REVIEW

Roles of CDKs in RNA polymerase II transcription of the HIV-1 genome

Andrew P. Rice

Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX, USA

ABSTRACT

Studies of RNA Polymerase II (Pol II) transcription of the HIV-1 genome are of clinical interest, as the insight gained may lead to strategies to selectively reactivate latent viruses in patients in whom viral replication is suppressed by antiviral drugs. Such a targeted reactivation may contribute to a functional cure of infection. This review discusses five Cyclin-dependent kinases – CDK7, CDK9, CDK11, CDK2, and CDK8 – involved in transcription and processing of HIV-1 RNA. CDK7 is required for Pol II promoter clearance of reactivated viruses; CDK7 also functions as an activating kinase for CDK9 when resting CD4⁺ T cells harboring latent HIV-1 are activated. CDK9 is targeted by the viral Tat protein and is essential for productive Pol II elongation of the HIV-1 genome. CDK11 is associated with the TREX/THOC complex and it functions in the 3' end processing and polyadenylation of HIV-1 transcripts. CDK2 phosphorylates Tat and CDK9 and this stimulates Tat activation of Pol II transcription. CDK8 may stimulate Pol II transcription of the HIV-1 genome through co-recruitment with NF-κB to the viral promoter. Some notable open questions are discussed concerning the roles of these CDKs in HIV-1 replication and viral latency.

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Current anti-HIV drugs are effective in suppressing viral replication in infected individuals. Upon cessation of these drugs, however, a reservoir of latent viruses spontaneously reactivates, making it necessary to resume anti-HIV drugs. The latent reservoir consists of long-lived memory CD4⁺ T cells with transcriptionally silent viruses integrated into the human genome. The study of cellular factors and mechanisms involved in RNA Polymerase II (Pol II) transcription of the HIV-1 genome is therefore a topic of considerable clinical interest, as it may allow development of small molecules and strategies that “shock” Pol II into the selective transcription of latent viral genomes. Cells with reactivated virus will subsequently express viral proteins, allowing the immune system to recognize and clear infected cells and hopefully, this will result in a functional cure of infection. This review discusses five CDK family members that play key roles in the Pol II transcription of the HIV-1 genome – CDK7, CDK9, CDK11, CDK2, and CDK8.

CDK7 and promoter clearance

The integrated HIV-1 genome contains long terminal repeat (LTR) sequences that are duplicated at the 5' and 3' ends [1]. The 5' LTR contains binding sites for

multiple cellular transcription factors, including notably NFAT, NF-κB, Sp1, and TFIID. Transcription factors that bind to these sites are responsible for directing Pol II to the 5' LTR and the initiation of transcription (Figure 1(a)). Stimulation of CD4⁺ T cells results in activation of NFAT and NF-κB and these transcription factors are believed to be critical in recruiting Pol II to the viral 5' LTR [2]. Because NFAT and NF-κB bind to overlapping sites in the viral LTR, they are believed to act sequentially during CD4⁺ T cell activation [3], with NF-κB acting initially and NFAT later [4–6]. For latent HIV-1, recruitment to Pol II of CDK7 in the TFIID general transcription factor complex is a rate-limiting step in reactivation of the virus [7]. Following this recruitment, CDK7 phosphorylates serine 5 in the carboxyl terminal domain (CTD) of Pol II, allowing promoter clearance and the commencement of elongation. As described below, CDK7 has an additional important role in transcription of the HIV-1 genome as an activating kinase for CDK9.

