Jones *et al. Epigenetics & Chromatin* (2025) 18:9
https://doi.org/10.1186/s13072-025-00573-x

Epigenetics & Chromatin

REVIEW Open Access

Impact of chromatin on HIV-1 latency: a multi-dimensional perspective

Joanna E. Jones[1,2], Chelsea E. Gunderson[1,2], Brian Wigdahl[1,2,3] and Michael R. Nonnemacher[1,2,3*]

Abstract

Human immunodeficiency virus type 1 (HIV-1) is a retrovirus that infects multiple immune cell types and integrates into host cell DNA termed provirus. Under antiretroviral control, provirus in cells is able to evade targeting by both host immune surveillance and antiretroviral drug regimens. Additionally, the provirus remains integrated for the life of the cell, and clonal expansion establishes a persistent reservoir. As host cells become quiescent following the acute stage of infection, the provirus also enters a latent state characterized by low levels of transcription and virion production. Proviral latency may last years or even decades, but stimuli such as immune activation, accumulation of viral proteins, and certain medications can trigger reactivation of proviral gene expression. Left untreated, this can lead to virema, development of pathogenic out comes, and even death as the immune system becomes weakened and dysregulated. Over the last few decades, the role of chromatin in both HIV-1 latency and reactivation has been characterized in-depth, and a number of host factors have been identified as key players in modifying the local (2D) chromatin environment of the provirus. Here, the impact of the 2D chromatin environment and its related factors are reviewed. Enzymes that catalyze the addition or removal of covalent groups from histone proteins, such as histone deacetylase complexes (HDACs) and methyltransferases (HMTs) are of particular interest, as they both alter the affinity of histones for proviral DNA and function to recruit other proteins that contribute to chromatin remodeling and gene expression from the provirus. More recently, advances in next-generation sequencing and imaging technology has enabled the study of how the higher-order (3D) chromatin environment relates to proviral latency, including the impacts of integration site and cell type. All together, these multi-dimensional factors regulate latency by influencing the degree of accessibility to the proviral DNA by transcription machinery. Finally, additional implications for therapeutics and functional studies are proposed and discussed.

Keywords HIV-1, HIV-1 cure, Latency, 3D chromatin, Chromatin remodeling

Brian Wigdahl and Michael Nonnemacher as co-senior authors.

*Correspondence:
Michael R. Nonnemacher
mrn25@drexel.edu
[1]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19102, USA
[2]Center for Molecular Virology and Gene Therapy, Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, Philadelphia, PA 19102, USA
[3]Sidney Kimmel Comprehensive Cancer Center, Thomas Jefferson University, Philadelphia, PA 19107, USA

Introduction

According to the World Health Organization (WHO), an estimated 39 million people worldwide were living with human immunodeficiency virus type 1 (HIV-1) in 2022. In the same year, approximately 630,000 deaths were attributed to HIV-1/acquired immunodeficiency syndrome (AIDS)-related illnesses. Of these deaths, 84,000 were children under the age of fifteen [1]. However, there has been a 70% reduction in the number of global HIV-1/AIDS-related deaths since 2003 due to the increased accessibility of antiretroviral therapy (ART) and testing [2].

Despite these improvements, people with HIV-1 (PWH) are at risk of developing non-AIDS HIV-1-associated pathologies, even with strict adherence to ART. It has recently been found that PWH are at greater risk of developing other diseases and health complications, including neurocognitive disorders, when compared with people without HIV-1 (PWoH) [3, 4]. In addition, cessation of ART results in viremia and the development of HIV-1-associated pathology within just a few weeks [5]. The inability of ART to completely abrogate these complications lies in the fact that current ART drugs are unable to target a critical phase of the HIV-1 replication cycle: the integrated provirus.

HIV-1 entry is facilitated by CD4 as the primary receptor, and CCR5 or CXCR4 as the coreceptor. Binding the coreceptor(s) triggers conformational changes in the viral surface proteins gp120 and gp41, which enables fusion with the cell membrane and deposition of the viral core into the host cell (reviewed in [6–9]). As the viral core is trafficked from the plasma membrane to the nucleus, the RNA genome of HIV-1 is reverse-transcribed to a linear, double-stranded DNA genome that is released into the nucleus after the capsid uncoats. Together with cellular factors such as Lens Epithelium-derived Growth Factor (LEDGF/p75), the viral enzyme integrase catalyzes the integration of the HIV-1 DNA genome into the host cell's own genome [10, 11]. This integrated form of HIV-1 is known as the provirus and remains within the host genome for the life of the cell. In addition, clonal expansion maintains the "reservoir": a chronically-infected population comprised of diverse cell types, including CD4 + T cells, macrophages and microglia, astrocytes, and others [12–17].

Activated, effector CD4 + T cells are generally considered to be the major source of viral gene products and new particles, as resting cells are less permissive to high levels of viral replication [18, 19]. Over the course of infection, however, a majority of these effector cells die (reviewed in [7]). In the absence of further stimulus by an antigen, the surviving T cells differentiate to a memory phenotype, where they remain metabolically quiescent yet poised for activation upon re-encountering the target antigen. This shift is driven in large part by epigenetic changes, particularly differential accessibility to certain genes by transcriptional machinery (for more detailed reviews on this topic, see references [20–22]).

Indeed, the average human cell contains approximately 2 m of genomic DNA, which must be compacted and condensed to fit into the 10-micron nucleus. This is accomplished through the assembly of chromosomes, which themselves are comprised of chromatin. The basic, "2D" subunit of chromatin is the nucleosome, an 11-nanometer nucleoprotein complex comprised of 145–147 base pairs of DNA – approximately one turn's worth – a core histone octamer, and the linker histone H1. The octamer core is assembled from two copies of each subunit: histones H2A, H2B, H3, and H4 [23, 24]. Histone proteins are rich in lysine and arginine residues, bestowing the core with an overall positive charge that is attracted to the negatively-charged DNA [23]. Each histone subunit also bears an N-terminal tail that protrudes from the core, and this tail can receive covalent modifications such as acetylation, methylation, phosphorylation, ubiquitinylation, and others; for example, histone H3 can become acetylated at its lysine-27 (H3K27ac). These modifications regulate the accessibility of the nucleosome-bound region by altering the charge of the histone core, and therefore its affinity for its associated DNA (reviewed in [25, 26]).

On a slightly larger scale, the position of the nucleosome itself may also be repressive or permissive to transcription, as it may overlie binding sites for transcription factors (TFs) or even RNA polymerase II (RNAPII) itself (for more detailed reviews on this topic, see references [26, 27]). Chromosomes also interact with one another and the environment of the nucleus, forming dynamic, higher-order, "3D" structures [28, 29]. Together, these "2D" and "3D" chromatin environments regulate the accessibility to DNA by transcriptional machinery, thus enabling epigenetic control of gene expression.

As the host cell transitions to a quiescent state, so too does the provirus enter a period of latency (reviewed in [30]), characterized by low levels of gene expression and virion production. The latent infection may last years or even decades, eventually progressing to AIDS in the absence of therapeutic intervention as the virus expands and infected cells die [31]. Accumulation of the viral trans-activator of transcription (Tat), T cell receptor engagement, and cytokine exposure can also trigger high levels of proviral gene expression as TFs become more plentiful and RNAPII processivity increases (reviewed in [16, 32, 33]). Reactivation of the provirus can lead to viremia, mass CD4 + T cell death, immunodeficiency and development of severe disease, and even death [5].

Unsurprisingly, because HIV-1 integrates into the host genome, the transitions into and out of latency are driven

by many of the same epigenetic mechanisms that regulate gene expression in the host cell. Multiple epigenetic factors have been implicated in proviral latency and reactivation, particularly the "2D" chromatin landscape, comprising elements such as nucleosomes and their modifications. More recently, the "3D" structure of chromatin, including higher-order chromosome architecture and nuclear organization, has also been identified as a key factor in both integration and proviral latency. Here, we review much of the literature surrounding the role of both "2D" and "3D" chromatin environments in maintaining the axis of proviral latency and reactivation.

3D genome architecture in virus latency and reactivation

The nuclear genomic architecture is a highly organized and dynamic structural network that allows for inter- and intra-chromosomal contacts and enhancer-promoter interactions that modulate gene expression (comprehensively reviewed in [34–36]). For the purposes of this review, we will briefly describe the key elements of the genomic hierarchy to contextualize the experimental results described here. At the highest order, chromosomes are located within specific chromosome territories (Fig. 1A). These territories are compartmentalized into transcriptionally active euchromatin or transcriptionally silent heterochromatin domains, termed compartments A and B, respectively (Fig. 1B). Currently, there are up to 6 subcompartments recognized within the A and B compartments (A1 – A2 and B1 – B4), classified by relative levels of transcriptional activation, spatial positioning within the nucleus (i.e. proximity to the repressive lamina-associated domains [LADs] or nucleolus-associated domains [NADs]), and association with certain regulatory nuclear bodies (e.g. active nuclear speckles or repressive polycomb bodies). Within these compartments are topologically associated domains (TADs), which are functional, self-associating stretches of the genome with boundaries that are delineated by the architectural proteins CCCTC-binding factor (CTCF) and cohesin (Fig. 1F). TADs may contain varying orders of sub-TADs down to the smallest scale of chromatin loops (Fig. 1G), which facilitate the actions of *cis*-regulatory

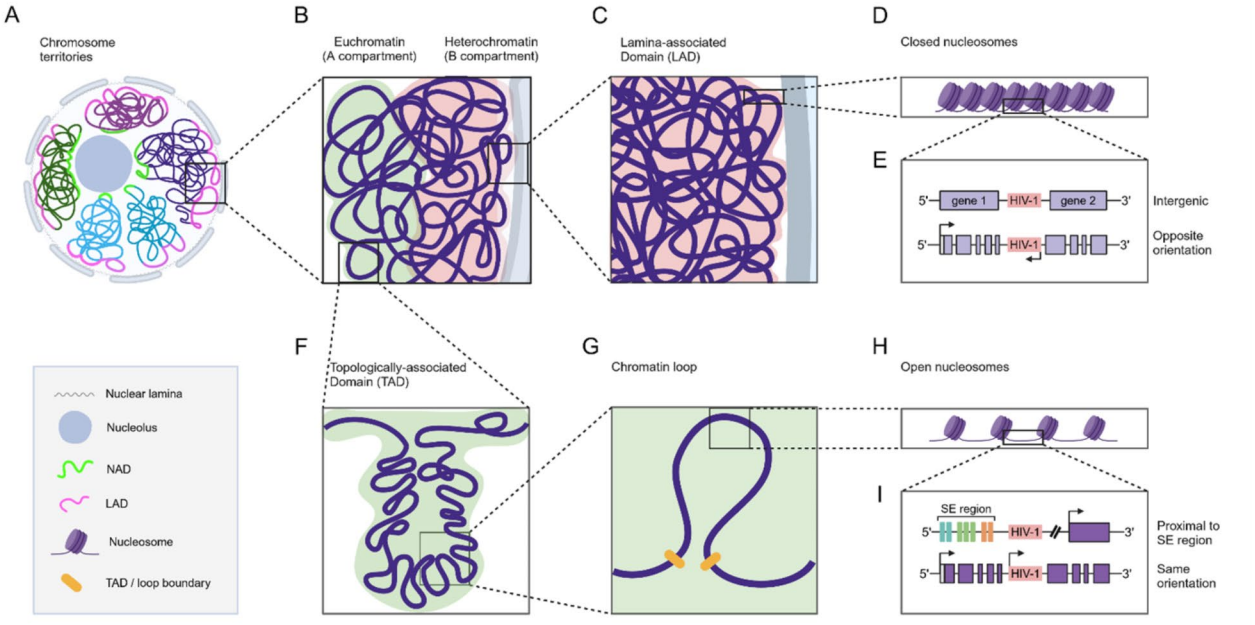

Fig. 1 3D genomic architecture and chromatin state support proviral transcription or latency. (**A**–**D**) Chromosomes are dynamic structures that occupy discrete territories within the nucleus **(A)**. The organization and spatial positioning of chromosomes within the nucleus allows inter- and intra-chromosomal contacts to occur. Chromosome territories are compartmentalized into transcriptionally active euchromatin or transcriptionally silent heterochromatin domains, termed compartments A and B, respectively **(B)**. Heterochromatin regions are closely associated with the nuclear lamina (LADs, shown in panel **C)** and the nucleolus (NADs, shown in panel A in bright green), and consist of tightly closed nucleosomes **(D)**. **(E**, **I)** HIV-1 integrates into both euchromatin and heterochromatin with varying affinity, and the orientation and location of the integration site further impacts the capacity for proviral transcription. Integration sites of intact proviruses from elite controllers and ART-suppressed PWH are typically found within intergenic regions or within introns and positioned opposite to the orientation of the host gene **(E)**. **(F–H)** Self-associating regions of chromatin are brought into closer proximity through the formation of TADs. TADs are the functional unit of chromatin, and as such contain varying orders of sub-TADs down to chromatin loops **(F**, **G)**. Euchromatin regions consist of open nucleosomes, allowing for transcription (**H**). In contrast to (**E**), integration sites ineuchromatin regions are typically found in close proximity to super enhancer regions or within genes and positioned in the same orientation of the host gene **(I)**

elements within this regional unit, as well as nucleosomes (Fig. 1D and H).

While the 3D chromatin architecture of the nucleus is known to play an important role in HIV-1 integration, recent studies have investigated how the chromatin landscape contributes to latency maintenance and reactivation of the integrated provirus. This area of study has expanded in recent years due to the utilization of new technologies that allow for investigation of chromatin conformation (e.g. chromatin conformation capture and Hi-C) and accessibility (e.g. ATAC-seq) [37, 38]. These methods and their derivatives are frequently used in combination with integration site analysis, RNA-seq, ChIP-seq, and other sequencing methods to provide a comprehensive representation of the chromatin state surrounding HIV-1 integration sites, and how this impacts proviral transcription. In this section, we will review the features of the 3D genome that have been shown to support proviral integration, the establishment of the latent reservoir and its subpopulations, and factors that influence reactivation.

Integration and productive infection

HIV-1 integration into the host cell genome allows for both proviral transcription and viral replication, and the establishment of the latent reservoir. Several features of the 3D genomic environment influence both where HIV-1 integrates and efficiency of proviral transcription. Here, we will review the studies that have focused on HIV-1 integration in the context of productive infection.

Multiple studies have shown that HIV-1 preferentially integrates into regions of permissive chromatin, near areas that undergo frequent interactions and have greater accessibility [39–41]; more specifically, the transcriptionally active A compartment (Fig. 1F-I) [39, 42–44]. This has been demonstrated through in vitro infection of T cell (e.g. Jurkat [42–44] and Sup-T1 [40]) and myeloid cell lines (e.g. U-937 [42] and C20 [41]), as well as primary cells including CD4 + T cells [41–43], monocyte-derived macrophages (MDMs) [41, 42], and iPSC-derived microglia [41].

Within the structure of the A compartment, integration has been linked to the accumulation of viral replication complexes near nuclear speckles – that is, hubs of gene expression and RNA processing – and their associated domains in cell lines (Jurkat, TZM-bl, and HEK293T), and primary MDMs and CD4 + T cells [45]. Furthermore, it has been shown in CD4 + T cells that integration is more likely to occur in the A1 subcompartment, which is enriched in super enhancer clusters associated with integration hotspots and recurrent integration targets [43]. This is in agreement with the observation that the majority of genes targeted for recurrent integration are localized outside of LADs [42]. At the functional level of chromatin, super enhancers are positively correlated with integration sites at TAD boundaries relative to TAD midpoints in both microglia and CD4 + T cells [41]. Overall, these findings demonstrate a similar pattern across cell types for HIV-1 integration into euchromatin, with preference for the A1 subcompartment and super enhancer regions localized at TAD boundaries (Fig. 1F).

Indeed, it is well known that HIV-1 preferentially integrates into introns of actively transcribed genes [46–49], which is in agreement with the observed preference for integration into euchromatin. Many studies have identified regions of the human genome that HIV-1 targets for integration, termed hotspots, along with specific genes that are recurrently targeted across PWH. Examples of such recurrently targeted genes include *BACH2* [43, 50–54], *MKL2* [43, 50, 51, 54], and *STAT5B* [43, 50, 52, 54], along with several other genes associated with cell cycle regulation and cancer [43, 50]. Hotspots have also been linked to regions enriched in *Alu* repeats [46, 54], with high rates of integration observed within chromosomes 11 [46], 16 [55], 17 [43, 55], and 19 [43, 55]. These recurrent integration targets and hotspots have been identified in cell lines (Jurkat [43, 55] and Sup-T1 [46, 55]), primary CD4 + T cells infected with HIV-1 reporter viruses in vitro [43], and CD4 + T cells isolated from PWH on ART [50–52, 54].

However, some differences in HIV-1 integration have been reported between cell types. Comparisons of integration hotspots in primary MDMs and CD4 + T cells have shown that integration sites in MDMs occur within more discrete gene clusters relative to the diverse range targeted in CD4 + T cells [56]. Further comparisons to CD4 + T cells have shown that MDMs undergo higher rates of integration within intergenic regions [57]. In addition to these differences, a recent study found differential integration site selection in archived brain tissues when compared with CD4 + T cells in the periphery, as lower rates of recurrent integration and higher rates of intergenic integration were observed in microglia [58]. These findings may be linked to altered expression of integration-mediators LEDGF and Cleavage and Polyadenylation Specific Factor 6 (CPSF6) in MDMs and microglia [56–58], demonstrating how cell type differences intersect with the 3D genome architecture to influence integration and subsequently shape important subpopulations of the latent reservoir.

Latency

The latent HIV-1 reservoir exists in different forms within the context of the 3D chromatin environment. In the initial stages of infection, HIV-1 integrates into both euchromatin and heterochromatin with variable affinity [39, 42–44, 52, 55, 59]. These integrated proviruses undergo host selective pressure over time, resulting in

latent subpopulations that have different capacities for reactivation [60]. These latent cell populations arise from integration into euchromatic regions that later become heterochromatic through changes in the cell phenotype (i.e. activated vs. quiescent), initial integration into heterochromatin, or the orientation of the provirus relative to the host gene's reading frame. Here, we will explore studies that have offered insight into the chromatin states that support HIV-1 latency and reactivation.

