JOURNAL OF VIROLOGY, Dec. 1994, p. 8180–8187
0022-538X/94/$04.00+0

Vol. 68, No. 12

Functional Domains of the Capsid Protein of Human Immunodeficiency Virus Type 1

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Received 6 June 1994/Accepted 16 September 1994

A series of deletions was introduced into the CA domain of the human immunodeficiency virus type 1 Gag polyprotein to examine its role in virus particle and core formation. The mutations resulted in two phenotypes, indicating the existence of two functionally distinct regions within the CA domain. Deletions within a conserved stretch of 20 amino acids referred to as the major homology region (MHR) and deletions C terminal to this region blocked virus replication and significantly reduced the ability to form viral particles. Deletions N terminal to the MHR also prevented virus replication, but the mutants retained the ability to assemble and release viral particles with the same efficiency as the wild-type virus. The mutant particles contained circular rather than cone-shaped cores, and while they were of a density similar to that of wild-type particles, they were more heterogeneous in size. These results indicate that CA domain sequences N terminal to the MHR are essential for the morphogenesis of the mature cone-shaped core.

Human immunodeficiency virus type 1 (HIV-1) particles assemble and bud simultaneously from the plasma membrane of infected cells (27, 45). The internal structural proteins of the virion are encoded by the *gag* gene and are synthesized as a polyprotein precursor ($Pr55^{gag}$) (27, 45). Viral gene products other than $Pr55^{gag}$ are not required for particle formation (20, 23, 37). Morphological maturation of newly released particles is characterized by the appearance of a condensed core and requires cleavage of $Pr55^{gag}$ by the viral protease (18, 22, 29, 34). The proteolytic cleavage sites define the different domains of $Pr55^{gag}$ which yield the matrix (MA), capsid (CA), and nucleocapsid (NC) proteins of the mature virion (25, 33). In addition to these products, which are common to all retroviruses, HIV-1 virions also contain a peptide ($p6^{gag}$) derived from the C terminus of $Pr55^{gag}$.

The N-terminal MA domain of $Pr55^{gag}$ has a signal sequence for the cotranslational attachment of myristic acid (11, 33), which is required for particle assembly at the cell membrane (4, 22). Additional sequences within the MA domain appear to determine the site of viral assembly, since a large internal deletion within the HIV-1 MA domain led to the redirection of viral particle assembly to intracellular membranes (16). Recent studies have shown that the HIV-1 MA domain is both essential and sufficient for incorporation of the viral envelope glycoprotein into nascent particles (13, 46). It has also been reported that the HIV-1 MA domain possesses a nuclear localization sequence that contributes to the ability of HIV-1 to replicate in nondividing cells (5).

The NC domain of $Pr55^{gag}$ contains two copies of an evolutionarily conserved sequence motif known as the cysteine-histidine box which are critical for efficient encapsidation of the viral genomic RNA (1, 12, 21). The HIV-1 NC domain was found to bind to HIV-1 RNA in vitro with a specificity similar to that of the entire Gag precursor, suggesting that specific RNA packaging during virion assembly is mediated primarily by the NC domain of $Pr55^{gag}$ (2). Direct evidence for a role of a retroviral NC domain in the specific uptake of genomic viral RNA was recently provided by a study of the packaging specificity of a mutant of Rous sarcoma virus in which the NC domain was replaced by that of Moloney murine leukemia virus (14). Retroviral NC domains also appear to be involved in annealing of the tRNA primer to the primer-binding site and dimerization of the genomic viral RNA (9, 35).

The role of the central CA domain of retroviral Gag polyproteins in viral morphogenesis and replication has been less well established than that of the MA and NC domains. The CA domain forms the shell which encases the condensed core in the mature virus particle (19). Mutations in the CA domain of different retroviruses have had remarkably different effects. Most deletions or linker insertion mutations in the CA domain of Moloney murine leukemia virus blocked the assembly of the mutant proteins into virions (24, 38). By contrast, most of the CA domain of Rous sarcoma virus was found to be dispensable for particle assembly and release, including a uniquely conserved stretch of 20 amino acids known as the major homology region (MHR) (45). In HIV-1, a 56-amino-acid deletion near the N terminus of the CA domain had no effect on particle formation (43) but specific residues within the MHR were shown to be crucial for this process (32).

To gain a more comprehensive understanding of the role of the CA domain in HIV-1 virion morphogenesis, we generated a series of small deletions throughout the CA coding region. All deletions that affected the MHR or CA sequences C terminal to this region significantly reduced viral particle formation. By contrast, deletions N terminal to the MHR had no effect on the assembly and release of viral particles. However, these deletions prevented the formation of cone-shaped virion cores, which appears to be essential for early steps in the viral life cycle.

