Review Article

Molecular Understanding of HIV-1 Latency

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The introduction of highly active antiretroviral therapy (HAART) has been an important breakthrough in the treatment of HIV-1 infection and has also a powerful tool to upset the equilibrium of viral production and HIV-1 pathogenesis. Despite the advent of potent combinations of this therapy, the long-lived HIV-1 reservoirs like cells from monocyte-macrophage lineage and resting memory CD4+ T cells which are established early during primary infection constitute a major obstacle to virus eradication. Further HAART interruption leads to immediate rebound viremia from latent reservoirs. This paper focuses on the essentials of the molecular mechanisms for the establishment of HIV-1 latency with special concern to present and future possible treatment strategies to completely purge and target viral persistence in the reservoirs.

1. Introduction

Infection with HIV-1, which was first isolated in 1983, causes AIDS, a syndrome that was first reported in 1981 [1]. The HIV-1 pandemic represents one of the great plagues in the history of mankind and a major challenge for medicine, public health, and medical research [2]. The majority of people living with HIV-1 belong to low- and middle-income countries. For example, sub-Saharan Africa accounts for two third of all infected people with HIV-1, where in few countries more than one in five adults are infected with HIV. South and south East Asia have second highest number of people living with HIV-1. Furthermore the epidemic is spreading most rapidly in Eastern Europe and central Asia, where the number of people living with HIV increased by 54.2% between 2001 and 2009. UNAIDS estimated that 33.3 million people were infected with HIV at the end 2009 compared to 26.2 million in 1999, a 27% increase in HIV infection. Each year 2.6 million people are infected with HIV-1 and 1.8 million die of AIDS (UNAIDS 2010). Much has been learned about the science of AIDS and continuous research has allowed the development of 25 different active compounds belonging to six different drug families shifting the HIV-1 infection from a fatal illness into a chronic disease [3, 4].

HIV-1 life cycle can be categorized into two phases. The early stage occurs between entry into host cells and integration into its genome (Figure 1). The late phase occurs from the state of integrated provirus to full viral replication [5]. Similarly two types of viral latency can be differentiated: preintegration latency results in generation of different forms of viral DNA before integration, whereas postintegration latency refers to the lack of viral replication after the insertion of viral DNA into host genome [6]. Virus enters through successive interactions with CD4 and CXC chemokine receptor type 4 (CXCR4) or CC chemokine receptor type 5 (CCR5); as a consequence HIV-1 core (diploid single strand positive sense RNA, tRNA primers, viral protease, retrotranscriptase, and integrase) is released into cytoplasm [7, 8]. After reverse transcription, the preintegration complex (linear dsDNA, integrase, matrix protein, retrotranscriptase, viral protein R and various host proteins) transportation into nucleus is mediated by microtubule and dynein, thereby allowing the infection of resting, nondividing cells. Linear dsDNA either integrates into host cell chromosomes or circulates as one or two long terminal repeat (LTR) containing circles [9, 10].

Activation of host cells induces the binding of transcriptional preinitiation complex to enhancer elements in the 5′LTR proximal promoter that gathers essential host
transcription factors, such as NF-κB, NFAT, AP-1 and SP1 which transmit activation signals to basal transcription machinery and promote the binding of RNA polymerase II to the TATA box to initiate transcription [11–13]. Transactivation response element (TAR), a 59-nucleotide stem loop structure, is then formed at 5’ end of nascent viral transcript that creates the binding site for viral transactivator (Tat) which promotes efficient elongation of viral transcripts by recruiting the positive transcription elongation factor b (PTEFb), thereby enhancing the functional capacity of RNAPII [14]. Viral regulatory protein (Rev) regulates the processing, nuclear cytoplasmic transport, and splicing of HIV mRNA. HIV-1 large precursor proteins assemble to create viable particles budding off the cell and are processed into mature proteins [15].

In 1996, the introduction of highly active antiretroviral therapy (a combination of three or more potent anti-HIV-1 drugs targeting different steps of viral life cycle) has greatly extended the survival and stabilized the AIDS pandemic on global scale (Table 1). This therapy can reduce the plasma virus levels below detection limits (<20 copies/mL) [16, 17]. However a residual viremia is still detected in patients on HAART with very sensitive methods. Furthermore, HIV-1 reverts to measurable plasma level in less than two weeks when HAART is interrupted [18]. These observations suggest that HAART cannot totally eliminate HIV. The virus persistence in cellular reservoirs because of viral latency, cryptic ongoing replication, or poor drug penetration represents the major obstacles for its eradication [19].

HIV-1 replicates preferentially in activated CD4+ T cells but these cells generally survive only for few days after infection. Hardly, an infected CD4+ lymphoblast survives long enough to revert back to a resting memory state [20–22]. Furthermore, HIV-1 gene expression is largely suppressed as
Table 1: Licensed antiretroviral drugs.

| Name                      | Trade name | Company                             | Launched |
|---------------------------|------------|-------------------------------------|----------|
| **Nucleoside/nucleotide reverse transcriptase inhibitors**                      |            |                                     |          |
| Zidovudine                | Retrovir   | GlaxoSmithkline                     | 1987     |
| Didanosine                | Videx      | Bristol-Myers Squibb                | 1991     |
| Zalcitabine               | HIVID      | Roche                               | 1992     |
| Stavudine                 | Zerit      | Bristol-Myers Squibb                | 1995     |
| Lamivudine                | Epivir     | GlaxoSmithkline/shire pharmaceuticals | 1998   |
| Abacavir                  | Ziagen     | GlaxoSmithkline                     | 1999     |
| Tenofovir                 | Viread     | Gilead                              | 2001     |
| Emtricitabine             | Emtriva    | Gilead                              | 2003     |
| **Non-nucleoside reverse transcriptase inhibitors**                             |            |                                     |          |
| Nevirapine                | Viramune   | Boehringer Ingelheim                | 1996     |
| Efavirenz                 | Sustiva, Stocrin | Bristol-Myers Squibb, Merck         | 1998     |
| Delavirdine               | Rescriptor | Pharmacia, Upjohn, Agouron, Pfizer  | 1999     |
| Rilpivirine               | Edurant    | Tibotec Therapeutics                | 2011     |
| Etravirine                | Intecence  | Tibotec Therapeutics                | 2008     |
| **Protease inhibitors**                                            |            |                                     |          |
| Saquinavir                | Invirase   | Hoffmann-La Roche                   | 1995     |
| Indinavir                 | Crixivan   | Merck                               | 1996     |
| Ritonavir                 | Norvir     | Abbott, GlaxoSmithkline             | 1996     |
| Nelfinavir                | Viracept   | Agouron, Pfizer                     | 1997     |
| Amprenavir                | Agenerase, Prozei | Vertex                      | 1999     |
| Lopinavir + Ritonavir     | Kaletra, Aluvia | Abbott                           | 2000     |
| Atazanavir                | Reyataz, Zrivada | Bristol-Myers Squibb, Novartis     | 2003     |
| Fosamprenavir             | Lexiva, Telzir | Vertex, GlaxoSmithkline           | 2003     |
| Tipranavir                | Aptivus    | Boehringer Ingelheim                | 2005     |
| Darunavir                 | Prezista   | Tibotec                             | 2006     |
| **Entry inhibitors**                                               |            |                                     |          |
| Enfuvirtide               | Fuzeon     | Trimeris, Roche                     | 2003     |
| Maraviroc                 | Celsentri, Selzenty | Pfizer                  | 2007     |
| **Integrase strand transfer inhibitors**                             |            |                                     |          |
| Raltegravir               | Isentress  | Merck & Co., Inc.                   | 2007     |
| **Multi-class combination inhibitors**                                |            |                                     |          |
| Efavirenz, Emtricitabine, and Tenofovir disoproxil fumarate             | Atripla    | Bristol-Myers Squibb and Gilead Sciences | 2006   |
| Emtricitabine, Rilpivirine, and Tenofovir disoproxil fumarate           | Complera   | Gilead Sciences                     | 2011     |

...and therefore contribute to the viral persistence [26–28]. Furthermore several features make cells from macrophages lineage potential HIV reservoir. The viral particles produced in macrophages are budding into intracytoplasmic compartments which may represent one of the favored sites for HIV replication [29]. Furthermore cells from macrophages lineage are also more resistant to apoptosis and cytopathic effects, harbor virus for longer period of time, and produce virus throughout their life span even in the microglial brain cells [30].

HAART results in a four-phase decline of viremia [31]: first an initial rapid loss of virus due to clearance of infected activated CD4+ T cells surviving about one day because...
of viral cytopathic effects or host cytolytic effector mechanism; the second slower phase of viral decay in infected macrophages, partially activated CD4+ T cells, and follicular dendritic cells (FDCs) due to the clearance of several cell population with a half-life of one to four weeks; the third phase of decay corresponding to cells with half-life of several weeks (memory cells); and the fourth phase with no appreciable decline, caused by the activation of resting memory CD4+ T cells [32]. During the fourth phase, HIV-1 plasma level normally ranges from 1 to 5 copies (RNA per mL) and can be detected by extremely sensitive RT-PCR assays [33].

