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Characterization of Human Immunodeficiency Virus Type 1 Vif Particle Incorporation

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The human immunodeficiency virus type 1 (HIV-1) Vif protein is necessary at the time of viral particle formation yet functionally manifests its effect after virions enter target cells. This suggests that Vif either acts on another viral protein or is itself incorporated into particles. In this study, we have examined the latter possibility. We confirm our previous observation that Vif is incorporated into human immunodeficiency virus type 1 virions at a ratio of approximately 1 molecule of Vif for every 75 to 220 molecules of p24, or 7 to 20 molecules per virion. Furthermore, we demonstrate that the relative concentration of Vif is much lower in particles than in infected cells, whereas the opposite is observed for the main virus components. The viral envelope, Nef, Vpr, Vpu, protease, reverse transcriptase, integrase, nucleocapsid, and p6^{gag} proteins as well as the viral genomic RNA are dispensable for Vif packaging. Furthermore, mutating several highly conserved residues (H-108, C-114, C-133, L-145, and Q-146) or deleting the C-terminal 18 amino acids of Vif, either of which severely impairs Vif function, does not abolish its incorporation into virions. Finally, Vif can be packaged into murine leukemia virus particles. On the basis of these data, we conclude that the specificity of Vif incorporation into virions remains an open question.

One of several so-called accessory proteins encoded by human immunodeficiency virus type 1 (HIV-1), Vif is common to all lentiviruses except equine infectious anemia virus (5, 13, 16, 26, 29, 32, 35, 37). Vif is highly conserved in viral isolates obtained from HIV-1-infected patients (38) and is essential for HIV-1, HIV-2, and simian immunodeficiency virus replication in peripheral blood mononuclear cells, the natural targets of these viruses (2, 7, 10, 23, 31, 36). HIV-1 Vif expression is Rev dependent and as such occurs late in the viral life cycle (12, 30). The 23-kDa Vif protein resides in the cell cytoplasm, with a fraction associating with the inner leaflet of the plasma membrane (14), and has been suggested to colocalize with the intermediate filament vimentin (19).

Early studies demonstrated that Vif significantly enhances the infectivity of HIV-1 particles (9, 34). Subsequently, it was clarified that this effect is cell type dependent (7, 10, 22, 28, 31, 36) and, furthermore, is determined exclusively by the nature of the cells producing the virions, not by the targets of the infection (10, 36). Cells have thus been classified as restrictive (peripheral blood lymphocytes, macrophages, H9), permissive (Jurkat, SupT1, C8166, 293, COS, HeLa), or semipermissive (CEM) for the growth of *vif*-defective (ΔVif) HIV-1. It has been speculated that permissive cell lines possess a factor which can substitute for Vif (10), although one cannot exclude the possibility that they lack an inhibitory element normally counteracted by the viral protein. ΔVif virions produced by semipermissive and restrictive cells exhibit an attenuated infectivity. This infectivity defect can be rescued by supplying Vif in *trans* in the producer cells, but not by doing the same in the target cells, suggesting that Vif function is required during particle formation (36).

The morphology of *vif*-defective HIV-1 particles produced by semipermissive and restrictive cells is abnormal and characterized by a nonhomogeneous packing of the core (18). These virions can enter target cells normally but subsequently fail to complete reverse transcription of the viral genome (33, 36). Because the activity of the reverse transcriptase (RT) enzyme in ΔVif virions is not impaired, it is tempting to postulate that the Vif defect results in the alteration of the uncoating or the stability of the virus nucleoprotein complex.

As Vif is required in the virus producer cell yet functionally manifests its effect in the target cell only after viral entry, two models have been proposed for its mechanism of action. One is that Vif acts indirectly by modifying some component of the RT complex; the other is that it is present in virions. Several independent groups have detected Vif in HIV-1 particles (4, 15, 19, 21), confirming our earlier report (39). In particular, Liu et al. (21) performed an extensive investigation of the association of Vif with virions and found it to be present not only in HIV-1 but also in simian immunodeficiency virus particles and to be associated with viral cores. Furthermore, by semiquantitative measurements, these authors detected significantly more Vif in HIV-1 virions (1 molecule of Vif per 20 to 30 molecules of p24) than we had previously reported (1 molecule of Vif per 100 to 300 molecules of p24) (39). On the basis of these findings, questions regarding the specificity and functional relevance of Vif virion incorporation have surfaced. The present work addresses these questions.

MATERIALS AND METHODS

DNA constructions. The HIV-1 HXB2 proviral constructs R7 and ΔVif have been described previously (36). The ΔVprΔE, ΔVprΔEΔGag, ΔVprΔEΔPol, and ΔVprΔEΔPolΔNCΔp6 constructs were obtained from Ned Landau and correspond to the previously described pNL4-3-R$^-$E$^-$, pNL4-3-R$^-$E$^-$-Gag$^-$, pNL4-3-R$^-$E$^-$-Pol$^-$, and pNL4-3-R$^-$E$^-$-NC$^-$ plasmids, respectively (27). Plasmid SNH-Vif places the pNLA3 *vif* gene (34) under the control of the HIV-1 5′ long terminal repeat and, as such, allows expression of wild-type HXB2 Vif. The SNH-Vif H^{108}D, SNH-Vif C^{114}S, SNH-Vif C^{133}S, SNH-Vif LQ^{146}RE, and SNH-Vif Δ174–192 plasmids were generated by PCR-mediated mutagenesis of *vif*.