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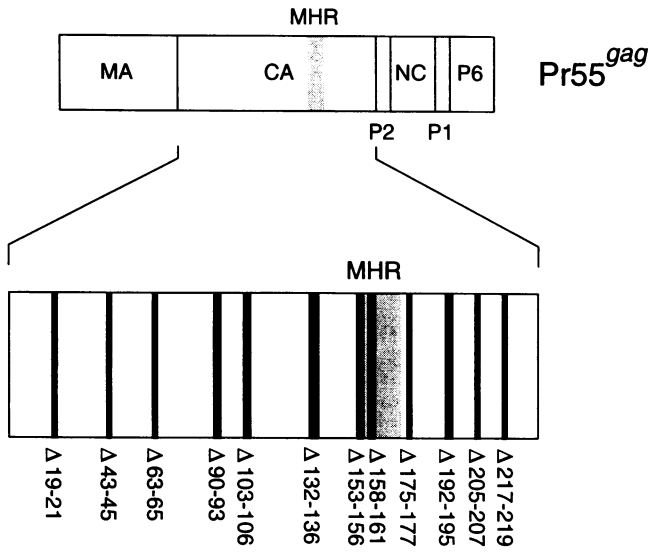

FIG. 1. Locations of deletions in the CA domain of HIV-1 Gag polyprotein Pr55^{gag}. The domain organization of Pr55^{gag} is shown at the top, and the position of the MHR in the CA domain is indicated. The CA domain is enlarged below. Numbers refer to the positions of the deleted amino acids relative to the N terminus of the CA domain.

MATERIALS AND METHODS

Proviral DNA constructs. For site-directed mutagenesis of the CA coding region, single-stranded DNA was prepared from plasmid pSK$^+$*gag* (22), which contains a 1.3-kb *Sac*I-*Apa*I *gag* fragment from the infectious HXBc2 proviral clone of HIV-1, and used as a template for annealing of oligonucleotides and primer extension with T4 DNA polymerase as previously described (30). Deletions introduced into the CA coding region are shown in Fig. 1. To regenerate full-length proviral clones after mutagenesis, 1.3-kb *Bss*HII-*Apa*I fragments (nucleotides 710 to 2009) carrying the desired mutations were inserted into the parental *vpu*$^+$ HXBH10 proviral construct (41) in exchange for the wild-type fragment. 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 (13).

Cell culture and transfections. Jurkat cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum. HeLa cells (CCL2) were obtained from the American Type Culture Collection (Rockville, Md.) and grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum. For virus replication studies, Jurkat cells (5×10^6) were transfected by the DEAE-dextran procedure (36) with 2.5 μg of proviral plasmid DNA. HeLa cells (10^6) were seeded into 80-cm^2 tissue culture flasks 24 h prior to transfection. The cells were transfected with 30 μg of proviral plasmid DNA by a calcium phosphate precipitation technique (8).

Viral protein analysis. HeLa cell cultures and aliquots of transfected Jurkat cells were metabolically labeled for 12 h with [^{35}S]cysteine (50 μCi/ml). Labeling of HeLa cells was begun 48 h posttransfection. Labeled cells were lysed in 1× 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 virus particles in cell-free supernatant fractions were disrupted by adding 5× RIPA buffer. HIV-1-encoded proteins were immunoprecipitated by using serum from an individual infected with HIV-1 and separated in SDS–12% polyacrylamide gels as previously described (10). To analyze particle-associated viral protein, particulate material released into the supernatant was pelleted through a 20% sucrose cushion (in phosphate-buffered saline [PBS]) for 2 h at 4°C and 27,000 rpm in a Beckman SW41 rotor. Pelleted virions were lysed in RIPA buffer, and viral proteins were either directly analyzed by electrophoresis through SDS–12% polyacrylamide gels or immunoprecipitated with a patient's serum prior to electrophoresis. For equilibrium centrifugation, pelleted virions were resuspended in 2 ml of Dulbecco's modified Eagle's medium and layered on top of preformed 10 to 60% sucrose gradients (in PBS). The samples were centrifuged at 40,000 rpm in an SW41 rotor for 16 h at 4°C, and 0.6-ml fractions were then collected from the bottom of the tubes. The fractions were diluted eightfold in Dulbecco's modified Eagle's medium–10% fetal calf serum, and virions were lysed by adding 5× RIPA buffer. Viral proteins were then immunoprecipitated with a patient's serum and analyzed by electrophoresis through SDS–12% polyacrylamide gels.