A 60-year uninterrupted HAART has been estimated being necessary to eradicate the latent reservoirs. The lifelong HAART treatment is today a necessary evil because of its association with many metabolic disorders and toxicities [34, 35]. Moreover, its interruption leads to rapid viral rebound, attributable to the persistence latently infected memory CD4+ T cells. Cells from latently infected reservoirs (cell where virus persists with more stable kinetics than main pool of actively replicating virus) are immunologically alike from uninfected cells and are insensitive to immune clearance and HAART [36]. To address the persistence of transcriptionally silent but replication competent HIV reservoirs, the first approach could be to strengthen and intensify HAART by introducing new viral inhibitors. Secondly, if therapeutic goal is virus eradication, then novel strategies need to be adopted to target and clear the latent reservoirs by inducing HIV-1 replication in latently infected cells, while maintaining or intensifying HAART in order to prevent spreading of new infections [37].

2. Molecular Insights into HIV-1 Latency

Two forms of viral latency have been seen on the basis whether or not HIV-1 genome has integrated into the host cell genomes: preintegration and postintegration latency [38]. Preintegration latency results in partial or complete blockade of viral life cycle prior to integration of virus genome into host genome. It could result from incomplete reverse transcription or from restriction by factors such as APOBEC3G (cellular deoxycytidinomaminase whose function can be counteracted by viral vif protein) [39, 40]. Further the preintegration latency does not appear to be of clinical significance because unintegrated forms persist in the cytoplasm for only one day and cannot account for long-term latently infected CD4+ T-cell reservoirs but this unintegrated form of DNA remains stable for at least one month in nondividing metabolically active macrophages [41, 42]. Postintegration latency occurs when HIV-1 genome integrated into host genome is reversibly silenced and is limited only by the life span of the infected cells and its progeny. Most mechanisms to maintain HIV-1 latency operate at transcriptional level.

2.1. The Site and Orientation of Integration. HIV-1 latency mostly operates at the transcriptional level; for example, the chromosome environment at the site of integration and availability of viral and host factors can have influence on viral latency [10]. HIV-1 integrates into the host chromosomal DNA in nonrandom manner. Specific sequences at the ends of dsDNA are required to target PIC predominantly to the intronic regions of the actively transcribed genes [43]. One study of the integration sites in purified resting CD4+ T cells from the patients on HAART found majority of provirus (93%) located within the coding regions of host gene, probably owing to the increased chromatin accessibility of these regions [44]. The finding that latent HIV-1 proviruses integrate in actively transcribed regions may seem paradoxical considering the establishment of transcriptional latent state [45]. However, the viral replication from these proviruses can suffer from intense transcriptional interference because of the orientation of the proviruses or their proximity to a stronger host gene promoter [46].

The steric hindrance occurs: when the provirus integrates in the same transcriptional orientation as the host gene, read-through transcription from upstream promoter displaces key transcription factors from HIV-1 promoter and prevents the assembly of the preinitiation complex on the viral promoter, thereby hindering HIV-1 transcription [47, 48]. These transcriptional interferences could be reversed by inhibiting the upstream transcription or by cooperatively activating viral transcription initiation and elongation [49]. Furthermore, integrated provirus suffering from transcriptional interference becomes transcriptionally active following Tat expression, and this provirus can switch off the transcription of the host genes within which it has integrated or can allow the coexistence of expression of both host and viral genes [50].

Promoter occlusion occurs when provirus integrated in opposite orientation to the host gene may lead to the collision of two RNP-R complexes during elongation, which can lead the premature termination of the transcription of one or both complexes [45, 51]. Convergent transcription may also allow for the elongation of both viral DNA strands which results in the formation of double-stranded RNAs, might lead to RNA-interference, RNA directed DNA methylation or generation of antisense RNA [52, 53]. Furthermore, the phenomenon of enhancer trapping can occur when enhancer of one gene is placed out of context near the promoter of the second gene. Taken together, the orientation-dependent regulation is highly variable and relies on the 5’LTR occupancy and on the rate of host gene elongation [54, 55].

2.2. Availability of Host Cell Transcription Factors and HIV-1 Viral Proteins. HIV-1 gene expression is strongly dependent on host cell transcription machinery and the lack of host transcriptional activator or the presence of host transcription repressors also influences the viral latency. The 5’LTR functions as an HIV-1 promoter and contains several DNA binding sites for various cellular transcription factors such as SP1 and NF-κB which are required for viral replication, whereas other sites, such as those binding NFAT, LEF-1, COUP-TF, ets-1, USF, and AP-1, enhance transcription without being indispensable [56–58]. The p50/p65 NF-κB heterodimer is sequestered into the cytoplasm in unstimulated cells through its interaction with an inhibitory protein of the family of NF-κB inhibitors (IκBs) [59]. Following cellular activation, phosphorylation of IκB by IκB kinase
(IKK) results in its dissociation from NF-κB, NF-κB translocation into the nucleus, and transcription of NF-κB-dependent genes [60]. On the contrary, the NF-κB p50/p50 homodimers, which lack the transactivation domain, recruit the histone deacetylase HDAC-1 to the LTR, leading to the local histone deacetylation and to a repressive chromatin environment on the HIV-1 5′LTR in HIV-infected cells [61, 62]. Following T-cell activation, p50/p50 homodimers are uprooted by the p50/p65 heterodimers, which recruit histone acetyltransferases (CBP and p300) to enhance the viral replication [24, 63]. Furthermore, the p65 subunit of NF-κB stimulates transcriptional elongation by interacting with RNAPII complexes of cdk7/TFIIH and pTEFb which in turn phosphorylate the serine-5 and serine-2 residues, respectively, in the carboxyl terminal domain (CTD) of RNAPII for the efficient transcription elongation [64, 65].

As far as NFAT is concerned, T-cell activation dephosphorylates cytoplasmic NFAT via PKC pathway and translocates into the nucleus where it interacts with 5′LTR at the sites overlapping the US NF-κB binding site and thus promotes the chromatin remodeling by recruiting transcriptional coactivator like CBP and p300 [66, 67]. Further the AP-1 complex, composed of Jun, Fos, and ATF family members, having three binding sites in HIV-1 5′LTR, cooperates with NFAT to activate HIV-1 transcription through US NF-κB/NFAT binding sites [68, 69]. In addition to host cell transcription factors, HIV-1 transcription is boosted by viral protein like Tat [70]. Tat interacts with the cis-acting RNA element TAR (transactivation response element) present at the 5′ of viral transcripts. The inhibition of Tat also induces latency because in its absence, transcription is initiated but blocked at the promoter in the early stage of elongation due to the repressive chromatin environment [71, 72]. Tat activity is regulated mainly through the acetylation of Lys28 and Lys50 [73]. Tat acetylation by PCAF on Lys28 enhances the recruitment of pTEFb at 5′ end of nascent viral transcripts promoting efficient elongation, whereas acetylation of Lys50 by CBP promotes the dissociation of Tat from Tat-cyclin T complex, allowing its interaction with PCAF and Tat-PCAF complex recruiting to the elongating RNAPII [74–76]. Some cellular protein affects the acetylation state of Tat modulating its activity. Sirptuin 1, a class III HDAC, acts as specific Tat deacetylase, thus increasing the quantity of Tat that is available to act as a transcriptional activator [77]. Further CDK9, a component of pTEFb, is acetylated by hGCN5 and PCAF, reducing the transcriptional activity of pTEFb and promoting HIV-1 latency [78].

In addition to transcription factors and their regulators, specific restriction factors exist to defend host cell against retroviral infection. For example, APOBEC3G impairs early phases of HIV-1 life cycle and may induce latency. APOBEC3G strongly inhibits HIV-1 replication in CD4+ T cells by inducing C to U conversions in the viral strand DNA during reverse transcription [79]. This viral replication inhibitory effect of APOBEC3G is only present in resting cells, where it exists as an active, low molecular mass ribonucleoprotein complex [80]. T-cell activation induces the shift from an active low molecular mass to inactive high molecular mass form of APOBEC3G that cannot restrict viral infection. This inactive form of APOBEC3G can be found in tissue resident naive or memory CD4+ T cells, which are permissive to HIV-1 infection [81].

2.3. The Chromatin Organization and Epigenetic Regulation of HIV-1 Promoter. HIV-1 promoter activity depends on the chromatin environment where two nucleosomes, namely, nuc-0 and nuc-1, are precisely positioned at the viral promoter in latently infected cell lines and impose a block to transcriptional elongation. Nuc-1 nucleosome, located immediately downstream the transcription initiation site, impedes the LTR activity [82, 83]. Epigenetic modification and disruption of nucleosomes, nuc-1, are required for LTR-driven transcription activation and viral gene expression [84]. The chromatin organization can be modulated through a variety of mechanisms, including posttranslational covalent modifications of histone tails and ATP-dependent, chromatin remodeling events [85, 86]. Histone modification (i.e., acetylation, methylation, phosphorylation, sumoylation, ADP-ribosylation and ubiquitination) can influence the gene expressions, which are all reversible and localized to N- and C-terminus of histone tails [87, 88]. Hypoacetylation of histones by histone deacetylase (HDACs) correlates with transcription repression, whereas hyperacetylation by histone acetyltransferase (HATs) induces the transcription activation [89].

