Incorporation of Pr160\textsuperscript{gag-pol} into Virus Particles Requires the Presence of both the Major Homology Region and Adjacent C-Terminal Capsid Sequences within the Gag-Pol Polyprotein

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The determinants critical for the incorporation of Pr160\textsuperscript{gag-pol} into human immunodeficiency virus type 1 (HIV-1) particles were examined by cotransfecting cells with (i) a plasmid expressing wild-type Gag protein and (ii) a series of chimeric Gag-Pol expression plasmids in which individual murine leukemia virus (MLV) Gag regions and subdomains precisely replaced their HIV-1 counterparts. The presence of the MLV MA and NC Gag regions in the chimeric Gag-Pol precursor had no detectable effect on the incorporation of Gag-Pol into progeny virions. In contrast, the entire HIV-1 CA region was required to achieve wild-type levels of Gag-Pol assembly into particles; both the CA major homology region and the adjacent C-terminal CA sequences play dominant roles in this process yet, when assayed in the context of a chimeric Gag-Pol polyprotein, restored the defect affecting Gag-Pol incorporation to approximately half of the wild-type level.

The human immunodeficiency virus type 1 (HIV-1) \textit{pol} gene is expressed as a high-molecular-weight, frameshifted Gag-Pol protein (Pr160\textsuperscript{gag-pol}) encoding four enzymatic activities (protease, reverse transcriptase [RT], RNase H, and integrase). During particle assembly, Pr160\textsuperscript{gag-pol} associates with the 55-kDa Gag precursor (Pr55\textsuperscript{gag}), becomes incorporated into nascent particles at the plasma membrane, and is processed into the mature Pol proteins during or subsequent to the release of progeny virions from infected cells.

The formation of retroviral particles is a self-assembly process requiring only the expression of the Gag precursor polyprotein (21, 22, 33). For HIV-1, particle production has been extensively studied with vaccinia virus, baculovirus, and simian virus 40 expression systems as well as with subgenomic HIV-1 constructs containing at least the 5′ long terminal repeat and adjacent gag sequences (12, 16, 19, 25, 29, 30, 35, 43). Many studies of retroviral virion production have typically evaluated the contributions of different \textit{gag} gene domains using in vitro mutagenesis in conjunction with assays in which the morphologic, biochemical, and physical properties of the resultant particles have been monitored (7, 14, 17, 32, 39, 43).

Expression of Gag-Pol precursor proteins (by permanently shifting the \textit{pol} gene into the gag reading frame in retroviral molecular clones) in some retroviral systems, including that of HIV-1, results in the appearance of processed Gag and Pol proteins intracellularly and detectable amounts of non-virion-associated RT activity in the supernatant medium in some instances but not in production of progeny virus particles (20, 26, 36). However, when Gag- and Gag-Pol-expressing plasmids are cotransfected into the same cell, the Gag-Pol precursor becomes incorporated into virus particles which may contain fully processed Gag and Pol proteins (27, 36). This cotransfection strategy has been exploited in the HIV-1 system to identify determinants within the Gag-Pol polyprotein that mediate its entry into virions (24, 27, 36, 37).

It was originally thought that Pr160\textsuperscript{gag-pol} was incorporated into budding particles via interactions with Pr55\textsuperscript{gag} (43). Moreover, it was subsequently shown that while N-terminal myristylation of Pr55\textsuperscript{gag} was required for particle formation, N-terminal myristylation of Pr160\textsuperscript{gag-pol} was not needed for Gag-Pol assembly into virions (5, 13, 27, 36). More recently, the incorporation of Pr160\textsuperscript{gag-pol} into virus particles has been investigated by mutating discrete domains within the Gag-Pol precursor, frequently in constructs containing an inactivated protease gene to minimize any processing effects during the assembly and budding steps. Unfortunately, the results of these studies have not unambiguously delineated the critical determinants mediating the capture of Pr160\textsuperscript{gag-pol} in virus particles. For example, mutagenesis of individual, highly conserved amino acid residues within the HIV-1 CA major homology region (MHR) (43) of Pr160\textsuperscript{gag-pol} had little if any effect on its incorporation into virions (24). This result is to be contrasted with those of another study in which extensive deletions within the Gag CA domain of Pr160\textsuperscript{gag-pol} or the removal of only the 21 residues comprising the entire MHR element prevented its assembly into particles (37).

