



P-TEFb: The master regulator of transcription elongation

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Abstract

The positive transcription elongation factor b (P-TEFb) is composed of cyclins T1 or T2 and cyclin dependent kinase 9 that regulate the elongation phase of transcription by RNA polymerase II. By antagonizing negative elongation factors and phosphorylating the C-terminal domain of RNA polymerase II, P-TEFb facilitates the elongation and co-transcriptional processing of nascent transcripts. This step is critical for the expression of most eukaryotic genes. In growing cells, P-TEFb is negatively regulated by its reversible associations with HEXIM1/2 in the 7SK snRNP and positively by a number of transcription factors as well as the super elongation complex. In resting cells, P-TEFb falls apart and cyclin T1 is degraded by the proteasome. This complex regulation of P-TEFb has evolved for the precise temporal and spatial regulation of gene expression in the organism. Its dysregulation contributes to inflammatory and neoplastic conditions.

eTOC

P-TEFb promotes transcription elongation by releasing the pause of RNAPII. Research on P-TEFb has expanded our understanding of eukaryotic transcription and diseases such as AIDS and cancer. In this review, Fujinaga et. al. focus on the structure, function and regulation of P-TEFb.

Control of RNAPII elongation.

Eukaryotic transcription of protein-coding genes by RNA polymerase II (RNAPII) is a multi-step process consisting of initiation, promoter clearance, elongation, co-transcriptional processing of nascent transcripts, termination, mRNA cleavage and polyadenylation (Fig. 1)¹. Initiation of transcription occurs when RNAPII pre-initiation complex (PIC) is recruited by general transcription factors and the mediator complex at the promoter to the transcription start site. Clearance of RNAPII from the promoter requires the phosphorylation of serines at position 5 (S5) in the tandemly repeated heptapeptide (52 repeats of Y1-S2-P3-T4-S5-P6-S7) of its C-terminal domain (CTD) by CDK7 from the transcription factor-II H

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Declaration of interests

The authors declare no competing interests.

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(TFIIH) ¹. Subsequently, to the 5'-end of each transcript is added 7-methylguanosine cap by the capping enzyme (CE), which protects it from exonuclease digestion ¹. Immediately after copying 20–100 nucleotides, RNAPII stalls and remains attached to the DNA template because of negative transcription elongation factors (NTEFs) such as the 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF) and the negative elongation factor (NELF) ². Although DSIF and NELF have little effect alone, their combination blocks the elongation of RNAPII ³. NELF binds to RNA ⁴ and a funnel region of RNAPII, which stabilizes the RNA-DNA hybrid in a tilted conformation and immobilizes RNAPII (Fig. 1 bottom left) ⁵. Interactions between NELF, DSIF and RNAPII also prevent the recruitment of the transcript cleavage factor TFIIS and the elongation promoting polymerase-associated factor (PAF) ⁶. Since TFIIS can relieve strong pauses and arrests, this inhibitory property stabilizes the confirmation of the paused RNAPII ⁶. Switching from this paused state to productive transcription elongation is achieved by P-TEFb, which contains cyclins T1 (CycT1), T2a (CycT2a) or T2b (CycT2b) and CDK9 ⁷. Upon recruitment to the paused RNAPII, CDK9 phosphorylates serines at position 2 (S2) in the CTD of RNAPII ⁸. CDK9 can also phosphorylate serines at positions 5 and 7 (S5 and S7) in the heptapeptide repeats ^{9,10}. Precise roles of these additional phosphorylations during transcription elongation need to be elucidated. NELF-A and E as well as multiple sites in DSIF are also substrates of CDK9 ^{5,11,12}. Upon phosphorylation, NELF dissociates from the RNAPII, and is replaced by TFIIS and PAF. SPT6 is recruited to phosphorylated CTD ^{3,13}. DSIF is converted to a positive elongation factor and remains associated with RNAPII ^{3,13,14}. In the RNAPII:PAF structure, the DNA-RNA hybrid is relaxed, which creates a permissive confirmation for RNA synthesis ⁵ (Fig.1, bottom right). This P-TEFb-dependent release of RNAPII is antagonized by the protein phosphatase (PP) 2A, PP4 and/or a noncanonical PP2A via the integrator complex, which fine-tunes transcription elongation ^{15–17}. The phosphorylated CTD acts as a scaffold for splicing (SR) and polyadenylation (pA) machineries ¹. In addition, chromatin-modifying enzymes and other elongation factors travel with the heavily phosphorylated RNAPII ¹⁸. Therefore, P-TEFb is the critical factor that releases the pause of RNAPII and promotes transcription elongation.

In early days, the control of transcription elongation was studied in specific transcription units such as *c-myc*, human immunodeficiency virus type 1 (HIV), and heat shock protein (HSP) genes ⁸. However, more recent genome-wide studies revealed that promoters of most inactive and inducible genes are already engaged by stalled RNAPII ^{19,20}. Therefore, the elongation step of transcription, which is regulated by P-TEFb, is the main gate keeper of eukaryotic gene expression. While early transcription elongation is mediated largely by P-TEFb, later stages are also impacted by other CDKs that also phosphorylate S2 in the CTD. For example, CycK:CDK12 are recruited to the phosphorylated CTD by SR proteins and/or PAF1C and mediate the cleavage and polyadenylation (CPA) of some genes ^{21,22}. On other genes, CycL:CDK11 are recruited by the TREX/THO (transcription/export // transcriptional defects of Hpr1 by over-expression) complex or FLASH (Fas-via death domain-associated-like ICE-associated huge protein) to facilitate their CPA and mRNA export ^{23,24}. Since P-TEFb is also involved in splicing and 3' end formation ^{25,26}, it remains to be investigated how all these different CDKs coordinate their effects during transcription.

P-TEFb was discovered in early 1990's as a nuclear factor that stimulates transcription elongation²⁷. First, the ATP analog DRB was demonstrated to inhibit greatly overall transcription with the recovery of only short, capped transcripts. In vitro transcription elongation was inhibited as well. Next, the use of a fly in vitro transcription system demonstrated that DRB did not inhibit RNAPII but a factor required for its elongation, most likely a kinase²⁷. Named P-TEFb, it was subsequently purified as a heterodimer, where its smaller subunit (~42K) was identified as CDK9²⁸. Next, its cyclin subunit, CycT1, was co-purified with the transactivator of transcription (Tat) from HIV²⁹. Thus, P-TEFb became central to our understanding of viral replication. CycT2a and CycT2b were cloned soon thereafter³⁰. Subsequently, many cellular transcription factors, chromatin remodeling machineries, and other proteins were demonstrated to interact with P-TEFb, leading to the current model of transcription elongation by RNAPII.

