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Molecular mechanisms of HIV latency

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HIV seeds reservoirs of latent proviruses in the earliest phases of infection. These reservoirs are found in many sites, including circulating cells, the lymphoid system, the brain, and other tissues. The “shock and kill” strategy, where HIV transcription is reactivated so that antiretroviral therapy and the immune system clear the infection, has been proposed as one approach to curing AIDS. In addition to many defective viruses, resting hematopoietic cells harbor transcriptionally latent HIV. Understanding basic mechanisms of HIV gene expression provides a road map for this strategy, allowing for manipulation of critical cellular and viral transcription factors in such a way as to maximize HIV gene expression while avoiding global T cell activation. These transcription factors include NF- κ B and the HIV transactivator of transcription (Tat) as well as the cyclin-dependent kinases CDK13 and CDK11 and positive transcription elongation factor b (P-TEFb). Possible therapies involve agents that activate these proteins or release P-TEFb from the inactive 75K small nuclear ribonucleoprotein (snRNP). These proposed therapies include PKC and MAPK agonists as well as histone deacetylase inhibitors (HDACis) and bromodomain and extraterminal (BET) bromodomain inhibitors (BETis), which act synergistically to reactivate HIV in latently infected cells.

Historical perspective

AIDS first came to public attention in 1981 (1) and represented an acquired immunodeficiency, which invariably led to the demise of the infected individual. Thus, whatever the transmissible agent, the immune system could not eliminate the infection. HIV, which proved to be a retrovirus of the lentivirus family, was identified as the causative agent two years later (2). The provirus was not sequenced until 1985 (3–6). The proviral genome contains genes encoding the viral structural proteins Gag, Pol, and Env, the regulatory proteins HIV transactivator of transcription (Tat) and the regulator of expression of virion proteins (Rev), which are required for HIV replication, and the accessory proteins negative factor (Nef), viral infectivity factor (Vif), viral protein U (Vpu), and viral protein R (Vpr). Vif, Vpu, and Vpr counteract host restriction factors and are not required for HIV replication in permissive cell lines.

Transcription of HIV initiates at the 5' long terminal repeat (LTR), which acts as a promoter and enhancer. Transcription from the 5' LTR generates a primary transcript, which is spliced into over 109 mRNAs to produce all viral proteins or is packaged into virions (7). Each LTR contains untranslated 3' (U3), repeat (R), and untranslated 5' (U5) regions. The U3 region (453 nt), located at the 5' end of the LTR, contains cis-acting DNA elements that serve as the binding sites for transcription factors (TFs) including nuclear factor of activated T cells (NFAT), NF- κ B, and specificity protein 1 (SP1). This region also contains the TATA box and the initiator element. The transcriptional start site (TSS) is located at the junction between the U3 and R regions. The R region (100 nt), which sits in the middle of the LTR, contains the transactivation response (TAR) element. The U5 region (80 nt) contains the HIV packaging sequences. The 3' end of the U5 region also contains a lysyl transfer RNA (tRNA) binding site, which serves as the primer for reverse transcription (RT).

By 1987, it was clear that the activation of infected cells increased HIV transcription, which followed the translocation of NF- κ B and/or NFAT into the nucleus (Figure 1 and refs. 8–10). Effects of these TFs are potentiated greatly by Tat, which binds to TAR RNA stem loop rather than to DNA (Figure 1). TAR, which forms after RNA polymerase II (RNAPII) stalls near the TSS, is located at the 5' end of all HIV transcripts and contains 60 nt (Figure 1). Thus, Tat requires some transcription before it can potentiate HIV gene expression. As such, Tat cannot initiate transcription, but elongates all viral transcripts that contain TAR (11). A new concept in eukaryotic biology was born, one that mirrored the prokaryotic world in which antitermination of transcription regulates, for example, the switch between lysogeny and lysis of bacteriophage λ (11).

