Simian Immunodeficiency Virus RNA Is Efficiently Encapsidated by Human Immunodeficiency Virus Type 1 Particles

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Packaging of retroviral RNA is attained through the specific recognition of a cis-acting encapsidation site (located near the 3' end of the viral RNA) by components of the Gag precursor protein. Human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV) are two lentiviruses that lack apparent sequence similarity in their putative encapsidation regions. We used SIV vectors to determine whether HIV-1 particles can recognize the SIV encapsidation site and functionally propagate SIV nucleic acid. SIV nucleic acid was replicated by HIV-1 proteins. Thus, efficient lentivirus pseudotyping can take place at the RNA level. Direct examination of the RNA contents of virus particles indicated that encapsidation of this heterologous RNA is efficient. Characterization of deletion mutants in the untranslated leader region of SIV RNA indicates that only a very short region at the 3' end of the SIV RNA is needed for packaging. Comparison of this region with the corresponding region of HIV-1 reveals that both are marked by secondary structures that are likely to be similar. Thus, it is likely that a similar higher-order RNA structure is required for encapsidation.

Human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV) are both members of the lentivirus subfamily of retroviruses. HIV-1 is an etiologic agent of AIDS in humans (7), and SIV can infect nonhuman primate hosts and induce an AIDS-like disease in some non-African primates, such as rhesus macaques (31). On the basis of the overall genome organization, nucleic and amino acid sequence homology, and immunological cross-reactivity of the major viral proteins, isolated SIV strains are closely related to HIV-2, and these viral isolates form a distinct lentivirus subfamily, while HIV-1 strains form a second subfamily (9, 13, 22, 25, 30).

As do other retroviruses, HIV-1 and SIV contain gag, pol, and env genes which encode viral structural proteins and proteins that function in nucleic acid replication (28). In addition, these lentiviruses possess at least six additional genes that modulate viral replication (28). Furthermore, as with other retroviruses, the terminal regions of HIV-1 and SIV DNA and RNA contain cis-acting sequences important for steps in nucleic acid replication, including transcription and polyadenylation, encapsidation, initiation of reverse transcription, and DNA integration (28, 31).

Efficient and specific encapsidation of viral RNA by the assembling virion particle is an essential step in the retrovirus life cycle. In this process, full-length, unspliced genomic RNA is preferentially packaged, whereas spliced viral RNA and cellular RNA are generally excluded from nascent particles (53, 56). Analysis of diverse retrovirus species, ranging from type C oncoviruses (2, 37, 42, 43, 55) to HIV-1 (3, 8, 10, 35), indicates that a necessary cis-acting sequence essential for packaging of viral RNA is located in the untranslated leader sequence of the viral RNA immediately proximal to the gag gene. In addition, for at least some retroviruses, the 5' end of the gag gene (2, 4), the 5' end of U5 (46), and a region near the 3' end of viral RNA (51) also facilitate encapsidation in conjunction with the untranslated leader. However, in the type C viruses these latter sequences are secondary to the untranslated leader and apparently serve to augment packaging, since the untranslated leader is sufficient to direct efficient packaging. The nucleocapsid and matrix domains of the Gag precursor protein appear to be required for recognition and encapsidation of viral RNA (17, 24, 29, 32, 33, 39, 45, 47, 48, 50, 52, 54).

HIV-1 and SIV share no obvious sequence homology in their putative encapsidation (E) regions. To investigate the nature of the cis-acting lentivirus encapsidation signal and to see whether RNA pseudotyping can take place, we asked whether HIV-1 particles can package and propagate SIV RNA. This analysis indicates that HIV-1 particles can package and functionally propagate SIV RNA. Surprisingly, a relatively short region encompassing only about half of the untranslated leader is sufficient for efficient encapsidation. This region includes the Tat-responsive element (TAR), the U5 sequence, the primer-binding site (PBS) used in the initiation of minus-strand reverse transcription, and only about 90 additional nucleotides. While there is little similarity in the primary sequences of HIV-1 and SIV in this region, similar secondary structures are highly likely.

MATERIALS AND METHODS

Nucleotide designations for SIVmac strain 239 are based on the published SIVmac 239 DNA sequence (31); the first nucleotide of U3 corresponds to nucleotide 1.