We have examined critical determinants mediating Pr160\textsuperscript{gag-pol} incorporation in cotransfection assays by using chimeric Gag-Pol precursor constructs in which individual HIV-1 Gag regions or subregions were precisely replaced with functionally analogous segments of Moloney murine leukemia virus (MLV), using a previously described PCR procedure (8). This approach, rather than deletion or site-specific mutagenesis, was chosen because it seemed plausible that incorporation of Gag-Pol is an evolutionarily conserved function shared among distantly related retroviruses. Our results show that the HIV-1 MA and NC Gag regions of HIV-1 Gag-Pol can be replaced by their MLV counterparts with no detectable effects on the capture of Gag-Pol in progeny virions. In contrast, the HIV-1 CA MHR and adjacent C-terminal CA sequences must be present within the Gag-Pol precursor for its incorporation into particles; the additional presence of the HIV-1 N-terminal CA domain restores Gag-Pol incorporation to wild-type levels.
MATERIALS AND METHODS

Construction of plasmids expressing HIV-1 Gag and the Gag-Pol polyprotein. In the experiments to be described, Gag and Gag-Pol expression constructs were derivatives of the full-length HIV-1 proviral DNA clone pNL4-3 (open rectangles) as described in Materials and Methods; the Gag regions within the parental HIV-1 and MLV Gag-Pol proteins are shown at the top. A similar group of protease-defective HIV-1 and HIV-1–MLV chimeric Gag-Pol expression plasmids were constructed by changing the Asp at HIV-1 protease residue 25 to Asn. The numbers above the diagrams of each Gag-Pol protein denote the amino acid residue located at the junctions between the different Gag regions, based on the originally reported sequence for the two parental viruses (1, 4). MA, matrix Gag protein; NC, Gag nucleocapsid protein; CA[N], amino-terminal portion of the Gag capsid protein; CA[C], carboxy-terminal portion of the Gag capsid protein; MHR, MHR of Gag capsid protein; p12, 12-kDa MLV Gag protein; p2, 2-kDa HIV-1 Gag protein; pol, HIV-1 pol-encoded proteins.

Construction of HIV-1–MLV chimeric Gag-Pol-expressing plasmids. The parental HIV-1 and MLV Gag-Pol as well as the different chimeric HIV-1–MLV Gag-Pol expression plasmids constructed for this study are shown in Fig. 1. A molecular clone of Moloney MLV DNA, originally isolated from the A9 mouse fibroblast cell line (4) and designated pMLV in this study, was used as the source of MLV gag sequences in the chimeric Gag-Pol constructs. Resequencing of the MLV gag gene (GenBank accession no. J02255 to J02257) revealed a single base change at position 1397 (T to C), which did not alter the amino acid residue specified.

A two-step PCR procedure, originally described by Deminie and Emerman (8), was used to precisely replace HIV-1 Gag regions and subdomains with analogous MLV Gag segments within the HIV-1 Gag-Pol expression plasmid pGP1. The initial amplification reaction utilized chimeric PCR primers (containing HIV-1 and MLV sequences) that precisely bridged desired junctions between the two gag genes. Subsequent to this initial PCR, specific amplified products were mixed, denatured, reannealed, and then subjected to a second PCR. In the second PCR, primer pairs that contained restriction sites for insertion of the final amplified product into the pGP1 expression plasmid were utilized. The primer sequences and restriction sites incorporated into the primers to generate the different chimeric Gag-Pol constructs are available from us. Vent DNA polymerase (New England Biolabs, Beverly, Mass.) was used for all PCRs as specified by the manufacturer. In addition, the denaturation and annealing conditions during the initial rounds of the second PCR were modified as follows: (i) 1 cycle of denaturation at 93°C for 4 min and annealing for 1 min at 90, 85, 80, 75, 70, 65, 60, 55, 50, and 45°C, followed by 72°C for 1 to 2 min, depending on fragment length; (ii) 5 cycles at 93, 90, 85, 80, 75, 70, 65, 60, 55, 50, and 45°C
for 1 min, followed by 72°C for 1 to 2 min, depending on fragment length; and (iii) 24 cycles at 93°C for 1 min, 60°C for 1 min, and 72°C for 1 to 2 min, the last again depending on fragment length.

