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HIV-1 Transcriptional modulation: Novel Host Factors and Prospective Therapeutic Strategies

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Abstract

Purpose of review—This review highlights advances in HIV transcription and epigenetic latency mechanisms and outlines current therapeutic approaches to eliminate or block the HIV-1 latent reservoir.

Recent findings—Novel host factors have been reported to modulate HIV-1 transcription and latency. Chromatin affinity purification strategies followed by mass spectrometry (ChAP-MS) identified the chaperone protein p32 to play an important role in HIV-1 transcriptional regulation via interactions with the viral transcriptional activator Tat. Similarly, an shRNA screen identified the methyltransferase SMYD5 contributing to HIV-1 transcriptional activation also by modulating Tat activity. These new factors, among others, represent potential druggable targets that could be explored in the “block-and-lock” or “shock-and-kill” approaches.

Summary—The HIV-1 latent reservoir is established early after infection, persists during antiretroviral therapy, and is the source of viral rebound after treatment interruption. An HIV cure requires either eliminating this reservoir or blocking latent proviral reactivation in the absence of antiretroviral therapy (ART). Understanding the mechanisms and key-players modulating HIV transcriptional and reactivation may facilitate therapeutic advancements. Here we summarize, the latest findings on host factors’ roles in HIV transcriptional regulation.

Keywords

HIV-1; transcription regulation; latency; host factors; chromatin regulation; block-and-lock; dCA; spironolactone; shock-and-kill

INTRODUCTION

In 2021 alone, 1.5 million new HIV-1 diagnoses added to the 38 million individuals worldwide living with the virus (1). Despite its life-saving benefits, ART is not curative and a pool of latently infected $CD4^+$T cells persists triggering widespread viral replication

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Conflict of interests

The authors declare no conflict of interest.

upon ART interruption (2–6). People living with HIV (PLWH) must remain on lifelong therapy since eradication of this pool of cells has proven extremely challenging and remains the primary HIV cure obstacle (7–10). A cure can entail either a total elimination of cells carrying HIV proviruses, or else, disabling the virus ability to reactivate without ART. In the latter, known as a functional cure, the provirus persists in cells, but is unable to make viral RNA/proteins, transmit or cause immunodepression without therapy. Further knowledge on mechanisms regulating transcription from integrated HIV-1 proviruses is needed to guide viral eradication or functional cure efforts. We review recent findings on HIV-1 transcriptional regulation, latency, and current HIV cure strategies hinging on HIV transcriptional modulation.

