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The p6^{gag} Domain of Human Immunodeficiency Virus Type 1 Is Sufficient for the Incorporation of Vpr into Heterologous Viral Particles

EISAKU KONDO,[1] FABRIZIO MAMMANO,[1] ERIC A. COHEN,[2] AND HEINRICH G. GÖTTLINGER[1]*

Division of Human Retrovirology, Dana-Farber Cancer Institute, and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115,[1] and Département de Microbiologie et Immunologie, Université de Montréal, Montréal, Québec, Canada H3C 3J7[2]

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The *vpr* gene of human immunodeficiency virus type 1 (HIV-1) encodes a virion-associated regulatory protein. Mutagenesis has shown that the virion association of Vpr requires sequences near the C terminus of the HIV-1 Gag polyprotein Pr55^{gag}. To investigate whether Vpr incorporation is mediated by a specific domain of Pr55^{gag}, we examined the ability of chimeric HIV-1/Moloney murine leukemia virus (MLV) Gag polyproteins to direct the incorporation of Vpr. Vpr expressed in *trans* did not associate with particles formed by the authentic MLV Gag polyprotein or with particles formed by chimeric Gag polyproteins that had the matrix (MA) or capsid (CA) domain of MLV precisely replaced by the corresponding domain of HIV-1$_{HXB2}$. By contrast, Vpr was efficiently incorporated upon replacement of the C-terminal nucleocapsid (NC) domain of the MLV Gag polyprotein with HIV-1 p15 sequences. Vpr was also efficiently incorporated into particles formed by a MLV Gag polyprotein that had the HIV-1 p6 domain fused to its C terminus. Furthermore, a deletion analysis revealed that a conserved region near the C terminus of the p6 domain is essential for Vpr incorporation, whereas sequences downstream of the conserved region are dispensable. These results show that a virion association motif for Vpr is located within residues 1 to 46 of p6.

In addition to the *gag*, *pol*, and *env* gene products, which are common to all replication-competent retroviruses, primate immunodeficiency viruses (human immunodeficiency virus types 1 and 2 [HIV-1 and -2] and simian immunodeficiency virus [SIV]) encode several accessory proteins. Two of these accessory proteins, Vpr and Vpx, have been found in virions in substantial quantities (2, 8, 16, 17, 34–36). Vpr is encoded by most if not all primate immunodeficiency viruses, while Vpx is unique to the HIV-2/SIV$_{mac}$ group and likely arose by duplication of an ancestral *vpr* gene (27). *vpr* is one of the best-conserved genes of primate immunodeficiency viruses (27) but is not essential for virus replication in cell culture. Although it has been reported that Vpr is dispensable in lymphoid cells (4), other functional studies indicate that Vpr is necessary for efficient virus replication in CD4$^+$ lymphocyte cell lines (24). It was also reported that Vpr profoundly increases the capacity of HIV-1 and HIV-2 to replicate in primary monocytes (1, 13, 30). Vpr appears to be critical for virus persistence in rhesus macaques infected with SIV$_{mac}$ (19), indicating that it has an important role in vivo.

Besides Vpr, the virally encoded components of the HIV-1 virion include the internal structural proteins, enzymes, and envelope glycoproteins. The internal structural proteins enter the nascent particle as components of a polyprotein precursor (Pr55^{gag}) (32). HIV-1 particle formation appears to be driven mainly by the self-association of Pr55^{gag} molecules at the cell membrane, since no other virally encoded products are required (9). Proteolytic cleavage of Pr55^{gag} after the completion of a nascent capsid structure yields the internal structural proteins of the mature virion. These include the matrix (MA), capsid (CA), and nucleocapsid (NC) proteins, which are common to all retroviruses, and a peptide derived from the C terminus of the Gag precursor (p6^{gag}), which is found only in primate immunodeficiency viruses (15, 22). The essential enzymatic components enter the virion in the context of the Pr160$^{gag\text{-}pol}$ polyprotein, which is thought to be incorporated through interactions of its amino-terminal Gag domain with Pr55^{gag} (32). The envelope glycoproteins are also synthesized in the form of a precursor which is cleaved into the surface and transmembrane glycoproteins (31). It was demonstrated recently that sequences within the MA protein domain of Pr55^{gag} are required for the virion association of HIV-1 envelope glycoprotein (7, 33).