Cell lines, transfections, and infections. All T-cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum. CEM-SS human T-lymphoid cells were infected with COS-derived R7 and ΔVif viral stocks as previously described (36). The MOLT IIIB cell line, a chronically infected hu-

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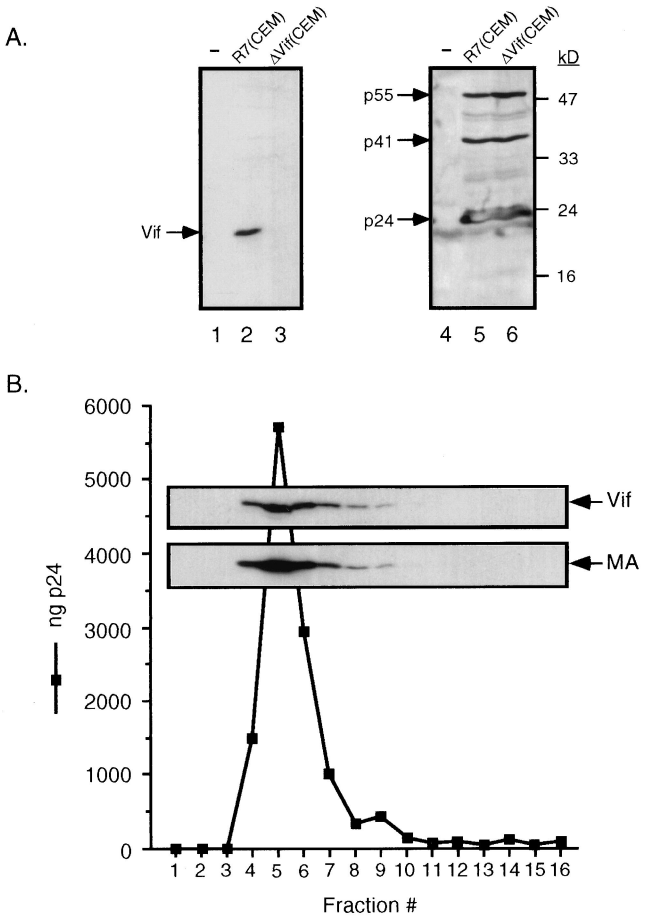

FIG. 1. Vif is associated with HIV-1 virions. (A) Cell-free supernatants from R7- and ΔVif-infected CEM cultures were ultracentrifuged, normalized for p24 content, and analyzed by Western blotting with an anti-Vif antibody (left panel). Lane 1, supernatant from uninfected CEM cells; lane 2, R7 virions; lane 3, ΔVif virions. The same blot was analyzed with an anti-p24/p55 antibody to verify that comparable amounts of virions had been loaded (right panel). Locations of molecular mass markers are shown on the right. (B) Molt IIIB-produced virions were ultracentrifuged and purified by gel filtration on a Sephacryl S-1000 column. Fractions (1 to 16) collected were analyzed for p24 content by ELISA and then for Vif and MA content by Western blotting with an anti-Vif antibody followed by an anti-MA antibody.

man T-cell line constitutively expressing the human T-cell leukemia virus IIIB strain of HIV-1 (8), was provided by Chris Farnet, Salk Institute. H9(ΔE.His) and H9(ΔVifΔE.His) cells are H9 human T-lymphoid cells constitutively expressing *env*-defective versions of R7 and ΔVif containing the histidinol D gene (17) in place of *nef*. Human kidney fibroblast 293 cells were grown in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum and transfected by the calcium phosphate method as described previously (3). Two days following transfection, supernatants were harvested for virus preparation. CRIP cells transduced with LXSN and LVifSN retroviral vectors as described previously (36) were maintained in Dulbecco's modified Eagle medium supplemented with 10% calf serum.

Preparation of virions. Virions were prepared from the supernatants of the various cell cultures by filtration through 0.45-μm-pore-size nitrocellulose membranes followed by ultracentrifugation through 20% (wt/vol) sucrose cushions at 23,000 rpm in an SW28 rotor (Beckman) for 2.5 h at 4°C. Each pellet was resuspended in 1 ml of phosphate-buffered saline (PBS), and its virion content was measured by $p24^{gag}$ enzyme-linked immunoabsorbent assay (ELISA) (DuPont). Concentrated viral stocks were stored at −80°C. When indicated, resuspended samples were further concentrated by centrifugation at 14,000 × *g* in a tabletop microcentrifuge for 1.5 h at 4°C, resuspended in 100 μl of TBS (20 mM Tris [pH 7.6], 150 mM NaCl), and then fractionated by gel exclusion chromatography on 2-ml columns of Sephacryl S-1000 (Pharmacia) as described previously (24, 25). Elution was done with 4 ml of TBS, and 4-drop fractions (average, 225 μl) were collected. A 5-μl aliquot of each fraction was analyzed for p24 content by ELISA or for RT content by an exogenous RT assay as described previously (1).