HIV-1 TRANSCRIPTION AND LATENCY REGULATION

HIV-1 transcriptional activation

A sequential network of viral and cellular factors, as well as the chromatin environment surrounding the provirus, regulate HIV-1 transcription. The viral promoter, the 5'-long terminal repeat (5'-LTR), irrespective of the host integration site, displays a typical nucleosomal (Nuc) organization with Nuc-0 and Nuc-1 framing the transcription start site (TSS). The 5'LTR contains multiple binding sites for host transcription factors (TFs) that help regulate transcription (11, 12). The general mechanism (Figure 1A) by which HIV-1 transcription is controlled has been described as follows: i) pre-initiation complex (PIC) formation at the HIV LTR starts with the assembly of the TATA-binding protein (TBP), other general TFs such as TFIIA, TFIIB and TFIIF at the core promoter (13, 14), also regulated by interactions with the nuclear factor kappa B (NF-κB) and specificity protein 1 (Sp1) (15–17). Then, RNA polymerase II (RNAPII) and the general transcription and DNA repair factor II human (TFIIH) are recruited (18). TFIIH's cyclin dependent kinase 7 (CDK7), phosphorylates the carboxy-terminal domain of RNAPII (RNAPII-CTD) at Ser5 residues triggering promoter clearance (19). ii) RNAPII typically stalls after transcribing the transactivation response element RNA (TAR), which forms a dynamic hairpin secondary structure (20). RNAPII is paused by its association with the negative elongation factor (NELF) and 5,6-dichloro-1-β-ribofuranosylbenzimidazol (DRB) sensitivity-inducing factor (DSIF), and Nuc-1 obstruction just downstream from the TSS. iii) Tat, the HIV-1 transactivator protein, releases RNAPII pausing by recruiting the positive transcriptional elongation factor b (P-TEFb), composed of CDK9 and cyclin T1 (21), to the nascent TAR. P-TEFb phosphorylates DSIF, NELF and RNAPII CTD at Ser2. Phosphorylated NELF dissociates from the complex, allowing DSIF to act as an elongation factor (22, 23). The production of full-length HIV mRNAs hinges on association/dissociation cycles between P-TEFb and Tat, CDK9 phosphorylation events, and chromatin remodeling by Tat-recruited polybromo-associated BRG1 or hbrm-associated factor (PBAF) (24–26). Furthermore, the Tat/P-TEFb complex interacts with the super elongation complex (SEC) composed of the scaffold protein (AFF1/4), the co-factors (ENL and AF9) and the positive elongation factor (ELL2) (27, 28). Tat binding to AFF4, increases binding affinity of SEC with Cyclin T1 and sequestration of ELL2 into the SEC. This cooperative association with P-TEFb triggers RNAPII pause release and transcriptional elongation (29–31). HIV-1 transcriptional

activation is a multistep complex process and further research is needed to understand this biological network and roles of each key host player.

Recent studies uncovered novel host factors involved in HIV-1 transcription. In a selective and unbiased approach, Li *et al.* used chromatin immunoprecipitation (ChIP) via dCas9/gRNA specific enrichment followed by mass-spectrometry ChAP-MS to identify host factors directly associated with latent and active HIV-1 promoters (32, 33). ASF/SF2 splicing factor-associated protein (p32), the far upstream element binding protein 3 (FUBP3), and the proliferation-associated 2G4 (PA2G4) (33) were found enriched in actively transcribing promoters. While research on FUBP3 and PA2G4 is ongoing (personal communication), p32 was found to directly bind the HIV-1 promoter and genome, in a Tat dependent manner (33). p32 is a multifunctional and multicompartmental protein involved in infection, inflammation and cancer (34), known to interact with some HIV-1 proteins, but had never been implicated in HIV-1 transcription (35–38). Li *et al.* showed p32 binds Tat's basic domain stabilizing Tat's half-life, and likely due to p32's scaffolding abilities, facilitates Tat-TAR/P-TEFb/RNAPII interaction enhancing HIV transcription.

A small short hairpin RNA (shRNA) screen identified the methyltransferase Su(var)3–9, enhancer-of-zeste, and trithorax (SET) and myeloid, Nervy, and DEAF-1 (MYND) domain-containing protein 5 (SMYD5) as a new host co-activator required for HIV transcription (39, 40). SMYD5 binds and activates the HIV-1 LTR significantly enhanced by Tat. This study suggests that SMYD5 assists Tat recruitment of P-TEFb to RNAPII and TAR, by prominently methylating Tat *in vitro*. The authors found deubiquitinating enzyme USP11 to increase SMYD5 expression, proposing that Tat and USP11 stabilizes SMYD5 protein levels and co-dependently SMYD5 methylates Tat, enhancing HIV-1 transcription. However, Tat methylation and SMYD5 ubiquitination sites remain unknown, and additional work is needed to elucidate SMYD5 participation in Tat- transactivation of the HIV-1 LTR. Recently, the tripartite-motif containing protein 24 (TRIM24) was also found to interact with TFII-I (41). This TF selectively regulates gene expression of TATA box-containing promoters and together with USF1 and USF2 plays a role during HIV-1 reactivation upon Jurkat T-cell activation (41–45). TFII-I seems to recruit TRIM24 to promote transcription elongation through enhanced CDK9 and Ser2 RNAPII CTD phosphorylation (41). Furthermore, inhibition of the TRIM24-C terminal bromodomain using the small molecule IACS-9571 (46, 47), in combination with the PKC agonist PEP005 (48), promotes HIV-1 reactivation in primary $CD4^+$ lymphocytes, suggesting their potential use as latency reversing agents (LRAs) (49). A CRISPR/Cas genome-wide screen in Jurkat T cells identified novel host factors and pathways contributing to HIV expression (50), including UBE2M, FBXW7, SLC39A7 or ING3 (50, 51), but their specific roles in the HIV-1 life cycle remains to be elucidated. In summary, numerous approaches are being used to gain insight into host protein involved in HIV transcriptional regulation and additional work is needed towards depth in their mechanism of action in cells and tissues.