In contrast to proviruses that undergo active transcription post-integration, latent proviruses generally reside in repressive heterochromatin (Fig. 2A-F). Comparisons of active and latent infection across in vitro models (Sup-T1 [40] and C20 [41]) and primary tissues (including CD4 + T cells, MDMs, and microglia [41]) have demonstrated differential chromatin accessibility across the genome. Studies utilizing primary CD4 + T cells infected with an HIV-1 reporter virus in vitro [39] or ex vivo samples from ART-treated PWH [52, 59] have shown that the integration sites of latent proviruses are typically found in non-coding regions (Fig. 1E) located further away from frequently interacting regions and TADs [59], and are often localized within LADs (Fig. 1C) [39, 52, 59]. For instances in which intact latent proviruses have been found in genic regions, these were more likely to be integrated in the opposite orientation of the host gene (Fig. 1E) [52] or within genes with relatively low levels of basal transcription [39]. Alternatively, intact latent proviruses in some ART-treated PWH have been found in close proximity to transcription start sites (TSSs) and regions of accessible chromatin, but likely remain silent due to repression by host transcriptional interference [52]. One study utilizing Jurkat T cells infected with a reporter virus in vitro found that latent proviruses were distributed throughout the genome but were generally integrated distal to enhancer regions [61]. Comparisons of intact proviruses in CD4 + T cells from individuals on long-term ART (LT-ART) and elite controllers (ECs) have shown that integration occurs in "disfavored" chromatin regions, which includes centromeric, micro-satellite, and non-coding DNA, and in locations distal to TSSs and accessible chromatin [62, 63]. Integration sites were also mostly found within the B compartment with similar distributions within the B subcompartments (Fig. 1B-E) [62, 63].

Recent studies focused on elucidating how the chromatin landscape shapes the latent reservoir and contributes to proviral reactivation have shown that latent proviruses reside in heterogeneously repressive chromatin environments. Studies comparing ECs and PWH on LT-ART have provided insight regarding host selective pressure and clonal expansion that results in latent proviruses contained within heterochromatin [62–64]. Longitudinal analysis of CD4 + T cells isolated from PWH on ART demonstrated a progressive increase in the numbers of proviral clones integrated in heterochromatin, likely reflecting the selective pressure that eliminates intact proviruses in transcriptionally active chromatin over time, resulting in subpopulations of intact proviruses that display characteristics of deep latency [63].

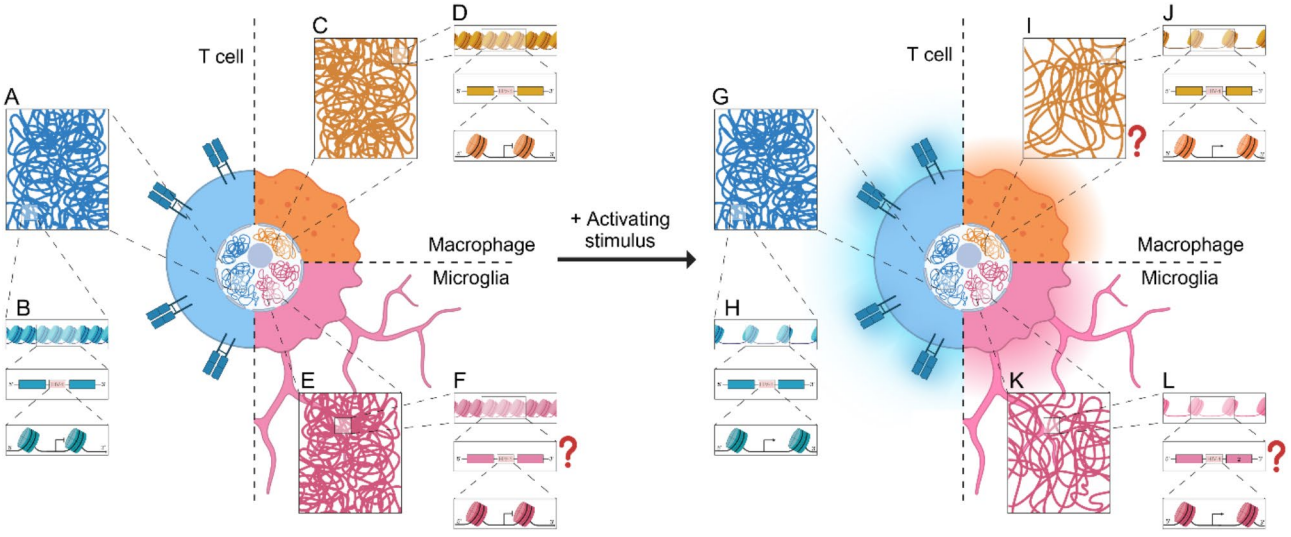

Fig. 2 Cell type influences 2D and 3D chromatin at the integration site under different activation conditions. **(A–F)** During latency, proviruses are found primarily in heterochromatin across different cell types found in the reservoir. In this state, 3D chromatin is highly compact **(A**, **C**, **E)** and nucleosomes are close together on the provirus and nearby genes **(B**, **D**, **F). (G–L)** When latency is reversed through an activating stimulus, different cell types in the reservoir undergo different levels of remodeling. T cells do not experience global chromatin opening **(G)**, but nucleosomes are repositioned to a more transcriptionally-favorable state **(H)**. While it is unknown whether macrophages undergo extensive chromatin remodeling **(I)**, nucleosomes are repositioned to increase access to HIV-1 DNA by transcriptional machinery **(J)**. When latently-infected microglia are reactivated, by contrast, chromatin is remodeled extensively **(K)**. In microglia, it is unknown but suspected that nucleosomes are repositioned to enable transcription **(L)**

More specifically, studies of ECs and PWH on LT-ART have demonstrated high rates of recurrent integration of intact proviruses within KRAB-ZNF genes located within a particularly heterochromatic region on chromosome 19 [62–64]. This is in agreement with earlier findings in CD4 + T cells infected in vitro, which showed that proviruses incapable of reactivation integrate into significantly fewer genic regions relative to reactivated proviruses [39].

Subpopulations of the latent reservoir that are refractory to reactivation exist in heterochromatin as described above and are enriched for repressive histone modifications, particularly H3K27me3 or H3K9me3 [65]. However, it has been demonstrated in CD4 + T cells infected with a reporter virus in vitro that a subset of latent proviruses with the potential for reactivation are contained within chromatin enriched in factors associated with enhancers, namely H3K4me1, H3K27ac, and RNAPII [65]. These proviruses are considered primed for reactivation, as depletion of enhancer-specific H3K27ac upon T cell activation allows for proviral transcription [65]. Like productive proviruses, the integration sites of proviruses with reactivation potential are enriched in transcription factor binding sites and have been found within genes with greater levels of basal expression relative to genes containing non-reactivated proviruses [39, 66]. Taken together, these studies demonstrate how proviral integration throughout the 3D genome and host selection pressures lead to the establishment of a complex and heterogenous latent reservoir.

Chromatin remodeling during reactivation

A limited number of recent studies have focused on HIV-1 reactivation and chromatin remodeling in the context of the 3D nuclear environment. One such investigation utilizing Hi-C and ATAC-seq in J-Lat cells demonstrated that while proviral activation resulted in increased transcription and chromatin accessibility immediately downstream of the 3' LTR, minimal changes occurred in chromatin structure globally and around the HIV-1 integration site upon proviral reactivation [67]. Similarly, another study that performed Hi-C and 4 C-seq on Jurkat clones infected with HIV-1 reporter viruses showed that the presence of the provirus resulted in increased chromatin contacts within 100–300 kb of the integration site without significantly affecting chromatin structure globally (compare Fig. 2A and G) [44]. These results therefore lead us to speculate that the majority of provirus-induced modifications to chromatin structure occur at the 2D level (compare Fig. 2B and H), reviewed in detail below.

Interestingly, recent evidence from primary microglia has shown slightly different results. HIV-1 reactivation in microglia is important with respect to HIV-1-associated neurocognitive disorder (HAND), pathogenesis in the CNS, and the overall reactivation of HIV-1 from latent reservoirs [68, 69]. Within HAND, there is a gradation of neurological disorders that range from asymptomatic to dementia with or without encephalitis (HIVE). While HIVE is uncommon in the ART era, a recent study examined the effects of HIV-1-induced encephalitis in primary microglia and in the HMC3 microglia cell line, showing that HIVE resulted in widespread remodeling of chromosomal conformations, with compartment alterations occurring over large portions of chromatin [58]. While the authors found that the remodeling may be due in part to IFN upregulation, as Hi-C showed genome-wide remodeling of chromatin loops and TADs occurred in HIVE microglia and IFN-treated HMC3, non-encephalitic HIV-1-infected primary microglia likewise displayed widespread alterations in chromatin conformation (Fig. 2K) [58]. While few studies have investigated these effects in peripheral macrophages, it is possible that they undergo similar levels of drastic chromosomal remodeling upon latency reversal (Fig. 2I).

Given the differential findings in these studies, it raises the question of the impact of cell type on chromatin remodeling during reactivation from latency. As discussed above, other studies have identified differences among cell types which impact proviral integration [56–58]. Together, these studies show that there may be differences between the lymphoid and myeloid lineages that contribute to the overall challenge of fully eliminating the latent reservoir. Further research into the dynamics underlying reactivation at the 3D level will be required to fully evaluate the differences imposed by cell type.

Conclusion & summary

Recent advances in sequencing methods have enabled the investigation of genomic architecture at different points of HIV-1 infection in cell lines and primary cells in vitro, as well as in ex vivo samples from PWH. The studies reviewed in this section represent our current understanding of how the host 3D genomic environment in part shapes the HIV-1 latent reservoir. Together, these works demonstrate that the HIV-1 provirus preferentially integrates into transcriptionally active euchromatin but also integrates into heterochromatic regions. This capacity for integration in combination with host selective pressures results in a heterogenous latent reservoir, in which subpopulations of proviruses may be locked in a state of deep latency while other subpopulations may become reactivated under the right conditions. These studies have provided an important perspective into the 3D factors that contribute to HIV-1 latency and reactivation, emphasizing the need for continued research in this area. It will be especially important to better understand how the 3D chromatin environment supports

subpopulations of the latent reservoir across cell types in the development of curative treatments.

The 2D chromatin landscape in latency and reactivation

Just as the host cell genome is comprised of individual genes, the 3D chromatin environment of the nucleus is comprised of "2D" elements. Of particular interest are nucleosomes and their covalent modifications, which regulate accessibility to local gene regions by promoting the formation of open or closed chromatin. The HIV-1 genome is one such local region, spanning approximately 9,200 to 9,600 nucleotides in length (reviewed in [70]). Because HIV-1 integrates into the host chromosome as a provirus, it is not surprising that the same factors that regulate cellular gene expression also maintain the axis of latency and reactivation.

The coding regions of the HIV-1 genome are flanked by identical 5' and 3' long terminal repeats (LTRs), which regulate gene expression and enable synthesis of mature mRNAs from viral genes (reviewed in [70]). The LTR contains 3 regions of interest (5' to 3'): U3, R, and U5 (reviewed in [16, 71]). Despite having identical sequences, however, each LTR has a different function. The 5' LTR acts as the viral promoter and contains binding sites for critical TFs, the TSS and trans-activation response element (TAR) sequence [72, 73]. By contrast, the 3' LTR terminates transcription and encodes the polyadenylation signal [74]. In addition, the provirus encodes Tat, which accumulates in the nucleus and facilitates proviral gene expression through several mechanisms (reviewed in [75]), including binding the TAR and recruiting factors that promote transcriptional processivity, chromatin-modifying factors, and other 2D epigenetic regulators described below.

The role of the 2D chromatin environment in HIV-1 latency and reactivation has been investigated in-depth over the last several decades. In this section, we summarize much of the research characterizing the enzymes, small molecules, and other factors that have been found to play a role in regulating the latent state of the HIV-1 provirus.

Nucleosome assembly and positioning on the HIV-1 provirus

The positions of nucleosomes on the proviral 5' LTR have been well-characterized in several cell lines, as well as primary models of HIV-1 latency (Fig. 3). In early studies, DNase I hypersensitive sites (HS or DHS) were identified at the 5' and 3' LTR. Of note are HS2, HS3 (collectively known as DHS1), and HS4 (also known as DHS2) (Fig. 3B, see T cell [immortalized]). Additional sites have also been identified on the 5' LTR, called HS1, and within gene coding regions in the monocytic cell line U1 (Fig. 3B, see Macrophage [immortalized]). Interestingly, the hypersensitive sites of the 3' LTR do not correspond to where they are found on the 5' LTR [76]. At the 5' LTR, the regions separating the hypersensitive sites are occupied by conserved nucleosomes, termed nuc-0 and nuc-1 (Fig. 3B); nuc-1 is of particular interest, as it overlies the R-U5 region just downstream of the TSS. Nuc-2, -3, and −4 have also been identified [77], but they are less well-characterized and may not be as highly conserved (Fig. 3B, compare T cell [immortalized] and T cell [primary]) [78, 79].

After the viral particle uncoats in the nucleus, histones are deposited onto the linear, unintegrated HIV-1 DNA; that is, integration into the host genome follows nucleosome assembly [80]. This timeline may be conserved among retroviruses, as inhibition of reverse transcriptase and nuclear import, but not integrase activity, prevented histones H2B and H3 loading in both HIV-1 and murine leukemia virus [80, 81]. In a Jurkat model of HIV-1 infection, nuc-0 and nuc-2 were only found at their known positions after integration; on unintegrated HIV-1 DNA, they were instead upstream of these sites (compare Fig. 3A and B). In primary cells, however, nuc-0 sliding was not observed, and nuc-2 was evicted after integration (Fig. 3B, see T cell [primary]) [80]. These differences in nucleosome positioning may suggest that host factors at the integration site influence the epigenetic landscape of the provirus. Recently, an additional 5' LTR nucleosome was identified on unintegrated HIV-1 DNA at DHS1, termed nucDHS (Fig. 3A). While its role has not been fully characterized, nucDHS may inhibit pre-integration transcription by establishing a repressive chromatin environment and reducing RNAPII recruitment. In support of this hypothesis, treating Jurkat T cells with histone deacetylase inhibitors early in infection stimulated HIV-1 transcription despite low levels of integrated HIV-1 at this stage. Upon integration, however, nucDHS was quickly evicted, exposing HS2 and HS3 in the provirus [80].

To date, the chromatin environment of the downstream coding regions of the provirus and 3' LTR is poorly characterized. Recently, a nucleosome positioned in the U3 region of the 3' LTR was identified, and may serve to regulate the expression of the HIV-1 antisense transcript *Ast* [82]. A "poorly-positioned" nucleosome at DHS1 has also been observed in J-Lat 11.1 cells (Fig. 3B, see J-Lat 11.1), indicated by incomplete protection from MNase digestion, but this has not been found in other systems to date [78, 79]. Whether this nucleosome is a result of a failure to evict nucDHS remains to be investigated. Additionally, ATAC-seq data has shown low levels of accessibility between the 5' and 3' LTRs in resting cells [83], indicating that other structures may be present.

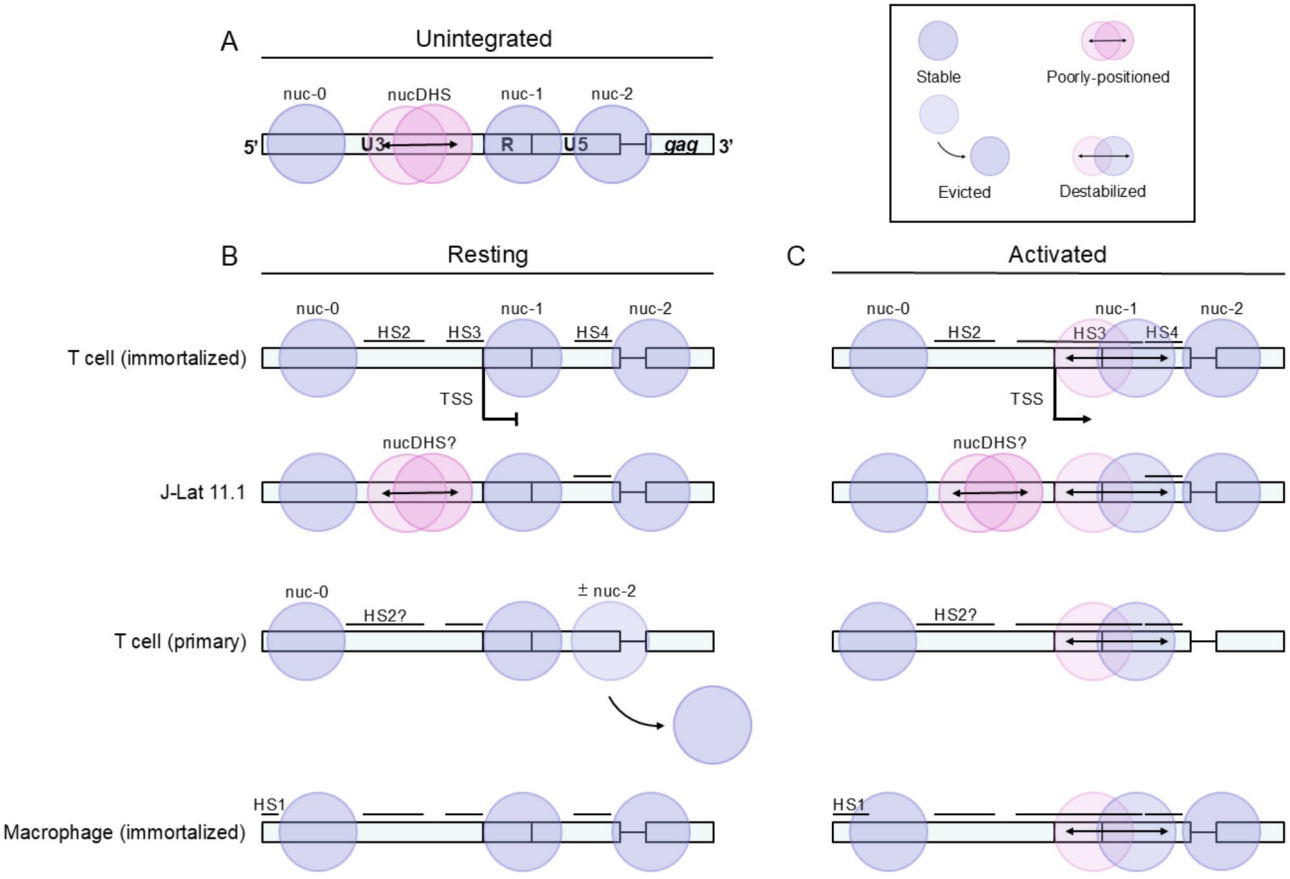

Fig. 3 The 2D chromatin environment of the 5′LTR is altered by cell type and activation state. **(A)** Nucleosomes on unintegrated HIV-1 DNA are positioned slightly upstream of where they are found on the integrated provirus. Additionally, nucDHS occupies the hypersensitive site between nuc-0 and nuc-1, preventing transcription factors from binding and stimulating gene expression prior to integration. **(B)** After integration and during latency in most immortalized cells, nucDHS is evicted and nuc-0, -1, and −2 occupy their canonical positions as HS2, HS3, and HS4 are formed between them. HS1 is also found in immortalized macrophages slightly upstream of nuc-0. In J-Lat 11.1 cells, HS2 and HS3 are slightly protected from digestion by MNase, indicating a poorly-positioned nucleosome in this region. In some primary T cells, nuc-0 remains slightly upstream of where it is found in other models, possibly leading to HS2 being larger than what is seen in immortalized cells. Additionally, nuc-2 is evicted. Across cell types, nuc-1 consistently occupies the R region, blocking the transcriptional start site (TSS) and TAR to block processive transcription. **(C)** When infected cells are activated with the appropriate stimulus, nuc-1 is quickly destabilized and repositioned to increase accessibility to the TSS (indicated by an arrow). In accordance with nuc-1 remodeling, HS3 also becomes larger and more sensitive to digestion with DNase I. In immortalized macrophages, HS1 undergoes similar alterations

Changes in the 5' LTR hypersensitive regions and nucleosomes following stimulation have been well-established. Indeed, the R-U5 region in U1 and ACH2 cells became more sensitive to DNase I and MNase digestion following treatment with PMA. In particular, HS3 became larger after stimulation with phorbol esters or histone deacetylase inhibitors; in U1 cells, HS1 also becamse more sensitive to digestion (Fig. 3C) [76, 77, 84]. Likewise, ATAC-seq data indicates increased accessibility at the R-U5 region in activated cells [83]. Taken together, these studies suggest that nuc-1 is destabilized during cell activation, establishing a more favorable chromatin environment for transcriptional machinery to assemble (Fig. 3C). Notably, inhibition of RNAPII does not prevent this process from occurring, indicating that nuc-1 remodeling is independent of, but critical to, efficient transcription from the provirus [77].