Western blot (immunoblot) analysis. For Western blot analysis, virions in PBS or TBS were pelleted by ultracentrifugation at 14,000 × *g* in a tabletop microcentrifuge for 1.5 h at 4°C and resuspended in protein lysis buffer (0.5% Triton X-100, 0.3 M NaCl, 12 mM Tris [pH 8.0], 1 mM $MgCl_2$, 0.5 mM dithiothreitol). Infected and transfected cells were washed twice with PBS and then lysed in the protein lysis buffer. Proteins were separated on sodium dodecyl sulfate (SDS)–15% polyacrylamide gels and transferred onto polyvinylidene fluoride membranes (Micron Separations Inc.) in a buffer containing 25 mM Tris (pH 8.0), 192 mM glycine, and 20% methanol. Membranes were incubated with a 1:1,000 dilution of rabbit anti-Vif antiserum (obtained from Dana Gabuzda through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases) (14), a 1:500 dilution of monoclonal anti-p17 matrix (MA) (Beckman), a 1:200 dilution of monoclonal anti-p24 capsid (CA) (Genzyme), or a 1:200 dilution of serum from an AIDS patient. The membranes were washed in a buffer containing 10 mM Tris (pH 8.0), 150 mM NaCl, and 0.05% Tween 20. Detection was performed with horseradish peroxidase-conjugated anti-rabbit or anti-mouse immunoglobulin by enhanced chemiluminescence (ECL Western Blotting Kit, Amersham) according to the manufacturer's instructions. For detection of murine leukemia virus (MLV) p15 MA, membranes were incubated with a 1:500 dilution of goat anti-Rauscher leukemia virus p15 antiserum (obtained through the National Cancer Institute from Quality Biotech Inc.), washed as described above, incubated with swine anti-goat alkaline phosphatase, and subjected to an alkaline phosphatase reaction.

Quantitation of virion-associated proteins. Recombinant Vif lacking the N-terminal 28 amino acids (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases) and recombinant histidine-tagged p17 MA (11) were serially diluted and used as standards. The protein concentration and purity of each of the two standards were determined by Coomassie blue staining of the proteins following SDS-polyacrylamide gel electrophoresis. Molt IIIB virions were concentrated by ultracentrifugation and quantitated by ELISA. Panels of serially diluted virions

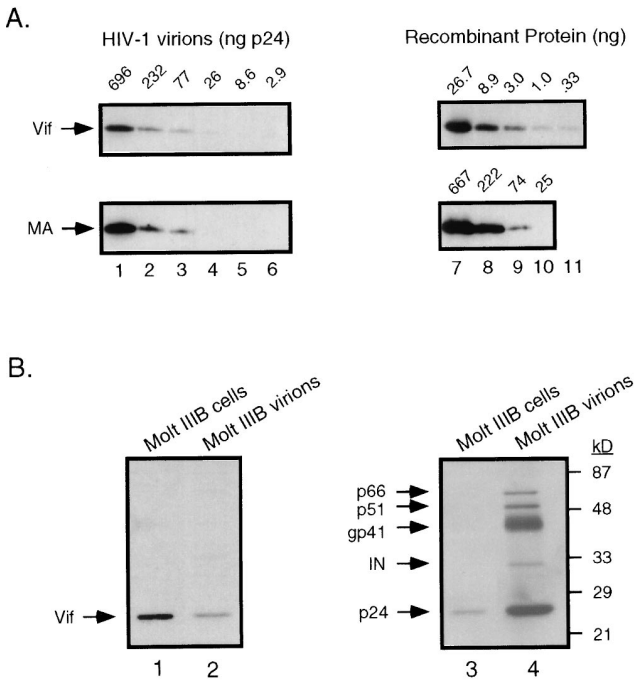

FIG. 2. Quantitation of Vif in Molt IIIB virions. (A) Top panels: the Vif content of serially diluted virions (lanes 1 to 6, 696 to 2.9 ng of p24) was assessed by immunoblot analysis with an anti-Vif antibody. Signal intensities were compared with those generated by recombinant Vif (lanes 7 to 11; 26.7 to 0.33 ng). Bottom panels: as for Vif, the MA content of virions (lanes 1 to 6; concentrations of p24 as for upper panel) was estimated from a dilution panel of recombinant p17 (lanes 7 to 10 ; 667 to 25 ng) with an anti-MA antibody. (B) Extracts of Molt IIIB-infected cells and virions were normalized for total protein content (bicinchoninic acid assay, Pierce). Samples each containing 13 μg of protein were compared by Western blotting for content of viral proteins. Left: Vif protein profile in virions versus that in cells as detected with an anti-Vif antibody; right: the same blot probed with AIDS patient serum detecting p66 and p51 RT, gp41 Env, p33 IN, and p24 CA; molecular mass marker locations are shown on the right.