Quantitation of virion-associated viral RNA. HeLa cell cultures were metabolically labeled with [^{35}S]cysteine (10 μCi/ml) from 48 to 60 h posttransfection. Virus particles released into the culture supernatant during the labeling period were pelleted through 20% sucrose cushions and resuspended in 0.2 ml of TNE (10 mM Tris hydrochloride [pH 7.5], 150 mM NaCl, 5 mM EDTA). Aliquots were removed and analyzed directly by SDS-polyacrylamide gel electrophoresis (PAGE). *Saccharomyces cerevisiae* tRNA (50 μg/ml) was added to the rest of the pelleted material, and virions were lysed by adding SDS to a final concentration of 0.5%. The viral RNA was extracted with phenol-chloroform and ethanol precipitated. A uniformly labeled RNA probe synthesized from *Mro*I-linearized pTZ18U/MB was hybridized to RNA extracted from the viral particles, and unhybridized portions of the probe were degraded by RNase as previously described (12). RNase-resistant fragments were separated on a 4.5% polyacrylamide–8.3 M urea gel, and the dried gel was subjected to autoradiography and analysis with a Betascope 603 Blot Analyzer (Betagen Corporation, Waltham, Mass.). The pTZ 18U/MB template (12) contains an *Mro*I-*Bss*HII fragment from pHXB-SV (nucleotides 308 to 713) in the antisense orientation with respect to a T7 RNA polymerase promoter. The *Mro*I-*Bss*HII fragment represents 5′ long terminal repeat and untranslated leader sequences upstream of the HIV-1 major splice donor site. The probe can distinguish between viral RNA and contaminating plasmid DNA (12).

Electron microscopy. Culture supernatants were harvested 60 h after transfection of HeLa cells with proviral plasmid DNAs, and particulate material in the supernatant fractions was pelleted at 20,000 × *g* for 1 h at 4°C. The pelleted material and the transfected HeLa cell monolayers were fixed in fresh 2.5% glutaraldehyde in PBS at pH 7.0 and postfixed with 1% OsO_4. After agar block enclosure, the fixed material was embedded in Epon. Thin sections were made approximately 60 to 80 nm thick and poststained with 1% uranyl acetate. Specimens were analyzed at an accelerating voltage of 80 kV with a Zeiss CEM 902 electron microscope equipped with a goniometer stage for analysis at different tilt angles.

RESULTS

Effects of deletions in CA on virus replication. We introduced a series of deletions into the CA coding region to remove between three and five codons in each mutant and alter regions which are identical or highly conserved between

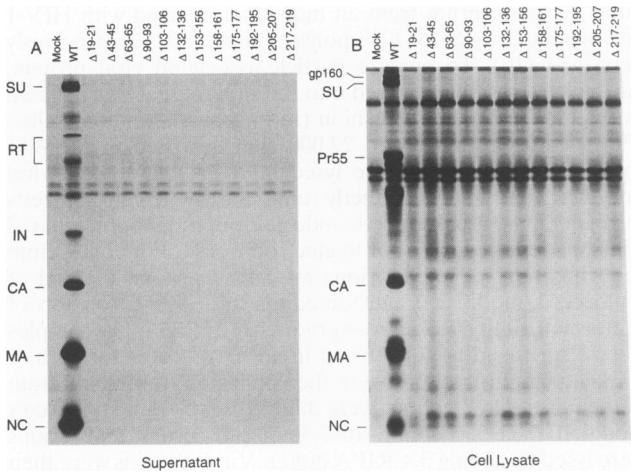

FIG. 2. Effects of deletions in the CA domain on viral replication. Jurkat cells were transfected with the parental HXBH10 provirus (wild type [WT]) or the deletion mutants indicated. Seven days posttransfection, aliquots were labeled with [^{35}S]cysteine and viral proteins were immunoprecipitated from the culture supernatants (A) and cell lysates (B) and resolved on SDS–12% polyacrylamide gels. Mock, mock transfection; SU, surface glycoprotein; RT, reverse transcriptase; IN, integrase.