Opposing roles for SWI/SNF complexes in HIV-1 latency and reactivation

In eukaryotes, nucleosome positioning is highly conserved and mediated by the activity of chromatin modifying complexes (CMCs). In humans, there are two broad categories of CMCs: complexes that affect nucleosome structure through covalent modifications, and those that use the energy of ATP hydrolysis to directly reposition the nucleosome (reviewed in [85]).

The SWItch/Sucrose Non-fermentable (SWI/SNF) family belongs to the latter category of CMCs and is of particular interest due to its role in HIV-1 latency and reactivation alike. In humans, there are two members of this family: BRG-/BRM-associated Factor (BAF) and Polybromo-associated BAF (PBAF) (reviewed in [85]). BAF may use Brahma-related Gene 1 (BRG1, also known as SMARCA4) or Brahma (BRM, also known as

SMARCA2) as a core catalytic subunit, while PBAF only uses BRG1 [86]. Additionally, there are other subunits that are exclusive to one member or the other. PBAF contains BAF180, BAF200, and Brd7, but lacks BAF250 [85, 86], while BAF does contain BAF250 and lacks the other PBAF-associated subunits. Otherwise, they share core components, such as BAF155/170, INI-1/BAF47, β-actin, and others (reviewed in [85]).

Notably, the sites occupied by nuc-0, -1, and – 2 are not what would be expected by DNA sequence alone [78, 79, 87]. Using the NuPoP algorithm to predict nucleosome positioning based on the LTR sequence [88], Rafati et al. found that the 5' LTR nucleosome positions were actually negatively correlated with histone affinity [78, 79], indicating a role for external factors in establishing the canonical epigenetic landscape of the provirus. Indeed, the BAF complex has been shown to be essential to this process early in infection, in the absence of Tat. Depletion of BRG1 and BAF250, but not BRM or BAF180, was associated with de-repression of the proviral promoter, and BAF250-deficient infected Jurkat T cells were unable to maintain latency. In these cells, the canonical epigenetic landscape of the 5' LTR was disrupted and the nucleosomes instead assumed the positions that would be predicted by the sequence's histone affinity; that is, accessibility at nuc-1 increased while DHS1 and DHS2 became more protected [78]. Both BAF200 and BAF250 have also been shown to be present at nuc-1 in resting cells; however, after the cells were activated, BAF250 was removed [89]. Taken together, in the absence of Tat, the BRG1-containing BAF (BRG1-BAF) complex facilitates HIV-1 latency through the positioning of nucleosomes at the 5' LTR (Table 1) [78].

Bromodomain-containing Protein 4 (BRD4) is a member of the Bromodomain and Extraterminal Domain (BET) family of proteins, and has long (L) and short (S) isoforms. BRD4L is known to inhibit Tat-mediated transactivation by binding with Positive Transcription Elongation Factor b (P-TEFb) [90, 91], but BRD4S seems to exert its own repressive action through recruiting the BRG1-BAF complex to the proviral promoter. Indeed, BRG1 and BRD4S immunoprecipitated with one another from the 5' LTR, but treating the cells with the BET inhibitor JQ1 disrupted this interaction. Additionally, knockdown of BRD4S reduced BRG1 occupancy at nuc-1 and induced HIV-1 RNA production. Taken together, these results indicate that BRG1-BAF is recruited to the proviral promoter by BRD4S [91] and has a repressive effect on HIV-1 transcription (Table 1).

Interestingly, while BRG1-containing BAF complexes are associated with repression of HIV transcription early in infection, BRM-containing BAF complexes seem to facilitate proviral transcription later in infection. In the presence of Tat, BRM and BAF155 were enriched at nuc-1, and overexpression of BRM in a HeLa model of infection could increase transactivation by Tat. In agreement with this, knockdown of the gene had the opposite effect. In the absence of Tat, however, BRM-deficient cells had no difference in basal promoter activity when compared to BRM-sufficient cells [92], indicating the synergistic relationship between Tat and BRM-containing BAF complexes (BRM-BAF). However, in a model of latency utilizing SW13 and C33A cells, some clones had greater dependence on BRM-mediated chromatin remodeling than others, while BRM-deficient ACH2 cells had impaired virion production when compared with controls [93]. Importantly, though, BRG1 and BRM have been demonstrated to have differential expression between cell and tissue types [94]. Taken together, it is possible that the host cell type and integration site of the provirus determine the degree to which proviral reactivation depends on BRM (Table 1). Further studies will be necessary to elucidate how BRM-BAF may play a role in maintaining latency in different anatomical reservoirs.

The other member of the SWI/SNF family, PBAF, has also been implicated in the activation of proviral gene

Table 1 Summary of enzymes known to remodel nucleosomes at the 5' LTR

Mechanism	Effect on HIV-1 Transcription	Factor	Cofactor	Target histone/residue	Cell line(s) used	Reference(s)
Nucleosome remodeling	Repressive	BRG1-BAF	BRD4S	nuc-0, nuc-1	J-Lat 11.1/A2, Jurkat 1G5	[80, 81]
					HEK293T, J-Lat A2/A72	[93]
		FACT	?	nuc-1/H2A/H2B	293T, A2, MT-2, primary CD4+	[106]
	Activating	BRM-BAF	Tat	nuc-1	HEK293, HeLa/LTR-luc/S3	[94]
					293FT, A204, AZ521, C33A, H1299, S3, SW13, primary CD4+	[95]
		PBAF	Tat-ac	nuc-1	A2/11.1, 1G5	[80, 81]
					J1.1, Jurkat	[91]
					C33A, Jurkat	[97]
					293T, CEM, HCC1143, TZM-bl	[98]
					8E5, ACH2, HLM-1	[99]
					A2/A72, C33A, G401, HCT116, MON, Jurkat	[100]

expression. In cells treated with PMA, depletion of BAF250 did not affect recruitment of PBAF subunits to nuc-1 [78, 95], but BAF200 was present at the promoter in both latent and active cells [89, 95, 96], indicating a role for PBAF in HIV-1 reactivation. Indeed, in several latently-infected T cell lines as well as stably-infected HEK293T cells, BRG1 knockdown inhibited viral gene expression and virion production [89, 97]. Mahmoudi et al. also demonstrated through immunoprecipitation that Tat is associated with Integrase Interactor 1 (INI-1, also known as SMARCB1), β-actin, and BRG-1 [98]. Interestingly, in contrast to the findings implicating BRM-BAF in proviral reactivation [92], they did not find Tat to be associated with BRM [98].

While this discrepancy may be due to the use of different cell lines and integration sites, it may also be attributed to the acetylation state of Tat in these systems. BRG1 contains a C-terminal bromodomain with a binding motif for acetylated proteins [98, 99], and Tat may be acetylated at Lys-50 or -51 (Tat-ac) by the cellular acetyltransferase p300 [98]. Tat-ac actually failed to interact with BRM, and was associated with BAF200 (PBAF-specific) [92, 96]. By contrast, immunoprecipitates of BAF250 (BAF-specific) were enriched with unmodified Tat [96]. Taken together, it would appear that PBAF's capacity to act on the proviral nucleosomes relies on acetylated Tat, while BRM-BAF utilizes unmodified Tat (Table 1). Indeed, Tat-mediated transactivation was abrogated in cells deficient in BAF200 [96]. Likewise, Lys-50 and – 51 mutants of Tat were also unable to undergo Tat-mediated transactivation. In support of this, the region occupied by nuc-1 has been shown to have increased sensitivity to digestion in the presence of acetylated Tat [97]. Tat's association with BRG1 was also increased in the presence of p300, but this relationship was abolished when Tat or the catalytic site of p300 were mutated [98]. Interestingly, in the absence of INI-1 and p300, Tat alone still had weak transactivation activity [98], possibly due to its interaction with BRM-BAF in the unmodified state [92, 93].

Other nucleosome remodeling factors

Other components of the SWI/SNF complex have also been implicated in HIV-1 latency and reactivation. During integration, the core subunit INI-1 directly interacts with integrase and stimulates its activity in vitro [100]. Interestingly, INI-1-deficient, stably-infected HeLa cells also had impaired, but not completely abrogated, transactivation capability. After the reintroduction of INI-1 in the presence of Tat, however, transactivation was rescued. The Rpt1 and Rpt2 domains of INI-1 were further demonstrated to be required to achieve this effect [101]. While these domains are required for the formation of a functional SWI/SNF complex [101], Rpt1 has also been shown to mimic the TAR structure in silico and in vitro [102]. The degree to which this affects the ability of SWI/SNF complexes to interact with Tat remains to be studied, however, and may provide a unique target in altering the latent state of the provirus.

The effects of the core subunit BAF53 are also at least partly dependent on the activity of Tat. BAF53-deficient HEK293T and J1.1 cells had increased virion production, and these virions additionally had improved reverse transcriptase activity. To this end, BAF53 was evicted from the 5' LTR in active cells. Taken together, BAF53 seems to be inhibitive to proviral gene expression in a resting cell, possibly early in infection. In the presence of Tat, however, the Cyclin T: CDK9 complex could more effectively phosphorylate BAF53, which prevented its association with actin [89]. This may have the overall effect of preventing the formation and binding of BRG1-BAF, which in turn permits the binding and activity of other SWI/SNF complexes and TFs to facilitate proviral gene expression.

Another chromatin remodeler, Facilitates Chromatin Transcription (FACT), has also been implicated in HIV-1 latency. While FACT normally removes and re-deposits the H2A-H2B dimer of histones to facilitate transcription [103], it may also have a repressive role in the context of HIV-1 latency. Knocking down FACT components SUPT16H or SSRP1 in NL4-3-infected HEK293 cells enhanced viral replication and Tat-mediated transactivation. Additionally, while both FACT components were associated with the 5' LTR, only SUPT16H co-precipitated with Tat. In agreement with these findings, FACT-deficient J-Lat A2 cells and infected primary CD4 + T cells underwent spontaneous latency reversal, and were also sensitized to the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). Taken together, these results indicate that FACT is repressive to proviral gene expression (Table 1) [104]. However, certain FACT-targeting compounds called curaxins have been shown to have variable effects on HIV transcription [105, 106]. Further studies will be necessary to elucidate the precise relationship between FACT and the 5' LTR nucleosomes, and the mechanism by which it may repress or facilitate proviral gene expression.

Overall, these results highlight the importance of the 2D chromatin environment in maintaining the axis of latency and reactivation in the HIV-1 provirus. Interestingly, SWI/SNF-mediated reactivation is improved when coupled with covalent modifications to the 5' LTR histones' tails, particularly acetylation [84, 89, 95, 97]. Thus, while direct nucleosome repositioning is highly important in regulating proviral latency and reactivation, additional mechanisms are also occurring at other levels.

Histone modifications

Besides direct repositioning of nucleosomes on the chromosome, one of the key mechanisms eukaryotic cells use to regulate gene expression is through post-translational modification (PTM) of the histone tails. As we have described previously, these PTMs alter the charge of the histone and therefore the strength of its interactions with DNA. Generally, positive charges are associated with tighter interactions and transcriptional repression; by contrast, neutral and negative charges are associated with transcriptional activation, as the negatively-charged DNA is repelled from the histone core (reviewed in [25]). Unsurprisingly, proviral latency and reactivation are influenced in large part through PTMs of the 5' LTR nucleosomes. Currently, acetylation and methylation are the best-characterized in both latent and reactivated HIV-1 infection (reviewed in [73, 107]).

Acetylation

Histone acetylation is mediated by two classes of enzyme: histone acetyltransferases (HATs), and histone deacetylases (HDACs). The former functions as the "writers" of histone acetylation, depositing the negatively-charged acetyl groups on the ε-amino groups of the histone tails' lysine or arginine residues; the latter are "erasers" in that they remove these acetyl groups and restore the repressive chromatin environment (reviewed in [108]). As we will describe in the sections below, several regions of the 5' LTR and their binding proteins are associated with the recruitment of HATs and/or HDACs to regulate the latent state of the provirus.

HDACs and the 5' LTR In humans, four classes of HDACs have been identified. Class I, II, and IV are zinc-dependent, whereas class III HDACs are NAD^+-dependent [109]. In resting T cells, class I members HDAC1 and HDAC3 transcripts are highly expressed [110]. In the context of HIV-1 infection, chromatin immunoprecipitation assays have shown that class I HDACs are localized to the latent 5' LTR, while other classes do not have significantly different distribution from uninfected cells [110, 111].

It has long been observed that inhibition of HDAC activity with chemical agents such as trichostatin A (TSA) or trapoxin (TPX) is associated with reactivation of the provirus and the formation of permissive chromatin at the 5' LTR in multiple cell types [84, 112], indicating that their activity represses proviral gene expression. Additionally, HDACs are often assembled into higher-order protein complexes that interact with a target site, enabling their regulatory activity [113, 114]. The 5' LTR – and, by extension, the 3' LTR – contains several binding sites for TFs and other proteins that are known to associate with HDACs, thus maintaining a repressive chromatin state and preventing proviral reactivation.

The 5' LTR contains two binding sites for the transcription factor LBP-1 (also known as LSF or UBP): the high affinity site I at nucleotides (nt) -16 to +27 (relative to the TSS), and the low affinity site II at nt -38 to -16. The latter lies over the TATA box and thus may impede interactions with TFs and RNAPII [115]. It has been demonstrated that mutants of LBP-1 lacking the DNA-binding domain fail to repress transcription [116, 117]. Additionally, in a HeLa model of infection utilizing LTR-driven chloramphenicol acetyltransferase (CAT) activity (HeLa-CAT), an LBP-1 mutant lacking site II binding capacity failed to repress CAT transcript expression [115]. Taken together, these results indicate that the ability of LBP-1 to directly bind to site II on the 5' LTR is essential for its ability to repress LTR-driven transcription. However, in the presence of Tat, the repressive capacity of LBP-1 seems to be diminished [115], and other studies have noted that LBP-1 alone is not sufficient to inhibit basal and Tat-activated transcription [118].

The sequence recognized by LBP-1 is also associated with a protein complex containing Yin Yang 1 (YY1) [118]. In the absence of HIV-1 DNA, LBP-1 and YY1 have been shown to interact, and indeed complex together at a region known as the repressor complex sequence (RCS) (nt -10 to +27) [119, 120]. The formation of this complex was disrupted when LBP-1 was unable to bind to the LTR [118, 120], or either component was otherwise sequestered from the other [112, 118]. In another HeLa model of infection, expression of both factors was sufficient to inhibit HIV-1 gene expression and virion production [118]. Similar results have also been observed in latently-infected cell lines [119]. Taken together, these studies indicate that YY1 must complex with LBP-1 to be able to exert its repressive action on the proviral promoter.

Along with its ability to complex with LBP-1, YY1 also contains a glycine/alanine-rich domain that has been demonstrated to recruit HDACs [121], but mutants lacking the recruitment domain were unable to inhibit LTR-driven transcription [112, 120]. By contrast, overexpression of YY1 was associated with increased HDAC1 occupancy and decreased acetylation of histone H4 at nuc-1, and could inhibit transactivation by Tat [112]. In agreement with this finding, Coull et al. found that HDAC1 indeed copurified with the YY1:LBP-1 complex [120].

Taken all together, these studies indicate that YY1 indirectly interacts with the 5' LTR through complexing with LBP-1. This complex then inhibits LTR-driven transcription – including Tat transactivation – by recruiting HDACs, which create a repressive chromatin environment at the proviral promoter (Table 2).

Interestingly, c-Myc has also been found in association with nuc-1 in resting cells [122]. In uninfected Jurkat T cells, c-Myc precipitated in a ternary complex with LBP-1

Table 2 Summary of factors known to modify histone acetylation at the 5′ LTR

Mechanism	Effect on HIV-1 Transcription	Factor	Cofactor	Target histone/residue	Cell line(s) used	Reference(s)
HDAC recruitment	Repressive	YY1	LBP-1	nuc-1	HeLa-CD4-LTR-CAT	[114]
					HeLa, Jurkat	[117]
					HeLa extract	[118]
					HeLa extract	[119]
					CEM, HeLa, U937	[120]
					8E5, ACH2, CEM, COS-1, HeLa	[121]
					HeLa-CD4-LTR-CAT	[122]
		c-Myc	LBP-1/YY1/Sp1	nuc-1	HeLa-CAT-CD4, J89, primary CD4+	[124]
					Jurkat	[125]
		p50/p50	?	nuc-0, nuc-1	J-Lat 6.3	[113]
					Jurkat, *Drosophila* SL2	[130]
		CBF-1	?	H3, H4	293T, CEM, HeLa, Jurkat	[132]
		AP-4	?	nuc-1	ACH2, CEM, HL-60, Jurkat, U1	[136]
		BCL11B	NuRD/MTA2	H3	COS7, HeLa, MOLT4, Jurkat, Raji	[138]
					A293T, GHOST-X4/R5, Jurkat/IG5, Sup-T1	[139]
					293T, U1, TZM-bl, human microglia	[140]
HAT recruitment	Activating	Tat	p300/PCAF	H3, H4	ACH2, J49, Jurkat, OM10.1, U1	[86]
					293, HeLa/LTR-luc/S3	[94]
					HL3T1, U1	[142]
					CHO, HL3T/extract	[143]
					Jurkat	[144]
					HeLa/LTR-CAT, NIH3T3	[145]
		p65?	Sp1	H4	Jurkat, *Drosophila* SL2	[130]
					S3 extract	[146]
					HeLa extract, *Drosophila* S-190 extract	[149]
					COS-7/extract, HUVEC, *Drosophila* SL-2	[150]

and YY1, indicating that they assemble together independent of the 5' LTR and are recruited to the integration site. Co-expression of the three factors was able to greatly reduce basal and Tat-activated transcription, while constructs lacking the low-affinity LBP-1 site or YY1 binding site had diminished c-Myc-mediated repression [123].

