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REVIEW

Molecular Mechanisms of HIV-1 Latency from a Chromatin and Epigenetic Perspective

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Abstract

Purpose of Review The main obstacle to an HIV-1 cure is the reservoir of HIV-1 infected cells. While antiretroviral therapy (ART) eliminates the HIV-1 virus effectively, it does not target the reservoir. To eliminate infected cells, we need an improved understanding of the reservoir maintenance and reactivation mechanisms, including the influence of chromatin.

Recent Findings The last years' technological advances enable an in-depth study of the reservoir, uncovering subsets of infected cells, proviral integration sites, and single-cell nucleosome histone modifications. These revelations illustrate how the immune system and cell proliferation shape reservoirs under long-term ART. These forces create highly individual reservoirs that will require personalized treatment for their eradication.

Summary A greater understanding of HIV-1 latency mechanisms, focusing on chromatin features, proviral reservoir dynamics, and inter-individual differences, can drive the development of more precise HIV-1 treatment strategies, ultimately achieving a globally available HIV-1 cure.

Keywords HIV-1 latency · Chromatin structure · Epigenetics · Proviruses · HIV-1 reservoir · Inter-individual variability

Introduction

An HIV-1 cure is prevented by the reservoir of infected cells. Whereas antiretroviral therapy is highly effective at eliminating the HIV-1 virus, it does not target the reservoir. Most people currently living with HIV-1 are under antiretroviral therapy and virally suppressed [1]. In these individuals, free infectious virus is virtually eliminated, but the reservoir persists. In the latent reservoir of HIV-1 infected cells, the provirus is silent, which makes these cells challenging to detect, both for the immune system and for therapeutic interventions. It is recognized that chromatin structure and composition play a crucial role in the formation and maintenance of proviral latency, as well as the reactivation capability of latently infected cells [2–5].

An understanding of the molecular mechanisms regulating HIV-1 latency from a chromatin and epigenetic perspective has the potential of opening avenues for novel therapeutic strategies targeting the reservoir of HIV-1 infected cells. Key aspects include the role of chromatin structure in proviral latency establishment, reactivation ability, proliferation potential of infected cells, and evasion of the immune system. Technical advances in the last years have enabled single cell determination of individual proviral sequences together with the integration sites [6–8], in combination with cell characteristics and epigenetic characterization of individual proviruses in single cells [9•, 10•]. Here, we summarize some of the last year's findings regarding HIV-1 latency mechanisms, focusing on chromatin features, the dynamics of the proviral reservoir, and inter-individual differences that may be exploited for personalized medicine.

Initial Infection and Establishment of the Reservoir

The acute phase of the HIV-1 infection, signifying the initial weeks post-HIV-1 acquisition, is associated with a rapid rise in plasma viremia and is critical to the long-term development of the infection [11, 12]. During this period, the virus inflicts substantial immunological damage and establishes a persistent reservoir of infected cells. HIV-1 rapidly targets

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a diverse pool of CD4 + T cells to establish both productive and latent infections [13•]. While HIV-1 mainly infects activated CD4 + T cells, it can also infect cells that do not actively proliferate [14–16]. The set of cells being infected develops throughout the acute stages of HIV-1 infection (Fiebig I-V), exhibiting differences between cells in blood and lymphatic compartments [17••]. Early antiretroviral therapy (ART) has a significant effect on the reservoir size in Fiebig stage II/III [17••]. As the infection transits from the acute to the chronic phase, target cell types diverge [13•, 18]. Ultimately, the latent HIV-1 reservoir persists mainly in a small population of long-lived memory CD4 + T cells and macrophages [19–21, 22••].