The mechanism by which Vpr is incorporated into virions remains to be elucidated. Vpr is incorporated in a selective manner, since other accessory viral proteins are excluded. Unlike other virally encoded components of the virion, Vpr is not synthesized as part of a polyprotein precursor. Since the C-terminal portion of Vpr is rich in arginine residues, it has been suggested that Vpr may enter the virion by binding to the genomic viral RNA (36). However, mutations that reduce the genomic RNA content of virions had no effect on the virion association of Vpr (20, 25), suggesting that incorporation of Vpr is independent of the encapsidation of viral RNA.

It was recently reported that Vpr is incorporated into viral particles formed in the absence of the *pol* or *env* gene product, indicating that the virion association of Vpr is mediated by a direct or indirect interaction with Pr55^{gag} (20, 21, 25). It was also reported that the incorporation of Vpr is prevented by truncations that remove the C-terminal p6 domain of Pr55^{gag} (21, 25). However, the possibility that other domains of Pr55^{gag} are also required was not excluded.

To determine the role of individual domains of Pr55^{gag} in the virion association of Vpr, we replaced domains of the *gag* gene

* Corresponding author. Mailing address: Division of Human Retrovirology, Dana-Farber Cancer Institute, Jimmy Fund Building, Room 824, 44 Binney St., Boston, MA 02115. Phone: (617) 632-3067. Fax: (617) 632-3113. Electronic mail address: Heinrich_Gottlinger@DFCI.harvard.edu.

of Moloney murine leukemia virus (MLV) with the corresponding sequences from HIV-1. The results obtained demonstrate that the presence of the HIV-1 p6^{gag} domain is sufficient for the association of Vpr with heterologous viral particles that do not normally incorporate Vpr.

MATERIALS AND METHODS

Plasmid construction. HXBH10-PR$^-$, which was used to express the HIV-1 Gag polyprotein Pr55^{gag}, is identical to the full-length HXBH10 molecular clone of HIV-1 except for a point mutation that inactivates the HIV-1 protease (10). HXBH10-*gag*$^-$ is a variant of HXBH10 that is unable to express Pr55^{gag} because of a premature termination codon in place of codon 8 of the *gag* gene and an additional frameshift mutation in the CA coding region (7). To obtain HXBH10/NCA$_{gag}$-PR$^-$, which served to express the authentic MLV Gag polyprotein Pr65^{gag}, the MLV protease gene in the previously described MLV Gag expression construction HXBH10/NCA$_{gag}$ (10) was disrupted by inserting 4 bp at an *Age*I site (nucleotide [nt] 2445 of MLV), using the Klenow fragment of DNA polymerase I.

The MLV/HIV-MA Gag expression construct is identical to HXBH10/NCA$_{gag}$-PR$^-$ except that the 5′ untranslated and MA protein-coding regions are from HIV-1$_{HXB2}$. To obtain the MLV/HIV-MA construct, an *Eco*47III site was introduced by site-directed mutagenesis into the HIV-1 *gag* gene immediately downstream of the MA coding region. A PCR-generated *Eco*47III site at the equivalent position in the MLV *gag* gene was then used to join HIV-1 and MLV *gag* coding sequences. The resulting chimeric *gag* gene construct contains one translationally silent mutation (C to G) at position 1019 of the MLV-derived sequence and has the MA coding region of MLV precisely replaced by that of HIV-1.

