New Findings on Transcription Regulation Across Different HIV-1 Subtypes

Eva Ramírez de Arellano, Vincent Soriano, José Alcami[1] and África Holguín
Department of Infectious Diseases, Hospital Carlos III, Madrid. [1]National Center for Microbiology, Instituto de Salud Carlos III, Majadahonda, Spain

Abstract

Transcriptional activation of gene expression in HIV-1 is controlled by the interaction of sequence-specific transcription factors with the long terminal repeat (LTR) of the provirus. The identification and characterization of cellular proteins involved in the process has provided a basic understanding about both general eukaryotic and HIV-1 proviral transcription regulation. The HIV-1 epidemic is expanding worldwide with an increasing number of distinct viral subtypes as well as intersubtype recombinant viruses. LTR-specific sequence variability among different HIV-1 variants could affect LTR binding to cellular and/or viral factors, influencing the extent of transcription.* **In vitro** *assays have demonstrated subtype-specific functional differences between the LTR regions of distinct HIV-1 subtypes. This observation could have consequences on the biology of the different HIV-1 clades and influence HIV-1 disease progression. Finally, the knowledge of the molecular mechanisms of transcription regulation events could help in the search for new compounds targeting the critical steps of viral transcription. **(AIDS Reviews 2006;8:9-16)**

Key words

HIV-1. Subtypes. Long terminal repeat. LTR. Transcription.

Introduction

Transcription is a crucial step for HIV-1 expression in all infected host cells. Multiple cell type-specific interplays between cellular and viral factors lead the virus to leave latency and to replicate in a great diversity of cells, despite the variability of its long terminal repeat (LTR) region in different HIV-1 strains. After fusion-mediated entry within host cells, un-coating, reverse transcription of the RNA genome, and nuclear entry of the pre-integration complex, the proviral DNA is integrated into the host-cell genome. Transcription of the HIV-1 provirus is then regulated by a combination of distinct viral and cellular transcription factors. A growing list of transcription factors interacting with the LTR region, their binding sites, and other viral and cellular factors are involved in the regulation of LTR activity[1].

Correspondence to:
África Holguín
Hospital Carlos III
Molecular Biology Laboratory
Department of Infectious Diseases
Calle Sinesio Delgado 10
28035 Madrid, Spain
E-mail: aholguin.hciii@salud.madrid.org

The LTR region of HIV-1 is similar to the eukaryotic promoter complex, with transcriptional enhancers and regulatory elements responsive to either viral or specialized cellular transactivation factors. HIV-1 gene expression directed by the viral LTR signals is carried out entirely by host-cell enzymes as RNA pol II (Fig. 1).

This review focuses on LTR of distinct HIV-1 subtypes, variations in different HIV-1 strains, regulation of their virologic function, and roles in disease progression. New advances in the molecular mechanisms of viral transcription could be helpful to identify potential targets to be used as antivirals.

LTR structure and function

LTR is the control center for gene expression and is also involved in retro-transcription and integration. The HIV-1 LTR is approximately 640 bp in length. The LTR structure of the viral RNA genome has the R-U5 region