Initial HIV-1 infection triggers a potent immune response. However, the establishment of the viral reservoir precedes seroconversion (Fiebig I-II) and the maturation of the adaptive immune response. Instead, the innate immune response is triggered at these earliest stages of infection [23]. Here, dendritic cells recognize viral RNA via Toll-like receptors (TLRs), specifically TLR7 and TLR8, that trigger the release of proinflammatory cytokines and type I interferons. This in turn stimulates CD4 + T cells [24], promoting their infection and establishment of the reservoir. TLR7 agonists have emerged as promising HIV-1 latency reversal agents, with efficacy demonstrated in primate studies [25, 26•]. Once the adaptive immune response is trained, the initial reservoir is already established [13•, 17••].

The infection creates a diverse initial reservoir of cells with unique integration sites. Within the reservoir, only a few cells have an inducible and intact provirus, capable of reseeding the infection. These cells are the crucial targets for a potential HIV-1 cure. During the initial Fiebig stages when the reservoir emerges, CD4 + T cells responsive to the innate immune activation are likely to be infected [27]. As the infection progresses, HIV-1 specific CD4 + T cells develop to recognize the virus, and these cells are more likely to be re-activated upon novel HIV-1 exposure and therefore infected. This could be a reason why the reservoirs formed at the acute and chronic stages of HIV-1 infection are distinct, and thus differentially targeted by ART [17••]. A current challenge in the field relates to most studies employing a universal reactivation that indiscriminately stimulates all CD4 + T cells, potentially overshadowing discrete effects of different mechanisms of physiological latency reversal.

HIV-1 Integration Affects Proviral Plasticity

Following the viral entry into the cell and nucleus [28], the HIV-1 RNA is reverse transcribed into double-stranded DNA. Histones then bind to the linear DNA before integration to form nucleosomes [29]. Normally, integration into the host genome follows rapidly upon nuclear entry [28]. HIV-1 integration is favoured in active genes [30], primarily at genomic loci with an open accessible structure [31, 32]. These regions are associated with histone modifications such as H3K36me3 [33], recognized by the HIV-1 integrase and its host interactors [34]. Some latency is induced when T cells and their active genes transition to a resting state [35]. Other mechanisms of latency establishment have also been explored. For example, a delay between viral nuclear entry and integration leads to the histones acquiring silencing marks via interaction with the SMC5/6 complex [36•]. The SMC5/6 belongs to the structural maintenance of chromosome (SMC) complexes, including condensin and cohesin, which topologically fold chromatin. However, the ability of the silenced pre-integration HIV-1 complexes to integrate into the host genome remains uncertain.

Chromatin activity is confined within chromatin domain boundaries. Through position effects, the provirus is assumed to take the form of the integration site [37], where chromatin marks spread over adjacent regions, until a boundary is reached, such as a CCCTC-binding factor (CTCF) binding site. Whereas CTCF boundaries act as insulators between chromatin domains, within these domains, smaller compartments are delineated by transcription and 3D interactions. These transcriptionally induced local microenvironments may be maintained by the SMC5/6 [38], providing a second function for SMC5/6 in controlling HIV-1 transcription. SMC5/6 has a role in resolving transcriptionally induced topological stress [39, 40].

Given that HIV-1 integration can occur in both directions of host gene transcription, topological stress following convergent transcription necessitate either silencing of the provirus or the host gene. This suggests that despite integration in active regions, proviruses can still be silenced by host mechanisms. Notably, a gradual accumulation of silencing marks on proviral chromatin has been observed even in in vitro cultures [41•]. This could be attributed to an array of factors, such as selective proliferation, toxicity of HIV-1 proteins, or other host mechanisms. This plasticity complicates the reservoir analysis. Technically, most studies infer the chromatin structure from the integration site, but it is unclear if the proviruses adopt this inferred epigenetic state. There is also the possibility that the epigenetic state of the host genome is altered by integration—a significant issue that merits further exploration.

To characterize the proviral chromatin in individual cells, techniques are currently being developed. Using our recently published technique, based on the proximity ligation assay, we could identify the chromatin composition of HIV-1 in single cells [9•, 42]. This technique may be combined with the determination of the transcriptional activity of the proviruses, resulting in viral RNA and proteins (Fig. 1) [9•, 42]. By linking chromatin composition and transcriptional output in single cells, we may identify mechanisms underlying latency maintenance and reversal.