The MLV/HIV-CA Gag expression construct (26) was obtained by replacing the CA coding region of the MLV *gag* gene (nt 1266 to 2054) in HXBH10/NCA$_{gag}$-PR$^-$ with the corresponding region from HIV-1$_{HXB2}$ (nt 1185 to 1919), using blunt-end cloning sites at the domain boundaries, which were generated by site-directed mutagenesis. The resulting chimeric *gag* gene contains one translationally silent mutation (C to T) at position 1265 of the MLV-derived sequence.

The MLV/HIV-p15 Gag expression construct has MLV *gag* sequences on an *Aat*II-*Bal*I fragment (nt 367 to 2055) inserted into HXBH10-PR$^-$ΔBal between engineered *Aat*II (nt 771) and *Pvu*II (nt 1879) sites. The resulting construct encodes an MLV *gag* gene that has the NC coding region precisely replaced by the p2 and p15 coding regions of HIV-1$_{HXB2}$. HXBH10-PR$^-$ΔBal is a variant of HXBH10-PR$^-$ with a deletion in the *pol* gene (nt 2621 to 4552).

The MLV/HIV-p6 Gag expression construct was obtained by replacing nt 771 to 2132 of the HIV-1-derived sequence in HXBH10-PR$^-$ΔBal with a fragment from MLV (nt 367 to 2234). The resulting construct contains the entire MLV *gag* coding sequence joined precisely at its 3′ end to the p6 coding sequence of HIV-1$_{HXB2}$. The 3′ end of the MLV *gag* coding sequence and the 5′ end of the HIV-1 p6 coding sequence were fused by a PCR-based strategy described previously (5). The MLV/HIV-p6(1-41) and MLV/HIV-p6(1-46) Gag expression constructs are variants of the MLV/HIV-p6 construct that carry premature termination codons generated by site-directed mutagenesis in place of codons 42 and 47 of the p6 coding sequence, respectively.

Cell culture and transfections. COS-7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Cells (1.4×10^6) were seeded into 80-cm^2 tissue culture flasks 24 h prior to transfection. The cultures were transfected with 25 μg of Gag expression plasmid by using a calcium phosphate precipitation technique (3) or cotransfected with 25 μg each of Gag and Vpr expression constructs.

Viral protein analysis. COS-7 cells were metabolically labeled with [^{35}S]cysteine or [^{35}S]methionine (50 μCi/ml) from 48 to 60 h posttransfection. Viral particles released during the labeling period were pelleted through sucrose cushions (in phosphate-buffered saline) for 90 min at 4°C and 26,000 rpm in a Beckman SW28 rotor. Pelleted virions were lysed in radioimmunoprecipitation assay (RIPA) buffer (140 mM NaCl, 8 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.05% sodium dodecyl sulfate [SDS]), and viral proteins were either directly analyzed by electrophoresis through SDS–12.5% polyacrylamide gels or immunoprecipitated prior to electrophoresis with a rabbit anti-Vpr serum (20).

RESULTS

Expression of chimeric Gag polyproteins. While the Gag polyproteins of HIV-1 and MLV exhibit very little sequence homology, the order in which the major Gag domains are linked is preserved (32). To identify HIV-1 Gag domains involved in the incorporation of Vpr, the sequences coding for the MA, CA, and NC domains of the MLV *gag* gene were individually replaced by corresponding HIV-1 sequences as illustrated in Fig. 1. MLV and HIV-1 *gag* sequences were joined precisely at domain boundaries by using blunt-end cloning sites generated by mutagenesis. The mutagenic primers were designed so that the predicted amino acid sequence of the fused domains remained unaltered. To examine the role of the HIV-1 p6 domain, which has no equivalent in the MLV Gag polyprotein, the HIV-1 p6 coding sequence was fused to the 3′ end of the MLV *gag* gene by using a PCR-based cloning strategy.