The structure of P-TEFb

In human cells, different P-TEFb complexes exist⁸. They contain CycT1, CycT2a or CycT2b and shorter or longer forms of CDK9 (42 kDa and 55 kDa)⁸. The two isoforms of CDK9 are produced from different transcription start sites in the first exon of the CDK9 gene³¹. For purposes of this review, only CycT1 and the shorter CDK9 are diagrammed in Fig. 2 (left). The N-terminal lobe (residues from positions 16 to 108), and C-terminal lobe (residues from positions 109–330) of CDK9 form a cleft which is highly conserved among CDKs (Fig. 2, right). CycT1 binds to CDK9 at the N-terminal lobe. The N-terminal lobe also contains the G-loop domain required for ATP-binding⁹. The aspartate at position 104 (D104) and cysteine at position 106 (C106) interact directly with ATP. The adjacent phenylalanine at position 103 (F103) determines the specificity of nucleotide binding and is therefore called the gatekeeper. A mutation of this residue (F103A) sensitizes CDK9 for bulkier ATP analog inhibitors, which do not inhibit other cellular kinases. Such an analog-sensitive CDK9 protein was utilized to identify additional substrates of CDK9^{32,33}. The PITALRE motif which is unique for each CDK is also located within the N-terminal lobe. The substrate-binding site is located in the cleft formed by N- and C-terminal lobes. It targets consensus CDK substrate motifs consisting of serine/threonine-proline-X-arginine/lysine (S/T-P-X-K/R) residues⁹. Next is the DFG (aspartate, phenylalanine, glycine) motif that binds to Mg⁺⁺. When CycT1 is absent, the cleft is masked by the self-inhibitory domain called the T-loop. The kinase activity of CDK9 requires that the threonine at position 186 (T186) in the T-loop be phosphorylated. CDK7 and CDK9 are candidates for the responsible CDK-activating kinase (CAK)^{9,34}. The serine at position 175 (S175) is also phosphorylated, which can affect the function of P-TEFb^{35,36} (Fig. 2, right). Additional phosphorylation of the C-terminus of CDK9 improves interactions between P-TEFb, Tat and its target, the transactivation response (TAR) RNA³⁷.

The N-termini of CycT1, CycT2a and CycT2b consist of two highly conserved cyclin boxes (residues from positions 30 to 248), which are essential for CDK9 binding (Fig. 2)³⁰. Phosphorylation at the two evolutionally conserved threonine residues at positions 143 (T143) and 149 (T149) by the protein kinase C (PKC) potentiates interactions between CDK9 and CycT1 (P-TEFb assembly)³⁸. In the absence of CDK9, cyclin boxes bind to ubiquitin E3 ligases Siah (seven in absentia homolog)1/2, which mediate the

polyubiquitination of CycT1 and lead to its rapid degradation³⁹. In CycT1, these cyclin boxes also serve as a platform for binding to Tat and other transcription factors⁸. A short stretch of lysines and arginines adjacent to these cyclin boxes serves as the nuclear localization signal (NLS)²⁹. This region also includes the Tat-TAR recognition motif (TRM) (residues from positions 253 to 270). Interestingly, the unique cysteine at position 261 (C261) in the TRM of the human CycT1 is critical for its ternary complex with Tat and TAR⁴⁰. Thus, CycT2a and CycT2b as well as the mouse CycT1 fail to mediate Tat transactivation due to the absence of this C261. Next, there is a histidine-rich (H-rich) region from positions 480 to 550 that mediates direct interactions between P-TEFb and the CTD of RNAPII⁴¹. Recent studies also revealed that it is required for the tight association between P-TEFb and chromatin and for creating functional phase-separated compartments with RNAPII in nuclear speckles. There, kinases and substrates are densely packed, providing an optimal environment for transcription elongation⁴². Interestingly, poly ADP-ribosylation of the H-rich region in CycT1 upon DNA-damage prevents the transition of P-TEFb into these phase-separated compartments, which can shut down transcription globally⁴³. In contrast to consensus N-termini, C-termini of CycT proteins are less well conserved. The unique C-terminus of CycT1 from positions 709 to 726 resembles the canonical PEST (proline, glutamate, serine, threonine) motif (Fig. 2)²⁹. It can be targeted by Skp2 (S-phase kinase associated protein 2), which mediates the subsequent ubiquitination of CDK9⁴⁴. However, the removal of this PEST motif does not affect the degradation of P-TEFb in cells³⁹.

Several crystal structures of P-TEFb have been reported^{45,46}. They contain only the N-terminal 266 residues of CycT1 and 351 residues of CDK9 (Fig. 2, right). These structures confirm previously mapped surfaces between CycT1 and CDK9 and conform to those found in other CDKs. Tat was also co-crystallized with P-TEFb. Its residues from positions 1 to 49 bind tightly to CycT1 and also to sequences near the T-loop in CDK9. Finally, subunits of the super elongation complex (SEC) were found to interact with P-TEFb, especially AFF1 and AFF4 (AF4/FMR2 family members 1 and 4)⁴⁷. From the N- to the C-terminus, different surfaces on AFF1/4 bind to CycT1, ELL2 (elongation factor for RNAPII 2) and ENL/AF9 (eleven-nineteen-leukemia protein/ALL1-fused gene from chromosome 9). In these structures, Tat and SEC bind to non-overlapping surfaces on P-TEFb and create a more stable multi protein complex for transcription⁴⁷. These structures also suggest surfaces, where small molecules could interfere with Tat transactivation and HIV transcription⁴⁸.

The function of P-TEFb

P-TEFb is recruited to the paused RNAPII by various mechanisms including (1) DNA-tethering, (2) RNA-tethering, and (3) sequence-independent mechanisms (Fig. 3 top)⁸. Many DNA-bound activators mediate their enhancer function via P-TEFb. They include NF- κ B, c-Myc, the Signal Transducer and Activator of Transcription (STAT) 3, the Myoblast Determination protein (MyoD), Myocyte Enhancer Factor-2 (MEF2), as well as hormone receptors (estrogen and androgen receptors)⁴⁹. This P-TEFb-dependent transcription activation can be recapitulated by artificial DNA-tethering systems. For example, CycT1 fused to the yeast Gal4 DNA binding domain activates transcription of reporter genes containing its target sequences upstream or downstream of promoters and coding sequences⁴¹. This activity requires the H-rich region that binds to the CTD of RNAPII and NTEFs.

Therefore, interactions between distal sites, CycT1 (also CycT2a and CycT2b) and the CTD enable P-TEFb to act as a co-activator of transcription and P-TEFb can link activators at enhancers and PIC at promoters, which results in transcription elongation (Fig. 3, top) ⁴¹.

P-TEFb can also be recruited to RNAPII via RNA. The most prominent example is Tat from HIV, which brings P-TEFb to TAR RNA ⁴⁰. Such recruitment of transcription factors via RNA had not been observed previously in eukaryotic systems. Again, this RNA-enhancer function can be recapitulated by artificial RNA-tethering systems, including the coat protein of the bacteriophage MS2/R17, which binds to its operator RNA to block translation in bacteria, or to the regulator of viral gene expression (Rev), which binds to the Rev response element (RRE) RNA in the *env* gene of HIV ^{50,51}. CycT1 or CDK9 fused to Rev activate transcription from an RRE's Stem-Loop IIB (SLIIB)-containing reporter gene to levels that are similar to Tat-transactivation ⁵². This finding implies that interactions between P-TEFb and nascent RNA are sufficient for the stimulation of transcription elongation (Fig. 3, top). Since RNA passes the CTD co-transcriptionally, a shorter CycT1 (from positions 1 to 280) is equally effective as its wild type counterpart ⁵³. Importantly, studies indicate that only proteins that interact with P-TEFb can activate transcription in this assay ⁵⁴. Thus, RNA-tethering represents a genetic assay for activators that recruit P-TEFb for their effects.