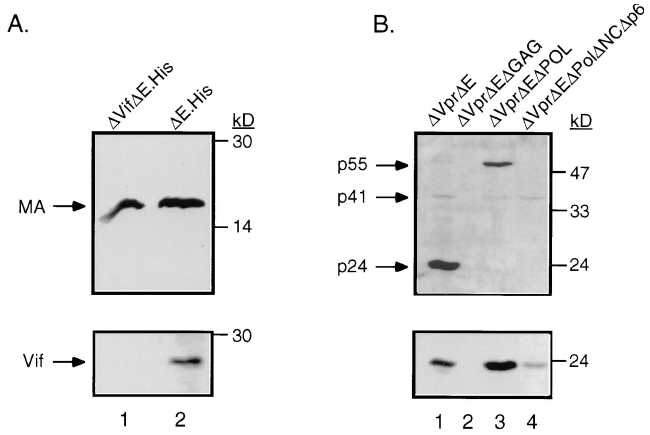

FIG. 3. Virion incorporation requires only MA and CA. All analyses were done by Western blotting with Vif-, Gag-, and/or MA-specific antibodies. (A) Viruses purified from H9 cells constitutively expressing *vif*-defective (ΔVifΔE.His) and *vif*-competent (ΔE.His) versions of an HIV-1-derived provirus from which *env* and *nef* had been detected. (B) Viruses purified from the supernatants of 293 cells transfected with HIV-1-derived constructs in which various genes had been inactivated: *vpr* + *env* (ΔVprΔE), *gag* + *pol* + *vpr* + *env* (ΔVprΔEΔGag), *pol* + *vpr* + *env* (ΔVprΔEΔPol), and *NC* + p6 + *pol* + *vpr* + *env* (ΔVprΔEΔPolΔNCΔp6). Locations of molecular mass markers are shown on the right for each panel.

were run alongside the recombinant Vif and MA standards and subjected to immunoblot analysis. Signal intensities generated from virions and standards were compared.

RESULTS

Detection of low levels of Vif protein in HIV-1 particles. *vif*-competent (R7) and *vif*-defective (ΔVif) particles produced by CEM cells and normalized by ELISA for p24 CA content were analyzed by Western blotting. A protein of approximately 23 kDa was detected in R7 virions but not in ΔVif virions (Fig. 1A, left panel). No differences in the amounts or processing of the p24, p41, and p55 Gag products were observed (Fig. 1A, right panel). To verify that the Vif protein thereby revealed was associated with virions and not simply released into the medium by lysed cells, particles produced by chronically infected Molt IIIB cells were purified by gel exclusion chromatography on a Sephacryl S-1000 column. Fractions harvested (approximately 250 μl) were analyzed for CA content, as it has previously been shown that the p24 peak eluting from such columns corresponds to the infectivity peak (24, 25). The bulk of the p24 antigen was recovered from the elution volume between 1 and 1.75 ml (fractions 4 through 7). Western blot analysis of the fractions revealed that the peaks of Vif and p17 MA paralleled that of p24 (Fig. 1B). Therefore, Vif present in the purified supernatant of HIV-1-infected cells is associated with viral particles.

To measure the relative concentration of Vif in virions, immunoblotting signals generated by given amounts of virions were compared with those obtained from known quantities of recombinant Vif protein (Fig. 2A). A virus sample containing 232 ng of p24 (lane 2) yielded a Vif signal equivalent to that of 1 to 3 ng of recombinant Vif (lanes 9 and 10). In view of the similar molecular weights of Vif and CA, this indicates that there is between 75 and 220 times less Vif than CA in virions. Assuming an average of 1,500 copies of CA per virion, there are correspondingly 7 to 20 copies of Vif per particle. To rule out the possibility that the absence of the N-terminal 28 amino acids in the Vif standard was causing it to be recognized less efficiently than the full-length Vif in virions, we also used a monoclonal anti-Vif antibody directed to the C terminus of Vif (residues 174 to 192). The monoclonal antibody gave the same immunodetection profile as the polyclonal antiserum (data not shown). To verify the accuracy of the method of measurement, the same type of quantitation was performed on MA (Fig. 2A, lower panels). The p17 signal present in a virion sample containing 696 ng of p24 (lane 1) should represent approximately 493 ng of MA, since we know that MA and CA are equimolar in virions. Indeed, the p17 signal intensity recovered from this sample was intermediate to those generated by 222 and 667 ng of purified recombinant MA. Therefore, the technique used here to quantitate Vif appears to be valid.

Only a small number of Vif molecules were detected in virions. The relative concentrations of this protein in cells and in particles were thus compared. Molt IIIB cells and Sephacryl S-1000-purified Molt IIIB virions were lysed and analyzed for total protein content. Both cell and viral lysates (13 μg each) were then examined by immunoblot analysis (Fig. 2B). There was considerably more p24 detected in virions than in cells as a function of total protein. Similarly, the p66 and p51 RT, gp41 envelope (Env), and p33 integrase (IN) proteins were easily detected in particles but not in cells. In contrast with these known virion-associated proteins, Vif was much more abundant in cells than in particles.

Determinants of Vif packaging. Next, the requirement for other viral components for Vif packaging was investigated. The R7 proviral clone used in previous experiments (Fig. 1A) is defective in the *vpu* and *vpr* genes, indicating that neither Vpu nor Vpr is necessary for this process. To test the roles of Env and Nef in Vif incorporation, virions produced from H9 cells stably transfected with an *env*- and *nef*-defective (ΔE.His) proviral construct were analyzed. Vif was easily detected in ΔE.His particles, indicating that its packaging depends neither on Env nor on Nef (Fig. 3A).

