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Cis–Acting RNA Elements of Human Immunodeficiency Virus

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1. Introduction

The World Health Organization has indicated that effective control of the HIV/AIDS pandemic is the world's most urgent public health challenge. The 2009 UNAIDS Global Facts and Figures report estimated that almost 60 million people have been infected with the virus and that 25 million AIDS-related deaths have occurred since the pandemic began in the early 1980s. In 2008, there were approximately 33.4 million people living with HIV, 2.7 million new infections and 2 million deaths from AIDS-related causes. The pathogen that causes this pandemic is the Major (M) group of HIV type 1 (HIV-1). Group M HIV-1 dominates the global pandemic with at least nine subtypes and multiple intersubtype recombinants have been identified to date (Leitner et al., 2005). Many of these recombinants are circulating in multiple geographical regions and are integral parts in the HIV-1 pandemic (McCutchan, 2006; Takebe et al., 2004).

HIV-1 has become the most studied virus in history. Our understanding of the replication mechanism of HIV-1 has allowed scientists to develop several classes of antiviral therapies targeting various steps of the virus life cycle (Gilliam et al., 2011; Liao et al., 2010; Perno et al., 2008). Current antiviral treatments target the functions of several HIV-1 proteins; available drugs include nucleoside and non-nucleoside reverse transcriptase inhibitors; protease inhibitors, which block the maturation of the nascent virus; and integrase inhibitors, which prevent the integration of viral DNA into the host genome. Fusion and entry inhibitors are newer classes of antiviral drugs and can prevent viral infection before the virus's entry into the cell.

In addition to the proteins encoded by the viral genome, RNA secondary structures play important roles in the replication of HIV-1 by acting *in cis* to regulate and facilitate different stages of viral replication. Indeed, these RNA secondary structures appear to be promising targets for next-generation antiviral drugs (Berkhout, 2009; Biswas et al., 2004; Daelemans et al., 2002; Haasnoot et al., 2007; Houghton et al., 2010; Reyes-Darias et al., 2008; Rossi et al., 2007). Here, we provide an overview of the functions of several important *cis*-acting RNA elements that are crucial to HIV-1 replication. We will also present our latest in-depth analysis of a multi-functional viral RNA element that participates in the dimerization of HIV

genomic RNA and virion packaging in producer cells, as well as reverse transcription (RT) and the recombination of viral RNA in infected cells.

2. The HIV-1 replication cycle

A schematic of the HIV life cycle is shown in Figure 1. The life cycle includes binding, entry, reverse transcription, integration, viral protein synthesis, assembly and budding.

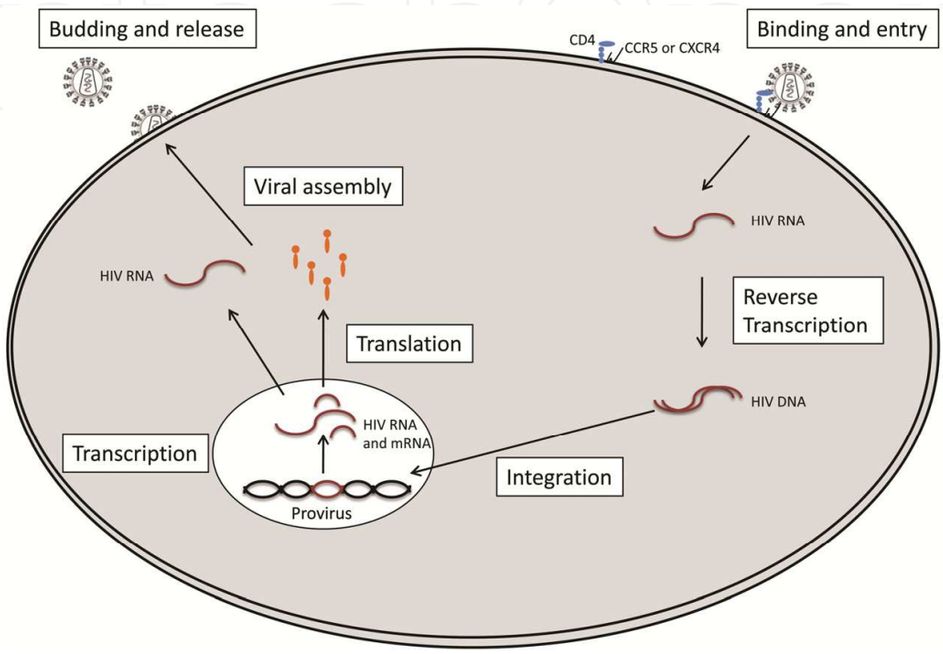


Fig. 1. Schematic representation of the life cycle of HIV-1.