The silent proviral 5′LTR can be activated from postintegration latency by cell treatment with a variety of stimuli, including cytokines like TNF-α and IL-6, antibodies (anti-CD3 and -CD28 stimulation) phorbol esters (PMA, PHA, prostratin), or by viral proteins (Tat and Nef). The nucleosome nuc-1, located immediately downstream of transcription start site, is specifically remodeled following IL-6, TNF, or PMA treatment, and this event is specifically correlated with the activation of HIV-1 gene expression [82, 84]. Furthermore, HIV-1 transcriptional activation was shown to occur following treatment with HDAC inhibitors (HDACIs) such as trichostatin A (TSA), trapoxin (TPX), valproic acid (VPA), and sodium butyrate (Na But), suggesting that during latency nuc-1 is constitutively deacetylated by HDACs [90, 91]. The HDACI-mediated transcriptional activation is accompanied by specific remodeling of nuc-1 and by an increased acetylation of H3K4 and H4K4 in the promoter region [92]. Several transcription factors such as ying and yang (YY1) and late SV40 factor (LSF; also known as TFCP2) repress the HIV-1 replication by recruiting HDAC1 to repressor complex sequence located at position −10 to +27 nucleotides in the LTR [93, 94]. Other host transcription factors, such as AP-4 (activating protein-4), NF-κB p50/p50 homodimers, and CBF-1 (C-promoter binding factor-1), can also recruit HDACs to the LTR and inhibit viral transcription [61, 95]. By contrast viral proteins like Tat and several cytokines and HDAC inhibitors decrease HDACs occupancy at the repressor complex sequence and activate the transcription at 5′LTR by recruiting factors with HAT activity such as CREB binding protein (CBP), CBP-associated factors (PCAFs), and human general control of amino acids synthesis protein 5, which induces nucleosome hyperacetylation in cell lines [49, 96].
Similarly, in the absence of Tat, LTR-associated nucleosomes are hypoacetylated, and viral gene expression is silenced, contributing to viral latency. HDAC inhibitors are not sole factors to induce transcription; host factors such as NF-kB, NF-AT, and SP-1 must also be recruited to the 5’LTR [97, 98].

Generally, the histone acetylation is associated with gene activation while histone methylation can be associated with both activation and silencing. For example, methylation of histone 3 at lysine 4 and histone 3 at lysine 36 is found in active genes whereas methylation of histone 3 at lysine 9 and 27 and histone 4 at lysine 20 is associated with gene silencing [99]. The histone 3 at lysine 9 methylation that is mediated by SUV39H1 (suppressor of variegation 3–9 homologue 1) has been correlated with heterochromatin assembly by recruiting HP1 (heterochromatin protein 1 homologue γ), resulting in HIV-1 silencing [96]. The transcription factor COUP-TF interacting protein 2 (CTIP2) plays an essential role in promoting viral latency in microglial cells by recruiting a chromatin modifying enzyme complex and by establishing a euchromatic environment at the HIV-1 promoter [100]. Actually, the CTIP2 recruits HDAC1, HDAC2, SUV39H1, and HP1 proteins to establish a euchromatin environment that leads to HIV-1 silencing in several cell lines [101]. Finally, by altering histones, recruiting other chromatin remodeling factors, and modifying the activity of certain transcription factors, HDACs appear to be critical for epigenetic repression of HIV-1 transcription and for the maintenance of viral latency [102].

2.4. Posttranscriptional Latency and MicroRNAs. MiRNAs are single-stranded noncoding RNAs of 19 to 25 nucleotides in length that function as posttranscriptional regulator and introduce a new level of complexity to virus-host interplay [103, 104]. Further miRNAs can also regulate the gene expression at the epigenetic level by remodeling chromatin surrounding [105]. Several cellular miRNAs (miR-28, miR-125b, miR-150, miR-223, and miR-382) control HIV-1 replication by targeting all spliced or unspliced HIV-1 mRNA except Nef coding mRNA [106]. These cellular miRNAs are enriched in resting CD4+ T cells and inhibit the translation of almost all HIV-1-encoded proteins contributing to viral latency [107]. Furthermore, viral genome produces viral interferences RNAs that can target the viral RNAs, cellular mRNAs, and host miRNAs. By targeting its own mRNA, HIV-1 induces its own latency [108, 109]. Moreover, HIV-1 can also suppress the miRNAs-mediated silencing pathway by reducing the expression of miRNA-17, miRNA-5p, and miRNA20a that results in increased expression of Tat cofactor PCAF ultimately enhancing the viral transcription [110].

HIV-1 products interfere directly with the cellular RNAi machinery through different mechanisms. Firstly, Tat physically interacts with the helicase domain of Dicer and partially represses the ability of Dicer to process precursor dsRNA into small interfering RNAs (siRNAs) [111, 112]. Further, the viral TAR sequence prevents the formation of a functional RNA-induced silencing complex (RISC) by sequestering the Dicer-interacting protein TAR RNA-binding protein 2 (TRBP2) [113]. Finally both cellular and viral miRNAs could be involved in maintaining HIV-1 latency or in controlling low ongoing viral replication [114].

3. Cellular Reservoirs in HIV-1 Pathogenesis

HIV-1 uses different strategies to survive within infected individuals. The macrophages, dendritic cells (DCs), and CD4+ T lymphocytes are considered reservoirs for HIV-1 infection. In CD4+ T cells, the viral replication is dependent upon the cell cycle of the host cell and HIV-1 entry into activated CD4+ T lymphocytes leads to productive infection. Virions found within monocyte-derived macrophages persist and retain infectivity for weeks, thus providing an environment for viral persistence. Dendritic cells capture and internalize extracellular virions via DC-SIGN which can be subsequently transmitted to T cells in trans. HIV-1 hidden in DCs and macrophages certainly play an important role for viral spread and cell-to-cell transmission, and its involvement in long-term viral persistence will be discussed here.

3.1. Monocyte-Macrophage Lineage as Viral Reservoirs. Cells of myeloid lineage including monocytes, macrophages, and dendritic cells are the first line of defense against pathogenesis, because these cells are critical immune cells responsible for a wide range of both innate and adaptive immune functions [28, 115]. These cells are important viral reservoirs and responsible for the dissemination of HIV-1 into sanctuaries such as brain.

Circulating monocytes are recruited to different tissues, differentiate into macrophages, and form the HIV-1 reservoirs. Furthermore, a minor monocyte subset, the CD16+ is more permissive to infection than the more abundant CD14+ CD16– monocytes subset, which account for less than 1% circulating monocytes [116]. Macrophages contain the CD4 receptor and CCR5 and CXCR4 coreceptors which are early cellular targets for HIV-1. These cells are able to produce and harbor the virus for longer period of time due to high resistance to cytopathic effects [117]. The resident macrophages of central nervous system like microglial cells are involved in the pathogenesis of HIV-1-associated dementia, survive for many years, and are potential reservoirs for HIV-1 [118, 119].

Macrophages can harbor large quantities of unintegrated viral DNA in circular form, which remains unstable for up to two months in nondividing macrophages [120, 121]. Further the viral protein Vpr is important for viral replication in monocyte macrophages lineage but not in nondividing CD4+ T cells. The deletion of Vpr decreases the transcription from unintegrated HIV-1 DNA up to 10 times [122, 123]. A recent finding shows that infected human macrophages can support persistent transcription from this unintegrated DNA which suggests that these circular forms of episomal DNA may therefore account for persistence and expression in non-dividing cells such as macrophages [124, 125]. However, several mechanisms generating HIV-1 postintegration latency have been described in the macrophages, including lack of functional Tat, availability of host transcription activator or repressors, influence of chromatin environment, and host antiviral processes such as miRNAs [126].
Another strategy that allows the virus to infect and persist in macrophages is the resistance to apoptosis. The NF-κB pathway is activated upon HIV-1 infection in primary monocytes and macrophages [127, 128]. It has been proposed that TNF-α-induced NF-κB activity might be involved in the inhibition of apoptosis and the survival of monocytes and macrophages. NF-κB-mediated resistance to TNF-α-induced apoptosis might result in a decreased susceptibility to apoptosis of macrophages versus T cells in the context of chronic immune activation during HIV-1 infection [129]. Further, the absence of apoptosis in HIV-1-infected primary macrophages has been correlated with an increase in antiapoptotic Bcl-2 and Bcl-XL proteins and a decrease of proapoptotic Bax and Bad proteins [130]. Furthermore, macrophages express 10 times lower number of CD4 receptor than CD4+ T cells and therefore are less susceptible to HIV-1 superinfection [131]. High number of CD4 receptors in HIV-1-infected CD4+ T cells induce a dramatic reduction in the infectivity of release virions by sequestering the viral envelope by CD4, while the less number of CD4 on the cell surface of the macrophages might favor the release of infectious virions from infected cells and thereby could optimize the transmission of virions to cells present in the vicinity [132]. Further the viral life cycle of HIV-1 or virion production is 6 times slower in MO than in primary T cells due to a slower reverse transcription process, allowing the MO to form long lasting viral reservoirs [133, 134].