Finally, there are mechanisms of P-TEFb recruitment independent of DNA- or RNA-sequences. They include co-activators such as the autoimmune regulator (AIRE) and the class II transactivator (CIITA) that require P-TEFb for their activities ⁸. Other factors directly associated with RNAPII, such as components of the mediator complex (Med26) also interact with P-TEFb and recruit it to the paused RNAPII ⁵⁵. Components of the SEC, such as AFF1 and AFF4, also bind to P-TEFb and form protein complexes containing multiple transcription factors ⁵⁶. Finally, P-TEFb can be recruited to chromatin by the bromodomain containing protein 4 (BRD4), whose bromodomain binds to the acetylated histone H3 (H3K27Ac) ⁵⁷. Although BRD4 interacts with the active histone code (H3K27Ac), the precise role of this recruitment of P-TEFb for the global or gene-specific transcription needs to be elucidated. Of note, the pharmacological degradation of BRD4 by a small molecule degrader of BET family proteins (dBET1) results in global inhibition of transcription elongation without affecting the chromatin localization of CDK9, suggesting that P-TEFb recruitment can occur independently of BRD4 ⁵⁸. Moreover, BRD4 is dispensable for effects of P-TEFb on NF- κ B and AIRE ⁵⁹ and some genes require other factors, such as JMJD6 (Jumonji Domain Containing 6) for their transcription ⁶⁰. Nevertheless, interactions between BRD4 and P-TEFb were reported to act as the bookmark of late mitosis, especially for the expression of genes required for the progression of the cell cycle ⁶¹.

Given that the recruitment of P-TEFb to paused RNAPII results in transcription elongation of target genes, active repression can be achieved simply by inhibiting the substrate recognition of CDK9 (Fig. 3, bottom). The best candidates contain sequences that resemble the CTD but cannot be phosphorylated. Indeed, when S2 and S5 in the heptad repeats are changed to alanines, this unmodifiable pseudosubstrate blocks the activity of P-TEFb. PIE-1 from *C. elegans* contains such a changed heptapeptide repeat and inhibits P-TEFb globally ⁶². Other repressors, such as Runx1, which binds to the CD4 silencer and represses the expression of the CD4 gene during T cell development in the thymus,

also contains such an inhibitory domain. Importantly, this Runx1-dependent inhibition occurs despite the engagement of RNAPII on the CD4 promoter (Fig. 3B)⁶³. Another mechanism of antagonizing P-TEFb during transcription elongation is to recruit specific protein phosphatases, which include canonical PP2A, PP4, and/or atypical PP2A that dephosphorylate P-TEFb substrates, to RNAPII¹⁵⁻¹⁷.

P-TEFb not only phosphorylates the CTD in RNAPII, but has additional protein targets. Many of them are involved in transcription, e.g. RNA processing and 3' end formation. For example, by phosphorylating and activating the exoribonuclease Xrn2, P-TEFb affects CPA³³. P-TEFb also phosphorylates and inhibits PP1, which stabilizes DSIF and RNAPII during transcription elongation. In addition, P-TEFb phosphorylates and activates UBE2A histone ubiquitin E2 ligase⁶⁴. The ubiquitinated histone H2B (H2BUb), regulates gene expression and DNA repair. Additional protein targets were revealed using an analogue sensitive CDK9 and mass spectrometry^{32,33}. They are involved in chromatin remodeling, RNA splicing, RNA turnover, cell cycle progression and apoptosis^{32,33}. Further validation and analysis of these substrates will reveal new cellular processes that are impacted P-TEFb.

The regulation of P-TEFb

7SK snRNP

In proliferating cells, there exists an equilibrium between the active (free) P-TEFb and its inactive (sequestered) form in the 7SK small nuclear ribonucleoprotein (7SK snRNP) complex (Fig. 4)^{65,66}. This small ribonuclear complex forms on the 7SK snRNA, which contains 332 nucleotides and is transcribed by RNAPIII⁸. It forms the scaffold for multiple protein:RNA and protein:protein interactions. The 5' end of 7SK snRNA is targeted by the methyl phosphate capping enzyme (MePCE), which adds a γ -monomethyl cap to the 5' end of this RNA. Via its N-terminal Lupus antigen (La) module, the La related protein 7 (LARP7) binds to the 3' terminal U residues in the 7SK snRNA⁶⁷. Its C-terminal xRRM motif binds to the 3' stem loop of 7SK snRNA. Both proteins stabilize 7SK snRNA⁶⁸. Without them, 7SK snRNA is rapidly degraded. Next, hexamethylene bisacetamide inducible mRNAs 1 and 2 (HEXIM1 and HEXIM2) proteins, also known as cardiac lineage protein (CLP) 1⁶⁹, estrogen-downregulated gene (EDG) 1⁷⁰, or menage a quatre (MAQ) 1⁷¹, bind to the GAUC bulge in the 5' stem loop of 7SK snRNA via their KHRR (lysine, histidine, arginine, arginine) repeats⁷¹. P-TEFb binds to the central loop of this hairpin structure⁷². Upon binding to RNA, conformational changes in HEXIM1/2 expose its PYNT (proline, tyrosine, arginine, threonine) motif that targets the ATP pocket in CDK9 and inhibits its kinase activity⁷¹. The inducible HEXIM1 is more abundant in most cells. These interactions resemble those between Tat, P-TEFb and TAR RNA⁸. Thus, it is not surprising that Tat can compete for this binding and release P-TEFb from the 7SK snRNP^{73,74}.

In different proliferating cells, 50% to 90% of P-TEFb is found within 7SK snRNP⁸. Only the active (free) P-TEFb turns on target genes, many of which are required for cell activation, growth and proliferation. Thus, a precise P-TEFb equilibrium is required for the proper homeostasis of cells and tissues in the organism⁷⁵. When P-TEFb is not incorporated into the 7SK snRNP, 7SK snRNA also interacts with heterologous nuclear RNP Q and/or R^{76,77}, or the high mobility group A1 (HMGA1) proteins⁷⁸. 7SK snRNA undergoes

large-scale conformational changes between these two states^{79,80}. When P-TEFb is released from the 7SK snRNP, the *hexim1* gene is one of its earliest targets. Newly synthesized HEXIM1 proteins then reassemble the 7SK snRNP (Fig. 4)⁸¹. This negative feedback loop ensures that the P-TEFb equilibrium is restored⁸². HEXIM1 and 7SK snRNP have also been observed at different transcription units, possibly for their repression or a more rapid deployment of P-TEFb to target genes following its release⁸³. Nevertheless, this association cannot be very tight, as 7SK snRNP elutes at low salt concentrations from the nucleus in cell fractionation experiments. In contrast, active P-TEFb requires very high salt concentrations for its removal from chromatin⁸.