2.1 Binding and entry

The HIV-1 replication cycle begins with a virion binding to a target cell. Both binding and entry depend upon the surface envelope proteins of the virus, which are trimeric glycoproteins composed of heterodimers of gp120 and gp41 (Checkley et al., 2011). Binding is mediated via the interaction between gp120 on the virion and CD4 on the T-lymphocyte (Yoon et al., 2010). Upon binding, the viral envelope glycoprotein undergoes a conformational change, exposing a specific domain capable of binding the CCR5 or CXCR4 chemokine receptors on the cell membrane (Trkola et al., 1996). The binding of gp120 to CD4 and one of the two chemokine receptors results in the fusion of gp41 on the viral envelope with the cellular membrane. After fusion of the viral envelope with the cell membrane, the virus core is released into the cytoplasm, and the viral RNA is uncoated from the viral core (Arhel, 2010). RT occurs in the cytoplasm, and the viral RNA is converted to a double-stranded cDNA by the polymerase and RNase H domains of the reverse transcriptase.

2.2 Reverse transcription and integration

Once HIV-1 genomic RNA is uncoated in the host cytoplasm, reverse transcriptase uses host tRNA as a primer for the viral primer binding site (PBS) to initiate minus-strand DNA

synthesis (Figure 2) (Jiang et al., 1993; Mak et al., 1994). RT proceeds to the 5′ end of the genomic RNA, creating a DNA/RNA hybrid. The RNA component of the hybrid is degraded by the RNase H activity of reverse transcriptase, generating minus-strand strong-stop DNA. The direct repeat (R) sequence allows the minus-strand strong-stop DNA to anneal to the identical R at the 3′ end of the viral genome (first-strand transfer). Once first-strand transfer is completed, minus-strand DNA synthesis continues. The RNase H domain of the reverse transcriptase degrades the RNA template when DNA is synthesized, but the degradation is incomplete.

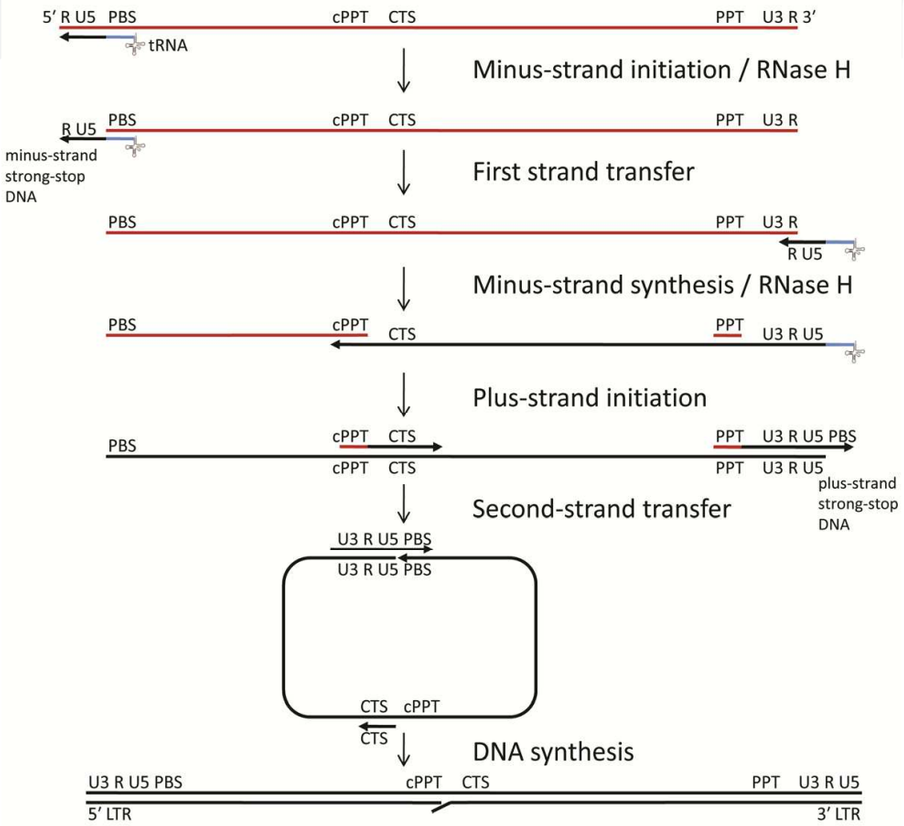


Fig. 2. HIV-1 reverse transcription. Reverse transcriptase uses host tRNA (blue line) bound to PBS as primer. Viral RNA is indicated as red lines. Black lines represent viral DNA. Approximate locations of *cis*-acting elements relevant to reverse transcription are shown. CTS, central termination signal.