HIV-1 latency state

The latent HIV-1 reservoir consists of long-lived memory $CD4^+$T cells harboring integrated latent proviruses. The switch between latent and active HIV transcription is highly

dependent on Tat's positive feedback loop (52, 53). Before Tat accumulates, viral transcription is very limited due to inefficient transcriptional elongation (54, 55). At this stage, the HIV promoter is heavily influenced by its local chromatin environment and availability of TFs. T cell activation triggers production of a few short transcripts that result in Tat production (54, 55), which results in exponential HIV transcription by recruitment of pTEFb to RNAPII CTD, and thus viral rebound (56–58). Latency usually correlates with limited Tat levels, but additional mechanisms contribute to HIV latency. For instance, the chromatin remodeler Brg1-associated factor (BAF) complex maintains Nuc-1 in an unfavorable position downstream of the TSS blocking transcription. Additionally, histone deacetylases (HDACs) and histone methyltransferases (HMTs) add repressive epigenetic modifications at the HIV promoter. Low-levels of TFs also limit transcription (Figure 1B) (26, 59, 60), e.g., during HIV latency, inactive P-TEFb associates with the 7SK RNP complex, composed of the small non-coding RNA 7SK (7SK snRNA), a homo- or heterodimer of the CDK9-inhibitory protein hexamethylene bisacetamide (HMBA)-inducible 1 or 2 (HEXIM 1/2), the methylphosphatase capping enzyme (MePCE), and the La ribonucleoprotein domain family member 7 (LARP7) (29). P-TEFb sequestration by 7SK RNP inhibits CDK9 activity blocking transcriptional elongation (61, 62), Tat releases P-TEFb by interacting with 7SK snRNA, which displaces HEXIM-1. HEXIM-1 and Tat share a similar RNA binding domain competing for 7SK RNA binding and thus P-TEFb (29). Tat then recruits free P-TEFb to TAR promoting HIV transcriptional elongation (21).

The chromatin environment surrounding HIV integration dictates transcriptional activity, since nucleosome positioning controlled by the activity of chromatin regulatory factors (CRFs) determines accessibility of TFs and PIC formation (63). CRFs have three main enzymatic activities: i) ATPase-driven chromatin remodeling that actively slide, deposit, or eject nucleosomes (64); ii) post-translational modification (PTM) of histone N-terminal tails including methylation, acetylation, crotonylation, and ubiquitination, that alter nucleosome dynamics and provide docking sites for other transcriptional modulators (24, 65); and iii) methylation of CpG islands, promoting recruitment of transcriptional repressors and blocking recruitment of positive TFs (66). A detailed review of these epigenetic modulations is provided elsewhere (12, 24).

The discovery of chromatin modulators involved in HIV transcription has gained significant attention. Recently the chromodomain helicase DNA-binding protein (CHD9) was shown to promote HIV-1 latency via direct association with the 5'LTR (67). Knock-down of CHD9 with shRNAs in J-Lat A2 and J-Lat 11.1 cells resulted in significant reversal from latency (67). ChIP experiments showed that CHD9 was enriched at the HIV-1 LTR in latent 11.1 J-Lat cells and displaced upon PMA stimulation suggesting a role in maintaining HIV-1 latency (67). How CHD9 is recruited to the HIV-1 LTR will require additional research.