P-TEFb releasing factors

Release of P-TEFb from the 7SK snRNP occurs in various ways⁸⁴. Most of them represent some form of stress to the cell⁷. They include strong intracellular signals, UV light, other forms of DNA damage and chromatin perturbations⁸. For example, TCR-mediated signaling can release P-TEFb from the 7SK snRNP⁸⁵. PKC agonists can mimic this effect⁸⁵. Other stimuli include small compounds such as histone deacetylase inhibitors (HDACis), bromodomain and extra-terminal (BET) bromodomain inhibitors (BETis) and hexamethylene bisacetamide (HMBA)⁸. For HMBA, this release is mediated by the phosphorylation of HEXIM1 by Akt⁸⁶. For others, different protein phosphatases (PP1a and PP2B) are involved. They dephosphorylate T186 in the T-loop of CDK9⁸⁷. Although T186 has to be rapidly re-phosphorylated for activity, S175 remains modified in the released P-TEFb³⁵. Other physiological stress-induced proteins include RNA-binding motif protein (RMB)7 and serine-arginine rich splicing factor (SRSF) 2^{88,89}. They can bind to the 7SK snRNP and displace P-TEFb. Released P-TEFb is then recruited to the paused RNAPII. The stronger the perturbation, the faster the release from and subsequent reassembly of the 7SK snRNP. Thus, increased levels of HEXIM1 commonly result in a P-TEFb disequilibrium that inhibits transcription⁸².

Degradation

P-TEFb is also regulated by levels of its subunits. This total amount is correlated to the growth and proliferation of cells. For example, levels of P-TEFb, especially of CycT1, are low to absent in most quiescent cells, such as resting, memory and naive T cells as well as terminally differentiated macrophages⁹⁰. Despite the loss of CycT1, levels of CDK9 remain high due to its binding to HSP70 and HSP90 in these cells⁹¹. Importantly, mRNA levels of CycT1 and CDK9 are not diminished⁹⁰. This finding implies that CycT1 is regulated post-transcriptionally in quiescent cells⁹⁰. Various mechanisms have been proposed, including micro (miRNA)-mediated inhibition of CycT1 translation⁹⁰. In addition, proteasomal inhibitors reverse the depletion of CycT1 in terminally differentiated macrophages, which implicates the proteasome⁹². This degradation of CycT1 is due to the dissociation of P-TEFb by PP1 (Fig. 5)³⁸. It removes phosphate groups from threonines at positions 143 and 149 in CycT1 (T143 and T149), which are required for optimal interactions between CycT1 and CDK9. The E3 ligases Siah1/2 then target the unbound CycT1 for degradation³⁹. The degron and ubiquitination sites were mapped to residues from positions 210 to 280 in the unbound CycT1³⁹. PKC α and PKC β phosphorylate T143 and T149, which explains how P-TEFb is reassembled in growing cells³⁹. In contrast, when PKC is inactive in resting cells

or degraded following strong activation signals or by the chronic administration of phorbol esters, P-TEFb falls apart in anergic, exhausted or terminally differentiated lymphocytes³⁸. However, to maintain cellular homeostasis, the extent of this degradation should vary between tissue resident, circulating and ex vivo cultured cells.

P-TEFb in HIV transcription and latency

Since P-TEFb is the cellular co-activator of HIV transcription, it plays a central role in the pathogenesis of AIDS. Indeed, viral replication was demonstrated early to be regulated by transcription elongation⁹³. Without Tat, RNAPII copies only short transcripts that form the TAR RNA structure. Tat then recruits P-TEFb to RNAPII via TAR. In addition, following T cell activation, NF- κ B also binds to the HIV LTR⁹⁴. Therefore, P-TEFb is recruited for Tat-independent (basal, NF- κ B) and Tat-dependent HIV transcription. In HIV-positive patients who receive antiretroviral therapy (ART), levels of HIV are kept low to undetectable in the peripheral blood. Although the majority of HIV-infected cells encode replication-defective HIV proviruses, a small population contain the intact HIV. Upon the cessation of ART, fully infectious progeny virions are produced from this reservoir, leading to the rebound of the disease. Several possible mechanisms of establishment and maintenance of HIV latency include integration into inactive heterochromatin, epigenetic silencing (i.e. DNA methylation, histone modifications), absence of required transcription factors (e.g. NF- κ B, Tat), transcriptional interference (i.e. integration into actively transcribed host cell genes), and increased levels of host restriction factors⁴⁰.

Importantly, levels of P-TEFb are low in resting CD4+ T cells and their memory subsets harboring silent HIV proviruses, which represents a major factor that maintains the latent viral reservoir⁹⁰. To achieve a functional cure of AIDS, attempts have been made to maintain proviral latency (“block and lock” strategy) or to reverse it by reactivating HIV and remove infected cells by host immune responses or via direct viral cytotoxic effects (“shock and kill” strategy)⁹⁵. To achieve this “shock and kill” of HIV, levels of active P-TEFb must be increased as well as those of NF- κ B and Tat. Proteasomal inhibitors, T cell receptor (TCR) or PKC agonists, HDACis and BETis alone or in combination have all been studied for HIV reactivation from latency⁹⁵. In this context, bortezomib induces the reactivation of latent HIV without global T cell activation⁹⁶. Of interest, even when 7SK snRNP has reassembled and the expression of most host cell genes is inhibited, HIV transcription continues because Tat competes with HEXIM1 for P-TEFb^{73,74}. The sequence of these events can be very rapid, resulting in waves of HIV transcription. Interestingly, since other viruses, such as HTLV-1, KSHV, Influenza virus, EBV, CMV, HSV as well as SIV, EIAV, and BIV also utilize P-TEFb for their replication, manipulating levels and activity of P-TEFb could also reactivate them from their reservoirs⁹⁷.

P-TEFb in health and disease

P-TEFb plays a critical role in many human diseases and conditions. In some, P-TEFb is not regulated by 7SK snRNP. In others, it is aberrantly recruited to specific loci via genetic rearrangements. One early example is cardiac hypertrophy, where treatment of cardiomyocytes with hypertrophic stimuli such as endothelin-1 (ET1) or mechanical stresses

releases P-TEFb from the 7SK snRNP⁹⁸. In addition, high levels of P-TEFb or the genetic inactivation of HEXIM1 leads to cardiac hypertrophy and heart failure in mice⁶⁹.

High levels of active P-TEFb have also been implicated in autoimmune diseases, where TNF α signaling releases it from the 7SK snRNP. NF- κ B and P-TEFb, or STAT3 and P-TEFb then increase the expression of their target genes, leading to inflammation⁴⁹. An important target are joints, e.g. rheumatoid arthritis, which is a condition ameliorated by TNF α inhibition. In addition, specific CDK9 inhibitors have beneficial effects in mouse models of the disease⁹⁹.