Dendritic cells are also involved in HIV-1 propagation, through capture of viruses by receptor DC-SIGN (DC-specific ICAM3-grabbing non integrin) as well as through efficient HIV-1 transmission to T cells at the virological synapse [135]. Follicular dendritic cells in lymphoid tissues are specialized in trapping and retaining the antigens, including HIV-1 virions, on their surface in the forms of immune complexes [136, 137]. Further, mature myeloid dendritic cells located in lymph nodes can sustain the very low virus replication and therefore have potential role in HIV-1 latency [138]. The mechanism of viral persistence in these cells is not yet clearly understood [139].

CD34+ haematopoietic cells (HPCs) also serve as a viral reservoir, since a subpopulation of CD34+ HPCs expresses CD4 and CCR5 and/or CXCR4 and these cells are susceptible to HIV-1 infection [140, 141]. Furthermore, HIV-1-infected CD34+ HPCs have been detected in some patients where these HPCs are associated with impaired growth and development [142]. Then these HPCs generate a subpopulation of monocyte which differentiates in dendritic cells, generating an infected cell lineage that may spread HIV-1 to sanctuaries [143].

3.2. Lymphocytes: Source of Latently Infected Cells. The most T lymphocytes in the body are in a resting G0 state, and following activation, these resting naïve T cells, in response to antigen, undergo a burst of proliferation and differentiation in response to antigen and give rise to effector T cells. Most of these cells die during the immune response, but a subset survive and return to G0 state and become memory T cells. These lymphocytes persist as memory cells with different pattern of gene expression for the long-term survival and rapid response to the relevant antigen in the future [144, 145].

Indeed, the activated CD4+ T cells are highly susceptible to HIV-1-infection and die quickly as a result of cytopathic effects either of the virus or of the host immune system. However, a subset of HIV-1-infected CD4+ T cells revert back to a resting state and survive for longer period of time [38]. Both naïve and memory subpopulation of resting lymphocytes provide an extremely restrictive environment for HIV-1 replication due to low CCR5 expression, low nucleotide pools and ATP level, and cytoplasmic APOBEC3G [79, 146]. Sometime, viral DNA cannot produce viable particles in this environment, but it can generate some RNA transcript and produce HIV-1 Nef (negative factor) in resting CD4+ T cells and macrophages that could increase cell activation and facilitate viral replication, either in the same cell or in the surrounding cells through production of soluble Nef from HIV-1-infected macrophages [147, 148].

In addition to macrophages or dendritic cells, a stable form of latency also occurs in CD4+ T cells that carry integrated provirus [149]. Certain chemokines CCL19, CXCL9/ CXCL10, and CCL20 activate the coflin and actin dynamics necessary for the development of latency in resting CD4 T cells [150]. Since the HIV-1 integration requires cell activation to allow efficient reverse transcription and nuclear import of preintegration complex [151], the postintegration latency occurs when infected activated T cells return to quiescent or memory cells. The phenotypes of these resting T cells carrying a nonproductive HIV-1 infection have specific set of surface markers such as CD4+, CD25−, CD69−, and HLA-DR− [152]. Further, it has been estimated to comprise 10⁶–10⁷ cells in asymptomatic patients, whose infected naïve CD4+ T cells can harbor an average of 3 to 4 copies of integrated HIV-1 per cell [153]. These cells do not allow to complete viral replication unless they are activated, and their stability and long half-lives represent major obstacle to HIV-1 eradication [154].

4. Targeting HIV-1 Reservoirs: A New Therapeutical Approach

The implementation of HAART therapy has improved the survival and quality of life of HIV-1-infected individual, but it has unable to eradicate the virus from latently infected reservoirs like memory CD4+ T cells and macrophages constituting a major obstacle in HIV-1 eradication [155]. The frequency of HIV-1-infected cells, in the patients on HAART, has been reduced to less than one cell per 10⁶ resting CD4 T cells, but after many years of treatment, the frequency of these infected cells is not decreasing further [152, 156]. Moreover, some reservoirs are found in tissue sanctuary sites, like the brain, that are protected from drug penetration [157].

Today, the current HIV-1 therapy has failed to demonstrate significant and persistent decline of these latent reservoirs, which appears small but stable and contains both wild type and drug resistant viral species. These considerations attract HIV-1 research to search for new and original anti HIV-1 treatment strategies. Furthermore, the efforts to tackle
HIV-1 latency fall into two keys: first blocking the development of latency and second reactivating the viral reservoirs in chronically infected individuals to clear the virus. These challenging targets could be achieved by targeting the viral reservoirs by HAART intensification or by using transcriptional regulators.

4.1. HAART Intensification. HIV-1 reservoirs are supposed to cause persistent low levels of HIV-1 RNA at a few copies/mL that are detected in HIV-1 patients on HAART. HIV-1 RNA from these reservoirs results from ongoing low level of viral replication conveying message to HIV-1 researcher that HAART is not hard enough [158, 159]. To tackle this problem, one possible solution is the HAART intensification. The objective of HAART intensification is to achieve complete suppression of residual viremia [160]. However, recent data on HAART intensification failed to decrease the residual viremia any more than normal HAART, suggesting that current regimen can halt ongoing cycles of viral replication effectively [161]. The approval of potent drugs targeting CCR5 and integrase (raltegravir) has raised the new hope for successfully decreasing the reservoir size particularly in patients with primary infection [162–164].

4.2. Strategies Based on Transcriptional Inhibitors to Control HIV. Beside the combination of HIV-1 gp41, reverse transcriptase, and protease inhibitors, new drugs should be developed to target other steps of HIV-1 life cycle [165]. For example, proteins involved in the transcription of proviral genome could be targeted. Further the drug could be designed to target cellular cofactors or viral protein like Tat that involves in the activation of transcription [166]. Several transcription inhibitors already characterized such as C-terminal truncated STAT5, Staf 50, prothymosin α, and thioredoxin reductase might be used to control the viral gene expression [167, 168]. In macrophages, the inhibition of NFAT and 5’LTR interaction by siRNA suppress the HIV-1 replication and therefore progression of AIDS also [169]. Furthermore, the treatment of HIV-1-infected lymphocyte with O-GlcNAcylation enhancing agent glucosamine represses viral transcription thus opening the way to metabolic treatment [170]. Further new approaches based on engineered transcription factors are now emerging with zinc finger protein as an attractive therapy for HIV-1 since their binding to HIV-1 LTR in a sequence-specific manner is associated with repression of LTR activation [171, 172]. For example, OKT18, a zinc finger protein, can reduce the HIV-1 replication by targeting the Tat-induced HIV-1 LTR activity. Interestingly, zinc-finger protein also has the ability to influence the chromatin and nuclear organization through protein involved in epigenetic regulation [173, 174].

The HIV-1 proteins (Tat, Nef, gp 120) should be also targeted, as these proteins have critical functions in different steps of viral life cycle and also in the acquired resistance to apoptosis. A better understanding of mechanism involved in resistance to apoptosis, has also allowed to devise new drugs against host factors which render the cells susceptible to die [175]. For example, the chemokine receptor CCR5, involved in virus entry and apoptosis could be targeted [176]. Further a chemotherapeutic drug, Imatibib, restored apoptotic sensitivity of HIV-1 macrophages through inhibition of activity of the prosurvival cytokine macrophages colony stimulating factor [177]. Finally, the addition of Akt inhibitors (Miltefosine) is also promising molecules for targeting long-lived viral reservoirs [178].

4.3. HIV-1 Reactivation from Latent Reservoirs. A new strategy so-called shock and kill has been recently proposed to eradicate the virus from infected patients. The main objective is to facilitate the reactivation of viruses from the latent reservoirs, naturally (via host immune system or viral cytopathic effects), which are then destroyed by HAART [16, 179]. Many factors have been involved in reactivation including physiological stimuli, chemical compounds (phorbol esters), HDACIs (histone deacetylase inhibitors), p-TEFb activators, and activation with antibodies (anti-CD3). Several eradication protocols passed through preclinical studies but to date all failed in clinical trials [180]. The combined therapy with IL-2 and HAART has not reduced the HIV-1 reservoirs, and viral rebound has been systematically observed. A combination of antibodies (anti-CD3) and IL-2 has proved to be highly toxic and is not further advised for HIV-1 treatment [181]. In addition, IL-7 can reactivate HIV-1 from latency in vitro through the induction of JAK/STAT signaling pathways. IL-7 increases the TCR repertoire and induces the proliferation of both naïve and memory T cells, making this cytokine an attractive candidate for future study [182].