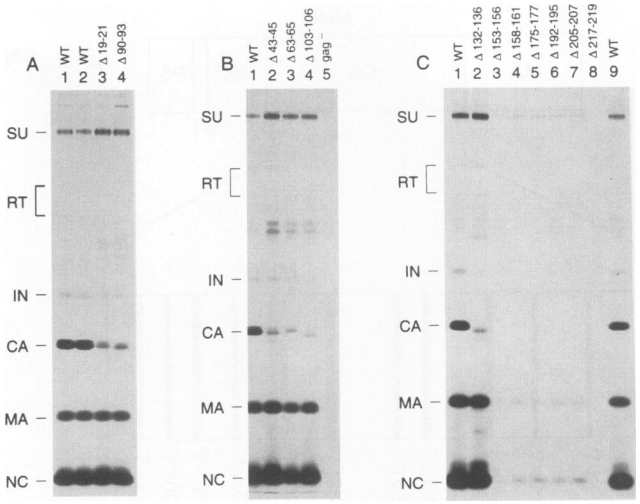

FIG. 3. Effects of deletions in the CA domain on viral particle formation. HeLa cells were transfected with the parental provirus (wild type [WT]) or the deletion mutants indicated and then metabolically labeled with [^{35}S]cysteine from 48 to 60 h posttransfection. Virions released into the supernatant were pelleted through 20% sucrose, and viral proteins in the pellets were immunoprecipitated with serum from an individual infected with HIV-1. Lanes 1 and 2 in panel A are duplicate samples. In panel B, the HXBH10-*gag*$^-$ construct was used as a negative control (lane 5). For definitions of abbreviations, see the legend to Fig. 2.

the CA domains of HIV-1 and HIV-2. The deletion mutations are shown in Fig. 1. Each deletion was introduced into the HXBH10 proviral construct, a *vpu*$^+$ variant of HXB2 (41).

To examine the effects of the deletions on virus replication, Jurkat cells were transfected with parental HXBH10 proviral DNA or mutant proviral DNAs. Equivalent numbers of cells from the transfected cultures were metabolically labeled with [^{35}S]cysteine at 1 week and 1 month posttransfection, and viral proteins were immunoprecipitated with serum from an individual infected with HIV-1. At 1 week posttransfection, *gag*-, *pol*-, and *env*-encoded proteins were abundant in the supernatant of cells transfected with the parental construct but could not be detected after transfection of any of the mutant proviral DNAs (Fig. 2A). Viral proteins were readily detected 1 week posttransfection in the lysate of cells transfected with parental proviral DNA but, with the possible exception of a faint band comigrating with NC protein, not in the lysate of cells transfected with any of the mutant DNAs (Fig. 2B). At 1 month posttransfection, *gag*- and *env*-encoded proteins were still abundant in the wild-type lysate but absent from the mutant lysates (data not shown). These results indicate that all 12 deletions in the CA domain prevented sustained expression of viral proteins in transfected Jurkat cells, which requires virus replication (12).

Effects of deletions on particle production. To determine the effects of the deletions on particle production, the mutant proviruses were transfected into HeLa cells. The amount of viral protein that is transiently expressed upon transfection of HeLa cells with HIV-1 proviral DNA is sufficient for analysis of the particle-forming ability of replication-defective mutants (12). After metabolic labeling with [^{35}S]cysteine, particulate material released into the supernatant of the transfected cells was pelleted through 20% sucrose. Pelleted virions were lysed in RIPA buffer, and viral proteins were immunoprecipitated with serum from an individual infected with HIV-1.

The six mutants with deletions N terminal to the MHR (Δ19-21, Δ43-45, Δ63-65, Δ90-93, Δ103-106, and Δ132-136) all yielded amounts of particle-associated MA and NC proteins which were comparable to those obtained with the parental construct (Fig. 3A to C). These results revealed that the ability to form viral particles was not reduced as a consequence of the deletions. However, the deletions caused a significant decrease in the amount of CA protein relative to that of MA or NC protein in immunoprecipitates from purified virion preparations (Fig. 3). This result was due only in part to inefficient immunorecognition of the mutant CA proteins, since reduced levels of CA relative to MA and NC proteins were also observed when the virion lysates were directly analyzed by SDS-PAGE (see Fig. 5; data not shown).

In contrast to the deletions N terminal to the MHR, the six deletions within or C terminal to the MHR (Δ153-156, Δ158-161, Δ175-177, Δ192-195, Δ205-207, and Δ217-219) all caused a major defect in particle formation (Fig. 3C). As measured by densitometry, the amount of MA and NC proteins in the pelletable fractions was reduced by about 10- to 15-fold as a consequence of the Δ158-161, Δ175-177, Δ192-195, and Δ205-207 mutations (Fig. 3C, lanes 4 to 7). Particle formation was almost completely blocked by the Δ153-156 mutation, which deletes the N-terminal four amino acids of the MHR (lane 3), and by the Δ217-219 mutation, which deletes three amino acids near the C terminus of the CA domain (lane 8). To determine whether the decrease in particle production was secondary to a decrease in the intracellular levels of the mutant proteins, the steady-state levels of wild-type and mutant Gag products in the transfected cells were compared. Immunoprecipitation after a 12-h labeling period revealed only small differences among the levels of Pr55^{gag} in the lysates of cells transfected with the wild-type and mutant proviruses (Fig. 4). However, the levels of processed Gag products were significantly reduced by the mutations.