The P-TEFb equilibrium is also shifted in various solid tumors and hematopoietic malignancies. In particular, mutations in LARP7 have been observed in gastric, breast and ovarian cancers¹⁰⁰. In these malignant cells, 7SK snRNA is degraded and 7SK snRNP cannot form¹⁰¹. Thus, all P-TEFb is in the active form. An example of cancer where P-TEFb is recruited to specific loci is mixed-lineage leukemia (MLL)⁵⁶. Here, the translocations and genetic rearrangements occur between the MLL gene and components of the SEC, which then recruits P-TEFb⁵⁶. A majority of MLL rearrangements (~50% of infant cases and ~75% of adult cases of acute lymphoblastic leukemia) result in in-frame fusion proteins between MLL and AFF4. MLL rearrangements also occur with ENL and AF9 genes⁵⁶. These MLL rearrangements lead to aberrant transcription elongation by P-TEFb. A different example is c-Myc, whose levels are increased in many cancers. The expression and activity of c-Myc are regulated by P-TEFb and BRD4^{102,103}, and the removal of BRD4 from the *c-myc* locus by BETi results in potent anti-tumor effects¹⁰⁴. In addition, P-TEFb binds to and regulates the activities of many other transcription factors involved in cancer, which includes estrogen and androgen receptors⁴⁹. For the treatment of autoimmune diseases and cancer, many chemical CDK9 inhibitors have been developed with varying degrees of success in clinical trials. Their use has been limited primarily because of off target effects (inhibiting other CDKs) or severe side effects of inhibiting P-TEFb globally. However, their development, uses and clinical trials go beyond the scope of this manuscript and have been covered extensively in other reviews⁴⁹.

The opposite situation, where P-TEFb is absent, pertains to anergic, exhausted and terminally differentiated lymphocytes. T cell exhaustion impairs greatly the effectiveness of cancer immunotherapy, including the check point blockade with antibodies that inhibit negative lymphocyte signaling and the chimeric antigen receptor (CAR)-T cell therapy¹⁰⁵. Exhausted T cells occur with chronic infections or tumors where constant antigen stimulation via TCR depletes PKC and other signaling molecules, leading to the degradation of CycT1³⁹. Since P-TEFb is required for the expression of most activated genes, which include those coding for cytokines and lymphokines, its absence in exhausted T cells could contribute greatly to their unresponsiveness¹⁰⁶. Increasing levels of active P-TEFb in these cells could potentially mitigate T cell exhaustion and improve the effectiveness of these therapies.

Future Directions

Although P-TEFb has been studied extensively, we still do not know many aspects of its structure and function. For example, we have no structure of the full length CycT1 and CDK9 or the inhibitory 7SK snRNP complex. What roles do different P-TEFb components (such as CycT2a and CycT2b as well as CDK9-55) play in development and tissues? How many transcription factors interact with P-TEFb directly or via the SEC? What roles do interactions between BRD4 and P-TEFb play in transcription elongation? Indeed, how well does chromatin bound BRD4 interact with P-TEFb? Finally, how are three different Cyclin/CDK complexes that phosphorylate S2 of RNAPII CTD (P-TEFb, CycK:CDK12 and CycL:CDK11) co-ordinated for the precise temporal and spatial regulation of specific genes, for elongation, RNA processing and 3' end formation?

Besides targeted questions concerning the structure and function of P-TEFb, what do low levels of P-TEFb mean for quiescent, anergic and exhausted T cells? Indeed, how are they controlled between different differentiated cells in the organism? How much P-TEFb is required for their simple housekeeping, vegetative functions? If levels of P-TEFb could be manipulated artificially, how would that affect cellular functions and the establishment or reversal of proviral latency?

The other side of the coin addresses the inhibition of P-TEFb in inflammation, autoimmunity and cancer. Since many cancers have mutations in LARP7, could the introduction of the WT protein act as a tumor suppressor? Alternatively, would specific inhibition or targeted degradation of P-TEFb decrease inflammation, ameliorate autoimmunity and arrest the growth of malignant cells. Possibly, new, more specific CDK9 inhibitors could be used in smaller, less toxic doses. Alternatively, one could target surfaces on P-TEFb that interact with specific transcription factors or use pseudo-substrate decoys that would inhibit CDK9 only for the transcription of specific genes? Thus, the field of transcription elongation and P-TEFb in particular remains a very fruitful area of future research, where most answers will benefit not only our knowledge of gene expression but of growth, development and disease.

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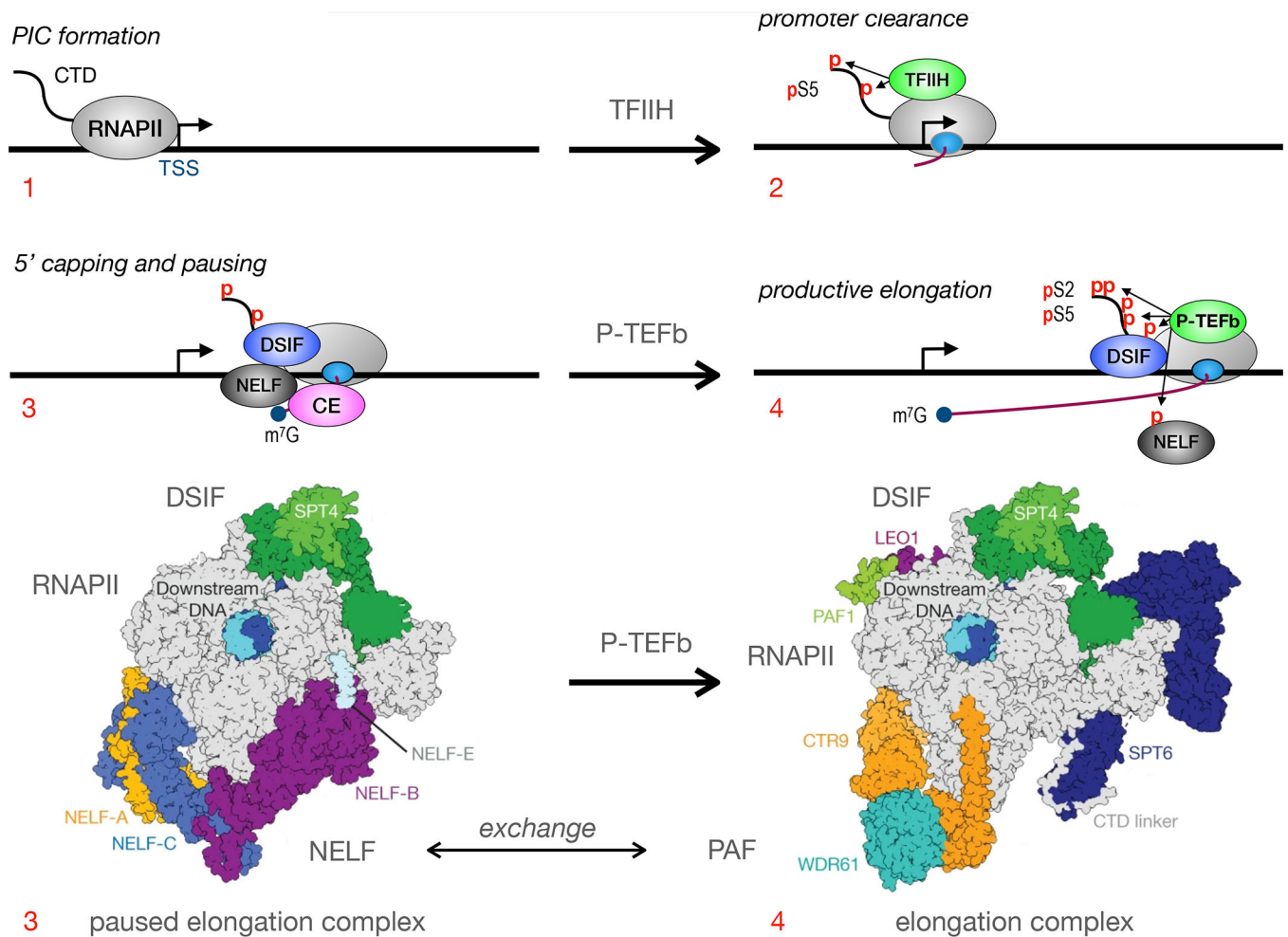
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**Figure 1.**