Vector construction. All plasmid constructions were made by standard molecular cloning techniques (41). Construction of pTR150 and pTR150ΔKpn was achieved through several cloning steps. First, an SphI-to-PstI fragment from p239SpSp5 (31) was subcloned into pUC20H (44) that had been cleaved with SphI and PstI to give rise to pTR148. Next, to generate a functional long terminal repeat (LTR) and to eliminate extraneous cellular DNA, a BglII-HincII

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fragment (1,076 bp) from p239SpE3' was cloned into the 3' end of pTTR148 that had previously been cleaved with BglII and NruI to generate pTTR149. Then a ClaI-ClaI fragment from pGB108 (20) containing the simian virus 40 (SV40) early promoter-enhancer and the hygromycin B phosphotransferase gene (Hyg') was inserted into the ClaI site of pTTR149 to give rise to pTTR150. Finally, all of the leader and gag sequences downstream to the KpnI site were deleted by digestion of pTTR150 with KpnI followed by ligation to make pTTR150A\(\Delta \)Kpn. This construct contains approximately 110 bp leader sequence between the end of U5 and the beginning of the gag gene. Neither pTTR150 nor pTTR150A\(\Delta \)Kpn has a rev-responsive element (RRE). In order to make similar constructs with the rev, a ClaI-BglII fragment containing the RRE from p239SpE3' (31) was separately cloned into ClaI-BglII sites of pTTR150 and pTTR150A\(\Delta \)Kpn to yield pTTR170 and pTTR170\(\Delta \)Kpn, respectively. To test the role of the 110-bp leader region in encapsidation and to assess the importance of the primer-binding site (PBS) in replication of the vector, a derivative of pTTR170 was made by deleting all of the sequences downstream of an NsiI site located at the 5' end of the PBS, and this plasmid was designated pTTR173. To test the possibility of nonspecific packaging of RNA containing the Hyg' gene through "retrofection" (16, 38), a derivative of pTTR150 which lacks all of the viral sequences at the 5' end of the RNA was made. This plasmid is called pTTR174, and it contains the SV40 promoter-enhancer and Hyg' gene at the 5' end and viral sequences from BglII-HincII at the 3' end. The HIV-1 expression construct pKT81 was constructed by digestion of the HIV-1 proviral clone NL4-3 (1) with BssHII, treatment with the Klenow fragment of Escherichia coli DNA polymerase, and the addition of an XbaI linker. Following digestion of the fragment with XbaI and XhoI, an 8,178-bp fragment containing all of the intact HIV-1 genes except nef was inserted into the XbaI and XhoI sites of p763 (kindly provided by Bill Sugden, University of Wisconsin-Madison). In pKT81, all of the HIV-1 gene products are expressed from the human cytomegalovirus immediate early enhancer-promoter.

To test the relative efficiencies of SIV and HIV-1 RNA packaging into HIV-1 particles, we constructed an HIV-1 vector (pTR167) that is similar to the SIV vector TR170. pTTR167 was made by deleting the entire region between the NsiI sites located at nucleotides 1237 and 6738 of the proviral clone NL4-3 (1) and ligating the ends to produce the intermediate clone pTTR166. Next, an Nhel-Nhel fragment containing the SIV vector early promoter-enhancer and Hyg' gene at the 5' end and viral sequences from BglII-HincII at the 3' end. For the HIV-1 TAR element, this was accomplished by pairing the bases starting at nucleotide 1 (nucleotide 1 is the transcription start site or the beginning of R) to those bases ending at nucleotide 124 over a length of 11 bases, those starting at nucleotide 18 to those ending at nucleotide 52 for 5 bases, those starting at nucleotide 29 to those ending at nucleotide 44 for 5 bases, those starting at nucleotide 54 to those ending at nucleotide 85 for 8 bases, and those starting at nucleotide 64 to those ending at nucleotide 77 for 4 bases. For the U5 leader stem and loop of SIV, this was achieved by pairing bases beginning at nucleotide 248 to those ending at nucleotide 303 for 10 nucleotides and those beginning at nucleotide 262 to those ending at nucleotide 289 for 12 bases. For the HIV-1 TAR element, the bases beginning at nucleotide 5 were paired with those ending at nucleotide 53 over a length of 11 bases. For the HIV-1 U5 leader, the bases beginning at nucleotide 289 for 12 bases.
117 were paired with those ending at nucleotide 238 over 5 bases, and those beginning at nucleotide 133 were paired with those ending at nucleotide 177 over 8 bases. The nucleotides that compose the PBS were excluded from base pairing by the prevent parameter. The data from fold were plotted with the SQUIGGLES program. Both programs were run on a VAX computer.