The construct GP<sub>MLV</sub> Gag</sub> which contains the entire MLV gag</sub> segment, was made from the previously constructed pNL3, pMLV, pGP<sub>MLV</sub> MA</sub>, and pGP<sub>MLV</sub> NC</sub> plasmid DNAs by standard recombinant DNA techniques. To make the GP<sub>MLV</sub> gag</sub>HIV CA</sub> and GP<sub>MLV</sub> gag</sub>HIV MHR/C</sub> constructs, the two-step PCR procedure described in the preceding paragraph was used to precisely replace MLV gag</sub> subdomains with analogous HIV-1 gag</sub> segments within the GP<sub>MLV</sub> Gag</sub> construct. A PCR-based, site-specific mutagenesis approach (9, 15) was used to create a myristylation-negative form of GP<sub>MLV</sub> gag</sub>HIV CA</sub> by introducing a Gly-to-Ala change at codon 2 in the Gag ORF.

All of the chimeric constructs were sequenced to confirm the presence of desired mutations.

Cell culture, transfection, and RT assay. HeLa cells were maintained in Dulbecco's modified Eagle's medium containing 5% fetal bovine serum. Calcium phosphate transfections were carried out as described previously (10). In cotransfection experiments, the Gag-Pol- and Gag-expressing DNAs were present at a ratio of 1:9 (a total of 25 μg of DNA), respectively; when Gag-Pol plasmids were transfected alone, the reaction mixture was supplemented with nine times more pUC19 DNA. RT assays were performed as reported previously (42) with reaction mixtures containing 0.8 mM EDTA. Assays measuring the release of particle-associated or free RT activity into the medium of transfected HeLa cells have been previously described (11).

Metabolic labeling and immunoprecipitation. The procedure used for metabolic labeling of transfected HeLa cells has been described previously (18). Immunoprecipitation was conducted (41) with AIDS patient sera or rabbit anti-HIV-1 RT polyclonal antibody, obtained from the National Institute of Allergy and Infectious Diseases AIDS Research and Reference Reagent Program (human HIV immune globulin, catalog no. 192; rabbit HIV-1 RT antiserum, catalog no. 654). All precipitates were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis analysis in 8% or 10% acrylamide-AcrylAide (FMC) gels. The gels were fixed in methanol and acetic acid, stained with 1 M salicylic acid, dried at 100°C, and exposed on X-Omat AR film (Kodak).