However, c-Myc alone was also able to repress LTR-driven CAT activity in Jurkat T cells [123]. c-Myc has been shown to co-precipitate with HDAC1 at the nuc-1 region [122], indicating that this may be the mechanism through which it is able to repress proviral transcription. Indeed, knocking down c-Myc in J89 and HeLa-CAT cells reduced the amount of HDAC1 at the 5' LTR (Table 2) [122]. Interestingly, c-Myc and HDAC1 were also present in immunoprecipitates of the transcription factor Sp1 [122], which is unsurprising as c-Myc and Sp1 are known to interact with one another [124, 125]. Knockdown of Sp1 significantly decreased the levels of c-Myc/HDAC1 at the 5' LTR, and resulted in increased transcription [122]. Because c-Myc can bind directly to proviral DNA through the "E-box" motif [126, 127], it is possible that Sp1 strengthens this interaction or otherwise helps recruit c-Myc to the 5' LTR.

The 5' LTR also contains binding sites for NF-κB subunits such as p50 and p65 [128], which may be repressive or permissive to transcription. In resting cells, p50 homodimers are predominant in the nucleus, while heterodimers – such as p50/p65 – are sequestered in the cytoplasm through interactions with IκBα (reviewed in [129]). Importantly, the p50 subunit lacks a transcriptional activation domain, and has been shown to assemble with HDAC1 [111]. p50 homodimers were found to be constitutively bound to the 5' LTR in resting J-Lat cells, and indeed precipitated with HDAC1 (Table 2). In agreement with this finding, p50-deficient J-Lat cells had increased acetylation of 5' LTR nucleosomes, but this was only sufficient to promote moderate increases of HIV-1 gene expression [111].

Interestingly, the NF-κB binding sites at the 5' LTR (AGGGAC and GGGGAC) are related to the CBF-1 consensus sequence (TGGGAA). Mutation of the GGG motif blocked binding of both NF-κB subunits and CBF-1

alike. CBF-1 was enriched at the 5' LTR in latently-infected Jurkat T cells, and was associated with high levels of HDAC1 and low levels of acetylation of histones H3 and H4 [130]. CBF-1 is also known to recruit HDAC-containing complexes as part of its normal regulatory activity (reviewed in [131]). To this end, knockdown of CBF-1 increased proviral gene expression, and unsurprisingly also decreased HDAC1 recruitment to the 5' LTR (Table 2). The same effect was seen when cells were activated by T cell receptor (TCR) stimulation, or treatment with TNF-α or PMA/PHA; interestingly, stimulated cells also had decreased levels of both CBF-1 protein and transcripts [130].

The 5' LTR also contains a site for Adapter Protein 4 (AP-4) binding near the TATA box (-21 to -16 and – 27 to -23). AP-4 is a ubiquitously expressed transcription factor that binds to DNA through its basic helix-loop-helix (bHLH) domain and dimerizes through its LR1 and LR2 motifs [132]. AP-4 may act as a transcriptional activator or repressor, depending on the context (reviewed in [133]. In HIV-1 infection, AP-4 could block Transcription Factor II D (TFIID) from interacting with the TATA box and inhibit Tat-activated gene expression, but DNA binding domain (DBD) mutants were unable to achieve this effect. In particular, deletion of the bHLH domain increased expression of proviral genes. LR2 mutants also failed to repress transcription, indicating that AP-4's contributions to latency are dependent on its ability to dimerize and bind to DNA. LTR constructs bearing distal AP-4 binding sites also had repressed transcription, indicating that obstruction of the TATA box may not be the only repressive mechanism. In fact, AP-4 was found to be complexed with HDAC1 at the promoter in latently-infected Jurkat E4 cells (E4) (Table 2), but HDAC1 recruitment was decreased when the AP-4 binding site was mutated in the LTR. Importantly, HDAC1 recruitment was not abrogated in AP-4 mutants [134], likely due to the activity of the other HDAC-recruiting factors.

The Nucleosome Remodeling and Deacetylase (NuRD) complex is a high-order ATP-dependent chromatin remodeling complex that is known for transcriptional activation and repression alike. While it can directly reposition nucleosomes, it also contains HDAC activity (reviewed in [135]). The factor B-cell lymphoma/leukemia 11B (BCL11B, also called CTIP2) is known to associate with the NuRD complex in Jurkat T cells through its MTA1 and MTA2 components, with MTA1 serving as the preferred binding partner [136, 137]. Indeed, knocking down BCL11B was permissive to proviral gene expression in Jurkat 1G5 and HeLa cells expressing LTR-driven luciferase (HeLa-luc). BCL11B also was enriched at the 5' LTR of resting cells, and was repressive to virion production in NL4-3-infected Jurkat T cells [137]. Marban et al. additionally found that BCL11B was associated with active HDAC1 and HDAC2 in microglia; interestingly, they were unable to detect NuRD complex components in HDAC immunoprecipitates [138]. In agreement with previous findings, however, BCL11B knockdown was associated with increased virion production and histone H3 acetylation [137, 138], while overexpression had the opposite effect [138]. Taken together, these studies indicate that BCL11B acts as a corepressor of proviral transcription through recruitment of HDACs, either directly or through association with the NuRD complex (Table 2).

Overall, these studies reveal that several factors interact with the 5' LTR and recruit HDACs, which promotes the formation of heterochromatin at the 2D level and represses proviral gene expression (summarized in Table 2). Many of these factors are found to be upregulated and enriched at the 5' LTR in resting T cells, and ART adherence is also associated with increased HDAC expression [139], indicating that the gene expression profiles of quiescent cells contributes to the repressive chromatin environment in latent proviruses. While these factors may appear to have redundant roles, many of them have also been found in association with one another at the 5' LTR, and may in fact work in concert to recruit higher-order repressive complexes that contain HDAC activity. However, further studies will be necessary to elucidate the interconnectedness of these factors. Additionally, these interactions and their associated histone modifications have been shown to be disrupted by chemical agents, which indicates potential therapeutic targets to affect proviral latency.

HATs and the 5' LTR The precise role of HATs in latency reversal is less well characterized than that of HDACs. However, it has long been observed that proviral gene expression is associated with increased levels of acetylation at histones H3 and H4 [84, 140]. Particularly, HATs p300/CBP, GCN5, and PCAF have been detected at the 5' LTR just a few hours after stimulation with PMA [140]. Importantly, this process is independent of transcriptional initiation [77, 140], further indicating that the formation of euchromatin is an important "first step" in proviral reactivation.

Tat has been found to associate with p300/CREB-binding protein (CBP) [92, 141–143], which is unsurprising as Tat can be acetylated by this HAT [97, 98] as we have outlined previously. When Tat was added exogenously to lysates from HeLa-CAT cells, the resulting immunoprecipitate had histone acetyltransferase activity [141]. Likewise, Tat has been found in immunoprecipitates of p300 [142, 143] and was associated with increases in the acetylation of histones H3 and H4 even in the absence of active transcription [140]. This interaction appears to be direct, and requires Tat's arginine-rich domain (ARD);

ARD mutants were unable to associate with p300, and were poor transactivators [141, 142]. In further support of this relationship, p300/CBP was enriched at the 5' LTR in HeLa-CAT cells treated with Tat. By contrast, without Tat, p300/CBP was absent [141]. It has also been observed that p300 alone is unable to induce LTR-driven transcription [142, 143]. Taken together, these studies indicate that Tat recruits p300/CBP to the 5' LTR, which enables acetylation of proviral nucleosomes – particularly nuc-1 – and facilitates transcriptional activation. As we have discussed previously, Tat-ac can also recruit PBAF to the 5' LTR to induce nucleosomal remodeling [93, 96, 98]. Thus, p300/CBP facilitates both direct nucleosomal positioning and histone modification to induce proviral gene expression (Table 2).

Interestingly, Benkirane et al. observed that Tat also precipitated with p300/CBP-associated factor (PCAF) [143]. This is not surprising, due to PCAF's innate ability to interact with p300/CBP. p300 mutants with abrogated PCAF-binding activity were unable to support Tat-mediated transactivation, and addition of PCAF alone was unable to induce proviral transcription in a murine cell system. However, in the presence of p300, Tat-mediated transactivation was significantly increased. Surprisingly, enzymatic mutants of PCAF, but not p300, were unable to facilitate proviral gene expression, indicating that p300 may serve as an adapter for PCAF in its interaction with Tat [143]. Further studies will be necessary to confirm this relationship in latently-infected T cells and myeloid cells.

As we have discussed previously in this section, NF-κB is associated with proviral latency by way of p50 homodimers, which recruit HDAC1 to the promoter. In contrast to this, dimers containing p65, together with Sp1, were associated with proviral transcription [128, 144], possibly because Sp1 improves the binding strength of p65 to the LTR [144]. Indeed, NF-κB and Sp1 binding sites were required for proviral reactivation in TF-1 cells after stimulation with PMA [145]. Importantly, however, this effect requires a chromatinized provirus, as p65 was unable to activate transcription from naked HIV-1 DNA templates. Sp1 and p65 together were also able to disrupt the periodicity of the 5' LTR nucleosomes, inducing DNase hypersensitive sites corresponding to those observed during transcriptional activation [76, 144]; interestingly, Sp1 and p50/p65 heterodimers appear to be necessary for canonical positioning of nucleosomes at the 5' LTR in the *Xenopus laevis* chromatin assembly system [146]. Additionally, inclusion of p65 in HeLa extracts induced acetylation of H4 [147]. p65 is known to associate with p300/CBP in other systems [148]; while p300 has not been found to directly associate with p65 at the LTR, the current evidence suggests that this is one of the mechanisms by which HATs are recruited to the 5' LTR (Table 2). This may also explain why proviral activation via NF-κB is independent of, but synergistic with, inhibition of HDACs [84, 147].

Overall, these studies indicate that acetylation of the nucleosomes at the 5' LTR is highly important in reactivation of the provirus, and multiple factors may interact to facilitate histone acetyltransferase recruitment to the proviral promoter. Similar to the recruitment of HDACs, the recruitment of HATs is influenced by the activation state of the host cell. As such, these factors may serve as effective targets for therapeutics aimed at maintaining or reversing latency.

Histone methylation and methyltransferases

Similar to acetylation, the methylation status of histone tails is mediated by "writers" and "erasers": histone methyltransferases (HMTs) and demethylases, respectively. These enzymes are generally specific to a particular lysine or arginine residue, as well as its methylation status. Methylation is slightly more complex than acetylation, as residues may be mono-, di-, or even tri-methylated, and each combination of residue and methylation status has its own implications for transcriptional activation or repression. For example, H3K4me3 is considered permissive to transcription, while H3K9me3 is repressive (reviewed in [149]). This dynamic relationship is reflected in the HIV-1 provirus, as specific methylation patterns, HMTs, and demethylases have been implicated in both latency and reactivation.

H3K27/Polycomb repressive complexes Some lysine residues, such as H3K27, may be acetylated or methylated. H3K27ac promotes the formation of euchromatin and facilitates transcription, but this residue can also be recognized by other enzymes for methylation, which promotes heterochromatin formation and inhibits transcription [150, 151]. As such, this residue is an important center of transcriptional regulation. Methylation of H3K27 is mediated in large part by Polycomb Repressive Complex 2 (PRC2), which contains intrinsic methyltransferase activity through its Enhancer of Zeste 1 (EZH1) and EZH2 subunits. This catalytic core also contains other proteins, including EED and SUZ12 (reviewed in [150]). PRC2 can additionally bind to YY1 and HDAC1/2, which further promotes heterochromatin formation [152, 153].

EZH2 has been found to be enriched in latent Jurkat E4 and 2D10 cells, and this is associated with increased levels of H3K27me3 at the 5' LTR [154–156]. This was also observed in the Venus-expressing Jurkat line developed by Matsuda et al. [157] (Jurkat-Venus) and primary cells [155]. As we have discussed previously, CBF-1 is enriched at the promoter in resting cells and is known to recruit HDACs [130, 156]. However, CBF-1 knockdown in infected Jurkat T cells was also associated with

removal of EZH2. Unsurprisingly, this was also correlated with a loss of H3K27me3 [156]. These factors were also lost from the 5' LTR following TNF-α stimulation or Tat induction, which was associated with a decrease in H3K27me3 levels [154, 156, 158]. Likewise, knockdown of EZH2 or other PRC2 components caused significant increases in proviral gene expression across multiple cell lines (Table 3) [154–157, 159], as well as Tat-mediated transactivation [158].

Additionally, EZH2 knockdown in E4 cells sensitized them to stimulation through the TCR, as well as to treatment with small molecule activators of HIV-1 transcription. Cells that failed to reactivate following TNF-α treatment had enriched H3K27me3 at the 5' LTR, with lower levels of acetylation, and were resistant to further stimulation [154]. Long-term culture of E4 and Jurkat-Venus cells revealed a progressive enrichment of H3K27me3 at the 5' LTR as the cells returned to quiescence [154, 157], further supporting EZH2's role in repressing proviral transcription (Table 3). Interestingly, EZH2 seems to exhibit its repressive function early in infection. Jurkat T cells deficient in EZH2 that were then infected with HIV-1 failed to enter the latent state [157, 159]. A similar effect was seen when cells were treated with the EZH2 inhibitor DZNep [154, 157, 159].

In the cell, EZH2's activity is partially regulated through phosphorylation of its Ser-21 by Protein Kinase B (PKB, also known as Akt). This inhibits its methyltransferase activity and promotes gene expression [160]. In the context of HIV-1, EZH2 mutants mimicking phosphorylated Ser-21 were dissociated from the 5' LTR, which was correlated with a decrease in H3K27me3 levels and increased proviral gene expression in TZM-bl cells. By contrast, a mutant mimicking unphosphorylated Ser-21 was enriched at the 5' LTR, while Tat-mediated transactivation was decreased [158]. Taken together, these results indicate that EZH2's ability to repress proviral gene expression is dependent on the phosphorylation state of its Ser-21.

In contrast to PRC2, UTX-1 is a demethylase of H3K27me3, and thus promotes the formation of euchromatin. When Tat was induced in TZM-bl cells, H3K27me2 and me3 levels decreased, which is unsurprising as Tat-mediated transactivation promotes euchromatin formation at the 5' LTR. However, in UTX-1-deficient cells, H3K27me3 was increased even in the presence of Tat. Interestingly, p65 recruitment to the NF-κB site of

Table 3 Summary of factors known to alter histone methylation at the 5' LTR

Mechanism	Effect on HIV-1 Transcription	Factor	Cofactor	Target histone/residue	Cell line(s) used	Reference(s)
Methyltransferase	Repressive	PRC2/EZH2	CBF-1	nuc-1/H3K27	Jurkat E4/E6/G4	[156]
					J89, Jurkat 2D10, primary CD4+	[157]
					CEM, Jurkat, primary CD4+/ Tyagi-Sahu model	[158]
					CCRF-CEM, HeLa/LTR-luc Jurkat, MOLT4, SupT1, U1, primary CD4+	[159]
					TZM-bl	[160]
					E4, primary CD4+	[161]
		EHMT2	?	H3K9	E4, primary CD4+	[161]
					293, ACH2, HeLa, OM10.1	[165]
		SUV39H1	HP1g/BCL11B	H3K9	293T, U1, TZM-bl, human microglia	[140]
					HeLa/LTR-luc, Jurkat, U1, primary CD4+	[166]
		SMYD2	L3MBTL1	H4K20	293T, A72, primary CD4+	[175]
		CARM1	?	H3R17, H3R26	2D10, S3, TZM-bl	[186]
	Activating				293, CV1, Jurkat, MEFs, primary murine macrophages	[184]
					2D10, S3, TZM-bl	[186]
		SMYD5	USP11/Tat	H1, H2B, H3, H4	293T, J-Lat 5A8/A2/A72, HeLa	[180]
		PRMT1	p65/CARM1	H4R3	293, CV1, Jurkat, MEFs, primary murine macrophages	[184]
Demethylase	Repressive	LSD1	BCL11B/NuRD	H3K4	COS7, HeLa, MOLT4, Jurkat, Raji	[138]
					A293T, GHOST-X4/R5, Jurkat/IG5, Sup-T1	[139]
					293T, U1, human microglia	[171]
	Activating	UTX-1	p65/Tat	H3K27	TZM-bl	[163]

the 5' LTR was inhibited in these cells. Catalytic mutants of UTX-1 were also unable to facilitate transactivation, indicating that its demethylase activity is required to achieve proviral reactivation (Table 3) [161].

Overall, these studies suggest that the methylation status of H3K27 is highly important in maintaining and reversing proviral latency. Because this residue may be recognized and modified by a number of different enzymes, including HATs, it may serve as an effective therapeutic target.

H3K9 H3K9 methylation is catalyzed by at least five HMTs: SUV39H1, SUV39H2, EHMT2 (also known as G9a), G9a-like protein (also known as GLP), and SETDB1 (also known as ESET). These enzymes are characterized by their Su(var), Enhancer of Zeste, and Trithorax (SET) domains, which confer methyltransferase activity [162]. Of these, Suppressor of Variegation 3–9 Homolog 1 (SUV39H1) and Euchromatic histone-lysine N-methyltransferase 2 (EHMT2) have been identified as regulators of HIV-1 latency.

The latter, EHMT2, catalyzes mono- and dimethylation of H3K9, which promotes the formation of heterochromatin and represses transcription. As one may expect, this has been implicated in proviral latency. Chemical inhibition or knockdown of EHMT2 induced viral protein expression in ACH2 and OM10.1 cells. Mutants with nonfunctional catalytic domains were also unable to repress viral gene expression. By contrast, both basal and Tat-activated transcription were potently inhibited in cells overexpressing EHMT2 [163]. Interestingly, Nguyen et al. found that EHMT2 knockdown alone in infected Jurkat T cells was insufficient to reactivate the provirus [159]. Importantly there was no significant change in H3K9me3 or H3K27me2 in EHMT2 inhibition or knockdown, but H3K9me2 was removed from the promoter [159, 163]. Taken together, these results indicate that H3K9me2 plays a role in maintaining viral latency, and this modification is specifically mediated by the action of EHMT2 (Table 3).

