JOURNAL OF VIROLOGY, July 1993, p. 4386–4390
0022-538X/93/074386-05$02.00/0

Vol. 67, No. 7

Vpx of Simian Immunodeficiency Virus Is Localized Primarily outside the Virus Core in Mature Virions

XIAOFANG YU, ZENE MATSUDA, QIAN-CHUN YU, TUN-HOU LEE, AND MAX ESSEX*
Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115

Received 6 November 1992/Accepted 31 March 1993

Human immunodeficiency virus type 2 and the related simian immunodeficiency virus (SIV) contain a unique regulatory gene, *vpx*. The Vpx protein is packaged in mature virions and is required for efficient viral replication in peripheral blood lymphocytes and macrophages. To study the localization of Vpx in mature virions, conical and bar-shaped core structures of SIV from macaques (SIV_{mac}) were purified. The SIV_{mac} core has a density of approximately 1.25 g/cm^3, compared with 1.16 g/cm^3 for an intact virion. The relative proportions of major capsid protein (p27) and reverse transcriptase activity were similar for intact virions and core structures. The majority of matrix protein (p14) was removed from the purified core structure, suggesting its association with the viral membrane. Similarly, most of the Vpx protein was absent from the purified core structure. This result suggests that as with the matrix protein, the majority of Vpx proteins are localized outside the virus core. The localization of Vpx suggests that it may be involved in virus entry such as penetration or uncoating.

Human immunodeficiency virus type 2 (HIV-2), simian immunodeficiency virus (SIV) from macaques (SIV_{mac}), and SIV from sooty mangabeys (SIV_{sm}) belong to a closely related group of primate lentiviruses (2, 6, 10, 12). Unlike other retroviruses, virions of this group of primate lentiviruses package two regulatory proteins, Vpx (7, 11, 16, 26) and Vpr (27), in addition to viral structural proteins. The *vpx* gene is unique to this group of SIV and HIV-2 and is absent from other groups of primate lentiviruses such as HIV-1. Vpx is dispensable for viral replication in established human lymphocyte cell lines (9, 14, 15, 18, 26) but is required for efficient viral replication in fresh lymphocytes (9, 15, 28) and macrophages (28). The exact function of Vpx is unclear. It is not known how Vpx is packaged into mature virions or where it is localized in the mature virions. It has been reported that Vpx can bind to single-stranded nucleic acids in vitro (11) and may therefore be associated with the viral RNA genome in mature virions. However, the location of Vpx in the mature virion has not been directly determined. In this study, we sought to isolate and characterize the core structure of SIV_{mac} and to determine the subviral localization of Vpx in virions to better understand the role of this gene in viral replication.

SIV_{mac} (17) was propagated in CEMX174 cells as previously described (28). The supernatant of infected CEMX174 cells was centrifuged at 1,000 × *g* for 30 min to remove cells and cell debris. The supernatant was vacuum filtered through a 0.2-μm-pore-size filter unit (Nalgene Co., Rochester, N.Y.) and centrifuged at 20,000 rpm (Beckman SW28 rotor) for 2 h through a 20% (wt/vol) sucrose cushion. Virus pellets were dissolved in TNE buffer (0.01 M Tris-HCl [pH 7.2], 0.1 M NaCl, 0.001 M EDTA), and this solution was overlaid on a discontinuous sucrose gradient. The discontinuous sucrose gradient was prepared by sequentially overlaying 2 ml of sucrose of the following concentrations: 70, 67.5, 65, 62.5, 60, 57.5, 55, 52.5, 50, 47.5, 45, 42.5, 40, 37.5, 35, 32.5, and 30% (wt/vol). The concentrations of sucrose were prepared by mixing 70% (wt/vol) sucrose in deuterium oxide (Sigma, St. Louis, Mo.) and 30% sucrose in water. Both solutions contained TNE buffer and 0.4% dithiothreitol, which have previously been used for the purification of Rous sarcoma virus core (3).