The use of antibodies coupled to drugs and treatment with immunotoxins are also proposed strategies for selective killings of infected cells. The combination of immunotoxins and viral reactivation agents has cleared HIV-1 in cultures of lymphocyte from patients and also in animal model. Unfortunately the toxic side effects of this treatment precluded it for further development [183]. Furthermore, HDAC inhibitors or DNA methylation inhibitors are an attractive potential means of inducing broad reactivation of HIV-1 reservoirs. The combination of TSA (an HDACI) and TNF-α (NF-κB inducer) synergistically activates the HIV-1 promoter. However, toxicity of these compounds undermines their clinical interest for HIV-1 therapy [184]. Although promising results in the reduction of HIV-1 reservoirs were reported using HDAC inhibitor VPA (valproic acid), more recent studies did not confirm these results [185, 186]. However, the inability of VPA (a week HDACI) to reactivate the latent reservoirs, when used alone, might have impact on the decay of HIV-1 reservoirs, when combined with other HIV-1 inducers (prostratin) [187]. Prostratin, a nontumorigenic phorbol ester, increases HIV-1 transcription through PKC activation and induction of NF-κB and SP 1. Prostratin also downregulates HIV-1 receptors, which has the additional advantage of decreasing the risk of reinfection [188]. This compound has been advanced in clinical development, and recent synthesis made this drug available for clinical trials [189].
5. Conclusion
HIV-1 infection is currently controlled by HAART but it has long-term toxicity and does not eradicate HIV-1 latent reservoir. It is now increasingly clear that epigenetic restriction poses an initial hurdle to viral transcription and cause of maintenance of viral latency. HIV-1 latency is regulated by both cellular and viral factors. A better understanding of epigenetic regulation of HIV-1 latency and identification of new pharmacological targets would open the doors to clear the viral reservoirs.

References
[1] F. Barre-Sinoussi, J. C. Chermann, F. Rey et al., “Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS),” Science, vol. 220, no. 4599, pp. 868–871, 1983.
[2] L. O. Kallings, “The first postmodern pandemic: 25 years of HIV/AIDS,” Journal of Internal Medicine, vol. 263, no. 3, pp. 218–243, 2008.
[3] R. I. Pomerantz and D. L. Horn, “Twenty years of therapy for HIV-1 infection,” Nature Medicine, vol. 9, no. 7, pp. 867–873, 2003.
[4] D. D. Richman, D. M. Margolis, M. Delaney, W. C. Greene, D. Hazuda, and R. I. Pomerantz, “The challenge of finding a cure for HIV infection,” Science, vol. 323, no. 5919, pp. 1304–1307, 2009.
[5] M. Stevenson, “HIV-1 pathogenesis,” Nature Medicine, vol. 9, no. 7, pp. 853–860, 2003.
[6] K. Lassen, Y. Han, Y. Zhou, J. Siliciano, and D. A. Jans, “Nuclear import of the T-cell factor to direct the P-TEFb CTD kinase complex at the nuclear pore,” Science, vol. 323, no. 5919, pp. 1304–1307, 2009.
[7] P. Loetscher, B. Moser, and M. Baggiouni, “Chemokines and their receptors in lymphocyte traffic and HIV infection,” Advances in Immunology, no. 74, pp. 127–180, 2000.
[8] P. Loetscher, A. Pellegrino, J. H. Gong et al., “The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3,” The Journal of Biological Chemistry, vol. 276, no. 5, pp. 2986–2991, 2001.
[9] N. J. Arhel, S. Souquere-Besse, S. Munier et al., “HIV-1 DNA flap formation promotes uncoating of the pre-integration complex at the nuclear pore,” The EMBO Journal, vol. 26, no. 12, pp. 3025–3037, 2007.
[10] S. C. Piller, L. Caly, and D. A. Jans, “Nuclear import of the pre-integration complex (PIC): the Achilles heel of HIV?” Current Drug Targets, vol. 4, no. 5, pp. 409–429, 2003.
[11] B. Corthesy and P. N. Kao, “Purification by dna affinity chromatography of two polypeptides that contact the NF-AT DNA binding site in the interleukin 2 promoter,” The Journal of Biological Chemistry, vol. 269, no. 32, pp. 20682–20690, 1994.
[12] K. A. Jones, J. T. Kadonaga, P. A. Luciw, and R. Tjian, “Activation of the AIDS retrovirus promoter by the cellular transcription factor, Sp1,” Science, vol. 232, no. 4751, pp. 755–759, 1986.
[13] G. Nabel and D. Baltimore, “An inducible transcription factor activates expression of human immunodeficiency virus in T cells,” Nature, vol. 326, no. 6114, pp. 711–713, 1987.
[14] M. E. Garber, P. Wei, and K. A. Jones, “HIV-1 Tat interacts with cyclin T1 to direct the P-TEFb CTD kinase complex to TAR RNA,” Cold Spring Harbor Symposia on Quantitative Biology, vol. 63, pp. 371–380, 1998.
[15] V. W. Pollard and M. H. Malim, “The HIV-1 Rev protein,” Annual Review of Microbiology, vol. 52, pp. 491–532, 1998.
[16] L. Geeraert, G. Kraus, and R. J. Pomerantz, “Hide-and-seek: the challenge of viral persistence in HIV-1 infection,” Annual Review of Medicine, vol. 59, pp. 487–501, 2008.
[17] G. Dornadula, H. Zhang, B. VanUitert et al., “Residual HIV-1 RNA in blood plasma of patients taking suppressive highly active antiretroviral therapy,” Journal of the American Medical Association, vol. 282, no. 17, pp. 1627–1632, 1999.
[18] P. R. Harrigan, M. Whaley, and J. S. G. Montaner, “Rate of HIV-1 RNA rebound upon stopping antiretroviral therapy,” AIDS, vol. 13, no. 8, pp. F59–F62, 1999.
[19] L. Zhang, B. Ramratnam, K. Tenner-Racz et al., “Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy,” The New England Journal of Medicine, vol. 340, no. 21, pp. 1605–1613, 1999.
[20] J. B. Margolick, D. J. Volkman, T. M. Folks, and A. S. Fauzi, “Amplification of HTLV-III/LAV infection by antigen-induced activation of T cells and direct suppression by virus of lymphocyte blastogenic responses,” The Journal of Immunology, vol. 138, no. 6, pp. 1719–1723, 1987.
[21] J. A. Zack, A. J. Cann, J. P. Lugo, and I. S. Y. Chen, “HIV-1 production from infected peripheral blood T cells after HTLV-1 induced mitogenic stimulation,” Science, vol. 240, no. 4855, pp. 1026–1029, 1988.
[22] D. D. Ho, A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz, “Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection,” Nature, vol. 373, no. 6510, pp. 123–126, 1995.
[23] E. J. Duh, W. J. Maury, T. M. Folks, A. S. Fauzi, and A. B. Rabson, “Tumor necrosis factor α activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF-κB sites in the long terminal repeat,” Proceedings of the National Academy of Sciences of the United States of America, vol. 86, no. 15, pp. 5974–5978, 1989.
[24] Y. K. Kim, C. F. Bourgeois, R. Pearson et al., “Recruitment of TFIIH to the HIV LTR is a rate-limiting step in the emergence of HIV from latency,” The EMBO Journal, vol. 25, no. 15, pp. 3596–3604, 2006.
[25] Y. Han, M. Wind–Rotolo, H. C. Yang, J. D. Siliciano, and R. F. Siliciano, “Experimental approaches to the study of HIV-1 latency,” Nature Reviews Microbiology, vol. 5, no. 2, pp. 95–106, 2007.
[26] T. W. Chun, R. T. Davey Jr., M. Ostrowski et al., “Relationship between pre-existing viral reservoirs and the re-emergence of plasma viremia after discontinuation of highly active anti-retroviral therapy,” Nature Medicine, vol. 6, no. 7, pp. 757–761, 2000.
[27] D. D. Ho and L. Zhang, “HIV-1 rebound after anti-retroviral therapy,” Nature Medicine, vol. 6, no. 7, pp. 736–737, 2000.
[28] C. M. Coleman and L. Wu, “HIV interactions with monocytes and dendritic cells: viral latency and reservoirs,” Retrovirology, vol. 6, article 51, 2009.
[29] D. D. Ho, T. R. Rota, and M. S. Hirsch, “Infection of monocyte/macrophages by human T lymphocyte virus type III,” The Journal of Clinical Investigation, vol. 77, no. 5, pp. 1712–1715, 1986.
[30] K. C. Williams, S. Corey, S. V. Westmoreland et al., “Peripheral macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS,” The Journal of Experimental Medicine, vol. 193, no. 8, pp. 905–915, 2001.
Advances in Virology

[31] V. Dahl, L. Josefsson, and S. Palmer, “HIV reservoirs, latency, and reactivation: prospects for eradication,” Antiviral Research, vol. 85, no. 1, pp. 286–294, 2010.