Because only short transcripts were observed in cells in the absence of Tat and these transcripts were elongated efficiently in the presence of Tat, we proposed that HIV mimicked bacteriophage λ and that the virus could lie undetected in cells. Sequential steps of initiation and elongation of transcription also explain the synergy between NF- κ B and Tat. Short, TAR-containing transcripts were also used to detect latently infected cells in patients. Surprisingly, all circulating peripheral blood monocytes (PBMCs) only contained short transcripts at seroconversion (12). However, they could be converted into long transcripts by incubating infected lymphoid cells with allogeneic cells, which activated the latently infected cells via the mixed lymphocyte reaction. In these latently infected cells, HIV replication was induced and fully infectious virus was recovered (12). The reason that circulating lymphocytes did not express any viral proteins lies in the resting nature of these cells, which home to lymphoid organs or sites of inflammation only after activation.

Although these findings clearly demonstrated that latently infected cells could be reactivated, at that time, RT inhibitors were not able to completely suppress HIV replication. A few years later, the addition of protease inhibitors to the antiretroviral therapy (ART) regimen allowed for the suppression of viral RNA below the

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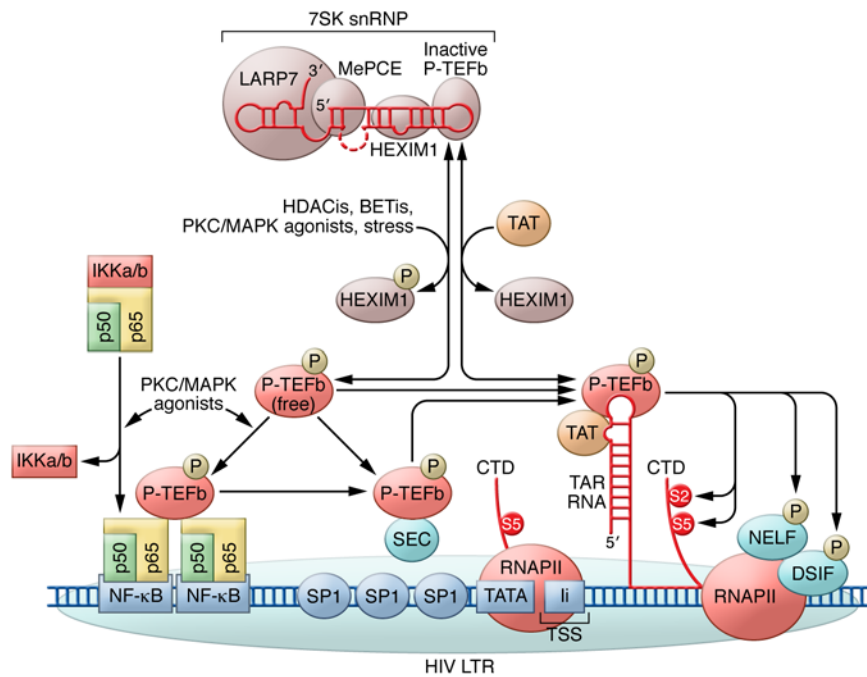


Figure 1. HIV LTR, TFs, and their activation. HIV transcription starts at the TSS, where the initiator element binds. The compact promoter contains a TATA box and 3 SP1 sites. The enhancer contains overlapping NF-κB and nuclear factor of activated T cells (NF-AT) binding sites (NF-AT sites not shown). The promoter recruits RNAPII. During promoter clearance, CTD Ser5 (red circles) is phosphorylated by CytH/CDK7. P-TEFb is recruited to the HIV LTR via NF-κB (p50/p65 heterodimer), the super elongation complex (SEC), and Tat. Upon recruitment, P-TEFb phosphorylates Ser2. Tat binds to TAR RNA from positions +1 to +60 in all HIV transcripts. Tat and P-TEFb bind to the 5' bulge and central loop in TAR, respectively. P-TEFb also phosphorylates Spt5 in DSIF and NELF-E, which releases the arrested RNAPII for elongation. PKC or MAPK agonists promote the translocation of NF-κB to the nucleus and increase the synthesis of P-TEFb. LRAs as well as stress (apoptosis, UV light, transcriptional blockers) release P-TEFb from the 7SK snRNP, where P-TEFb is inactivated by HEXIM1. Tyr271 in HEXIM1 binds and occludes the ATP pocket in CDK9. Active CDK9 is phosphorylated on Thr186 in the T loop and Ser175. Note the similarity between TAR and the first stem loop in 7SK snRNA, which allows Tat and HEXIM1 to bind to both structures. Tat also competes with HEXIM1 for P-TEFb. Thus, when sufficient amounts of Tat are made, HIV transcription continues despite increased levels of 7SK snRNP.