The roles of Pol and Gag in Vif incorporation were assessed by examining virions produced from 293 cells transfected with proviral constructs further mutated in the genes encoding these two polyproteins (Fig. 3B). Plasmid ΔVprΔEΔGag served as a negative control that produced no Gag and therefore no particles. Vif could be detected in cells transfected with this construct (data not shown) but not in the extracellular medium (lane 2). The ΔVprΔEΔPol plasmid produces virions which lack the protease, RT, and IN Pol proteins as well as Vpr and Env; particles produced from ΔVprΔEΔPolΔNCΔp6 are further defective in the nucleocapsid (NC) and p6 proteins. Vif was still incorporated into these mutant virions despite the

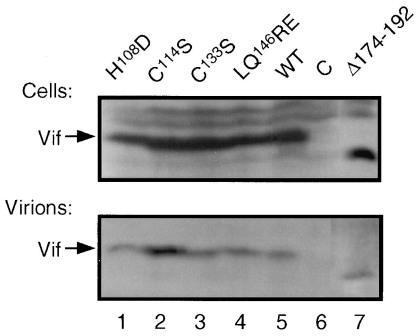

FIG. 4. A number of Vif mutants are packaged into virions. Cell line 293 cells were transfected with the R7ΔVif proviral construct, together with SNH vectors expressing the various mutant *vif* alleles (SNH-Vif $H^{108}D$, SNH-Vif $C^{114}S$, SNH-Vif $C^{133}S$, SNH-Vif $LQ^{146}RE$, and SNH-Vif Δ174–192), wild-type *vif* (WT), or a control vector (C). Top: Western blot analysis of cell extracts, using a Vif-specific antibody. Bottom: same analysis but on purified virions.

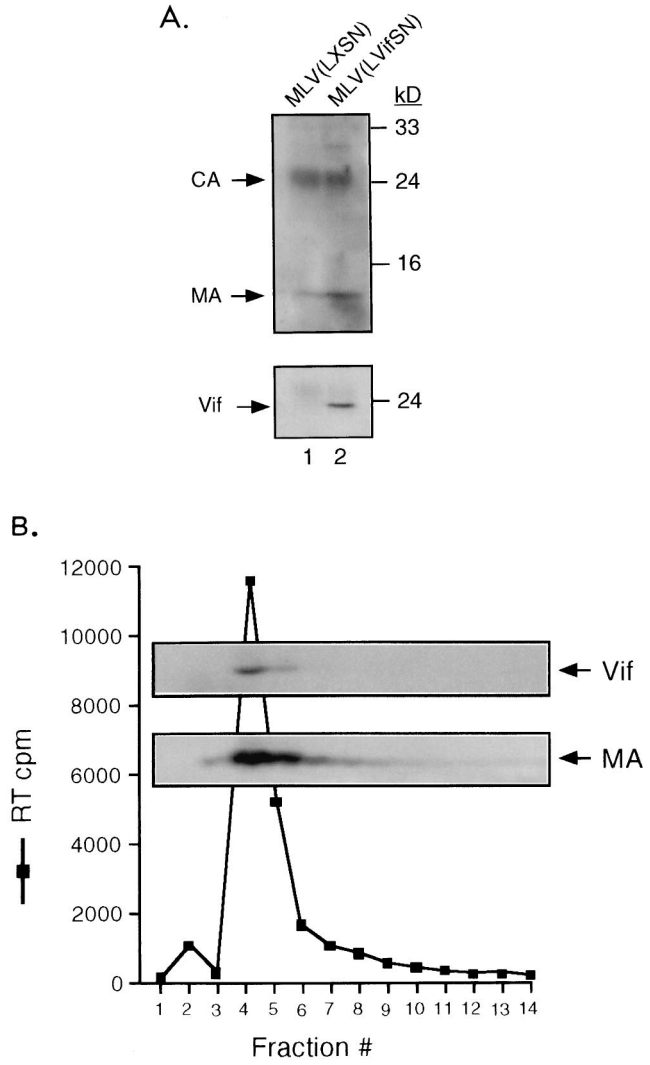

FIG. 5. Vif is incorporated into MLV particles when expressed in the producer cell. (A) MLV particles produced from the packaging cell lines CRIP(LXSN) and CRIP(LVifSN) were prepared by ultracentrifugation, normalized by RT assay, and subjected to Western blot analyses with anti-Vif and anti-Rauscher leukemia virus p15 antibodies. Lane 1, MLV(LXSN) particles; lane 2, MLV(LVifSN) particles. Positions of molecular mass markers are on the right. (B) MLV(LVifSN) virions were purified by gel exclusion chromatography with a Sephacryl S-1000 column, and fractions (1 to 14) collected were analyzed for RT content with an exo-RT assay and for Vif and p15 MA contents by immunodetection with an anti-Vif antibody and an anti-Rauscher leukemia virus p15 antibody, respectively.

absence of the Pol enzymes or of the Gag cleavage products NC and p6 (lanes 3 and 4). Taken together, these results indicate that the only viral proteins necessary for Vif particle incorporation are those required for the efficient formation of virions, that is, MA and CA. Furthermore, since NC mediates the packaging of the retroviral genome (20), one can conclude that Vif is not recruited in particles through an interaction with the viral RNA.