The purine-rich region of the RNA genome is called the poly-purine tract (PPT) and central PPT (Charneau et al., 1992; Huber and Richardson, 1990). PPT acts as a primer for plus-strand DNA synthesis. The PPT and central PPT are relatively resistant to RNase H digestion and can prime plus-strand DNA synthesis. Plus-strand synthesis from the PPT continues to the 3′ end of the viral genome and the portion of the primer tRNA yielding plus-strand strong-stop DNA. RNase H removes the primer tRNA, allowing the PBS on the plus-strand strong-stop DNA to anneal to the upstream complementary PBS (second-strand transfer). DNA synthesis from the central PPT provides an additional primer for plus-strand synthesis. Plus- and minus-strand syntheses are then completed, with each strand of DNA

serving as the template for the other. The resulting double-stranded HIV-1 cDNA is imported into the nucleus and integrated into the cell genome by integrase (Li et al., 2011). The virus then resides permanently in the genome as a provirus.

2.3 Viral protein synthesis, assembly and budding

When the host cell receives a signal to become active, cellular RNA polymerase uses the promoter and enhancer in the 5'LTR to initiate transcription of proviral DNA into viral RNA (Kingsman and Kingsman, 1996). The full-length unspliced viral RNA serves two purposes: it expresses Gag and Pol, and becomes incorporated into newly generated viral particles. Upon maturation, Gag forms the three structural proteins of the virion: the matrix, capsid and nucleocapsid (NC) (Freed, 1998). The protease, reverse transcriptase and integrase are encoded by the pol gene. Other viral mRNA encodes the remaining viral proteins. Gag expressed from the unspliced viral RNA recognizes viral genomic RNA that contains the major packaging signal and packages two copies of RNA into a virion (Clever et al., 1995). Virion assembly takes place at the cellular membrane, and the assembly process gives rise to immature viral particles (Adamson and Freed, 2007). The viral protease cleaves the Gag-Pol polyproteins into matrix, capsid, NC, reverse transcriptase and integrase proteins, producing mature and infectious virus particles. The mature particle buds through the infected cell membrane and acquires viral envelope glycoproteins that are encoded by the env gene and expressed on the cell membrane.

3. HIV-1 secondary RNA structure

The viral genome of HIV contains several secondary RNA structures that are important for the regulation of viral replication (Watts et al., 2009; Wilkinson et al., 2008). The known secondary RNA structures with well-defined functions are the trans-activation responsive (TAR) element, stem-loop (SL) 1 to SL4, ribosomal frameshift signal, PPT, central PPT, and Rev response element (RRE).

3.1 Trans-activation responsive element

The TAR element primarily resides in an approximately 45-nucleotide region of the 5' R of the HIV-1 genome. TAR RNA forms a hairpin stem-loop structure with a side bulge. The viral transactivator protein, Tat, binds to the bulge of the *cis*-acting TAR to activate transcription. The absence of Tat severely impairs viral replication, highlighting the importance of this protein in the viral life cycle. Transcription from the LTR is enhanced several hundred-fold in the presence of Tat. Upon binding to TAR, Tat promotes the binding of cellular proteins to form the ribonucleoprotein complex, a positive transcription elongation factor (P-TEFb) complex that contains Cyclin T1, cdk9 and Brd4 and ensures efficient transcription of the full-length HIV genome (Jang et al., 2005; Marshall and Price, 1992). The interactions of TAR with Tat and P-TEFb allow it to bind RNA polymerase II and increase its processivity (Isel and Karn, 1999; Parada and Roeder, 1996).

3.2 Stem-loops in the 5' untranslated region

In addition to TAR, HIV-1 possesses RNA secondary structures at the 5' end of the HIV-1 RNA in the untranslated region. This region forms a series of four SLs preceding and

overlapping the Gag start codon that are important for the regulation of viral replication (Figure 3A) (Berkhout and van Wamel, 2000; Clever et al., 1995; Watts et al., 2009; Wilkinson et al., 2008). Despite some sequence variation, different subtypes of HIV-1 all have similar secondary structures in this region (Berkhout and van Wamel, 1996; Laughrea et al., 1997). SL1 contains the dimerization initiation sequence that controls partner selection during viral RNA dimerization in the cytoplasm (Figure 3B and see Section 4 below). In the absence of SL1, HIV-1 cannot replicate in human T cell lines, highlighting the crucial role of this element in HIV-1 replication. SL2 is the splice donor that directs the splicing of viral mRNA transcripts such as tat and rev. SL3 is the major packaging signal that allows Gag to recognize and package viral genomic RNA into the virion. HIV-1 RNA is encapsidated into virions through Gag–RNA interactions involving the recognition of SL3 by zinc finger motifs in the Gag NC. In addition, SL3 is present in unspliced genomic HIV-1 RNA but absent from spliced viral mRNAs, ensuring efficient packaging of the full length HIV-1 viral genome. Moreover, SL1, SL2 and SL4 are integral components of the packaging signal (Amarasinghe et al., 2000; Clever et al., 1995; Clever and Parslow, 1997; Damgaard et al., 1998; McBride and Panganiban, 1996, 1997; Sakaguchi et al., 1993). Biochemical analysis has indicated that short RNAs possessing HIV-1 SL2 or SL3 have the highest affinity for NC, whereas those with SL1 or SL4 have lower affinity for NC (Shubsda et al., 2002). Mutation analyses have shown that all of these structures are important for RNA packaging (Berkhout and van Wamel, 1996; Clever and Parslow, 1997; Laughrea et al., 1997; Shankarappa et al., 2001).