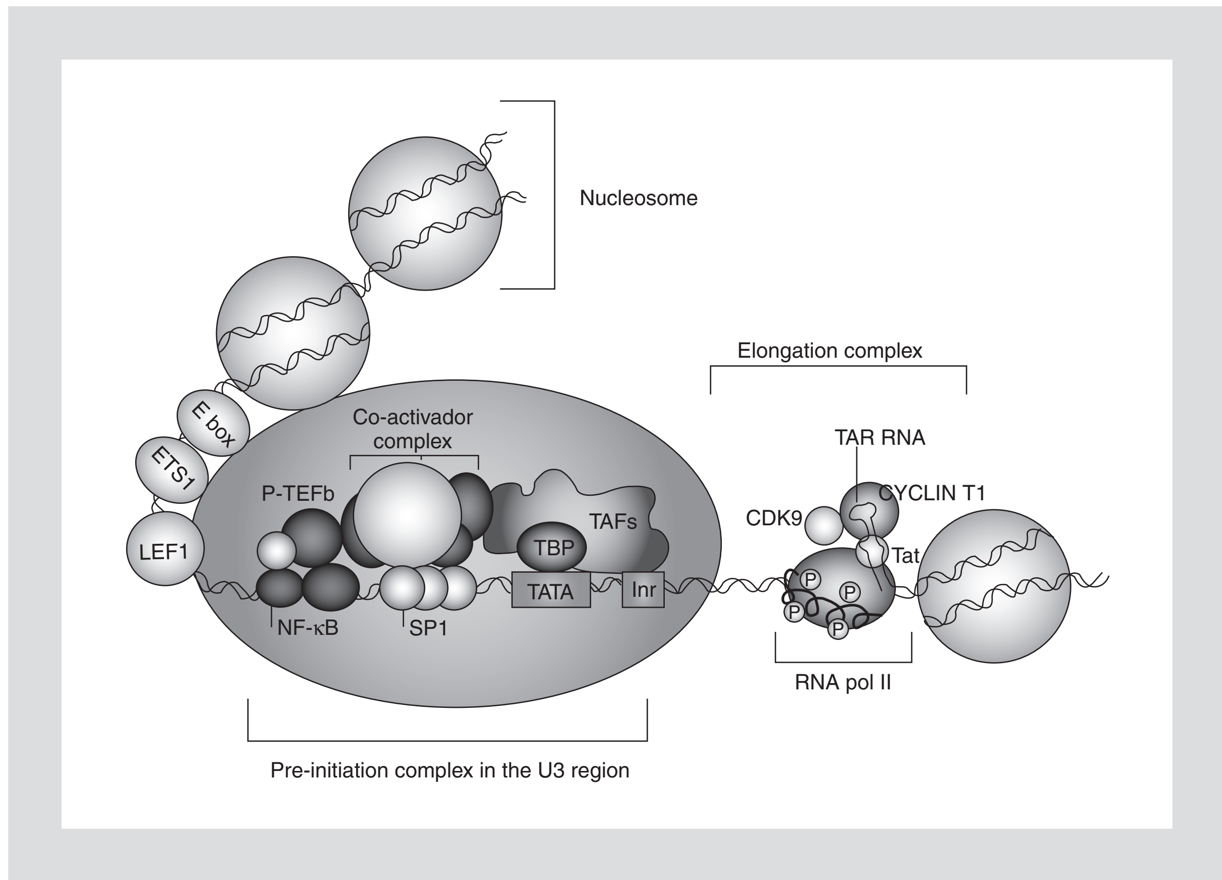

Figure 1. *Initiation and elongation complexes necessary for transcription activity of HIV-1 promoter* (modified from Peterlin M, Nature Reviews Immunology 2003).

in the 5' extreme and the R-U3 region in the 3' extreme. By contrast, the LTR harbors three regions (U3, R and U5) in both extremes of the proviral doubled stranded DNA (Fig. 2). The U3 region (–454 to –1 position) is subdivided into three elements: promoter or core, enhancer, and modulatory regions. The promoter region contains the TATAA box and Sp1 sites, both necessary for transcription initiation. Upstream of the core, is located the enhancer element with important NF-κB binding motifs involved in the control of a variety of cellular processes such as the immune and inflammatory responses, development, cellular growth, and apoptosis[2]. The modulatory region, which is comprised of sequences upstream of the NF-κB sites, contains binding sites for numerous cellular factors such as cyclic AMP response element-binding protein (CREB), Ets, lymphocyte enhancer factor (LEF-1), nuclear factor of activated T-cells (NF-AT) and nuclear hormone receptors. Indeed, this region has also been proposed to contain a negative regulatory element (NRE) between nt –340 and –163 because deletions within this region increase HIV-1 LTR-directed transcription and viral replication[1]. The presence and position of specific sequence signals used for transcription and binding sites are shown in figure 2.

The R region (positions +1 to +100) contains the TAR hairpin, which binds to the viral protein Tat, being essential for the production of viable viral transcripts. The transactivation function of the viral Tat protein requires the presence of Sp1 and NF-κB sites. Moreover, Tat recruits to the LTR viral promoter diverse transcriptional factors, including enzymes with histone and factor acetyltransferase activity, which modify chromatin conformation at the proviral integration site[3]. After the initiation of HIV-1 transcription and subsequent synthesis of the TAR RNA, HIV-1 Tat interacts with cyclin T1/CDK9, and this interaction strongly enhances the affinity and specificity of the binding of Tat to the bulge region of the TAR RNA. Consequently, the CTD of the large subunit of RNA polymerase II can be hyper-phosphorylated, which is necessary for the efficient elongation of HIV-1 mRNA[4] (Fig. 3). Although most Tat-associated proteins function as positive factors, others act as repressors[5].

