman TD, Garcia BA, Reinberg D, Siebenlist U, O'Shea JJ, Ozato K (2014) BRD4 assists elongation of both coding and enhancer RNAs by interacting with acetylated histones. *Nat Struct Mol Biol* 21(12):1047–1057. <https://doi.org/10.1038/nsmb.2912>
 256. Winter GE, Mayer A, Buckley DL, Erb MA, Roderick JE, Vittori S, Reyes JM, di Iulio J, Souza A, Ott CJ, Roberts JM, Zeid R, Scott TG, Paulk J, Lachance K, Olson CM, Dastjerdi S, Bauer S, Lin CY, Gray NS, Kelliher MA, Churchman LS, Bradner JE (2017) BET Bromodomain proteins function as master transcription elongation factors independent of CDK9 recruitment. *Mol Cell* 67(1):5–18. <https://doi.org/10.1016/j.molcel.2017.06.004>
 257. Itzen F, Greifenberg AK, Bosken CA, Geyer M (2014) Brd4 activates P-TEFb for RNA polymerase II CTD phosphorylation. *Nucleic Acids Res* 42(12):7577–7590. <https://doi.org/10.1093/nar/gku449>
 258. Barboric M, Nissen RM, Kanazawa S, Jabrane-Ferrat N, Peterlin BM (2001) NF-kappaB binds P-TEFb to stimulate transcriptional elongation by RNA polymerase II. *Mol Cell* 8(2):327–337. [https://doi.org/10.1016/s1097-2765\(01\)00314-8](https://doi.org/10.1016/s1097-2765(01)00314-8)
 259. Giraud S, Hurlstone A, Avril S, Coqueret O (2004) Implication of BRG1 and cdk9 in the STAT3-mediated activation of the p21 waf1 gene. *Oncogene* 23(44):7391–7398. <https://doi.org/10.1038/sj.onc.1207972>
 260. Nojima M, Huang Y, Tyagi M, Kao HY, Fujinaga K (2008) The positive transcription elongation factor b is an essential cofactor for the activation of transcription by myocyte enhancer factor 2. *J Mol Biol* 382(2):275–287. <https://doi.org/10.1016/j.jmb.2008.07.017>
 261. Takahashi H, Parmely TJ, Sato S, Tomomori-Sato C, Banks CA, Kong SE, Szutorisz H, Swanson SK, Martin-Brown S, Washburn MP, Florens L, Seidel CW, Lin C, Smith ER, Shilatifard A, Conway RC, Conway JW (2011) Human mediator subunit MED26 functions as a docking site for transcription elongation factors. *Cell* 146(1):92–104. <https://doi.org/10.1016/j.cell.2011.06.005>
 262. He N, Chan CK, Sobhian B, Chou S, Xue Y, Liu M, Alber T, Benkirane M, Zhou Q (2011) Human polymerase-associated factor complex (PAF_c) connects the super elongation complex (SEC) to RNA polymerase II on chromatin. *Proc Natl Acad Sci USA* 108(36):E636–645. <https://doi.org/10.1073/pnas.1107107108>
 263. Gardini A, Baillat D, Cesaroni M, Hu D, Marinis JM, Wagner EJ, Lazar MA, Shilatifard A, Shiekhhattar R (2014) Integrator regulates transcriptional initiation and pause release following activation. *Mol Cell* 56(1):128–139. <https://doi.org/10.1016/j.molcel.2014.08.004>
 264. Zaborowska J, Isa NF, Murphy S (2016) P-TEFb goes viral. *BioEssays* 38(Suppl 1):S75–85. <https://doi.org/10.1002/bies.201670912>
 265. Zhang Z, Klatt A, Gilmour DS, Henderson AJ (2007) Negative elongation factor NELF represses human immunodeficiency virus transcription by pausing the RNA polymerase II complex. *J Biol Chem* 282(23):16981–16988. <https://doi.org/10.1074/jbc.M610688200>
 266. Karn J (1999) Tackling tat. *J Mol Biol* 293(2):235–254. <https://doi.org/10.1006/jmbi.1999.3060>
 267. Tahirov TH, Babayeva ND, Varzavand K, Cooper JJ, Sedore SC, Price DH (2010) Crystal structure of HIV-1 Tat complexed with human P-TEFb. *Nature* 465(7299):747–751. <https://doi.org/10.1038/nature09131>
 268. Flores O, Lee G, Kessler J, Miller M, Schlieff W, Tomassini J, Hazuda D (1999) Host-cell positive transcription elongation factor b kinase activity is essential and limiting for HIV type 1 replication. *Proc Natl Acad Sci USA* 96(13):7208–7213. <https://doi.org/10.1073/pnas.96.13.7208>