RESULTS

Successful replication of SIV nucleic acid mediated by HIV-1 would require the recognition of cis-acting sites on SIV RNA by HIV-1 proteins. These sites include the E region (required for efficient incorporation of viral RNA into assembling virion particles), the PBS, and the polypurine-tract (PPT) (required for initiation of reverse transcription following infection) and attachment (ATT) sites (required for integration of the viral DNA into the host cell genome). Figure 1 indicates the relative locations of these cis-acting sites in the viral RNA and DNA for HIV-1 and SIV. Comparison of the primary nucleic acid sequences of SIVmac 239 (31) and HIV-1(NL4-3) (1) indicate sequence identity in the PBS and PPT of both viruses (Fig. 1B). In addition, the ATT sites of both viruses exhibit extensive identity. However, the U5 ATT component of SIV contains either one or two additional nucleotides at the terminus (Fig. 1B). In contrast, there is no obvious sequence similarity in the region corresponding to the E region. However, the overall lengths of the leader region of the RNA between the PBS and the gag gene are similar.

We constructed parallel HIV-1 and SIV vectors, examined the propagation of these vectors over a single cycle of replication following trans complementation with HIV-1 proteins, and analyzed the encapsidation of the viral RNA by direct methods. Plasmid KT81 is an expression construct containing all of the HIV-1 genes except nef (Fig. 2). Expression is attainted from the human cytomegalovirus

![Diagram](image)

FIG. 1. cis- and trans-acting elements of HIV-1 and SIV DNA and RNA. (A) The locations of the viral open reading frames for HIV-1 are indicated. Although SIV lacks a vpu open reading frame and contains a vpr open reading frame not found in HIV-1, the overall spatial organization of the trans-acting regions of the two viruses is similar. (B) The relative locations of the principal cis-acting regions which function in different steps in retrovirus nucleic acid replication are indicated. The nucleotide sequences that compose the PBS and the PPT and ATT sites are shown in alignment. The T residue at the end of the US ATT site is shown in parentheses, since it is not clear whether this nucleotide is present at the end of the viral DNA or whether it is the last nucleotide of the PBS.

![Diagram](image)

FIG. 2. HIV-1 and SIV vectors used in analysis of a cis-acting encapsidation site for packaging of SIV RNA into HIV-1 particles. pKT81 is an expression construct for the HIV-1 proteins. The construction of the vectors is described in Materials and Methods. CMV, cytomegalovirus promoter-enhancer.
promoter-enhancer rather than the upstream HIV-1 LTR, and transcription termination and RNA polyadenylation are mediated by SV40 signals rather than by a downstream HIV-1 LTR. Thus, KT81 can be used for the expression of HIV-1 proteins, but the mRNA encoding those proteins lacks cis-acting sequences needed for retroviral nucleic acid replication. TR167 is an HIV-1 vector that expresses the Hyg" gene (confering resistance to hygromycin B) from an internal SV40 promoter-enhancer (Fig. 2). This vector contains all sequences known to be required in cis for replication, including the E region, PBS, PPT, ATT, as well as the RRE. TR150 and TR170 are SIV vectors analogous to the HIV-1 vector; however, TR150 lacks an RRE region (Fig. 2).

To determine whether SIV RNA can be successfully propagated via HIV-1 particles, CosM6 cells were transiently cotransfected with KT81 along with TR150 or TR170, virus was harvested, CD4+ HeLa (HeLa T4) cells were infected, and these cells were selected with hygromycin B to identify successful infection events. As expected, trans-complementation of the HIV-1 vector TR167 by KT81 was readily observed over a single round of replication (Table 1). In addition, the SIV vectors TR150 and TR170 were also propagated through HIV-1 particles. However, the efficiency of propagation of both of these heterologous vectors was about 10% of that of HIV-1. These data indicate that SIV RNA can be encapsidated and transferred to cells by way of HIV-1 particles but less efficiently than HIV-1 RNA.