[32] A. S. Perelson, P. Essunger, Y. Cao et al., “Decay characteristics of HIV-1-infected compartments during combination therapy,” Nature, vol. 387, no. 6629, pp. 188–191, 1997.

[33] S. Palmer, A. P. Wiegand, F. Maldarelli et al., “New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma,” Journal of Clinical Microbiology, vol. 41, no. 10, pp. 4531–4536, 2003.

[34] G. Barbaro, “Metabolic and cardiovascular complications of highly active antiretroviral therapy for HIV infection,” Current HIV Research, vol. 4, no. 1, pp. 79–85, 2006.

[35] T. Pierson, J. McArthur, and R. F. Siliciano, “Reservoirs for HIV-1: mechanisms for viral persistence in the presence of antiviral immune responses and antiretroviral therapy,” Annual Review of Immunology, vol. 18, pp. 665–708, 2000.

[36] G. Nunnari, D. Leto, J. Sullivan et al., “Seminal reservoirs of HIV-1 proviral latency are required for in vitro basal and Tat-activated transcription,” BMC Medicine, vol. 7, article 48, 2009.

[37] A. Marcello, “Latency: the hidden HIV-1 challenge,” Retrovirology, vol. 3, article 7, 2006.

[38] D. Bisgrove, M. Lewinski, F. Bushman, and E. Verdin, “Molecular mechanisms of HIV-1 proviral latency,” Expert Review of Anti-infective Therapy, vol. 3, no. 5, pp. 805–814, 2005.

[39] K. Strebel, J. Luban, and K. T. Jeang, “Human cellular restriction factors that target HIV-1 replication,” BMC Medicine, vol. 7, article 48, 2009.

[40] S. A. Williams and W. C. Greene, “Regulation of HIV-1 latency by T-cell activation,” Cytokine, vol. 39, no. 1, pp. 63–74, 2007.

[41] Y. Zhou, H. Zhang, J. D. Siliciano, and R. F. Siliciano, “Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4+ T cells,” Journal of Virology, vol. 79, no. 4, pp. 2199–2210, 2005.

[42] R. S. Mitchell, B. F. Beitzel, A. R. W. Schroder et al., “Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences,” PLoS Biology, vol. 2, no. 8, article E234, 2004.

[43] Y. Han, K. Lassen, D. Monie 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,” Journal of Virology, vol. 78, no. 12, pp. 6122–6133, 2004.

[44] M. K. Lewinski, D. Bisgrove, P. Shinn et al., “Genome-wide analysis of chromosomal features repressing human immunodeficiency virus transcription,” Journal of Virology, vol. 79, no. 11, pp. 6610–6619, 2005.

[45] Y. Han, Y. B. Lin, W. An et al., “Orientation-dependent regulation of integrated HIV-1 expression by host gene transcriptional readthrough,” Cell Host and Microbe, vol. 4, no. 2, pp. 134–146, 2008.

[46] I. H. Greger, F. Demarchi, M. Giacca, and N. J. Proudfoot, “Transcriptional interference perturbs the binding of Sp1 to the HIV-1 promoter,” Nucleic Acids Research, vol. 26, no. 5, pp. 1294–1300, 1998.

[47] B. P. Callen, K. E. Shearwin, and J. B. Egan, “Transcriptional interference between convergent promoters caused by elongation over the promoter,” Molecular Cell, vol. 14, no. 5, pp. 647–656, 2004.

[48] T. Lenasi, X. Contreras, and B. M. Peterlin, “Transcriptional interference antagonizes proviral gene expression to promote HIV latency,” Cell Host and Microbe, vol. 4, no. 2, pp. 123–133, 2008.

[49] A. de Marco, C. Biancotto, A. Knezevich, P. Mairui, C. Vardabasso, and A. Marcello, “Intragenic transcriptional cis-activation of the human immunodeficiency virus 1 does not result in allele-specific inhibition of the endogenous gene,” Retrovirology, vol. 5, article 98, 2008.

[50] N. Crampton, W. A. Bonass, J. Kirkham, C. Rivetti, and N. H. Thomson, “Collision events between RNA polymerases in convergent transcription studied by atomic force microscopy,” Nucleic Acids Research, vol. 34, no. 19, pp. 5416–5425, 2006.

[51] K. V. Morris, S. W. L. Chan, S. E. Jacobsen, and D. J. Looney, “Small interfering RNA-induced transcriptional gene silencing in human cells,” Science, vol. 305, no. 5688, pp. 1289–1292, 2004.

[52] W. Y. Hu, F. D. Bushman, and A. C. Siva, “RNA interference against retroviruses,” Virus Research, vol. 102, no. 1, pp. 59–64, 2004.

[53] K. J. Perkins and N. J. Proudfoot, “An ungracious host for an unwelcome guest,” Cell Host and Microbe, vol. 4, no. 2, pp. 89–91, 2008.

[54] A. Mazo, J. W. Hodgson, S. Petruk, Y. Sedkov, and H. W. Brock, “Transcriptional interference: an unexpected layer of complexity in gene regulation,” Journal of Cell Science, vol. 120, part 16, pp. 2755–2761, 2007.

[55] N. D. Perkins, N. L. Edwards, C. S. Duckett, A. B. Agranoff, R. M. Schmid, and G. J. Nabel, “A cooperative interaction between NF-xB and Sp1 is required for HIV-1 enhancer activation,” The EMBO Journal, vol. 12, no. 9, pp. 3551–3558, 1993.

[56] C. Sune and M. A. Garcia-Blanco, “Sp1 transcription factor is required for SP1 basal and Tat-activated transcription from the human immunodeficiency virus type 1 long terminal repeat,” Journal of Virology, vol. 69, no. 10, pp. 6572–6576, 1995.

[57] R. Weil and A. Israel, “T-cell-receptor- and B-cell-receptor-mediated activation of NF-xB in lymphocytes,” Current Opinion in Immunology, vol. 16, no. 3, pp. 374–381, 2004.

[58] M. Coiras, M. R. Lopez-Huertas, J. Rullas, M. Mittelbrunn, and J. Alcamí, “Basal shuttle of NF-xB/IκB alpha in resting T lymphocytes regulates HIV-1 LTR dependent expression,” Retrovirology, vol. 4, article 56, 2007.

[59] A. J. Henderson and K. L. Calame, “CCAAT/enhancer binding protein (C/EBP) sites are required for HIV-1 replication in primary macrophages but not CD4+ T cells,” Proceedings of the National Academy of Sciences of the United States of America, vol. 94, no. 16, pp. 8714–8719, 1997.

[60] S. A. Williams, L. F. Chen, H. Kwon, C. M. Ruiz-Jarabo, E. Verdin, and W. C. Greene, “NF-xB p50 promotes HIV latency through HDAC recruitment and repression of transcriptional initiation,” The EMBO Journal, vol. 25, no. 1, pp. 139–149, 2006.

[61] D. M. Gerritsen, A. J. Williams, A. S. Neish, S. Moore, Y. Shi, and T. Collins, “CREB-binding protein/p300 are transcriptional coactivators of p65,” Proceedings of the National Academy of Sciences of the United States of America, vol. 94, no. 7, pp. 2927–2932, 1997.
Advances in Virology

[63] H. Zhong, M. J. May, E. Jimi, and S. Ghosh, “The phosphorylation status of nuclear NF-κB determines its association with CBP/p300 or HDAC-1,” *Molecular Cell*, vol. 9, no. 3, pp. 625–636, 2002.

[64] V. Quivy and C. van Lint, “Regulation at multiple levels of NF-kB-mediated transactivation by protein acetylation,” *Biochemical Pharmacology*, vol. 68, no. 6, pp. 1221–1229, 2004.

[65] M. Calao, A. Burny, V. Quivy, A. Dekoninck, and C. van Lint, “A pervasive role of histone acetyltransferases and deacetylases in an NF-kB-signaling code,” *Trends in Biochemical Sciences*, vol. 33, no. 7, pp. 339–349, 2008.

[66] H. Okamura, J. Aramburu, C. Garcia-Rodriguez et al., “Concerted dephosphorylation of the transcription factor NFATc4 forms a conformational switch that regulates transcriptional activity,” *Molecular Cell*, vol. 6, no. 3, pp. 539–550, 2000.

[67] R. Q. Cron, S. R. Bartz, A. Clausell, S. J. Bort, S. J. Klebanoff, and D. B. Lewis, “NFAT1 enhances HIV-1 gene expression in primary human CD4 T cells,” *Clinical Immunology*, vol. 94, no. 3, pp. 179–191, 2000.

[68] C. van Lint, C. A. Amella, S. Emiliani, M. John, T. Jie, and E. Verdin, “Transcription factor binding sites downstream of the human immunodeficiency virus type 1 transcription start site are important for virus infectivity,” *Journal of Virology*, vol. 71, no. 8, pp. 6113–6127, 1997.

