Retroviral Latency and Transcription Balance

Subjects: Pathology

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The representative of the Lentivirus genus is the human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS). To date, there is no cure for AIDS because of the existence of the HIV-1 reservoir. HIV-1 infection can persist for decades de-spite effective antiretroviral therapy (ART), due to the persistence of infectious latent viruses in long-lived resting memory CD4+ T cells, macrophages, monocytes, microglial cells, and other cell types. However, the biology of HIV-1 latency remains incompletely understood. Retroviral long terminal repeat region (LTR) plays an indispensable role in controlling viral gene expression. Regulation of the transcription initiation plays a crucial role in establishing and maintaining a retro-virus latency. Whether and how retroviruses establish latency and reactivate remains unclear.

retroviruses human immunodeficiency virus type 1

1. Introduction

The human immunodeficiency virus type 1 (HIV-1) belongs to the family of Retro-viridae, subfamily Orthoretrovirinae, and genus Lentivirus. HIV¬-1 is firmly associated with the acquired immunodeficiency syndrome (AIDS) [1]. Highly pathogenic lentivi-ruses, after integration of double-stranded viral DNA into cellular genome, activate transcription of the viral genome. After synthesis of viral nucleic acid and formation of several viral proteins, to complete the viral life cycle, progeny virions are produced [2]. The efficiency of the initial transcription of integrated DNA from 5′ long terminal re-peat (LTR) region promoter determines the level of viral RNA in an infected cell. Pro-viral 5′ LTR promoter contains numerous cis-regulatory elements, which modulate the rate of viral transcription initiation. However, certain cell types and the cell differen-tiation processes with respect to diversity of cell activation signals may contribute to substantial variations in transcriptional activity of LTR [3]. All these variables gener-ate a remarkably broad range in HIV-1 gene expression level. Contrary to simple ret-roviruses (avian leukemia virus and murine leukemia virus), regulation of lentivirus gene expression involves both cellular and virally encoded regulatory factors. Conse-quently, RNA production in HIV-1 infection is highly variable.

The latently infected cells are a source of viral reactivation and lead to marked increase of the viral load after a pause of highly active antiretroviral therapy (HAART). In this context, a better understanding of the molecular mechanisms re-sponsible for the regulation of proviral latency and reactivation would define rational strategies aimed at purging the HIV-1 reservoirs in treated patients. The regulation of gene expression in HIV-1 is complex and requires multiple steps, including chromatin organization, allowance of transcription machinery, mRNA processing and its transport to the cytoplasm, translation and posttranslational processes.

2. LTR Regulatory Elements

Retroviruses integrate into host DNA as proviruses that are flanked by LTRs at each end of the viral DNA. Transcription of proviral DNA is catalyzed by cellular RNA polymerase II (RNAPII) and initiated at the U3 end of 5′ LTR. Each LTR is composed of three regions: unique 3′ (U3), repeated (R), and unique 5′ (U5). U3 occupies most of the LTR and plays an important role in the induction of retroviral transcription, since it contains the viral promoters and other cis-active elements required for the modulation of promoter activity. The TATA box, located within the LTR promoter element, provides the binding site for RNAPII, determining the site of initiation and also affecting the efficiency of the initiation of transcription [4].

The U3 region of HIV-1 LTR contains the crucial regulatory elements for the core promoter region: three *specific protein* 1 (Sp1) sites and TATA box; for the enhancer region: two nuclear factor-κB sites (NF-κB) and one nuclear factor of activated T-cells (NF-AT) site; for the modulatory region: three CCAAT/enhancer binding protein (C/EBP) sites, the activating transcription factor/cyclic AMP response element binding (ATF/CREB) region, two NF-AT sites, two activator protein *1 (*AP-1) sites, one upstream stimulatory factor Ets/PU.1, and one T-cell specific *transcription factor/*lymphoid enhancer binding factor (TCF/LEF-1) [5][6][7][8].

In HIV-1, the following regulatory sequences downstream of the transcription start site are as follows: the initiator (Inr), the inducer of short transcripts (ITS), and trans-activation responsive element (TAR). TAR forms an RNA stem-loop structure, which recruits the virally encoded transactivator protein (Tat) to the LTR to modulate the activity of the viral promoter [1]. In addition, HIV-1 LTR consists of several substantial transcription factor (TF) binding sites including AP-1 sites, an AP-3-like (AP-3L) sequence, C/EBP/NFAT (nuclear factor for activated T cells) downstream binding site (DS3), two downstream sequence element (DSE) sites, one downstream binding factor (DBF-1) in R region, and two Sp1 binding sites and gag leader sequence (GLS) in the U5 [9]. Enhancer functions have been also mapped to the *gag–pol* regions of simian immunodeficiency *virus (*SIV) and HIV, but their role in the virus replication has yet to be established.