Early steps in RNAPII transcription. Depicted in top panels are: 1. the formation of the pre-initiation complex (PIC); 2. promoter clearance and early phosphorylation of serines at position 5 (pS5) in the C-terminal domain (CTD) of RNAPII by CDK7; 3. RNAPII pausing via DRB sensitivity (DSIF) and negative elongation (NELF) factors and 5' capping (m7G) of early transcripts by the capping enzyme (CE); 4. recruitment of P-TEFb, the phosphorylation of S2 (pS2) in the CTD, DSIF and NELF by CDK9, followed by transcription elongation. As presented in bottom panels, paused and released elongation complexes now have greater structural understanding. In the paused state, NELF stabilizes the tilted RNA:DNA hybrid state, inhibits the diffusion of nucleotides and obstructs the binding of TFIIS and PAF. For the release of RNAPII to elongation, P-TEFb phosphorylates CTD as well as subunits of NELF and DSIF. NELF dissociates from RNAPII. TFIIS and PAF can now bind to RNAPII. Together with DSIF, they help to relieve the tilt of the RNA-DNA hybrid. They also stabilize the transcription funnel. Co-transcriptional processing of nascent transcripts ensues, i.e. their splicing, termination, polyadenylation and export. TSS is the transcription start site, where the arrow points in the direction of transcription. Other markings: the gray ovals represent RNAPII; its inner smaller oval is where early transcripts emerge; the S shaped line is the CTD with red small ps indicating phosphorylated residues;

the blue circle depicts capped mRNA species. Depicted are NELF subunits A, B, C and E, SPT4 and 6, as well as PAF subunits PAF1, LEO1, CTR9 and WDR61. Bottom panels are adapted from Vos et al ⁵.

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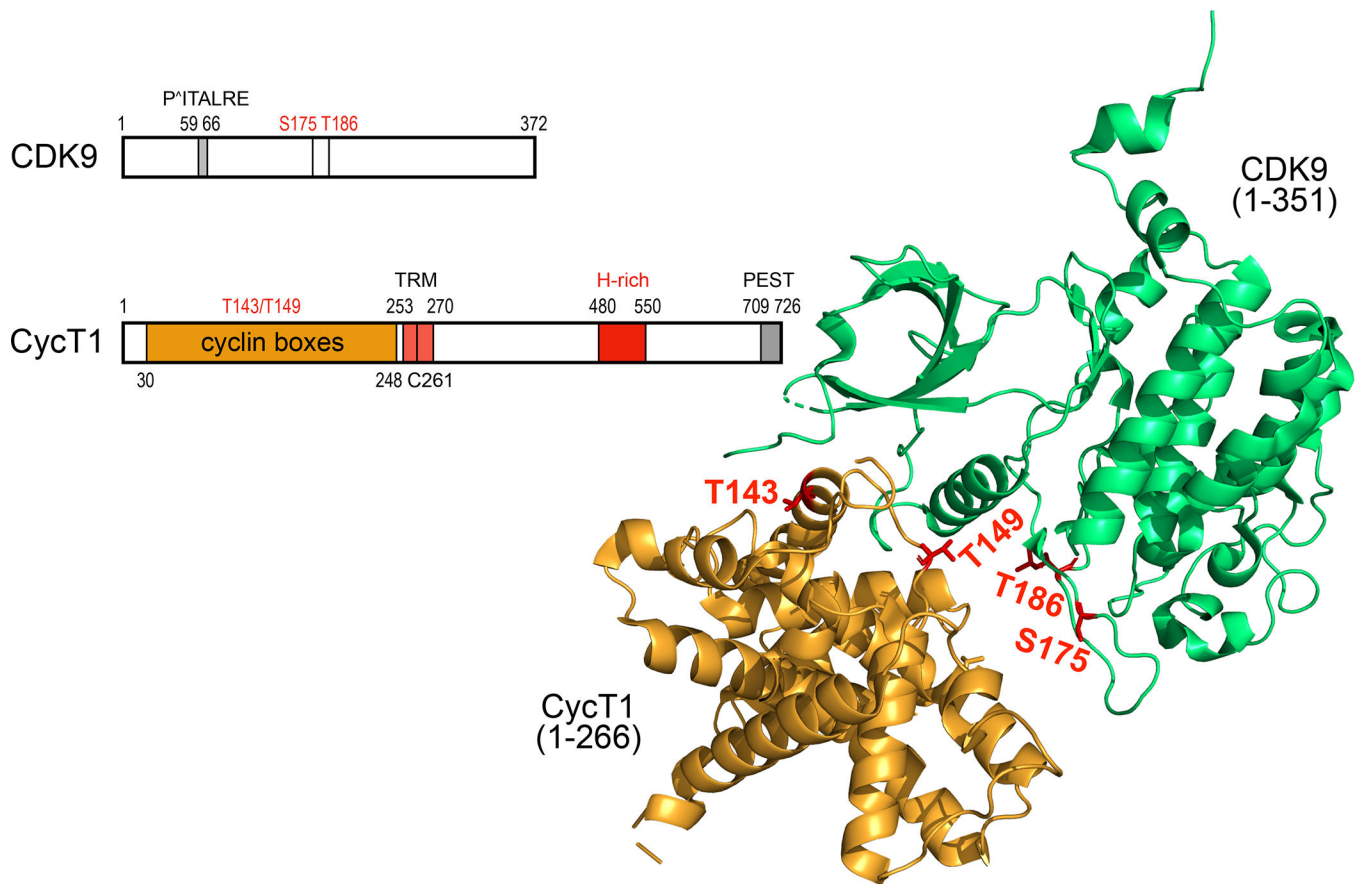


Figure. 2.