For preparation of the virus cores, several conditions were tested, including 1 and 0.5% Triton X-100 and 0.25% Nonidet P-40 treatments at room temperature for 10 min. It appeared that 0.5% Triton X-100 gave the highest yield of SIV_{mac} core structure (data not shown). Ninety percent of the virus pellets dissolved in TNE buffer (concentrated from 500 ml of culture supernatant) were treated with 0.5% Triton X-100 for 10 min at room temperature and were immediately overlaid on a discontinuous sucrose gradient. The remaining 10% of the dissolved untreated virus pellets were overlaid on another discontinuous sucrose gradient. Both gradients were spun at 20,000 rpm (Beckman SW28 rotor) for 20 h at 4°C. Two-milliliter fractions were collected dropwise from each gradient from the bottom of the centrifuge tubes. Fifty microliters of each fraction was tested for reverse transcriptase (RT) activity as previously described (28). Fractions with high RT activity from each gradient were layered onto separate second sucrose gradients. Specifically, fraction 12 from the sucrose gradient containing the intact virions and pooled fractions 5 and 6 of the treated virions were diluted with TNE buffer and sublayered onto separate sucrose gradients, respectively. Centrifugation was performed, and fractions were collected as described for the first sucrose gradients. The RT activity of each sucrose gradient fraction (both the intact virions and the 0.5% Triton X-100-treated virions) was analyzed (Fig. 1). A peak value of RT activity was detected in fraction 12 of the sucrose gradient with the intact virions (Fig. 1A). In contrast, a peak value of RT activity was detected in fraction 5 of the 0.5% Triton X-100-treated virions (Fig. 1B). The density of each sample was determined by weighing 0.1 ml of each fraction. The density corresponding to the RT peak of the intact virions was approximately 1.16 g/cm^3 (Fig. 1A). In contrast, the density corresponding to the RT peak of the 0.5% Triton X-100-treated virions was approximately 1.25 g/cm^3 (Fig. 1B).

To confirm that the SIV_{mac} core structures were purified, samples were taken from the RT peak fractions of each gradient and examined by electron microscopy. Round and

* Corresponding author.

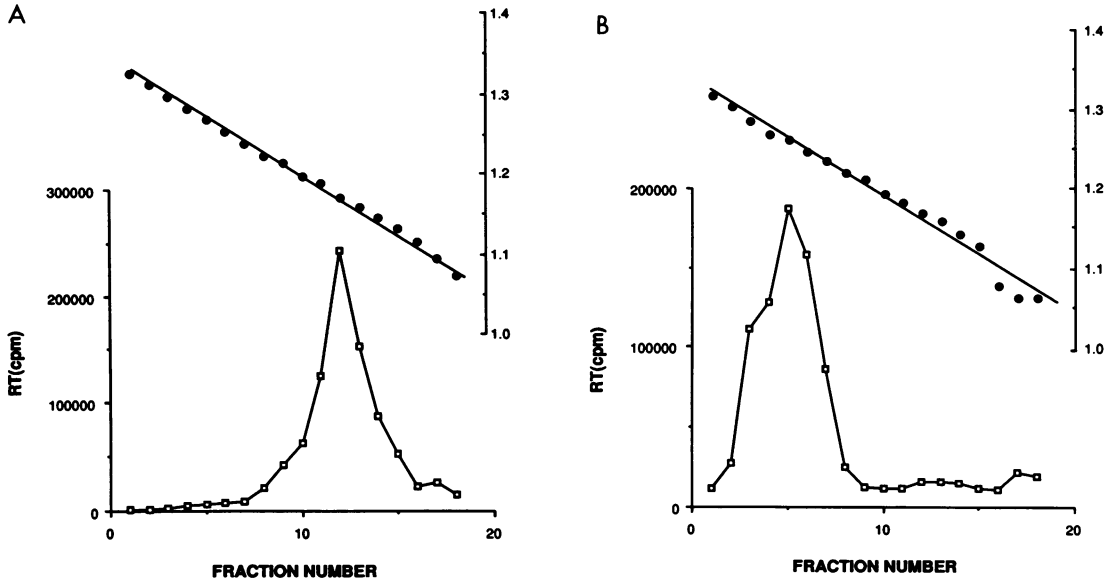

FIG. 1. Sucrose density gradient centrifugation of intact (A) and 0.5% Triton X-100-treated (B) SIV_{mac} virions. A 50-μl sample from each sucrose gradient fraction was analyzed for RT activity (squares) as previously described (28); 100 μl of sample from each sucrose gradient fraction was weighed to determine virion and core density in grams per cubic centimeter (circles).