SUV39H1 associates with Heterochromatin Protein 1 (HP1) orthologs and catalyzes trimethylation of H3K9, which also promotes the formation of heterochromatin. As we have discussed previously, BCL11B is associated with proviral latency through its recruitment of HDAC1 to the promoter. However, Marban et al. also found that, in microglia, BCL11B occupancy was also correlated with SUV39H1 and HP1 orthologs. Overexpression of BCL11B was associated with HP1 recruitment to nuc-1 and an increase in H3K9me3. In accordance with this finding, knocking down BCL11B reduced SUV39H1 and HP1γ occupancy at nuc-1, decreased H3K9me3 levels, and stimulated proviral transcription (Table 3) [138].

Similar effects were observed when SUV39H1 itself was knocked down in HeLa-luc cells. Interestingly, basal transcription was unaffected, while transactivation by Tat was enhanced; however, knocking down HP1γ significantly increased both transcriptional stages. This was also seen in HIV-1-infected PBMCs, which additionally experienced increased viral rebound. Further, SUV39H1 and HP1γ – but not HP1α or HP1β – were enriched at the promoter in the absence of Tat, and this was associated with an increase in H3K9me3 levels. However, in the presence of Tat, the opposite was true and H3 acetylation was enhanced (Table 3) [164]. This is not surprising, as H3K9ac is a mark of euchromatin and transcriptional activation.

Additionally, BCL11B seemed to become dissociated from the promoter in transcriptionally-active U1 cells, and H3K9me3 levels decreased [138]. Likewise, knockdown of HP1γ was associated with a reduction of SUV39H1 recruitment and H3K9me3 levels, as well as increased H3 acetylation and LTR-driven transcriptional activation. However, when Sp1 was knocked down concomitantly, this activation was reduced five-fold [164], likely due to the requirement for Sp1 in reactivation [128, 144]. Taken together, these results implicate SUV39H1 and HP1γ in the repression of HIV-1 transcription through trimethylation of H3K9 at the 5' LTR (Table 3).

H3K4 Methylation of H3K4 is catalyzed by the Complex Associated with Set1 (COMPASS), specifically its Set1 domain [165, 166] and is associated with active or poised transcription (reviewed in [167]). Indeed, H3K4me3 has been shown to be enriched at the 5' LTR during proviral gene expression and in the presence of Tat [157, 161].

As we have discussed previously, the NuRD complex represses transcription from the LTR [136, 137]. Along with HDACs and direct nucleosome remodeling enzymes, the NuRD complex has also been found to contain the H3K4 demethylase Lysine-specific Demethylase 1 (LSD1) [168]. Indeed, BCL11B, which is known to associate with the NuRD complex and recruit it to the LTR [137], has also been demonstrated to co-precipitate with LSD1. LSD1 knockdown in microglia was associated with significantly increased virion production, and an expected increase of H3ac. In U1 cells, stimulation with PMA caused LSD1 to be released from the promoter (Table 3). However, whether LSD1's ability to repress proviral gene expression was connected to its demethylase activity remains undetermined [169]. Interestingly, LSD1-deficient cells also had diminished BCL11B recruitment to the promoter, and BCL11B knockdowns actually had slightly *increased* recruitment of LSD1 and COMPASS to the promoter [169]. While it is possible that COMPASS and BCL11B may compete for LSD1

binding, this relationship has not been fully elucidated in the context of HIV-1.

SMYD family methyltransferases Like SUV39H1 and EHMT2, the enzymatic activity of the SET and MYND Domain-containing (SMYD) family of methyltransferases is facilitated by their SET domains. SMYD2 is generally known to act on H3K4, H3K20, H3K36 [166, 170, 171], but some evidence suggests that H4 may in fact be a more efficient substrate for its methyltransferase activity [172]. As such, it may function as a transcriptional activator or repressor depending on the substrate.

In the context of HIV-1, SMYD2 repressed proviral transcription and promoted latency. Knocking down SMYD2 in J-Lat 5A8, A2, and A72 cells induced transcriptional activation; in A72 cells, this even occurred in the absence of Tat. Likewise, treatment with the SMYD2-specific inhibitor AZ505 induced proviral gene expression in both J-Lat cells and ex vivo participant samples (Table 3). Interestingly, while chemical inhibition synergized with JQ1 in cell lines, this effect was not seen in participant samples [173], likely due to other mechanisms of transcriptional repression in PWH.

Interestingly, H4K20 was the predominantly methylated residue in nuclear extracts. H4K20me was also enriched at the 5' LTR in J-Lat A72 cells, but this decreased after stimulation with TNF-α [173], indicating a suppressive role for this modification. Lethal (3) malignant Brain Tumor-like Protein 1 (L3MBTL1) is a "reader" of H4K20me [174], and was found to be enriched at the 5' LTR in latent cells. In agreement with this finding, TNF-α stimulation and knockdown of SMYD2 caused L3MBTL1 to become dissociated from the 5' LTR. Additionally, knocking down or chemically inhibiting this factor directly increased basal transcription in A72 cells [173]. Taken together, L3MBTL1 represses proviral gene expression through interacting with H4K20me, and this is facilitated by the methyltransferase activity of SMYD2 (Table 3).

Another member of the SMYD family, SMYD5, is less well-characterized. It is known to monomethylate H3K36 and H3K37, as well as trimethylate H3K36 and H4K20 [175–177]. Thus, SMYD5 may play a role in activating or repressing gene expression. Recently, Boehm et al. have identified a potential role for SMYD5 in HIV-1 latency. They found that SMYD5 knockdown in J-Lat 5A8, A2, and A72 cells prevented latency reversal. By contrast, overexpression of SMYD5 caused a significant increase in proviral gene expression regardless of Tat in HeLa-luc cells. In agreement with this finding, stimulation by TNF-α resulted in increased recruitment of SMYD5 to the 5' LTR, but it was absent when cells were latent. Interestingly, SMYD5-deficient cells had decreased levels of H3K4me3 [178], suggesting that H3K4 may be a novel target for the enzyme's activity. Additionally, SMYD5 could also methylate Tat, as well as histones H1, H2B, H3, and H4 (Table 3) [178]. Future studies will be necessary to fully elucidate SMYD5's substrate selection and activity, both in the context of normal cellular function and HIV-1 infection. The de-ubiquitinylating enzyme USP11 was also found to be associated with SMYD5 regardless of Tat expression. While TNF-α stimulation increased the level of SMYD5 protein in the cell, knocking down USP11 caused a significant decrease, suggesting that USP11 may normally protect SMYD5 from ubiquitinylation and subsequent degradation [178].

Overall, these results indicate that SMYD5 methylates Tat to facilitate transactivation, and may also methylate multiple histones of the 5' LTR nucleosomes to further promote proviral gene expression. (Table 3). Further studies will be necessary to determine the details of this relationship, and may reveal unique therapeutic targets to prevent proviral reactivation.

Arginine methylation

Besides lysines, arginine residues on histone tails can also become methylated. There are at least nine enzymes that catalyze this reaction, known as protein arginine methyltransferases (PRMTs) (reviewed in [179]). The H3R17/36 methyltransferase Coactivator-associated Arginine Methyltransferase 1 (CARM1, also known as PRMT4 [180]) has been demonstrated to act as a co-activator of NF-κB-dependent genes [181]. In fact, CARM1 associates with p300 and interacts directly with p65. In mammalian cells, however, the H4R3 methyltransferase PRMT1 is the predominant one [182, 183].

Indeed, upon stimulation with TNF-α, coupled with co-expression of PRMT1 and CARM1 or p65, HEK293T cells expressing an HIV-1-luciferase reporter had strong induction of transcription. However, constructs with mutant NF-κB binding sites failed to achieve this effect, indicating that PRMT1 may act as a co-activator of NF-κB-dependent gene expression in the context of HIV-1 infection. Similar results were seen in mouse embryo fibroblasts expressing the reporter construct. In further support of this, p65 and PRMT1 were complexed in Jurkat T cells. Importantly, enzymatic mutants of PRMT1 or CARM1 were unable to achieve a synergistic activating effect [182], indicating that their methyltransferase activity is critical to activating proviral gene expression (Table 3). Further studies in latently-infected cells will be necessary to fully elucidate PRMT1's role in latency reversal.

More recently, CARM1's transcriptional activation has been connected to its interactions with other histone modifications. Zhang et al. found that methylation of H3R26 was enhanced when H3K27 was also acetylated; however, H3R17me3 was unaltered. Mutating Lys-27 to

methionine, thus giving it a neutral charge, reproduced this effect, indicating that acetylation at H3K27 may act as a "priming event" to boost H3R26 methylation [184].

In the context of HIV-1, CARM1 overexpression reduced Tat's ability to transactivate in TZM-bl cells. By contrast, CARM1 knockdown moderately increased proviral gene expression, and enzymatic mutants were unable to suppress transactivation [184], further supporting the importance of CARM1's methyltransferase activity in maintaining latency. Additionally, chemical inhibition of CARM1 induced proviral gene expression in both TZM-bl and resting primary T cells, and this effect was amplified when the latency reversal agents SAHA or JQ1 were added [184]. In contrast with previous findings, these results indicate that CARM1 also has the capacity to repress proviral gene expression (Table 3). Further studies will be necessary to elucidate the role of arginine methylation in HIV-1 latency and reactivation, and may provide new therapeutic targets that synergize well with current strategies.

Other histone modifications

As we have illustrated in this section, histone acetylation and methylation are the most well-characterized modifications in HIV-1 latency and proviral reactivation to date (reviewed in [107]). To this end, modifications to histones H3 and H4 are by far the best-studied. However, as we have noted previously, other histone PTMs and subunits are involved in regulating the expression of normal eukaryotic genes.

In a resting cell, histone H1 normally binds the histone core and its associated DNA to facilitate compaction and maintain patterns of chromatin during differentiation and development of the cell. Importantly, H1 becomes phosphorylated by CDK2 during the G1/S transition, which promotes dissociation of the nucleosome during replication fork progression [185]. O'Brien et al. found that H1 could also be phosphorylated by P-TEFb in a cell-free system. Indeed, immunoprecipitation of Cyclin T1, but not CDK2, also pulled down H1. Additionally, co-transfection with C22G Tat, which is unable to bind Cyclin T1, reduced the level of H1 phosphorylation in uninfected HeLa cells, while wild type Tat increased H1 phosphoryation; likewise, HIV-1-infected H9 cells had much higher levels of H1 phosphorylation than controls. Importantly, they also found that MAGI cells had decreased H1 binding at the LTR in the presence of wild type Tat, but not C22G Tat [186]. Taken together, these results indicate that Tat's recruitment of P-TEFb to the 5' LTR facilitates phosphorylation of H1 and its dissociation from the promoter region, possibly nuc-1, thus promoting HIV-1 gene expression. Additional studies will be necessary to characterize how phosphorylation of other histones, as well as other modifications of H1, affect the latency of the HIV-1 provirus.

Similar to acetylation, histone crotonylation is suggested to increase gene transcription, and may even be more potent than acetylation in some cases [187, 188]. Crotonylation can be "written" by typical HATs like p300, but is less efficient than acetylation. Likewise, "erasers" of crotonylation include class I and III HDACs. The metabolic enzyme Acyl-CoA Synthetase Short Chain Family Member 2 (ACSS2) could also be considered a "writer" of crotonylation, as it converts crotonate groups to crotonyl-CoA, which is used as a co-substrate for acetyltransferases (reviewed in [188]).

Jiang et al. have recently implicated this process in HIV-1 reactivation. Sodium crotonate (Na-Cro) induced both global and LTR-specific levels of H3K4cr, H3K4ac, and H3K18ac; by contrast, the repressive modification H3K27me3 was decreased [189]. These modifications together suggest a relaxed chromatin state and derepressed proviral transcription. Indeed, exposure to Na-Cro induced virion production in both infected immortalized cell lines and primary cells from well-suppressed participants, and induction of ACSS2 had similar levels of proviral reactivation as HDAC inhibition in J-Lat A1 cells. In agreement with this finding, inhibition of ACSS2 led to a substantial decrease in both proviral gene expression and crotonylation at the LTR. Likewise, Na-Cro exposure failed to achieve high levels of reactivation in ACSS2-deficient cells [189]; together, these results suggest the importance of crotonyl-CoA as a substrate in this process.

Notably, Jiang et al. also observed that the effects of crotonylation synergize with latency reversal agents. Treating J-Lat A1, U1, and participant cells with Na-Cro prior to stimulation with the latency reversal agents ingenol-3-angelate, bryostatin-1, or SAHA led to a much higher degree of proviral reactivation than either treatment alone. Interestingly, treating cells with SAHA *before* Na-Cro exposure failed to achieve this same level of synergy [189]. While this mechanism has not been fully elucidated, it may be that the cascade of transcriptional activation following HDAC inhibition simply outcompetes the machinery required to crotonylate the histone tails at the 5' LTR.

Conclusion & summary

As we have described in this section, the 2D chromatin environment of the integrated provirus is regulated by a myriad of TFs, chromatin-modifying enzymes and their products, and additional nonenzymatic proteins that facilitate their interaction (summarized in Tables 1 to 3). Some promote the formation of heterochromatin and repress transcription from the 5' LTR, which promotes latency (Fig. 4A). Others have the opposite function, and

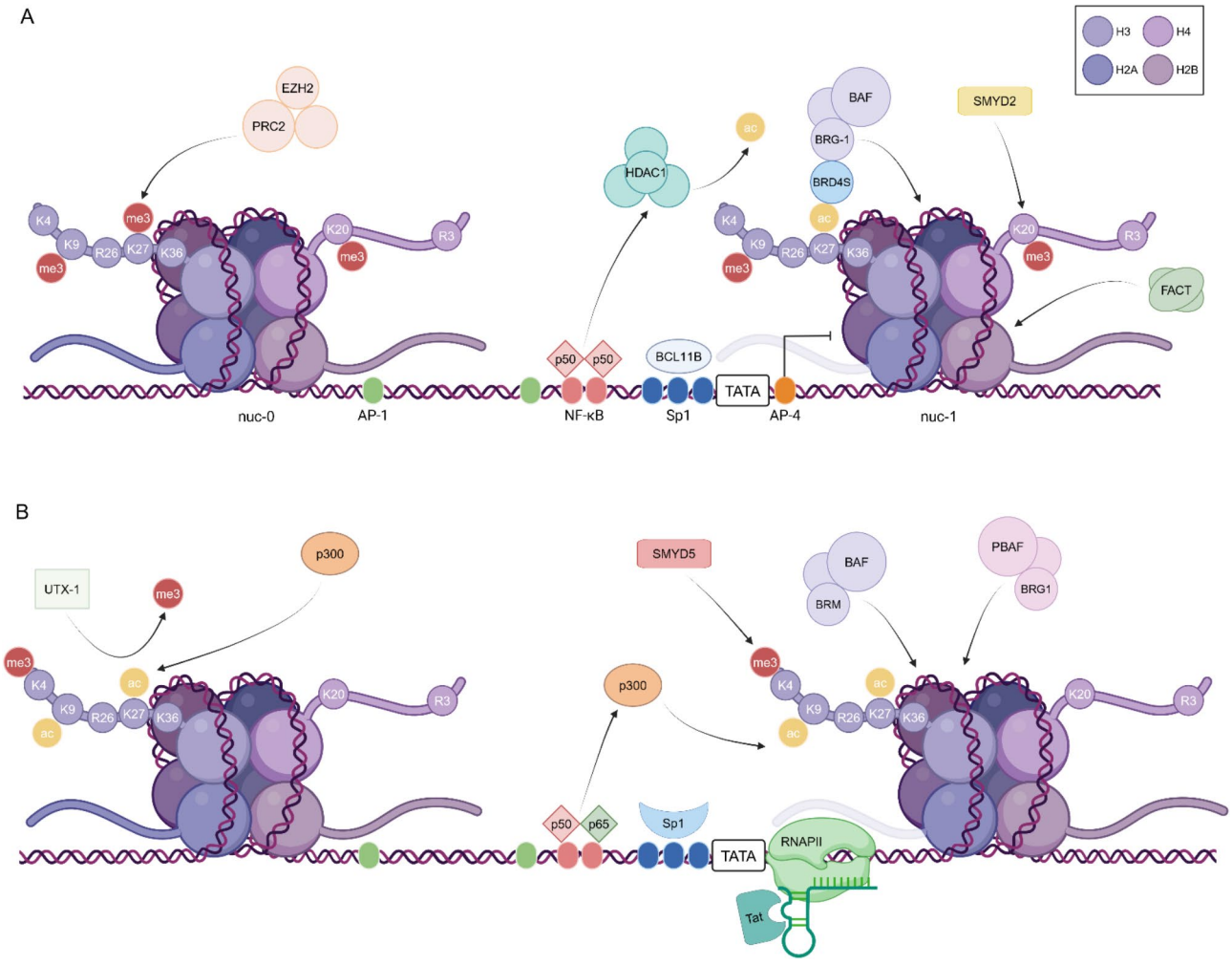

Fig. 4 2D chromatin factors at the 5′ LTR promote or reverse latency. **(A)** During latency, the 5′ LTR exists within a repressive chromatin environment. HDAC1 activity maintains low levels of acetylation, while methyltransferases such as PRC2 and SMYD2 catalyze the addition of repressive methylation patterns. These enzymes are recruited by factors that bind to sites on the 5′ LTR, such as NF-κB p50 homodimers and BCL11B. Nuc-1 is also positioned over the TSS by the BRG1-BAF and FACT complexes. **(B)** When latency is reversed, new histone modification patterns and nucleosome positions emerge. Acetylation by p300 loosens DNA interactions with nucleosomes, while PBAF and BRM-BAF position nuc-1 slightly downstream to enable RNAPII binding and activity. Tat binding to the TAR RNA increases transcriptional processivity and promotes high levels of HIV-1 gene expression

reverse latency by promoting the formation of euchromatin (Fig. 4B).

Many of these factors appear to have redundant function, such as HDAC recruitment (Table 2). However, several of them are also known to associate with one another, including in the context of HIV-1. While many of these individual components or smaller complexes (such as the YY1/c-Myc/LBP-1 ternary complex [123]) have been identified and characterized, additional studies will be necessary to determine whether they act independently or assemble into higher-order multifunctional structures.

Discussion & current gaps in knowledge

HIV-1 is a lentivirus that integrates into the host genome as a provirus. While many other viral infections are cleared by host immune mechanisms, HIV-1 establishes a latent, persistent reservoir within infected cells. This latent infection is characterized by low levels of gene expression and viral particle production, and is regulated in large part by epigenetic mechanisms that remodel the chromatin landscape of the integrated provirus (summarized in Tables 1, 2 and 3).