A genome-wide CRISPR inhibition screen in Jurkat T cell clones identified 18 new HIV-1 latency factors including the scaffold attachment factor B-like transcription modulator (SLTM) (68). SLTM is an epigenetic and transcriptional modulator known to inhibit estrogen receptor signaling but not previously reported to affect HIV-1 transcription (68, 69). SLTM knockdown in HIV-1-infected T Jurkat cell clones increased HIV transcription and chromatin accessibility, highlighting SLTM repressor functions in HIV transcription. The

chromatin regulatory mechanisms that control HIV-1 latency hold promise as therapeutic targets, and further research is needed to understand detailed mechanisms of action and develop of specific small molecule modulators.

A recent longitudinal study focused on the transformation of the HIV-1 reservoir at the chromatin level in individuals on long-term ART revealed the role of chromatin compaction in HIV transcription (70). This analysis of cells from PLWH on long-term ART revealed that intact HIV-1 proviruses integrated in repressive chromatin regions are more likely to persist and evade immune recognition and elimination compared to those integrated in permissive chromatin (70). This selection becomes more prominent in the second decade of suppressive ART with an attenuated viral reservoir profile with reduced potential for viral rebound, potentially contributing to ART free control of HIV-1 (70). Clinical studies are now needed to fully understand the extent of these findings and its clinical implications. Similarly, single-cell latent reservoir studies uncovered novel insights into the mechanisms of HIV-1 latency. For instance, we know that HIV-1 integrates in various genomic locations resulting in diverse proviral transcriptional activities (71, 72). Einkauf *et al.* suggest that ART positively selects proviruses with lower transcriptional activity, since highly active proviruses are vulnerable to host immune clearance (73). Modulation of proviral transcriptional behavior may thus enhance susceptibility to immune-mediated elimination offering novel avenues for HIV-1 control without continuous ART (73). Additional studies are needed to understand the effects of transcriptional modulation on infected cells to determine the vulnerabilities and susceptibilities of HIV-1 reservoir cells.

CURRENT THERAPEUTIC STRATEGIES TO CURE HIV

The block-and-lock strategy

The realization that HIV proviruses act as a hibernating threat, triggering rapid viral rebound when ART is interrupted sparked research efforts in functional or remission cures, akin approaches used in cancer treatment (74, 75). An HIV remission entails the long-term, durable suppression of viral expression without therapy, effectively preventing disease progression and transmission despite the presence of integrated proviruses (76). Over the years, our group has provided evidence that contributed to the introduction of the “block-and-lock” concept for an HIV remission. This approach harnesses the combined power of ART and latency promoting agents (LPAs), such as HIV Tat inhibitors, transcription initiation inhibitors, or epigenetic modulators, to establish a state of deep and irreversible latency, which persists without treatment (Figure 2A) (77–79). Epigenetic silencing, a common phenomenon in the human genome, restricts expression of large fractions of our genetic repertoire, with only approximately 8,000 genes actively expressed per cell out of around 20,000 human genes (80, 81). Similarly, most human endogenous retroviruses (HERVs) which make up approximately 8% of our genome, are persistently silenced (81–87).

The nexus for this approach was demonstrated with the Tat inhibitor, didehydro-Cortistatin A (dCA) (88, 89). By specifically binding the basic domain of Tat, dCA disrupts Tat’s interaction with TAR, blocking HIV transcriptional elongation at nanomolar concentrations without cell-associated toxicity (88, 90). *In vitro* studies and humanized mice models of

HIV infection have shown that treatment with ART and dCA significantly reduces HIV transcription levels that persist under ART by inhibiting RNAPII recruitment to the HIV promoter (78, 89). As such, inhibition of HIV transcription by dCA gradually leads to epigenetic silencing (91). Notably, even in the presence of potent LRAs, dCA effectively blocks or delays viral reactivation observed after treatment interruption (78, 89). The lack of clinically available HIV transcriptional inhibitors has hindered clinical testing of block-and-lock approaches. It remains thus uncertain whether long-term transcriptional inhibition can permanently epigenetically suppress HIV *in vivo* and whether multiple latency-promoting agents (LPAs) targeting different aspects of HIV transcription are necessary to achieve this goal. Nevertheless, trickling transcription from HIV reservoirs during suppressive ART contribute to immune activation, inflammation, comorbidities, and accelerated aging in PLWH, thus combining transcriptional inhibitors to ART may improve these clinical outcomes (92, 93).