slightly deformed structures with a diameter of approximately 120 nm were detected from the intact virion samples by negative staining with 1% uranyl acetate (Fig. 2B). Conical and bar-shaped structures of 100 nm in length were detected in pooled fractions (fractions 4 and 5) of 0.5% Triton X-100-treated virus preparations (Fig. 2C). These structures resembled the SIV_{mac} core structures detected in the intact virions by transmission electron microscopy (Fig. 2A), suggesting that the SIV_{mac} core structures were successfully purified.

Viral proteins of intact virions and purified core structures were analyzed by immunoblotting. Samples from each fraction were centrifuged at 40,000 rpm (Beckman 70 Ti rotor) for 60 min at 4°C and analyzed by immunoblotting with HIV-2 sera and a specific anti-Vpx serum as previously described (26). The ratio of RT activity and the intensity of the major capsid protein p27 were similar for intact virions and core structures (Fig. 1 and 3). This finding suggests that RT was packaged in the virus core. Viral Gag proteins p27 (capsid) and p14 (matrix), a small Gag protein of 7 or 6 kDa (p7/6), and a small amount of Gag precursor p55 were detected in the RT peak fractions of intact virions with a pooled human HIV-2 serum (Fig. 3A). A protein of 34 kDa was also detected with the pooled HIV-2 serum (Fig. 3A). This protein could also be detected by a specific serum (a gift from D. Grandgenett, Institute of Molecular Virology, St. Louis University Medical Center, St. Louis, Mo.) raised against SIV_{mac} integrase (data not shown). A significant amount of Vpx was detected in the intact virions by the HIV-2 serum (Fig. 3A) or a goat anti-Vpx serum (26) (Fig. 3B).

Similar to findings for the intact virions, comparable amounts of p27, p7/6, p55, and p34 were detected in the core fractions (Fig. 3B). In sharp contrast to the intact virions, the majority of matrix protein p14 was not detected in the core structures with the same HIV-2 serum (Fig. 3B). No Vpx was detected with the HIV-2 serum (Fig. 3A), and only a small amount was detected with the more sensitive goat anti-Vpx serum (Fig. 3B). To test whether these results were reproducible, the experiments described above were repeated with a different preparation of intact virions and purified core structures. Comparable amounts of p27, p7/6, p55, and p34 were detected with human HIV-2 serum in the RT peak fractions of the sucrose gradient for intact virions (Fig. 4, lane 1) or the core structures (lane 4). p27 and p55 were also recognized by a monoclonal antibody to HIV-2 p27 (Du Pont, Wilmington, Del.). The majority of the matrix protein and Vpx, which were detected in the intact virions, were not detected in the purified core (Fig. 4).

The conical and bar-shaped core structures of SIV_{mac} have a density of approximately 1.25 g/cm^3, which is similar to that of type B and type C retrovirus spherical cores (3, 22, 23). Unlike avian myeloblastosis virus core (1), the SIV_{mac} core was not infectious when added to T-cell cultures (data not shown). The dominant structural protein detected in the SIV_{mac} core was the capsid protein p27 (Fig. 3 and 4), which is consistent with the assumption that capsid proteins form the shell of the retrovirus core. Other structural proteins that were detected with the purified core are RT, integrase p34, a small amount of Gag polyprotein p55, and a protein of 7 or 6 kDa, which is probably the nucleic acid-binding protein (p7/6). The majority of matrix protein p14 was absent from the SIV_{mac} core, which supports the finding that matrix proteins associate with the inner surface of the virus membrane (8, 20). However, a small amount of p14 was still associated with the virus core after two rounds of sucrose gradient purification. Although it is unlikely, this may be due to incomplete disruption of intact virions. No viral proteins could be detected in fractions 4, 5, and 6 from the intact virion sucrose gradient (Fig. 3A). Examination of the core fraction also did not reveal intact virions (Fig. 2). Therefore, an alternative explanation is that there is an interaction between the matrix protein and the virus core. A recent morphological study of HIV-1 virions suggests that the narrow end of the core is attached to the virus envelope membrane (13), which is consistent with this interpretation. The interaction between the matrix protein and the virus core may play some role during virus core internalization