The transcription of *Lentiviruses* is regulated by the interactions between numerous and different viral proteins and transcription factors with binding sites located in the 5′ LTR. Most of regulatory elements encompass the U3 region. Regulatory elements situated in R and U5 regions may improve the promoter and enhancer strengths and provide a broad viral response for stimulating factors and control transcription in cell-type-dependent manner.

3. A Variety of Enhancers with Regulatory Functions

The HIV-1 mainly infects CD4+ T cells, monocytes, and macrophages, and in a lower proportion also dendritic cells (DCs) and microglial cells. HIV-1 enhancer sequence consists of two NF-κB binding sites and three adjacent Sp1 binding sites that are required for viral transcription [5]. Other factors shown to bind the enhancer include Ets, PU.1, NF-AT, C/EBP, AP-1, cAMP response element-binding protein/ activating transcription factor (CREB/ATF), *upstream* stimulatory *factor* (USF), Sp1, Sp3 and chicken ovalbumin upstream promoter transcription factor (COUP-TF) and they play role in enhancing the transcription (Table 1).

Table 1. Key transcription factors involved in regulation of human immunodeficiency virus type 1 (HIV-1) transcription in different cell types.

Transcription Factor	Cell Type
NF-κB	T cells*, monocytes, macrophages, iDC, microglial cells
NF-AT	T cells
Sp1	microglial cells, T cells, monocytes, macrophages, iDC
Sp3	microglial cells, monocytes, macrophages
AP-1	microglial cells, monocytes, T cells
COUP-TF	microglial cells, T cells
Ets-1	T cells
USF	monocytes, macrophages, iDC, T cells, microglial cells
C/EBP (NF-IL-6)	monocytes, macrophages, iDC, T cells, microglial cells
CREB/ATF	T cells, microglial cells, monocytes, macrophages

* transcription factors required for transcriptional activation in cell-type-specific expression of HIV-1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NF-AT, nuclear factor of activated T-cells; Sp1, 3, *specific protein* 1, 3; AP-1, activator protein *1; COUP-TF,* chicken ovalbumin upstream promoter transcription factor; Ets-1, E26 transformation-specific (ETS) transcription factor; USF, *upstream* stimulatory *factor*; C/EBP, CCAAT/enhancer-binding protein; NF-IL-6, transcription factor nuclear factor interleukin 6; CREB/ATF, cAMP response element-binding protein/ activating transcription factor.

This variety of binding sites may result in maintenance reverse latency in some cells. As an example, NF-κB transcription factor binding to enhancer sites within LTR activate viral transcription in most HIV-1-infected types of cells [10]. The transcriptional activity of the NF-κB and other transcription factors in primary immune cells versus transformed cell lines is listed in Table 2.

Table 2. Regulation of HIV-1 gene transcription in primary immune cells and transformed cell lines.

Transcription Factor	Cell Type	Primary Cells	Transformed Cell Line
NF-κB	T cells	• activates transcription in dopamine-stimulated PBMCs [11]	• activates transcription in dopamine-stimulated lymphoid Jurkat T cell line [11]
		• activates transcription in CD4+ T cells by direct occupancy of enhancer by NF-κB p50/p65 [12]	• activates transcription in Jurkat T cell line that stably expresses the Tat [14]
		• activates transactivation in TNF-, IL-1-, and IL-7-stimulated TEC co-cultured with thymocytes [13]	• activates transcription in latently HIV-1-infected established T lymphoid cell line J1.1 promoted by MRPs [15]
	monocytes/macrophages	• activates transcription in macrophages by direct occupancy of enhancer by NF-κB p50/p65 [16] • involved in efficient activation of viral transcription in monocytes isolated from PBMC [17]	• activates HIV gene transcription in monocytic cell line U937 and promonocytic cell U1 by direct occupancy of enhancer by NF-κB p50/p65 [18] [19]
	microglial cells	nd	• activates transcription in human microglial MC-3 cell line and embryonic microglial cell