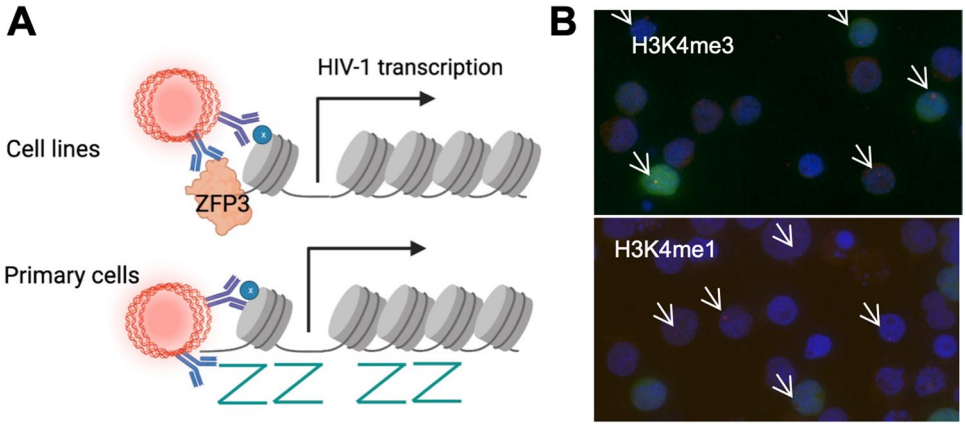

Fig. 1 Techniques enabling single cell characterization of the chromatin surrounding the HIV-1 provirus. **A** The provirus is marked by an engineered zinc-finger protein (ZFP3) [40] that specifically recognizes a sequence of the HIV-1 LTR. This protein is introduced in cell lines (upper panel). In primary cells, the provirus is marked by sense-RNAscope probes (lower panel). The proximity ligation assay (PLA) allows detection of histone modifications at the provirus locus by an antibody against the ZFP3 protein (cell lines) or RNAscope probes (primary cells) together with an antibody against the histone modification of interest. When antibodies are in proximity, rolling circle amplification results in a spot, visible under microscopy. **B** Micrograph of H3K4me3 and H3K4me1 after activation of J-lat cells. Simultaneous detection of HIV-1 activity can be detected by immunofluorescence of proteins expressed under the HIV-1 promoter (here GFP) or RNAscope for transcripts. White arrows point towards red PLA spots, indicating the proviral chromatin marked by H3K4me3 (upper) or H3K9me1 (lower). Nuclei are counterstained with DAPI (blue). Image adapted from ref [9•]

Latent Cells with Reactivation Ability

When proviral integration occurs in activated loci in stimulated CD4 + T cells, the activity of the provirus typically aligns with the status of the cell. However, uncoupling of cell state and proviral state is observed [43, 44]. Nevertheless, the proviral chromatin in resting cells affects the proviral activity after T cell stimulation (Fig. 2A). Deep viral latency is marked by low or no viral transcription even in activated cells. Nevertheless, irrespective of cell activation status, promoter-proximal transcription is frequently detected [41•, 45, 46]. These short transcripts are important as they correlate with time to rebound after ART interruption [47, 48•].

Some chromatin compartments are not clearly defined as active or silent. Enhancer regions are open regions that are transcribed, but the resulting transcripts are not translated. HIV-1 integration occurs frequently in these regions [32, 49].