To test which region of Vif might mediate its packaging, Vif variants with mutations in highly conserved residues (H-108→D, C-114→S, C-133→S, and LQ-146→RE) or with a C-terminal truncation (Δ174–192) were tested. All of these mutants were profoundly defective in a functional assay (results not illustrated). Plasmids expressing these *vif* alleles were cotransfected with the R7ΔVif proviral construct into 293 cells. The resulting virions were subjected to immunoblot analysis with a Vif-specific antibody (Fig. 4). Despite their attenuation, all of the mutants were incorporated into particles in amounts that directly reflected their levels of intracellular expression (Fig. 4). These results indicate that neither the conserved residues H-108, C-114, C-133, L-145, and Q-146 nor the C-terminal 18 amino acids of Vif are critical for its packaging into virions. The deletion mutant is of interest because it lacks a region apparently involved in localizing Vif to the cell membrane (15).

Vif is incorporated into MLV particles. In the absence of obvious restrictions for Vif incorporation into HIV-1 virions, the possibility that it might be recruited into heterologous retroviral particles was addressed. MLV is a simple retrovirus that does not encode a Vif-like protein. Particles produced from CRIP packaging cells (6) transduced with a *vif*-expressing or a control vector (36) were concentrated, normalized for RT activity, and subjected to Western blot analyses. Virions from the *vif*-expressing CRIP cells contained readily detectable quantities of Vif, whereas those harvested from the control cells did not (Fig. 5A). To ensure that the Vif detected was indeed virion associated, MLV particles were purified by gel exclusion chromatography on a Sephacryl S-1000 column. The fractions collected (approximately 250 μl) were then analyzed for RT activity and subjected to immunoblot analysis with Vif- and p15 MA-specific antibodies (Fig. 5B). The Vif profile paralleled that of the RT and MA proteins, with all peaks occurring in fractions 4 and 5. Hence, expression of HIV-1 Vif in MLV-producing cells can result in the uptake of Vif into MLV particles.

DISCUSSION

In this work, we confirmed our previous finding that Vif is very inefficiently incorporated into HIV-1 virions. Indeed, Vif levels in particles are from 75- to 220-fold lower than those of the viral CA, and the relative concentration of Vif is much higher in cells than in virions. Liu et al. found significantly higher levels of Vif in HIV-1 virions (on average, between 60 and 100 molecules per particle) (21). The reason for this discrepancy between their results and ours is unclear, since we both used methodologies based on immunoblot analyses of serially diluted virions with recombinant Vif protein as a standard. In both cases, another viral protein was evaluated through the same technique to ensure the validity of the method. Liu et al. measured RT concentrations and determined via the immunodetection measurement that there were approximately 10 to 20 times more p24 molecules than RT molecules per virion, a result which is in agreement with previously reported estimates. We chose to assay MA levels and found, as predicted, that MA and CA were equimolar within particles. Nevertheless, while we measured a Vif-to-CA ratio of between 1:75 and 1:200, Liu et al. found 1 molecule of Vif for every 20 to 30 molecules of p24. We do not think that this difference is due to the fact that we used virions produced from a chronically infected cell line (Molt IIIB) while Liu et al. produced particles from an acute infection. Indeed, we tested virions from acutely infected SupT1 and H9 cells as well as from transfected 293 cells and observed no significant deviation from our original Vif calculations.

To investigate the requirements for Vif packaging, we examined viruses derived from a number of mutated proviral constructs. We thus found that MA and CA are sufficient for Vif recruitment. In contrast, the viral RNA, the Env, protease, RT, IN, NC, and p6 structural components, as well as the

accessory proteins Nef, Vpr, and Vpu are dispensable for this process. Because it is not possible to produce virions efficiently in the absence of either MA or CA, we could not determine if these two Gag products participate directly in Vif particle incorporation. Karczewski and Strebel recently reported that Vif binds to CA and suggested that this interaction might be responsible for Vif packaging (19). Certainly, our data do not allow us to rule out this hypothesis. However, an implication of our results is that if Vif recruitment is dependent upon an interaction with either CA or MA, then it must involve a region of these proteins that is well conserved between HIV-1 and MLV. Indeed, Vif is readily incorporated into MLV particles produced from a cell line expressing the HIV-1 protein.

We also tested several Vif mutants in an effort to identify which residues of this protein, if any, were critical determinants for its packaging. The amino acids targeted for mutation (H-108, C-114, C-133, L-145, and Q-146) were chosen because of their high conservation among HIV-1 isolates (38) as well as among Vif proteins from other lentiviruses (26). Although all of the Vif mutants generated were severely compromised in a functional assay, they were incorporated into virions. Furthermore, deleting the C terminus of Vif, a region previously shown to mediate Vif association with membranes, did not significantly impair its packaging.