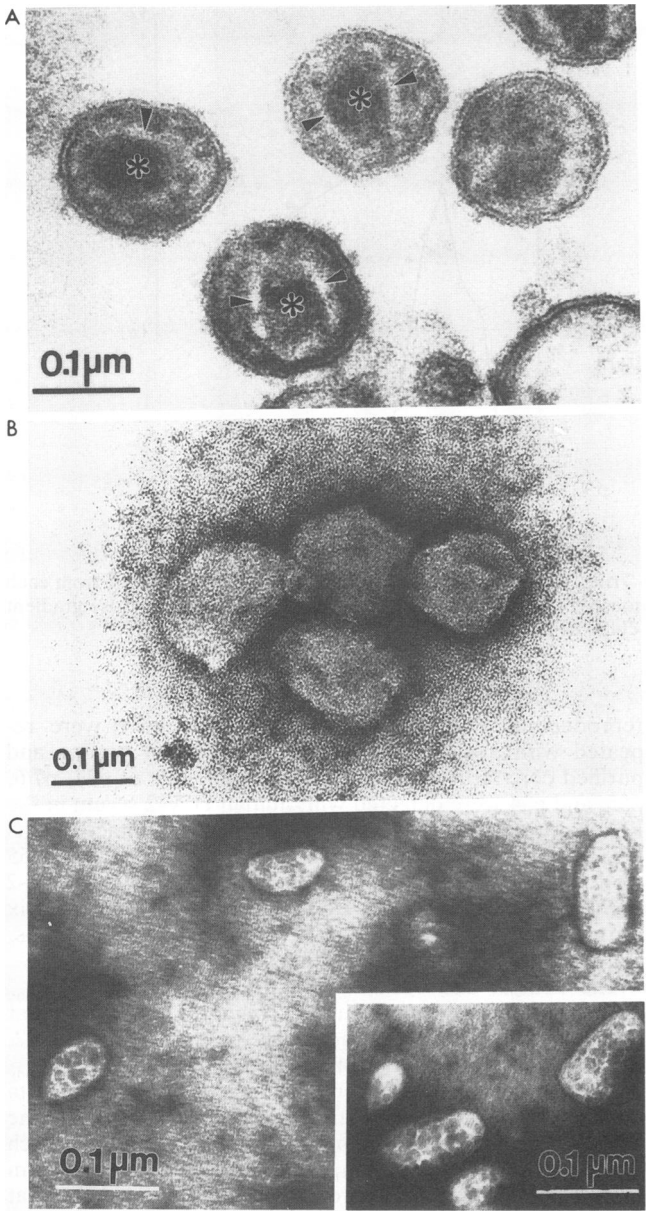

FIG. 2. (A) SIV_{mac} virions from infected CEMX174 cells as visualized by transmission electron microscopy; (B) purified SIV_{mac} intact virions as observed by negative staining; (C) purified SIV_{mac} cores as observed by negative staining.