[69] M. Karin, “The regulation of AP-1 activity by mitogen-activated protein kinases,” *The Journal of Biological Chemistry*, vol. 270, no. 28, pp. 16483–16486, 1995.

[70] A. M. Hidalgo-Estvez, E. Gonzalez, C. Punzon, and M. Fresno, “Human immunodeficiency virus type 1 Tat increases cooperation between AP-1 and NFAT transcription factors in T cells,” *The Journal of General Virology*, vol. 87, no. 6, pp. 1603–1612, 2006.

[71] J. Brady and F. Kashanchi, “Tat gets the ‘green’ light on transcription initiation,” *Retrovirology*, vol. 2, article 69, 2005.

[72] M. Barboric and B. M. Peterlin, “A new paradigm in eukaryotic biology: HIV Tat and the control of transcriptional elongation,” *PloS Biology*, vol. 3, no. 2, article e76, 2005.

[73] R. E. Kiernan, C. Vanhulle, L. Schiltz et al., “HIV-1 Tat transcriptional activity is regulated by acetylation,” *The EMBO Journal*, vol. 18, no. 21, pp. 6106–6118, 1999.

[74] E. Col, C. Caron, D. Seigneurin-Berny, J. Gracia, A. Favier, and S. Khochbin, “The histone acetyltransferase, hGCN5, interacts with and acetylates the HIV transactivator, Tat,” *The Journal of Biological Chemistry*, vol. 276, no. 30, pp. 28179–28184, 2001.

[75] A. Dorr, V. Kiernmer, A. Pedal et al., “Transcriptional synergy between Tat and PCAF is dependent on the binding of acetylated Tat to the PCAF bromodomain,” *The EMBO Journal*, vol. 21, no. 11, pp. 2715–2723, 2002.

[76] M. Ott, A. Dorr, C. Hetzer-Egger et al., “Tat acetylation: a regulatory switch between early and late phases in HIV transcription elongation,” *Novartis Foundation Symposium*, vol. 259, pp. 182–193, 2004.

[77] S. Pagana, A. Pedal, B. J. North et al., “SIRT1 regulates HIV transcription via Tat deacetylation,” *PloS Biology*, vol. 3, no. 2, article e41, 2005.

[78] A. Sabo, M. Lusie, A. Cereseto, and M. Giacca, “Acetylation of conserved lysines in the catalytic core of cyclin-dependent kinase 9 inhibits kinase activity and regulates transcription,” *Molecular and Cellular Biology*, vol. 28, no. 7, pp. 2201–2212, 2008.

[79] Y. L. Chiu, V. B. Soros, J. F. Kreisberg, K. Stopak, W. Yonemoto, and W. C. Greene, “Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells,” *Nature*, vol. 435, no. 7038, pp. 108–114, 2005.

[80] H. Xu, E. Chertova, J. Chen et al., “Stoichiometry of the antiviral protein APOBEC3G in HIV-1 virions,” *Virology*, vol. 360, no. 2, pp. 247–256, 2007.

[81] B. Mangeat, P. Turelli, S. Liao, and D. Trono, “A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action,” *The Journal of Biological Chemistry*, vol. 279, no. 15, pp. 14481–14483, 2004.

[82] C. van Lint, S. Emiliani, M. Ott, and E. Verdin, “Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation,” *The EMBO Journal*, vol. 15, no. 5, pp. 1112–1120, 1996.

[83] C. van Lint, “Role of chromatin in HIV-1 transcriptional regulation,” *Advances in Pharmacology*, vol. 48, pp. 121–160, 2000.

[84] E. Verdin, P. Paras Jr., and C. van Lint, “Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation,” *The EMBO Journal*, vol. 12, no. 8, pp. 3249–3259, 1993.

[85] J. L. Workman and R. E. Kingston, “Alteration of nucleosome structure as a mechanism of transcriptional regulation,” *Annual Review of Biochemistry*, vol. 67, pp. 545–579, 1998.

[86] V. K. Gangaraju and B. Bartholomew, “Mechanisms of ATF dependent chromatin remodeling,” *Mutation Research*, vol. 618, no. 1–2, pp. 3–17, 2007.

[87] X. J. Yang and E. Seto, “HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention,” *Oncogene*, vol. 26, no. 37, pp. 5310–5318, 2007.

[88] X. J. Yang and E. Seto, “Lysine acetylation: codified crosstalk with other postranslational modifications,” *Molecular Cell*, vol. 31, no. 4, pp. 449–461, 2008.

[89] G. Legube and D. Trouche, “Regulating histone acetyltransferases and deacetylases,” *EMBO Reports*, vol. 4, no. 10, pp. 944–947, 2003.

[90] A. El Kharroubi, G. Piras, R. Zensen, and M. A. Martin, “Transcriptional activation of the integrated chromatin-associated human immunodeficiency virus type 1 promoter,” *Molecular and Cellular Biology*, vol. 18, no. 5, pp. 2535–2544, 1998.

[91] A. Jordan, D. Biggrove, and E. Verdin, “HIV reproducibly establishes a latent infection after acute infection of T cells in vitro,” *The EMBO Journal*, vol. 22, no. 8, pp. 1868–1877, 2003.

[92] G. He and D. M. Margolis, “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,” *Molecular and Cellular Biology*, vol. 22, no. 9, pp. 2965–2973, 2002.

[93] S. Gordon, G. Akopyan, H. Garban, and B. Bonavida, “Transcription factor YY1: structure, function, and therapeutic implications in cancer biology,” *Oncogene*, vol. 25, no. 8, pp. 1125–1142, 2006.

[94] L. Ylisastigui, J. J. Coull, V. C. Rucker et al., “Polyamides reveal a role for repression in latency within resting T cells of HIV-infected donors,” *Advances in Virology*, vol. 21, no. 11, pp. 2715–2723, 2002.
D. Avram, A. Fields, K. Pretty On Top, D. J. Nevrivy, J. E. Y. Han and R. F. Siliciano, “Keeping quiet: microRNAs in...” Nature, vol. 19, no. 8, pp. 12218–12226, 2007.

[113] H. S. Christensen, A. Daher, K. J. Soye et al., “Small interfering RNAs against the TAR RNA binding protein, TRBP, a Dicer cofactor, inhibit human immunodeficiency virus type 1 long terminal repeat expression and viral production,” Journal of Virology, vol. 81, no. 10, pp. 5121–5131, 2007.

[114] A. Gatignol, S. Laine, and G. Clerzius, “Dual role of TRBP in HIV replication and RNA interference: viral diversion of a cellular pathway or evasion from antiviral immunity?” Retrovirology, vol. 2, article 65, 2005.

[115] G. Herbein and A. Varin, “The macrophage in HIV-1 infection: from activation to deactivation?” Retrovirology, vol. 7, article 33, 2010.

[116] P. J. Ellery, E. Tippett, Y. L. Chiu et al., “The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo,” The Journal of Immunology, vol. 178, no. 10, pp. 6581–6589, 2007.

[117] C. F. Perno, V. Svircher, D. Schols, M. Polliccita, J. Balzarini, and S. Aquaro, “Therapeutic strategies towards HIV-1 infection in macrophages,” Antiviral Research, vol. 71, no. 2-3, pp. 293–306, 2006.

[118] G. A. Garden, “Microglia in human immunodeficiency virus-associated neurodegeneration,” Glia, vol. 40, no. 2, pp. 240–251, 2002.

[119] H. Lassmann, M. Schmied, K. Vass, and W. F. Hickey, “Bone marrow derived elements and resident microglia in brain inflammation,” Glia, vol. 7, no. 1, pp. 19–24, 1993.

[120] L. Gillim-Ross, A. Cara, and M. E. Klotman, “HIV-1-extrachromosomal 2-LTR circular DNA is long-lived in human macrophages,” Viral Immunology, vol. 18, no. 1, pp. 190–196, 2005.

[121] S. Pang, Y. Koyanagi, S. Miles, C. Wiley, H. V. Vinsters, and I. S. Y. Chen, “High levels of unintegrated HIV-1 DNA in brain tissue of AIDS dementia patients,” Nature, vol. 343, no. 6253, pp. 85–89, 1990.

[122] D. A. Eckstein, M. P. Sherman, M. L. Penn et al., “HIV-1 Vpr enhances viral burden by facilitating infection of tissue macrophages but not nondividing CD4+ T cells,” The Journal of Experimental Medicine, vol. 194, no. 10, pp. 1407–1419, 2001.

[123] B. Poon and I. S. Y. Chen, “Human immunodeficiency virus type 1 (HIV-1) vpr enhances expression from unintegrated HIV-1 DNA,” Journal of Virology, vol. 77, no. 7, pp. 3962–3972, 2003.