To date, most of the focus on HIV-1 epigenetics and chromatin has been directed towards 2D factors. However, several gaps remain in our current understanding. First, it is not well known how the host cell type affects the 2D chromatin environment of the provirus. HIV-1's tropism includes CD4 + T cells, macrophages and

microglia; non-leukocytes such as astrocytes can also be infected in a CD4-independent manner [12–17, 32, 190, 191]. It has also been found that HIV-1 DNA can persist in the hepatocytes of PWH who also have hepatitis B virus (HBV) [192]. The enzymes that catalyze nucleosome positioning and histone modifications are expressed differently between cell types and activation states, and this is further affected by individuals' lifestyle choices and habits, such as ART adherence and substance use [33, 94, 110, 111, 139, 193, 194]. Likewise, results from brain tissue and other neuronal cells suggests that the 3D chromatin environment is cell type-specific [195–197]. Additionally, while nuc-0 and nuc-1 are found consistently across proviruses and cell types, several studies have provided evidence that other nucleosomes, such as nuc-2, may be present or absent depending on the individual cell [78–80]. Taken together, these findings suggest that the 2D and 3D chromatin environments may vary between each provirus and cell type found in the HIV-1 reservoir. However, this has not been characterized in full, as a majority of investigations into HIV-1 latency have been conducted in T cells.

Additionally, the processes of reverse transcription and integration are highly error-prone, leading to the development of diverse viral quasispecies within and between PWH, even with ART adherence [198, 199]. These quasispecies may contain large insertions and/or deletions (indels) in the genome that can render the provirus transcriptionally defective or replication-incompetent. However, the extent to which these indels affect the 2D and/or 3D chromatin environment of the provirus is currently unknown. Quasispecies may also have single-nucleotide mutations in transcription factor binding sites in the 5' LTR that alter proviral gene expression and affect disease severity [73, 200, 201]. As we have reviewed in this work, critical TFs such as p65 and Sp1 are associated with enzymes that promote euchromatin formation (Tables 2 and 3). Additional studies will be necessary to elucidate how the 2D chromatin environment is affected in these mutant LTRs, and how this correlates with clinical outcomes.

As ART is unable to clear the latent HIV-1 reservoir, much of the field has shifted focus to cure strategies that target and inactivate the latent provirus. In fact, two of these propose targeting proviral chromatin to repress or promote gene expression. "Block and Lock," as the name suggests, purports to act as a functional cure for HIV-1 by inducing a state of deep latency, rather than clearing the proviral reservoir. This is accomplished through the use of latency promoting agents (LPAs), which often induce a repressive 2D chromatin environment at the integration site by promoting modifications such as H3K27me3, sequestering Tat and its cofactors, and other mechanisms (reviewed in [202]). "Shock and Kill," by contrast, utilizes latency reversal agents (LRAs) to reactivate the provirus and promote immune-mediated killing of infected cells. Current and historical LRAs are diverse in their mechanisms of action, but generally promote an accessible chromatin environment at the integration site to enable transcription factor binding and RNAPII activity (reviewed in [68, 203]).

To date, most of the studies investigating the efficacy of various small molecule LPAs or LRAs have focused primarily on the 2D chromatin environment of the integrated provirus, especially histone modifications – for example, HDAC inhibitors and Protein Kinase C (PKC) agonists are major players in the Shock and Kill strategy (reviewed in [68]). While many of the current LPAs and LRAs have shown great promise in vitro and ex vivo, their efficacy in in vivo clinical trials has been limited (reviewed in [202, 203]. It has been shown that different CD4 + T cell-based models of HIV-1 latency are variable in their sensitivity to reactivation [204]. As we have outlined previously, however, CD4 + T cells do not undergo changes in global chromatin conformation upon proviral reactivation [44, 67], whereas microglia do [58, 69]. Could the 3D chromatin environment be a gatekeeper for these chemicals and their effects? Depending on the integration site and cell type, the targets of LPAs/LRAs may be unable to effectively penetrate the intra- and interchromosomal interactions to access the latent provirus. Current LPAs and LRAs may also not be potent enough to induce sufficient remodeling of 3D chromatin. Future studies should therefore also seek to understand how the 2D chromatin environment of the provirus influences the 3D, and vice versa.

Gene editing has also been proposed as a cure for HIV-1 infection. CCR5 is an attractive target for such a strategy, as individuals encoding a nonfunctional form of the receptor (CCR5Δ32) are naturally resistant to HIV-1 infection and disease progression as entry from R5-tropic strains is compromised [8, 9, 205, 206]. However, because the provirus integrates into the host genome, it too can be targeted by gene editing strategies.

The clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) system has recently been investigated as one such gene editing strategy to target the HIV-1 provirus. In this system, the Cas9 endonuclease and associated guide RNA (gRNA) are delivered to infected cells. The gRNA targets Cas9 to a site of interest, and recognition of the protospacer-adjacent motif (PAM) activates Cas9 to induce a double-stranded break in the DNA. The break is repaired by nonhomologous end-joining (NHEJ) in the absence of additional donor DNA. Because NHEJ is highly error-prone, this often leads to the production of indels; in the context of HIV-1, complete excision is also possible as each LTR could be targeted simultaneously

due to their identical sequences (reviewed in [206, 207]). Indeed, CRISPR/Cas9-based gene editing is highly effective at inactivating HIV-1 gene expression and preventing latency reversal in vitro, particularly when the LTR is targeted [208–211]. gRNAs directed near the TAR sequence are predicted to be nearly 100% effective in silico [212], but their ability to inactivate HIV-1 in the latently-infected J-Lat 10.6 system was more limited [208]. Importantly, the site targeted by these gRNAs is also occupied by nuc-1 [77–79, 84, 212], and nucleosomes and other host proteins are known to inhibit Cas9's ability to cleave DNA [213–216]. While the effect of the 3D chromatin environment on Cas9's activity is currently not well understood, it is also possible that target sequences found in the B subcompartment are protected from cleavage in a similar way. This is especially important to consider, as HIV-1 is often found integrated into heterochromatic regions in PWH who adhere to ART long-term [62, 63]. To maximize efficacy, the CRISPR/Cas9 strategy should account not only for quasispecies diversity [207, 212, 217], but also integration site and the epigenetic factors therein. This may involve utilizing other Cas proteins of different sizes and PAM requirements [218], multiplexed gRNAs (reviewed in [207]), and/or deliberate alteration to the chromatin environment of the provirus.

Indeed, as we have discussed here, LRAs are presently used to reactivate latent HIV-1 and trigger an immune response against infected cells. Many small molecules used as LRAs have direct effects on the chromatin environment of the 5' LTR, generally acting to increase accessibility to the core promoter region and transcription start site (reviewed in [68]). However, computational analysis of data from GUIDE-seq, CIRCLE-seq, and RNA-seq experiments suggests that the amount of DNA accessibility required by Cas9 for its activity is less than what is required for transcriptional initiation and processivity [213]. To this end, small molecules with mechanisms of action associated with latency reversal, such as HDAC inhibition, have been demonstrated to improve Cas9's ability to cleave target sequences by facilitating the formation of euchromatin [219–221]. Taken together, a strategy incorporating elements of both Shock and Kill *and* CRISPR/Cas9 gene editing emerges: one that uses LRAs to "tickle" proviral chromatin enough to increase accessibility at the 5' LTR without full latency reversal, then the CRISPR/Cas9 system to "tweeze" key regions to inactivate HIV-1 gene expression and replication.

In conclusion, while HIV-1 remains a global health concern, advances in technology and analytical tools have led to a greater understanding of the molecular mechanisms underlying latency and reactivation, to the point where these mechanisms may now serve as therapeutic targets. However, several gaps remain in our current knowledge of HIV-1 chromatin dynamics, such as the influence of cell type, quasispecies, and integration site. By understanding how these factors affect the axis of proviral latency and reactivation, the field can identify new and effective LPAs/LRAs, and investigate novel combinatorial strategies to improve the current clinically-relevant agents.

Acknowledgements
Thank you to Jacqueline Barker and William Dampier at Drexel University for providing feedback towards the initial draft of this manuscript. Figures 1 and 2, and 4 were created in BioRender. Figure 3 was created in Microsoft PowerPoint.

Author contributions
JEJ contributed significantly to the writing and figure preparation of the introduction, 2D chromatin, and discussion sections of this manuscript. CEG contributed significantly to the writing and figure preparation of the 3D chromatin section of this manuscript. All authors provided editorial feedback and approved the final manuscript.

Funding
This work was supported by National Institute of Mental Health (NIMH) R01 MH110360 (Contact PI, BW), NIMH Comprehensive NeuroAIDS Center (CNAC) P30 MH092177 (Kamel Khalili, PI; Brian Wigdahl, PI of the Drexel subcontract), and the Ruth L. Kirschstein National Research Service Award T32 MH079785 (Dr. Brian Wigdahl, Principal Investigator of the Drexel University College of Medicine component). The contents of the paper were solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

Received: 29 January 2025 / Accepted: 4 February 2025
Published online: 08 March 2025

References
1. WHO. HIV data and statistics [Web]. 2023 [Available from: https://www.who.int/teams/global-hiv-hepatitis-and-stis-programmes/hiv/strategic-information/hiv-data-and-statistics
2. CHAI. 2023 HIV Market Report: The state of the HIV market in low- and middle-income countries. 2023.
3. Marcus JL, Leyden WA, Alexeeff SE, Anderson AN, Hechter RC, Hu H, Infection HIV, et al. JAMA Netw Open. 2020;3(6):2000–16.
4. Saylor D, Dickens AM, Sacktor N, Haughey N, Slusher B, Pletnikov M, et al. HIV-associated neurocognitive disorder–pathogenesis and prospects for treatment. Nat Rev Neurol. 2016;12(4):234–48.
5. Li JZ, Aga E, Bosch RJ, Pilkinton M, Kroon E, MacLaren L, et al. Time to viral Rebound after interruption of modern antiretroviral therapies. Clin Infect Dis. 2022;74(5):865–70.
6. Aiamkitsumrit B, Sullivan NT, Nonnemacher MR, Pirrone V, Wigdahl B. Human immunodeficiency virus type 1 Cellular Entry and exit in the T lymphocytic and monocytic compartments: mechanisms and Target opportunities during viral disease. Adv Virus Res. 2015;93:257–311.
7. Moir S, Chun TW, Fauci AS. Pathogenic mechanisms of HIV disease. Annu Rev Pathol. 2011;6:223–48.
8. Mohamed H, Gurrola T, Berman R, Collins M, Sariyer IK, Nonnemacher MR, et al. Targeting CCR5 as a component of an HIV-1 therapeutic strategy. Front Immunol. 2021;12:816515.
9. Aiamkitsumrit B, Dampier W, Antell G, Rivera N, Martin-Garcia J, Pirrone V, et al. Bioinformatic analysis of HIV-1 entry and pathogenesis. Curr HIV Res. 2014;12(2):132–61.

10. Brown PO. Outline of the integration process. In: Coffin JM, Hughes SH, Varmus HE, editors. Retroviruses. New York, NY: Cold Spring Harbor Laboratory Press; 1997.
11. Ciuffi A, Llano M, Poeschla E, Hoffmann C, Leipzig J, Shinn P, et al. A role for LEDGF/p75 in targeting HIV DNA integration. Nat Med. 2005;11(12):1287–9.
12. Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA, Axel R. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell. 1986;47(3):333–48.
13. Glatzová D, Cebecauer M. Dual role of CD4 in Peripheral T lymphocytes. Front Immunol. 2019;10:618.
14. Jordan CA, Watkins BA, Kufta C, Dubois-Dalcq M. Infection of brain microglial cells by human immunodeficiency virus type 1 is CD4 dependent. J Virol. 1991;65(2):736–42.
15. Liu Y, Liu H, Kim BO, Gattone VH, Li J, Nath A, et al. CD4-independent infection of astrocytes by human immunodeficiency virus type 1: requirement for the human mannose receptor. J Virol. 2004;78(8):4120–33.
16. Kilareski EM, Shah S, Nonnemacher MR, Wigdahl B. Regulation of HIV-1 transcription in cells of the monocyte-macrophage lineage. Retrovirology. 2009;6:118.
17. Pirrone V, Mell J, Janto B, Wigdahl B. Biomarkers of HIV susceptibility and Disease Progression. EBioMedicine. 2014;1(2–3):99–100.
18. Stevenson M, Stanwick TL, Dempsey MP, Lamonica CA. HIV-1 replication is controlled at the level of T cell activation and proviral integration. Embo j. 1990;9(5):1551–60.
19. Zhang Z, Schuler T, Zupancic M, Wietgrefe S, Staskus KA, Reimann KA, et al. Sexual transmission and propagation of SIV and HIV in resting and activated CD4 + T cells. Science. 1999;286(5443):1353–7.
20. Tough DF, Rioja I, Modis LK, Prinjha RK. Epigenetic regulation of T cell memory: recalling therapeutic implications. Trends Immunol. 2020;41(1):29–45.
21. van der Windt GJ, Pearce EL. Metabolic switching and fuel choice during T-cell differentiation and memory development. Immunol Rev. 2012;249(1):27–42.
22. Cuddapah S, Barski A, Zhao K. Epigenomics of T cell activation, differentiation, and memory. Curr Opin Immunol. 2010;22(3):341–7.
23. Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 a resolution. Nature. 1997;389(6648):251–60.
24. Richmond TJ, Davey CA. The structure of DNA in the nucleosome core. Nature. 2003;423(6936):145–50.
25. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res. 2011;21(3):381–95.
26. Paranjape SM, Kamakaka RT, Kadonaga JT. Role of chromatin structure in the regulation of transcription by RNA polymerase II. Annu Rev Biochem. 1994;63:265–97.
27. Jiang C, Pugh BF. Nucleosome positioning and gene regulation: advances through genomics. Nat Rev Genet. 2009;10(3):161–72.
28. Ou HD, Phan S, Deerinck TJ, Thor A, Ellisman MH, O'Shea CC. ChromEMT: visualizing 3D chromatin structure and compaction in interphase and mitotic cells. Science. 2017;357(6349).
29. Guelen L, Pagie L, Brasset E, Meuleman W, Faza MB, Talhout W, et al. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature. 2008;453(7197):948–51.
30. Mbonye U, Karn J. Transcriptional control of HIV latency: cellular signaling pathways, epigenetics, happenstance and the hope for a cure. Virology. 2014;454–455:328–39.
31. HIVinfo. The Stages of HIV Infection: National Institutes of Health; 2021 [Available from: https://hivinfo.nih.gov/understanding-hiv/fact-sheets/stages-hiv-infection
32. Alexaki A, Liu Y, Wigdahl B. Cellular reservoirs of HIV-1 and their role in viral persistence. Curr HIV Res. 2008;6(5):388–400.
33. Shirazi J, Shah S, Sagar D, Nonnemacher MR, Wigdahl B, Khan ZK, et al. Epigenetics, drugs of abuse, and the retroviral promoter. J Neuroimmune Pharmacol. 2013;8(5):1181–96.
34. Hildebrand EM, Dekker J. Mechanisms and functions of chromosome compartmentalization. Trends Biochem Sci. 2020;45(5):385–96.
35. Bonev B, Cavalli G. Organization and function of the 3D genome. Nat Rev Genet. 2016;17(11):661–78.
36. Rowley MJ, Corces VG. Organizational principles of 3D genome architecture. Nat Rev Genet. 2018;19(12):789–800.
37. Kempfer R, Pombo A. Methods for mapping 3D chromosome architecture. Nat Rev Genet. 2020;21(4):207–26.
38. Moar P, Premeaux TA, Atkins A, Ndhlovu LC. The latent HIV reservoir: current advances in genetic sequencing approaches. mBio. 2023;14(5):e0134423.
39. Battivelli E, Dahabieh MS, Abdel-Mohsen M, Svensson JP, Tojal Da Silva I, Cohn LB et al. Distinct chromatin functional states correlate with HIV latency reactivation in infected primary CD4(+) T cells. Elife. 2018;7.
40. Le-Bury G, Chen Y, Rhen JM, Grenier JK, Singhal A, Russell DG, et al. HIV-1 active and latent infections induce disparate chromatin reorganization and transcriptional regulation of mRNAs and lncRNAs in SupT1 cells. mBio. 2023;14(6):e0261923.
41. Rheinberger M, Costa AL, Kampmann M, Glavas D, Shytaj IL, Sreeram S, et al. Genomic profiling of HIV-1 integration in microglia cells links viral integration to the topologically associated domains. Cell Rep. 2023;42(2):112110.
42. Marini B, Kertesz-Farkas A, Ali H, Lucic B, Lisek K, Manganaro L, et al. Nuclear architecture dictates HIV-1 integration site selection. Nature. 2015;521(7551):227–31.
43. Lucic B, Chen HC, Kuzman M, Zorita E, Wegner J, Minneker V, et al. Spatially clustered loci with multiple enhancers are frequent targets of HIV-1 integration. Nat Commun. 2019;10(1):4059.
44. Collora JA, Ho YC. Integration site-dependent HIV-1 promoter activity shapes host chromatin conformation. Genome Res. 2023;33(6):891–906.
45. Francis AC, Marin M, Singh PK, Achuthan V, Prellberg MJ, Palermino-Rowland K, et al. HIV-1 replication complexes accumulate in nuclear speckles and integrate into speckle-associated genomic domains. Nat Commun. 2020;11(1):3505.
46. Schröder ARW, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. HIV-1 integration in the Human Genome favors active genes and local hotspots. Cell. 2002;110(4):521–9.
47. Wang GP, Ciuffi A, Leipzig J, Berry CC, Bushman FD. HIV integration site selection: analysis by massively parallel pyrosequencing reveals association with epigenetic modifications. Genome Res. 2007;17(8):1186–94.
48. Brady T, Agosto LM, Malani N, Berry CC, O'Doherty U, Bushman F. HIV integration site distributions in resting and activated CD4 + T cells infected in culture. Aids. 2009;23(12):1461–71.
49. Han Y, Lassen K, Monie D, Sedaghat AR, Shimoji S, Liu X, et al. Resting CD4 + T cells from human immunodeficiency virus type 1 (HIV-1)-infected individuals carry integrated HIV-1 genomes within actively transcribed host genes. J Virol. 2004;78(12):6122–33.
50. Wagner TA, McLaughlin S, Garg K, Cheung CYK, Larsen BB, Styrchak S, et al. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. Science. 2014;345(6196):570–3.
51. Maldarelli F, Wu X, Su L, Simonetti FR, Shao W, Hill S, et al. Specific HIV integration sites are linked to clonal expansion and persistence of infected cells. Science. 2014;345(6193):179–83.
52. Einkauf KB, Lee GQ, Gao C, Sharaf R, Sun X, Hua S, et al. Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. J Clin Invest. 2019;129(3):988–98.
53. Ikeda T, Shibata J, Yoshimura K, Koito A, Matsushita S. Recurrent HIV-1 integration at the BACH2 locus in resting CD4 + T cell populations during effective highly active antiretroviral therapy. J Infect Dis. 2007;195(5):716–25.
54. Cohn LB, Silva IT, Oliveira TY, Rosales RA, Parrish EH, Learn GH, et al. HIV-1 integration landscape during latent and active infection. Cell. 2015;160(3):420–32.
55. Vansant G, Chen HC, Zorita E, Trejbalova K, Miklik D, Filion G, et al. The chromatin landscape at the HIV-1 provirus integration site determines viral expression. Nucleic Acids Res. 2020;48(14):7801–17.
56. Kok YL, Vongrad V, Shilaih M, Di Giallonardo F, Kuster H, Kouyos R, et al. Monocyte-derived macrophages exhibit distinct and more restricted HIV-1 integration site repertoire than CD4 + T cells. Sci Rep. 2016;6(1):24157.
57. Barr SD, Ciuffi A, Leipzig J, Shinn P, Ecker JR, Bushman FD. HIV Integration Site Selection: Targeting in macrophages and the effects of different routes of viral entry. Mol Ther. 2006;14(2):218–25.
58. Plaza-Jennings AL, Valada A, O'Shea C, Iskhakova M, Hu B, Javidfar B, et al. HIV integration in the human brain is linked to microglial activation and 3D genome remodeling. Mol Cell. 2022;82(24):4647–e638.
59. Einkauf KB, Osborn MR, Gao C, Sun W, Sun X, Lian X, et al. Parallel analysis of transcription, integration, and sequence of single HIV-1 proviruses. Cell. 2022;185(2):266–82. e15.
60. Dufour C, Gantner P, Fromentin R, Chomont N. The multifaceted nature of HIV latency. J Clin Invest. 2020;130(7):3381–90.
61. Chen HC, Martinez JP, Zorita E, Meyerhans A, Filion GJ. Position effects influence HIV latency reversal. Nat Struct Mol Biol. 2017;24(1):47–54.