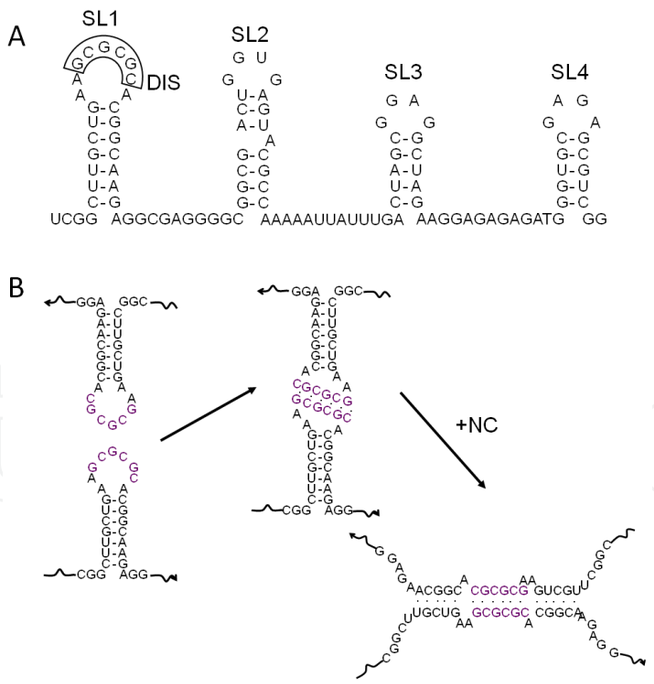


Fig. 3. Stem-loops of the HIV-1 5' untranslated region. (A) RNA structure of the four stem-loops. The dimerization initiation signal (DIS) sequence is shown in box. (B) Mechanism of viral RNA dimerization. Dimerization is initiated by base-pairing of the DIS forming a kissing-loop complex. Gag nucleocapsid (NC) promotes the formation of a more stable RNA dimer.

3.3 Ribosomal frameshift signal

The compactness of the HIV-1 genome makes it challenging for the virus to express multiple viral proteins. HIV-1 overcomes this problem by incorporating a ribosomal frameshift signal between the gag and pol transcripts (Jacks et al., 1988). The signal has a slippery sequence (UUUUUUA) that causes a frameshift, and the sequence immediately downstream forms a stem-loop structure (Dinman et al., 2002; Dulude et al., 2002; Jacks et al., 1988; Parkin et al., 1992). The stem-loop RNA structure of the signal is hypothesized to stall the ribosome, resulting in a switch from the zero reading frame to the minus-one frame in the 5' direction; translation continues in the new frame (Jacks et al., 1988). The signal is a translational control mechanism that is responsible for a minus-one ribosomal frameshift that, in turn, produces a specific ratio of Gag and Gag-Pol polyproteins from the overlapping Gag-Pol open reading frames.

3.4 The polypurine and central polypurine tracts

The RNA genome of HIV-1 contains two short PPTs that are involved in the initiation of plus-strand DNA synthesis (Figure 2). The 3′ PPT is a purine-rich sequence (AAAAGAAAAGGGGGGA) located just upstream of U3 (Huber and Richardson, 1990). The central copy of the PPT, which has an additional function in the nuclear import of HIV-1 cDNA, is an exact copy of the 3′ PPT (Charneau et al., 1992; Zennou et al., 2000). PPT acts as a primer for plus-strand DNA synthesis because it is relatively resistant to RNase H degradation. Downstream plus-strand synthesis is primed by the central PPT. Mutations in the central PPT significantly reduce viral replication as a result of reduced plus-strand initiation (Charneau et al., 1992). The 3′ end PPT primes the synthesis of the 3′ LTR, which is paused after the primer tRNA is degraded to produce a plus-strand strong-stop DNA. After strand transfer of the plus-strand strong-stop DNA, DNA synthesis continues to the center of the viral genome, which is defined by a central termination signal (CTS) located approximately 68 nucleotides downstream of the central PPT (Charneau and Clavel, 1991). The central initiation of the plus-strand DNA at the central PPT and the downstream termination at the CTS generate a linear DNA molecule with a three-stranded DNA structure called the central DNA flap (Charneau et al., 1992; Charneau and Clavel, 1991; Zennou et al., 2000). This central DNA flap promotes HIV-1 DNA nuclear import *in cis*. Absence of the central DNA flap results in the accumulation of unintegrated linear DNA in the cytoplasm of infected cells.