Since there is no obvious primary sequence similarity between the E regions of HIV-1 and SIV, we attempted to identify the region of SIV RNA that can serve as a recognition site for packaging into HIV-1 particles. In addition, we directly examined SIV RNA incorporation into HIV-1 particles and characterized SIV proviruses that arose through RNA pseudotyping.

The 5' leader region of retroviral RNA has been invoked to function as the primary cis-acting region necessary for RNA encapsidation into assembling virus particles. Therefore, derivatives of TR150 and TR170 that contain deletions of this region were constructed (Fig. 2). TR150ΔKpn and TR170ΔKpn are derivatives of TR150 and TR170, respectively, and they retain only the 5' 422 nucleotides of the SIV leader RNA: these vectors contain R, U5, PBS, and only an additional 92 nucleotides. Thus, these vectors retain the PBS and PPT and ATT sites but lack much of the putative E region. TR173 contains only the 5' 304 nucleotides of SIV RNA, so this vector retains the PPT and ATT sites but lacks both the putative E region and an intact PBS (Fig. 2). Finally, TR174 lacks all proximal SIV sequences and is deficient in the PBS and the ATT site as well as the putative E region (Fig. 2). This last construct was used to determine whether mRNA that was initiated from the SV40 promoter-enhancer and that expresses the Hyg" gene was propagated through HIV-1 particles by retrofection (16, 38).

The overall propagation of these SIV vector derivatives over a single replicative cycle was analyzed by trans complementation with HIV-1 proteins expressed from KT81 as previously described. TR173 and TR174 did not yield detectable Hyg" colonies (Table 1), indicating that they are not efficiently propagated by HIV-1 particles. In contrast, and somewhat surprisingly, TR150ΔKpn and TR170ΔKpn were successfully propagated at an efficiencies similar to those for TR150 and TR170, even though they lack much of the leader region, including a segment of the region encompassing the putative E region. These data indicate that there is a cis region within the 5' 422 nucleotides of the SIV genome that functions in encapsidation and propagation of SIV RNA by HIV-1 but that the remaining distal leader region is dispensable.

Comparison of the infectious titers of HIV-1 and SIV vectors packaged by HIV-1 particles provides only an indirect assessment of RNA encapsidation. If there are differences in the efficiencies of SIV and HIV-1 nucleic acid replication other than in packaging, then this would also be manifested in the assay measuring titer. Moreover, the overall titer of the vectors was relatively low. Therefore, we sought to measure directly the relative packaging efficiencies of the vector RNA by physical methods and to compare physical particle production with that in a system in which the virus titer is higher.

To ensure that the Hyg" gene could be expressed from each of the expression constructs, the DNAs were transfected into HeLa cells and the cells were treated with hygromycin B. Similar numbers of Hyg" colonies arose from transfection with each of these DNAs, whereas control transfections with DNA lacking the Hyg" gene resulted in no detectable colonies (data not shown). Moreover, transient transfection and direct analysis of intracellular RNA by slot blot hybridization with a DNA probe to the Hyg" gene revealed that the steady-state levels of RNA from each of these DNAs were similar (Fig. 3A).

To directly examine packaging, cells were again transfected, virus was isolated (see Materials and Methods), and purified virus RNA was analyzed by RNA hybridization analysis with a DNA probe to the Hyg" gene. This analysis indicated that RNAs from the HIV-1 vector TR167 and the SIV vector TR150ΔKpn were packaged at equivalent efficiencies (Fig. 3B). Therefore, the terminal 5' end of the SIV genome serves, either intrinsically or in conjunction with sequences in the 3' end of the genome, as a recognition sequence for encapsidation by HIV-1. Although TR173 and TR174 contain multiple deficiencies in cis-acting sites that are required for replication steps following infection, both were also defective in encapsidation, as evidenced by the absence of detectable viral RNA in virus preparations. This observation indicates that the region of viral RNA immediately downstream from the PBS is necessary for encapsidation of SIV RNA by HIV-1 particles.