level of detection. Yet viral DNA persisted, and upon treatment interruption, HIV returned with a vengeance (13–15). Thus, the concepts of proviral latency and HIV reservoir were born and found to be the intractable problem preventing HIV eradication and cure. In subsequent decades, more parameters of the reservoir were defined, i.e., which cells harbor latent proviruses, how many of these are replication competent, the location of the sites of residual replication, etc. Importantly, this knowledge of hidden reservoirs revealed why the immune system and ART cannot eliminate HIV. Only certain HLA haplotypes provide some protection against fulminant disease (16). As to the cure, only heroic measures, such as multiple bone marrow transplants with inhospitable cells, appear to have eliminated the reservoir in a single patient (17).

Basic mechanisms of HIV latency and reservoir

The latent HIV reservoir is estimated to comprise one in a million resting CD4⁺ T cells in optimally treated individuals (18), although the total number of these cells could be much higher (19) and is dependent on how soon ART is initiated after infection as well as the duration of the infection (20). In other words, the reservoir expands with HIV replication. In latently infected cells, most provi-

rus are found in actively transcribed genes (21). Several mechanisms could silence HIV gene expression and replication, including (a) deleterious mutations in the viral genome (some of which could be repaired by recombination if more than one virus integrates in the same cell; refs. 22–24), (b) transcriptional interference (25–28), (c) changes in chromatin structure (29, 30), (d) epigenetic silencing (such as increased DNA methylation), (e) the presence of negative TFs (31, 32), (f) the absence of positive TFs (33–35), and (g) problems with RNA processing and transport (36). These mechanisms could contribute individually and in different combinations to silencing HIV and rendering it invisible to the immune system. Transcriptional interference, heterochromatin and epigenetic alterations, and TF regulation are discussed in greater detail below.

Transcriptional interference. Replication-competent HIV can be silenced in activated as well as in resting cells. The most likely cause of this lack of HIV gene expression is transcriptional interference (Figure 2). In this scenario, HIV is its own worst enemy. Retroviruses have two identical LTRs, one for initiation of transcription (5' LTR), the other for termination of transcription (3' LTR) (37). In the case of HIV, the 5' LTR initiates transcription 20 times more often than the 3' LTR, which is occluded by the elongating RNAPII (Figure 2A and ref. 38). Transcriptional interference is partly due to the low-affinity binding between the DNA at the 3' LTR and SP1, TFIID, and the initiator element (25, 26). The elongating RNAPII from the 5' LTR displaces these TFs from DNA at the 3' LTR, then continues to the polyA site in the R region and terminates transcription. Notably, when the virus integrates in the same orientation as the host gene, the elongating RNAPII from the host gene terminates at the polyA site in the 5' LTR, curtailing HIV transcription (25–28). In this case, the 3' LTR is not occluded and initiates transcription, producing sterile transcripts that contain TAR (Figure 2B). When the virus integrates in the opposite orientation, RNAPII copies HIV antisense transcripts, ignoring both polyA sites in the HIV sense orientation and producing long hybrid mRNA species (Figure 2C). If the provirus integrates in introns of host genes, the HIV antisense transcripts are spliced out and rapidly degraded. Nevertheless, HIV antisense transcripts have been detected (39, 40), and they could provide an estimate of transcriptional interference in latently infected cells.