3.5 Rev response element

HIV-1 genomic RNA and unspliced mRNA are blocked from nuclear export and are retained in the nucleus. To overcome nuclear retention, HIV-1 expresses the Rev protein and harbors an RRE in its RNA genome. The RRE is an approximately 200-nucleotide RNA element located at the junction between the surface (gp120) and transmembrane (gp41) domains of the env gene. The RRE has multiple high-affinity binding sites for the Rev viral protein (Dillon et al., 1990; Zapp and Green, 1989). Rev contains the nuclear export signal and is expressed from a fully spliced HIV-1 mRNA that can be exported from the nucleus normally. After expression and nuclear entry, Rev binds to RRE and facilitates the nuclear export of viral genomic RNA and viral unspliced mRNA via the Crm1 nuclear export

pathway (Fridell et al., 1996; Fukuda et al., 1997; Neville et al., 1997). The Rev-RRE interaction is an essential regulatory switch in the viral life cycle. At the beginning of viral replication, Rev concentration is low, and only fully spliced viral mRNA, e.g., Rev mRNA, is exported to the cytoplasm. Following expression and nuclear entry, Rev concentration increases, and the protein binds to and multimerizes with the RRE to recruit nuclear export complexes (Olsen et al., 1990; Zapp et al., 1991). This process results in the export of viral genomic and unspliced mRNA from the nucleus to the cytoplasm, which marks the late stage of viral replication.

4. Multiple functions of stem-loop 1

Unlike most *cis*-acting elements that participate in a defined step of viral replication, SL1 has multiple well-defined functions in the virus life cycle (Berkhout and van Wamel, 1996). Studies have shown that SL1 directs the dimerization of HIV genomic RNA and its packaging into the virion in producer cells, as well as RT and recombination of the viral RNA in infected cells (Chin et al., 2007; Chin et al., 2008; Chin et al., 2005; Moore et al., 2007; Moore and Hu, 2009).

4.1 Stem-loop 1 directs viral RNA dimerization and controls genetic recombination

HIV-1 virions contain two copies of the viral RNA genome. The genomic RNA is held together as a dimer by a noncovalent linkage at the 5′ end (Hoglund et al., 1997; Song et al., 2007). The dimerization process occurs in the cytoplasm, and the dimeric RNA is then packaged through Gag-RNA interactions, as described above (Chen et al., 2009; Moore et al., 2007; Moore et al., 2009). The viral element that directs the dimerization process is a 6-nucleotide palindromic sequence called the dimerization initiation signal (DIS), located at the SL1 loop in the 5′ untranslated region (Figure 3A) (Berkhout and van Wamel, 1996; Chin et al., 2005; Laughrea and Jette, 1994; Moore et al., 2007; Moore and Hu, 2009; Muriaux et al., 1995; Skripkin et al., 1994; Song et al., 2007). The DIS sequences of HIV-1 are either subtype B-like, i.e., GCGCGC, or subtype C-like, i.e., GTCGAC (Leitner et al., 2005). Once full-length HIV-1 genomic RNAs are exported into the cytoplasm, the DIS sequences of two viral RNAs interact through Watson-Crick base-pairing (Figure 3B) (Clever et al., 1996; Muriaux et al., 1996b; Paillart et al., 1996b). The dimerization process is then initiated and produces a kissing loop complex (Clever et al., 1996; Kieken et al., 2006; Laughrea and Jette, 1994; Muriaux et al., 1996b; Skripkin et al., 1994). The NC domain of Gag then promotes the conversion of the kissing loop complex to a more stable extended dimer (Feng et al., 1996; Muriaux et al., 1996a). Gag then packages the viral RNA dimer into the virion by interacting with the major packaging signal in SL2.