Spironolactone (SP), an FDA approved drug for the treatment of hypertension and heart failure, was reported to degrade the XPB subunit of TFIIH and inhibit HIV transcription (94–96). TFIIH is involved in DNA opening at the TSS and RNAPII promoter escape during transcriptional initiation (97, 98). SP inhibits both HIV-1 and HIV-2 infection in primary cells which correlates with its ability to degrade XPB (95). SP treatment or shRNA knockdown of XPB limits RNAPII recruitment to the HIV locus blocking viral transcription, without affecting cellular mRNA expression (96). However, contrary to what was observed for dCA, SP interruption and the reemergence of XPB jumpstarts HIV transcription (96). This study identified XPB as an important host factor for HIV transcription and SP as a novel LPA. HIV transcriptional inhibitors have unique potential in block-and-lock approaches, and exploring the newly discovered roles of host factors in HIV transcription regulation opens novel avenues to be explored for HIV functional cure.

The shock-and-kill strategy

The shock-and-kill strategy aims to fully eradicate all latently infected cells (99). This approach involves induction of viral protein expression with LRAs (“shock”), with ART to blocks viral reinfection, and subsequent elimination (“kill”) of infected cells through cytopathic mechanisms or by cytotoxic $CD8^+$T cells (Figure 2B). LRAs can be classified into four categories: i) epigenetic modulators (e.g. DNA methyltransferase inhibitors); ii) TF activators (e.g. protein kinase C activators); iii) bromodomain and extra-terminal domain inhibitors (e.g. BRD4 inhibitors); and iv) inhibitor of apoptosis proteins (IAPs) (e.g. second mitochondrial-derived activator of caspases mimetics) (100–102). Clinical trials testing this approach have unfortunately been unsuccessful in reducing the proviral reservoir (103). Reasons for this failure include immune dysfunction in PLWH and thus limited clearance of reactivated cells, inadequate LRA efficacy and LRA toxicity in immune cells (104–109). Furthermore, as discussed above, many factors affect transcriptional reactivation such as proviral integration sites, host factor availability and immune selection during ART. Work is ongoing to develop novel potent and specific LRAs, without off target activity, and strategies to enhance $CD8^+$T cell competence that declines in PLWH over years on ART.

A combination of LRAs is likely needed to improve specific reactivation of proviruses and host immune response. *In vitro* studies using cell line models of HIV-1 latency and *ex vivo* studies of patient derived $CD8^+$ and $CD4^+$T cells has shown promise (110–112). For instance, immune check point inhibitors and cytokine (IL15) associated with protein kinase C agonist (prostratin) led to efficient HIV reactivation followed by natural killer (NK) cell clearance (113). A similar study using prostratin in combination with a pan-caspase inhibitor successfully induced HIV-1 latency reversal in NK infected cells (114). However, though the “shock” increased cell associated viral RNAs, the ‘kill’ may have been limited by the caspase inhibitor block of cell death. Finally, nanomedicine drastically improves delivery and efficacy of drugs, and may be applied in both shock-and-kill and block-and-lock approaches to treat HIV-1 (115).