CDK9 and transcriptional elongation

Following promoter clearance, transcriptional elongation of the HIV-1 genome is repressed by the

Figure 1. Role of CDKs in RNA Polymerase II transcription of the HIV-1 genome. 1. CDK7 (magenta) in the TFIID complex is recruited to the transcriptional complex and phosphorylates the CTD of Pol II, resulting in promoter clearance. 2. The viral Tat protein recruits CDK9/Cyclin T1 (P-TEFb) to TAR RNA and the Super Elongation Complex assembles, allowing CDK9 (green) to phosphorylate the CTD of Pol II, DSIF, and NELF and thereby activation transcriptional elongation. 3. CDK11 (purple), associated with the TREX/THOC complex, is recruited to the 3' end of the integrated viral genome where it phosphorylates the CTD to enhance 3' cleavage and polyadenylation of the viral transcript.

action of two negative elongation factors, DSIF and NELF, which limit elongation to only a few hundred nucleotides [8,9]. DSIF is composed of two subunits, Spt4 and Spt5, while NELF is composed of four subunits, NELF-A, NELF-B, NELF-C/NELF-D, and NELF-E. The first ~ 60 nucleotides of the nascent viral transcript form a stem-loop RNA structure, termed TAR RNA, which serves as a binding site for Tat and the assembly of a large protein complex that activates transcriptional elongation (Figure 1 (b)). Initially, Tat binds to TAR RNA along with the general elongation factor P-TEFb that consists of CDK9 and Cyclin T1. In metabolically active cells such as activated CD4⁺ T cells, approximately 90% of CDK9/Cyclin T1 is sequestered in a kinase-inactive state in the 7SK RNP. This RNP contains the non-coding 7SK RNA and three additional proteins – HEXIM1/2, LARP7, and MEPCE [10]. Tat is able to extract CDK9/Cyclin T1 from the 7SK RNP [11–13], and Tat/CDK9/CyclinT1 binding to TAR RNA allows the assembly of a larger Super Elongation Complex (SEC) composed of ELL1/ELL2, AFF4, and ENL/AF9 [14–16]. Following assembly of the SEC on TAR RNA, CDK9 phosphorylates multiple substrates in the Pol II complex – the

E subunit of NELF [17–19], the Spt5 subunit of DSIF [20,21], and Serine 2 and 5 of the CTD of Pol II [22–24]. Phosphorylation of the E subunit causes NELF to dissociate from Pol II, phosphorylation of Spt5 converts DSIF into a positive elongation factor, and phosphorylation of the CTD provides binding sites for factors involved in RNA processing [25–27]. Of relevance to HIV-1 replication and latency, a portion of the CDK9/Cyclin T1 heterodimer in cells is bound by BRD4, a bromodomain protein that binds acetylated histones H3 and H4 and thereby directs P-TEFb to active cellular genes [28]. As Brd4 is an abundant cellular protein, it competes with Tat for binding to P-TEFb and can inhibit Pol II transcription of the HIV-1 genome, thereby contributing to viral latency [29–31].

CDK7 activation of CDK9 in resting CD4⁺ T cells

In resting CD4⁺ T cells that harbor latent HIV-1, Cyclin T1 protein levels are low and T cell activation leads to an induction of Cyclin T1 by a post-transcriptional mechanism [32–34]. Several miRNAs have been identified that are expressed at high levels in

resting CD4⁺ T cells and appear to repress translation of Cyclin T1 mRNA in these cells [35]. Unlike Cyclin T1, CDK9 is generally expressed at a high basal level in resting CD4⁺ T cells and is found in an inactive cytoplasmic complex with the chaperones Hsp70 and Cdc37 [36,37]. For CDKs, phosphorylation of a protein structure termed the T-loop is essential for kinase activity; phosphorylation of a serine or threonine residue in this structure displaces the loop and allows substrates access to the catalytic core of the enzyme [38]. Threonine 186 in the CDK9 T-loop is not phosphorylated in the Hsp90/Cdc37 complex and CDK9 is therefore in an off-state. Following T cell activation, there is rapid (within 15 minutes) phosphorylation of the CDK9 T-loop [39]. CDK7 was identified through a chemical genetics approach as the kinase that can phosphorylate the T-loop of CDK9 [40]. Hence, CDK7 appears to play two critical roles in reactivation of latent HIV-1 – it acts in Pol II promoter clearance and as an activating kinase for CDK9. CDK7 also phosphorylates serine 175 in CDK9, a modification that stabilizes the interaction between CDK9 and Cyclin T1 [37]. A number of phosphatases – PPM1A, PP2B, PP1 α , PPM1G – have been identified that can dephosphorylate the CDK9 T-loop and inhibit kinase function and consequently Pol II transcription of the HIV-1 genome [41–44]. Additionally, PPM1G has been reported to be involved in extracting CDK9/Cyclin T1 from the 7SK RNP [43]. The identification of multiple phosphatases capable of dephosphorylating the CDK9 T-loop suggest redundancy in this activity, or alternatively, specific phosphatases may function in distinct CDK9 regulatory pathways.