 269. Budhiraja S, Famiglietti M, Bosque A, Planelles V, Rice AP (2013) Cyclin T1 and CDK9 T-loop phosphorylation are down-regulated during establishment of HIV-1 latency in primary resting memory CD4+ T cells. *J Virol* 87(2):1211–1220. <https://doi.org/10.1128/JVI.02413-12>
 270. Zhu J, Gaiha GD, John SP, Pertel T, Chin CR, Gao G, Qu H, Walker BD, Elledge SJ, Brass AL (2012) Reactivation of latent HIV-1 by inhibition of BRD4. *Cell Rep* 2(4):807–816. <https://doi.org/10.1016/j.celrep.2012.09.008>
 271. Shortridge MD, Wille PT, Jones AN, Davidson A, Bogdanovic J, Arts E, Karn J, Robinson JA, Varani G (2019) An ultra-high affinity ligand of HIV-1 TAR reveals the RNA structure recognized by P-TEFb. *Nucleic Acids Res* 47(3):1523–1531. <https://doi.org/10.1093/nar/gky1197>
 272. Sano M, Schneider MD (2003) Cyclins that don't cycle—cyclin T/cyclin-dependent kinase-9 determines cardiac muscle cell size. *Cell Cycle* 2(2):99–104
 273. Espinoza-Derout J, Wagner M, Shahmiri K, Mascareno E, Chaour B, Siddiqui MA (2007) Pivotal role of cardiac lineage protein-1 (CLP-1) in transcriptional elongation factor P-TEFb complex formation in cardiac hypertrophy. *Cardiovasc Res* 75(1):129–138. <https://doi.org/10.1016/j.cardiores.2007.03.019>
 274. Sano M, Abdellatif M, Oh H, Xie M, Bagella L, Giordano A, Michael LH, DeMayo FJ, Schneider MD (2002) Activation and function of cyclin T-Cdk9 (positive transcription elongation factor-b) in cardiac muscle-cell hypertrophy. *Nat Med* 8(11):1310–1317. <https://doi.org/10.1038/nm778>

275. Bywater MJ, Burkhardt DL, Straube J, Sabo A, Pendino V, Hudson JE, Quaife-Ryan GA, Porrello ER, Rae J, Parton RG, Kress TR, Amati B, Littlewood TD, Evan GI, Wilson CH (2020) Reactivation of Myc transcription in the mouse heart unlocks its proliferative capacity. *Nat Commun* 11(1):1827. <https://doi.org/10.1038/s41467-020-15552-x>
276. Anand P, Brown JD, Lin CY, Qi J, Zhang R, Artero PC, Alaiti MA, Bullard J, Alazem K, Margulies KB, Cappola TP, Lemieux M, Plutzky J, Bradner JE, Haldar SM (2013) BET bromodomains mediate transcriptional pause release in heart failure. *Cell* 154(3):569–582. <https://doi.org/10.1016/j.cell.2013.07.013>
277. Martin RD, Sun Y, MacKinnon S, Cuccia L, Page V, Hebert TE, Tanny JC (2020) Differential activation of P-TEFb complexes in the development of cardiomyocyte hypertrophy following activation of distinct G protein-coupled receptors. *Mol Cell Biol*. <https://doi.org/10.1128/MCB.00048-20>
278. Stratton MS, Lin CY, Anand P, Tatman PD, Ferguson BS, Wickers ST, Ambardekar AV, Sucharov CC, Bradner JE, Haldar SM, McKinsey TA (2016) Signal-dependent recruitment of BRD4 to cardiomyocyte super-enhancers is suppressed by a MicroRNA. *Cell Rep* 16(5):1366–1378. <https://doi.org/10.1016/j.celrep.2016.06.074>
279. Sunagawa Y, Morimoto T, Takaya T, Kaichi S, Wada H, Kawamura T, Fujita M, Shimatsu A, Kita T, Hasegawa K (2010) Cyclin-dependent kinase-9 is a component of the p300/GATA4 complex required for phenylephrine-induced hypertrophy in cardiomyocytes. *J Biol Chem* 285(13):9556–9568. <https://doi.org/10.1074/jbc.M109.070458>
280. Sano M, Wang SC, Shirai M, Scaglia F, Xie M, Sakai S, Tanaka T, Kulkarni PA, Barger PM, Youker KA, Taffet GE, Hamamori Y, Michael LH, Craigen WJ, Schneider MD (2004) Activation of cardiac Cdk9 represses PGC-1 and confers a predisposition to heart failure. *EMBO J* 23(17):3559–3569. <https://doi.org/10.1038/sj.emboj.7600351>
281. Huang F, Wagner M, Siddiqui MA (2004) Ablation of the CLP-1 gene leads to down-regulation of the HAND1 gene and abnormality of the left ventricle of the heart and fetal death. *Mech Dev* 121(6):559–572. <https://doi.org/10.1016/j.mod.2004.04.012>