Studies have shown that the DIS-mediated base-pairing of two viral RNA molecules is a major determinant in the selection of the copackaged RNA partners (Chin et al., 2005; Moore et al., 2007; Moore and Hu, 2009). Using an assay that measures recombination rate as a proxy for the efficiency of packaging of genotypically distinct HIV-1 RNA molecules, studies have found that the copackaging of two subtype B or subtype C HIV-1 RNAs is very efficient (Chin et al., 2005; Rhodes et al., 2005). However, the copackaging of a subtype B RNA with a subtype-C RNA occurs with much lower efficiency (9-fold reduction) compared to the copackaging of homologous sequences. Therefore, HIV-1 copackaging of

genotypically different genomic RNAs is restricted. The major element that restricts the copackaging of subtype B and subtype C RNAs was mapped to the DIS on SL1. Subtype B and subtype C HIV-1 possess different palindromic sequences in their DIS sequences. This sequence difference reduces the co-packaging of subtype B and subtype C viral RNA molecules. Although the frequency of template-switching or recombination by reverse transcriptase is unchanged, now only a small population of virions contains two different subtypes of RNA is present. Because genotypically distinct recombinants can only be generated from viruses containing two RNA molecules that encode different sequences (heterozygous virions) but not from viruses containing two identical RNAs (homozygous virions) (Hu and Temin, 1990), sequence differences in the DIS result in a drastic decrease in recombinant HIV-1 formation.

Based on the numbers and prevalence of circulating and unique recombinant forms of HIV-1, it is evident that recombination has played a significant role in generating the diversity of virus strains in the infected population. Recombination can occur during reverse transcription, generating DNA that contains genetic information from each co-packaged RNA (Coffin, 1979). The studies described above showed that the DIS sequence identity plays a pivotal role in determining the packaging efficiency of RNAs from different HIV-1 strains and thus governs the opportunities for recombination to occur. It has been suggested that the recombination potential between two HIV-1 subtypes can be predicted from their DIS sequences. One study explored this possibility by measuring the recombination rate between subtype B, subtype C and circulating recombinant forms 01_AE (AE) strains of HIV-1 (Chin et al., 2007). In that study, the recombination rate between AE and subtype B viruses, which have different DIS sequences, was four-fold lower than the rate between AE and subtype C viruses, which have identical DIS sequences. Moreover, the lower recombination rate between the AE and B viruses could be recovered by changing the subtype B DIS to a subtype C DIS. Therefore, mismatches that affect base-pairing within the DIS can severely disrupt recombination between HIV-1 subtypes. Although the intersubtype HIV-1 recombination rate is much lower than the intrasubtype rate, HIV-1 has exceedingly high recombination rates, approximately 10-fold higher than those of murine leukemia virus or spleen necrosis virus (Anderson et al., 1998; Hu and Temin, 1990). Therefore, even for different DIS sequences, intersubtype HIV-1 recombination still occurs at levels similar to gammaretrovirus recombination rates.

4.2 Stem-loop 1 maintains proper nucleic acid structures in the reverse transcription complex

Studies have suggested that the multi-functional SL1 of HIV-1 helps to facilitate RT. It was shown that SL1 deletion impairs plus-strand HIV-1 DNA transfer in RT (Paillart et al., 1996a; Shen et al., 2000). In addition, template-switching is restricted in a 2-kb region immediately downstream of SL1 mutations (Chin et al., 2008), which affects the efficiency of RT and the synthesis of full-length HIV-1 DNA (King et al., 2008). The second observation is intriguing because most HIV-1 RNA secondary structures that are thought to stall RT and thereby increase recombination are limited to a very short region (Derebail and DeStefano, 2004; Galetto et al., 2004; Moumen et al., 2001). Unlike these RNA structures, the SL1 mutations that cause improper base-pairing between two HIV-1 RNA molecules have a

long-range effect on the template-switching tendency of reverse transcriptase (Chin et al., 2008). In that study, viruses that packaged two RNAs containing different DIS sequences were examined. The lack of perfect base-pairing between the two DIS regions caused an apparent recombination gradient with far fewer recombination events immediately downstream from the DIS compared to the pol region, which is more than 2 kb downstream from the DIS.

The long-range effect can be corrected when there is perfect base-pairing between the DIS of the two viral RNAs, indicating that the long-range effect is caused by the DIS rather than by other local sequences. These results suggest that the two RNA molecules in the RT complex are organized in a particular structure(s) and that the base-pairing of the DIS sequences has an important role in forming this structure. It is possible that the DIS serves as a nucleation point to allow proper arrangement of the dimeric RNA structures immediately downstream from it. Without this nucleation point, the 2 kb region immediately following the DIS is not structurally suitable for recombination. The effect of DIS base-pairing diminishes after approximately 2 kb; most of the pol regions had similar numbers of recombination events regardless of whether the DIS could base-pair perfectly. This observation suggests that the remainder of the RNA sequences adopt the proper dimer structure. This result is consistent with the conclusion generated by several studies that, despite the importance of the DIS, the base-pairing of DIS sequences is not absolutely essential for the generation of virion RNA dimers (Berkhout and van Wamel, 1996; Laughrea and Jette, 1996; Moore et al., 2007; Muriaux et al., 1996b). These findings reveal that the DIS plays a critical role in maintaining proper nucleic acid structure in the RT complex.