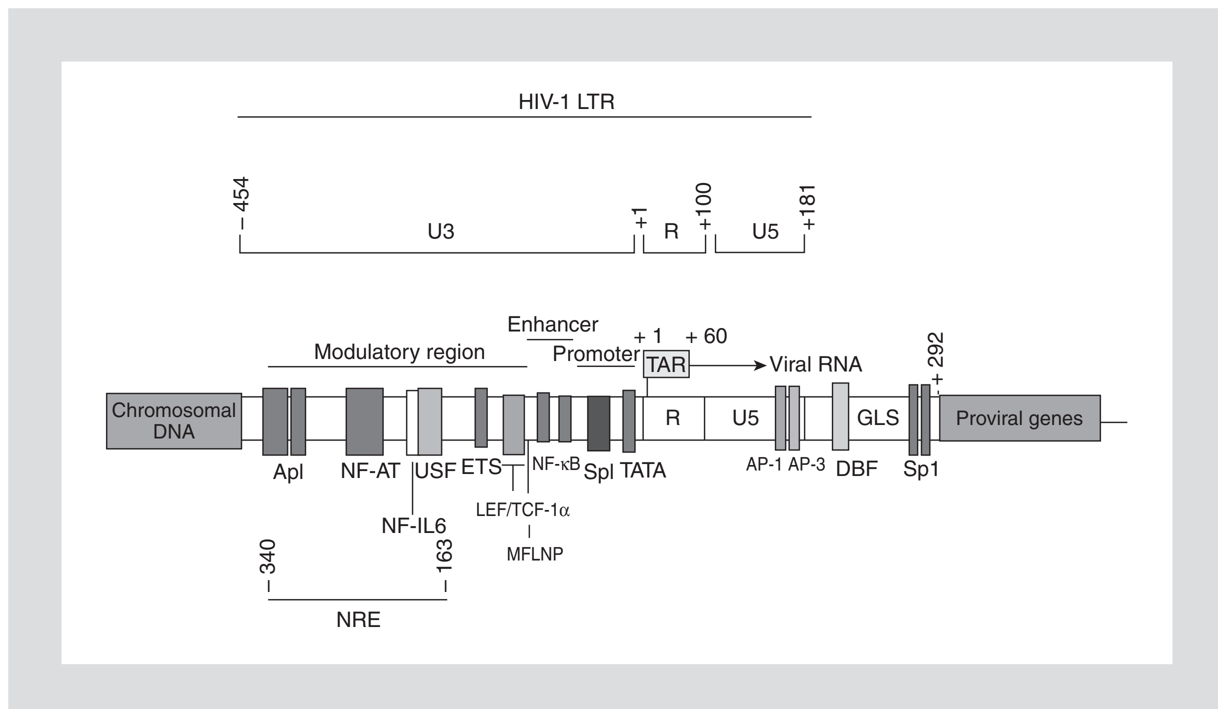

Figure 2. *Structure of the 5' LTR and gag leader sequence (GLS) of HIV-1. Schematic representation of the binding sites for cellular transcription factors in the promoter, enhancer and modulatory regions of U3, R and U5. Numbering of the boxed region is relative to the transcription start site nucleotide +1.*

The U5 region (positions +100 to +181) contains other binding sites for cellular transcription factors such as AP-1, AP3-like (corresponding to an NF-AT site) and Sp1 that are important for virus infectivity (Fig. 2)[6,7].

The transcript begins, by definition, at the beginning of R, and proceeds through U5 and the rest of the provirus, usually terminating by the addition of a poly A tract just after the R sequence in the 3' LTR. The 3' LTR is not normally functional as a promoter, although it has exactly the same sequence arrangement as the 5' LTR. Instead, the 3' LTR acts in transcription termination and polyadenylation and the 5' LTR has dominant control as a promoter. When the integrity of the 5' LTR is disrupted, the 3' LTR can act as a promoter.

Variability of LTR across distinct HIV-1 subtypes

The wide genetic diversity of HIV-1 is mostly due to its high mutation rate and recombination events, favored by a rapid virus turnover. Specific environmental pressure could favor the selection of different viral variants at any time and location. According to the genetic similarity, HIV-1 may be further subdivided into three distinct groups: M (major), O (outlier) and N (non-M, non-O). Nine subtypes (A, B, C, D, F, G, H, J, K), at least 21 circulating recombinant forms (CRF) and multiple intersubtype unique recombinant forms (URF) are further recognized within HIV-1 group M.

Classification of HIV-1 is based primarily on the analysis of genetic sequences coding for the envelope *(env)* and other structural and functional proteins *(gag, pol)*, even in LTR, with sequencing being the best tool for the assignment of HIV-1 isolates. While subtype B is predominant in North America and Western Europe, HIV-1 non-B subtypes and recombinants forms are responsible for more than 90% of the estimated 40 million HIV-1 infections that have occurred worldwide since the beginning of the pandemic[8]. As a consequence of immigration, international travel, and risky behavior with individuals infected in countries where other HIV-1 variants are highly prevalent, the picture of HIV-1 infection is gradually changing in industrialized countries due to the rapid spreading of non-B clades and recombinants.

Sequence variability in the LTR binding sites within the promoter, enhancer, modulatory and TAR regions have been examined in several HIV-1 subtypes[9-15]. However, it remains unclear for the majority of clades and recombinants whether the genetic differences observed in LTR sequences of distinct HIV-1 subtypes could influence its replicative efficiency.