Schematic representation and the 3-D structure of human CDK9 and CycT1 proteins. CDK9 contains 372 residues. From N- to C-termini, important elements contain the PITALRE sequence. serine at positions 175 (S175) and threonine at position 186 (T186, T-loop). S175 and T186 are phosphorylated in the active P-TEFb. CycT1 contains 726 residues. From the N-terminus, there are 2 cyclin boxes (yellow), the Tat-TAR recognition motif (TRM), the histidine-(H)-rich region (red) and the putative PEST sequence (gray). Threonines at positions 143 and 149 (T143, T149) are targets of PKC and facilitate the binding between CDK9 and CycT1. In the unbound CycT1, the degron, where Siah1/2 bind, is located between position 210 and 260. The TRM is the binding site for the HIV transcriptional transactivator Tat. The H-rich sequence binds to targets of CDK9, i.e. the CTD of RNAPII, DSIF and NELF, among others. The function of the PEST sequence remains enigmatic. Some of these features are presented on the 3-D structure of P-TEFb^{45,46}. The ordered structure (ribbons) contains 266 residues from CycT1 and 351 residues from CDK9. T143 and T149 in CycT1 form intra- and inter-molecular contacts with CycT1 and CDK9, respectively. T186 (T-loop) and S175 in CDK9 are phosphorylated in the active P-TEFb.

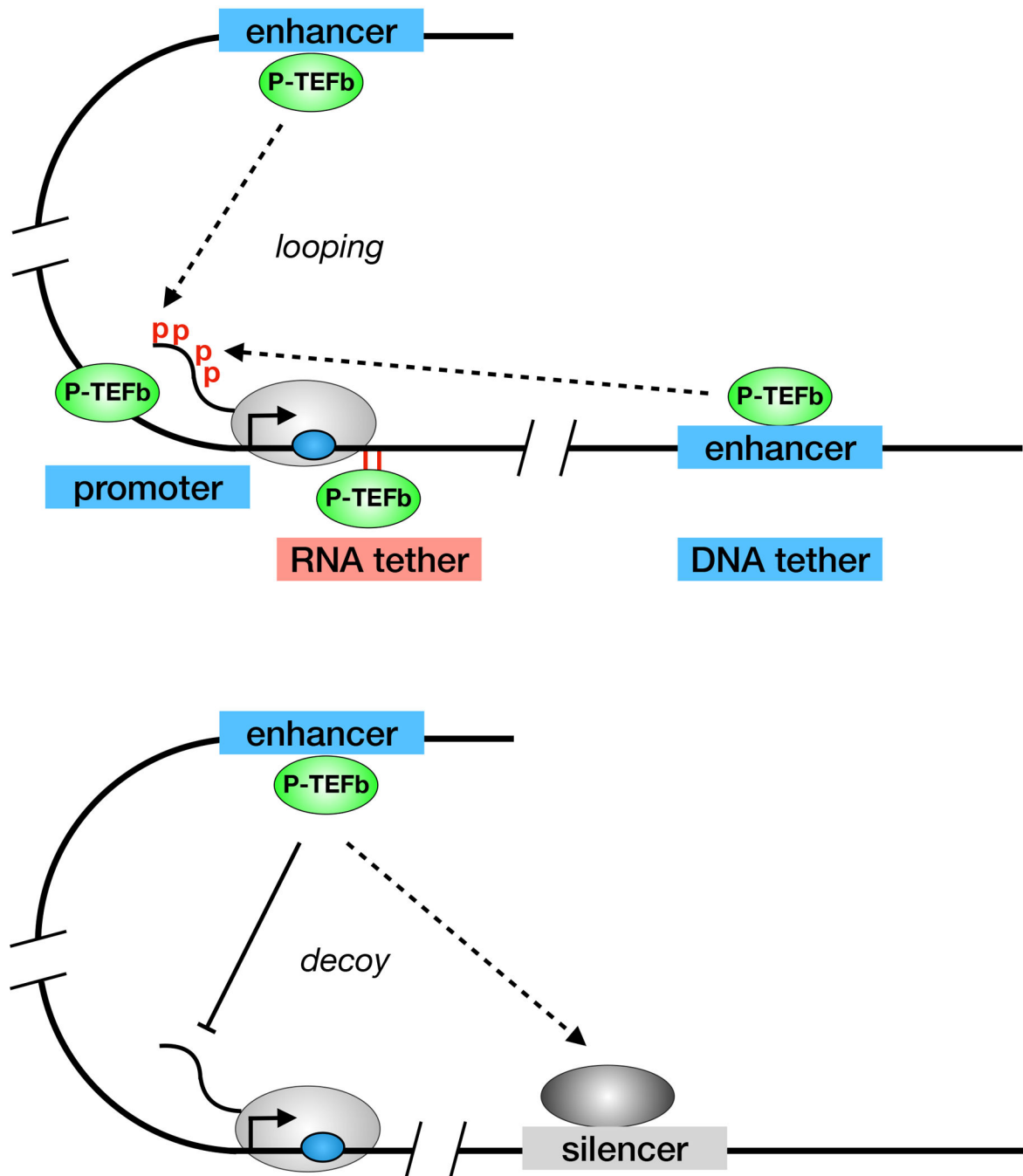


Figure 3. P-TEFb is critical for transcription elongation of most eukaryotic genes. Top panel depicts how P-TEFb can be recruited to RNAPII from promoters, 5' and 3' enhancers (via looping), and even RNA, as is the case with Tat from HIV. Bottom panel depicts how some silencers and/or repressors of transcription decoy P-TEFb away from RNAPII. Such is the case with global transcriptional repression by PIE1 and temporary silencing of the CD4 gene by RunX1. The other markings are as in Fig. 1.