The study of transcriptional interference has been facilitated by the use of stably integrated reporter proviruses in Jurkat T cells, including the J-Lat cell lines (41). If transcriptional interference is robust and persists for the lifetime of the cell, HIV will not be reactivated. However, if the host gene is silenced, then HIV will reactivate. There are several mechanisms by which HIV transcrip-

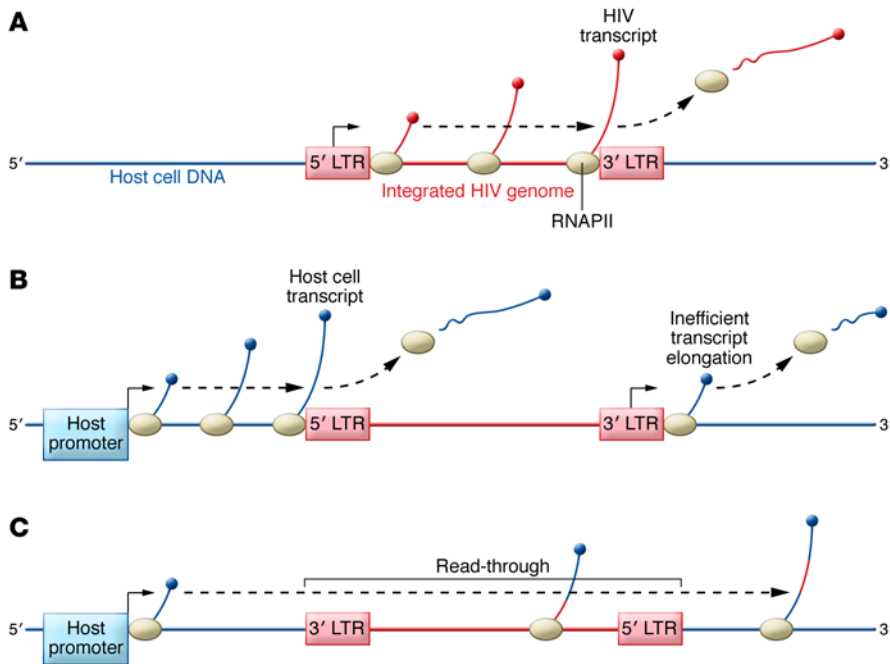


Figure 2. Transcriptional interference. (A) HIV utilizes transcriptional interference for its own replication. The 5' LTR initiates and elongates transcription. The 3' LTR is occluded because RNAPII displaces TFs from its DNA. Thus, RNAPII terminates past TAR near the polyA site in the 3' LTR, where CPA of HIV transcripts takes place. However, the same RNAPII transcribes HIV sequences and host cell genes. Thus, when HIV integrates in the sense orientation within a host cell gene, the transcribing RNAPII terminates in the 5' LTR (B). The 3' LTR now initiates transcription, which, in the absence of Tat, does not elongate efficiently. When HIV integrates in the antisense orientation within a host gene, RNAPII reads through without stopping (C), and HIV antisense transcripts are released. When integration occurs in introns, HIV RNAs are degraded rapidly. The strength of transcriptional interference depends on rates of transcription of host cell genes. High levels of NF- κ B, which bind to DNA tightly, counteract transcriptional interference. Blue and red circles represent 5' caps; rising blue and red lines represent mRNA; squiggles represent polyA tails of host cell and HIV mRNAs.

tion can be reactivated. In J-Lat 9.2 cells, HIV is integrated in the same orientation as the estrogen-dependent *PP5* gene and reactivates upon removal of the hormone (25). Cytokines and the activation state of the infected T cell also contribute to HIV reactivation via NF- κ B. Elevated levels of nuclear NF- κ B bind tightly to DNA, thereby impeding the progress of upstream RNAPII and initiating some transcription from the 5' LTR. Depending on the degree of transcriptional interference, these processes compete with each other, such that low levels of host gene transcription are overcome more rapidly by cellular activation and vice versa.

