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trans-Acting Proteins Involved in RNA Encapsidation and Viral Assembly in Human Immunodeficiency Virus Type 1

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The human immunodeficiency virus type 1 *gag* gene product Pr55^{gag} self-assembles when expressed on its own in a variety of eukaryotic systems. Assembly in T lymphocytes has not previously been studied, nor is it clear whether Pr55^{gag} particles can package genomic RNA or if the Gag-Pol polyprotein is required. We have used a series of constructs that express Gag or Gag-Pol proteins with or without the viral protease in transient transfections in COS-1 cells and also expressed stably in CD4$^+$ T cells to study this. Deletion of the p6 domain at the C terminus of protease-negative Pr55^{gag} did not abolish particle release, while truncation of the nucleocapsid protein reduced it significantly, particularly in lymphocytes. Gag-Pol polyprotein was released from T cells in the absence of Pr55^{gag} but did not encapsidate RNA. Pr55^{gag} encapsidated human immunodeficiency virus type 1 RNA whether expressed in a protease-positive or protease-negative context. p6 was dispensable for RNA encapsidation. Marked differences in the level of RNA export were noted between the different cell lines.

Specific encapsidation of retroviral RNA involves interactions between the viral structural proteins and the genomic RNA. *cis*-Acting sequences involved in retroviral RNA encapsidation have been located at the 5′ end of the genomic RNA, known as ψ sites (1, 2, 4, 6, 12, 32, 36, 38, 58). In human immunodeficiency virus type 1 (HIV-1), deletions between the major splice donor and the *gag* initiation codon reduce encapsidation of genomic RNA (2, 12, 36). This region has been demonstrated to form a stable secondary structure which presumably acts as a packaging signal (5, 27, 28, 49).

HIV-1 encodes three major genes, *gag*, *pol*, and *env*, which are common to all retroviruses. The *gag* gene is synthesized as a 55,000-molecular-weight (55K) precursor, Pr55^{gag}, and forms the structural basis of the viral core. During or after budding, Pr55^{gag} is cleaved by the viral protease to yield the major *gag* proteins (p17 [matrix; MA], p24 [capsid; CA], p7 [nucleocapsid; NC], and p6). Approximately 5 to 10% of the ribosomes translating the *gag* gene undergo a frameshift near the p7/p6 junction, resulting in the deletion of the p6 domain and production of a much larger Gag-Pol fusion protein precursor (Pr160$^{gag\text{-}pol}$). In this way, the *pol* gene products (protease, reverse transcriptase, integrase, and endonuclease H) are incorporated into the assembling virus particle.

The HIV-1 *gag* gene self-assembles when expressed alone in a variety of eukaryotic systems (20, 31, 53, 59), even when substantial deletions are introduced (57a). In contrast, cells overproducing the Pr160$^{gag\text{-}pol}$ polyprotein are reported not to produce viruslike particles (39, 44, 52). Both Pr55^{gag} and Pr160$^{gag\text{-}pol}$ are modified posttranslationally by the addition of *N*-myristic acid to the amino-terminal glycine residue (9, 24, 44). Myristylation of Pr55^{gag} is required for the assembly and release of immature virus particles (20, 24, 57a); however, myristylation of Pr160$^{gag\text{-}pol}$ is not required for the interaction with Pr55^{gag} necessary for incorporation into viruslike particles (45, 54).

The NC proteins of all known retroviruses, with the exception of spumaretroviruses, contain one or two copies of the sequence Cys-X_2-Cys-X_4-His-X_4-Cys, termed Cys-His motifs (14). Each Cys-His motif is thought to chelate one zinc ion and to function in a way analogous to those of the zinc finger domains found in many DNA-binding proteins (7, 41, 43, 55). Mutations affecting the Cys-His motifs disrupt packaging of genomic RNA into virions (2, 12, 17, 21, 22, 29, 40). Purified Gag as a Gag-GST fusion protein and NC proteins of HIV-1 have been shown to bind specifically to the postulated HIV-1 ψ site in vitro (8, 13, 15, 30, 37, 49).

In a number of retroviruses, the Gag precursor has been found to be sufficient for RNA packaging (42, 51). In HIV-1, MA protein is not required (34a). NC protein alone has been proposed to be responsible for genomic RNA dimer linkage and RNA annealing through the dimerization-encapsidation site (16). Another report studying the yeast (*Saccharomyces cerevisiae*) L-A virus identified the Gag-Pol polyprotein as essential for RNA packaging (19).

To study the *trans*-acting factors involved in HIV-1 genomic RNA encapsidation, we have used a series of constructs that express cleaved or uncleaved Gag with or without uncleaved Gag-Pol protein. The abilities of the constructs to form viruslike particles and to encapsidate viral RNA were evaluated. Truncations of Pr55^{gag} removing either p6 or both p6 and the C-terminal region of p7 were used to further define protein requirements for encapsidation and particle release.