282. Matrone G, Wilson KS, Maqsood S, Mullins JJ, Tucker CS, Denvir MA (2015) CDK9 and its repressor LARP7 modulate cardiomyocyte proliferation and response to injury in the zebrafish heart. *J Cell Sci* 128(24):4560–4571. <https://doi.org/10.1242/jcs.175018>
283. Krystof V, Chamrad I, Jorda R, Kohoutek J (2010) Pharmacological targeting of CDK9 in cardiac hypertrophy. *Med Res Rev* 30(4):646–666. <https://doi.org/10.1002/med.20172>
284. Modur V, Singh N, Mohanty V, Chung E, Muhammad B, Choi K, Chen X, Chetal K, Ratner N, Salomonis N, Weirauch MT, Waltz S, Huang G, Privette-Vinnedge L, Park JS, Janssen EM, Komurov K (2018) Defective transcription elongation in a subset of cancers confers immunotherapy resistance. *Nat Commun* 9(1):4410. <https://doi.org/10.1038/s41467-018-06810-0>
285. Gabay M, Li Y, Felsher DW (2014) MYC activation is a hallmark of cancer initiation and maintenance. *Cold Spring Harbor Perspect Med*. <https://doi.org/10.1101/cshperspect.a014241>
286. Chen R, Keating MJ, Gandhi V, Plunkett W (2005) Transcription inhibition by flavopiridol: mechanism of chronic lymphocytic leukemia cell death. *Blood* 106(7):2513–2519. <https://doi.org/10.1182/blood-2005-04-1678>
287. Loven J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, Bradner JE, Lee TI, Young RA (2013) Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell* 153(2):320–334. <https://doi.org/10.1016/j.cell.2013.03.036>
288. Mertz JA, Conery AR, Bryant BM, Sandy P, Balasubramanian S, Mele DA, Bergeron L, Sims RJ 3rd (2011) Targeting MYC dependence in cancer by inhibiting BET bromodomains. *Proc Natl Acad Sci USA* 108(40):16669–16674. <https://doi.org/10.1073/pnas.1108190108>
289. Lin CY, Loven J, Rahl PB, Paranal RM, Burge CB, Bradner JE, Lee TI, Young RA (2012) Transcriptional amplification in tumor cells with elevated c-Myc. *Cell* 151(1):56–67. <https://doi.org/10.1016/j.cell.2012.08.026>
290. Smith E, Lin C, Shilatifard A (2011) The super elongation complex (SEC) and MLL in development and disease. *Genes Dev* 25(7):661–672. <https://doi.org/10.1101/gad.2015411>
291. Biewenga P, Buist MR, Moerland PD, Loren V, van Themaat E, van Kampen AH, ten Kate FJ, Baas F (2008) Gene expression in early stage cervical cancer. *Gynecol Oncol* 108(3):520–526. <https://doi.org/10.1016/j.ygyno.2007.11.024>
292. Cheng Y, Jin Z, Agarwal R, Ma K, Yang J, Ibrahim S, Olaru AV, David S, Ashktorab H, Smoot DT, Duncan MD, Hutchison DF, Abraham JM, Meltzer SJ, Mori Y (2012) LARP7 is a potential tumor suppressor gene in gastric cancer. *Lab Invest* 92(7):1013–1019. <https://doi.org/10.1038/labinvest.2012.59>
293. Ji X, Lu H, Zhou Q, Luo K (2014) LARP7 suppresses P-TEFb activity to inhibit breast cancer progression and metastasis. *Elife* 3:e02907. <https://doi.org/10.7554/eLife.02907>
294. Devaraj SG, Fiskus W, Shah B, Qi J, Sun B, Iyer SP, Sharma S, Bradner JE, Bhalla KN (2016) HEXIM1 induction is mechanistically involved in mediating anti-AML activity of BET protein bromodomain antagonist. *Leukemia* 30(2):504–508. <https://doi.org/10.1038/leu.2015.142>
295. Liu P, Xiang Y, Fujinaga K, Bartholomeeusen K, Nilson KA, Price DH, Peterlin BM (2014) Release of positive transcription elongation factor b (P-TEFb) from 7SK small nuclear ribonucleoprotein (snRNP) activates hexamethylene bisacetamide-inducible protein (HEXIM1) transcription. *J Biol Chem* 289(14):9918–9925. <https://doi.org/10.1074/jbc.M113.539015>

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