Characterization of mutant particles. Since none of the deletions N terminal to the MHR significantly affected the efficiency of particle formation yet the mutants were unable to

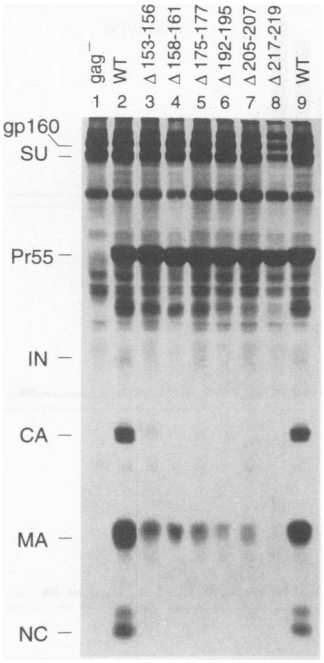

FIG. 4. Effects of deletions within or downstream of the MHR on cell-associated Gag protein levels. HeLa cells were transfected with HXBH10-*gag*⁻ (lane 1), the parental HXBH10 provirus (lanes 2 and 9), or the CA deletion mutants indicated (lanes 3 to 8). After metabolic labeling with [^{35}S]cysteine from 48 to 60 h posttransfection, the cells were lysed and viral proteins were immunoprecipitated with a patient's serum and analyzed by SDS-PAGE. For definitions of abbreviations, see the legend to Fig. 2. WT, wild type.

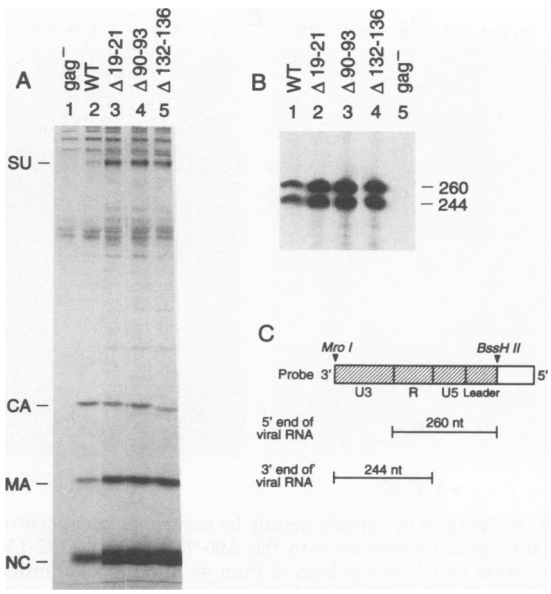

FIG. 5. Viral RNA contents of CA mutant virions. (A) Comparison of amounts of particle-associated viral protein present in wild-type (WT) and mutant virion preparations. HeLa cells were transfected with HXBH10-*gag*⁻ (lane 1), the parental HXBH10 provirus (lane 2), or the CA deletion mutants indicated (lanes 3 to 5). After metabolic labeling, particulate material released into the supernatant was pelleted through 20% sucrose and resuspended and equivalent aliquots were directly analyzed by SDS-PAGE. (B) RNase protection analysis of RNA extracted from the rest of the pelleted material. (C) Structure of the probe and expected sizes of fragments protected from RNase digestion by the 5′ and 3′ ends of the viral RNA. Hatched segments of the RNA probe refer to regions of complementarity between the probe and viral sequences. SU, surface glycoprotein. The numbers to the right of panel B indicate the lengths of protected fragments (in nucleotides).

replicate, mutant particle RNA content, density, and morphology were examined. To determine whether incorporation of genomic viral RNA was affected, HeLa cells transfected with parental proviral DNA or the Δ19-21, Δ90-93, and Δ132-136 mutants were metabolically labeled with [^{35}S]cysteine and virions released into the supernatant were pelleted through sucrose and resuspended. Aliquots were analyzed by SDS-PAGE to compare the amounts of viral protein in the virion pellets. RNA was then extracted from the rest of the pelleted material and subjected to quantitative RNase protection analysis (12). An excess of a radiolabeled probe complementary to the viral long terminal repeats was used to distinguish viral RNA from any contaminating proviral plasmid DNA in the preparations. In the experiment whose results are shown in Fig. 5, the mutant virion preparations contained more particles than the wild-type virion preparation as judged from the amounts of MA and NC proteins present (Fig. 5A). The mutant virion preparations also contained more viral RNA (Fig. 5B). However, the ratios of viral RNA to MA or NC protein in the wild-type and mutant virion preparations were similar, suggesting that the wild-type and mutant particles contained similar amounts of viral RNA.