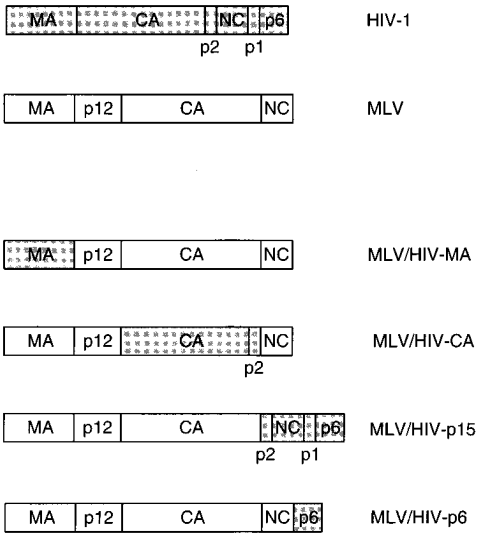

FIG. 1. Domain organization of the parental and chimeric *gag* gene products. Coding sequences for HIV-1 domains (shaded boxes) and MLV domains (open boxes) were fused without altering the predicted amino acid sequence of each domain.

The chimeric *gag* genes were inserted into the HXBH10 proviral construct in place of the HIV-1 *gag* and *pol* genes. An analogous construct that contains the authentic MLV *gag* gene has been described previously (10). Since it was shown that the processing of *gag* products is not required for the incorporation of Vpr (20, 21, 25), viral protease coding sequences flanking the chimeric *gag* genes were disrupted to simplify an analysis of the chimeric *gag* gene products. To examine the ability of the chimeric *gag* gene constructs to direct the production of viral particles, [^{35}S]cysteine-labeled particulate material released from transfected COS-7 cells was pelleted through 20% sucrose, solubilized in RIPA buffer, and analyzed directly by SDS-polyacrylamide gel electrophoresis (PAGE). As shown previously (10), transfection of the protease-defective HIV-1 proviral clone HXBH10-PR$^-$ resulted in the release of the HIV-1 Gag polyprotein precursor Pr55^{gag} in a particulate form (Fig. 2, lane 1). As expected, the appearance of the 55-kDa product in the particulate fraction depended on the presence of an open *gag* reading frame in the transfected DNA (Fig. 2, lane 6). For each of the chimeric *gag* gene constructs, predominant bands with electrophoretic mobilities that corresponded well with the expected sizes of the chimeric Gag polyproteins were obtained (Fig. 2, lanes 2 to 5). The intensities of the chimeric Gag polyprotein bands, taking into account the number of cysteine residues present in each molecule, indicated that each of the chimeric *gag* gene constructs had the ability to produce viral particles in COS-7 cells at least as efficiently as the parental HXBH10-PR$^-$ construct.

Association of Vpr with particles formed by chimeric Gag polyproteins. All HXBH10-based proviral constructs contained the defective *vpr* gene of the HXB2 molecular clone, which codes for a truncated Vpr product (2). Since Vpr can be

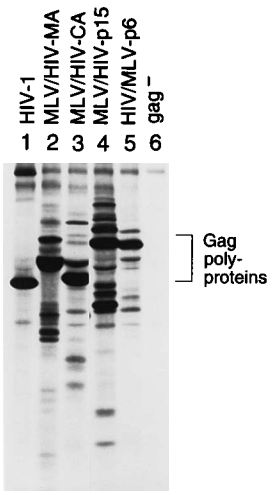

FIG. 2. Particle formation by chimeric Gag polyproteins. COS-7 cells were transfected with HXBH10-PR$^-$ (lane 1), the indicated chimeric Gag expression constructs (lanes 2 to 5), or HXBH10-*gag*$^-$ (lane 6) and metabolically labeled with [^{35}S]cysteine from 48 to 60 h posttransfection. Particulate material released into the supernatant was pelleted through 20% sucrose, disrupted in RIPA buffer, and directly analyzed by SDS-PAGE.

