Virion packaging determinants and reverse transcription of SRP RNA in HIV-1 particles

Chunjuan Tian[1,2], Tao Wang[1], Wenyan Zhang[1,2] and Xiao-Fang Yu[1,*]

[1]Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205, USA and [2]Jilin University, Jilin, China

ABSTRACT

Diverse retroviruses have been shown to package host SRP (7SL) RNA. However, little is known about the viral determinants of 7SL RNA packaging. Here we demonstrate that 7SL RNA is more selectively packaged into HIV-1 virions than are other abundant Pol-III-transcribed RNAs, including Y RNAs, 7SK RNA, U6 snRNA and cellular mRNAs. The majority of the virion-packaged 7SL RNAs were associated with the viral core structures and could be reverse-transcribed in HIV-1 virions and in virus-infected cells. Viral Pol proteins influenced tRNAlys,3 packaging but had little influence on virion packaging of 7SL RNA. The N-terminal basic region and the basic linker region of HIV-1 NCp7 were found to be important for efficient 7SL RNA packaging. Although Alu RNAs are derived from 7SL RNA and share the Alu RNA domain with 7SL RNA, the packaging of Alu RNAs was at least 50-fold less efficient than that of 7SL RNA. Thus, 7SL RNAs are selectively packaged into HIV-1 virions through mechanisms distinct from those for viral genomic RNA or primer tRNAlys,3. Virion packaging of both human cytidine deaminase APOBEC3G and cellular 7SL RNA are mapped to the same regions in HIV-1 NC domain.

INTRODUCTION

Retroviruses package two copies of viral genomic RNA per viral particle. The selective packaging of viral genomic RNA is mediated by the specific interaction between sequences in the viral RNA (ψ) and the nucleocapsid (NC) domain of Gag molecules (1,2). Although packaging of viral genomic RNA is essential for virus infectivity, viral genomic RNA is dispensable for virus assembly, which is mediated by the viral structural protein Gag. However,

RNAs of either viral or cellular origin are believed to be important for retroviral particle assembly (3,4).

In addition to viral genomic RNA, retroviruses also contain abundant copies of small RNA molecules ranging in size from 4S to 7S (1,2). Among these small RNAs, the tRNAs used by various retroviruses, and particularly the primer tRNAs, have been well characterized (1,2). Primer tRNAs are selectively packaged through an interaction with viral reverse transcriptase (5,6). In the case of HIV-1, tRNAlys,3 is also selected by means of an interaction between the capsid domain of Gag and tRNARS, which forms a complex with tRNAlys,3 (7).

Other small RNAs in retroviral particles that have been characterized include 7SL RNA (8–12), 5S rRNA (9), and U6 snRNA (9,13). A recent study of Moloney murine leukemia virus (MuLV) observed that 7SL RNA and viral genomic RNA were similarly enriched in MuLV virions (9). Several other cellular RNAs, including Y1 RNA, Y3 RNA, B1 RNA, 5S rRNA and U6 snRNA, were also found to be packaged with an efficiency similar to that of 7SL RNA (9). An earlier study detected three major species (7S, 5S and tRNAs) of small cellular RNAs in HIV-1 virions (14). Although 7SL RNA has been detected in HIV-1 virions (10,12), packaging of other Pol-III-transcribed RNAs into HIV-1 virions has not been well studied. The viral determinants for the packaging of various cellular small RNAs are also poorly defined.

In the present study, we have shown that 7SL RNA is packaged into HIV-1 particles at a much higher efficiency than are Y RNAs, 7SK RNA or U6 snRNA. Although Alu RNA was derived from 7SL RNA and shares the Alu domain with 7SL RNA, packaging of Alu RNAs was at least 50-fold less efficient than that of 7SL RNA. The majority of the virion-associated 7SL RNA molecules were associated with viral core structures. Viral Pol and Env proteins, as well as viral genomic RNA, were dispensable for the packaging of 7SL RNA. Although both the MA and NC domains of HIV-1 Gag polyproteins have been shown to interact with nucleic acids, we found that the NC domain, but not the MA domain or L domain (p6), played a critical role in

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mediating 7SL RNA packaging. The N-terminal basic region and the basic linker region of HIV-1 NC, but not the zinc finger motifs, were important for 7SL packaging.

MATERIALS AND METHODS

Plasmid constructs

The HIV-1 constructs Pr-, ΔPolΔEnv, Gag expression vectors pGAGINS, pNCS and pP2LZ have been previously described (15). B2FS- was generously provided by Dr Shan Cen. HIV-1Gag-myc, SrcΔMAGag-myc, Gag-CFP432, Gag-CFP411, Gag-CFP406 and Gag-CFP395 were generously provided by Dr Paul Spearman. Gag411ΔN8 and Gag411ΔZ1 were generated from Gag-CFP411. The HIV-1 NC mutant construct pBR653-47 was a generous gift of Dr Robert Gorelick.

Antibodies and cells

The following antibodies were used for this study: anti-HA mouse monoclonal antibody (Mab; Covance, Cat. #MMS-101R-10000), anti-myc mouse mouse Mab (Sigma, Cat. #M 5546), pooled HIV-1+ human sera, anti-NC antibody and anti-human ribosomal P antigen antibody (Immunovision, Cat. #HP0-0100). Anti-p24 Mab (Cat# 1513) and anti-gp120 antibody (Cat# 288) were obtained from the AIDS Research Reagents Program, Division of AIDS, NIAID, NIH. 293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) with 10% fetal bovine serum and gentamicin (5 μg/ml) (D-10 medium) and passaged upon confluence.

Transfections, virus and VLP purification, and virion-associated RNA extraction

DNA transfection was carried out using Lipofectamine 2000 (Invitrogen) as recommended by the manufacturer. Viruses in cell culture supernatants were cleared of cellular debris by centrifugation at 3000 r.p.m. for 15 min in a Sorvall RT 6000B centrifuge and filtration through a 0.2-μm-pore size membrane (Millipore). Virus particles were then concentrated by centrifugation through a 20% sucrose cushion by ultracentrifugation at 100 000g for 2 h at 4° in a Sorvall Ultra80 ultracentrifuge. Viral pellets were resuspended in lysis buffer (PBS containing 1% Triton X-100 and complete protease inhibitor cocktail [Roche] and RNase inhibitor [New England BioLabs]). Viral lysates were analyzed by immunoblotting, or virion-associated RNA was extracted with Trizol (Invitrogen) according to the manufacturer's instructions. Cell and virion-associated RNAs were extracted and analyzed by qRT-PCR. For sucrose density gradient purification of HIV-1 particles, viral pellets as described above were dissolved in PBS solution and loaded on top of 20 to 60% stepwise sucrose gradients. Samples were subjected to ultracentrifugation using a SW41 rotor in the Sorvall Discovery ultracentrifuge at 22 000 r.p.m. for 16 h at 4°C. After centrifugation, 12 fractions (0.85 ml each) were collected from the top of the gradient and analyzed by immunoblotting using anti-p24 Mab. Fractions 3 to 8 were also analyzed for viral genomic RNA and virion-associated cellular RNAs. Samples (100 μl) from each fraction were left untreated, treated with 8 μg/ml RNase (Roche, 11119915001) in the absence of detergent, or treated with 8 μg/ml RNase plus 0.1% Triton X-100 at 37°C for 30 min. Reactions were stopped by the addition of 1 mM EDTA plus 1 ml Trizol, and RNAs were extracted according to the manufacturer's instructions.

Immunoblot analysis

Cells were collected 48 h after transfection. Cell and viral lysates were prepared as previously described (16). Cells (1×10^5) were lysed in $1 \times$ loading buffer (0.08 M Tris, pH 6.8, with 2.0% SDS, 10% glycerol, 0.1 M dithiothreitol, and 0.2% bromophenol blue). The samples were boiled for 10 min, and proteins were separated by SDS-PAGE. Proteins were transferred onto two separate nitrocellulose membranes by passive diffusion for 16 h, producing identical mirror-image blots. Membranes were probed with various primary antibodies against proteins of interest. Secondary antibodies were alkaline phosphatase-conjugated anti-human, anti-rabbit, anti-mouse, or anti-goat IgG (Jackson Immunoresearch, Inc), and staining was carried out with 5-bromo-4-chloro-3indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT) solutions prepared from chemicals obtained from Sigma.

Quantitative real-time PCR (qRT-PCR)

RNA samples were derived from cell lysates or viral lysates and treated with DNase by incubation in 10 μl of DEPC-treated water with $1 \times$ RQ1 RNase-free DNase Buffer, 1 μl RQ1 RNase-free DNase (Promega) and 4 U RNase inhibitor (New England BioLabs) for 30 min at 37°C. DNase was inactivated by the addition of 1 μl of RQ1 DNase Stop Solution and incubated at 65°C for 10 min. RNA was reverse-transcribed using random primers and the Multiscribe reverse transcriptase from the High Capacity cDNA Archive Kit (Applied Biosystems) according to the manufacturer's instructions. The cDNA was either undiluted or serially diluted in DEPC-treated water before input into the qRT-PCR reaction to ensure that amplification was within the linear range of detection.

The ABI 7000 sequence detection system (Applied Biosystems) was used for qRT-PCR amplifications. All primers were synthesized by Invitrogen, and fluorescent-tagged probes were synthesized by Applied Biosystems. Agarose gel analysis was used to verify that each primer pair produced single amplicons, and the identities of the PCR products were verified by cloning and sequencing. qRT-PCR was performed using either Taqman fluorescent probes or SYBR Green methods. For the Taqman method, each 20 μl reaction contained 1 μl of each forward- and reverse-specific primers (10 μM), 1 μ of fluorescent TaqMan probes (5 μM), 10 μl of $2 \times$ Universal Taqman PCR Master Mix, 4 μl of RNase-free water, and 3 μl of template cDNA. The reactions were carried out under the following conditions: 50°C for 2 min,

95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min. The target sequences were amplified using the following primer pairs and probes:

Y1 RNA: forward, 5′-GGCTGGTCCGAAGGTAGTGA-3′; reverse, 5′-AAAAGACTAGTCAAGTGCAGT-3′; and probe 5′ FAM-TGATTGTTCACAGTCAGTTAC-TAMRA- 3′;

Y3 RNA: forward, 5′-GGCTGGTCCGAGTGCAGT-3′; reverse, 5′-AAAAGGCTAGTCAAGTGAAGC-3′; and probe 5′ FAM-CACAACCAGTTACAGATT-TAMRA- 3′;

7SL RNA: forward, 5′-ATCGGGTGTCCGCACTAAG-3′; reverse, 5′-CACCCCTCCTTAGGCAACCT-3′; and probe 5′ FAM-CATCAATATGGTGACCTCCTAMR A-3′;

HIV RNA: forward, 5′-TGTGTGCCCGTCTGTTGTGT-3′; reverse, 5′-GAGTCCTGCGTCGAGAGAGC-3′; and probe 5′ FAM-CAGTGGCGCCCGAACAGGGA-TAMRA- 3′;

beta-actin RNA: forward, 5′-TCACCCACACTGTGCCCATCTACGA-3′; reverse, 5′-CAGCGGAACCGCTCATTGCCAATGG-3′; and probe: 6FAM-ATGCCCTCCCCCATGCCATCCTGCGT-TAMRA-3′.