Heterochromatin and epigenetic alterations. Integrated proviruses in actively transcribed genes, gene deserts, and Alu-rich repeats can be silenced by heterochromatin. In this scenario, increased levels of DNA methylation, reduced overall cellular gene transcription, increased levels of chromatin silencing complexes, or insufficient positive TFs can potentially contribute to the recruitment of nucleosomes to the viral genome and the HIV LTR (42). Located at the U5 and R regions, nucleosomes inhibit the movement of RNAPII and thus block HIV replication (42). Nevertheless, this silencing can be overcome by activating cells and by increasing Tat synthesis. It is also possible that much of this silencing results from the absence of essential TFs, the levels of which are progressively diminished as activated lymphocytes transition to memory T cells (43–45).

Essential TFs in HIV transcription. In addition to NF- κ B and Tat, other TFs that are expressed at low levels in resting cells are essential for HIV gene expression, including positive transcription elongation factor b (P-TEFb). P-TEFb was identified by Tat-affinity chromatography (Figure 1 and refs. 33, 34, 46–48) and consists of a large cyclin, CycT1, and a cyclin-dependent kinase, CDK9 (49, 50). In the absence of Tat, RNAPII pauses after transcription of TAR. Tat recruits P-TEFb via CycT1 to TAR, allowing CDK9 to phosphorylate the C-terminal domain (CTD) of RNAPII. In addition to P-TEFb, Tat increases the activity of Cych/CDK7, which also

phosphorylates the RNAPII CTD (51). The RNAPII CTD contains 52 YSPSPS heptapeptide repeats in which all serines, the tyrosine, and the threonine can be phosphorylated by different kinases. These modifications, especially the phosphorylation of serines at position 2 (Ser2P) and 5 (Ser5P), play critical roles in the cotranscriptional processing of nascent mRNA species (52). Cych/CDK7 phosphorylates Ser5, which is required for capping of all viral transcripts (53). More importantly, P-TEFb phosphorylates Ser2, thereby promoting efficient elongation of viral transcription (50).

P-TEFb also modulates the activity of factors that impair transcript elongation. P-TEFb phosphorylates the transcription elongation factor SPT5, which is part of the 5,6-dichloro-1- β -D-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF) complex and the RD subunit of the negative elongation factor (NELF) complex (NELF-E); these two complexes arrest RNAPII at TAR (54–56). When SPT5 is phosphorylated, DSIF is converted to a positive elongation factor. When NELF-E is phosphorylated, NELF disengages from TAR so that the RNAPII can transition from initiation to elongation (57). Thus, it is not surprising that when levels of P-TEFb are low, HIV is not transcribed. Indeed, this is the case in resting lymphocytes in the periphery of infected individuals as well as in memory T cells (58). P-TEFb is also the coactivator of NF- κ B (59). Thus, both Tat and NF- κ B, the critical regulators of HIV transcription, depend on P-TEFb.

The regulation of P-TEFb itself is of some importance to HIV transcription. As mentioned above, levels of CycT1 are exceedingly low in resting cells. Regulation of CycT1 expression is post-transcriptional and depends on specific miRNAs that inhibit the translation of *CYCT1* mRNA by binding to its 3' UTR (45). Cell activation relieves this block, and levels of P-TEFb rise rapidly (45, 60); however, most P-TEFb is sequestered and inactivated in the 7SK small nuclear ribonucleoprotein (7SK snRNP) (Figure 1 and refs. 61, 62). Methylphosphate capping enzyme (MePCE) and La-related protein 7 (LARP7) bind and protect the 5' and 3' ends of