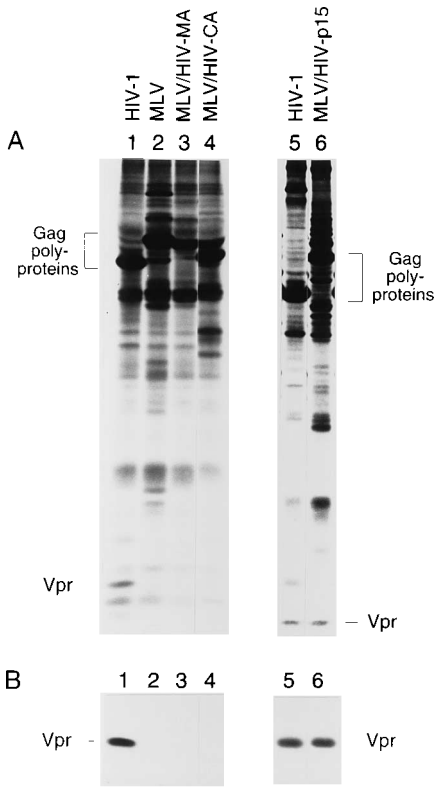

FIG. 3. Incorporation of Vpr into particles formed by chimeric Gag polyproteins. COS-7 cells were cotransfected with ptrENV and either the HIV-1 Gag polyprotein expression construct HXBH10-PR$^-$ (lanes 1 and 5), the MLV Gag polyprotein expression construct HXBH10/NCA$_{gag}$-PR$^-$ (lane 2), or the chimeric Gag expression constructs MLV/HIV-MA (lane 3), MLV/HIV-CA (lane 4), and MLV/HIV-p15 (lane 6). [^{35}S]methionine-labeled particulate material released into the supernatant was pelleted through 20% sucrose and disrupted in RIPA buffer. Aliquots were either analyzed directly by SDS-PAGE (A) or immunoprecipitated with rabbit anti-Vpr serum prior to SDS-PAGE (B).

incorporated in *trans* (20), a complete *vpr* gene was supplied by cotransfection of ptrENV, a variant of the HIV-1$_{BRU/LAI}$ molecular clone pBRU-1 with large deletions in the *gag*, *pol*, and *env* genes (25a). To determine the amount of Vpr protein that was incorporated into viral particles, the transfected COS-7 cells were labeled with [^{35}S]methionine from 48 to 60 h posttransfection and particulate material released during the labeling period was sedimented through 20% sucrose. Cotransfection of the protease-defective HIV-1 proviral clone HXB H10-PR$^-$ and ptrENV resulted in the appearance of a 14-kDa protein in the solubilized particulate fraction (Fig. 3A, lane 1) that was not detectable when only HXBH10-PR$^-$ was transfected (data not shown). The efficient immunoprecipitation of the 14-kDa product from the particulate fraction by a rabbit anti-Vpr peptide antiserum confirmed that this protein indeed was Vpr (Fig. 3B, lane 1). Consistent with previous results (21), the 14-kDa *vpr* gene product was not detectable in the particulate fraction when ptrENV was cotransfected with HXBH10-*gag*$^-$, an HIV-1 proviral construct that is unable to form particles because of the presence of premature termination codons in the *gag* gene (data not shown).

It was reported previously that a Vpr molecule fused to a nine-amino-acid epitope tag was not detectably incorporated into MLV particles (25). To examine whether the native Vpr protein associates with MLV particles, we used a protease-defective variant (HXBH10/NCA$_{gag}$-PR$^-$) of the previously described HXBH10/NCA$_{gag}$ chimera, which contains the *gag* gene of MLV in an HIV-1 background (10). We demonstrated previously that the transfection of the HXBH10/NCA$_{gag}$ construct into HeLa cells results in the release of viral particles that contain the MLV Gag proteins (10). The protein compositions of particles released from COS-7 cells cotransfected with HXBH10/NCA$_{gag}$-PR$^-$ and ptrENV are shown in Fig. 3A (lane 2). The particulate fraction contained the MLV Gag polyprotein Pr65^{gag} in molar amounts that exceeded by about fivefold the amounts of pelletable HIV-1 Pr55^{gag} obtained after transfection of the HXBH10-PR$^-$ proviral DNA (Pr65^{gag} has 3 methionines, and Pr55^{gag} has 15). Nevertheless, a band corresponding to Vpr was not detectable in the MLV particle preparation as verified by immunoprecipitation (Fig. 3B, lane 2). Since Vpr was expressed at comparable levels in cells cotransfected with ptrENV and either HXBH10-PR$^-$ or HXBH 10/NCA$_{gag}$-PR$^-$ (data not shown), these results confirmed that MLV particles are unable to incorporate Vpr.