The primer/probe set specific for A3G was a predesigned TaqMan gene expression assay (Applied Biosystems assay, identification number: Hs00222415).

For the SYBR Green method, each 20 µl reaction contained 1 µl of each forward- and reverse-specific primer (10 µM), 10 µl of 2 × SYBR Green PCR Master Mix, 5 µl of RNase-free water, and 3 µl of template cDNA. The reactions were performed with the following conditions: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min, followed by a dissociation protocol. Single peaks in the melting curve analysis indicated specific amplicons. The target sequences amplified by the SYBR Green method used the following primer pairs:

5S RNA: forward, 5′-TTCAGCGTCTACGGCCATAC-3′; reverse, 5′-AGCCAAAGAAAAAGCCTAC-3′;

Y4 RNA: forward, 5′-GGCTGGTCCGATGGTAGTG-3′; reverse, 5′-AAGCCAGTCAAATTTAGCAGTGGG-3′;

Y5 RNA: forward, 5′-AGTTGGTCCGAGTGTTGTGGGT-3′; reverse, 5′-ACAGCAAGCTAGTCAAGCGCG-3′;

tRNA-Phe: forward, 5′-GCCGAAATAGCTCAGTTGGGAGA-3′; reverse, 5′-TGGTGCCGAAACCCGG-3′;

tRNA-Lys,3: forward, 5′-GCCCGGATAGCTCAGTCG-3′; reverse, 5′-TGGCGCCCGAACAGG-3′;

GAPDH: forward, 5′-GCA AATTCCATGGCACCGT-3′; reverse, 5′-TCGCCCCACTTGATTTTG G-3′;

The copy numbers of the target cDNAs in the qRT-PCR assay were determined by using a standard curve of 10-fold serial dilutions of non-linearized plasmid DNA containing the target sequence (ranging from 5 or 10 copies to 5×10^6 or 10^6 copies). Absolute RNA copy numbers were calculated by using standard dilution curves of plasmids containing the target sequence. If the template cDNA was diluted before input into the reaction, the copy number of the target transcript was adjusted by the dilution factor. The sensitivity of the assay or limit of detection was determined by the lowest copy number that was consistently amplified within the linear portion of the standard curve. The detection limit ranged from 5 to 50 copies per reaction for the various primer sets. The qRT-PCR assay detected A3G, Y1, Y3, Y4, 7SL and GAPDH at 5 copies; 5S and tRNA-lys,3 at 10 copies; Alu1, tRNA-phe and Y5 at 50 copies per reaction. All standards at varying concentrations were amplified linearly over a range of at least 5 orders of magnitude, and the correlation coefficients (R^2) were greater than 0.99. Copy numbers of transcripts were calculated by using standard dilution curves of plasmids containing the target sequence.

Detection of 7SL RNA reverse transcription in HIV-1 virions and infected cells

293T cells were transfected with NL4-3 or control pcDNA3.1. Culture supernatant was clarified by low-speed centrifugation, filtered through a 0.22-µm membrane, and sedimented by ultracentrifugation over a cushion of 20% sucrose at 28 000 r.p.m. using an SW28 rotor for 2 h. The viral pellets were re-suspended in 200 µl of endogenous RT buffer (40 mM Tris-HCl, pH 8.0, 10 mM MgCl$_2$, 6 mM KCl, 2 mM DTT,0.02% Triton X-100) containing no dNTP or 0.2 mM dNTP. Samples were incubated at 37°C for16 h. The reaction mixtures were stopped by adding 1% SDS at 100°C for 1 min. The reaction products were extracted by adding chloroform/phenol and precipitated with isopropanol, washed with ethyl alcohol, and dissolved in water for PCR or qRT-PCR analysis.

To detect 7SL DNA in HIV-1-infected cells, a total of 10^6 MAGI cells were infected with NL4-3 viruses (equivalent to 1 µg of p24) or negative control supernatant for 14 h. Cells were then washed with PBS, dissolved in lysis buffer (0.5% Triton X-100, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl,1 mM EDTA) for 30 min at 37°C, and centrifuged at $3000 \times g$ for 10 min, and the pellets (containing nucleus and chromosomal DNA) were discarded. The DNA sample in the supernatant was extracted with chloroform/phenol, precipitated with ethanol (3- to 4-fold), and dissolved in water for qRT-PCR analysis.

RESULTS

The majority of 7SL RNA molecules in HIV-1 virions are associated with the viral cores

An earlier study has identified three major species of small cellular RNAs (7S, 5S and 4S tRNAs) in purified HIV-1 virions (14). Recently, 7SL RNA has also been detected in HIV-1 virions (10,12); however, the mechanism of 7SL packaging into HIV-1 virions is still unclear. It is possible that 7SL RNAs passively diffuse into budding particles as a result of the high local concentration at the site of virus assembly/budding. If this were the case, one would not expect 7SL RNAs to be enriched in any subviral structures. To address this question, we determined the localization of 7SL RNA in mature HIV-1 virions. Purified HIV-1 virions were subjected to brief treatment with the non-ionic detergent Triton
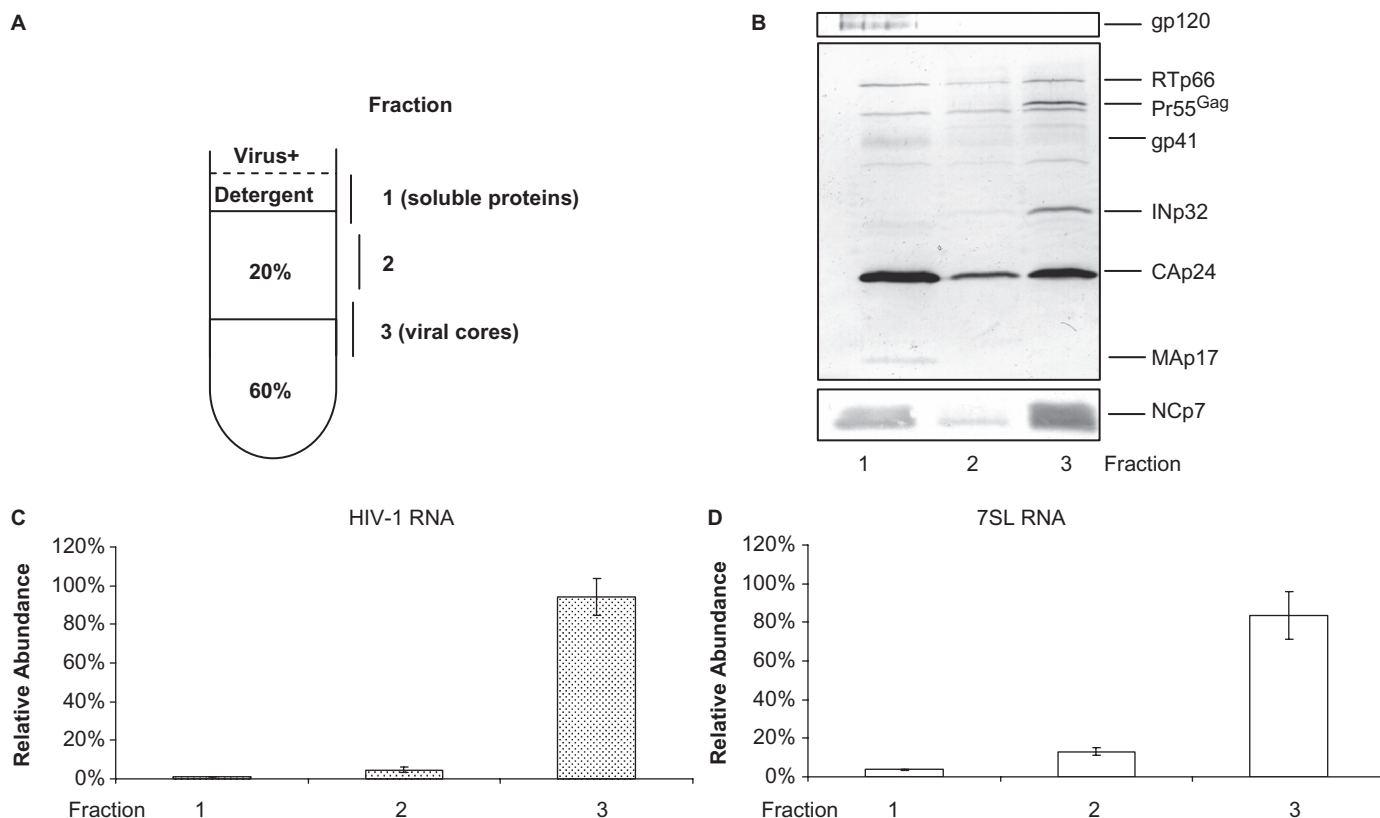
Figure 1. Association of 7SL RNA molecules with viral cores. Purified HIV-1 virions were treated briefly with detergent to separate cores from viral membranes, as illustrated in (**A**) and described previously (17). (**B**) Analysis of core-associated viral proteins. HIV-1 core-enriched fraction 3 contained HIV-1 INp32, NCp7, GagPr55 and CAp24. Viral membrane-associated proteins gp120, gp41 and MAp17 were mostly found in the soluble fraction 1. As previously described (18,19), significant amounts of CAp24 and RTp66 also disassociated from the cores. (**C** and **D**) Association of HIV-1 viral genomic RNA (C) and 7SL RNA molecules (D) with viral cores. RNAs in fractions 1, 2 and 3 were extracted and analyzed by qRT-PCR using specific primers for HIV-1 RNA or 7SL RNA. Results represent means ± standard deviations of at least three independent experiments.