CDK11

This CDK family member was first implicated in HIV-1 replication when it was identified in a screen for Cyclin T1-dependent genes in activated CD4⁺ T cells; siRNA expressions demonstrated that CDK11 has a positive role in HIV-1 gene expression [45]. Additionally, a genetic screen identified an amino terminal fragment of the translational initiation factor eIF3 as an inhibitor of HIV-1 RNA 3' end processing [46]. As eIF3 was known to interact with CDK11, it was demonstrated that CDK11 plays an important role in 3' end processing of HIV-1 RNA [47]. In an

unbiased proteomics study, CDK11 was found to associate with the TREX/THOC complex that is involved in mRNA 3' end processing [48]. When recruited to the HIV-1 genome via TREX/THOC, CDK11 phosphorylates the Pol II CTD and this increases cleavage and polyadenylation of the 3' end of viral transcripts.

CDK2

CDK2 contributes to an increase in Pol II transcription of the HIV-1 genome through phosphorylation of both the viral Tat protein and CDK9. CDK2 phosphorylates Tat serine 16 and this modification facilitates Tat binding to TAR RNA; molecular modeling further suggests that serine 16 phosphorylation rearranges the Tat/CDK9/Cyclin T1 complex [49,50]. CDK2 can also phosphorylate serine 90 in CDK9 both *in vitro* and *in vivo* [51]. Phosphorylation of serine 90 enhances the ability of CDK9 to stimulate Tat function, as a CDK9 mutant with aspartic acid substituted for serine to mimic constitutive phosphorylation increases Tat activation of a reporter plasmid [51].

CDK8

CDK8 is a component of the Mediator complex that functions as a general transcriptional coactivator module [52]. Although CDK8 generally functions in the Mediator as a co-repressor, it can function alternatively as an activator in some activation pathways, such as p53, Wnt/ β -catenin, and the serum response [53]. CDK8 has been shown to be co-recruited with NF- κ B to cellular genes to potentiate activation by NF- κ B [54]. Because the HIV-1 LTR contains NF- κ B sites, CDK8 may contribute to Pol II transcription of the viral genome through co-recruitment with NF- κ B to the LTR. Indeed, a fusion of CDK8 to the HIV-1 Rev protein can activate an HIV-1 LTR reporter plasmid containing the Rev-Response RNA element in place of the TAR RNA element [55].

Important open questions

Following T cell activation, it is not known what mechanisms are involved in the ability of CDK7 to phosphorylate the CDK9 T-loop. As phosphorylation

can be detected within 15 minutes of T cell activation [39], it is plausible that CDK7 activity for this substrate may be inhibited by sequestration of CDK9 in a complex that is not accessible to CDK7. Alternatively, CDK7 may be sequestered in a complex that does not allow access to CDK9. It is also possible, if not probable, that CDK7 itself lacks a phosphorylated T-loop in resting CD4⁺ T cells. The activating kinase that phosphorylates the CDK7 T-loop and converts it to an active kinase in quiescent cells is not known [56]. CDK1 and CDK2 phosphorylate the CDK7 T-loop in cycling cells, but these kinases are not active in resting CD4⁺ T cells. The identification of this activating kinase for CDK7 is a key issue for the mechanistic understanding of reactivation of latent HIV-1. Additionally, it is possible in resting T cells that phosphatase activities for the CDK9 T-loop dominate over CDK7 kinase activity, and this dominance might be overcome following cellular activation. In the case of CDK11, the function of this critical CDK is low in resting CD4⁺ T cells and is up-regulated following T cell activation (B.M. Peterlin, personal communication). Identification of the underlying mechanisms that regulate CDK11 function and 3' end processing of viral transcripts in resting CD4⁺ T cells will provide insight into reactivation of latent HIV-1.