These data are compatible with a model in which Vif is passively incorporated into retroviral particles. Its high levels in infected cells, as well as its cytoplasmic localization (14), certainly could allow its engulfment by budding virions. Such a nonspecific mechanism of incorporation would account for the very low concentration of Vif in particles. It would also explain why Vif is so much more abundant in cells than in virions, whereas the opposite is observed for all the virus-associated proteins examined in our experiments. Finally, a passive recruitment would be consistent with the packaging of Vif into MLV particles, as this virus, which does not encode a Vif-like protein, is not likely to possess a specific mechanism for Vif incorporation. Of note, we did not find that expression and packaging of wild-type HIV-1 Vif significantly enhanced the infectivity of MLV particles. However, this cannot be taken as proof of lack of functional significance, since it could be argued that the murine fibroblasts from which these particles were produced might not reveal Vif action.

With the same sensitive enhanced chemiluminescence system employed to detect Vif, we could detect various cellular proteins in HIV-1 virions, including p56Lck, GRB-2, and Ras (data not shown). Neither the functional significance nor the exact mechanism by which these cytoplasmic proteins are incorporated into particles is known, but we speculate that they might be passively recruited because of their presence at the site of virion formation. Until otherwise demonstrated, we are inclined to think that the same applies to Vif.

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REFERENCES

1. **Aiken, C., and D. Trono.** 1995. Nef stimulates human immunodeficiency virus type 1 proviral DNA synthesis. J. Virol. **69:**5048–5056.
2. **Akari, H., J. Sakuragi, Y. Takebe, K. Tomonaga, M. Kawamura, M. Fukasawa, T. Miura, T. Shinjo, and M. Hayami.** 1992. Biological characterization of human immunodeficiency virus type 1 and type 2 mutants in human peripheral blood mononuclear cells. Arch. Virol. **123:**157–167.
3. **Ausubel, F., R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith, J. G. Seidman, and K. Struhl.** 1987. Current protocols in molecular biology. John Wiley & Sons, New York.
4. **Borman, A. M., C. Quillent, P. Charneau, C. Dauguet, and F. Clavel.** 1995. Human immunodeficiency virus type 1 Vif$^-$ mutant particles from restrictive cells: role of Vif in correct particle assembly and infectivity. J. Virol. **69:**2058–2067.
5. **Chakrabarti, L., M. Guyader, M. Alizon, M. D. Daniel, R. C. Desrosiers, P. Tiollais, and P. Sonigo.** 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to the human and simian retroviruses. Nature (London) **328:**543–547.
6. **Danos, O., and R. C. Mulligan.** 1988. Safe and efficient generation of recombinant retroviruses with amphotropic and ecotropic host ranges. Proc. Natl. Acad. Sci. USA **85:**6460–6464.
7. **Fan, L., and K. Peden.** 1992. Cell-free transmission of vif mutants of HIV-1. Virology **190:**19–29.
8. **Farnet, C. M., and W. A. Haseltine.** 1991. Determination of viral proteins present in the human immunodeficiency virus type 1 preintegration complex. J. Virol. **65:**1910–1915.
9. **Fisher, A. G., B. Ensoli, L. Ivanoff, M. Chamberlain, S. Petteway, L. Ratner, R. C. Gallo, and F. Wong-Staal.** 1987. The *sor* gene of HIV-1 is required for efficient virus transmission in vitro. Science **237:**888–893.
10. **Gabuzda, D. H., K. Lawrence, E. Langhoff, E. Terwilliger, T. Dorfman, W. A. Haseltine, and J. Sodroski.** 1992. Role of *vif* in replication of human immunodeficiency virus type 1 in CD4$^+$ T lymphocytes. J. Virol. **66:**6489–6495.
11. **Gallay, P., S. Swingler, C. Aiken, and D. Trono.** 1995. HIV-1 infection of nondividing cells: C-terminal tyrosine phosphorylation of the viral matrix protein is a key regulator. Cell **80:**379–388.
12. **Garrett, E. D., L. S. Tiley, and B. R. Cullen.** 1991. Rev activates expression of the human immunodeficiency virus type 1 *vif* and *vpr* gene products. J. Virol. **65:**1653–1657.
13. **Garvey, K. J., M. S. Oberste, J. E. Elser, M. J. Braun, and M. A. Gonda.** 1990. Nucleotide sequence and genome organization of biologically active proviruses of the bovine immunodeficiency-like virus. Virology **175:**391–409.
14. **Goncalves, J., P. Jallepalli, and D. H. Gabuzda.** 1994. Subcellular localization of the Vif protein of human immunodeficiency virus type 1. J. Virol. **68:**704–712.
15. **Goncalves, J., B. Shi, X. Yang, and D. Gabuzda.** 1995. Biological activity of human immunodeficiency virus type 1 Vif requires membrane targeting by C-terminal basic domains. J. Virol. **69:**7196–7204.
16. **Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon.** 1987. Genome organization and transactivation of the human immunodeficiency virus type 2. Nature (London) **326:**662–669.
17. **Hartman, S. C., and R. C. Mulligan.** 1988. Two dominant-acting selectable markers for gene transfer studies in mammalian cells. Proc. Natl. Acad. Sci. USA **85:**8047–8051.
18. **Hoglund, S., A. Ohagen, K. Lawrence, and D. Gabuzda.** 1994. Role of vif during packing of the core of HIV-1. Virology **201:**349–355.
19. **Karczewski, M. K., and K. Strebel.** 1996. Cytoskeleton association and virion incorporation of the human immunodeficiency virus type 1 Vif protein. J. Virol. **70:**494–507.
20. **Lever, A., H. Gottlinger, W. Haseltine, and J. Sodroski.** 1989. Identification of a sequence required for efficient packaging of human immunodeficiency virus type 1 RNA into virions. J. Virol. **63:**4085–4087.
21. **Liu, H., X. Wu, M. Newman, G. M. Shaw, B. H. Hahn, and J. C. Kappes.** 1995. The Vif protein of human and simian immunodeficiency viruses is packaged into virions and associates with viral core structures. J. Virol. **69:**7630–7638.
22. **Lori, F., L. Hall, P. Lusso, M. Popovic, P. Markham, G. Franchini, and M. S. Reitz, Jr.** 1992. Effect of reciprocal complementation of two defective human immunodeficiency virus type 1 (HIV-1) molecular clones on HIV-1 cell tropism and virulence. J. Virol. **66:**5553–5560.
23. **Michaels, F. H., N. Hattori, R. C. Gallo, and G. Franchini.** 1993. The human immunodeficiency virus type 1 (HIV-1) vif protein is located in the cytoplasm of infected cells and its effect on viral replication is equivalent in HIV-2. AIDS Res. Hum. Retroviruses **9:**1025–1030.
24. **Moore, J. P., J. A. McKeating, W. A. Norton, and Q. J. Sattentau.** 1991. Direct measurement of soluble CD4 binding to human immunodeficiency virus type 1 virions: gp120 dissociation and its implications for virus-cell binding and fusion reactions and their neutralization by soluble CD4. J. Virol. **65:**1133–1140.
25. **Moore, J. P., J. A. McKeating, R. A. Weiss, and Q. J. Sattentau.** 1990. Dissociation of gp120 from HIV-1 virions induced by soluble CD4. Science **250:**1139–1142.
26. **Oberste, M. S., and M. A. Gonda.** 1992. Conservation of amino-acid sequence motifs in lentivirus vif proteins. Virus Genes **6:**95–102.
27. **Paxton, W., R. I. Connor, and N. R. Landau.** 1993. Incorporation of Vpr into human immunodeficiency virus type 1 virions: requirement for the p6 region of *gag* and mutational analysis. J. Virol. **67:**7229–7237.
28. **Sakai, H., R. Shibata, J.-I. Sakuragi, S. Sakuragi, M. Kawamura, and A. Adachi.** 1993. Cell-dependent requirement of human immunodeficiency vi-