MATERIALS AND METHODS

Plasmid construction. All constructs were derived from pSVC21, an infectious proviral clone of the human T-cell leukemia virus 3b (HTLV-3b) isolate originally isolated from a plasmid (pHXBc2) supplied by R. Gallo and F. Wong-Staal (18). pSVC21 contains a simian virus 40 origin of replication. The constructs are illustrated in Fig. 1. Restriction sites, where given, refer to positions in the HXBc2 genome (Los Alamos database numbering, where position 1 is the first base of the 5′ long terminal repeat [LTR]). HVP was previously described (47). HVP contains a deletion between the *Bal*I (position 2689) and *Eco*RI (position 5743) sites, removing the reverse transcriptase and integrase domains of *pol*; a *Bgl*II fragment within *env* (positions 7041 to 7621) was also removed, rendering the *env* gene nonfunctional (33). A *Not*I site was introduced at a previously created *Xba*I site near the 3′ end of the *env* gene (56). A promoterless *puro* gene was inserted in a position analogous to that of the *nef* gene, between the *Not*I site and an *Xho*I site at position 8897. HVPΔEB and HVPΔEA were constructed by deleting the sequences in HVP between *Eco*RI (position 5743) and either *Bgl*II (position 2096) or *Apa*I (position 2006), respectively. In HVPΔEB, the reverse transcriptase, integrase, and protease domains of the *pol* gene and the p6 domain at the C terminus of *gag* have been deleted. In HVPΔEA, the distal Cys-His motif

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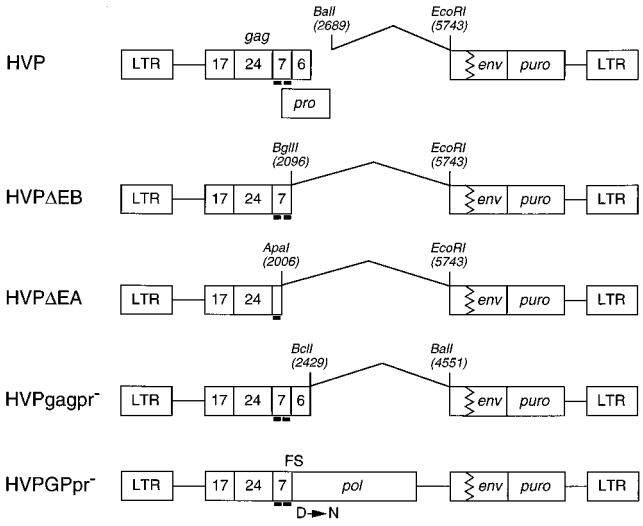

FIG. 1. Gag and Gag-Pol expression constructs. All constructs contain the 5′ and 3′ LTRs. A puromycin resistance gene (*puro*) is inserted in the *nef* position. Deletions were constructed by digestion with the relevant restriction enzymes, filling in the overhanging ends with Klenow polymerase, and religation. The positions of the relevant restriction enzyme sites are indicated in brackets. The Cys-His motifs in the NC domain are underlined. The *gag* and *pol* genes of HVPGPpr$^-$ were placed in the same translational reading frame by insertion of a single adenine residue at position 2091 (FS), as described in reference 44. The aspartic acid (D) at the active site of the protease in HVPGPpr$^-$ was changed to arginine (N) by site-directed mutagenesis, as described in reference 45.

of the NC domain has also been deleted. HVPgagpr$^-$ and HVPGPpr$^-$ were constructed by replacing the *Bss*HII-to-*Sal*I (positions 714 to 5786) fragment of HVP with the *Bss*HII-to-*Sal*I fragments of pGAG and pGPpr$^-$, respectively (a kind gift of C. Morrow [45]). HVPgagpr$^-$ contains a deletion between *Bcl*I (position 2429) and *Bal*I (position 4551), resulting in the loss of the coding region of the *pol* gene, so that no functional protease, reverse transcriptase, or integrase is expressed. HVPGPpr$^-$ contains a frameshift mutation (described in reference 45), so that permanently frameshifted Gag-Pol fusion protein (Pr160$^{gag\text{-}pol}$) is expressed. The protease enzyme was inactivated by mutating the aspartic acid at the active site to asparagine (described in reference 44).

Cell culture and transfections. COS-1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. Jurkat-*tat* cells (48) were maintained in RPMI 1640 supplemented with 10% fetal calf serum, penicillin, and streptomycin. Transient transfection of COS-1 cells was performed by the DEAE-dextran method (50). Cells and supernatant samples were harvested 72 h later. Jurkat-*tat* cells were stably transfected with plasmid DNA by electroporation (46). Puromycin (0.5 μg/ml) was used for selection of stable transfectants.