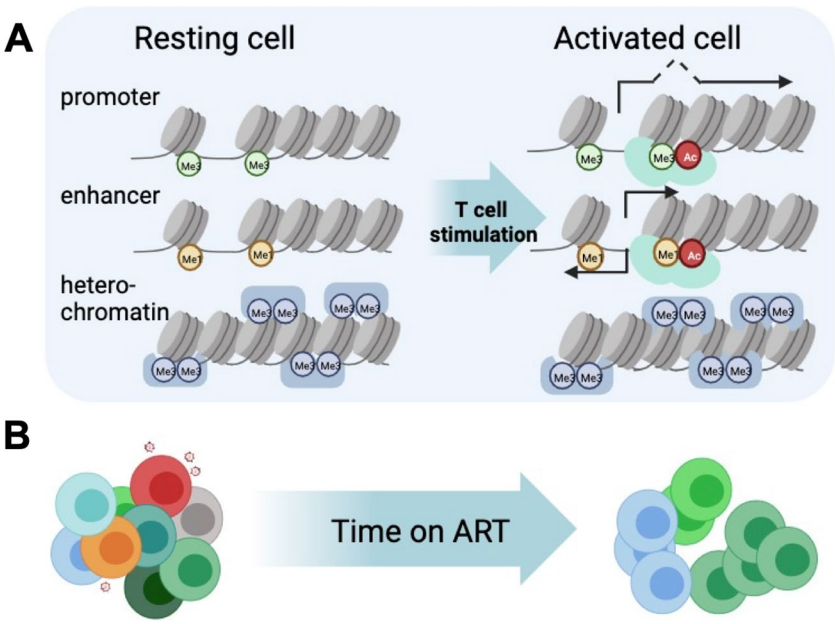

Fig. 2 Multiple factors contribute to dynamics of the HIV-1 reservoir. **A** The proviral reactivation potential depends largely on the local chromatin structure in the resting cell. T cell stimulation that activate proviruses in promoter-like structures lead to spliced and otherwise processed RNAs, whereas enhancer-like structures result in bi-directional, short, unspliced transcripts, and heterochromatin hinders transcription overall. **B** The initially heterogeneous HIV-1 reservoir evolves over time due to proliferation, cell death, and immune evasion. After long-term ART, the reservoir becomes highly clonal, with intact proviruses mainly found embedded in inert heterochromatin

Such integration offers a strategic hiding spot for the virus, enabling maintained activation potential, while avoiding immune recognition as no viral proteins are expressed. However, the viral Tat protein can alter this microenvironment by recruiting CBP/P300 and pTEFb to the HIV-1 promoter [50], changing the proviral chromatin into a gene-like structure that supports mRNA processing and protein production [9•, 41•]. A significant feature distinguishing enhancers from genes in host sequences is the presence of a splice donor within the first nucleosomes of protein coding genes [51]. HIV-1 encodes this splice donor, but its exposure can be modulated by nucleosome repositioning and Tat-recruitment [52, 53]. This ability allows HIV-1 to toggle between enhancer-like transcription and gene-like transcription [41•]. Although these cells are transcribing part of the provirus, they can be regarded as latent since they are not translated into functional proteins.

Proliferation of Infected Cells Shapes the HIV-1 Reservoir

The fate of an infected cell is influenced by various factors including its metabolic state, the chromatin activity at the integration site, the cell type, and external factors such as the state of the immune defense. Over time, the HIV-1 reservoir size is largely preserved under ART, attributed to cellular proliferation rather than de novo infections [54]. With prolonged time under ART, a selection for latent or immune evasive CD4 + T cells arises as other cells are eliminated. This selection pressure gradually results in a more clonal reservoir [55, 56] (Fig. 2B). Selective proliferation gradually transforms the initial heterogeneous and dynamic HIV-1 reservoir into a more homogeneous entity, resulting in significant inter-individual differences.

Both homeostatic or antigen-driven proliferation ensure maintenance and promote evolution of the HIV-1 reservoir [57••]. Homeostatic proliferation typically involves cellular division without proviral activation, allowing latent HIV-1 to persist [58]. Conversely, antigen-driven proliferation, stimulated by both HIV-1 and other external antigens, triggers the activation of CD4 + T cells [59, 60].