Similarly, Vpr was detectable in the cell lysates (not shown) but not in the particulate fractions when ptrENV was cotransfected with proviral constructs which expressed MLV Gag polyproteins that had the MA or CA domain replaced by that of HIV-1 (Fig. 3A and B, lanes 3 and 4). Products with the expected mobilities of the MLV/HIV-MA and MLV/HIV-CA Gag precursors were observed in molar amounts comparable to those of Pr55^{gag} (Fig. 3A), taking into account the number of methionine residues in each molecule. Thus, the transfer of the HIV-1 MA or CA domain was not sufficient to achieve Vpr incorporation.

By contrast, significant amounts of Vpr were detected in the particulate fraction when ptrENV was cotransfected with the MLV/HIV-p15 *gag* gene construct (Fig. 3A and B, lanes 6), which contains HIV-1 *gag* sequences coding for the region downstream of the scissile bond that defines the C terminus of the mature CA protein (Fig. 1). To further delimit the HIV-1 Gag sequences required for Vpr incorporation, the coding sequence for the C-terminal HIV-1 p6 domain was fused to the 3′ end of the MLV *gag* gene (Fig. 1). As shown in Fig. 4A and B (lanes 1), Vpr was found in the pelletable fraction when coexpressed with the MLV/HIV-p6 Gag product. The relative