Protein analysis. Particles released into the tissue culture supernatant were pelleted by polyethylene glycol (PEG) precipitation by the addition of 0.5 volumes of 30% PEG 8000 in 0.4 M NaCl for 16 h at 4°C. The precipitate was collected by centrifugation at 2,000 rpm in an MSE 43124-129 rotor at 4°C and resuspended in 0.5 ml of TNE (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA [pH 7.5]). This material was layered over an equal volume of TNE containing 20% sucrose and centrifuged at 98,000 × *g* for 2 h at 4°C in a Beckman TLA45 rotor.

Cell lysates and particles pelleted from tissue culture supernatants were analyzed by Western blotting (immunoblotting) onto Hybond-C extra hybridization membranes (Amersham). Anti-p24 monoclonal antibody (obtained from the Medical Research Council AIDS Reagent Programme) was incubated in 5% nonfat dried milk–1% fetal calf serum–1% bovine serum albumin in phosphate-buffered saline (PBS), and detection was performed with enhanced chemiluminescence Western blotting detection reagents (Amersham).

p24 antigen capture assay. p24 antigen was quantitated by the following assay. Affinity-purified sheep anti-p24^{gag} (Aalto Bioreagents, Dublin, Ireland) was applied to micro-enzyme-linked immunosorbent assay (ELISA) plates in 150 mM $NaHCO_3$ (pH 9.0) for 16 h at room temperature. After being washed with TBS (144 mM NaCl, 0.05% Tween 20, 25 mM Tris-HCl [pH 7.5]), the wells were blocked with 2% nonfat dried milk in TBS for 30 min at room temperature. Tissue culture supernatants were adjusted to 0.1% empigen and incubated in the micro-ELISA wells for 16 h at room temperature. p24 standards (baculovirus-derived p24 obtained from the Medical Research Council AIDS Reagent Programme) were included. After being washed with TBS, biotinylated mouse monoclonal anti-p24^{gag} (obtained from the Medical Research Council AIDS Reagent Programme) was diluted 1:1,000 in TBS containing 2% nonfat milk, 20% fetal calf serum, and 0.5% Tween 20 and added to each well, and then the wells were incubated at room temperature for 2 h. The wells were washed with TBS and incubated with extravidin-alkaline phosphatase (Sigma) diluted 1:1,000 in PBS containing 0.05% Tween 20 for 1 h at room temperature. After the washing with TBS, 1 mg of the chromogenic substrate *p*-nitrophenyl phosphate (Sigma 104 phosphatase substrate) per ml in reaction buffer (10% ethanolamine, 0.5 mM $MgCl_2$ [pH 9.8]) was added. The A_{405} was read after approximately 30 min.

RNA isolation. Cytoplasmic RNA was obtained by rapid lysis at 4°C in Nonidet P-40 buffer (50 mM Tris-HCl [pH 8.0], 100 mM NaCl, 5 mM $MgCl_2$, 0.5% [vol/vol] Nonidet P-40). Cell debris and nuclei were removed by a 2-min centrifugation step in a microcentrifuge. The supernatant was adjusted to 0.2% sodium dodecyl sulfate (SDS) and 125 μg of proteinase K per ml, incubated at 37°C for 15 min, and extracted twice with phenol-chloroform and once with chloroform. Nucleic acids were collected by ethanol precipitation, and RNA was stored at −70°C. For RNA extraction from virions, particles were purified from tissue culture supernatant through 20% sucrose cushions as described above. Virus particles were lysed in proteinase K buffer (50 mM Tris-HCl [pH 7.5], 100 mM NaCl, 10 mM EDTA, 1% SDS, 100 μg of proteinase K per ml, 100 μg of tRNA per ml) for 30 min at 37°C. After two phenol-chloroform extractions and one chloroform extraction, the RNA was precipitated with ethanol and stored at −70°C.

RNase protection assay. A DNA template for synthesis of radiolabelled RNA probes was constructed as follows. A *Sca*I-to-*Cla*I fragment (positions 313 to 830) of HXBc2 was inserted into *Eco*RV and *Cla*I sites in the polylinker of Bluescript KSII (Stratagene). The resulting construct, KSIIψCS, was used to synthesize antisense riboprobes for use in RNase protection assays. KSIIψCS was linearized with *Xba*I, and ^{32}P-labelled antisense riboprobes were synthesized with T3 RNA polymerase with an in vitro transcription kit (Promega). Riboprobes were purified from 5% polyacrylamide–8 M urea gels prior to use in RNase protection assays.