SUMMARY AND CONCLUSION

The viral latent reservoir forms early during acute HIV infection and virus promoter activity and transcriptional activity is regulated via a complex network of viral and host factors. Elimination of this reservoir has been extremely challenging and is the main obstacle to an HIV cure. We reviewed recent discoveries of host factors and CRFs involved in HIV-1 transcription, as well as discoveries on the chromatin environment modulating HIV expression in PLWH. Recent advances on cure strategies exploiting HIV transcriptional modulation, such as the block-and-lock and the shock-and-kill approaches, were also summarized. We propose that a combinatorial approach may be needed for a successful HIV cure, first easy to reactivate provirus may be cleared via shock-and-kill, followed by the irreversibly silencing of proviruses already buried in unfavorable chromatin regions and difficult to reactivate by block-and-lock approaches. By expanding our knowledge on HIV transcription, we can pave the way for more effective therapeutic interventions and ultimately HIV-1 eradicate.

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KEY POINTS

- Transcription from the HIV-1 latent reservoir, formed early after infection, is regulated by complex mechanisms that involve cellular transcription factors, the viral Tat protein, chromatin remodelers and epigenetic modifications.
- Recent studies using ChAP-MS, shRNA or CRISPR-based screening approaches identified host factors p32, SLTM or SMYD5 playing important roles in HIV transcription and latency.
- Multiple therapeutic strategies take advantage of HIV transcriptional modulation to tackle HIV: the block-and-lock approach aims to epigenetically silence HIV-1 and the shock-and-kill strategy to activate and promote immune clearance of the viral reservoir.