Wild-type and mutant particle densities were examined by equilibrium centrifugation in sucrose gradients. HeLa cells were transfected with parental proviral DNA or the Δ90-93 and Δ132-136 mutants and metabolically labeled with [^{35}S]cysteine from 48 to 60 h posttransfection. Virions released into the supernatants were pelleted through 20% sucrose and resuspended in culture medium. The samples were then layered on top of 10 to 60% sucrose gradients. After centrifugation to equilibrium, fractions were collected from the bottom of the gradients and immunoprecipitated with serum from an individual infected with HIV-1. Most of the *gag*- and *pol*-encoded protein was found in three consecutive fractions, indicating that both the wild-type and mutant particles were of rather uniform density (Fig. 6 and data not shown). Surface glycoprotein (gp120) was also detected in fractions of lower density that lacked the Gag proteins, possibly because of an association with cellular vesicular material (28). For both mutants, the fractions that contained the largest amount of viral protein had a density of 1.16 g/ml, which is similar to the density of wild-type HIV-1 virions (43).

To examine whether virion morphology was affected, HeLa cells transfected with the parental and mutant proviruses were analyzed by transmission electron microscopy. The morphology of virus particles was also examined by goniometer analysis at different tilt angles. Wild-type virions varied in diameter between 95 and 175 nm (Fig. 7). Mature wild-type virions exhibited a conical core structure in vertical sections and a round, dense core in horizontal sections (Fig. 8A). Viral particles produced by the Δ19-21, Δ90-93, and Δ132-136 mutants were more heterogeneous in size and varied between 75 and 315 nm (Fig. 7 and 8B to E). Mutant particles with a conical core structure were not detected. Instead, mutant particles with a morphologically mature appearance contained round, dense core structures in all section planes. Particles produced by all three mutants occasionally contained two dense core structures along the inner surface of the virion

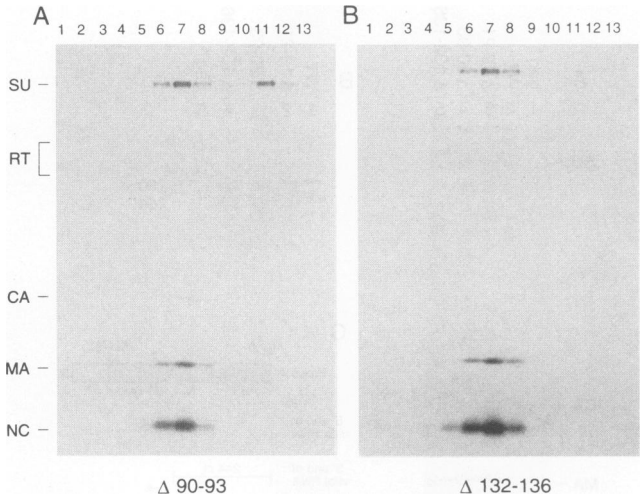

FIG. 6. Analysis of particle density by sucrose gradient centrifugation. HeLa cells transfected with the Δ90-93 (A) and Δ132-136 (B) mutants were metabolically labeled from 48 to 60 h posttransfection. Particulate material released into the supernatant was pelleted through 20% sucrose, resuspended in Dulbecco's modified Eagle's medium, and subjected to density equilibrium centrifugation in 10 to 60% sucrose gradients. Fractions were collected and analyzed by immunoprecipitation with serum from an individual infected with HIV-1. For definitions of abbreviations, see the legend to Fig. 2.

envelope (Fig. 8C). Neither released virions nor budding structures were detected in cultures transfected with the Δ153-156 or Δ217-219 mutant (data not shown), indicating that the defect in particle production displayed by these mutants was due to a defect at the level of particle assembly rather than release.

DISCUSSION

Although it is well established that expression of retroviral Gag polyproteins in the absence of any other viral proteins is sufficient to induce the formation of enveloped virus-like particles (20, 23, 27, 37), the roles of the different domains of Gag precursors in viral particle formation are poorly understood. The genetic analysis presented in this study demonstrates that sequences within the C-terminal third of the HIV-1 CA domain are crucial for particle formation. By contrast, mutants with deletions in the remainder of the HIV-1 CA domain retained the ability to produce viral particles with wild-type efficiency. However, the mutant particles lacked cone-shaped cores, indicating that these deletions affected the assembly of the core shell which surrounds the viral nucleoid in mature wild-type virions.

The important role of the MHR for HIV-1 particle formation was confirmed by the phenotype of the Δ153-156 and Δ158-161 deletion mutants. We had shown previously that even conservative single-amino-acid substitutions at two invariant positions of the MHR (glutamine 155 and glutamic acid 159) significantly impaired HIV-1 particle formation (32). Similarly, particle formation can be blocked by single-amino-acid substitutions in the MHR of Mason-Pfizer monkey virus (40). However, despite its unique evolutionary conservation, the MHR does not appear to have the same role in all retroviruses, since mutants of Rous sarcoma virus that lacked the MHR were shown to retain the ability to form particles with wild-type efficiency (45).