Reagents for RNase protection assays were obtained from a commercially available kit (Ambion, Austin, Texas). Cytoplasmic RNA or RNA extracted from pelleted particles was incubated with 2×10^5 cpm of ^{32}P-labelled probe in 20 μl of hybridization buffer (80% formamide, 100 mM sodium citrate [pH 6.4], 300 mM sodium citrate [pH 6.4], 1 mM EDTA) for 16 h at 42°C. Unhybridized regions of the probe were then degraded by the addition of 0.5 U of RNase A and 20 U of RNase T_1 in 200 μl of RNase digestion buffer (Ambion). Protected fragments were precipitated in ethanol, resuspended in RNA loading buffer, and separated on 5% polyacrylamide–8 M urea gels. For size determination, ^{32}P-labelled RNA markers synthesized with the RNA Century Marker template set (Ambion) were run in parallel. The gels were subjected to autoradiography, and the radioactivity was counted by real-time analysis with an Instant Imager (Packard).

RESULTS

Effect of *gag* mutations on particle formation and release. To study the protein requirements for HIV-1 RNA encapsidation, Gag and Gag-Pol expressors, with or without viral protease, were constructed as described in Materials and Methods and illustrated in Fig. 1. The ability of these constructs to form viruslike particles was studied by transient transfection into COS-1 cells. The results are shown in Fig. 2. Transfection of COS-1 cells with HVP resulted in the expression of Pr55^{gag} precursor and cleaved p24 in the cells and release of these products into the supernatant in the form of pelletable viruslike particles. Cells transfected with HVPΔEB, in which the *pol* gene and p6 have been deleted, resulted in expression of uncleaved Gag precursor, slightly smaller than Pr55^{gag}. This Gag protein was released into the supernatant in viruslike particles. Cells transfected with HVPΔEA, in which the *pol* gene, p6, and the C-terminal portion of NC protein, including the distal Cys-His motif, have been deleted, produced uncleaved Gag product slightly smaller than the product produced by HVPΔEB. This product was released into the supernatant in pelletable form. Cells transfected with HVPgagpr$^-$ expressed uncleaved Pr55^{gag}, which was released in viruslike particles. Cells transfected with HVPGPpr$^-$ expressed Pr160$^{gag\text{-}pol}$; however, this was not released into the supernatant. This is in agreement with previous findings that Pr160$^{gag\text{-}pol}$ expressed alone in COS-1 cells does not form viruslike particles (39, 44, 52). Cotransfection of HVPGPpr$^-$ with HVP, HVPΔEB, HVPΔEA, or HVPgagpr$^-$ rescued the

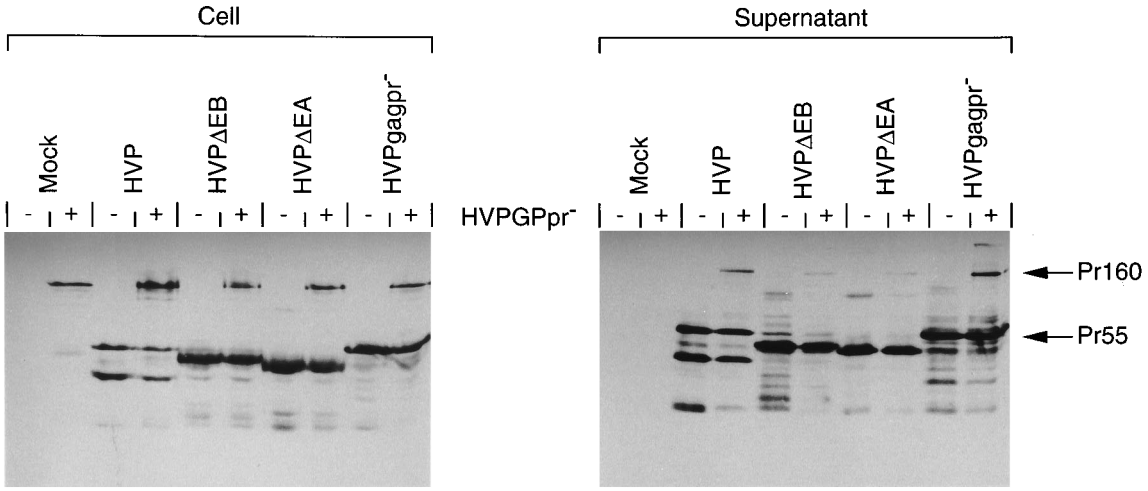

FIG. 2. Analysis of protein expression in COS-1 cells. COS-1 cells were transfected with the Gag expression constructs, with (+) or without (−) the Gag-Pol expression construct HVPGPpr$^-$. Cell lysates and particles pelleted from the tissue culture supernatant were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting with an anti-p24 monoclonal antibody. The positions of Pr160$^{gag\text{-}pol}$ and Pr55^{gag} are indicated.

ability of the Gag-Pol protein to be incorporated into viruslike particles, indicating that the interaction between Gag and Gag-Pol precursors must involve regions upstream of the *Apa*I site in the NC protein.