RESULTS

The strategy used to assess determinants within the HIV-1 Gag-Pol protein that facilitate its incorporation into virus particles was to coexpress, in the same cell, HIV-1 Pr<sub>55</sub> and a series of HIV-1–MLV chimeric Pr<sub>160</sub> proteins containing MLV substitutions for HIV-1 gag</sub> regions (24, 27, 36, 37). The chimeric HIV-1–MLV Gag-Pol proteins used in these analyses are shown in Fig. 1; each contains MLV gag</sub> sequences precisely introduced into HIV-1 Pr<sub>160</sub> and was constructed by a modification of the procedure originally described by Deminie and Emerman (8). In the experiments to be described, HeLa cells were cotransfected with a plasmid expressing HIV-1 Pr<sub>55</sub> (designated G<sub>HIV</sub>) plus one of the Gag-Pol constructs shown in Fig. 1. The RT activity released into the medium was monitored by RT assay (42), and the radiolabeled viral proteins present in cells or associated with pelleted particulate virus particles released from the cells were analyzed by immunoprecipitation (18). As expected (26, 36), when G<sub>HIV</sub> alone was transfected into HeLa cells, RT activity was detected in the medium but was not particle associated (Fig. 2A). In contrast, cotransfection of G<sub>HIV</sub> with the HIV-1 Pr<sub>160</sub> expression plasmid (G<sub>P</sub>HIV) resulted in substantial amounts of particle-associated RT activity in the HeLa cell supernatant, as previously reported with such constructs (26, 36). When the same cotransfection was conducted with a protease-deficient Pr<sub>160</sub> (G<sub>P</sub>HIVPR–), the released particle-associated RT activity was approximately 10 to 15% of that observed in cells transfected with the protease-competent G<sub>P</sub>HIV, also in agreement with previous reports (26, 36). In all experiments of this type, the ratio of Pr<sub>G</sub>HIV to Pr<sub>P</sub>HIV was 9:1 in the transfection reaction mixture; when G<sub>P</sub>HIV was assayed alone, the total DNA concentration was maintained by supplementing the mixture with a ninefold excess of pUC19 DNA.

Replacement of the Gag portion of HIV-1 Gag-Pol with MLV analogs. In an initial experiment, the entire Gag region of HIV-1 Gag-Pol was replaced with MLV gag</sub> sequences to generate GP<sub>MLV</sub> Gag</sub>. Transfection of GP<sub>MLV</sub> Gag</sub> alone into HeLa cells resulted in the release of ample amounts of RT into the medium (Fig. 2B, left). However, only a small fraction of this secreted RT activity became virion associated when GP<sub>MLV</sub> Gag</sub> was cotransfected with G<sub>HIV</sub> (Fig. 2B, middle). Because (i) the RT assay used to detect Gag-Pol incorporation is dependent on the efficient processing of the chimeric GP<sub>MLV</sub> Gag</sub> and (ii) HIV-1 protease may be less active in cleaving chimeric Gag-Pol than the HIV-1 Gag-Pol, the cotransfection experiment was repeated with a protease-deficient GP expression plasmid. As shown in Fig. 2B (right), a similar result was obtained: compared with those of G<sub>P</sub>HIVPR–, very low levels of RT activity were detected in the culture supernatant when GP<sub>MLV</sub> Gag</sub> was used.

To rule out the possibility that contemporaneous synthesis of chimeric Gag-Pol negatively affects nascent particle production, thereby reducing the RT activity detected, radioimmunoprecipitation experiments were carried out with an AIDS patient serum or a rabbit polyclonal antibody directed against HIV-1 RT (Fig. 2C). Furthermore, protease-deficient GP expression plasmids were used in these assays because (i) they permitted an assessment of Gag-Pol incorporation to be made by the visualization of uncleaved Pr<sub>160</sub> in the gel and (ii) they provided unambiguous information regarding Pr<sub>55</sub> synthesis. Forty-eight hours postcotransfection with G<sub>HIV</sub> plus G<sub>P</sub>HIVPR– or GP<sub>MLV</sub> Gag</sub>PR–, HeLa cells were labeled with [<sup>35</sup>S]methionine for 12 h and viral proteins were immunoprecipitated from cell lysates or pelleted particles. In addition to the Gag and Env proteins immunoprecipitated from the cell lysate with patient serum, the HIV-1 and chimeric Gag-Pol precursor proteins were also visualized (Fig. 2C, left); their identity was confirmed with polyclonal anti-RT antibody (Fig. 2C, top). In contrast, HIV-1 Pr<sub>160</sub> was not incorporated into pelleted particles following immunoprecipitation with either antibody (Fig. 2C, right). Taken together, these results show that although the GP<sub>MLV</sub> Gag</sub> precursor was stably expressed, it was not incorporated into HIV-1 particles.