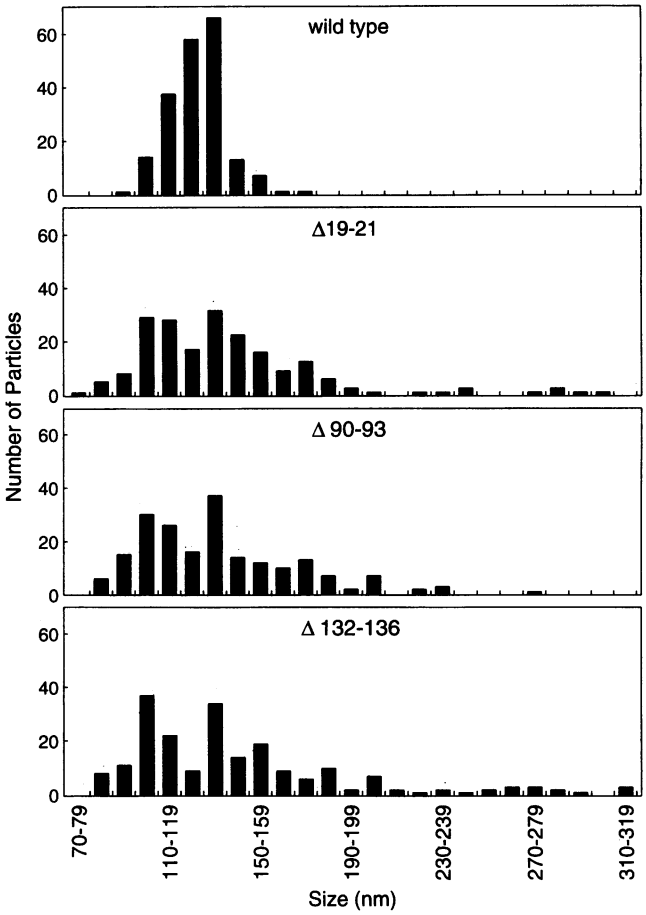

FIG. 7. Comparison of the size distribution of wild-type and mutant virions (200 of each) by electron microscopy.

It is likely that the defect in particle formation caused by deletions in or C terminal to the MHR was at the level of assembly rather than release, since no cell-associated viral structures could be detected for the Δ153-156 and Δ217-219 mutants. Also, the appearance of cell-associated Gag cleavage products was impaired, which may reflect effects on polyprotein multimerization, as suggested previously (31). The presence of an assembly domain near the carboxy terminus of retroviral CA domains was indicated previously by the observation that C-terminal truncations extending into the CA domain of Rous sarcoma virus disrupted particle formation (45). In Moloney murine leukemia virus, C-terminal CA domain sequences were required for efficient incorporation of Gag–β-galactosidase fusion proteins into virus particles (28). In HIV-1, a recent analysis of a series of linker insertion mutants suggested that a region within the C-terminal two-thirds of the CA domain is important for viral particle assembly (7). In addition, a region of 12 amino acids near the C terminus of the CA domain that is conserved among lentiviruses was found to be critical for HIV-1 particle formation (42). Our results are consistent with this finding and show that additional sequences within the C-terminal third of the HIV-1 CA domain are also critical.

It is likely that the defect in assembly was caused, at least in part, by a defect at the level of Gag protein multimerization. It was demonstrated in vitro that the isolated CA domain pos-