X-100 to remove the viral membrane and isolate viral cores (Figure 1A) as previously described (17). The majority of the viral-membrane-associated proteins, such as the Env proteins gp120 and gp41 and the viral Map17 proteins (Figure 1B, lane 1), were separated from the core structure (lane 3). In contrast, most of the NCp7 and INp32 were located inside the cores (Figure 1B, lane 3). Consistent with the observations of Forshey *et al.* (18) and Tang *et al.* (19), we also found that some CAp24 and RTp66 molecules could be separated from the core structures (Figure 1B). As expected, HIV-1 genomic RNA was located mainly in the cores (Figure 1C, lane 3). Similarly, the majority of the 7SL RNAs were also detected with the cores (Figure 1C, lane 3). These results suggest that 7SL RNAs are likely to be recruited into HIV-1 virions by interacting with viral components inside the core structures.

Selective packaging of 7SL RNAs into HIV-1 virions

Although packaging of 7SL RNA and tRNAs into HIV-1 virions has been studied (6,10,12), the packaging of other Pol-III-derived RNAs into HIV-1 virions has not been fully characterized. To examine the packaging of Pol-III-derived RNAs into HIV-1 particles, HIV-1 virions

in the culture supernatants of NL4-3-transfected 293T cells were separated from cell debris by filtration and ultracentrifugation through a 20% sucrose cushion, then further purified on sucrose density gradients (20–60% wt/ vol). As a control for non-specific secretion of cellular RNAs in the culture supernatants, culture supernatants from mock-transfected 293T cells were also prepared side-by-side. Sucrose-gradient-purified HIV-1 virions were pelleted by ultracentrifugation, and virion-associated RNAs were analyzed by real-time PCR (qRT-PCR) using specific primers for various Pol-III RNAs.

Packaging of various Pol-III RNAs within HIV-1 virions was indeed detected, and there was only minimal non-specific secretion of most of these RNAs into the culture supernatant in the absence of HIV-1 virions (Figure 2A). For example, the level of particle-associated 7SL RNA in purified HIV-1 virions was more than 50-fold higher than that of the mock control (Figure 2A). High copy numbers of 7SL RNA and 5S rRNA were detected in HIV-1 virions (Figure 2A), consistent with the earlier observation of 7S and 5S RNA species in purified HIV-1 virions (14).

The packaging of various RNAs could potentially be influenced by the relative abundances of these RNAs in
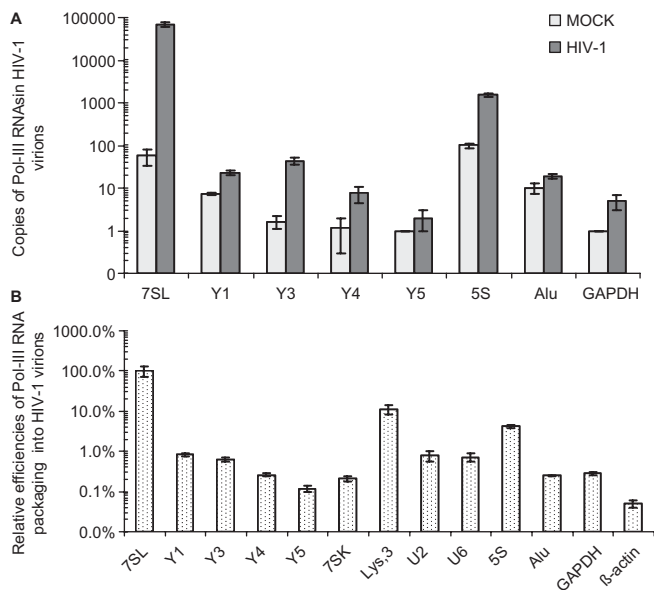
Figure 2. Analysis of the packaging of cellular RNAs into HIV-1 virions. (**A**) Detection of 7SL RNA and 5S rRNA in purified HIV-1 virions. HIV-1 virions in the culture supernatants of NL4-3-transfected 293T cells were purified by sucrose density gradient centrifugation. Virion-associated RNAs were analyzed by qRT-PCR using specific primers for Y1, Y3, Y4, Y5 RNAs, 5S rRNA, GAPDH mRNA, Alu RNAs or 7SL RNA. Similar samples from mock-transfected 293T cells were prepared and analyzed side-by-side to determine non-virus-mediated secretion of various RNAs. The copy numbers of RNAs detected in HIV-1 virions from 60 pg of p24 equivalent culture supernatant are shown. (**B**) Relative efficiency of virion packaging of RNAs, as analyzed by qRT-PCR. The efficiency of RNA packaging was evaluated by comparing the ratio of the number of copies of a particular RNA in HIV-1 virions to the number of copies of the same RNA in virus-producing cells. The efficiency of 7SL RNA packaging was set to 100%. Results represent means ± standard deviations of at least three independent experiments.

virus-producing cells. We therefore compared the relative efficiency of virion packaging of the various cellular RNAs. For comparison purposes, the efficiency of 7SL RNA packaging (copies of 7SL RNA in HIV-1 virions/copies of 7SL RNA in virus-producing cells) was set to 100%. Selective enrichment of primer tRNAlys,3 and 5S rRNA within HIV-1 virions was also observed (Figure 2B). Virion packaging of U6 snRNA, 7SK RNA, Y1, Y3, Y4 and Y5 RNAs was much less efficient than that of 7SL RNA (Figure 2B). Abundant cellular mRNAs such as β-actin and GAPDH mRNAs were also packaged much less efficiently into HIV-1 virions than was 7SL RNA (Figure 2B). Since the intracellular abundance of the various RNAs was taken into consideration, the inefficient packaging of these RNAs when compared to 7SL RNA could not be attributed to their lower intracellular levels. In MuLV, a 7SL RNA-derived SINE RNA, B1 RNA, was packaged as efficiently as 7SL RNA (9). Alu RNAs are also derived from 7SL RNA and share the Alu domain structures with it (20). However, Alu RNAs were also packaged into HIV-1 virions at least 50-fold less efficiently than was 7SL RNA (Figure 2B).

While 7SL RNA was clearly packaged within HIV-1 virions (Figure 2A), HIV-1 virion-associated 5S rRNA was only a few-fold higher than the mock control (Figure 2A). To further investigate whether 5S rRNA was indeed packaged into HIV-1 virions, sucrose-gradient-purified HIV-1 virions were pelleted by ultracentrifugation, and virion-associated RNAs were analyzed by real-time PCR (qRT-PCR) using specific primers for 7SL RNA or 5S rRNA. Fractions 3–8 contained detectable HIV-1 virions, with the peak occurring in fractions 5–7, as determined by analyzing viral CAp24 (Figure 3A), viral genomic RNA (Figure 3B) or 7SL RNA (Figure 3C). Surprisingly, both HIV-1 virions and samples from mock-transfected cells contained 5S rRNA peaks that co-migrated with CAp24 (Figure 3D). Virion-associated 7SL RNAs (Figure 3E) and 5S rRNA (Figure 3F) were resistant to RNase treatment unless the viral membrane was first disrupted with the detergent Triton X-100, indicating that these RNAs were packaged within viral particles. In repeated experiments, the level of HIV-1 virion-associated 5S rRNA was always a few-fold higher than the mock control, suggesting that some 5S rRNA was indeed packaged into HIV-1 virions. These results also suggest that 293T cells secreted some unidentified structures that contained 5S rRNA and had a density similar to that of HIV-1 virions.

To further examine the packaging of 7SL RNA and 5S rRNA into HIV-1 virions, HIV-1 (NL4-3)-infected CD4+ Jurkat T cells were established. HIV-1 virions in the culture supernatants of NL4-3-infected Jurkat cells were separated from cell debris by filtration and ultracentrifugation through a 20% sucrose cushion, then further purified on sucrose density gradients (20–60% wt/vol). As a control for non-specific secretion of cellular RNAs in the culture supernatants, culture supernatants from uninfected Jurkat cells were also prepared side by side. Sucrose-gradient-purified HIV-1 virions were pelleted by ultracentrifugation, and virion-associated RNAs were analyzed by real-time PCR (qRT-PCR) using specific primers for 7SL RNA, 5S rRNA and HIV-1 genomic RNA. Fractions 3–8 contained detectable HIV-1 virions, with the peak occurring in fraction 5, as determined by analyzing viral CAp24 (Figure 4A) and viral genomic RNA (Figure 4B). Packaging of 7SL RNA (Figure 4C) or 5S rRNA (Figure 4D) into HIV-1 virions produced from HIV-1-infected Jurkat cells, but not uninfected Jurkat cells, was clearly observed.