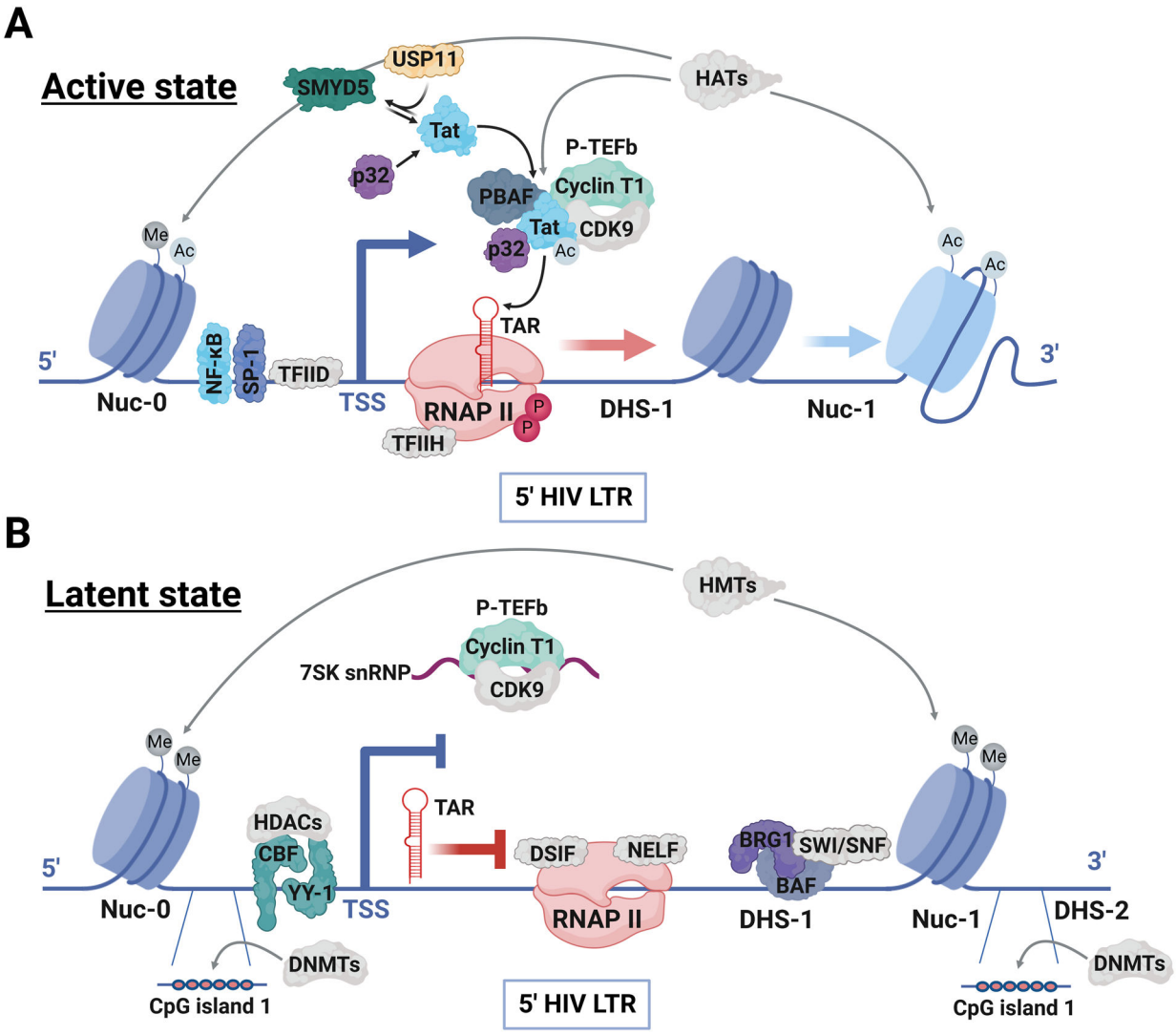

Figure 1. Summary of HIV-1 transcriptional activation and latency regulation.

A) Initially, transcriptional activators (such as NF-κB, SP1, etc.) are recruited to form the pre-initiation complex (PIC). Histone acetyltransferases (HATs) are then responsible for inducing chromatin opening and recruiting the PBAF complex. The PBAF complex, in turn, facilitates the displacement of Nuc-1, relocating it downstream from the transcription start site (TSS), thereby enhancing accessibility for host factors at the TAR RNA-RNAPII complex. TFIIH phosphorylates the serine residues at position 7 and 5 of the RNA polymerase II (RNAPII) C-terminal domain (CTD), which activates transcription. CDK9, associated with P-TEFb, further phosphorylates the CTD of RNAPII at serine 2, inducing full-length transcription of HIV-1 and the expression of Tat. Additionally, HATs acetylate Tat at lysine 50, facilitating its binding to the secondary structure of the newly formed TAR RNA. Furthermore, it is believed that SMYD5 plays a role in HIV-1 transcription both independently and through its interaction with Tat and USP11. p32 serves to stabilize Tat's interaction with RNAPII, P-TEFb, and TAR. Finally, Tat recruits additional factors, thereby further promoting transcription and creating a positive feedback loop that increases

HIV transcription and consequently enhances Tat expression. B) The chromatin environment contributes to latency through various mechanisms. It enhances nucleosome-DNA affinity, reduces DNA accessibility, and recruits repressive factors like the SWI/SNF chromatin remodeling BAF complex. Factors such as CBF and YY-1 bind to DNA, enabling the recruitment of HDACs and HMTs. HDACs remove acetyl groups from nucleosomes, while HMTs replace them with methyl groups. DNMTs are believed to hypermethylate the two CpG islands, leading to HDAC recruitment. Lastly, P-TEFb, crucial for HIV transcription, is sequestered through its association with 7SK snRNP. Abbreviations: DHS, DNase hypersensitive regions; DNMTs, DNA methyltransferases.