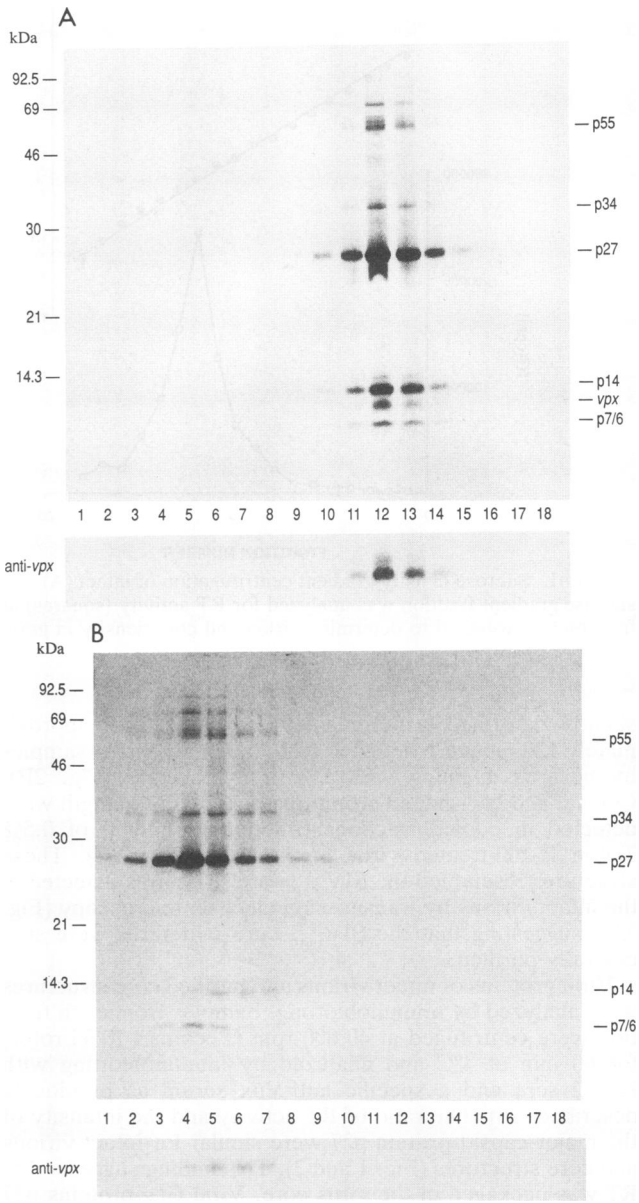

FIG. 3. Immunoblot analysis of viral proteins from sucrose gradient-purified intact virions (A) or core structure (B). Samples were reacted with human HIV-2 sera (A) or goat anti-Vpx serum (B).

and uncoating. Recent observations have suggested that the HIV-1 matrix protein plays a role in early steps of viral replication (29).

The *vpx* gene is uniquely present in HIV-2, SIV_{mac}, and SIV_{sm} and is required for efficient viral replication in primary lymphocytes (9, 15, 28) and macrophages (28). It has been suggested that the *vpx* gene arose by duplication of the *vpr* gene after the divergence of the HIV-2 group from the other primate lentivirus groups (24, 25). Vpr and Vpx proteins are both virion associated (4, 7, 11, 15, 26, 27, 30). It has been proposed that a conserved motif between Vpx and Vpr provides the virion packaging signal (19, 24). The fact that HIV-2 and SIV_{mac} Vpx can be packaged into HIV-1 virions (19) supports such a theory. The mechanism by which Vpx is packaged into mature virions is not clear. The Vpx protein contains a conserved cysteine-histidine motif that can bind to single-stranded nucleic acids in vitro (11). It is possible that Vpx binds to the viral RNA genome to be packaged into virions. However, our study suggests that this is unlikely. Retroviral RNA genomes are packaged inside the virus core (1, 3, 21, 22, 23), while the majority of the SIV_{mac} Vpx were detected outside of the core structure (Fig. 3 and 4). The localization of Vpx in the virion is very similar to that of the matrix protein (Fig. 3 and 4). It is possible that Vpx, like the matrix protein (8, 20), forms an association with the inner surface of the virus membrane. This hypothesis is consistent with a previous observation that Vpx was detected with the cellular membrane fraction (7). It is also possible that Vpx

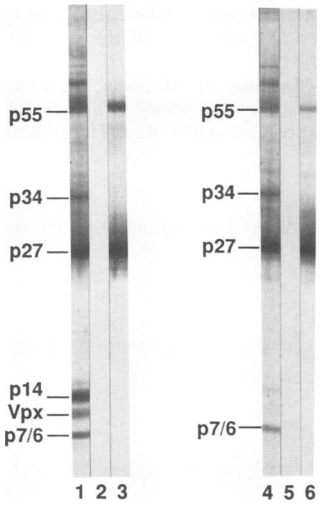

FIG. 4. Immunoblot analysis of viral proteins from purified intact virions (lanes 1 to 3) or core structure (lanes 4 to 6), detected by human HIV-2 sera (lanes 1 and 4), sera from HIV-negative donors (lanes 2 and 5), or a monoclonal antibody against HIV-2 p27 (lanes 3 and 6).