HIV-1 Gag NC zinc-binding domains, viral genomic RNA, Pol and Env proteins are not essential for 7SL RNA packaging

With the exception of primer tRNAlys,3, the determinants of Pol-III RNA packaging into HIV-1 virions are poorly defined. The influence of the viral Pol and Env proteins on 7SL RNA packaging was first examined using the constructs Pr-, B2FS- and ΔPolΔEnv. The construct Pr- contains an active site mutation in the protease gene and expresses the Gag, Gag-Pol and Env proteins (Figure 5A). The B2FS- construct contains mutations that destroy the frameshifting sequences in the gag coding region that are essential for the generation of Gag-Pol
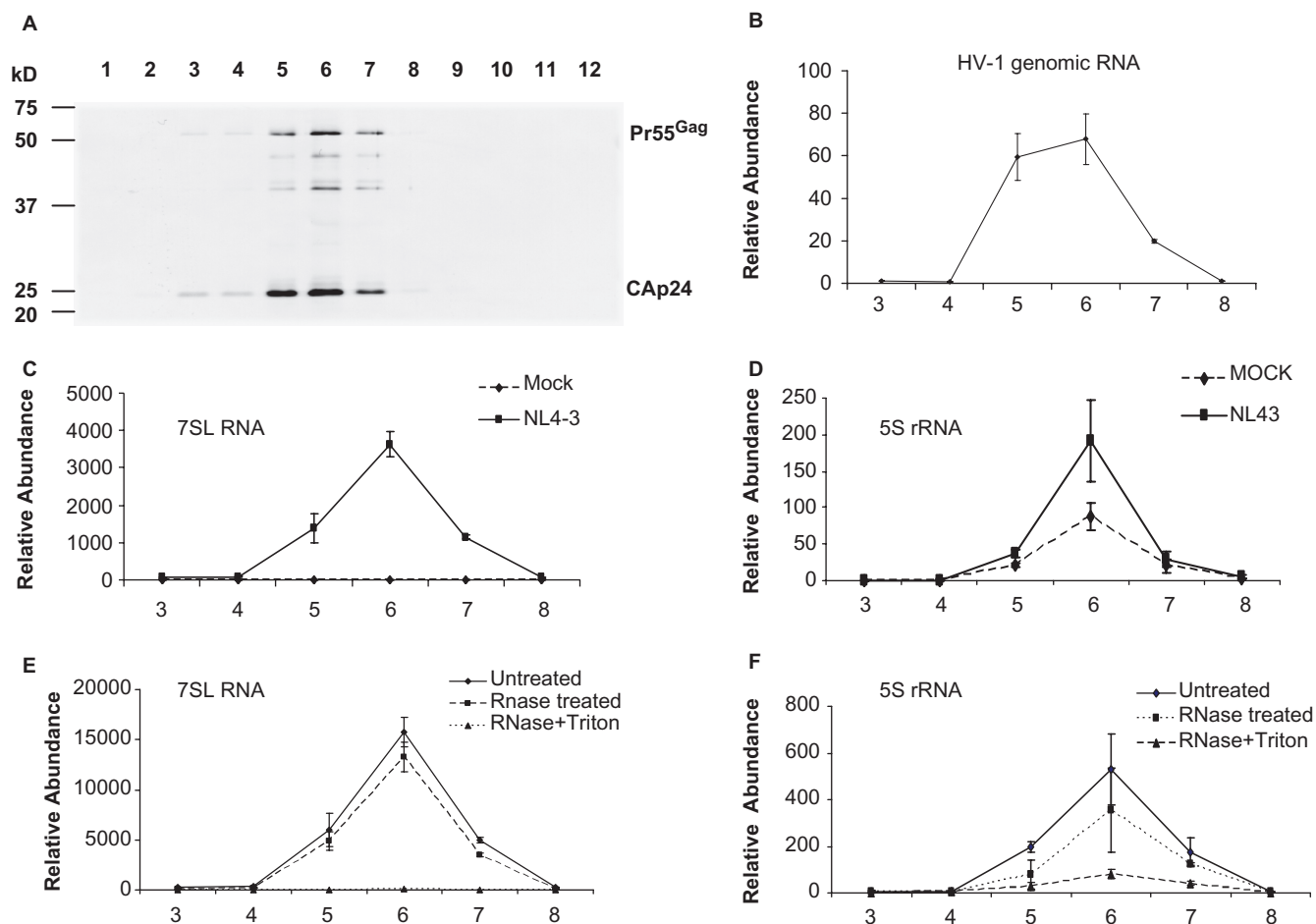
Figure 3. Characterization of 7SL RNA and 5S rRNA in HIV-1 virions. (**A**) NL4-3 viruses were produced from transfected 293T cells and purified by sucrose-density gradient centrifugation. Viral proteins in each fraction were analyzed by immunoblotting using the anti-p24 antibody. Virion-associated RNAs were extracted from fractions 3 to 8 of the sucrose-density gradient and analyzed by qRT-PCR using HIV-1 viral RNA- (**B**), 7SL RNA- (**C**) or 5S rRNA- (**D**) specific primers. Samples from mock-transfected 293T cells were also prepared side-by-side and analyzed to detect the non-specific secretion of 7SL RNA or 5S rRNA. Virion-associated 7SL RNA (**E**) or 5S rRNA molecules (**F**) were resistant to RNase treatment unless the virions were pretreated with Triton X-100. Results represent means ± standard deviations of at least three independent experiments.

proteins (Figure 5A). The construct ΔPolΔEnv contains a deletion of most of the protease, the whole RT, and the IN coding sequences and an internal deletion of the Env coding sequence that abolished most of the Env protein expression (Figure 5A). VLPs from transfected 293T cells were separated from cell debris by filtration and ultracentrifugation through a 20% sucrose cushion. As a control for non-specific secretion of cellular RNAs into the culture supernatants, supernatants from mock-transfected 293T cells were also prepared side by side. Pelleted particles were analyzed by immunoblotting using a monoclonal antibody against CAp24 (Figure 5B). As expected, Pr- particles contained both Pr55^{Gag} and Pr160$^{Gag-Pol}$, while B2FS- and ΔPolΔEnv particles contained Pr55^{Gag} but not Pr160$^{Gag-Pol}$ (Figure 5B). Particle-associated RNAs were isolated and analyzed by qRT-PCR using specific primers for 7SL RNA or tRNAlys,3. Packaging of 7SL RNAs was clearly detected in Pr-, B2FS- and ΔPolΔEnv particles (Figure 5C). Non-specific secretion of 7SL RNA from the mock-transfected 293T cells was less than 9.0% of that for the

Pr- particles (Figure 5C). Packaging of 7SL RNAs was essentially comparable in Pr-, B2FS- and ΔPolΔEnv particles (Figure 5C). In contrast, packaging of tRNAlys,3 was reduced in B2FS- and ΔPolΔEnv particles when compared to that in Pr- particles (Figure 5D), consistent with previous reports (5,6) that viral Pol proteins influence the packaging of tRNAlys,3.

The role of viral genomic RNA in the packaging of 7SL RNAs was also examined. We compared the level of virion-associated 7SL RNAs in ΔPolΔEnv particles and in virus-like particles (VLP) containing only Gag molecules (pGAGINS) (15). The ΔPolΔEnv construct contains all the 5′RNA sequences upsteam of the gag coding sequence, which are important for viral genomic RNA packaging (Figure 6A). The Gag amino acid sequence of pGAGINS is identical to the Gag of ΔPolΔEnv. However, pGAGINS lacks all the 5′ viral RNA sequences, including the viral RNA packaging sequences upstream of the Gag coding region (Figure 6A). Although VLP produced by pGAGINS contained less than 1% of the viral RNA present in
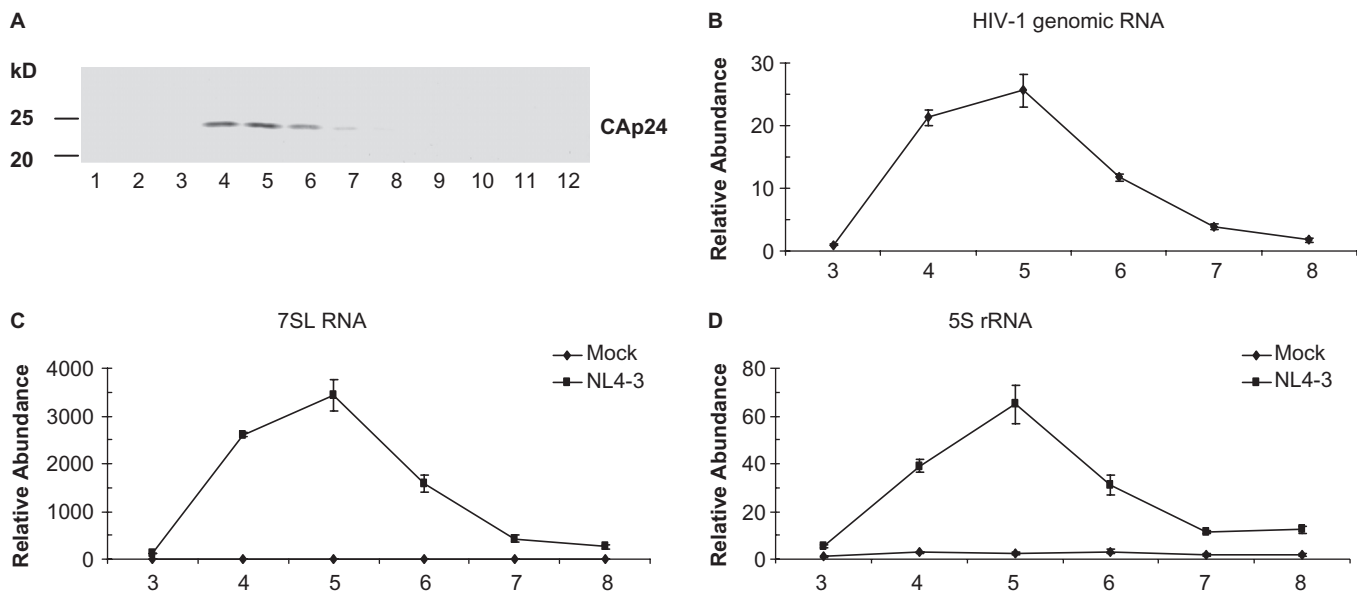
Figure 4. Packaging of 7SL RNA and 5S rRNA into HIV-1 virions produced from infected CD4+ Jurkat T cells. (**A**) NL4-3 viruses were produced from infected CD4+ Jurkat T cells and purified by sucrose-density gradient centrifugation. Viral proteins in each fraction were analyzed by immunoblotting using the anti-p24 antibody. Virion-associated RNAs were extracted from fractions 3 to 8 of the sucrose-density gradient and analyzed by qRT-PCR using HIV-1 viral RNA- (**B**), 7SL RNA- (**C**), or 5S rRNA- (**D**) specific primers. Samples from mock-transfected 293T cells were also prepared side-by-side and analyzed to detect the non-specific secretion of 7SL RNA or 5S rRNA. Results represent means ± standard deviations of at least three independent experiments.

particles produced by ΔPolΔEnv (data not shown), packaging of 7SL RNAs was readily detected in VLP produced by pGAGINS (Figure 6B). When the amount of Gag was normalized, the level of 7SL RNAs was similar in VLP produced by pGAGINS and in particles produced by ΔPolΔEnv (Figure 6B). Thus, VLP containing the Gag molecules alone could efficiently package 7SL RNA, suggesting that the Gag molecule but not viral genomic RNA contains a major determinant for the packaging of 7SL RNA.