Introduction of individual MLV Gag regions or subdomains into HIV-1 Gag-Pol. Previous studies of Gag-Pol incorporation into nascent HIV-1 particles concluded that portions of the CA region present in HIV-1 Pr<sub>160</sub> were required for optimal and stable protein-protein interaction between the two precursor molecules (37, 40). The failure to incorporate GP<sub>MLV</sub> Gag into HIV-1 particles was consistent with these reports and prompted us to evaluate six additional chimeric Gag-Pol precursors containing individual MLV Gag regions or subdomains. As shown in Fig. 3A (top), all of the chimeric Gag-Pol constructs except GP<sub>MLV</sub> MA</sub> and GP<sub>MLV</sub> NC</sub> transferred alone, released levels of non-particle-associated RT activity into the medium comparable to that directed by the parental G<sub>HIV</sub>. This result suggests that the synthesis, intracellular transport, and release of five of the seven chimeric Gag-Pol proteins were roughly equivalent to that of HIV-1 Pr<sub>160</sub>. However, when assayed by cotransfection with G<sub>HIV</sub>, only GP<sub>MLV</sub> MA</sub> and GP<sub>MLV</sub> NC</sub> secreted substantial amounts of RT activity (Fig. 3A, middle). The five chimeric Gag-Pol constructs containing replacements for all or parts of the HIV-1 CA region (viz., GP<sub>MLV</sub> CA</sub> and GP<sub>MLV</sub> CA</sub>HR; GP<sub>MLV</sub> CA</sub>N</sub> and GP<sub>MLV</sub> CA</sub> and GP<sub>MLV</sub> CA</sub>HR</sub>C</sub>) released much smaller amounts of virion-associated RT activity into the medium. Similar results were obtained when the same chimeric Gag-Pol constructs were assayed in a protease-deficient background (Fig. 3A, bottom). Radioimmunoprecipitation of labeled proteins in transfected cells or pelleted from the culture supernatant confirmed these results and revealed that although high-molecular-weight chimeric Gag-Pol proteins were synthe-
sized in cells cotransfected with GP<sub>MLV</sub><sub>CA[MHR]</sub><sup>PR</sup> and GP<sub>MLV</sub><sub>CA[C]</sub><sup>PR</sup>, the amount of chimeric precursor that became incorporated into particles was markedly reduced (Fig. 3B). Thus, the results of radioimmunoprecipitation experiments are consistent with the RT activities measured in the medium of cells transfected with the chimeric Gag-Pol constructs. In the case of GP<sub>MLV</sub><sub>Gag[N]</sub>, however, the absence of a high-molecular-weight intracellular protein (Fig. 3B) or significant amounts of virion-associated RT activity (Fig. 3A) suggests that this chimeric Gag-Pol protein may not be synthesized or is very unstable. Consequently, the role of the N-terminal half of the CA region in mediating Gag-Pol incorporation into virions cannot be directly evaluated in this experiment.

Rescue of defective GP<sub>MLV</sub><sub>Gag</sub> incorporation. The experiments described thus far show that determinants for Gag-Pol incorporation into nascent particles are not located in the MA or NC regions but reside in the CA region and include elements located in the MHR and C-terminal half of this Gag segment. To confirm and extend these results, gain of function experiments were carried out with two constructs in which HIV-1 CA sequences were added back to the chimeric GP<sub>MLV</sub><sub>Gag</sub> construct, shown in Fig. 2 to be markedly deficient for Gag-Pol incorporation. As shown in Fig. 4A, the presence of the entire HIV-1 CA domain, in a background of an otherwise MLV Gag region (viz., in GP<sub>MLV</sub><sub>Gag[HIV CA]</sub>), restored the particle-associated RT activity to wild-type levels. Inclusion of only the HIV-1 PR plus the adjacent C-terminal CA sequences (in GP<sub>MLV</sub><sub>Gag[HIV MHR]</sub>) resulted in the release of approximately 60% of the wild-type RT levels. These results were confirmed by radioimmunoprecipitation (Fig. 4B), which revealed that wild-type levels of GP<sub>MLV</sub><sub>Gag[HIV CA]</sub> were in-
corporated, compared to only 56% of wild-type levels for GPMLV Gag[HIV MHR/C], as determined by phosphorimager analysis of the ratio of cell-associated to virion-associated Gag-Pol. Taken together, the data presented in Fig. 3 and 4 show that the MHR and adjacent C-terminal CA sequences are able to mediate Gag-Pol incorporation but that the complete HIV-1 CA region is required to achieve wild-type levels.