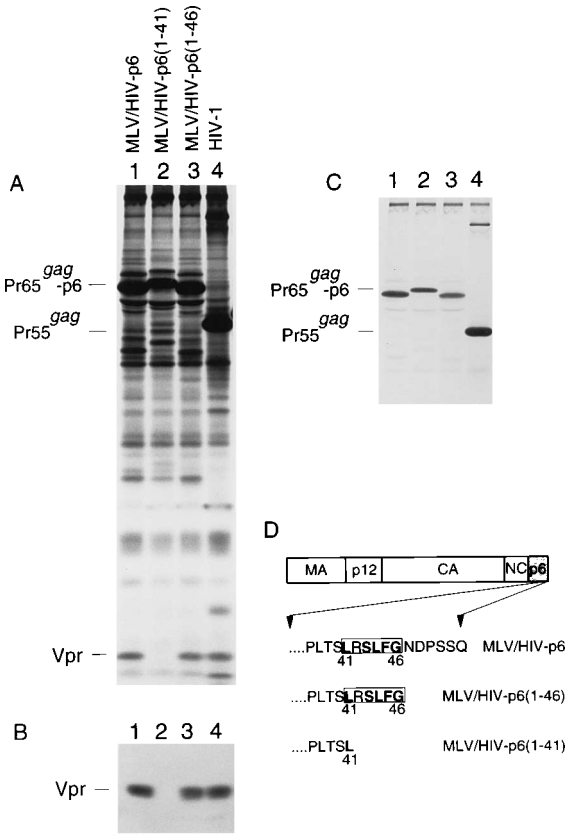

FIG. 4. Role of HIV-1 p6 sequences in Vpr incorporation. COS-7 cells were cotransfected with ptrENV and either the chimeric Gag expression constructs MLV/HIV-p6 (lane 1), MLV/HIV-p6(1-41) (lane 2), and MLV/HIV-p6(1-46) or with the HIV-1 Gag polyprotein expression construct HXBH10-PR$^-$. [^{35}S]methionine-labeled particulate material released into the supernatant was pelleted through 20% sucrose and disrupted in RIPA buffer, and aliquots were either analyzed directly by SDS-PAGE (A) or immunoprecipitated with rabbit anti-Vpr serum prior to SDS-PAGE (B). Panel C shows a shorter autoradiographic exposure of panel A for the positions occupied by Gag polyproteins. Panel D illustrates the domain organization and the C-terminal amino acid sequence of the Pr65^{gag}-p6 products expressed by MLV/HIV-p6-based constructs. A conserved six-amino-acid motif near the C terminus of the p6 domain is boxed. Residues that are invariant among the HIV-1, HIV-2/SIV$_{mac}$, and SIV$_{agm}$ groups of primate immunodeficiency viruses are highlighted in boldface. Numbers refer to the positions of amino acids in the HIV-1$_{HXB2}$ p6 domain.

intensities of the Vpr and Gag protein bands in Fig. 4A and C, taking into account the number of methionines in Pr55^{gag} and in the MLV/HIV-p6 Gag product (15 versus 3), indicated that the amounts of Vpr associated with particles formed by Pr55^{gag} (lanes 4) and by the MLV/HIV-p6 Gag product (lanes 1) were comparable. Thus, the presence of the HIV-1 p6 domain was sufficient for Vpr incorporation.

Role of a C-terminal conserved region in p6 in Vpr incorporation. Among the Gag domains of different subtypes of HIV-1, the p6 domain is by far the most variable, both in length and in sequence (23). However, two short regions within the p6 domain are highly conserved among primate immunodeficiency viruses. One of these conserved motifs (P-T/S-A-P-P) is located near the N terminus of the p6 domain, and the other (L-X-S-L-F-G) is located near its C terminus. Both motifs are nearly invariant among viruses that are known to possess a *vpr*-like gene.

To examine the role of the conserved motif near the C terminus of p6, premature termination codons which truncate the p6 domain were introduced into the MLV/HIV-p6 *gag* gene construct. The MLV/HIV-p6(1-46) construct harbors a premature termination codon which deletes only the six variable residues immediately downstream of the conserved motif (Fig. 4D). By contrast, the chimeric Gag polyprotein encoded by the MLV/HIV-p6(1-41) construct lacks most of the conserved motif as a result of an 11-amino-acid deletion from the C terminus of the p6 domain (Fig. 4D). As shown in Fig. 4A to C, the six-amino-acid deletion in the MLV/HIV-p6(1-46) construct did not affect the molar ratio between *vpr* and *gag* gene products in the particulate fraction (lanes 3). By contrast, the deletion of an additional five amino acids from the C terminus of the p6 domain prevented the appearance of Vpr in the particulate fraction, while the release of pelletable Gag protein was not affected (lanes 2). These results indicate that the conserved motif near the C terminus of p6 is essential for the association of Vpr with viral particles, whereas sequences C terminal to the conserved motif are dispensable.

DISCUSSION

The results presented here provide evidence that the p6 domain of the HIV-1 Gag polyprotein mediates the virion association of Vpr. Previous studies had shown that Vpr was not incorporated into viral particles formed by C-terminally truncated HIV-1 Gag precursors that lacked the p6 domain (21, 25). However, these observations did not exclude the possibility that the requirement for the p6 domain arose from distal conformational effects of the truncations rather than the loss of a Gag domain directly involved in the virion association of Vpr. Our study demonstrates that the p6 domain alone, when fused to the C terminus of the MLV Gag polyprotein, is sufficient for the association of Vpr with viral particles. The association of Vpr with the hybrid particles was dependent on p6 sequences, as Vpr was not incorporated into particles formed by the authentic MLV Gag polyprotein. Moreover, since the molar amount of Vpr relative to Gag that was present in the hybrid particles was comparable to that found in authentic HIV-1 particles, our results demonstrate that HIV-1 Gag regions other than the p6 domain are dispensable for the efficient incorporation of Vpr. These observations are in agreement with the recent proposal that Vpr binds directly to the p6 domain of the Gag polyprotein during HIV-1 virion assembly (25). A direct interaction of Vpr with the HIV-1 Gag polyprotein was also suggested by experiments in which Vpr coprecipitated with Gag products (20).