Furthermore, packaging of viral genomic RNA into HIV-1 mutant pBR653-47 viruses (21), with mutations of the cysteine residues in the zinc fingers of the HIV-1 NC domain (Figure 6C), was reduced by about 87% when compared to that of NL4-3 viruses (Figure 6D). However, these mutations had little effect on 7SL RNA packaging into these NC mutant viruses, when compared to the parental HIV-1 NL4-3 viruses (Figure 6E). Thus, the zinc fingers of HIV-1 Gag NC domain and the viral genomic RNA do not play a major role in mediating 7SL RNA packaging.

The N-terminal basic region and the basic linker region of the HIV-1 Gag NC domain are required for 7SL RNA packaging

To examine which Gag domain(s) is(are) required for 7SL RNA packaging, the HIV-1 full-length Gag construct (pGAGINS), the Gag construct missing p6 and p1 (pNCS) and the Gag construct missing NC (P2LZ) were used (Figure 7A). P2LZ contains a deletion of NC, which is replaced by the leucine zipper (LZ) domain of the yeast GCN4 (Figure 7A), and is assembly-competent (15,22). Viral particles were produced from transfected 293T cells and separated from cell debris by filtration

and ultracentrifugation through a 20% sucrose cushion. Particle-associated RNAs were extracted and analyzed by qRT-PCR using 7SL RNA-specific primers. 7SL RNAs were detected in both GagINS and NCS VLPs. When the amount of Gag was normalized (Figure 7B), there was no significant difference in the levels of these RNAs in the two types of VLPs (Figure 7C). On the other hand, 7SL RNAs were poorly packaged into P2LZ VLPs lacking NC (Figure 7C). Thus, the p6 domain of HIV-1 Gag is not essential for the packaging of 7SL RNAs, but the NC domain is apparently important.

The MA domain of HIV-1 Gag has also been reported to mediate RNA binding (23). To examine whether the MA domain of Gag is required for 7SL RNA packaging, HIV-1 Gag VLP with or without MA (Figure 7D) were produced from transfected 293T cells. The membrane targeting function in the MA deletion mutant was restored by the v-src myristylation signal (24). Viral particles were separated from cell debris by filtration and ultracentrifugation through a 20% sucrose cushion (Figure 7E). Particle-associated RNAs were extracted and analyzed by qRT-PCR using 7SL RNA-specific primers. 7SL RNAs were packaged into Gag VLPs containing MA and Gag VLPs lacking MA (Figure 7F). 7SL RNA packaging was only slightly reduced in Gag VLPs lacking MA (Figure 7F). Thus, the NC domain is more important than the MA domain for the packaging of 7SL RNAs.

To further map the region of the NC domain that is required for 7SL RNA packaging, HIV-1 Gag-CFP expression vectors (25) containing various C-terminal NC sequence truncations (Figure 8A) were transfected into 293T cells. Gag particles were isolated and analyzed
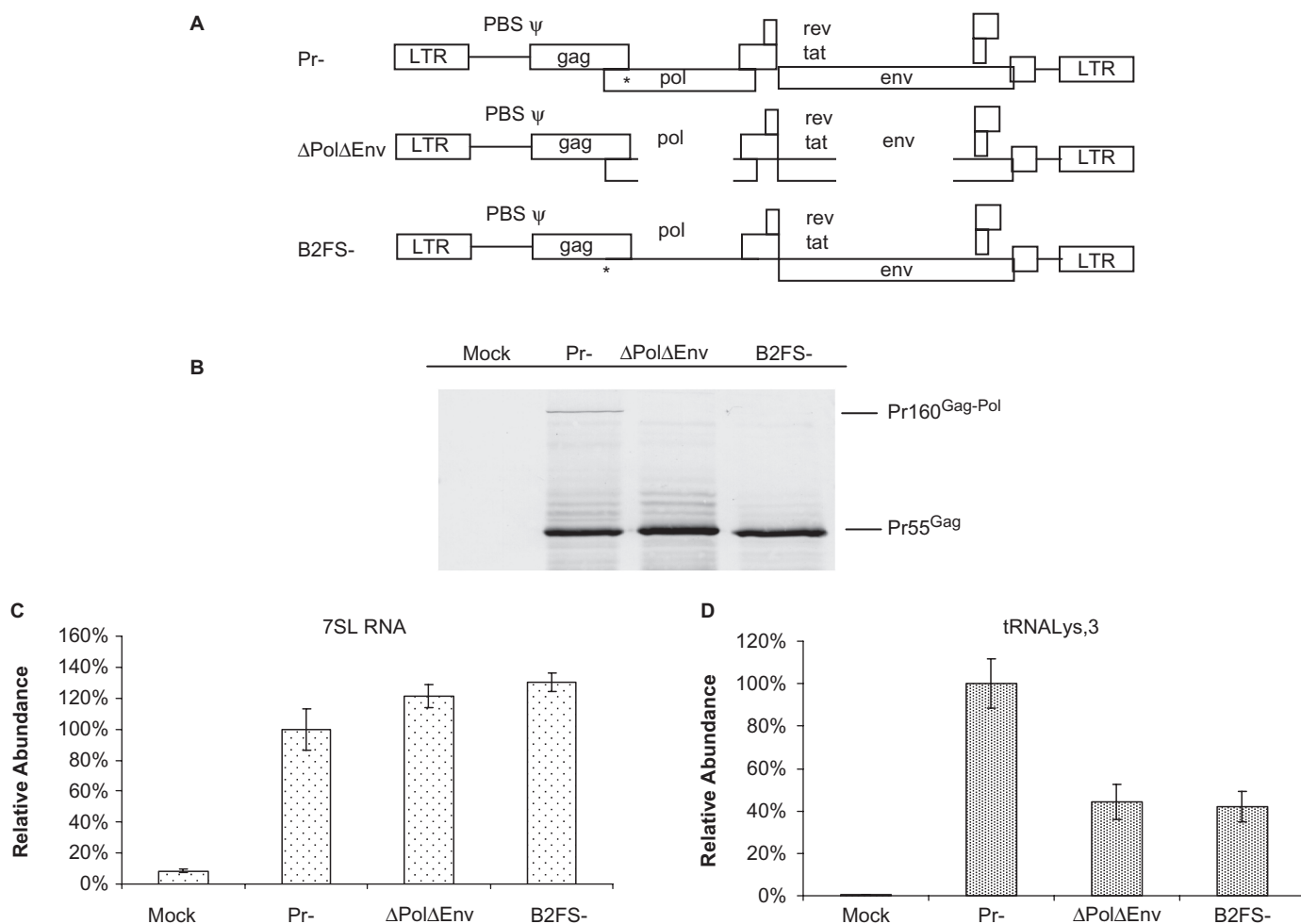
Figure 5. Pol or Env is not required for 7SL RNA packaging. (**A**) Illustration showing Pr- (which expresses viral Env proteins and uncleaved Gag and Gag-Pol proteins), B2FS- (which expresses viral Env proteins and uncleaved Gag proteins) and ΔPolΔEnv (which only expresses uncleaved Gag proteins) constructs. (**B**) Immunoblot analysis of Pr-, B2FS- or ΔPolΔEnv particles using an anti-p24 antibody. Detection of 7SL RNA (**C**) or tRNAlys,3 (**D**) in Pr-, B2FS- or ΔPolΔEnv particles by qRT-PCR using 7SL RNA- or tRNAlys,3-specific primers. Packaging of 7SL RNA or tRNAlys,3 in Pr- particles was set to 100%. Results represent means ± standard deviations of at least three independent experiments.

by immunoblotting using an anti-p24 antibody (Figure 8B). Particle-associated RNAs were extracted and analyzed by qRT-PCR using primers specific for 7SL RNA. The packaging of 7SL RNAs into Gag432 (containing full-length NC) particles was set to 100%. Gag411, which contains the C-terminal zinc finger deletion, was able to package 7SL RNA efficiently when compared to Gag432 (Figure 8C). However, removal of the basic linker region of the NC domain (Gag405) significantly reduced 7SL RNA packaging (Figure 8C).

The N-terminal basic region, the first zinc finger, and the basic linker region (Gag411) are the minimal determinants for 7SL RNA packaging. To examine the role of each of these three domains in 7SL RNA packaging, several NC mutant constructs were generated and compared (Figure 9A). Viral particles were produced from transfected 293T cells (Figure 9B). Particle-associated RNAs were extracted and analyzed by qRT-PCR using primers specific for 7SL RNA. The packaging of 7SL RNAs into Gag411 particles was set to 100%.

Deletion of the N-terminal basic region (Gag411ΔN8) or removal of the basic linker region of the NC domain (Gag405) significantly reduced 7SL RNA packaging (Figure 9C). Deletion of the first zinc finger (Gag411ΔZF1) had a lesser effect on 7SL RNA packaging (Figure 9C). Thus, these data indicate that the N-terminal basic region and the basic linker region of the NC domain play an important role in mediating 7SL RNA packaging in HIV-1.