			line upon stimulation with IFNγ, IL1β, and TNFα [20][21][22]
NF-AT	T cells	• enhances activation of transcription in CD4+ T cells • NF-AT1,2 enhances activation of transcription in PMA/ionomycin stimulated CD4+ T cells [23] • NFAT1, 2 positive effect on transcription in PMA-, PHA-, bpV-stimulated PBMC [24]	• efficient binding to the HIV-1 LTR enhancer in Jurkat-derived CD4+ T cells isoform CD45(−), stimulated with PMA/PHA/α-CD3 [25] • represses Tat-mediated transactivation in PMA/ionomycin-stimulated Jurkat T-cells [26] • NFAT1, 2 enhances transcription in Jurkat T cells stimulated with PMA, PHA and bpV [24]
Sp1, 3	microglial cells	nd	• Sp1 interaction with COUP-TF leads to activation of HIV gene transcription in microglial cell line [18] • binding CTIP-2 to Sp1 represses Tat-mediated transcriptional activation HIV promoter [27] • Sp3 represses Sp1 and COUP-TF-induced activation in human microglial cell line [18]
	T cells	• Sp1 associated with Tat activates transcription in CD4+ T cells and PBMCs [28]	• Tat-induced Sp1 activates promoter in MT-2 cell line and Jurkat T cells [28]

			• Sp1 assembly pre-initiation complex at the LTR TATA box and cooperatively interacts with NF-κB to activate transcription in Jurkat T cells stimulated with PMA[29]
	monocytes/macrophages	• Sp1-to-Sp3 ratio increases during monocyte lineage differentiation, resulting in increased HIV-1 transcription [30]	• Sp1 activates LTR-driven transcription in U1 monocytic cells [31][35] • Sp1 has moderate impact on transcription activation in human monocytic line U-937 [32]
	iDC	• Sp1 activates HIV gene transcription in DC differentiated from monocytes derived from PBMCs [35]	nd
AP-1	microglial cells	nd	• c-jun and c-fos interact with TRE sequence and enhance HIV-1 gene transcription in glial cells [34]
	monocytes/ macrophages	• Vpr-activated AP-1 enhances viral transcription in macrophages differentiated from PBMCs [35]	• Vpr-activated AP-1 enhances viral transcription in U937 cells [35] • Nuclear complex of c-fos and c-jun binds directly to the HIV LTR and enhances NF-κB activity in human monocytic cell lines U1 and U937 [36] • AP-1 activated by Nef stimulates HIV transcription in

			U1 and U937 cells [37]
	T cells	• enhances HIV-1 gene expression in CBMCs more than in PBMCs [38]	• c-jun and c-fos do not interact with TRE sequence and do not enhance HIV-1 transcription in Jurkat T cells [39]
COUP-TF	microglial cells	• cooperates with Tat to promote NF-κB- and Sp1-independent transactivation HIV-1 transcription in human fetal microglial cells [40]	• cooperates with Tat and promotes NF-κB and Sp1-independent activation HIV-1 transcription in microglial cell line [40] • COUP-TF Sp1 interaction stimulates HIV transcription in microglial cell line • COUP-TF, Sp1, and CTIP2 cooperation suppresses HIV transcription initiation in microglial cells [41]
	T cells	nd	• COUP-TF interaction with Sp1 synergistically stimulates viral transcription in Jurkat T cells in response to cAMP and dopamine [42]
Ets	T cells	• Ets in cooperation with NF-kB/NFAT activates HIV-1 enhancer in human peripheral blood T cells [43]	• Ets in cooperation with USF-1 enhances transcriptional activity of HIV-1 LTR in Jurkat T cells [44]
	monocytes/macrophages	• regulates HIV transcription by recruiting HATs to the LTR in primary macrophages [45]	• recruits HATs to LTR and mediates initiation of transcription in promonocytic U937 cells [46]

C/EBP			
(NF-IL-6)	T cells	nd	• is not required in HIV transcription in Jurkat CD4+ T cell line [45] • cooperates with CREB and mediates prostaglandin E2-induced stimulation of LTR-driven transcription Jurkat E6.1 [47]
	microglial cells	nd	• in presence of IL-1, IL-6, and TNF- α, activates LTR-driven transcription versus C/EBPγ that acts as inhibitor [48]
CREB	T cells	• phospho-CREB recruits CBP and basal transcription factors, which increases promoter activation in primary lymphocytes [49]	• phospho-CREB recruits CBP and basal transcription factors, which increases promoter activation in MT-4 human T cell line [50] • mediates cAMP and dopamine-induced transcriptional stimulation through indirect interactions with LTR in Jurkat T cells [42] • cooperates with COUP-TF in the presence of forskolin, cAMP, and dopamine to activate HIV-1 gene transcription in Jurkat T cells [42]
	monocytes/macrophages	nd	• CREB homodimers bind to their DNA site, interact with C/EBPs, and lead to increase HIV promoter activation in U-