interacts with other viral proteins such as the transmembrane protein or the matrix protein. Such an interaction would facilitate the packaging of Vpx into virions. Preliminary immunoprecipitation analysis indicates that the matrix protein could be coprecipitated with Vpx by an anti-Vpx serum (data not shown). It has been reported that an HIV-2 Vpx mutant had a defect prior to the synthesis of viral DNA after infection (15). The localization of Vpx in the virion suggests that Vpx may participate in virus entry (e.g., penetration and uncoating) and that a direct involvement in reverse transcription is less likely.

We thank P. Kanki of the Harvard School of Public Health for HIV-2 sera, J. Mullins of Stanford University for pBK-28, X. Yuan and M. F. McLane for helpful discussions, and E. Conway for editorial assistance.

This work was supported by Public Health Service grants CA-39805, HL-33774, and HL-43561 from the National Institutes of Health.

REFERENCES

1. **Bolognesi, D. P., H. Gelderblom, H. Bauer, K. Molling, and G. Huper.** 1972. Polypeptides of avian RNA tumor viruses. V. Analysis of the virus core. Virology **47**:567–578.
2. **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 other human and simian retroviruses. Nature (London) **328**:543–547.
3. **Coffin, J. M., and H. M. Temin.** 1971. Comparison of Rous sarcoma virus-specific deoxyribonucleic acid polymerases in virions of Rous sarcoma virus and in Rous sarcoma virus-infected chicken cells. J. Virol. **7**:625–634.
4. **Cohen, E. A., G. Dehni, J. G. Sodroski, and W. A. Haseltine.** 1990. Human immunodeficiency virus *vpr* product is a virion-associated regulatory protein. J. Virol. **64**:3097–3099.
5. **Cohen, E. A., E. F. Terwilliger, Y. Jalinoos, J. Proulx, J. G. Sodroski, and W. A. Haseltine.** 1990. Identification of HIV-1 vpr product and function. J. Acquired Immune Defic. Syndr. **3**:11–18.
6. **Dietrich, U., M. Adamski, R. Kreutz, A. Seipp, H. Kuhnel, and H. Rubsamen Waigmann.** 1989. A highly divergent HIV-2-related isolate. Nature (London) **342**:948–950.
7. **Franchini, G., J. R. Rusche, T. J. O'Keeffe, and F. Wong-Staal.** 1988. The human immunodeficiency virus type 2 (HIV-2) contains a novel gene encoding a 16 kD protein associated with mature virions. AIDS Res. Hum. Retroviruses **4**:243–249.
8. **Gelderblom, H. R., E. H. S. Hausmann, M. Özel, G. Pauli, and M. A. Koch.** 1987. Fine structure of human immunodeficiency virus (HIV) and immunolocalization of structural proteins. Virology **156**:171–176.
9. **Guyader, M., M. Emerman, L. Montagnier, and K. Peden.** 1989. VPX mutants of HIV-2 are infectious in established cell lines but display a severe defect in peripheral blood lymphocytes. EMBO J. **8**:1169–1175.
10. **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.
11. **Henderson, L. E., R. C. Sowder, T. D. Copeland, R. E. Beneveniste, and S. Oroszlan.** 1988. Isolation and characterization of a novel protein (x-orf product) from SIV and HIV-2. Science **241**:199–201.
12. **Hirsch, V. M., R. A. Olmsted, M. Murphey-Corb, R. H. Purcell, and P. R. Johnson.** 1989. An African primate lentivirus (SIV_{sm}) closely related to HIV-2. Nature (London) **339**:389–392.