HIV-1 Gag interacts with 7SL RNA in virus-producing cells

To determine whether HIV-1 Gag interacts with 7SL RNAs and therefore mediates its virion packaging, we transfected the HIV-1 Gag-myc or untagged Gag expression vector into 293T cells. Gag-myc, but not untagged Gag, was immunoprecipitated with the anti-myc antibody (Figure 10A). RNA samples of Gag-myc co-precipitated with the anti-myc antibody from transfected 293T cells were analyzed by qRT-PCR using primers specific for 7SL RNA. Cell lysates from Gag-transfected 293T
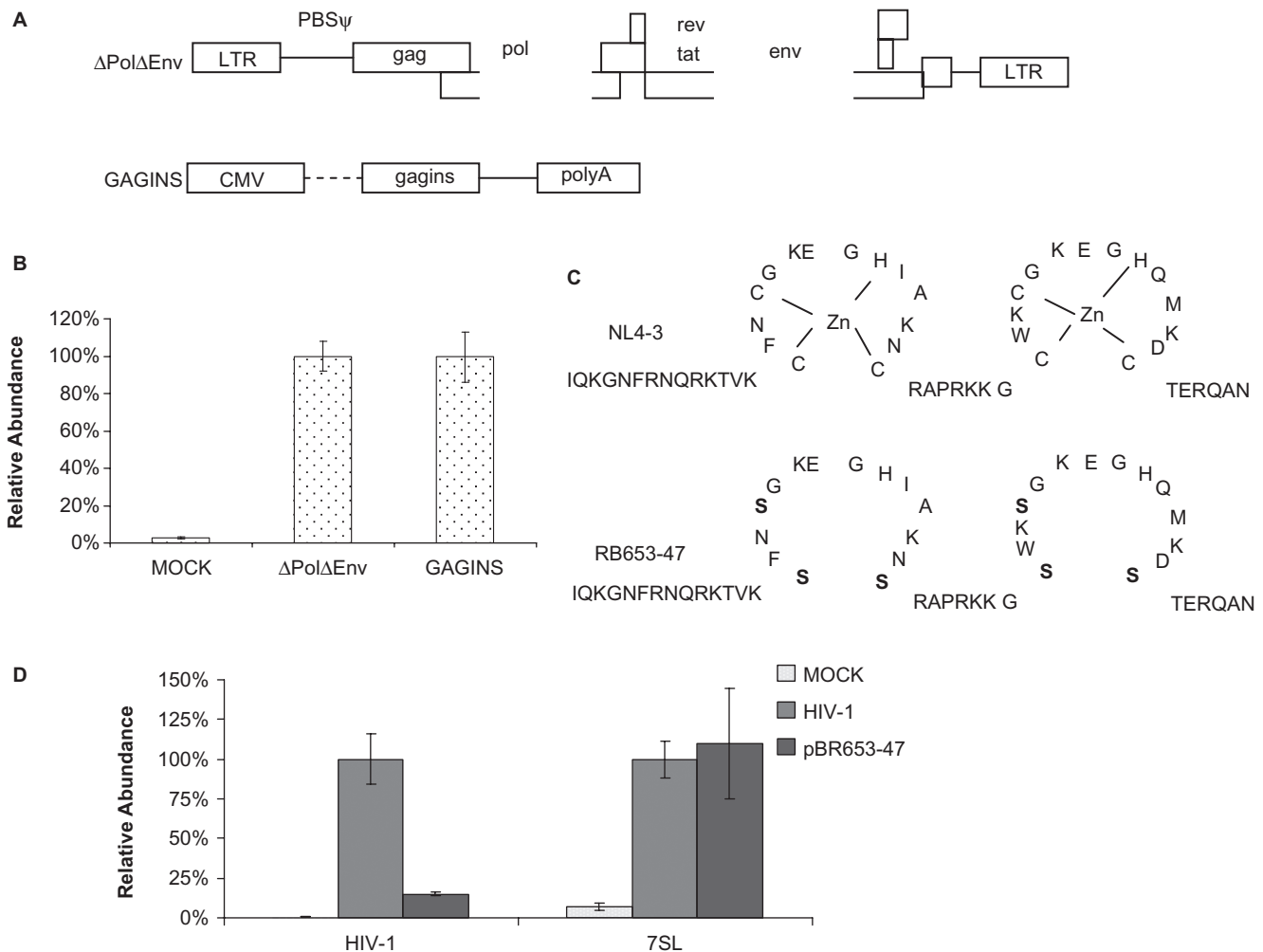
A

B

C

D

Figure 6. HIV-1 Gag, but not viral genomic RNA, is required for 7SL RNA packaging. (**A**) Illustration showing ΔPolΔEnv (which package viral RNA) and pGAGINS constructs. (**B**) Detection of 7SL RNA in ΔPolΔEnv and pGAGINS particles. Virion-associated RNAs were extracted from purified virions and analyzed by qRT-PCR using HIV-1 viral RNA- or 7SL RNA-specific primers. (**C**) Illustration of NC sequences in NL4-3 or pBR653-47 constructs. (**D**) HIV-1 NC zinc finger mutant viruses (pBR653-47) are defective for viral genomic RNA packaging but still package 7SL RNA. Results represent means ± standard deviations of at least three independent experiments.

cells, which could not be immunoprecipitated by the anti-myc antibody, were also examined side-by-side as the negative control. A specific interaction of 7SL RNA with Gag-myc, as compared to the control Gag sample, was clearly detected (Figure 10B). Thus, 7SL RNA is likely packaged into HIV-1 virions through an interaction with HIV-1 Gag molecules during virus assembly.

A3G interacts with 7SL RNA in HIV-1 virions

The virion packaging of 7SL RNA, as well as that of A3G, requires NC, suggesting that 7SL RNA could mediate A3G packaging. To examine whether A3G interacts with 7SL RNA in released HIV-1 virions, we immunoprecipitated A3G-HA in purified HIV-1 virions with an anti-HA antibody conjugated to agarose beads (Figure 11A). RNAs co-precipitated with A3G-HA were analyzed by qRT-PCR using primers specific for HIV-1 genomic RNA, tRNAlys,3, or 7SL RNA. As a control, HIV-1 virions lacking A3G-HA were also examined side

by side to detect non-specific binding of these virion-associated RNAs to the assay system (set as 1). A specific interaction between 7SL RNA and A3G-HA was observed; this interaction was approximately 18-fold higher than that observed for control HIV-1 virion samples lacking A3G-HA (Figure 11B). Interaction of A3G-HA with viral genomic RNA was also observed (Figure 11B). The level for tRNAlys,3 co-precipitated with A3G-HA in purified HIV-1 virions was about 5-fold higher than that for the control HIV-1 virion sample lacking A3G-HA (Figure 11B), suggesting that there may be a weak interaction between A3G-HA and tRNAlys,3 in HIV-1 virions.

7SL RNA could be reverse-transcribed in HIV-1 virions

Various cellular RNAs packaged into retroviruses have been shown to be reverse-transcribed (8,13,26,27). To determine whether virion-packaged 7SL RNA could be reverse-transcribed in HIV-1 virions, HIV-1 particles
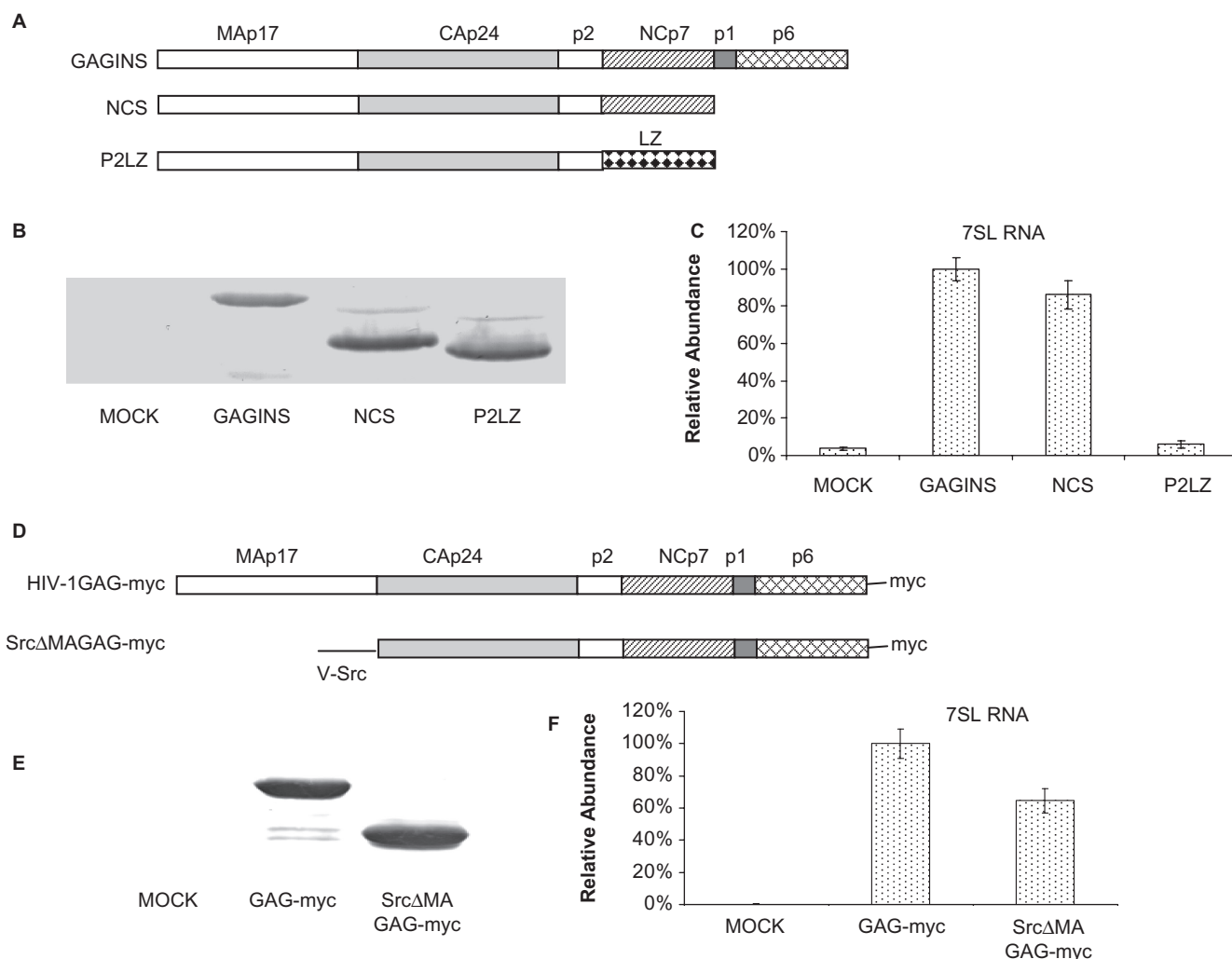
Figure 7. The NC domain of HIV-1 Gag is important for 7SL RNA packaging. (**A**) Schematic representation of HIV-1 C-terminal truncation mutant constructs. (**B**) The full-length HIV-1 Gag (pGAGINS), Gag lacking p6 and p1 (pNCS), or Gag lacking p6, p1 and NCp7 (pP2LZ) particles were produced from transfected 293T cells and analyzed by immunoblotting using the anti-p24 antibody. (**C**) Influence of NC deletion on 7SL RNA packaging. RNAs associated with pGAGINS, pNCS or pP2LZ particles were extracted and analyzed by qRT-PCR. Packaging of 7SL RNA in pGAGINS particles was set to 100%. (**D**) Schematic representation of the HIV-1 MA deletion mutant and its parental constructs. (**E**) The full-length HIV-1 Gag-myc or or SrcΔMAGag-myc particles were produced from transfected 293T cells and analyzed by immunoblotting using the anti-p24 antibody. (**F**) Influence of MA deletion on 7SL RNA packaging. RNAs associated with HIV-1 Gag-myc or SrcΔMAGag-myc particles were extracted and analyzed by qRT-PCR. Packaging of 7SL RNA in HIV-1 Gag-myc particles was set to 100%. Results represent means ± standard deviations of at least three independent experiments.