Incorporation of unmyristylated chimeric Gag-Pol proteins.

Previous studies have shown that unmyristylated HIV-1 Gag-Pol can be incorporated into nascent HIV-1 particles (27, 36). Although analogous experiments have not been performed in the MLV system, two previous reports are relevant to this issue. In one, the incorporation of unmyristylated Moloney MLV Pr65⁰⁰⁰ into particles was measured in cells dually infected with wild-type amphotropic MLV (isolate 4070A) and the Moloney MLV myristylation mutant. Under conditions in which 3% of virion-associated Moloney MLV p12 was expected to be detected, none was measured (31). This result suggests that both the MLV Pr65⁰⁰⁰ and the MLV Gag-Pol precursor proteins were not incorporated into the nascent amphotropic MLV particles. In the other study, which monitored the incorporation of MLV Gag–β-galactosidase (not MLV Gag-Pol) into virions, the myristylated, but not the unmyristylated, fusion protein was assembled into MLV particles (14).

To ascertain whether myristylation was required for the incorporation of GPMLV Gag[HIV CA]myr, which contains the MLV MA region, into HIV-1 particles, the second amino acid residue was changed from Gly to Ala, a mutation previously shown to block MLV MA myristylation (14, 28). Following cotransfection of GPMLV Gag[HIV CA]myr with G HIV, levels of particle-associated RT activity comparable to those of G HIV were detected (Fig. 4A). Preliminary experiments (not shown) revealed that Gag-Pol incorporation did not increase significantly when the ratio of the Gag plasmid to the Gag-Pol plasmid varied from 1:1 to 10:1 (keeping pGag constant), indicating that the amount of HIV-1 Gag present in our assay system was limiting. Incorporation of Gag-Pol, as assayed under standard conditions in our system, therefore reflected the presence of specific Gag-Pol determinants rather than the intracellular concentration of Gag-Pol. Thus, the chimeric unmyristylated Gag-Pol polyprotein behaved like HIV-1 Pr160⁰⁰⁰ even though its MA region was of MLV origin.

FIG. 3. Mapping the domains in the Gag region of Gag-Pol required for its incorporation into particles. (A) HeLa cells were transfected (top graph) or cotransfected (middle and bottom graphs) with the indicated Gag-Pol expression plasmids. Protease deficient (PR−) Gag-Pol constructs were used in the cotransfection shown in the bottom panel. Cells were harvested, the medium was fractionated, and RT activity was measured and normalized as described in the legend to Fig. 2B. The total RT activity in the culture medium (top graph) or associated with pelleted virus particles (middle and bottom graphs) was measured. (B) HeLa cells were transfected with G HIV (the left-most lane in each gel) or cotransfected with G HIV plus the indicated PR-defective Gag-Pol expression plasmids. The transfected cells were labeled with [³⁵S]methionine, fractionated, immunoprecipitated with AIDS patient sera, and resolved on SDS–8% polyacrylamide-AcrylAide gels as described in the legend to Fig. 2C. Molecular mass markers are noted at the right in kilodaltons (K).
Gag-Pol lacking these sequences than with a similar construct containing the entire HIV-1 CA region, which displayed the wild-type pattern. Nonetheless, the presence of both the MHR and the adjacent C-terminal segment of the HIV-1 CA region was sufficient to effect partial restoration of a Gag-Pol incorporation defect. The Gag MA and NC regions, also present in HIV-1 Pr160\(^{p60-pol}\), play little if any role in the inclusion of Pr160\(^{p60-pol}\) in progeny virions as monitored in these assays.