The ability of the Gag and Gag-Pol expression constructs to produce particles was also studied with CD4$^+$ T cells. Jurkat-*tat* cells were transfected with the constructs by electroporation, and stable transfectants were selected in the presence of puromycin. Cell lysates and particles pelleted from stably transfected cells were analyzed by Western blotting with an anti-p24 monoclonal antibody. The results are shown in Fig. 3. Cells stably transfected with HVP produced Pr55^{gag} and cleaved p24; these products were also detected in the supernatant. Cells stably transfected with HVPΔEB produced uncleaved Gag product, which was also released in viruslike particles. Cells transfected with HVPΔEA produced a Gag product slightly smaller than that produced by HVPΔEB. Although this Gag product was detected in the supernatant, deletion of the C-terminal portion of the NC protein appears to reduce the release of viruslike particles from Jurkat-*tat* cells. Cells stably transfected with HVPgagpr$^-$ produced uncleaved Pr55^{gag}, which was also released into the supernatant in the form of viruslike particles. Cells transfected with HVPGPpr$^-$ produced Pr160$^{gag\text{-}pol}$. This was detectable in the supernatant after PEG precipitation and sucrose gradient centrifugation, presumably in particulate form.

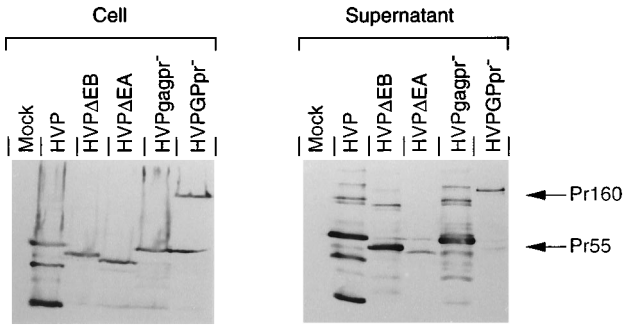

FIG. 3. Analysis of protein expression in T cells. Jurkat-*tat* cells stably expressing the Gag or Gag-Pol constructs were selected by the addition of puromycin to the culture medium. Cell lysates and particles pelleted from the tissue culture supernatant were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting with an anti-p24 monoclonal antibody. The positions of Pr160$^{gag\text{-}pol}$ and Pr55^{gag} are indicated.

Viral RNA content of viruslike particles. To determine the amounts of viral RNA packaged into the viruslike particles, a quantitative RNase protection assay was used. This method allows the measurement of particle-associated RNA in the presence of viral DNA. This approach was necessary, because particles released from transiently transfected cells may be coated with input plasmid DNA. An antisense riboprobe was designed that spanned the U3-R region of the 5′ LTR, allowing differentiation of viral RNA and plasmid DNA. The probe also spanned the major splice donor, allowing differentiation of full-length genomic RNA and spliced transcripts. The predicted sizes of the protected fragments are illustrated in Fig. 4.

COS-1 cells were transiently transfected with HVP, HVPΔEB, HVPΔEA, HVPgagpr$^-$, and HVPGPpr$^-$. Cytoplasmic RNA and particle-associated RNA were extracted 72 h later, as described in Materials and Methods, and then were annealed to probe KSIIψCS. Figure 5 shows a representative RNase protection assay of COS-1 cells. The input probe is the expected size, and no signal was detected with the control tRNA. A fragment corresponding to full-length viral RNA (labelled 5′ LTR; 375 nucleotides [nt]) was detected in all cytoplasmic RNA samples, except for mock-transfected cells. Spliced RNA (289 nt) and RNA corresponding to the 3′ LTR (238 nt) were also detected. Full-length viral RNA was detected in particles released from cells transfected with HVP, HVPΔEB, HVPΔEA, and HVPgagpr$^-$. No particles were released from cells transfected with HVPGPpr$^-$, so as expected, no viral RNA was detected for this sample. The level of RNA detected in particles released from cells transfected with HVPΔEA was reduced compared with that of the other constructs; however, this is probably due to a reduction in the level of particles released from the cells (Table 1). Where possible, particle-associated RNA levels were compared by using p24 estimates from ELISA, except where these were too low, in which case only qualitative comparisons are possible. The levels of full-length RNA were quantitated with the Packard Instant Imager. The ratios of particle-associated RNA to cytoplasmic RNA are shown in Table 1. The uncleaved Gag