were produced from transfected 293T cells. We then subjected purified HIV-1 virions to detergent-permeabilization and endogenous RT reactions, followed by PCR analysis using 7SL-specific primers that could detect full-length or near-full-length 7SL DNA. 7SL DNA was clearly detected in the HIV-1 virion sample after endogenous RT reaction in the presence of dNTP (Figure 12A, lane 1) but not in the absence of dNTP (Figure 12A, lane 2). Reverse transcription of 7SL RNA in HIV-1 virions was also analyzed by qRT-PCR using HIV-1 and 7SL DNA-specific primers. As controls we used samples from mock-transfected 293T cells and HIV-1 virion samples without the addition of dNTPs. Neither HIV-1 DNA nor 7SL DNA was detected in samples from mock-transfected 293T cells (Figure 12B). Low levels of 7SL DNA and HIV-1 DNA were detected in HIV-1

virions without the addition of dNTPs (Figure 12B), presumably as a result of a low level of reverse transcription in released HIV-1 virions (add ref.). Significant increased levels of 7SL DNA and HIV-1 DNA were detected in endogenous RT-treated HIV-1 virion samples (Figure 12B). The PCR DNA products were sequenced and confirmed to be 7SL or HIV-1 sequences. To further examine whether virion-packaged 7SL RNA could be reverse-transcribed during virus infection, we infected MAGI cells (CD4+ HeLa cells) with NL4-3 virions. Cytoplasmic DNA samples from uninfected or HIV-1 infected MAGI cells were extracted and analyzed by qRT-PCR using 7SL- or HIV-1-specific primers. 7SL DNA was clearly detected in the HIV-1 infected MAGI cell extracts when compared to those from uninfected MAGI cells (Figure 12C). Thus, these data
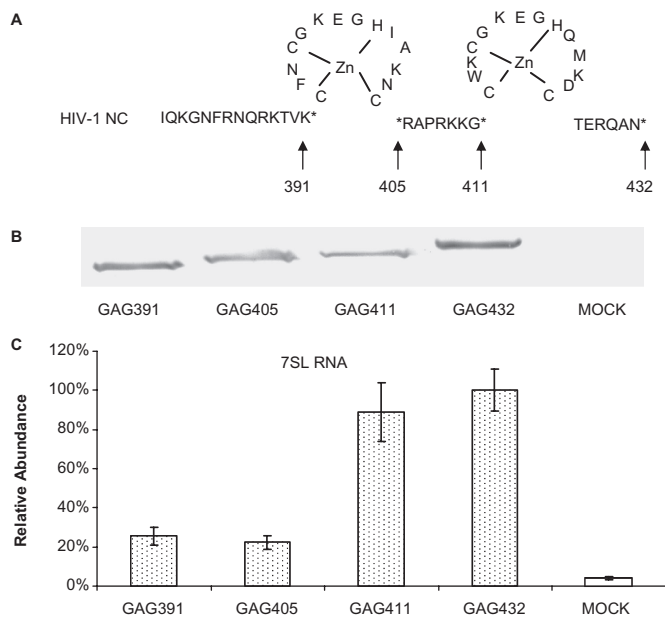
Figure 8. Identification of minimal regions in the HIV-1 Gag NC domain that are important for 7SL RNA packaging. (**A**) Schematic representation of Gag-CFP constructs. Asterisks indicate the location of the stop codons in the NC truncation constructs. (**B**) Immunoblot analysis of Gag-CFP particles. Gag-CFP particles were produced from transfected 293T cells and analyzed by immunoblotting using the anti-p24 antibody. (**C**) Influence of NC deletion on 7SL RNA packaging. RNAs associated with various Gag-CFP particles were extracted and analyzed by qRT-PCR using 7SL RNA-specific primers. The packaging of 7SL RNA within Gag-CFP particles containing full-length NC domain (Gag432) was set to 100%. Results represent means ± standard deviations of at least three independent experiments.

Figure 9. Comparison of the role of the N-terminal basic region, first zinc finger and the basic linker region of HIV-1 Gag NC in 7SL RNA packaging. (**A**) Schematic representation of NC deletion Gag-CFP constructs. (**B**) Immunoblot analysis of NC mutant particles. Gag-CFP particles were produced from transfected 293T cells and analyzed by immunoblotting using the anti-p24 antibody. (**C**) Influence of NC deletion on 7SL RNA packaging. RNAs associated with various NC mutant particles were extracted and analyzed by qRT-PCR using 7SL RNA-specific primers. The packaging of 7SL RNA within Gag411 particles was set to 100%. Results represent means ± standard deviations of at least three independent experiments.

indicate that virion-packaged 7SL RNA can be reverse-transcribed during HIV-1 infection.

DISCUSSION

In addition to viral genomic RNA, which accounts for >50% of the total RNA mass, small cellular RNAs of 4S to 7S have been detected in diverse retroviruses (1,2). These small RNAs are more abundant on a molar basis than the viral genomic RNA, and some have been identified as tRNAs, 7SL RNA, 5S rRNA and U6 snRNA in RSV (28), MuLV (5,9,29–31), FeLV (32), visna virus (33) and HIV-1 (10,14). With the exception of primer tRNAs, the viral determinants of Pol-III RNA packaging into retroviruses are poorly understood.

Our study has demonstrated that the NC domain, and particularly the basic linker region of this domain, plays an important role in mediating 7SL RNA packaging into HIV-1 virions. Viral genomic RNA and the viral structural proteins Pol and Env were dispensable for the packaging of 7SL RNAs, consistent with previous reports (10,12). The majority of the virion-packaged 7SL RNAs were associated with viral cores. Furthermore, interactions of 7SL RNAs with HIV-1 Gag proteins could be detected in virus-producing cells (Figure 10). These data su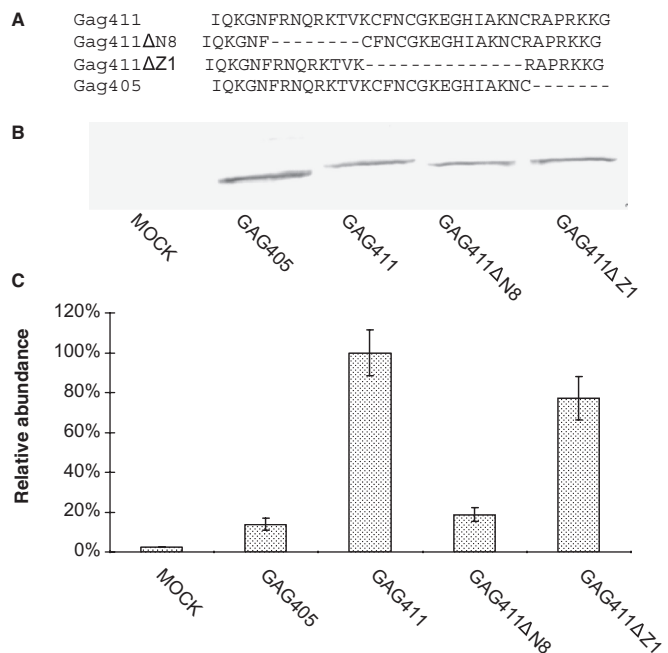ggest that 7SL RNAs are not passively included into budding particles but are instead packaged through interactions with HIV-1 Gag molecules during virus assembly.

Our data further demonstrate that virion-packaged 7SL RNA could be reverse-transcribed in HIV-1 virions and in HIV-1-infected cells. Reverse transcription of cellular RNA packaged into retroviruses such as mRNA (26,27), U6 snRNA (13), VL30 RNA (34,35) and 7SL RNA (8) has been previously reported. Primers annealing to the ends of 7SL sequences amplified an approximately 300-bp PCR product (confirmed to be 7SL sequence), suggesting full-length or nearly full-length 7SL RNA was being reverse-transcribed. Future study will be required to determine whether 7SL RNA was reverse-transcribed as a single DNA product using unidentified primer(s) or as a product of viral/7SL recombination.

The NC domain of retroviral Gag molecules plays important roles in viral genomic RNA packaging, RNA dimerization and annealing of primer tRNA to the viral genomic RNA through RNA chaperoning activity (1,2,36–39). Results presented here indicate that HIV-1 NC is important for the packaging of both viral genomic RNA and 7SL RNAs. Mutations of cysteine residues in the zinc fingers of the HIV-1 NC domain largely abolished viral genomic RNA packaging. However, these NC mutations had little effect on the packaging of 7SL RNAs.
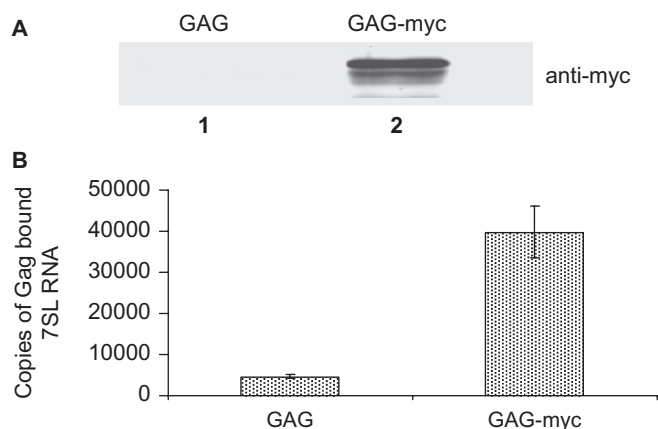
Figure 10. Interaction of 7SL RNA with HIV-1 Gag. (**A**) HIV-1 Gag-myc, or Gag was expressed in transfected 293T cells. Cell lysates were prepared and immunoprecipitated with the anti-myc Mab, and Gag-myc, but not Gag, was precipitated as analyzed by immunoblotting using the anti-myc antibody. (**B**) RNAs were extracted from co-precipitated samples and analyzed by qRT-PCR using primers specific for 7SL. Non-specific binding of 7SL RNA to the assay system was represented by the Gag sample. Results represent means ± standard deviations of at least three independent experiments.