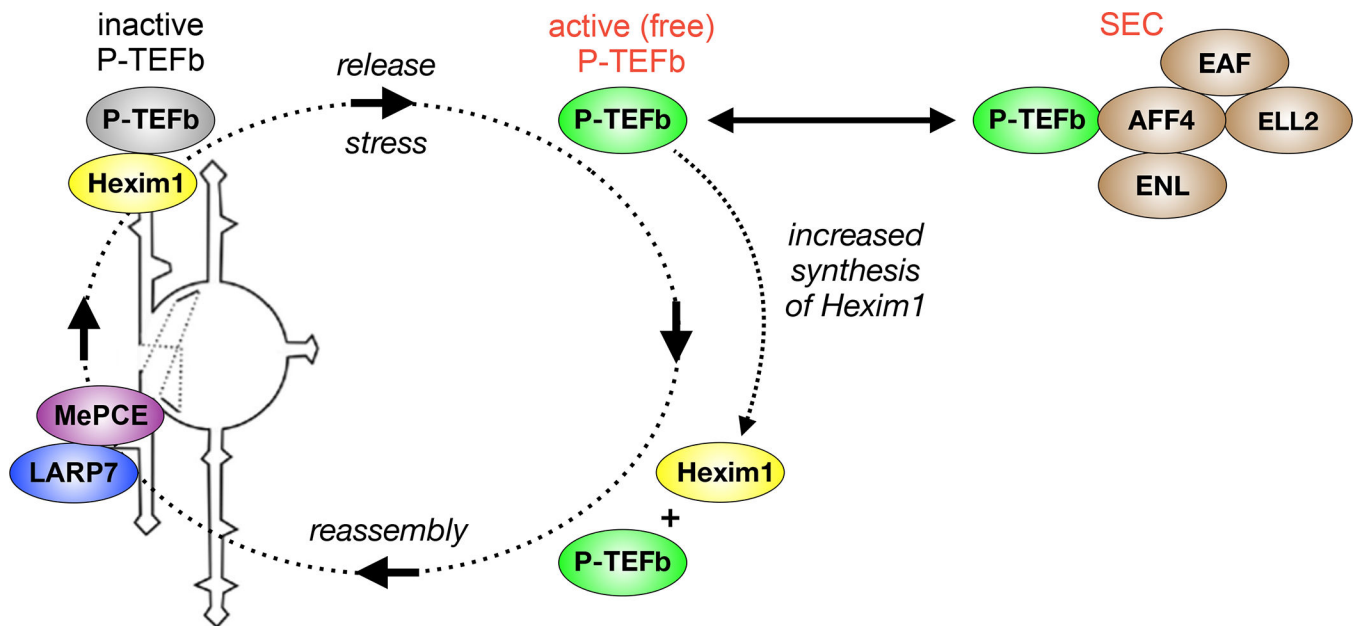


Figure 4.

The regulation of P-TEFb in growing cells. In growing, dividing cells, P-TEFb partitions between the active (free) state and the inactive (sequestered) form. In the former, it binds other transcription factors (TFs) or is incorporated into the super elongation complex (SEC). In the latter, it is incorporated into the 7SK small nuclear ribonucleoprotein (7SK snRNP) complex by 7SK snRNA and HEXIM1. 7SK snRNA is stabilized by the La related protein 7 (LARP7) and methyl phosphate capping enzyme (MePCE) at its 3' and 5' ends, respectively. P-TEFb is released by stress from the 7SK snRNP, which increases the synthesis of HEXIM1. Thus, 7SK snRNP reassembles quickly. Stress can come in many forms, from cellular signaling pathways, certain signaling molecules (cytokines and lymphokines), DNA damage (e.g. UV light) to chromatin stress (HDAC and BET bromodomain protein inhibition), differentiation agents (HMBA) and viral infections. Of interest, HEXIM1 and P-TEFb bind to the 5' bulge and central loop in stem loop 1 of 7SK snRNA, which is analogous to how Tat interacts with TAR RNA. Indeed, Tat can displace P-TEFb from the 7SK snRNP.

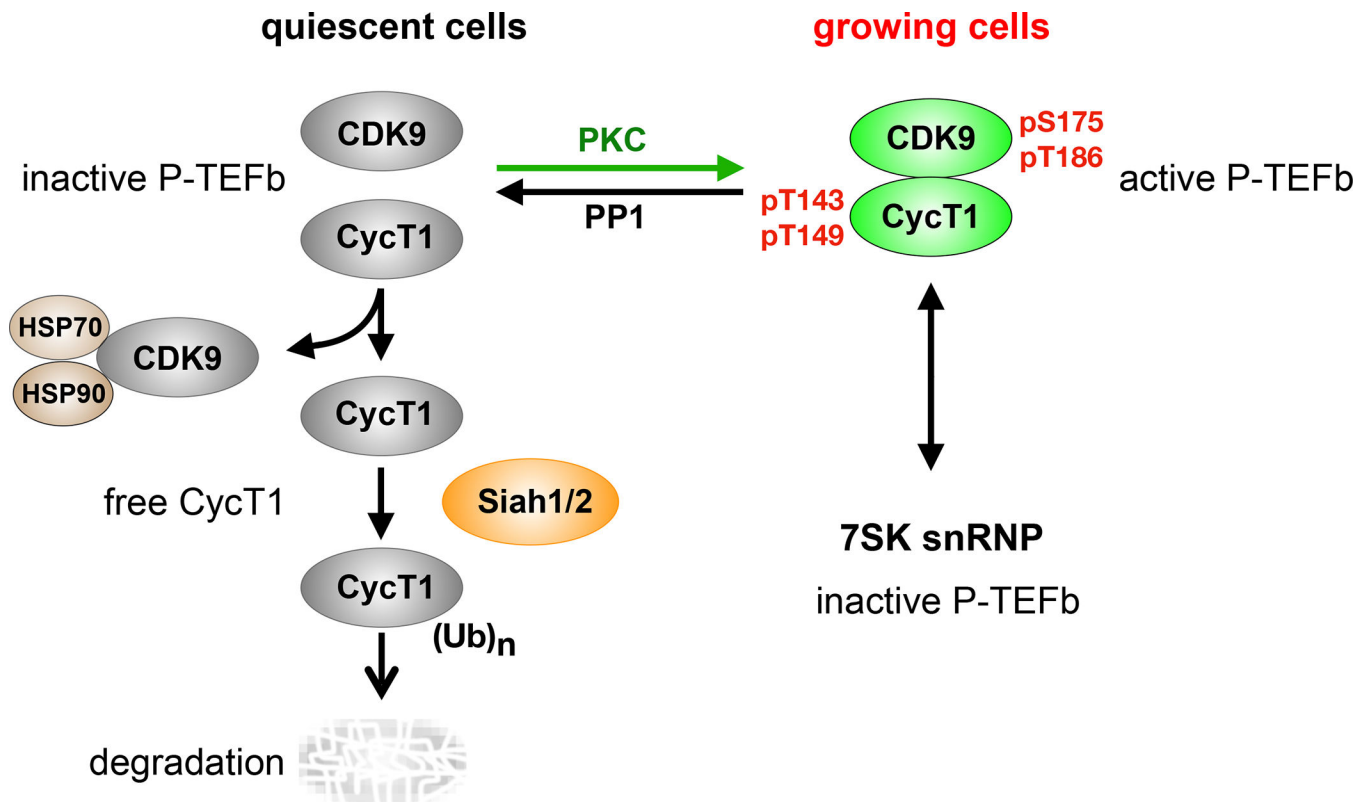


Figure 5.

The regulation of P-TEFb in quiescent cells. In contrast to growing cells, P-TEFb is degraded in quiescent cells. In these cells, the lack of active PKC allows protein phosphatase 1 (PP1) to dephosphorylate threonines at positions 143 and 149 (T143 and T149) in CycT1, thus dissociating it from CDK9. HSP 70 and HSP90 bind and stabilize the free CDK9. Siah1/2 bind to and ubiquitinate the unbound CycT1, which is quickly degraded. CDK9 and CycT1 transcripts remain high in these cells. Upon cell activation, PKC phosphorylates T143 and T149 and reverses this process. Arrows point in the direction of the unbound CycT1 degradation. However, upon PKC activation, the first step is reversed and P-TEFb is stabilized. Phosphorylated serine and threonine at positions 175 and 186 (pS175 and pT186) in CDK9 and phosphorylated T143 and T149 (pT143 and pT149) are found in CycT1 in the active P-TEFb.