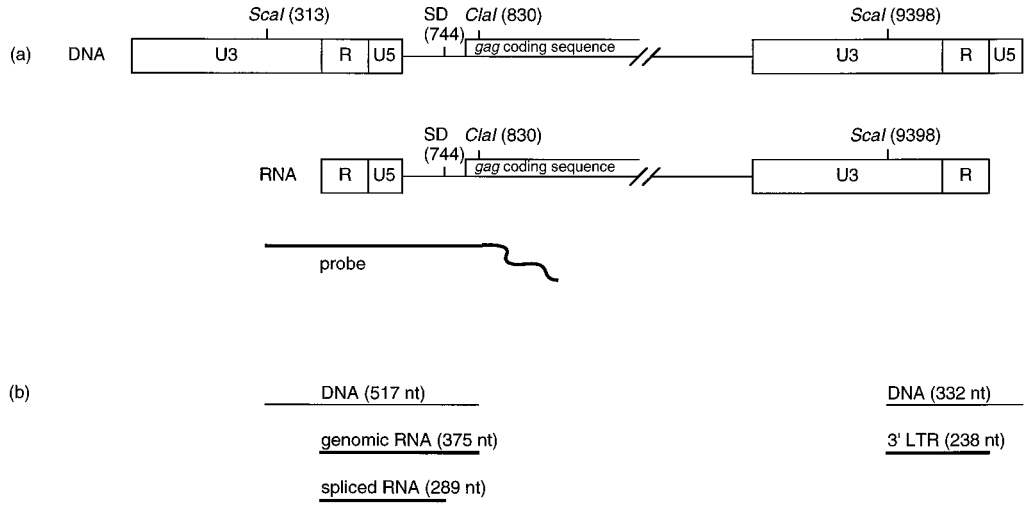

FIG. 4. Predicted sizes of the protected fragments for the RNase protection assay. (a) KSIIψCS riboprobe is complementary to HIV-1 nt 313 to 830. (b) Predicted sizes of KSIIψCS fragments protected by DNA and RNA. SD, Shine-Dalgarno sequence.

precursor, $Pr55^{gag}$, expressed by HVPgagpr$^-$ is capable of encapsidating viral RNA. The ratio of particle-associated RNA to cytoplasmic RNA is approximately three times higher for the uncleaved Gag than for the cleaved Gag expressed by HVP. This is despite similar levels of particles being released from both constructs (Table 1). The uncleaved $Pr55^{gag}$ is therefore sufficient to encapsidate genomic viral RNA. The $Pr160^{gag\text{-}pol}$ precursor is not required for the incorporation of viral RNA into particles. Deletion of the p6 domain in HVPΔEB did not abolish RNA encapsidation. The ratio of particle-associated RNA to cytoplasmic RNA was comparable to that for HVP. Although the level of RNA detected in particles released from cells transfected with HVPΔEA was relatively low compared with those of the other constructs, the ratio of particle-associated RNA to cytoplasmic RNA was similar to that for cleavable Gag (3.0 versus 2.7). This is due to a lower abundance of RNA present in the cytoplasm of these cells.

The ability of the particles released from stably transfected Jurkat-*tat* cells to package viral RNA was also studied. Cytoplasmic RNA and particle-associated RNA were prepared 4 days after flasks of stably transfected cells were set up. RNase protection assays of the RNA were performed, and the results are shown in Fig. 6. Full-length viral RNA (labelled 5′ LTR; 375 nt) was detected in the cytoplasm of cells stably transfected with all of the constructs. Although equivalent amounts of cytoplasmic RNA were used for each construct, the levels of full-length RNA vary among the different constructs. Spliced RNA (289 nt) and RNA corresponding to the 3′ LTR (238 nt)

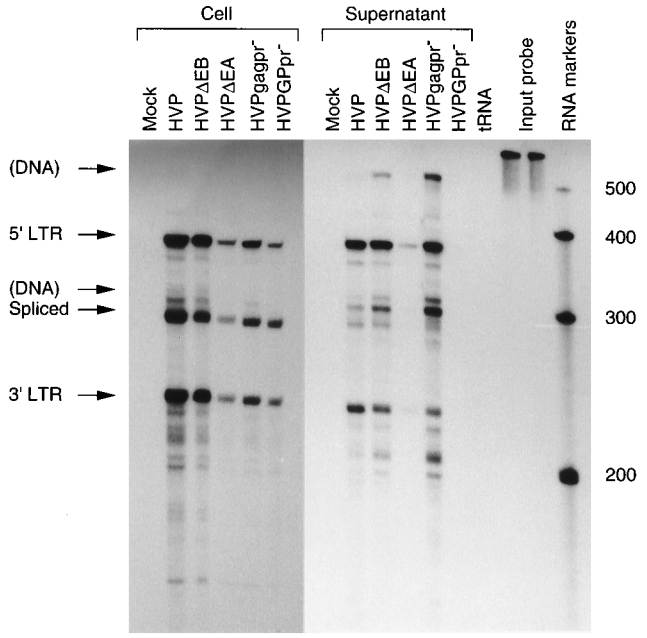

FIG. 5. RNase protection assay of transiently transfected COS-1 cells. Shown is an autoradiogram of gel-separated fragments of ^{32}P-labelled riboprobe KSIIψCS resulting from RNase protection with cytoplasmic RNA and particle-associated RNA prepared from COS-1 cells transfected with Gag and Gag-Pol expression constructs. Protection with tRNA alone and with the riboprobe without RNase treatment (input probe) is shown. The sizes of ^{32}P-labelled RNA size markers (in base pairs) are indicated to the right of the panel. The positions of the protected fragments are indicated.