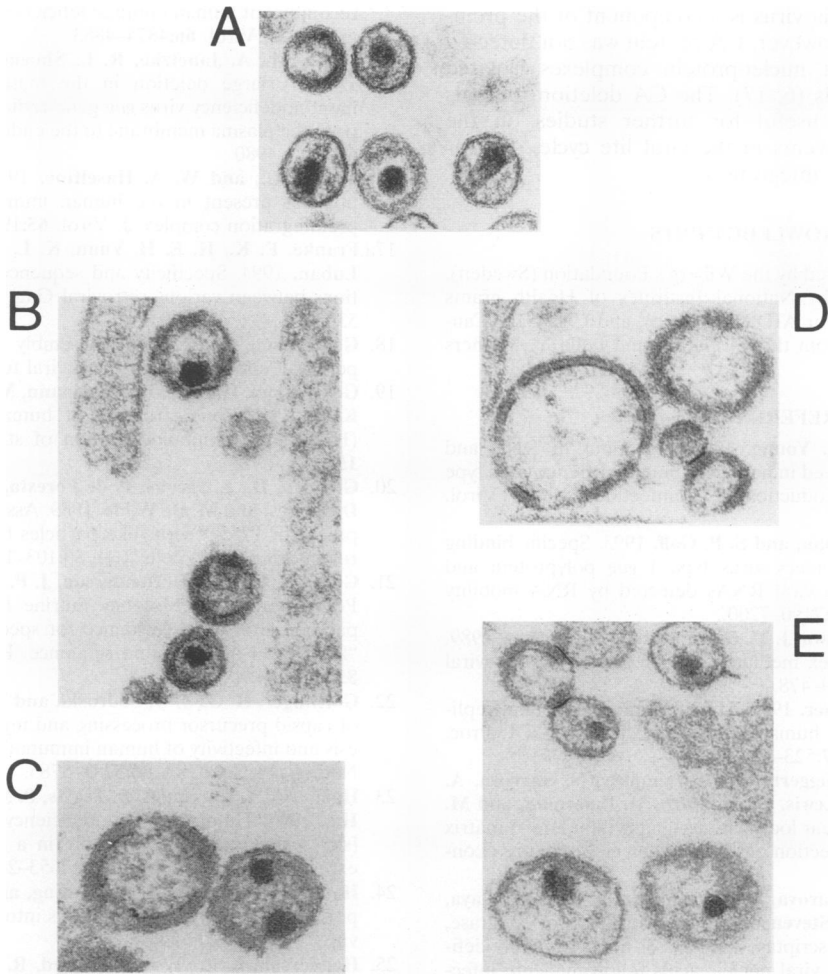

FIG. 8. Electron micrographs of virions produced after transfection of HeLa cells with parental proviral DNA (A) or CA deletion mutant Δ19-21 (B), Δ90-93 (C), or Δ132-136 (D and E). Magnification, ×75,000.

sesses self-associative properties (15). Furthermore, a recent study has shown that apart from the NC domain, sequences other than the second half of the CA domain are dispensable for HIV-1 Gag polyprotein multimerization in the yeast two-hybrid system (17a). Our results could, alternatively, be explained by an effect of the mutations on transport of the mutant Gag proteins to the site of assembly. A defect in intracellular transport was recently reported for mutant HIV-1 Gag precursors with altered MA domains (16, 39, 47). However, large deletions in the MA domain led to the formation of virus-like particles at intracellular sites (16, 39), a phenotype which was not observed for assembly-defective CA domain mutants.

Deletions upstream of the MHR prevented virus replication but did not significantly affect particle formation. Despite marked size heterogeneity, the mutant virions had a uniform density similar to that of wild-type virions. Particles of wild-type density but heterogeneous size were also observed for mutants of Rous sarcoma virus with large deletions in the N-terminal half of the CA domain (44), indicating that sequences within this region are required in different retroviruses to produce particles of uniform size. In HIV-1, it was recently reported that a mutant with a 56-amino-acid deletion near the N terminus of the CA domain can efficiently produce viral particles of wild-type density (43). Our results, together with these observations, suggest that most of the CA domain upstream of the MHR is dispensable for the formation of particles of wild-type density.

Although the efficiency of particle formation was unaffected, deletions N terminal to the MHR resulted in the formation of aberrant virion cores. The core material condensed into spherical rather than cone-shaped structures. Formation of spherical core structures was previously observed when proteolytic liberation of the CA domain from the envelope-associated MA domain was prevented by a cleavage site mutation (22). This finding demonstrated that the viral nucleoid has the ability to condense in the absence of a surrounding core shell. The similar morphology of the core of the CA deletion mutants suggests that the mutant capsid proteins were also unable to encase the viral nucleoid in a core shell.

Mutations that prevent the formation of a cone-shaped core shell are usually lethal (22, 32), suggesting that the core shell has an essential function in the early stages of the virus replication cycle. In Moloney murine leukemia virus, it was reported that a mutant with substitutions in the N-terminal half of the CA domain produced viral particles that were defective at a stage prior to reverse transcription of the viral RNA (26). It has also been reported that the CA protein of

Moloney murine leukemia virus is a component of the preintegration complex (3). However, CA protein was not detected in integration-competent nucleoprotein complexes isolated from HIV-1-infected cells (6, 17). The CA deletion mutants described here will be useful for further studies on the requirements for early events in the viral life cycle, such as reverse transcription and integration.

ACKNOWLEDGMENTS

S.H. and Å.Ö. are supported by the Wiberg's Foundation (Sweden). This work was supported by National Institutes of Health grants AI29873, AI28691 (Center for AIDS Research), and CA06516 (Cancer Center) and by a gift from the G. Harold and Leila Y. Mathers Charitable Foundation.

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