4.3 Stem-loop 1 regulates the packaging of spliced and unspliced viral RNA

The zinc finger motifs of NC recognize the major packaging signal within the SL3 in a full-length unspliced genomic HIV-1 RNA to promote packaging into virions. Partially spliced and completely spliced viral RNAs, which do not contain SL3, are largely excluded from packaging. An SL1 deletion mutant of HIV-1 is non-viable and has an abnormal packaging preference for full-length unspliced HIV-1 genomic RNA and singly and fully spliced viral mRNA (Clever and Parslow, 1997; Clever et al., 2000; Houzet et al., 2007; McBride and Panganiban, 1997; Ristic and Chin, 2010; Russell et al., 2003). The ΔSL1 mutant packaged genomic RNA two-fold less efficiently than the wildtype (Figure 4A) (Ristic and Chin, 2010). This result is not surprising because the SL1 has been suggested to have a role in binding Gag during packaging (Clever et al., 1995; Clever and Parslow, 1997; Shubsda et al., 2002). In contrast, three- to four-fold more spliced viral mRNA is packaged into the virion when SL1 is deleted (Figure 4B). The deletion of SL1 increased the amount of spliced viral mRNA relative to HIV-1 genomic RNA by seven- to nine-fold (Ristic and Chin, 2010). This aberrant packaging of genomic and spliced viral RNA is caused by an abnormal interaction between the RNA and Gag; three-fold less ΔSL1 genomic RNA co-immunoprecipitates with Gag compared to wildtype RNA. This result indicates that the decreased packaging efficiency of ΔSL1 genomic RNA is caused by a reduced association of Gag with the ΔSL1 RNA. In accordance with this observation, Gag showed an enhanced association with ΔSL1 spliced viral mRNA, immunoprecipitating approximately four-fold more singly spliced and fully spliced viral mRNA (Ristic and Chin, 2010).

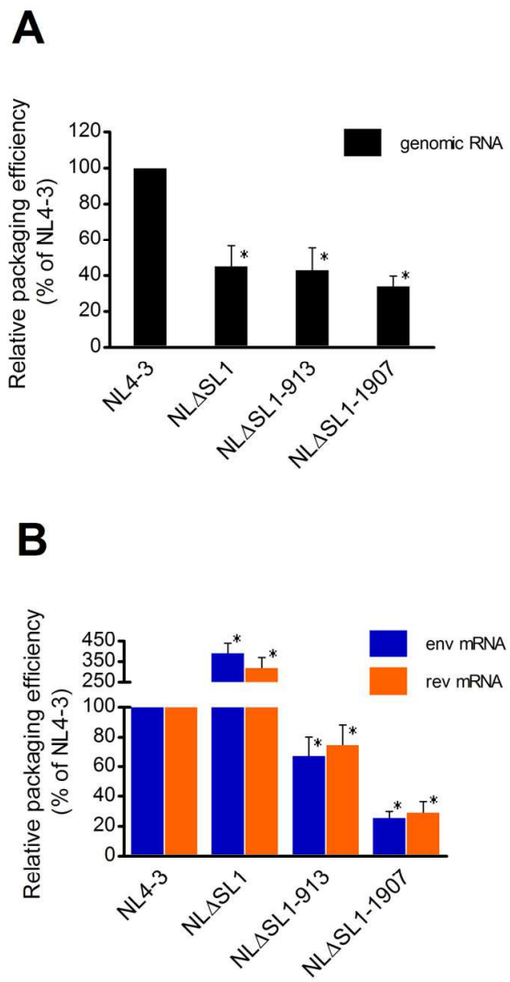


Fig. 4. Quantification of HIV-1 RNA content in the virion by real-time PCR. (A) Efficiency of HIV-1 genomic RNA packaging. NL4-3, wildtype HIV-1; NLΔSL1, SL1 deletion mutant; NLΔSL1-913, ΔSL1 with compensatory mutation in matrix; NLΔSL1-1907, ΔSL1 with compensatory mutation in SP1. The amount of NL4-3 genomic RNA was set at 100%. *, indicates $p < 10^{-4}$ and significant deviation from the wild-type copy number as determined by Student's *t* test. (B) Efficiency of spliced HIV-1 RNA (env and rev mRNA) packaging. The amount of NL4-3 spliced mRNA was set at 100%. *, indicates significant deviation from the wild-type copy number as determined by Student's *t* test; $p < 10^{-4}$, except for NLΔSL1-913, $p < 10^{-3}$.