TABLE 1. Cytoplasmic and particle-associated RNA levels

RNA	Ratio packaged[a]	Amt of p24 (ng/ml)
COS-1		
Mock	ND[b]	<0.3
HVP	2.7	316
HVPΔEB	3.3	158
HVPΔEA	3.0	2.5
HVPgagpr$^-$	6.7	316
HVPGPpr$^-$	ND	<0.3
Jurkat-*tat*		
Mock	ND	<0.3
HVP	0.1	63
HVPΔEB	0.3	2
HVPΔEA	ND	<0.3
HVPgagpr$^-$	0.8	25
HVPGPpr$^-$	ND	<0.3

[a] Ratio of particle-associated RNA to cytoplasmic RNA.
[b] ND, value not determined because of the low level of p24 released into the supernatant.

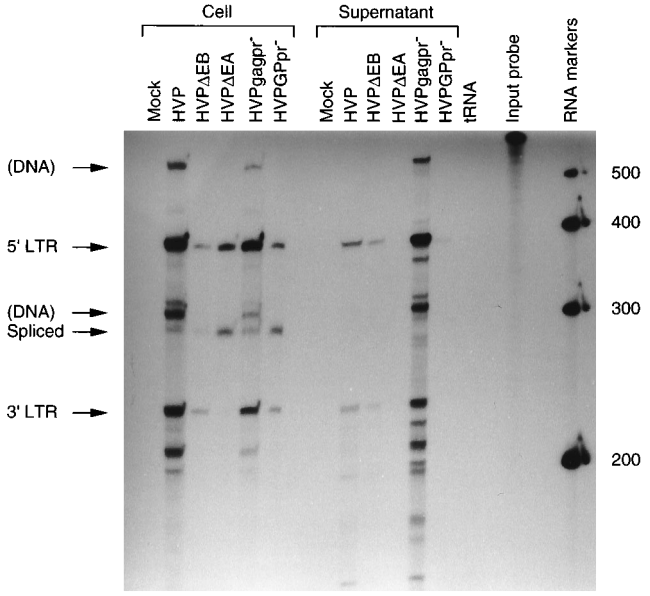

FIG. 6. RNase protection assay of stably transfected Jurkat-*tat* cells. Shown is an autoradiogram of gel-separated fragments of ^{32}P-labelled riboprobe KSIIψCS resulting from RNase protection with cytoplasmic RNA and particle-associated RNA prepared from Jurkat-*tat* cells stably transfected with Gag and Gag-Pol expression constructs. Protection with tRNA alone and with the riboprobe without RNase treatment (input probe) is shown. The sizes of ^{32}P-labelled RNA size markers (in base pairs) are indicated to the right of the panel. The positions of the protected fragments are indicated.

were also detected in the cytoplasm of the stably transfected cells. Full-length viral RNA was detected in particles released from HVP, HVPΔEB, and HVPgagpr$^-$. The level of RNA packaged by the uncleaved Pr55^{gag} expressed by HVPgagpr$^-$ was much greater than that released from cleavable Gag expressed from HVP. The ratio of particle-associated RNA to cytoplasmic RNA was 0.8 compared with 0.1. No viral RNA was detected for HVPΔEA; however, no particles were released from these cells, so it is not possible to comment on the ability of these constructs to encapsidate viral RNA. Cells expressing and exporting HVPGPpr$^-$ did not export genomic RNA, confirming the dispensability of Gag-Pol for RNA encapsidation.

In a number of samples from COS and Jurkat-*tat* cells, a low level of a protected band corresponding to that of the DNA provirus is seen. This is likely due to either partial reverse transcripts arising in viral particles or, possibly, transcripts arising from tandem integrated viruses.

Cell type variations in particle formation and RNA packaging. For these studies, both transient transfection of COS-1 cells and stable transfection of Jurkat-*tat* cells were used. Several differences between the cell lines in their ability to release particles and to encapsidate RNA were seen. Transiently transfected COS-1 cells released higher levels of particles than stably transfected Jurkat-*tat* cells (Table 1). The construct HVPΔEA, in which the *pol* gene, p6, and the C-terminal portion of the NC protein have been deleted, showed reduced release from both COS-1 cells and Jurkat-*tat* cells; however, this effect was greater in Jurkat-*tat* cells than in COS-1 cells (Fig. 3 and Table 1).