		937 and THP-1 human monocytic cell lines; sequence variations at the CREB site affect LTR activity [51]

nd, not determined; TEC, human thymic epithelial cell; MRPs, proinflammatory myeloid-related proteins; PBMC, peripheral blood mononuclear cell; NF-AT, nuclear factor of activated T cells; PMA, Phorbol 12-myristate 13-acetate; NF-κB, nuclear factor-kappa B; PHA, phytohemagglutinin; bpV, bis-peroxovana-dium a protein tyrosine phosphatases (PTP) inhibitor; COUP-TF, chicken ovalbumin upstream promoter transcription factor; MT-2, cell line derived from normal human cord leukocytes cocultivated with leukemic cells from an adult T cell leukemia (ATL) patient; CBMCs, umbilical cord blood mononuclear cells; CTIP2, Chicken ovalbumin upstream promoter transcription factor interacting protein 2; cAMP, cyclic AMP, adenosine 3',5'-cyclic monophosphate; HATs, histone acetylotransferase; Ets, erythroblast transformation specific transcription factor; Sp1, transcription factor specificity protein 1; AP-1, activator protein; C/EBP, CCAAT/enhancer-binding protein; NF-IL-6, transcription factor nuclear factor interleukin 6; CREB, cAMP response element-binding protein; CBP, CREB binding protein.[53]

In activated CD4+ T lymphocytes, the Sp1 transcription factors are not sufficient to mediate transcription and further binding NF-κB and NF-AT cellular factors to the LTR enhancer region is required to activate transcription. In addition, the USF, Ets, NF-IL-6 and CREB proteins facilitate efficient transcription. In long-lived latently infected CD4+ T cells, NF-κB and NF-AT, as key factors for initiation of HIV-1 transcription in these cells, are present in very low nuclear concentrations. In addition, Cyclin T1 protein levels are also very low in comparison to activated T cells. For that reason, the above mechanisms have been proposed to be probably involved in CD4+ T cell latency [52].

In monocyte–macrophage lineage cells, regulation of HIV-1 transcription varies considerably during macrophage differentiation, as numerous transcription factors are expressed in a differentiation-dependent manner. In monocytes, LTR activity may be regulated during their differentiation stages by changes in the Sp1 (activator):Sp3 (repressor) ratio.

Increased permissiveness of macrophages for HIV-1 replication leads to expression of the cofactors utilized for Tat transactivation of the LTR, and this leads to a high level of HIV-1 transcription. There are numerous studies supporting that microglial cells are susceptible to HIV-1 infection and can be latently infected, constituting a major reservoir in the brain. In contrast to the monocytes, NF-κB, AP-1, and NFAT proteins are constitutively localized in the nucleus of microglial cells, and the Sp1 expression predominates over the Sp3. Interestingly, latently infected microglial cells can be reactivated by cytokine stimulation. In contrast to other reservoirs, the NF-kB and Sp1 binding sites are sufficient for HIV-1 transcription in microglial cells [53]. Contrary to CD4+ T cells, which express only Sp1, microglial cells produce both Sp1 and Sp3; the latter acting as transcriptional repressor. In addition, C/EBPɤ is expressed and acts as repressor by competing with the transcriptional activator C/EBP (Table 2) [54].

To conclude, the LTRs play a significant role in cell-type-specific expression of the proviral genome. HIV-1 enhancer sequences contain many binding sites providing mechanisms for a broad viral response to extracellular factors and regulate transcription in the cell-type-dependent manner. These observations thus emphasize the differences in mechanisms underlying HIV-1 latency between infected cells.