rus type 1 Vif protein for maturation of virus particles. J. Virol. **67:**1663–1666.
29. **Saltarelli, M., G. Querat, D. A. Konings, R. Vigne, and J. E. Clements.** 1990. Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus. Virology **179:**347–364.
30. **Schwartz, S., B. K. Felber, and G. N. Pavlakis.** 1991. Expression of human immunodeficiency virus type 1 vif and vpr mRNAs is rev-dependent and regulated by splicing. Virology **183:**677–686.
31. **Shibata, R., M. Kawamura, H. Sakai, M. Hayami, A. Ishimoto, and A. Adachi.** 1991. Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. J. Virol. **65:**3514–3520.
32. **Sonigo, P., M. Alizon, K. Staskus, D. Klatzmann, S. Cole, O. Danos, E. Retzel, P. Tiollais, A. Hasse, and S. Wain-Hobson.** 1985. Nucleotide sequence of the visna lentivirus: relationship to the AIDS virus. Cell **49:**659–668.
33. **Sova, P., and D. J. Volsky.** 1993. Efficiency of viral DNA synthesis during infection of permissive and nonpermissive cells with *vif*-negative human immunodeficiency virus type 1. J. Virol. **67:**6322–6326.
34. **Strebel, K., D. Daugherty, K. Clouse, D. Cohen, T. Folks, and M. A. Martin.** 1987. The HIV 'A' (*sor*) gene product is essential for virus infectivity. Nature (London) **328:**728–730.
35. **Talbott, R. L., E. E. Sparger, K. M. Lovelace, W. M. Fitch, N. C. Pedersen, P. A. Luciw, and J. H. Elder.** 1989. Nucleotide sequence and genomic organization of feline immunodeficiency virus. Proc. Natl. Acad. Sci. USA **86:**5743–5747.
36. **von Schwedler, U., J. Song, C. Aiken, and D. Trono.** 1993. *vif* is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. J. Virol. **67:**4945–4955.
37. **Wain-Hobson, S., P. Sonigo, O. Danos, S. Cole, and M. Alizon.** 1985. Nucleotide sequence of the AIDS virus, LAV. Cell **40:**9–17.
38. **Wieland, U., J. Hartmann, H. Suhr, B. Salzberger, H. J. Eggers, and J. E. Kuhn.** 1994. In vivo genetic variability of the HIV-1 vif gene. Virology **203:**43–51.
39. **Zombek, D., N. R. Landau, and D. Trono.** 1994. Vif is incorporated into HIV-1 particles: mapping of its packaging requirements, p. 233. *In* Retroviruses '94. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.