13. **Höglund, S., L. G. Öfverstedt, A. Nilsson, P. Lundquist, H. Gelderblom, M. Özel, and U. Skoglund.** 1992. Spatial visualization of the maturing HIV-1 core and its linkage to the envelope. AIDS Res. Hum. Retroviruses **8**:1–7.
14. **Hu, W., N. Vander Heyden, and L. Ratner.** 1989. Analysis of the function of viral protein X (VPX) of HIV-2. Virology **173**:624–630.
15. **Kappes, J. C., J. A. Conway, S.-W. Lee, G. M. Shaw, and B. H. Hahn.** 1991. Human immunodeficiency virus type 2 vpx protein augments viral infectivity. Virology **184**:197–209.
16. **Kappes, J. C., C. D. Morrow, S. W. Lee, B. A. Jameson, S. B. H. Kent, L. E. Hood, G. M. Shaw, and B. H. Hahn.** 1988. Identification of a novel retroviral gene unique to human immunodeficiency virus type 2 and simian immunodeficiency virus SIV_{MAC}. J. Virol. **62**:3501–3505.
17. **Kornfeld, H., N. Riedel, G. A. Viglianti, V. Hirsch, and J. I. Mullins.** 1987. Cloning of HTLV-4 and its relation to simian and human immunodeficiency viruses. Nature (London) **326**:610–613.
18. **Marcon, L., F. Michaels, N. Hattori, K. Fargnoli, R. C. Gallo, and G. Franchini.** 1991. Dispensable role of the human immunodeficiency virus type 2 Vpx protein in viral replication. J. Virol. **65**:3938–3942.
19. **Matsuda, Z., X.-F. Yu, Q.-C. Yu, T.-H. Lee, and M. Essex.** 1993. A virion-specific inhibitory molecule with therapeutic potential for human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **90**:3544–3548.
20. **Pepinsky, R. B., and V. M. Vogt.** 1979. Identification of retrovirus matrix proteins by lipid-protein cross-linking. J. Mol. Biol. **131**:819–837.
21. **Roberts, M. M., and S. Oroszlan.** 1989. The preparation and biochemical characterization of intact capsids of equine infectious anemia virus. Biochem. Biophys. Res. Commun. **160**:486–494.
22. **Stromberg, K.** 1972. Surface-active agents for isolation of the core component of avian myeloblastosis virus. J. Virol. **9**:684–697.
23. **Teramoto, Y. A., R. D. Cardiff, and J. K. Lund.** 1977. The structure of the mouse mammary tumor virus: isolation and characterization of the core. Virology **77**:135–148.
24. **Tristem, M., C. Marshall, A. Karpas, and F. Hill.** 1992. Evolution of the primate lentiviruses: evidence from *vpx* and *vpr*. EMBO J. **11**:3405–3412.
25. **Tristem, M., C. Marshall, A. Karpas, J. Petrik, and F. Hill.** 1990. Origin of vpx in lentiviruses. Nature (London) **347**:341–342.
26. **Yu, X. F., S. Ito, M. Essex, and T. H. Lee.** 1988. A naturally immunogenic virion-associated protein specific for HIV-2 and SIV. Nature (London) **335**:262–265.
27. **Yu, X.-F., M. Matsuda, M. Essex, and T.-H. Lee.** 1990. Open

reading frame *vpr* of simian immunodeficiency virus encodes a virion-associated protein. J. Virol. **64**:5688–5693.
28. **Yu, X.-F., Q.-C. Yu, M. Essex, and T.-H. Lee.** 1991. The *vpx* gene of simian immunodeficiency virus facilitates efficient viral replication in fresh lymphocytes and macrophages. J. Virol. **65**:5088–5091.
29. **Yu, X.-F., Q.-C. Yu, T.-H. Lee, and M. Essex.** 1992. The C terminus of human immunodeficiency virus type 1 matrix protein is involved in early steps of the virus life cycle. J. Virol. **66**:5667–5670.
30. **Yuan, X., Z. Matsuda, M. Matsuda, M. Essex, and T.-H. Lee.** 1990. Human immunodeficiency virus *vpr* gene encodes a virion-associated protein. AIDS Res. Hum. Retroviruses **6**:1265–1271.