These data indicate that the packaging mechanism of viral genomic RNA is distinct from that of 7SL RNA packaging.

By analyzing the packaging of 7SL RNA into a series of C-terminal truncation and internal deletion mutants of the HIV-1 NC domain, we were able to demonstrate that the N-terminal basic region and the basic linker region, but not the N-terminal or C-terminal zinc finger, was the most critical for 7SL RNA packaging. Further analysis will be required to identify the amino acids in the basic linker region of the HIV-1 NC domain that are critical for this packaging process. The N-terminal basic region and the basic linker region of HIV-1 NC have also been proposed to be critical for viral genomic RNA packaging, virus production and virion stability (40–43). The role of 7SL RNA in virus production, virion stability, and viral infectivity requires further investigation.

Various Pol-III-derived RNAs are packaged into retroviruses, but their packaging mechanisms are apparently different from that of 7SL RNAs. Primer tRNA packaging requires viral reverse transcriptase (5,6) and, at least in the case of HIV-1, an interaction between tRNAlys synthetase and the viral capsid domain (6,7). In contrast, the packaging of 7SLRNA is more dependent on the NC domain of HIV-1 Gag. The apparent differences between retroviruses in terms of the packaging efficiencies of the various Pol-III-derived RNAs are also worth noting. In the case of MuLV, Y1 and Y3 RNAs are packaged as efficiently as 7SL RNA (9). On the other hand, Y RNA packaging into HIV-1 virions was much less efficient than that of 7SL RNA. The packaging efficiency of U6 snRNA and 7SK RNA into MuLV particles is only moderately lower than that of 7SL RNA (9). However, in HIV-1, the packaging of U6 snRNA or 7SK RNA was approximately 100-fold less efficient
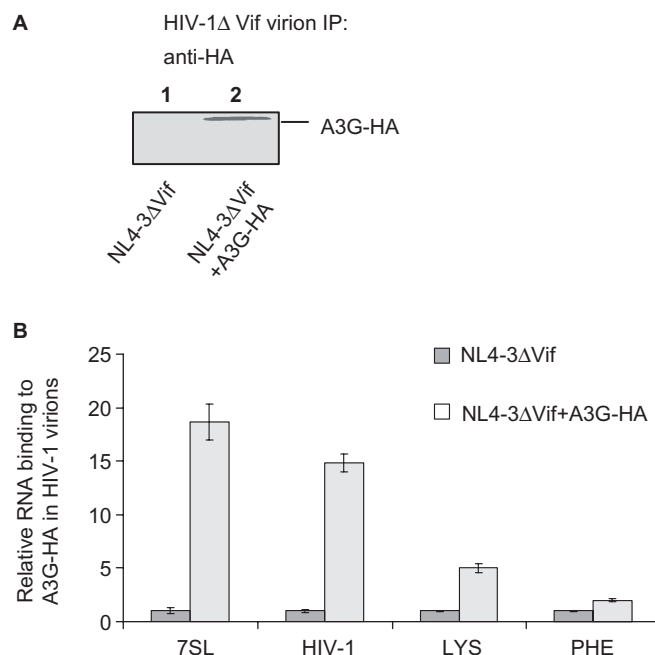

Figure 11. Interaction of 7SL RNA with A3G-HA in HIV-1 virions. (**A**) Immunoprecipitation and immunoblot analysis of A3G-HA from HIV-1ΔVif virions produced from transfected 293T cells in the presence or absence of A3G-HA. A 50 μg NL4-3ΔVif was transfected into 293/A3G-HA or 293 cells in T175 flasks. HIV-1 virions in the culture supernatants were purified by sucrose-density gradient centrifugation. Virion lysates were immunoprecipitated with the anti-HA antibody conjugated to agarose beads and analyzed by immunoblotting using the anti-HA antibody. (**B**) RNAs were extracted from co-precipitated samples and analyzed by qRT-PCR using primers specific for 7SL RNA, HIV-1 genomic RNA, tRNAphe or tRNAlys,3. The RNA detected in the control NL4-3ΔVif sample lacking A3G-HA was set to 100%. Results represent means ± standard deviations of at least three independent experiments.

than that of 7SL RNA. It has been proposed that Alu RNAs were originally derived from 7SL RNAs (20). Although Alu RNAs share the Alu RNA domain with 7SL RNA (20), we found that the packaging of Alu RNAs into HIV-1 virions was much less efficient than that of 7SL RNA.

An interesting observation from the current study is that the virion packaging of both human cytidine deaminase A3G and cellular 7SL RNA mapped to the HIV-1 NC domain. Although it is still controversial whether HIV-1 viral RNA plays an important role in mediating virion packaging of A3G (17,44), many groups have reported that HIV-1 Gag can mediate efficient A3G packaging in the absence of viral genomic RNA (15,17,44–50). Previous studies have observed that the packaging of A3G proteins into Gag particles that contain a leucine zipper domain from the yeast GCN4 in place of the HIV-1 NC domain is much less efficient than that of Gag particles containing an intact NC domain (15,45–50). Gag particles containing the leucine zipper domain also packaged 7SL RNA significantly less efficiently than did Gag particles containing an intact NC domain (Figure 7). Furthermore, we have demonstrated that the N-terminal basic region and the basic
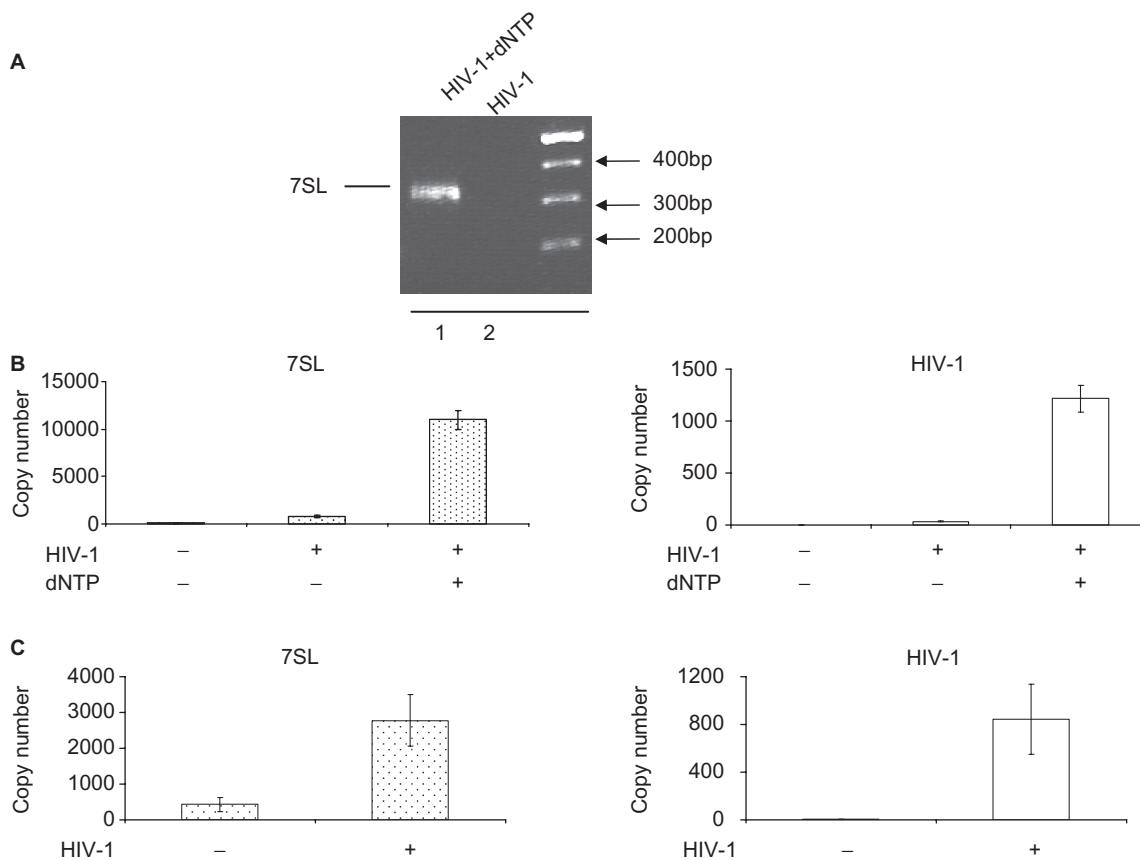
Figure 12. Reverse transcription of 7SL RNA in purified HIV-1 virions. (**A**) Detection of near-full-length 7SL DNA by PCR from HIV-1 virions subjected to endogenous RT reaction. Purified HIV-1 virions (NL4-3) were partially permeabilized with detergent and incubated in the presence (lane 1) or absence (lane 2) of dNTPs. Samples were then analyzed by PCR using 7SL-specific primers. (**B**) Detection of 7SL DNA by qRT-PCR from HIV-1 virions subjected to endogenous RT reaction. Purified HIV-1 virions (NL4-3) were partially permeabilized with detergent and incubated in the presence (lane) or absence (lane) of dNTPs. Samples were then analyzed by qRT-PCR using 7SL- or HIV-1-specific primers. The copy numbers of RNAs detected in HIV-1 samples from 2 ng of p24 equivalent virions are shown. (**C**) Detection of 7SL DNA by qRT-PCR from HIV-1-infected cells. MAGI cells (10^6) were infected with 1 μg of p24 equivalent NL4-3 virions. Cytoplasmic DNA samples from uninfected or HIV-1-infected MAGI cells were extracted and analyzed by qRT-PCR using 7SL- or HIV-1-specific primers. The copy numbers of DNAs detected from samples equivalent to 600 HIV-1 virion-infected MAGI cells are shown. Results represent means ± standard deviations of at least three independent experiments.

linker region of HIV-1 NC domain are important for 7SL RNA packaging (Figure 8). It is interesting that these regions were also identified as being important for efficient A3G packaging (15,25). We have observed that 7SL RNA is one of the most abundant RNAs that interacts with A3G in HIV-1 virions (Figure 11). Collectively, these observations support a role for 7SL RNA in mediating A3G packaging into retroviruses, including HIV-1. Studies mapping the regions in 7SL RNA that are important for A3G and HIV-1 Gag binding/virion packaging are currently underway.

ACKNOWLEDGEMENTS

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