Comparison of the physical particles by Western blot

<table>
<thead>
<tr>
<th>Table 1. Propagation of SIV and HIV-1 vectors by HIV-1 particles</th>
<th>Hyg' transforming region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector source of DNA</td>
<td>U/ml*</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>KT81</td>
<td>0</td>
</tr>
<tr>
<td>TR167</td>
<td>0</td>
</tr>
<tr>
<td>TR150</td>
<td>0</td>
</tr>
<tr>
<td>TR170</td>
<td>0</td>
</tr>
<tr>
<td>KT81 + TR167</td>
<td>460 ± 15</td>
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<tr>
<td>KT81 + TR150</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>KT81 + TR170</td>
<td>45 ± 2</td>
</tr>
<tr>
<td>TR173</td>
<td>0</td>
</tr>
<tr>
<td>TR174</td>
<td>0</td>
</tr>
<tr>
<td>TR150ΔKpn</td>
<td>0</td>
</tr>
<tr>
<td>TR170ΔKpn</td>
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</tr>
<tr>
<td>KT81 + TR173</td>
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<tr>
<td>KT81 + TR150ΔKpn</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>KT81 + TR170ΔKpn</td>
<td>42 ± 2</td>
</tr>
</tbody>
</table>

* Data are the means of three independent experiments with two duplicate samples per experiment.
analysis with anti-p24 antiserum indicated that similar amounts of virus were present in parallel samples (Fig. 3C). Moreover, the amount of physical virus produced from these transfections appeared to be similar to that of virus produced from transfections in which the infectious titer was high, as assayed by pseudotyping with envelope glycoprotein from an amphotropic murine leukemia virus ($\sim 5 \times 10^6$ infectious units/ml) (data not shown). (The reason for the difference in titer between the two assays is probably that the infectious titer can be increased by pseudotyping with envelope glycoprotein from an amphotropic murine leukemia virus [23].) In any case, the results of the Western blot indicate that KT81 is able to direct the efficient production of physical particles.

To ensure that proviruses that arose from pseudotyping and subsequent replication of SIV RNA by HIV-1 exhibit the expected overall structure, proviruses from five independent infection events were analyzed. Genomic DNA from individual Hyg$^R$ colonies resulting from infection by TR150\DeltaKpn was digested with NdeI and examined by Southern blot analysis with a vector-specific probe (Fig. 4). There is one NdeI site in both SIV LTRs as well as an internal site in the vector. Hybridization analysis detected two fragments of the expected sizes for all five SIV proviruses. Thus, the proviruses are of the overall expected structure and size, and propagation of TR150\DeltaKpn did not occur through anomalous recombination with HIV-1 sequences present on KT81.

Since only a small segment of the 5′ end of SIV RNA is necessary for encapsidation and since the corresponding region of the HIV-1 genome lacks obvious primary sequence similarity, we searched for similarity in secondary structure. Previous extensive analysis of the TAR element has provided strong evidence for TAR stem-and-loop structures in HIV-1 and SIV (6, 14, 19, 26, 34). In addition, mutational analysis of the avian leukosis virus leader region indicates that there is likely to be a U5-leader stem in that virus which augments the initiation and elongation of minus-strand reverse transcription (11). In this structure, the PBS is not intramolecularly base paired but is instead available for hydrogen bonding with the tRNA that serves as a primer for reverse transcription. A similar structure has been proposed for the corresponding region of HIV-1 on the basis of results with avian leukosis virus (11).

A free-energy minimization program was used to predict secondary structure in the SIV RNA leader region present in TR150\DeltaKpn; the program was specified to form the TAR stem-and-loop structure as well as the putative U5-leader stem (see Materials and Methods). This yielded the overall secondary structure shown in Fig. 5. The region features three general stem-and-loop structures. From the 5′ end of the viral RNA, these are the TAR element, a second loop designated the R-U5 loop in Fig. 5, and a long U5-leader stem-loop. Analysis of the corresponding region of the HIV-1 genome by using the same constraints as for SIV again yielded three stems and loops derived from the same segments of the viral leader RNA (Fig. 5). Thus, the leader of both viral RNAs can be subdivided into three sections on the basis of potential secondary structure, and one or all...
might function in encapsidation of SIV RNA by HIV-1. Since TR173 and TR174 both lack nucleotides at the 3' side of the base of the long U5-leader stem-loop, and since both are deficient in encapsidation, the results are consistent with a role for the U5-leader secondary structure in encapsidation.

**DISCUSSION**

Previous analysis of type C retroviruses indicated that under some conditions the avian retrovirus spleen necrosis virus can package heterologous viral RNA from a related murine leukemia virus (18). The data presented here indicate that HIV-1 can similarly package and propagate SIV RNA. However, functional RNA pseudotyping of retroviral RNA by HIV-1 does not appear to be completely promiscuous, since HIV-1 does not efficiently propagate RNA from an amphotropic murine leukemia virus strain (15).