4. Transactivation of LTR by Virus-Encoded Tat Protein

Lentiviruses are capable of promoting the rate of their gene expression through virus-encoded transactivator proteins. Activation occurs by binding of Tat HIV-1 protein to a specific sequence adjacent to 5′ trans-activation response (TAR) element RNA transcript [55][56][57]. Tat protein of HIV-1 (and related *Lentiviruses*) interacts with the viral RNA transcript, through a unique RNA regulatory segment of the LTR termed transactivation-responsive element (TAR). The TAR secondary RNA structure is formed from transcription of the +19–43 tract in the LTR R region [58]. Various mechanisms of HIV-1 Tat transactivation have been proposed. One model suggests overriding transcription terminations, since in the absence of Tat transcripts that initiate in LTR pause after synthesis of about 70 nucleotides. It has also been proposed that in early steps of viral transcription, the complex of positive transcription elongation factor b (P-TEFb) composed of Cyclin T1 (CycT1) and cyclin-dependent kinase 9 (CDK9) is recruited to the LTR via nuclear factor kappa B (NF-κB). The recruitment of Tat and P-TEFb to the TAR hairpin facilitates phosphorylation of RNAP II, which increases their combined effectiveness and prevents premature termination [58][59]. On the other hand, several investigations revealed that NF-κB can promote both transcription initiation and elongation complex, at a similar level to that of Tat, in a manner independent of Tat. The NF-κB transcription factors induce LTR regulation via interaction with binding sites located within the enhancer region [59]. Deletion of the NF-κB binding sites strongly reduces basal, as well as Tat-transactivated, LTR activity. The Tat proteins activate NF-κB through a IκB kinase (IKK), which accelerates the degradation of IκB, a protein that regulates NF-κB activity by binding NF-κB and translocating to the nucleus [59][60]. In vitro model systems support an alternative hypothesis where Tat initiates transcription through a protein–protein interaction with the Sp1 transcription factor. This paradigm is supported by findings that nucleotide changes within the cis-acting elements recruiting Sp factors to the HIV-1 LTR reduce Tat-mediated LTR activity [60].

Additionally,viral protein R (Vpr) is another viral accessory protein capable of enhancing the activity of the HIV-1 LTR. Vpr can bind to histone acetyltransferases (HAT) CREB-binding protein and p300, glucocorticoid receptor, CycT1, and Tat to activate transcription [61][62]. Vpr can also activate NF-κB-directed transcription (reviewed in [1]). HIV-1 LTR C/EBP and NF-κB complex demonstrates a high affinity for Vpr and a low affinity for C/EBPβ during late-stage HIV in brain cells from patients with HIV-associated dementia (HAD) [61][62]. In addition, Kilareski and co-workers identified specific Tat variants derived from HAD brain, which were defective in LTR transactivation, however still were able to activate promoters of the other proinflammatory cytokine genes. Collectively, in the tissues of the brain, Tat may become less transcriptionally competent, however, in this situation, Vpr may facilitate HIV-1 replication by enhancing transcription in the absence of a fully active Tat. On the other hand, Razooky and co-workers suggested that Tat can control a viral reservoir in infected resting and memory CD4+ T cells, even if the Tat level in these cells is low. They found that Tat mutants exaggerated lower levels of HIV-1 expression in the

resting cells [63][64]. In addition, Chakraborty and co-workers data indicated that Tat promotes latency by generating a negative feedback loop at later stages of infection, which leads to the silencing of HIV-1 promoter [65].

The primary function attributed to Tat is the transactivation of HIV-1 promoter. Additionally, it has been demonstrated that Tat enhances HIV-1 virulence by interacting with various cellular proteins in order to induce T cell apoptosis, co-receptor regulation, and cytokine induction in the host cells [66][67][68]. The effect of Tat on many viral activities in the host cell contributes to the pathogenesis of HIV-1, pointing to this molecule as a potential target for HIV-1 therapy, for example, by blocking viral replication by targeting Tat [69][70][71][72]. The Tat naturally occurring polymorphisms are usually caused by viral mutational escape from CD8+ cytotoxic T lymphocyte (CTL) recognition. The host immune responses mediated by CTLs and less by CD4+ T lymphocytes and B lymphocytes may potentially force selective pressure towards Tat diversity and affect its activity [73][74]. It has been proposed that variations in Tat sequence could modulate transactivation and have implications on HIV-1 latency and the reactivation phase. Ronsard and co-workers reported that the Tat variants with a change of S46F were able to significantly enhance LTR transactivation compared with wild-type Tat [75]. Additionally, the change of S46F caused strong Tat interaction with TAR in in vitro and in silico models. In contrast, a naturally occurring change of the C22S in HIV-1 Oyi strain reduced Tat transactivation activity and was linked with long-term nonprogressive infections [76]. Furthermore, other naturally occurring polymorphisms within Tat identified in HIV-infected patients at acute and/or early infection phase (i.e., P10S, W11R, K19R, A42V, and Y47H) have been shown to significantly impair transactivation activity in the infected CD4+ T lymphocytes [77]. These data suggest that certain naturally occurring changes can change Tat transactivation activity.

The infected lymphocytes *rapidly* produce great numbers of viral particles, and it is clear that Tat protein triggers this process. Clones with nonsense changes are unable to replicate and thereby disappear from the spectra in vivo. However, as the infection progresses, some naturally occurring changes in Tat can change its immunogenic properties, prevent transactivation, and may influence viral latency. Nevertheless, it remains unclear to what extent CTL escape changes occurring in the Tat epitope may affect the HIV-1 latency kinetic from *establishment* to reversal *stages* [78].

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