The balance of the HIV-1 reservoir following T cell activation is maintained through the opposing forces of clonal expansion and toxicity or activation-induced cell death. Cells infected with an activatable provirus, capable of producing functional viral proteins, face a relatively high risk of elimination. Consequently, under an extended period of ART when the reservoir is maintained by proliferation, the intact—or mildly defective—and activatable proviral reservoir declines. In contrast, epigenetically repressed intact proviruses remain silent and consequently are protected from elimination [61••]. This reduces the potential for viral rebound in time.

Defective vs. Intact Proviruses in HIV-1 Reservoir Dynamics

The HIV-1 reservoir of intact inducible proviruses capable of regenerating rebound viruses after treatment interruption constitutes a minute fraction of the reservoir. However, this fraction is critical to cure the HIV-1 infection, whereas other segments of the reservoir can still profoundly impact chronic immune activation, carcinogenesis, psychological issues, and stigma among other issues [62•].

Reservoir cells with intact proviruses decay faster but become more clonal over time [54]. Conversely, cells harboring defective proviruses have been reported to decay at a slower rate [53, 63–65, 66•]. Yet, the nature and impact of these defective proviruses vary widely. For instance, some defective proviruses express proteins that are both toxic and immunogenic [62•, 67, 68•], whereas proviruses with detrimental mutations have lost this ability. Read-through transcription of downstream host genes has been observed [69] that also may correlate with proliferation depending on the proviral integration site [70]. These various defects contribute to large variation in reservoir decay kinetics [56]. Defective proviruses containing intact internal coding sequences are under different selective pressure than those that are not translation competent, i.e., proviruses missing internal genes decay faster than noncoding proviruses. The viral proteins Nef and Gag are among the most immunogenic [71], and consequentially *gag* is the viral gene most often missing in defective proviruses [63]. Intriguingly, some defective proviruses can produce viral particles, potentially contributing to persistent immune activation [60]. If defective viruses express Nef to downregulate immune response or to boost anti-apoptotic mechanisms, their rate of decline may be effectively stalled.

Drawing Parallels to Cancer Dynamics

When considering the HIV-1 reservoir in virally suppressed individuals under long-term ART, cancer may serve as an analogy. In oncology, only a minority of cells possess the capability to divide indefinitely and propagate the disease. The majority of cancer cells have limited proliferative potential and therefore do not directly contribute to disease progression. Likewise, within the HIV-1 reservoir, only the small fraction of intact, inducible proviruses can generate rebound viruses to reseed the infection should ART be interrupted. The bulk of the reservoir, although larger, harbors defective proviruses which are less critical to viral rebound and unable to propagate the infection. In cancer treatment, the objective is to eradicate every single cell exhibiting stem-like properties due to their potential to regenerate the

tumor. Similarly, in the pursuit of an HIV-1 cure, the challenge is to eliminate all cells capable of producing infectious viruses that spread or reseed the infection [72].

Immune Evasion Affecting the HIV-1 Reservoir

The survival of infected cells can be attributed to a multitude of mechanisms, such as immune evasion [73], upregulation of anti-apoptotic genes [74], induction of cell survival pathways [70, 75], and the downregulation of anti-proliferative genes [76]. Host responses can also directly repress HIV-1 transcription, further complicating the elimination of infected cells [77].

The presence of active viral transcription and protein production renders HIV-1-infected cells more prone to recognition and elimination by the host immune system [78]. Interestingly, a small subset of cells can exhibit continuous HIV-1 expression, even under ART [46]. At least some of these cells are likely to be clones of cytotoxic CD4 + T cells [79••]. This cell population expands during HIV-1 infection, and cytotoxic CD4 + T cells can release cytolytic granules to eliminate CD8 + T cells targeting them [80].