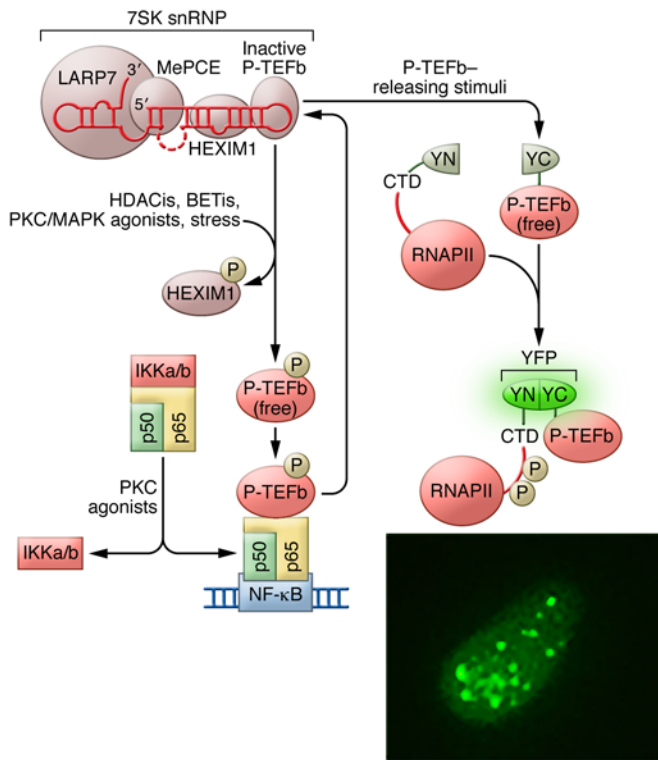


Figure 3. Combinatorial activation of TFs for HIV reactivation from latency. Mechanisms of action of PKC agonists and LRAs for increased HIV gene expression as well as the V-PAC assay are shown. PKC agonists activate NF-κB and increase levels of transcriptional CDKs, P-TEFb, CycK/CDK13, and CycL/CDK11. LRAs release P-TEFb from the 7SK snRNP. This rapid release is followed by the reassembly of the 7SK snRNP, which returns activated cells to their resting states. Tat interrupts this P-TEFb cycle (see Figure 1). V-PAC measures the release of P-TEFb from the 7SK snRNP in living cells; thus, it measures the strength and kinetics of LRA effects. In this BiFC, the two halves of YFP are linked to the CTD of RNAPII and P-TEFb. When P-TEFb is released and phosphorylates the CTD, cells turn green, especially in nuclear speckles, which are sites of active transcription (inset).

7SK small nuclear RNA 7SK (snRNA) from degradation. The 5' RNA stem loop of 7SK snRNP resembles TAR (57, 63) and is bound by cellular hexamethylene bis-acetamide-inducible proteins (HEXIM1/2), which undergo a conformational change upon RNA binding. RNA-associated HEXIMs then bind and inhibit P-TEFb in the 7SK snRNP. Notably, the rapid release of P-TEFb from the 7SK snRNP also increases the synthesis of HEXIM1, which reassembles the 7SK snRNP and sequesters free P-TEFb (64). The ratio of free and bound P-TEFb (the P-TEFb equilibrium) then determines the state of cell growth, proliferation, and differentiation (65). Although cell activation increases levels of P-TEFb sufficiently to support HIV transcription (58), this effect is augmented when P-TEFb is released rapidly from the 7SK snRNP. This release follows cell stress, which can come in the form of apoptosis, radiation, UV light, and compounds that block transcription (actinomycin D, DRB, or flavopiridol) as well as chemicals that cause changes in chromatin (histone deacetylase inhibitors [HDACis] and bromodomain and extraterminal [BET] bromodomain inhibitors [BETis]) and affect DNA methylation (5-azacytidine) (43, 61, 62). Importantly, these latency reversing agents (LRAs) only work when P-TEFb is abundant and present in the 7SK snRNP (43).