[124] A. Cara and M. E. Klotman, “Retroviral E-DNA: persistence and gene expression in nondividing immune cells,” Journal of Leukocyte Biology, vol. 80, no. 5, pp. 1013–1017, 2006.

[125] J. Kelly, M. H. Beddall, D. Yu, S. R. Iyer, J. W. Marsh, and Y. Wu, “Human macrophages support persistent transcription from unintegrated HIV-1 DNA,” Virology, vol. 372, no. 2, pp. 300–312, 2008.

[126] V. le Douce, G. Herbein, O. Rohr, and C. Schwartz, “Molecular mechanisms of HIV-1 persistence in the monocyte-macrophage lineage,” Retrovirology, vol. 7, article 32, 2010.

[127] S. Asin, J. A. Taylor, S. Trushin, G. Bren, and C. V. Paya, “IkκB mediates NF-κB activation in human immunodeficiency virus-infected cells,” Journal of Virology, vol. 73, no. 5, pp. 3893–3903, 1999.

[128] W. Choe, D. J. Volsky, and M. J. Potash, “Activation of NF-κB by R5 and X4 human immunodeficiency virus type 1 induces macrophage inflammatory protein 1α and tumour necrosis factor alpha in macrophages,” Journal of Virology, vol. 76, no. 10, pp. 5274–5277, 2002.
Advances in Virology

[129] G. Herbein and K. A. Khan, “Is HIV infection a TNF receptor signalling-driven disease?” Trends in Immunology, vol. 29, no. 2, pp. 61–67, 2008.

[130] E. Guillemand, C. Jacquemot, F. Aillet, N. Schmitt, F. Barre-Sinoussi, and N. Israel, “Human immunodeficiency virus 1 favors the persistence of infection by activating macrophages through TNF,” Virology, vol. 329, no. 2, pp. 371–380, 2004.

[131] B. Lee, M. Sharron, L. J. Montaner, D. Weissman, and R. W. Doms, “Quantification of CD4, CCR5, and CXCR4 levels on lymphocyte subsets, dendritic cells, and differentially conditioned monocyte-derived macrophages,” Proceedings of the National Academy of Sciences of the United States of America, vol. 96, no. 9, pp. 5215–5220, 1999.

[132] J. Lama, A. Mangasarian, and D. Trono, “Cell-surface expression of CD4 reduces HIV-1 infectivity by blocking Env incorporation in a Nef- and Vpu-inhibitable manner,” Current Biology, vol. 9, no. 12, pp. 622–631, 1999.

[133] M. Collin, P. Illei, W. James, and S. Gordon, “Definition of the range and distribution of human immunodeficiency virus macrophage tropism using PCR-based infectivity measurements,” The Journal of General Virology, vol. 75, part 7, pp. 1597–1603, 1994.

[134] W. A. O’Brien, A. Namazi, H. Kalhor, S. H. Mao, J. A. Zack, and I. S. Y. Chen, “Kinetics of human immunodeficiency virus type 1 reverse transcription in blood mononuclear phagocytes are slowed by limitations of nucleotide precursors,” Journal of Virology, vol. 68, no. 2, pp. 1258–1263, 1994.

[135] A. I. Spira, P. A. Marx, B. K. Patterson et al., “Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques,” The Journal of Experimental Medicine, vol. 183, no. 1, pp. 215–225, 1996.

[136] A. Smed-Sorensen, K. Lore, J. Vasudevan et al., “Differential susceptibility to human immunodeficiency virus type 1 infection of myeloid and plasmacytoid dendritic cells,” Journal of Virology, vol. 79, no. 14, pp. 8861–8869, 2005.

[137] K. Lore, A. Smed-Sorensen, J. Vasudevan, J. R. Mascola, and R. A. Koup, “Myeloid and plasmacytoid dendritic cells transfer HIV-1 preferentially to antigen-specific CD4+ T cells,” The Journal of Experimental Medicine, vol. 201, no. 12, pp. 2023–2033, 2005.

[138] A. T. Haase, K. Henry, M. Zupancic et al., “Quantitative image analysis of HIV-1 infection in lymphoid tissue,” Science, vol. 274, no. 5289, pp. 985–989, 1996.

[139] M. Otero, G. Nunnari, D. Leto et al., “Peripheral blood dendritic cells are not a major reservoir for HIV type 1 in infected individuals on virally suppressive HAART,” AIDS Research and Human Retroviruses, vol. 19, no. 12, pp. 1097–1103, 2003.

[140] J. M. Carr, H. S. Ramshaw, P. Li, and C. J. Burrell, “Cd34 cells and their derivatives contain mRNA for CD4 and human immunodeficiency virus (HIV) co-receptors and are susceptible to infection with M- and T-tropic HIV,” The Journal of General Virology, vol. 79, no. 1, pp. 71–75, 1998.

[141] A. Aiuti, L. Turchetto, M. Cota et al., “Human CD34 cells express CXCR4 and its ligand stromal cell-derived factor-1. Implications for infection by T-cell tropic human immunodeficiency virus,” Blood, vol. 94, no. 1, pp. 62–73, 1999.

[142] G. J. Randolph, K. Inaba, D. F. Robbiani, R. M. Steinman, and W. A. Muller, “Differentiation of phagocytic monocytes into lymph node dendritic cells in vivo,” Immunity, vol. 11, no. 6, pp. 753–761, 1999.

[143] G. J. Randolph, S. Beaulieu, S. Lebecque, R. M. Steinman, and W. A. Muller, “Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking,” Science, vol. 282, no. 5388, pp. 480–483, 1998.

[144] J. Sprent and C. D. Surh, “Normal T cell homeostasis: the conversion of naive cells into memory-phenotype cells,” Nature Immunology, vol. 12, no. 6, pp. 478–484, 2011.

[145] R. D. Michalek and J. C. Rathmell, “The metabolic life and times of a T-cell,” Immunological Reviews, vol. 236, no. 1, pp. 190–202, 2010.

[146] M. I. Bukrinsky, N. Sharova, M. P. Dempsey et al., “Active nuclear import of human immunodeficiency virus type 1 preintegration complexes,” Proceedings of the National Academy of Sciences of the United States of America, vol. 89, no. 14, pp. 6580–6584, 1992.

[147] S. Swingler, B. Brichacek, J. M. Jacque, C. Ulich, J. Zhou, and M. Stevenson, “HIV-1 Nef intersects the macrophage CD40L signalling pathway to promote resting-cell infection,” Nature, vol. 424, no. 6945, pp. 213–219, 2003.

[148] Y. Wu and J. W. Marsh, “Selective transcription and modulation of resting T cell activity by preintegrated HIV DNA,” Science, vol. 293, no. 5534, pp. 1503–1506, 2001.

[149] D. Persaud, T. Pierson, C. Ruff et al., “A stable latent reservoir for HIV-1 in resting CD4+ T lymphocytes in infected children,” The Journal of Clinical Investigation, vol. 105, no. 7, pp. 995–1003, 2000.

[150] Y. Wu, “Chemokine control of HIV-1 infection: beyond a binding competition,” Retrovirology, vol. 7, article 86, 2010.

[151] J. A. Zack, S. J. Arrigo, R. S. Weisman, A. S. Go, A. Haislip, and I. S. Y. Chen, “HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure,” Cell, vol. 61, no. 2, pp. 213–222, 1990.

[152] T. W. Chun, D. Finzi, J. Margolick, K. Chadwick, D. Schwartz, and R. F. Siliciano, “Long-term follow-up of resting CD4+ T lymphocytes in infected children,” Nature Medicine, vol. 1, no. 12, pp. 1284–1290, 1995.

[153] T. W. Chun, L. Carruth, D. Finzi et al., “Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection,” Nature, vol. 387, no. 6629, pp. 183–188, 1997.

[154] J. D. Siliciano, J. Kajdas, D. Finzi et al., “Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells,” Nature Medicine, vol. 9, no. 6, pp. 727–728, 2003.

[155] D. D. Richman, “Antiviral drug resistance,” Antiviral Research, vol. 71, no. 2–3, pp. 117–121, 2006.

[156] D. Finzi, J. Blankson, J. D. Siliciano et al., “Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy,” Nature Medicine, vol. 5, no. 5, pp. 512–517, 1999.

[157] O. Lambotte, K. Deiva, and M. Tardieu, “HIV-1 persistence, viral reservoir, and the central nervous system in the HAART era,” Brain Pathology, vol. 13, no. 1, pp. 95–103, 2003.

[158] M. Coiras, M. R. Lopez-Huertas, M. Perez-Olmeda, and J. Alcami, “Understanding HIV-1 latency provides clues for the eradication of long-term reservoirs,” Nature Reviews, vol. 7, no. 11, pp. 798–812, 2009.

[159] L. M. Frenkel, Y. Wang, G. H. Learn et al., “Multiple viral genetic analyses detect low-level human immunodeficiency virus type 1 replication during effective highly active antiretroviral therapy,” Journal of Virology, vol. 77, no. 10, pp. 5721–5730, 2003.