One question that emerges here is whether these perpetually HIV-1-expressing cells contribute to the phenomenon of non-suppressible viremia (NSV). The rare occurrence of NSV has made this phenomenon challenging to study. In individuals exhibiting NSV, large clones of HIV-1-expressing cells have been identified [81•]. However, these clones have been associated with defects in the 5′ RNA, resulting in defective, non-infectious virions observed as NSV [82••]. These RNAs most often lack the strongly immunogenic *gag* [63] and therefore have the possibility to persist even in non-cytotoxic CD4 + T cells. Residual viremia does not correlate with inflammation under ART, and a recent study even concluded that HIV-1 persistence under ART does not drive or respond to inflammation or immune activation [65].

Infected Cells with Non-reactivatable Provirus

Infected cells in which the intact provirus is permanently silenced can be viewed as eliminated from the reservoir, given their incapacity to generate viruses. “Elite controllers” (ECs), a small subset of people living with HIV-1 who maintain undetectable levels of plasma viremia in the absence of ART, may serve as a model to achieve a functional cure. A significant proportion of ECs have the protective HLA-B*57 allele, which also can be found in non-ECs [83]. ECs tend to have a small reservoir size, but this does not automatically imply infection control [84]. These factors do not allow identification of ECs. Recently, it was discovered that the HIV-1 integration sites in ECs are biased towards transcriptionally inert heterochromatin regions [85]. Interestingly, such bias towards heterochromatin regions also appears to occur after long-term ART that extends beyond 20 years [61••]. Even though HIV-1 integration only rarely occurs in silent chromatin regions, mutations in the viral integrase protein can stimulate heterochromatin targeting [86•]. Cells with proviruses integrated into heterochromatic regions are detected already in the acute stages of infection [13•]. While cells with proviruses in heterochromatin are expected to be scarce initially, their proportion gradually increases over time, largely due to their evasion from selection pressure exerted by viral protein toxicity or immune recognition [61••].

This raises an intriguing question: could post-treatment controllers have their reservoirs evolved so that only heterochromatin-integrated proviruses remain? It is conceivable that post-treatment control could arise from the reservoir gradually assuming characteristics similar to those of ECs’ reservoirs after extended treatment periods.

Reservoir Location

While the core insights about the HIV-1 reservoir are primarily derived from studies on peripheral blood and CD4 + T cells, the HIV-1 reservoir persists in various tissues, including lymph nodes, gut, spleen, and brain [87, 88]. However, peripheral blood serves as the main source of dispersal, giving it a reflection of the most compartments of the reservoir [89••]. Macrophages represent another key cell type involved in the long-term maintenance of the HIV-1 reservoir, either through direct infection, engulfment, or cell fusion [22••, 90, 91]. Although the macrophage reservoir is distinct from that of CD4 + T cells and generally lower in viral content, its potential to facilitate viral spread renders it a critical component to consider in reservoir studies [22••]. The anatomical source of the rebound viruses after ART interruption remains elusive but peripheral blood contains traces of most reservoir cells found in tissues [92]. This underscores the importance of a comprehensive understanding of the reservoir’s distribution across different tissue types for successful HIV-1 eradication strategies.

Conclusions

In conclusion, recent insights into the HIV-1 reservoir have revealed intricate details of the infected cells, the influence of proviral integration sites and the role of single-cell histone modifications. This has led to an understanding of how cell proliferation together with the immune system sculpts the reservoir in the absence of free virus (Fig. 2). An emphasis on chromatin features, proviral reservoir dynamics, and inter-individual differences offers a comprehensive perspective that could significantly enhance HIV-1 treatment

precision and effectiveness. To manipulate these molecular mechanisms to eradicate the reservoir, a detailed understanding of the single cells capable of instigating new viral production is crucial. In single cells, we need to identify the mechanisms that keep the provirus latent and reactivatable, and the triggers that activate it. Long-term ART has led to a high diversity in HIV-1 reservoirs among individuals. These unique HIV-1 reservoirs will require specific treatment for their eradication, making personalized medicine a necessity to achieve a global HIV-1 cure.

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Declarations

Human and Animal Rights and Informed Consent All reported studies/experiments with human subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

Competing Interests The authors declare no competing interests.

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- • Of importance
- •• Of major importance

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