62. Jiang C, Lian X, Gao C, Sun X, Einkauf KB, Chevalier JM, et al. Distinct viral reservoirs in individuals with spontaneous control of HIV-1. Nature. 2020;585(7824):261–7.
63. Lian X, Seiger KW, Parsons EM, Gao C, Sun W, Gladkov GT, et al. Progressive transformation of the HIV-1 reservoir cell profile over two decades of antiviral therapy. Cell Host Microbe. 2023;31(1):83–96. e5.
64. Huang AS, Ramos V, Oliveira TY, Gaebler C, Jankovic M, Nussenzweig MC et al. Integration features of intact latent HIV-1 in CD4 + T cell clones contribute to viral persistence. J Exp Med. 2021;218(12).
65. Lindqvist B, Svensson Akusjarvi S, Sonnerborg A, Dimitriou M, Svensson JP. Chromatin maturation of the HIV-1 provirus in primary resting CD4 + T cells. PLoS Pathog. 2020;16(1):e1008264.
66. Wu VH, Nordin JML, Nguyen S, Joy J, Mampe F, Del Rio Estrada PM, et al. Profound phenotypic and epigenetic heterogeneity of the HIV-1-infected CD4(+) T cell reservoir. Nat Immunol. 2023;24(2):359–70.
67. Shah R, Gallardo CM, Jung YH, Clock B, Dixon JR, McFadden WM, et al. Activation of HIV-1 proviruses increases downstream chromatin accessibility. iScience. 2022;25(12):105490.
68. Datta PK, Kaminski R, Hu W, Pirrone V, Sullivan NT, Nonnemacher MR, et al. HIV-1 latency and eradication: past, Present and Future. Curr HIV Res. 2016;14(5):431–41.
69. Eggers C, Arendt G, Hahn K, Husstedt IW, Maschke M, Neuen-Jacob E, et al. HIV-1-associated neurocognitive disorder: epidemiology, pathogenesis, diagnosis, and treatment. J Neurol. 2017;264(8):1715–27.
70. Human Immunodeficiency Virus (HIV). Transfus Med Hemother. 2016;43(3):203–22.
71. Münch J, Rajan D, Rücker E, Wildum S, Adam N, Kirchhoff F. The role of upstream U3 sequences in HIV-1 replication and CD4 + T cell depletion in human lymphoid tissue ex vivo. Virology. 2005;341(2):313–20.
72. Klaver B, Berkhout B. Comparison of 5' and 3' long terminal repeat promoter function in human immunodeficiency virus. J Virol. 1994;68(6):3830–40.
73. Shah S, Nonnemacher MR, Pirrone V, Wigdahl B. Innate and adaptive factors regulating human immunodeficiency virus type 1 genomic activation. J Neuroimmune Pharmacol. 2010;5(3):278–93.
74. Cherrington J, Ganem D. Regulation of polyadenylation in human immunodeficiency virus (HIV): contributions of promoter proximity and upstream sequences. Embo j. 1992;11(4):1513–24.
75. Li L, Dahiya S, Kortagere S, Aiamkitsumrit B, Cunningham D, Pirrone V, et al. Impact of Tat Genetic Variation on HIV-1 Disease. Adv Virol. 2012;2012:123605.
76. Verdin E. DNase I-hypersensitive sites are associated with both long terminal repeats and with the intragenic enhancer of integrated human immunodeficiency virus type 1. J Virol. 1991;65(12):6790–9.
77. Verdin E, Paras P Jr., Van Lint C. Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. Embo j. 1993;12(8):3249–59.
78. Rafati H, Parra M, Hakre S, Moshkin Y, Verdin E, Mahmoudi T. Repressive LTR nucleosome positioning by the BAF complex is required for HIV latency. PLoS Biol. 2011;9(11):e1001206.
79. Rafati H, Parra M, Hakre S, Moshkin Y, Verdin E, Mahmoudi T. Correction: repressive LTR nucleosome positioning by the BAF Complex is required for HIV latency. PLoS Biol. 2015;13(11):e1002302.
80. Machida S, Depierre D, Chen HC, Thenin-Houssier S, Petitjean G, Doyen CM, et al. Exploring histone loading on HIV DNA reveals a dynamic nucleosome positioning between unintegrated and integrated viral genome. Proc Natl Acad Sci U S A. 2020;117(12):6822–30.
81. Wang GZ, Wang Y, Goff SP. Histones are rapidly loaded onto unintegrated retroviral DNAs soon after Nuclear Entry. Cell Host Microbe. 2016;20(6):798–809.
82. Li R, Caico I, Xu Z, Iqbal MS, Romerio F. Epigenetic regulation of HIV-1 sense and antisense transcription in response to latency-reversing agents. Noncoding RNA. 2023;9(1).
83. Jefferys SR, Burgos SD, Peterson JJ, Selitsky SR, Turner AW, James LI, et al. Epigenomic characterization of latent HIV infection identifies latency regulating transcription factors. PLoS Pathog. 2021;17(2):e1009346.
84. Van Lint C, Emiliani S, Ott M, Verdin E. Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. Embo j. 1996;15(5):1112–20.
85. Tang L, Nogales E, Ciferri C. Structure and function of SWI/SNF chromatin remodeling complexes and mechanistic implications for transcription. Prog Biophys Mol Biol. 2010;102(2–3):122–8.
86. Xue Y, Canman JC, Lee CS, Nie Z, Yang D, Moreno GT, et al. The human SWI/SNF-B chromatin-remodeling complex is related to yeast rsc and localizes at kinetochores of mitotic chromosomes. Proc Natl Acad Sci U S A. 2000;97(24):13015–20.
87. Steger DJ, Workman JL. Stable co-occupancy of transcription factors and histones at the HIV-1 enhancer. Embo j. 1997;16(9):2463–72.
88. Xi L, Fondufe-Mittendorf Y, Xia L, Flatow J, Widom J, Wang JP. Predicting nucleosome positioning using a duration hidden Markov Model. BMC Bioinformatics. 2010;11:346.
89. Van Duyne R, Guendel I, Narayanan A, Gregg E, Shafagati N, Tyagi M, et al. Varying modulation of HIV-1 LTR activity by Baf complexes. J Mol Biol. 2011;411(3):581–96.
90. Bisgrove DA, Mahmoudi T, Henklein P, Verdin E. Conserved P-TEFb-interacting domain of BRD4 inhibits HIV transcription. Proc Natl Acad Sci U S A. 2007;104(34):13690–5.
91. Conrad RJ, Fozouni P, Thomas S, Sy H, Zhang Q, Zhou MM, et al. The short isoform of BRD4 promotes HIV-1 latency by engaging repressive SWI/SNF chromatin-remodeling complexes. Mol Cell. 2017;67(6):1001–e126.
92. Tréand C, du Chéné I, Brès V, Kiernan R, Benarous R, Benkirane M, et al. Requirement for SWI/SNF chromatin-remodeling complex in Tat-mediated activation of the HIV-1 promoter. Embo j. 2006;25(8):1690–9.
93. Mizutani T, Ishizaka A, Tomizawa M, Okazaki T, Yamamichi N, Kawana-Tachikawa A, et al. Loss of the Brm-type SWI/SNF chromatin remodeling complex is a strong barrier to the Tat-independent transcriptional elongation of human immunodeficiency virus type 1 transcripts. J Virol. 2009;83(22):11569–80.
94. Reisman DN, Sciarrotta J, Bouldin TW, Weissman BE, Funkhouser WK. The expression of the SWI/SNF ATPase subunits BRG1 and BRM in normal human tissues. Appl Immunohistochem Mol Morphol. 2005;13(1):66–74.
95. Henderson A, Holloway A, Reeves R, Tremethick DJ. Recruitment of SWI/SNF to the human immunodeficiency virus type 1 promoter. Mol Cell Biol. 2004;24(1):389–97.
96. Easley R, Carpio L, Dannenberg L, Choi S, Alani D, Van Duyne R, et al. Transcription through the HIV-1 nucleosomes: effects of the PBAF complex in Tat activated transcription. Virology. 2010;405(2):322–33.
97. Agbottah E, Deng L, Dannenberg LO, Pumfery A, Kashanchi F. Effect of SWI/SNF chromatin remodeling complex on HIV-1 Tat activated transcription. Retrovirology. 2006;3:48.
98. Mahmoudi T, Parra M, Vries RG, Kauder SE, Verrijzer CP, Ott M, et al. The SWI/SNF chromatin-remodeling complex is a cofactor for Tat transactivation of the HIV promoter. J Biol Chem. 2006;281(29):19960–8.
99. Shen W, Xu C, Huang W, Zhang J, Carlson JE, Tu X, et al. Solution structure of human Brg1 bromodomain and its specific binding to acetylated histone tails. Biochemistry. 2007;46(8):2100–10.
100. Kalpana GV, Marmon S, Wang W, Crabtree GR, Goff SP. Binding and stimulation of HIV-1 integrase by a human homolog of yeast transcription factor SNF5. Science. 1994;266(5193):2002–6.
101. Ariumi Y, Serhan F, Turelli P, Telenti A, Trono D. The integrase interactor 1 (INI1) proteins facilitate Tat-mediated human immunodeficiency virus type 1 transcription. Retrovirology. 2006;3:47.
102. Dixit U, Bhutoria S, Wu X, Qiu L, Spira M, Mathew S, et al. INI1/SMARCB1 Rpt1 domain mimics TAR RNA in binding to integrase to facilitate HIV-1 replication. Nat Commun. 2021;12(1):2743.
103. Gurova K, Chang HW, Valieva ME, Sandlesh P, Studitsky VM. Structure and function of the histone chaperone FACT - resolving FACTual issues. Biochim Biophys Acta Gene Regul Mech; 2018.
104. Huang H, Santoso N, Power D, Simpson S, Dieringer M, Miao H, et al. FACT proteins, SUPT16H and SSRP1, are transcriptional suppressors of HIV-1 and HTLV-1 that facilitate viral latency. J Biol Chem. 2015;290(45):27297–310.
105. Jean MJ, Hayashi T, Huang H, Brennan J, Simpson S, Purmal A, et al. Curaxin CBL0100 blocks HIV-1 replication and reactivation through inhibition of viral transcriptional elongation. Front Microbiol. 2017;8:2007.
106. Jean MJ, Zhou D, Fiches G, Kong W, Huang H, Purmal A, et al. Curaxin CBL0137 has the potential to reverse HIV-1 latency. J Med Virol. 2019;91(8):1571–6.
107. Turner AW, Margolis DM. Chromatin regulation and the histone code in HIV latency Yale. J Biol Med. 2017;90(2):229–43.
108. Gillette TG, Hill JA. Readers, writers, and erasers: chromatin as the whiteboard of heart disease. Circ Res. 2015;116(7):1245–53.
109. Park SY, Kim JS. A short guide to histone deacetylases including recent progress on class II enzymes. Exp Mol Med. 2020;52(2):204–12.
110. Keedy KS, Archin NM, Gates AT, Espeseth A, Hazuda DJ, Margolis DM. A limited group of class I histone deacetylases acts to repress human immunodeficiency virus type 1 expression. J Virol. 2009;83(10):4749–56.

111. Williams SA, Chen LF, Kwon H, Ruiz-Jarabo CM, Verdin E, Greene WC. NF-kappaB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation. Embo j. 2006;25(1):139–49.
112. He G, Margolis DM. Counterregulation of chromatin deacetylation and histone deacetylase occupancy at the integrated promoter of human immunodeficiency virus type 1 (HIV-1) by the HIV-1 repressor YY1 and HIV-1 activator Tat. Mol Cell Biol. 2002;22(9):2965–73.
113. Tie F, Prasad-Sinha J, Birve A, Rasmuson-Lestander A, Harte PJ. A 1-megadalton ESC/E(Z) complex from Drosophila that contains polycomblike and RPD3. Mol Cell Biol. 2003;23(9):3352–62.
114. Zhang Y, Ng HH, Erdjument-Bromage H, Tempst P, Bird A, Reinberg D. Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. Genes Dev. 1999;13(15):1924–35.
115. Kato H, Horikoshi M, Roeder RG. Repression of HIV-1 transcription by a cellular protein. Science. 1991;251(5000):1476–9.
116. Yoon JB, Li G, Roeder RG. Characterization of a family of related cellular transcription factors which can modulate human immunodeficiency virus type 1 transcription in vitro. Mol Cell Biol. 1994;14(3):1776–85.
117. Parada CA, Yoon JB, Roeder RG. A novel LBP-1-mediated restriction of HIV-1 transcription at the level of elongation in vitro. J Biol Chem. 1995;270(5):2274–83.
118. Romerio F, Gabriel MN, Margolis DM. Repression of human immunodeficiency virus type 1 through the novel cooperation of human factors YY1 and LSF. J Virol. 1997;71(12):9375–82.
119. Margolis DM, Somasundaran M, Green MR. Human transcription factor YY1 represses human immunodeficiency virus type 1 transcription and virion production. J Virol. 1994;68(2):905–10.
120. Coull JJ, Romerio F, Sun JM, Volker JL, Galvin KM, Davie JR, et al. The human factors YY1 and LSF repress the human immunodeficiency virus type 1 long terminal repeat via recruitment of histone deacetylase 1. J Virol. 2000;74(15):6790–9.
121. Dong S, Ma X, Wang Z, Han B, Zou H, Wu Z, et al. YY1 promotes HDAC1 expression and decreases sensitivity of hepatocellular carcinoma cells to HDAC inhibitor. Oncotarget. 2017;8(25):40583–93.
122. Jiang G, Espeseth A, Hazuda DJ, Margolis DM. c-Myc and Sp1 contribute to proviral latency by recruiting histone deacetylase 1 to the human immunodeficiency virus type 1 promoter. J Virol. 2007;81(20):10914–23.
123. Stojanova A, Caro C, Jarjour RJ, Oster SK, Penn LZ, Germinario RJ. Repression of the human immunodeficiency virus type-1 long terminal repeat by the c-Myc oncoprotein. J Cell Biochem. 2004;92(2):400–13.
124. Parisi F, Wirapati P, Naef F. Identifying synergistic regulation involving c-Myc and sp1 in human tissues. Nucleic Acids Res. 2007;35(4):1098–107.
125. Kyo S, Takakura M, Taira T, Kanaya T, Itoh H, Yutsudo M, et al. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). Nucleic Acids Res. 2000;28(3):669–77.
126. Blackwell TK, Huang J, Ma A, Kretzner L, Alt FW, Eisenman RN, et al. Binding of myc proteins to canonical and noncanonical DNA sequences. Mol Cell Biol. 1993;13(9):5216–24.
127. Ou SH, Garcia-Martínez LF, Paulssen EJ, Gaynor RB. Role of flanking E box motifs in human immunodeficiency virus type 1 TATA element function. J Virol. 1994;68(11):7188–99.
128. Perkins ND, Edwards NL, Duckett CS, Agranoff AB, Schmid RM, Nabel GJ. A cooperative interaction between NF-kappa B and Sp1 is required for HIV-1 enhancer activation. Embo j. 1993;12(9):3551–8.
129. Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol. 2009;1(4):a000034.
130. Tyagi M, Karn J. CBF-1 promotes transcriptional silencing during the establishment of HIV-1 latency. Embo j. 2007;26(24):4985–95.
131. Lai EC. Keeping a good pathway down: transcriptional repression of notch pathway target genes by CSL proteins. EMBO Rep. 2002;3(9):840–5.
132. Hu YF, Lüscher B, Admon A, Mermod N, Tjian R. Transcription factor AP-4 contains multiple dimerization domains that regulate dimer specificity. Genes Dev. 1990;4(10):1741–52.
133. Wong MM, Joyson SM, Hermeking H, Chiu SK. Transcription factor AP4 mediates cell fate decisions: to divide, Age, or die. Cancers (Basel). 2021;13(4).
134. Imai K, Okamoto T. Transcriptional repression of human immunodeficiency virus type 1 by AP-4. J Biol Chem. 2006;281(18):12495–505.
135. Basta J, Rauchman M. The nucleosome remodeling and deacetylase complex in development and disease. Transl Res. 2015;165(1):36–47.
136. Cismasiu VB, Adamo K, Gecewicz J, Duque J, Lin Q, Avram D. BCL11B functionally associates with the NuRD complex in T lymphocytes to repress targeted promoter. Oncogene. 2005;24(45):6753–64.
137. Cismasiu VB, Paskaleva E, Suman Daya S, Canki M, Duus K, Avram D. BCL11B is a general transcriptional repressor of the HIV-1 long terminal repeat in T lymphocytes through recruitment of the NuRD complex. Virology. 2008;380(2):173–81.
138. Marban C, Suzanne S, Dequiedt F, de Walque S, Redel L, Van Lint C, et al. Recruitment of chromatin-modifying enzymes by CTIP2 promotes HIV-1 transcriptional silencing. Embo j. 2007;26(2):412–23.
139. Bogoi RN, de Pablo A, Valencia E, Martín-Carbonero L, Moreno V, Vilchez-Rueda HH, et al. Expression profiling of chromatin-modifying enzymes and global DNA methylation in CD4 + T cells from patients with chronic HIV infection at different HIV control and progression states. Clin Epigenetics. 2018;10:20.
140. Lusic M, Marcello A, Cereseto A, Giacca M. Regulation of HIV-1 gene expression by histone acetylation and factor recruitment at the LTR promoter. Embo j. 2003;22(24):6550–61.
141. Marzio G, Tyagi M, Gutierrez MI, Giacca M. HIV-1 tat transactivator recruits p300 and CREB-binding protein histone acetyltransferases to the viral promoter. Proc Natl Acad Sci U S A. 1998;95(23):13519–24.
142. Hottiger MO, Nabel GJ. Interaction of human immunodeficiency virus type 1 Tat with the transcriptional coactivators p300 and CREB binding protein. J Virol. 1998;72(10):8252–6.
143. Benkirane M, Chun RF, Xiao H, Ogryzko VV, Howard BH, Nakatani Y, et al. Activation of integrated provirus requires histone acetyltransferase. p300 and P/CAF are coactivators for HIV-1 Tat. J Biol Chem. 1998;273(38):24898–905.
144. Pazin MJ, Sheridan PL, Cannon K, Cao Z, Keck JG, Kadonaga JT, et al. NF-kappa B-mediated chromatin reconfiguration and transcriptional activation of the HIV-1 enhancer in vitro. Genes Dev. 1996;10(1):37–49.
145. Alexaki A, Quiterio SJ, Liu Y, Irish B, Kilareski E, Nonnemacher MR, et al. PMA-induced differentiation of a bone marrow progenitor cell line activates HIV-1 LTR-driven transcription. DNA Cell Biol. 2007;26(6):387–94.
146. Widlak P, Gaynor RB, Garrard WT. In vitro chromatin assembly of the HIV-1 promoter. ATP-dependent polar repositioning of nucleosomes by Sp1 and NFkappaB. J Biol Chem. 1997;272(28):17654–61.
147. Sheridan PL, Mayall TP, Verdin E, Jones KA. Histone acetyltransferases regulate HIV-1 enhancer activity in vitro. Genes Dev. 1997;11(24):3327–40.
148. Gerritsen ME, Williams AJ, Neish AS, Moore S, Shi Y, Collins T. CREB-binding protein/p300 are transcriptional coactivators of p65. Proc Natl Acad Sci U S A. 1997;94(7):2927–32.
149. Miller JL, Grant PA. The role of DNA methylation and histone modifications in transcriptional regulation in humans. Subcell Biochem. 2013;61:289–317.
150. Fischer S, Weber LM, Liefke R. Evolutionary adaptation of the polycomb repressive complex 2. Epigenetics Chromatin. 2022;15(1):7.
151. Sankar A, Mohammad F, Sundaramurthy AK, Wang H, Lerdrup M, Tatar T, et al. Histone editing elucidates the functional roles of H3K27 methylation and acetylation in mammals. Nat Genet. 2022;54(6):754–60.
152. Satijn DP, Hamer KM, den Blaauwen J, Otte AP. The polycomb group protein EED interacts with YY1, and both proteins induce neural tissue in Xenopus embryos. Mol Cell Biol. 2001;21(4):1360–9.
153. Wilkinson F, Pratt H, Atchison ML. PcG recruitment by the YY1 REPO domain can be mediated by Yaf2. J Cell Biochem. 2010;109(3):478–86.
154. Friedman J, Cho WK, Chu CK, Keedy KS, Archin NM, Margolis DM, et al. Epigenetic silencing of HIV-1 by the histone H3 lysine 27 methyltransferase enhancer of Zeste 2. J Virol. 2011;85(17):9078–89.
155. Tripathy MK, McManamy ME, Burch BD, Archin NM, Margolis DM. H3K27 demethylation at the Proviral promoter sensitizes latent HIV to the effects of Vorinostat in Ex vivo cultures of resting CD4 + T cells. J Virol. 2015;89(16):8392–405.
156. Sharma AL, Hokello J, Sonti S, Zicari S, Sun L, Alqatawni A et al. CBF-1 promotes the establishment and maintenance of HIV latency by Recruiting Polycomb Repressive Complexes, PRC1 and PRC2, at HIV LTR. Viruses. 2020;12(9).
157. Matsuda Y, Kobayashi-Ishihara M, Fujikawa D, Ishida T, Watanabe T, Yamagishi M. Epigenetic heterogeneity in HIV-1 latency establishment. Sci Rep. 2015;5:7701.
158. Zhang HS, Liu Y, Wu TC, Du GY, Zhang FJ. EZH2 phosphorylation regulates Tat-induced HIV-1 transactivation via ROS/Akt signaling pathway. FEBS Lett. 2015;589(24 Pt B):4106–11.
159. Nguyen K, Das B, Dobrowolski C, Karn J. Multiple histone lysine methyltransferases are required for the establishment and maintenance of HIV-1 latency. mBio. 2017;8(1).
160. Cha TL, Zhou BP, Xia W, Wu Y, Yang CC, Chen CT, et al. Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. Science. 2005;310(5746):306–10.