As stated in the beginning of this review, current anti-HIV drugs are capable of suppressing viral replication in infected individuals. However, the reservoir of transcriptionally silent virus in memory CD4⁺ T cells spontaneously reactivates when the drugs are discontinued. There is considerable clinical interest in developing strategies to shock latent viruses out of transcriptional latency and thereby allow viral antigens to be expressed and recognized by the immune system. With a decreased viral reservoir and development of parallel strategies to enhance infected individuals' immune systems against HIV-1, it is hoped that a functional cure can be achieved. Although the mechanisms involved in latency in CD4⁺ T cells are incompletely understood, multiple mechanisms are known to be involved in the establishment and maintenance of latency [57–59]. Transcriptional interference by cellular genes at the site of integration contributes to latency [60,61]. Repressive chromatin is established for latent proviruses [62]. Limiting levels of cellular

transcription factors make important contributions to latency, especially P-TEFb [33,63]. Shock strategies to reactivate latent HIV-1 will require the induction of CDK9 T-loop phosphorylation and Cyclin T1 protein levels. However, it is critical that agents that induce P-TEFb do not lead to generalized immune activation. This may be achievable as a combination of cytokines (TNF- α , IL-2, IL-6) or the PKC agonist prostratin have been shown to induce P-TEFb activity in resting CD4⁺ T cells without inducing the T cell activation marker CD25 or cellular proliferation [32,34]. A synthetic prostratin derivative is potent in reactivating latent HIV-1 in patient cells *ex vivo* and does not induce the activation marker CD25 [64]. Additional PKC agonists termed Ingenol esters effectively reactivate latent HIV-1 in patient cells *ex vivo* and also up-regulate P-TEFb [65–67]. An important component of the shock to reactivate latent HIV-1 is an activity that can relieve repressive chromatin of the integrated virus, such as histone deacetylase inhibitors (HDACis) [68]. Interestingly, broad spectrum HDACis (vorinostat and panobinostat) that reactivate latent HIV-1 through relief of repressive chromatin also up-regulate P-TEFb through elevated levels of T-loop phosphorylation, suggesting that some HDACis have multiple mechanisms of action [69,70].

A recent publication reported that CDK9/Cyclin T1 utilizes a biomolecular condensate to enhance phosphorylation of the Pol II CTD [71]. Biomolecular condensates are subcellular structures that form through interactions between nucleic acids and proteins with low complexity domains – that is, a domain consisting of predominantly a single or very limited number of amino acids [72]. Biomolecular condensates are thought to compartmentalize and accelerate biochemical reactions [73]. Examples of biomolecular condensates in the nucleus include speckles, paraspeckles, PML bodies, and Cajal bodies. Cyclin T1 contains a histidine-rich low complexity domain that increases binding to CDK9 and the Pol II CTD. The histidine-rich region also promotes the formation of biomolecular condensates *in vitro* and localization of CDK9/Cyclin T1 to nuclear speckles *in vivo* [71]. Thus, CDK9/Cyclin T1 activation of Pol II elongation of the HIV-1 genome may

involve the formation of a biomolecular condensate in which the integrated HIV-1 genome, CDK9/Cyclin T1, and Pol II are concentrated to facilitate the phosphorylation of NELF, DSIF, and the CTD. The study of the role biomolecular condensates in Pol II transcription is currently a highly active area of research [73]. If CDK9 activation of elongation of the HIV-1 genome does utilize a biomolecular condensate, we can anticipate mechanistic insight into this process in the near future. Such insight may provide the opportunity to develop novel strategies to either inhibit Pol II transcription of the HIV-1 genome or selectively reactivate latent virus.

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