Consistent with our observation that the HIV-1 p6 domain encompasses a virion association motif for Vpr, the presence both of a p6 domain and of a Vpr-like gene product is a common feature of primate immunodeficiency viruses (23, 27). It therefore seems likely that the p6 domains of primate immunodeficiency viruses other than HIV-1 are similarly involved in the incorporation of Vpr and, in cases of HIV-2 and SIV$_{mac}$, of the homologous virion-associated gene product Vpx. The p6 domain also appears to have a function distinct from its role in Vpr incorporation, since a p6 domain is required for HIV-1 replication in tissue culture (11) while Vpr is not (4, 24). We reported previously that alterations that affect the p6 domain of the HIV-1 Gag polyprotein can cause a defect at a late step of viral budding (11).

The p6 domain exhibits considerable sequence variability among different groups of primate immunodeficiency viruses. Even within the HIV-1 group, the p6 domains of the most divergent subtypes share less than 35% identical residues (23). This low level of overall sequence conservation may reflect some flexibility of the Vpr association motif and points to two conserved regions within the p6 domain as potential sites of

interaction. Our results show that a short, highly conserved motif near the C terminus of the p6 domain is essential for the incorporation of Vpr. By contrast, the region C terminal to the conserved motif, which is variable in length, is dispensable. However, other nonconserved regions within the p6 domain are likely to contribute to the interaction, since particles formed by the Gag polyprotein of HIV-2_{ROD} incorporate the *vpr* gene product of the HIV-1_{BRU} strain with very low efficiency (18).

In contrast to the p6^{gag} domain, the Vpr protein is well conserved among different HIV-1 genotypes (27). It appears likely that the evolutionary pressure for the conservation of the Vpr sequence arises only in part from the need to preserve a virion association motif, while other sequences are needed to exert its function in the infected cell. It was reported recently that the deletion of 13 amino acids from the C terminus of Vpr resulted in a relatively stable protein that was not efficiently incorporated into virions (25). However, additional sequences within Vpr appear to be required for incorporation, since the deleted region alone was not sufficient to direct the virion association of a fusion protein.

It is noteworthy that each of the three major domains of the MLV Gag precursor could be individually replaced by HIV-1 sequences while retaining the ability to efficiently produce viral particles. We reported previously that the MA domain of HIV-1 can substitute for the MA domain of the more closely related visna virus without significant effects on the efficiency of particle formation (7). The results of the present study suggest that the MA, CA, and NC domains of even widely divergent retroviruses are functionally equivalent with respect to their roles in viral particle assembly and release. It was reported previously that viral particles were formed only at low levels when the MA or CA domain in an MLV provirus was replaced precisely by the corresponding HIV-1 sequence (5). However, the chimeric Gag products could be rescued into virions by the coexpression of wild-type MLV Gag protein (5). Discrepancies in the observed efficiency of particle formation by chimeric Gag polyproteins may, at least in part, be due to the use of different expression systems. Furthermore, the region coding for the p2 peptide, which is located between the HIV-1 CA and NC domains, was included in the present study to replace the MLV CA region. It is conceivable that the presence of the 14-amino-acid spacer peptide facilitated the correct folding of the adjacent HIV-1 CA sequences in the context of the chimeric Gag polyprotein, as HIV-1 particle formation is particularly sensitive to alterations in the vicinity of the CA-p2 cleavage site (6, 12, 28).

Although several studies have demonstrated that Vpr facilitates virus replication in cells of the macrophage lineage (1, 13, 30), it is unclear whether the incorporation of Vpr into virions is essential for this effect. A requirement for incorporation for the function of Vpr is suggested by a recent study which shows that Vpr enhances the ability of HIV-1 nucleic acids to access the nucleus in newly infected nondividing cells such as monocyte-derived macrophages (14). The characterization of a specific virion association motif(s) in the p6 domain may help to further elucidate the functional relevance of Vpr incorporation.

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