CycH/CDK7 and P-TEFb are not sufficient for optimal cotranscriptional processing of viral transcripts, which is necessary for HIV gene expression; additional transcriptional CDKs, including CycK/CDK13 and CycL/CDK11, are required (66–68). Both of these complexes increase levels of Ser2P at HIV coding and 3' end sequences (69, 70). Levels of CDK13 affect HIV splicing, and high and low levels of CDK13 increase and decrease splicing of HIV mRNA, respectively (66). Only the unspliced transcripts lead to the production of infectious viral particles. In contrast, CDK11 does not significantly affect HIV mRNA splicing (68); instead, it affects

the cleavage and polyadenylation (CPA) of all HIV viral transcripts. By increasing levels of Ser2P near the HIV 3' end, CDK11 helps to recruit CPA factors to RNAPII, which directs the appropriate CPA of all viral transcripts. Longer polyA tails lead to greater stability of these mRNA species and their improved export and translation in the cytoplasm. Importantly, CDK11 levels are also very low in resting cells and increase following T cell activation (67).

All of these RNAPII CTD kinases must work in harmony with each other, and their imbalances can have deleterious effects. For example, when LARP7 or 7SK snRNA are reduced in stem cells, levels of free P-TEFb rise and overall transcription is increased (71). However, RNAPII does not terminate transcription efficiently, allowing for read-through of megabases of DNA, which results in extremely long transcripts that contain many genes (72). Protein products of these transcripts are not expressed, as only proper RNA processing ensures the stability, transport, and translation of genes. Thus, levels of other necessary CTD kinases are not sufficiently high for appropriate mRNA splicing and CPA in these cells. This scenario may also occur if P-TEFb alone is increased by specific interventions. In addition, because the stalled RNAPII requires P-TEFb for elongation, levels of P-TEFb must rise before those for CDK11 or CDK13. Thus, at least in the initial phases of HIV reactivation, most viral RNA species could represent read-through transcripts from host cell genes.

To ensure proper transcription and cotranscriptional processing of HIV, CDK expression must be coordinately upregulated. T cell activation, which occurs via antigen stimulation of the T cell receptor and engagement of costimulatory receptors, accomplishes this task efficiently. CDK expression is required to overcome T cell quiescence and reactivate HIV in these previously resting cells (58); however, such activation also results in the release of deleterious

rious lymphokines and cytokines, resulting in cytokine storm with concomitant hypotension and shock. Thus, more gentle methods of T cell activation are required. Indeed, specific PKC agonists can also increase levels of CTD kinases. These compounds work synergistically with LRAs that release P-TEFb from the 7SK snRNP (Figure 3 and refs. 73, 74). Release of P-TEFb by LRAs induces the synthesis of HEXIM1, which reassembles the 7SK snRNP and inhibits free P-TEFb (64). Because lymphokine and cytokine genes require active P-TEFb for their expression, HDACis and BETis also ensure that there is no cytokine storm (75).

Diagnostic and therapeutic implications

Given these mechanistic details about HIV and eukaryotic transcription, it would be useful to translate them to clinical strategies for the cure of AIDS. First, there are diagnostic considerations. Stalled transcription at 5' or 3' LTRs and the release of short transcripts that contain TAR can serve as surrogate marker for measurements of the viral reservoir. For example, one can detect these short RNA species in the blood, in PBMCs, and in exosomes that are released from infected cells (12, 76, 77). In one study, TAR exosomes were even more abundant in elite controllers, infected individuals with undetectable levels of viral RNA species in their plasma (77). Short HIV transcripts also reveal the presence of latently infected circulating cells (12). Comparing transcript ratios in tissues and blood could yield an approximation of the viral reservoir in the infected host. Importantly, once those levels are established, it should be possible to monitor these ratios and assess the clinical effectiveness of any HIV anti-latency therapy (HALT).