The observation that HIV-1 can pseudotype and replicate SIV RNA is significant for several reasons. First, co-infection of the same cell with two disparate lentiviruses might be expected to result in the propagation of viral nucleic acid via RNA pseudotyping. Thus, pseudotyping can take place both by this mechanism and by the incorporation of heterologous envelope glycoprotein onto the lentivirus envelope (15). Secondly, the cis-acting sequences of one lentivirus can apparently be recognized by a second virus, so a wide variety of genetically chimeric viruses derived from HIV-1 and SIV might be expected to replicate. The examination of HIV-1-SIV chimeras in nonhuman primates has already been initiated in an attempt to characterize the important determinants of HIV-1 replication and pathogenesis in an animal model (36). Finally, the observation that HIV-1 proteins can recognize the SIV encapsidation sequence indicates that the encapsidation signals of these two viruses are similar in some way.

The primary nucleic acid sequence that corresponds to the encapsidation of SIV is not similar to that of HIV-1. However, on the basis of the known secondary structure of the TAR element in the leader (6, 14, 19, 26, 34), the likely presence of a U5-leader stem and loop (11), and a free-energy minimization computer program, it is likely that a similar secondary structure is present in both viruses (Fig. 5). This region in both SIV and HIV-1 contains three distinct putative hairpin loop regions. A likely secondary structure for a different portion of the HIV-1 leader RNA has recently been reported (27). However, this structure is derived from a region of the HIV-1 leader that is wholly downstream from the corresponding region of SIV which we have shown to
facilitate RNA encapsidation. We think that it is likely that this downstream region may also facilitate encapsidation, but such a structure cannot be responsible for the recognition of SIV RNA by the HIV-1 proteins that we observed.

Since the terminal 422 nucleotides (including only 92 nucleotides downstream of the PBS) of SIV RNA facilitate encapsidation, we believe that intramolecular interaction within this region alone is sufficient to form an encapsidation signal. However, we think that it is very likely that sequences downstream from the U5-leader region do augment encapsidation, since the region that we have identified as an E region lies upstream of the splice donor site in the leader; efficient retroviral nucleic acid replication would be expected to mandate specific packaging of full-length RNA and general exclusion of spliced viral mRNA, so there is almost certainly a mechanism to exclude the spliced lentivirus mRNA. Nonetheless, SIV RNA containing only the upstream signal in the untranslated leader is efficiently incorporated into HIV-1 particles (Fig. 3). We cannot formally rule out the possibility that the 3′ end of the genome also contains signals necessary for encapsidation. However, since the 5′ end of the genome of some type C retroviruses is sufficient for the encapsidation of RNA (16, 38), we do not think that it is currently warranted to invoke a role of the 3′ end in lentivirus encapsidation.

Deletion of nucleotides that encroach on the downstream stem of the putative U5-leader abrogated encapsidation of SIV RNA (see the TR173 and TR174 virion preparations in Fig. 3). Therefore, this component of the viral RNA appears to contain a crucial element that serves as a packaging signal.

SIV and HIV-1 RNA both appear to be encapsidated at similar efficiencies by HIV-1 proteins (Fig. 3). However, full replication of the HIV-1 nucleic acid was significantly more efficient for HIV-1 RNA than for SIV RNA (Table 1). This likely indicates that some other step(s) in nucleic acid replication is less efficient for the heterologous genome. Since the PBS and PPT sequences for the two viruses are identical, it is unlikely that efficiencies of minus- and plus-strand initiation are different, unless subtle differences in the U5-leader stem and loop (Fig. 5) affect the efficiency of reverse transcription (11). The ATT sequences for the two viruses, present at the LTR termini, are similar but not identical. Therefore, it is possible that integration of SIV DNA, mediated by HIV-1 proteins, is not as efficient as that of HIV-1 DNA.

Since we have identified a relatively short region that can facilitate encapsidation of SIV RNA, and since the corresponding region of HIV-1 RNA potentially forms a similar structure, it should be possible to identify and characterize in detail this determinant of lentivirus RNA that is important for encapsidation. Experiments to determine whether the putative higher-order structures form and whether they are functionally important for encapsidation are under way.

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REFERENCES