161. Zhang HS, Du GY, Liu Y, Zhang ZG, Zhou Z, Li H, et al. UTX-1 regulates Tat-induced HIV-1 transactivation via changing the methylated status of histone H3. Int J Biochem Cell Biol. 2016;80:51–6.
162. Padeken J, Methot SP, Gasser SM. Establishment of H3K9-methylated heterochromatin and its functions in tissue differentiation and maintenance. Nat Rev Mol Cell Biol. 2022;23(9):623–40.
163. Imai K, Togami H, Okamoto T. Involvement of histone H3 lysine 9 (H3K9) methyltransferase G9a in the maintenance of HIV-1 latency and its reactivation by BIX01294. J Biol Chem. 2010;285(22):16538–45.
164. du Chéné I, Basyuk E, Lin YL, Triboulet R, Knezevich A, Chable-Bessia C, et al. Suv39H1 and HP1gamma are responsible for chromatin-mediated HIV-1 transcriptional silencing and post-integration latency. Embo j. 2007;26(2):424–35.
165. Shilatifard A. The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. Annu Rev Biochem. 2012;81:65–95.
166. Hyun K, Jeon J, Park K, Kim J. Writing, erasing and reading histone lysine methylations. Exp Mol Med. 2017;49(4):e324.
167. Zardo G. The role of H3K4 trimethylation in CpG islands Hypermethylation in Cancer. Biomolecules. 2021;11(2).
168. Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, et al. The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet. 2009;41(1):125–9.
169. Le Douce V, Colin L, Redel L, Cherrier T, Herbein G, Aunis D, et al. LSD1 cooperates with CTIP2 to promote HIV-1 transcriptional silencing. Nucleic Acids Res. 2012;40(5):1904–15.
170. Brown MA, Sims RJ 3rd, Gottlieb PD, Tucker PW. Identification and characterization of Smyd2: a split SET/MYND domain-containing histone H3 lysine 36-specific methyltransferase that interacts with the Sin3 histone deacetylase complex. Mol Cancer. 2006;5:26.
171. Abu-Farha M, Lambert JP, Al-Madhoun AS, Elisma F, Skerjanc IS, Figeys D. The tale of two domains: proteomics and genomics analysis of SMYD2, a new histone methyltransferase. Mol Cell Proteom. 2008;7(3):560–72.
172. Wu J, Cheung T, Grande C, Ferguson AD, Zhu X, Theriault K, et al. Biochemical characterization of human SET and MYND domain-containing protein 2 methyltransferase. Biochemistry. 2011;50(29):6488–97.
173. Boehm D, Jeng M, Camus G, Gramatica A, Schwarzer R, Johnson JR, et al. SMYD2-Mediated histone methylation contributes to HIV-1 latency. Cell Host Microbe. 2017;21(5):569–e796.
174. Trojer P, Li G, Sims RJ 3rd, Vaquero A, Kalakonda N, Boccuni P, et al. L3MBTL1, a histone-methylation-dependent chromatin lock. Cell. 2007;129(5):915–28.
175. Stender JD, Pascual G, Liu W, Kaikkonen MU, Do K, Spann NJ, et al. Control of proinflammatory gene programs by regulated trimethylation and demethylation of histone H4K20. Mol Cell. 2012;48(1):28–38.
176. Aljazi MB, Gao Y, Wu Y, He J. SMYD5 is a histone H3-specific methyltransferase mediating mono-methylation of histone H3 lysine 36 and 37. Biochem Biophys Res Commun. 2022;599:142–7.
177. Zhang Y, Fang Y, Tang Y, Han S, Jia J, Wan X, et al. SMYD5 catalyzes histone H3 lysine 36 trimethylation at promoters. Nat Commun. 2022;13(1):3190.
178. Boehm D, Lam V, Schnolzer M, Ott M. The lysine methyltransferase SMYD5 amplifies HIV-1 transcription and is post-transcriptionally upregulated by Tat and USP11. Cell Rep. 2023;42(3):112234.
179. Di Lorenzo A, Bedford MT. Histone arginine methylation. FEBS Lett. 2011;585(13):2024–31.
180. Shishkova E, Zeng H, Liu F, Kwiecien NW, Hebert AS, Coon JJ, et al. Global mapping of CARM1 substrates defines enzyme specificity and substrate recognition. Nat Commun. 2017;8:15571.
181. Covic M, Hassa PO, Saccani S, Buerki C, Meier NI, Lombardi C, et al. Arginine methyltransferase CARM1 is a promoter-specific regulator of NF-kappaB-dependent gene expression. Embo j. 2005;24(1):85–96.
182. Hassa PO, Covic M, Bedford MT, Hottiger MO. Protein arginine methyltransferase 1 coactivates NF-kappaB-dependent gene expression synergistically with CARM1 and PARP1. J Mol Biol. 2008;377(3):668–78.
183. Tang J, Frankel A, Cook RJ, Kim S, Paik WK, Williams KR, et al. PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. J Biol Chem. 2000;275(11):7723–30.
184. Zhang Z, Nikolai BC, Gates LA, Jung SY, Siwak EB, He B, et al. Crosstalk between histone modifications indicates that inhibition of arginine methyltransferase CARM1 activity reverses HIV latency. Nucleic Acids Res. 2017;45(16):9348–60.
185. Alexandrow MG, Hamlin JL. Chromatin decondensation in S-phase involves recruitment of Cdk2 by Cdc45 and histone H1 phosphorylation. J Cell Biol. 2005;168(6):875–86.
186. O'Brien SK, Cao H, Nathans R, Ali A, Rana TM. P-TEFb kinase complex phosphorylates histone H1 to regulate expression of cellular and HIV-1 genes. J Biol Chem. 2010;285(39):29713–20.
187. Sabari BR, Tang Z, Huang H, Yong-Gonzalez V, Molina H, Kong HE, et al. Intracellular crotonyl-CoA stimulates transcription through p300-catalyzed histone crotonylation. Mol Cell. 2015;58(2):203–15.
188. Ntorla A, Burgoyne JR. The regulation and function of histone crotonylation. Front Cell Dev Biol. 2021;9:624914.
189. Jiang G, Nguyen D, Archin NM, Yukl SA, Méndez-Lagares G, Tang Y, et al. HIV latency is reversed by ACSS2-driven histone crotonylation. J Clin Invest. 2018;128(3):1190–8.
190. Lutgen V, Narasipura SD, Barbian HJ, Richards M, Wallace J, Razmpour R, et al. HIV infects astrocytes in vivo and egresses from the brain to the periphery. PLoS Pathog. 2020;16(6):e1008381.
191. Marino J, Maubert ME, Mele AR, Spector C, Wigdahl B, Nonnemacher MR. Functional impact of HIV-1 Tat on cells of the CNS and its role in HAND. Cell Mol Life Sci. 2020;77(24):5079–99.
192. Zerbato JM, Avihingsanon A, Singh KP, Zhao W, Deleage C, Rosen E, et al. HIV DNA persists in hepatocytes in people with HIV-hepatitis B co-infection on antiretroviral therapy. EBioMedicine. 2023;87:104391.
193. Srikanth S, Ramachandran S, Mohan SS. Construction of the gene regulatory network identifies MYC as a transcriptional regulator of SWI/SNF complex. Sci Rep. 2020;10(1):158.
194. Wang W, Xue Y, Zhou S, Kuo A, Cairns BR, Crabtree GR. Diversity and specialization of mammalian SWI/SNF complexes. Genes Dev. 1996;10(17):2117–30.
195. Song M, Pebworth MP, Yang X, Abnousi A, Fan C, Wen J, et al. Cell-type-specific 3D epigenomes in the developing human cortex. Nature. 2020;587(7835):644–9.
196. Winick-Ng W, Kukalev A, Harabula I, Zea-Redondo L, Szabó D, Meijer M, et al. Cell-type specialization is encoded by specific chromatin topologies. Nature. 2021;599(7886):684–91.
197. Takei Y, Zheng S, Yun J, Shah S, Pierson N, White J, et al. Single-cell nuclear architecture across cell types in the mouse brain. Science. 2021;374(6567):586–94.
198. Dampier W, Nonnemacher MR, Mell J, Earl J, Ehrlich GD, Pirrone V, et al. HIV-1 Genetic Variation resulting in the development of New quasispecies continues to be encountered in the Peripheral blood of well-suppressed patients. PLoS ONE. 2016;11(5):e0155382.
199. Bruner KM, Murray AJ, Pollack RA, Soliman MG, Laskey SB, Capoferri AA, et al. Defective proviruses rapidly accumulate during acute HIV-1 infection. Nat Med. 2016;22(9):1043–9.
200. Nonnemacher MR, Pirrone V, Feng R, Moldover B, Passic S, Aiamkitsumrit B, et al. HIV-1 promoter single nucleotide polymorphisms are Associated with Clinical Disease Severity. PLoS ONE. 2016;11(4):e0150835.
201. Nonnemacher MR, Irish BP, Liu Y, Mauger D, Wigdahl B. Specific sequence configurations of HIV-1 LTR G/C box array result in altered recruitment of sp isoforms and correlate with disease progression. J Neuroimmunol. 2004;157(1–2):39–47.
202. Ahlenstiel CL, Symonds G, Kent SJ, Kelleher AD. Block and Lock HIV cure strategies to Control the Latent Reservoir. Front Cell Infect Microbiol. 2020;10:424.
203. Kim Y, Anderson JL, Lewin SR. Getting the kill into shock and kill: strategies to Eliminate Latent HIV. Cell Host Microbe. 2018;23(1):14–26.
204. Spina CA, Anderson J, Archin NM, Bosque A, Chan J, Famiglietti M, et al. An in-depth comparison of latent HIV-1 reactivation in multiple cell model systems and resting CD4 + T cells from aviremic patients. PLoS Pathog. 2013;9(12):e1003834.
205. Allen AG, Chung CH, Atkins A, Dampier W, Khalili K, Nonnemacher MR, et al. Gene editing of HIV-1 co-receptors to prevent and/or cure virus infection. Front Microbiol. 2018;9:2940.
206. Atkins AJ, Allen AG, Dampier W, Haddad EK, Nonnemacher MR, Wigdahl B. HIV-1 cure strategies: why CRISPR? Expert Opin Biol Ther. 2021;21(6):781–93.
207. Berman RE, Dampier W, Nonnemacher MR, Wigdahl B. What's in a cure: designing a broad-spectrum HIV gene therapy. Curr Opin HIV AIDS. 2024;19(3):150–6.
208. Allen AG, Chung CH, Worrell SD, Nwaozo G, Madrid R, Mele AR, et al. Assessment of anti-HIV-1 guide RNA efficacy in cells containing the viral target sequence, corresponding gRNA, and CRISPR/Cas9. Front Genome Ed. 2023;5:1101483.
209. Kaminski R, Chen Y, Fischer T, Tedaldi E, Napoli A, Zhang Y, et al. Corrigendum: elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. Sci Rep. 2016;6:28213.

210. Kaminski R, Chen Y, Fischer T, Tedaldi E, Napoli A, Zhang Y, et al. Elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. Sci Rep. 2016;6:22555.
211. Chung CH, Allen AG, Atkins AJ, Sullivan NT, Homan G, Costello R, et al. Safe CRISPR-Cas9 inhibition of HIV-1 with high specificity and broad-spectrum activity by targeting LTR NF-κB binding sites. Mol Ther Nucleic Acids. 2020;21:965–82.
212. Sullivan NT, Dampier W, Chung CH, Allen AG, Atkins A, Pirrone V, et al. Novel gRNA design pipeline to develop broad-spectrum CRISPR/Cas9 gRNAs for safe targeting of the HIV-1 quasispecies in patients. Sci Rep. 2019;9(1):17088.
213. Chung CH, Allen AG, Sullivan NT, Atkins A, Nonnemacher MR, Wigdahl B, et al. Computational analysis concerning the impact of DNA accessibility on CRISPR-Cas9 cleavage efficiency. Mol Ther. 2020;28(1):19–28.
214. Isaac RS, Jiang F, Doudna JA, Lim WA, Narlikar GJ, Almeida R. Nucleosome breathing and remodeling constrain CRISPR-Cas9 function. Elife. 2016;5.
215. Chen X, Rinsma M, Janssen JM, Liu J, Maggio I, Gonçalves MA. Probing the impact of chromatin conformation on genome editing tools. Nucleic Acids Res. 2016;44(13):6482–92.
216. Yarrington RM, Verma S, Schwartz S, Trautman JK, Carroll D. Nucleosomes inhibit target cleavage by CRISPR-Cas9 in vivo. Proc Natl Acad Sci U S A. 2018;115(38):9351–8.
217. Chung CH, Allen AG, Atkins A, Link RW, Nonnemacher MR, Dampier W, et al. Computational design of gRNAs Targeting Genetic variants across HIV-1 subtypes for CRISPR-Mediated antiviral therapy. Front Cell Infect Microbiol. 2021;11:593077.
218. Dampier W, Berman R, Nonnemacher MR, Wigdahl B. Computational analysis of cas proteins unlocks new potential in HIV-1 targeted gene therapy. Front Genome Ed. 2023;5:1248982.
219. Liu B, Chen S, Rose A, Chen D, Cao F, Zwinderman M, et al. Inhibition of histone deacetylase 1 (HDAC1) and HDAC2 enhances CRISPR/Cas9 genome editing. Nucleic Acids Res. 2020;48(2):517–32.
220. Liu N, Zhou L, Lin G, Hu Y, Jiao Y, Wang Y, et al. HDAC inhibitors improve CRISPR-Cas9 mediated prime editing and base editing. Mol Ther Nucleic Acids. 2022;29:36–46.
221. Park H, Shin J, Choi H, Cho B, Kim J. Valproic acid significantly improves CRISPR/Cas9-Mediated gene editing. Cells. 2020;9(6).

Publisher's note