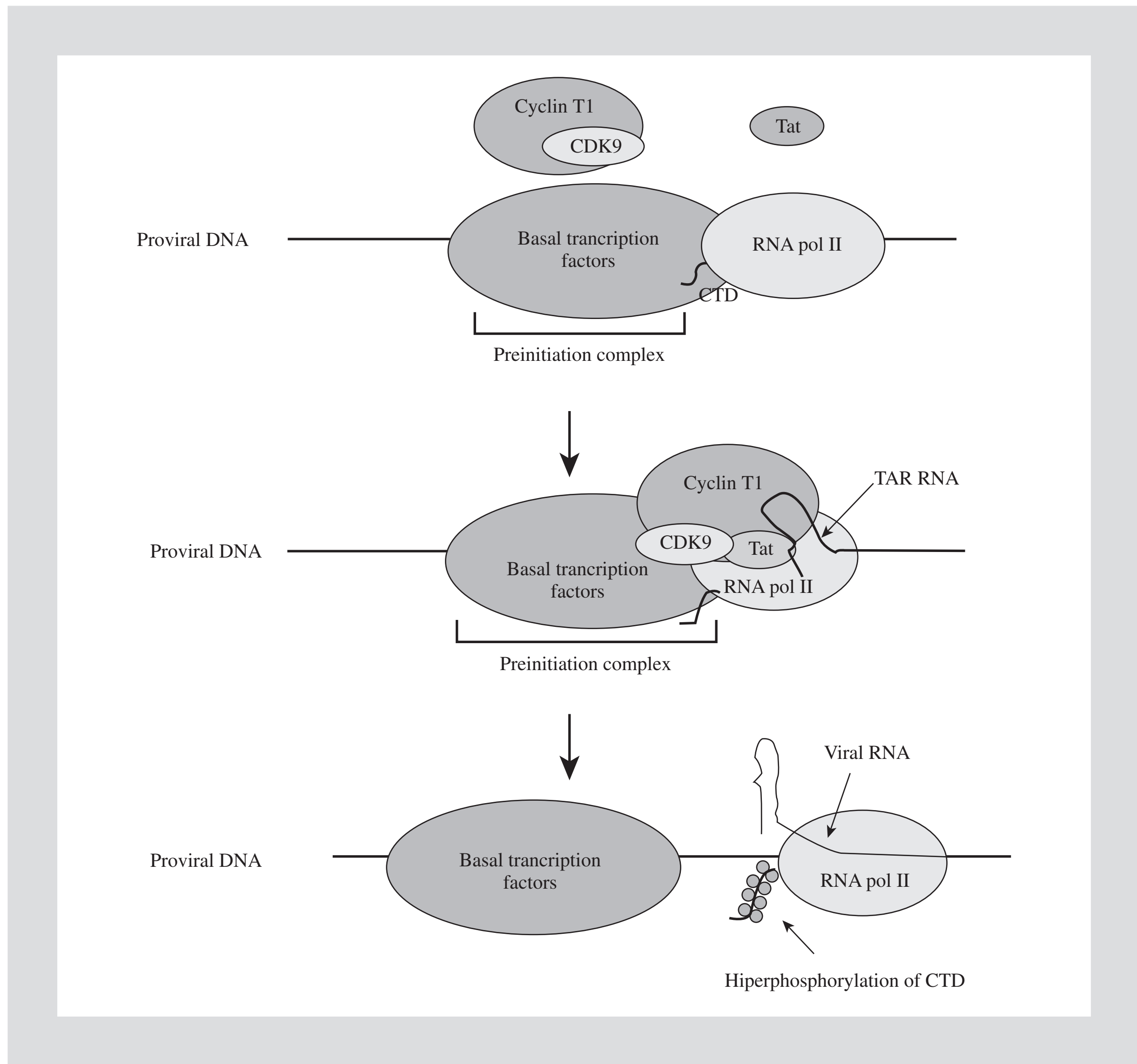

Figure 3. *Mechanism for the transcriptional elongation by Tat.*

Functional effects of HIV-1 LTR sequence variation

Extensive studies have been performed on LTR from some HIV-1 subtypes. Despite their role in the binding of regulatory factors, a high genetic heterogeneity in the sequences involved in transcription regulation located at the U3/R region of LTR has been found in non-B viruses. In one of these studies, sequence variability was significantly higher ($p = 0.001$) in the Core-NRE, LEF-1, NF-κB (I), Sp-1 (II, III) sites, and TAR region when comparing non-B viruses and clade-B specimens[15]. Of note, the most conserved LTR sites in non-B viruses were NF-AT1, RBF-1, RBF-2, Sp-1 (I), and NF-κB (II)[15]. The effects of those changes on transcriptional levels have been tested in functional studies carried out using LTR from distinct HIV-1 clades.

Consequences of LTR variation within the U3 region

Several subtype-specific sequence motifs in the U3 LTR region have been identified (Table 1). The sequence TATAA at position –28 is involved in transcription initiation. While all HIV-1 clades show the TATAA box, the recombinant CRF01-AE and subtype J contain a TAAAA functional sequence[10,14,15].

Three upstream binding sites for the transcription factor Sp1 are usually included in the core element

Table 1. Difference published between LTRs sequences of distinct HIV-1

	A	B	C	D	E*	F	G	H	J
TATAA box (-28 position)	yes	yes	Yes	Yes	No (TAAAA)	yes	yes	yes	No (TAAAA)
Number of Sp1 sites	3	3†	3	3	3	3	3	3	3
Number of NF-kB sites	2	2	3	2	1	2	2	3	3
Number of AP-1 sites	2	0	1	0	1	2	1	nd	nd
USF interaction with Core-NRE	No	yes	Yes	No	No	nd	No	nd	nd
NRE subtype specific sequence	No	No	CGCA(Pu) ACACATC	TTTGAAY CAAAG	CGAAAA CACATA	No	No	No	CGAAGA CACATA
TAR sequence variation	1 nucleotide deletion (U25Δ)	No	C24T	C24T	1 nucleotide deletion (U25Δ)	C24T, A22G, T31C	C24T	No	T31C

*Subtype E, currently named as CRF01-AE, a recombinant form.
†Some B strains harbor four Sp1 sites.
nd: not determined.

within U3 of all HIV-1 subtypes. Meanwhile, however, the Sp1 (I) and (II) sites at positions –46 to –69 are responsible for LTR-promoter activity *in vitro*, the third Sp1 (III), located at positions –70 to –81, has minimal effect on viral transcription[16]. Some non-B subtypes (A, C, D, CRF01-AE and G) exhibit high variability in the three Sp1 sites being the distal Sp1 (III) the most variable[9-14]. By contrast, the second Sp1 (II) site is the most variable in subtypes A, CRF01-AE, G and J[15]. A correlation between the copy number of *cis*-acting transcription factors binding sites Sp1 and increased LTR function has been described. For instance, duplications in Sp1 binding sites in subtype B increase LTR activity, leading to greater viral replication rates[17,18].