HVP, which expresses cleavable Gag protein, and HVPgagpr$^-$, which expresses uncleaved Gag, were capable of encapsidating viral RNA in both cell types. However, there were differences in the selection of full-length genomic RNA versus spliced RNA for packaging. In COS-1 cells, while the particles were enriched for full-length viral RNA, spliced transcripts were still detected in virus particles (Fig. 5), but in Jurkat-*tat* cells, predominantly full-length RNA was detected in virus particles (Fig. 6), with little if any spliced message visible even after prolonged autoradiography. We have noted the high level of RNA encapsidated in COS cells compared with that in Jurkat T cells before, and this has now been quantitated.

DISCUSSION

In this study, we have sought to define the *trans*-acting factors involved in RNA encapsidation and to relate them to those required for particle formation. We have noted several phenomena which appear to distinguish both processes when occurring in T lymphocytes compared with previously tested cell lines. First, we were surprised to detect uncleaved Gag-Pol polyprotein being exported intact from T cells. This was not due to passive release from dying cells, because by trypan blue examination, the cells were viable. The Gag-Pol polyprotein was pelletable from PEG precipitates through sucrose gradients and was presumably particulate. Studies are under way to determine the physical characteristics of this exported protein. Second, deletion of the distal Cys-His motif in the construct HVPΔEA, while reducing particle release in both cell types, appeared to have a more profound effect in T lymphocytes.

Cotransfection of Gag and Gag-Pol expressors in COS-1 cells demonstrated rescue of Gag-Pol export; the protease-defective Gag-Pol was inefficiently cleaved in *trans* by the protease, which was clearly active in *cis* in the HVP vector. This suggests that after export of particles containing Gag plus rescued Gag-Pol, the latter protein either does not stay associated with the Gag particle or Gag-Pol is insensitive to cleavage in *trans*. The stability of Pr55^{gag} is consistent with the findings of others on the detergent stability of particles composed of uncleaved Gag precursor (26).

Our studies demonstrate that in Jurkat T cells and COS cells, the p6 subdomain of Pr55^{gag} is not required for particle export in the context of an uncleaved Gag. This is in contrast with mutagenic studies suggesting the essential nature of p6 for particle release (23), although these studies were performed in a protease-positive context. This suggests that the effect of p6 mutations in cleaved Gag is to unmask an inhibitory effect of another region of Gag normally inaccessible in uncleaved Pr55^{gag}. Cleaved *gag* products are known to have specific functions, such as nuclear targeting (10, 11) and enhancement of reverse transcription (3, 34).

Encapsidation of viral RNA can be achieved in both cell types by Pr55^{gag} without any apparent contribution from the Gag-Pol polyprotein. Our results clearly demonstrate the lack of requirement for isolated NC protein for RNA recognition, although from other studies the NC domain of the Pr55^{gag} is undoubtedly important (2, 12, 17, 21, 22, 29, 40). Truncation of the NC domain still permitted RNA encapsidation in COS cells, suggesting that the C-terminal zinc finger is dispensable in this cell type, as was suggested in the domain exchange studies of Gorelick et al. (20a). Most striking is the ability of COS cells to package nucleic acid somewhat promiscuously. Spliced messages are clearly detectable in particles derived from these cells (Fig. 5), and this probably reflects the overall volume of viral RNA exported from these cells (Table 1). This lack of specificity goes some way toward explaining the differences in results obtained by different groups in defining sequences necessary for genomic RNA encapsidation in HIV-1. These findings help explain discrepancies in the levels of spliced and unspliced RNA found in viral particles noted pre-

viously (37), confirming the findings of Luban and Goff (37) that spliced message is detectable in virions budding from COS cells.

The p6 protein is also dispensable for RNA recognition, and these studies confirm those of others that p6 is not involved significantly in intermolecular recognition of Pr55^{gag}.

Finally, where encapsidation in *cis* has been observed, this finding provides unequivocal evidence that the *pol* gene region of HIV-1 does not contain an important *cis*-acting packaging signal.

We have demonstrated that uncleaved Gag protein packages genomic RNA efficiently and that, as is the case in other retroviruses but not in the yeast L-A virus, the Gag-Pol polyprotein is dispensable for genomic RNA encapsidation. While involvement of the NC domain is still unquestioned, cleaved NC protein is not essential for encapsidation, and, if anything, in viral particles consisting of cleavable Gag, less RNA is detected, suggesting that particles composed of uncleaved Gag may protect the genomic RNA more efficiently. Protease-deficient Gag particles have previously been shown to be resistant to detergent lysis (26).

We have also demonstrated differences in encapsidation data between different cell lines, as found with other viral processes (25, 35, 57). While other groups have examined RNA encapsidation with RNase protection, this is the first report giving comparative data from different cell types and suggesting that studies of HIV-1 may yield misleading results if not performed with a cell type for which the virus is itself tropic. The situation in macrophage cell lines may be different again, and studies are under way to examine this.

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