We also need to engineer useful tools for developing effective HALT. Cell line models of latency, such as J-Lat cells, have traditionally been used to screen for potential compounds (78). Now, primary cells must also be examined. Activation and translocation of NF- κ B can be followed by reporter gene and immunofluorescent assays (IFAs) in living cells. Recently, we developed an assay for P-TEFb equilibrium in which we follow the recruitment of P-TEFb to the CTD of RNAPII. We call this assay visualization of P-TEFb activation in living cells (V-PAC) (Figure 3 and ref. 79). Using bimolecular fluorescence complementation (BiFC) (80), we follow the speed and extent of P-TEFb release from the 7SK snRNP in real time. The V-PAC assay can also be adapted for the screening of compounds that could be used for the "shock and kill" strategy of HIV elimination. Importantly, all compounds that score positive in the V-PAC assay also contribute to HIV reactivation in primary cells, including models of proviral latency or resting cells from optimally treated, HIV-infected individuals. As mentioned above, HEXIM1 is most sensitive to levels of free P-TEFb and is synthesized rapidly upon the disruption of the 7SK snRNP. Thus, V-PAC can be complemented by a reporter assay in which the promoter of *HEXIM1* is placed upstream of the firefly luciferase gene. Additionally, IFAs, Western blotting, and flow cytometry with antibodies that detect phosphorylation of S175 have been used to detect the activation of P-TEFb (81). Thus far, there are no assays to detect levels and activation of CDK11 or CDK13. Nevertheless, similar assays can be developed to follow their levels and activities in cells. Although specific targets have to be introduced into cells in all these reporter systems, these approaches are now possible with primary cells, including resting T cells. By using liposomes loaded with Vpx to eliminate the HIV

restriction factor SamHD1, reporter lentiviruses can be introduced into resting primary CD4⁺ lymphocytes (82).

Measurements of expression levels and activities of critical TFs will suggest combinations of compounds that could be used for HIV reactivation from latency (Figure 3). Because amounts of transcriptional CDKs are low in resting cells, they must be increased for HIV gene expression (58). PKC agonists, including phorbol esters, prostratin, bryostatin, and ingenol, can accomplish this task (74, 83–88). These compounds have appreciable toxicity, and most of them cannot be administered orally. An exception is ingenol B, which was modified from other ingenols obtained from the euphorbia plant. Oral administration of ingenol B to optimally treated, infected rhesus macaques activated cells and increased levels of SIV in the periphery (86, 87, 89, 90).

Combinations of PKC or MAPK agonists and LRAs will be needed to mitigate toxic effects and lower effective doses (73, 74, 91). Such LRAs include HDACis, BETis, and 5' azacytidine, among others. Indeed, vorinostat, romidepsin, panobinostat, and JQ1 act synergistically with PKC agonists to reactivate HIV transcription (43, 91–95). Whereas PKC agonists activate NF- κ B and increase overall levels of P-TEFb and CycL/CDK11, LRAs primarily affect the P-TEFb equilibrium. By releasing free P-TEFb, LRAs also induce the synthesis of HEXIM1, which results in the rapid reassembly of the 7SK snRNP. Thus, LRAs limit the concomitant production of cytokines induced by PKC agonists. Most importantly, lower concentrations of PKC agonists and LRAs can be used in combination to achieve optimal HIV reactivation (73, 91). Time of administration might also be an important therapeutic component, as PKC agonists and LRAs act through different mechanisms. By first increasing cellular levels of P-TEFb, PKC agonists could facilitate the ability of LRAs to release P-TEFb from 7SK snRNP. This one-two punch should be explored further in primary cells, patient cells *ex vivo*, and infected rhesus macaques. It is possible that sustained, low levels of ART and HALT will diminish the reservoir of HIV and lead to a functional cure of the infection, helping to convert most infected individuals to elite controllers.

Summary

Much has been learned about the replicative cycle of HIV. A critical component is its ability to establish proviral latency in infected cells, which was revealed by studies in transformed cell lines, primary cells, and infected individuals. Containing and/or removing this viral reservoir is a daunting problem for eukaryotic biology and clinical science. It is hoped that sustained reactivation of HIV in the presence of optimal ART and adequate immune responses will prevent *de novo* infections and remove HIV, albeit slowly, from the infected host.

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