With respect to NF-κB binding sites, the numbers of those sites in the enhancer region of U3 differs among different HIV-1 subtypes (Table 1). There are two in clade B and the majority of non-B subtypes, three in clade C, and only one in CRF02-AE. Studies examining the effect that NF-kB has on viral promoter activity have been carried out[9,11,19]. Transient expression analyses with clade-C LTR, which have three NF-κB sites, have shown increased activation compared to viruses with one or two NF-κB sites within LTR. In addition, the importance of this factor in Jurkat T-cells and in microglial cells was revealed by the drastic decrease of the transcription levels due to deletion of two NF-κB sites[20]. Interestingly, a new GABP binding site enhancing the transactivation mediated by Tat was found in all subtype E variants analyzed, replacing one of the two NF-κB sites[21].

Upstream of the NF-κB region is located the modulatory region. It has also been proposed to contain a negative regulatory element (NRE) between nucleotides –340 and –163 (Fig. 2) because deletions within this region increase the HIV-1 LTR-directed transcription and virus replication[22]. Although originally described as a negative regulatory region, the NRE also confers a positive regulation and contains binding sites for many cellular transcription factors including upstream stimulating factor (USF), Ap1, NF-AT and LEF/TCF-α[23]. The core NRE (positions –174 to –163) within the NRE region contain subtype-specific sequences (Table 1). Thus, a consensus sequence was established for subtypes C (CGC A[Pu]A CAC ATG), E (CGA AAA CAC ATA), D (TTT GAA YCA AAG) and J (CGA AGA CAC ATA)[11,15]. This site contains a characteristic E-Box motif, (CAC[A/G]TG), being a target for USF[24]. *In vivo,* USF was found to repress transcription of HIV-1 subtypes A, B, C, D, E and G in epithelial cells, although it activates the same transcription in T-cell lines[25].

The presence of AP-1 motifs (TGACTCA) just upstream of the NF-κB sites and near the core, represents another significant difference between distinct HIV-1 subtypes (Table 1), with one AP-1 site recognized in subtypes C, E and G, two adjacent AP-1 sites in subtypes A and F, and none in subtypes B and D[13]. The plasticity of the virus for using or not the AP-1 transcription factor could depend of the infected cell type[26]. Interestingly, the viral Nef protein has been shown to stimulate the AP-1 pathway[27].

The RBE III site (positions –122 to –130) is a binding site for the transcription factor RBF-2, and it is believed to be highly conserved across all HIV-1 subtypes[13]. However, we previously found a significant genetic variability in this region in specimens belonging to clades A, F and J with respect to the HXB2 clade B reference sequence[15].

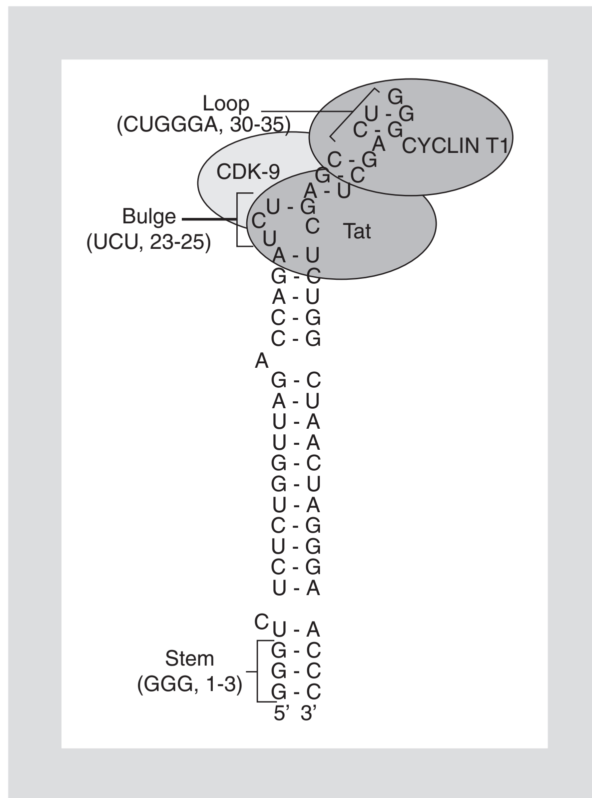

Figure 4. *TAR RNA secondary structure and Tat interactions with TAK (Tat-associated kinase), cyclin T1 and CDK9. The numbers indicate the position in the TAR RNA structure.*

The LTR region, including the most frequent naturally occurring length polymorphisms (MFNLP), can include from 15 to 34 bp long insertions upstream of the NF-κB site at position –120 in the HXB2 sequence (Fig. 2). The prevalence of these insertions is high within the MFNLP region in LTR belonging to clades A, C, D, F, H and J when compared to subtype B[15].