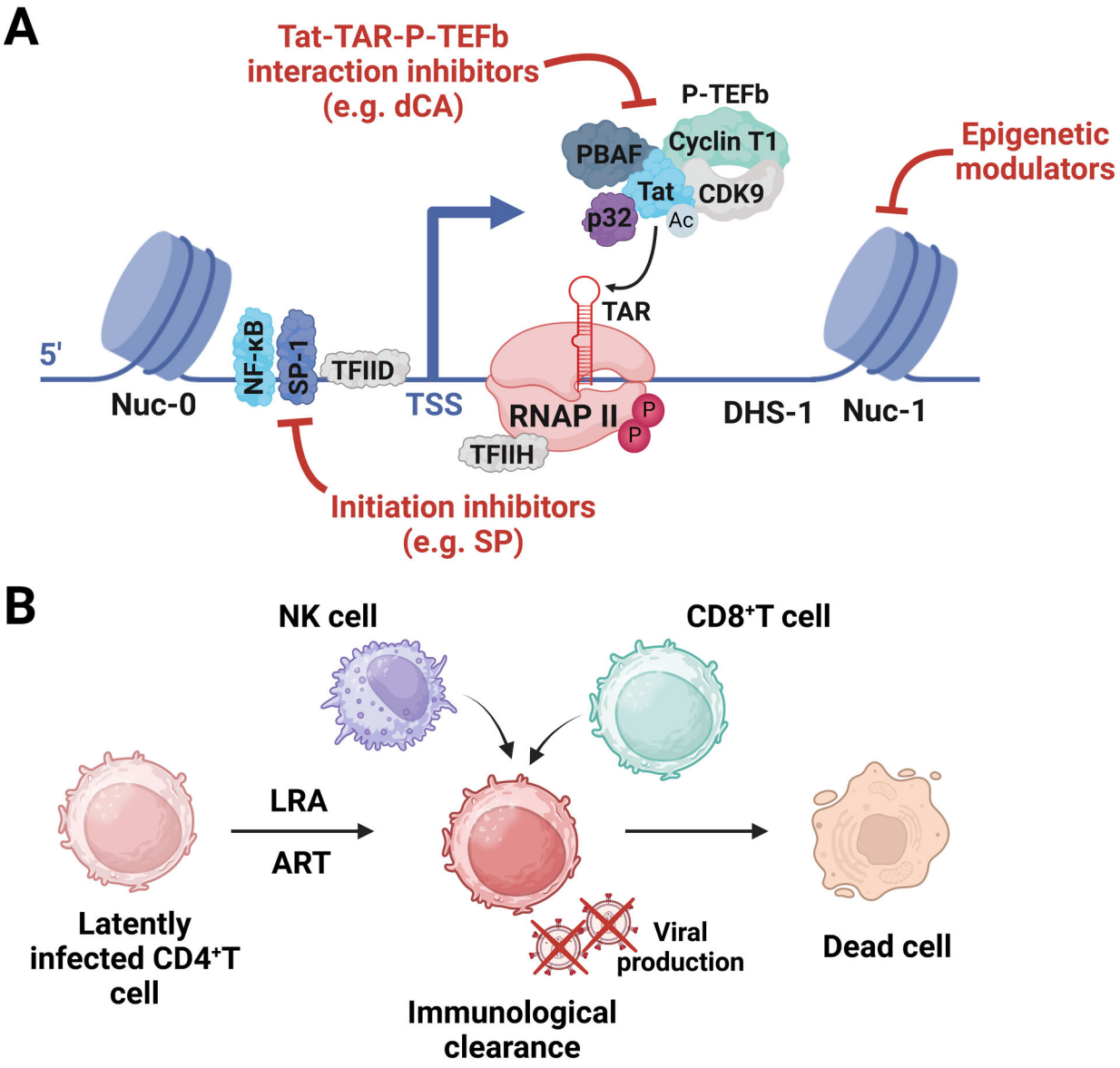

Figure 2. Principles of the block-and-lock and shock-and-kill approaches.

A) To trigger epigenetic silencing, various targets can be explored for inhibition or modulation. This may involve examining members of the PIC to impede transcription initiation, components of the Tat-transactivation complex to block transcription elongation, and ultimately, factors that regulate the chromatin environment to hinder and prevent HIV transcription. B) The objective of the shock-and-kill strategy is to completely eliminate the proviral latent reservoir. This is achieved by initially reactivating dormant infected cells using LRAs, followed by the elimination of these cells through either cell cytolysis or immune clearance. Simultaneously, ART is administered to prevent new infections.