These results complement and extend earlier studies of HIV-1 Pr160\(^{p60-pol}\) incorporation. Subtle alterations of the CA component of HIV-1 Gag-Pol (viz., individual point mutations that change three highly conserved residues in the HIV-1 MHR) had only modest effects on Gag-Pol incorporation (24). It should be noted that all three of these conserved MHR residues were present in the chimeric \(\text{GP}\text{MLV}_{\text{CA}}\text{MHR}\), which, nonetheless, failed to be incorporated into HIV-1 particles. In contrast, the introduction of multiple deletions into the CA region of HIV-1 Pr160\(^{p60-pol}\), including one that removed the 21 residues comprising the MHR, blocked Gag-Pol entry into progeny virus particles (37). One of these deletions, which eliminated about 100 amino acid residues of the CA region immediately upstream of the MHR but retained the MHR and the adjacent C-terminal portion of the CA region, produced a level of incorporation 50% of that of the wild type, as determined by the two-plasmid cotransfection assay. The results of this second study point to the importance of the MHR and possibly other portions of the CA region in mediating Gag-Pol incorporation. Our results should, of course, be tempered by the possibility that the defects in chimeric Pr160\(^{p60-pol}\) incorporation observed in fact reflect aberrant intracellular transport due to the presence of MLV CA elements. Altered trafficking of the chimeric Gag-Pol precursor away from the intracellular site where interaction with Pr55\(^{p55}\) occurs would register as defective incorporation.

Another earlier study examined the requirements for incorporating an HIV-1 Gag–β-galactosidase fusion protein (not Pr160\(^{p60-pol}\)) into HIV-1 particles (40). Deletion of the entire CA region blocked incorporation of the fusion protein into virions, whereas retention of the C-terminal two-thirds of HIV-1 CA resulted in wild-type levels of Gag–β-galactosidase capture, reiterating, yet again, the functional importance of CA elements in this process. However, in contrast to other reports demonstrating that the Pr160\(^{p60-pol}\) protein is able to enter progeny particles, even as an unmyristylated polypeptide (27, 36), unmyristylated Gag–β-galactosidase was not incorporated, thereby raising the issue of the suitability of this fusion protein as an HIV-1 Pr160\(^{p60-pol}\) surrogate. In a previous study evaluating the incorporation of an analogous Moloney MLV Gag–β-galactosidase fusion protein into MLV particles generated from Psi2 packaging cells, Hansen et al. reported that (i) linker insertion mutations affecting the CA domain blocked incorporation of the fusion protein and (ii) the unmyristylated Gag–β-galactosidase protein did not enter progeny MLV particles (14).

Although we did not examine the possible role(s) of Pol sequences in HIV-1 Pr160\(^{p60-pol}\) incorporation, there are numerous previous reports describing deleterious effects of RT and integrase mutations on virion formation and particle-associated RT activity (2, 3, 34, 38). For example, Engelman et al. (9) showed by radioimmunoprecipitation that a single point mutation in IN or an extensive deletion that removed all but the 4 N-terminal residues of IN markedly reduced the amounts of virion-associated p66 and p51 RT. More recently, Bukovsky and Göttlinger (6) observed the same phenomena with IN mutants and with a more extensive IN plus RT deletion mutant lacking all of IN as well as the adjacent C-terminal 134 amino acids.
acids of RT. However, the latter group noted that the block in HIV-1 particle production associated with mutations in the pol gene could be reversed by either mutating the viral protease or exposing the transfected cells to an HIV-1 protease inhibitor. In fact, their radioimmunoprecipitation analyses revealed that C-terminally truncated Gag-Pol proteins were present at wild-type levels in pelletted particles in the absence of active protease, implying that the incorporation of HIV-1 Pr160\(^{\text{gag-pol}}\) into particles is not dependent on IN or the C terminus of RT (6). Taken together with those of our experiments, these results again emphasize the importance of HIV-1 CA Gag sequences in mediating the incorporation of Gag-Pol into virions.

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REFERENCES