Koken, et al. described insertions within the MFNLP region as a CTG motif, due to the presence of a duplicated 5'-ACTGCTGA-3' sequence in some LTR belonging to subtype B[28]. Subsequently they demonstrated that LTR containing the monomeric CTG motif were the most active transcriptional promoters in HeLa or Jurkat cells[29]. Furthermore, mutant viruses with dimeric or deleted CTG motif were consistently out-competed by the wild-type virus in co-culture experiments[29]. Most likely, the MFNLP represents a partial or full TCF-1α/LEF-1 duplication[30], although it could bind a specific nuclear factor that appears to be identical to RBF-2, which could support that these polymorphisms represent a duplication of the RBE III site[31]. In fact, MFNLP deletions cause an increase in LTR-driven transcription in cells expressing RBF-2, but not in cells with undetectable RBE-2.

Consequences of LTR variation within the R region

The TAR motif is encoded by the transcribed R region of LTR sequences acting as an RNA enhancer though binding the viral Tat-transactivation protein (Tat), the cellular cyclin T factor (cyclin T1) and CDK9 Kinase (Fig. 4). Because the RNA secondary structure of this motif is critical for its correct function, it should be relatively conserved. The typical hairpin structure has been extensively studied in different HIV-1 variants[13-15]. TAR sequences from both subtypes A and E contain a 2-nucleotide bulge (sequence involved in the Tat binding site of TAR), known as U25Δ, as opposed to the 3-nucleotide bulge seen in clade-B TAR (Table 1)[10]. Moreover, all subtypes C, D, F, most G and half of A show a C24T change in the TAR bulge[15]. Two additional substitutions (A22G and T31C) within the TAR region have been described in all CRF01-AE viruses[10].

The Tat protein exhibits a high divergence across HIV-1 subtypes, which may influence its binding to TAR and transactivation functions[14,32]. The Tat protein has a basic domain which functions as a binding motif to TAR RNA. Variation in subtype C at two charged amino acid residues in Tat (Arg57Ser and Gln63Glu) within and close to the basic domain, respectively, could explain the higher activity recognized for the subtype-C Tat protein[32]. It could show a higher affinity for the TAR RNA[32]. However, functional assays in Jurkat cells have not confirmed these findings, showing that Tat E demonstrates a higher transactivation activity than Tat from clades B and C[10].

Consequences of LTR variation within the U5 region

In vivo and *in vitro* analyses of the region downstream of the transcription start site (U5) have identified recognition sites for several constitutive and inducible transcription factors: three AP-1 binding sites, one AP3-like site (corresponding to an NF-AT site), one DBF motif (corresponding to an interferon-responsive factor) and two juxtaposed Sp1 binding sites (nt 724 to 743) (Fig. 2). Functional studies have shown that these sites have a critical role in HIV-1 transcription and virus replication, defining a new positive transcriptional regulatory element in the HIV-1 provirus[6,7]. For instance, proviruses containing a mutation in the two adjacent Sp1-binding sites are totally defective for viral replication in T lymphoid cells[6].

HIV-1 gene regulation as a target for anti-HIV chemotherapy

The targets so far marketed to stop the replication of HIV-1 are acting at different stages of the virus life cycle. They are the reverse transcriptase (RT) enzyme, the viral protease (PR) and the gp41 envelope protein. Although highly active antiretroviral therapy (HAART) has been effective in dramatically reducing the mortality and morbidity of HIV-1-infected individuals, drug-related adverse side effects, poor treatment compliance, and selection of viral resistance remain major obstacles for a sustained benefit of therapy. Therefore, new drugs and treatment strategies are needed for a growing number of patients.

The possibility of repressing LTR-driven transcription using inhibitors for cellular LTR-binding factors such as NF-κB and Sp1 has been explored in recent years[33,34]. In a preliminary report, Reynolds, et al. have demonstrated that engineered zinc-finger transcription factors, designed to bind the -69/-51 Sp1 region within the LTR, could inhibit HIV replication in Jurkat T-cells[35].

Since Tat is the most potent transactivator of HIV-1 gene expression, is essential for viral replication, and has no similar homologous in human cells, it has been considered as one of the most attractive targets for inhibition of HIV-1 transcription. Several compounds have demonstrated to block the Tat/TAR RNA interaction. Among them, ALX40-4C is a peptidomimetic compound of the Tat basic domain able to specifically compete with the Tat/TAR RNA binding[36]. Another molecule, CGP64222, is a hybrid peptoid/peptide oligomer that inhibits the formation of the Tat/TAR RNA complex *in vitro*[37], although its antiretroviral activity seems to be primarily due to inhibition of viral entry[38]. All these compounds and others are in very early phases of clinical development.

As mentioned above, hyper-phosphorylation of the CTD of the RNA polymerase II is needed for transcriptional elongation. It is sensitive to the inhibition by protein kinase inhibitors, such as 5,6-dichloro-1-b-D-ribofuranosylbenzimidazoles (DRB). As expected, protein kinase inhibitors, including DRB derivatives, isoquinoline sulfonamides and flavonoids, could inhibit Tat-activated transcriptional elongation *in vitro*[39]. Other compounds could block Tat cofactors (such as CDK9 and Cyc T1) by interfering with Tat function. Identification of additional compounds that target the transactivation process remains a hot line of investigation that might provide an opportunity to block HIV-1 expression and therefore disease progression in infected persons.