4.4 Is stem-loop 1 a potential target for antiviral intervention?

Given the important role of SL1 in regulating multiple stages of the viral life cycle, it has been proposed as a target for RNA-based antiviral therapies including RNA interference and antisense approaches (Elmen et al., 2004; Ennifar et al., 2006; Sugiyama et al., 2009). However, mutation, sequence deletion and recombination are common mechanisms by which HIV-1 escapes antiviral intervention and continues to replicate in the host. Indeed, ΔSL1 HIV-1 replicated in human peripheral blood mononuclear cells (PBMCs), although it

was >10-fold less infectious than the wildtype (Hill et al., 2003; Jones et al., 2008). Several studies have shown that HIV-1 can replicate without SL1 by acquiring changes in the genome (Liang et al., 1998; Liang et al., 1999; Ristic and Chin, 2010; Russell et al., 2003). We have demonstrated that, despite the absence of a vital element regulating RNA dimerization, the ΔSL1 mutant still dimerized, copackaged and subsequently recombined with a recombination-competent HIV-1 and was restored to infectivity similar to that of wildtype. We also showed that, in PM-1 cells infected with ΔSL1 HIV-1, syncytia were observed 14 days post-infection; wildtype infected cells showed syncytia by seven days post-infection. Virus production by the infected PM-1 cells was detected by ELISA in the culture supernatant three to four days before cytopathogenicity was observed (Ristic and Chin, 2010).

Sequence analysis of the near full-length genome of the ΔSL1 virus at 14 days post-infection in PM-1 showed that HIV-1 variants still harbored the SL1 deletion found in the ΔSL1 input virus. Two independent mutations were identified in the matrix domain and the SP1 domain of Gag (Ristic and Chin, 2010). When these two mutations were separately placed into a ΔSL1 HIV-1 backbone, both were able to enhance the infectivity of the deletion mutant by partially restoring the packaging specificity of viral RNA (Figures 4A and 4B). These compensatory mutations allow Gag to exclude spliced viral mRNA from packaging, thus reducing interference with the production of infectious virus in the ΔSL1 mutant. Flow cytometry analysis of infected PBMCs showed that ΔSL1 HIV-1 carrying these compensatory mutations depleted CD4+ cells more rapidly than the original ΔSL1 mutant. These data indicate that more than one pathway can compensate for the loss of SL1 secondary RNA structure. HIV-1 adapts quickly to the deletion of SL1 by compensatory mutations or recombination with a variant. These results highlight the ever-changing nature of HIV-1. In addition to SL1, future antiviral drug design should also target essential and highly conserved gene coding sequences.

5. Conclusion

Despite advances in antiviral therapy against HIV and greater understanding of the biology of the virus, the eradication of HIV/AIDS remains elusive. HIV continues to evade drug interventions and vaccines by mutation and recombination, which allow rapid diversification of HIV population. Facing this challenge, antiviral development has expanded to target HIV replication at the RNA level. Viral *cis*-acting RNA elements play crucial roles in regulating various steps of viral replication; in particular, the SL1 participates in multiple stages of the virus's lifecycle. Indeed, RNA interference-based antivirals targeting these elements have been tested *in vitro* but is far from success mainly because of the high variability of the virus. To this end, we have demonstrated that HIV adapts quickly to a severe defect at the SL1 region and regains wild type-like infectivity and pathogenicity. Therefore, in the light of this urgent public health problem, scientists need to continue the endeavor to elucidate new viral *cis*-acting elements and the mechanisms by which they regulate replication, thereby revealing new targets for antiviral intervention and to develop combination therapy.

6. Acknowledgment

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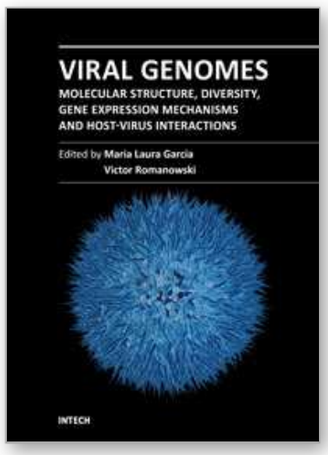
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Viruses are small infectious agents that can replicate only inside the living cells of susceptible organisms. The understanding of the molecular events underlying the infectious process has been of central interest to improve strategies aimed at combating viral diseases of medical, veterinary and agricultural importance. Some of the viruses cause dreadful diseases, while others are also of interest as tools for gene transduction and expression and in non-poluting insect pest management strategies. The contributions in this book provide the reader with a perspective on the wide spectrum of virus-host systems. They are organized in sections based on the major topics covered: viral genomes organization, regulation of replication and gene expression, genome diversity and evolution, virus-host interactions, including clinically relevant features. The chapters also cover a wide range of technical approaches, including high throughput methods to assess genome variation or stability. This book should appeal to all those interested in fundamental and applied aspects of virology.

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