Finally, another strategy proposes the induction of HIV expression through the lymphocyte activation to purge latent reservoirs. Thus, prostratin is a non-tumorigenic phorbol ester that delays HIV replication *in vitro*, but paradoxically activates HIV expression in latently infected cells[40, 41]. Recently, others authors corroborated that prostratin induces HIV activation in latently infected cells and a downregulation of HIV receptors in PBMCs[42]. These data support the potential use of prostratin to activate HIV from latency. Another article suggested that the combination of antiretroviral therapy with a histone deacetylase (HDAC) inhibitor (valproic acid), which induces HIV expression *ex vivo* from resting CD4– T-cells, safely accelerates the clearance of HIV from resting CD4- T-cells *in vivo*[43]. This strategy could represent a new and practical approach to eliminate HIV infection in viral reservoirs.

In summary, the LTR region of HIV-1 shows a high genetic variability across distinct HIV-1 subtypes. In fact, several subtype-specific markers exist in this region. Further studies should be conducted to assess whether these changes may determine distinct interactions with cellular transcription factors, leading to different transcriptional levels among distinct HIV-1 clades. In this regard, it is noteworthy that the differences noticed in the TAR region across HIV-1 subtypes could directly influence the extent of synthesis of viable HIV-1 transcripts. Finally, the LTR region may be of particular interest for the design of new drugs, which may block the interaction of HIV-1 LTR with cellular or viral proteins involved in transcription regulation.

Acknowledgments

This work was supported in part by grants from Fundación Investigación y Educación en SIDA (IES) and Red de Investigación en SIDA (RIS) project 60/173.

References

1. Pereira L, Bentley K, Peeters A, Churchill M, Deacon N. A compilation of cellular transcription factor interactions with the HIV-1 LTR promoter. Nucleic Acids Res 1999;28:663-8.
2. Krebs F, Hogan T, Quiterio S, Gartner S, Wigdahl B. Lentiviral LTR-directed expression, sequence variation, and disease pathogenesis. Los Alamos 2001:29-70.
3. Brigati C, Giacca M, Albini A, et al: HIV Tat, its targets and the control of viral gene expression. FEMS Microbiol Letters 2003; 220:57-65.
4 Masanori B. Inhibitors of HIV-1 gene expression and transcription. Curr Top Med Chem 2004;4:871-82.
5. Rohr O, Marban C, Aunis D, Schaeffer E. Regulation of HIV-1 gene transcription: from lymphocytes to microglial cells. J Leuk Biol 2003; 74:736-49.

6. Vant Lint C, Ann Amella C, Emiliani S, et al. Transcription factor binding sites downstream of the HIV-1 transcription start site are important for virus infectivity. J Virol 1997;71:6113-27.
7. El Kharroubi A, Martin M. Cis-acting sequences located downstream of the HIV-1 promoter affect its chromatin structure and transcriptional activity. Mol Cell Biol 1996;16:2958-66.
8. Peeters M, Toure-Kane C, Nkengasong J. Genetic diversity of HIV-1 in Africa: impact of diagnosis, treatment, vaccine development and trials. AIDS 2003;17:2547-60.
9. Montano M, Novitsky V, Blackard J, et al. Divergent transcriptional regulation among expanding HIV-1 subtypes. J Virol 1997; 71:8657-65.
10. Montano M, Nixon C, Essex M. Dysregulation through the NF-kB enhancer and TATA box of the HIV-1 subtype E promoter. J Virol 1998;72:8446-52.
11. Naghavi M, Schwartz S, Sönnerborg A, Vahlne A. LTR promoter/enhancer activity of different subtypes of HIV-1. AIDS Res Hum Retroviruses 1999;15:1293-303.
12. De Baar M, De Ronde A, Berkhout B, et al. Subtype-specific sequence variation of the HIV-1 type 1 LTR and primer-binding site. AIDS Res Hum Retroviruses 2000;16:499-504.
13. Jeeninga R, Hoogenkamp M, Armand-Ugon M, et al. Functional differences between the LTR transcriptional promoters of HIV-1 subtypes A through G. J Virol 2000;74:3740-51.
14. Roof P, Ricci M, Genin P, et al. Differential regulation of HIV-1 clade-specific B, C, and E long terminal repeats by NF-kB and the Tat transactivator. Virology 2002;296:77-83.
15. Ramirez de Arellano E, Soriano V, Holguín A. Genetic analysis of regulatory, promoter and Tar regions of LTR sequences belonging to HIV-1 non-B subtypes. AIDS Res Human Retroviruses 2005; 21:949-54.
16. Jones K, Kadonaga J, Luciw P, Tjian R. Activation of the AIDS retrovirus promoter by the cellular transcription factor, SP1. Science 1986;232:755-9.
17. Koken S, Van Wamel J, Goudsmit J, Berkhout B, Geelen J. Natural variants of the HIV-1 LTR: analysis of promoters with duplicated DNA regulatory motifs. Virol 1992;191:968-72.
18. Michael N, D'Arcy L, Ehrenberg P, Redfield R. Naturally occurring genotypes of the HIV-1 LTR display a wide range of basal and Tat-induced transcriptional activities. J Virol 1994;68:3163-74.
19. Rodenburg M, Li Y, Gao F, et al. Near full-length clones and reference sequence for subtype C isolates of HIV-1 from three different continents. AIDS Res Human Retroviruses 2001;17:161-8.
20. Rohr O, Sawaya B, Schaeffer E, et al. Dopamine stimulates expression of HIV-1 via NF-kB in cells of the immune system. Nucleic Acids Res 1999;27:3291-9.
21. Verhoef K, Sanders W, Berkhout B, et al. Evolution of the HIV-1 LTR promoter by conversion of an NF-kB enhancer element into a GABP binding sites. J Virol 1999;73:1331-40.
22. Rosen C, Sodroski J, Haseltine W. The location of cis-acting regulatory sequences in the human T-cell lymphotropic virus type III (HTLV-III/LAV) LTR. Cell 1985;41:813-23.
23. Gaynor R. Cellular transcription factors involved in the regulation of HIV-1 gene expression. AIDS 1992;6:347-63.
24. Sawadogo M, Roeder G. DNA-binding specificity of USF, a human gene-specificity transcription factor required for maximum expression of the major late promoter of adenovirus. In DNA Tumor Viruses: Control of Gene Expression and Replication. Cancer Cells 1985;4:147-54.
25. Naghavi M, Estable M, Schwartz S, Roeder R, Vahlne A. Upstream stimulating factor affects HIV-1 LTR-directed transcription in a cell-specific manner, independently of the HIV-1 subtype and the core-negative regulatory element. J Gen Virol 2001;82:547-59.
26. Canonne-Hergaux F, Aunis D, Schaeffer E. Interactions of the transcription factor AP-1 with the LTR of different HIV-1 strains in Jurkat, glial and neuronal cells. J Virol 1995;69:6634-42.
27. Varin A, Manna S, Aggarwal B, et al. Exogenous Nef protein activates NF-kB, AP-1 and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. J Biol Chem 2003;278:2219-27.
28. Koken S, van Wamel J, Goudsmit J, Berkhout B, Geelen J. Natural variants of the HIV-1 LTR: analysis of promoters with duplicated DNA regulatory motifs. Virology 1992;191:968-72.
29. Koken S, van Wamel J, Goudsmit J, Berkhout B, Geelen J. Functional analysis of the ACTGCTGA sequence motif in the HIV type 1 LTR promoter. J Biomed Sci 1994;1:83-92.
30. Zhang L, Huang.Y, Yuan H et al. Identification of a replication-competent pathogenic HIV-1 with duplication in the TCF-1 alpha region but lacking NF-κB binding sites. J Virol 1997;71:1651-6.
31. Estable M, Bell B, Hirst M, Sadowski I. Naturally occurring HIV-1 LTR have a frequently observed duplication that binds RBF-2 and represses transcription. J Virol 1998;72:6465-74.
32. Kurosu T, Mukai T, Komoto S, et al. HIV-1 subtype C exhibits higher transactivation activity of Tat than subtypes B and E. Microbiol Immunol 2002;46:787-99.
33. Daclemans D, Vandamme A, De Clerq E. HIV-1 gene regulation as a target for antiviral chemotherapy 1999;10:1-14.
34. Masanori B. Inhibitors of HIV-1 gene expression and transcription. Curr Topics Med Chem 2004;4:871-82.
35. Reynolds L, Ullman C, Choo Y. Repression of the HIV-1 5' LTR promoter and inhibition of HIV-1 replication by using engineered zinc-finger transcription factors. Proc Natl Acad Sci USA 2003; 100:1615-20.
36. O'Brien W, Summer-Smith M, Chen Y, et al. Anti-HIV-1 activity of an oligocationic compound mediated via gp120 V3 interactions. J Virol 1996;70:2825-31.
37. Hamy F, Felder R, Klimkait T, et al. An inhibitor of the Tat/TAR interaction that effectively suppresses HIV-1 replication. Proc Natl Acad Sci USA 1997;94:3548-53.
38. Daelemans D, Schols D, Vandamme A, et al. A second target for the peptoid Tat/transactivation response element inhibitor CGP64222: Inhibition of HIV-1 replication by blocking CXC-chemokine receptor 4-mediated virus entry. Mol Pharmacol 2000; 57:116-24.
39. Mancebo Y, Lee G, Flores O, et al. PTEFb kinase is required for HIV Tat transcriptional activation *in vivo* and *in vitro*. Genes Dev 1997;11:2633-44.
40. Kulkosky J, Culnan D, Pomerantz R, et al. Prostratin: activation of latent HIV-1 expression suggests a potential inductive adjuvant therapy for HAART. Blood 2001;98:3006-15.
41. Brooks D, Hamer D, Zack J, et al. Molecular characterization, reactivation, and depletion of latent HIV. Immunity 2003;19:413-23.
42. Rullas J, Bermejo M, Alcamí J, et al. Prostratin induces HIV activation and downregulates HIV receptors in peripheral blood. Antivir Ther 2005;9:397-406.
43. Lehrman G, Hogue L, Margolis D, et al. Depletion of latent HIV-1 infection *in vivo:* a proof-of-concept study. Lancet 2005;366: 549-55