JOURNAL OF VIROLOGY, June 1990, p. 2653–2659
0022-538X/90/062653-07$02.00/0

Vol. 64, No. 6

Human Immunodeficiency Virus-Like, Nonreplicating, *gag-env* Particles Assemble in a Recombinant Vaccinia Virus Expression System

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Received 29 November 1989/Accepted 28 February 1990

We report the assembly of human immunodeficiency virus (HIV)-like particles in African green monkey kidney cells coinfected with two recombinant vaccinia viruses, one carrying the HIV-1 *gag* and protease genes and the other the *env* gene. Biochemical analysis of particles sedimented from culture supernatants of doubly infected cells revealed that they were composed of *gag* proteins, primarily p24, as well as the *env* proteins gp120 and gp41. Thin-section immunoelectron microscopy showed that these particles were 100 to 120 nm in diameter, were characterized by the presence of cylindrical core structures, and displayed the mature gp120-gp41 complexes on their surfaces. Furthermore, thin-section immunoelectron microscopy analysis of infected cells showed that particle assembly and budding occurred at the plasma membrane. Nucleic acid hybridization suggested that the particles packaged only the *gag* mRNA but not the *env* mRNA. Therefore, the system we present is well suited for studies of HIV virion maturation. In addition, the HIV-like particles provide a novel and attractive approach for vaccine development.

Maturation of human immunodeficiency virus type 1 (HIV-1) is thought to involve the association of the envelope glycoprotein gp160 with the core proteins in a manner similar to that of other retroviruses (34). This association may occur either intracellularly or at the cell surface and correlates with the site of virus budding from target cells. For instance, HIV-1 has been shown to bud from the Golgi apparatus of infected macrophage-monocyte cell lines (11), suggesting that the *gag-env* interaction occurs in intracellular membranes. In contrast, the virus buds from the cell surface of infected CD4 T lymphocytes and lymphoblastoid cell lines (22), suggesting that the process of maturation and encapsidation may be initiated at the plasma membrane.

Previous studies directed to analysis of retrovirus assembly have shown that encapsidation and maturation of virions were dependent on membrane anchoring of both the core and envelope proteins (34). Direct association between the *gag* and *env* proteins has been described for two C-type retroviruses, the Rous sarcoma virus (8) and murine leukemia virus (32). This association occurred at the plasma membrane and was mediated, for Rous sarcoma virus, by the transmembrane domain of the *env* protein (30).

The HIV envelope precursor gp160 is anchored in membranes primarily by the transmembrane domain located in gp41 (3, 23). In addition, biochemical as well as structural analysis suggested that the gp160 formed a secondary association with the membrane bilayer mediated by sequences in its cytoplasmic tail (16, 37). Although the exact domains of p55 and gp160 involved in the maturation of HIV virions have not been elucidated to date, mutational analysis of the cytoplasmic tail region of gp160 suggested that it plays a role in this process. For instance, various sequence deletions from the C terminus of gp41 were reported to alter the infectivity (25) and pathogenicity (7, 25) of the virus and to modulate the intracellular transport and proteolytic processing of gp160 in mammalian cells (16a). The HIV p55 protein is anchored in cellular membranes by a myristic acid moiety added to the N-terminal penultimate glycine residue (2). Similar to other retrovirus systems (33), anchoring of the HIV-1 core protein has been shown to be necessary for production of virions (14).

Development of an in vitro recombinant expression system that mimics the HIV-1 virus synthesis and maturation pathways would be advantageous for studying the role of *env* and *gag* protein association in regulating virus formation and release. We (S.-L. Hu, B. M. Travis, J. Garrigues, P. Sridhar, J. M. Zarling, J. W. Eichberg, and C. E. Alpers, *in Vaccine*, in press) and others (12, 14) have previously shown that HIV-1 *gag* proteins synthesized in mammalian and insect cells by a variety of expression systems spontaneously assembled into viruslike particles that were released from cells. Myristoylation but not proteolytic processing of p55 was required for the assembly and production of the recombinant-made core particles (12, 14). Therefore, we hypothesized that coexpressing the *env* proteins in the same cell would result in *gag-env* interaction, followed by envelopment of the *gag* particles.

In this report, we present evidence for the assembly of HIV-like particles, generated in mammalian cells coinfected with recombinant vaccinia viruses carrying either the complete envelope gene of HIV-1 (v-*env5*) (21) or the complete *gag* and protease genes (v-*gag2*) (S.-L. Hu et al., in press). These recombinant particles bud from the plasma membrane and were similar to HIV-1 virion by morphology and protein content.

MATERIALS AND METHODS

Cells and viruses. African green monkey kidney cells (BSC-40) were used for recombinant vaccinia virus infection studies. The parental vaccinia virus used for generating the recombinant viruses was from the New York strain. The HIV-1 (BRU isolate) DNA fragment used to generate the

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v-*gag2* recombinant virus was of coordinates 258 to 3317 (S.-L. Hu et al., in press), while that used to generate the v-*env5* recombinant virus was of coordinates 5,671 to 8572 (21). The numbering system adopted was with respect to the start of the transcription initiation codon. The psoralen-inactivated HIV-1 virus was the LAV-1 strain.

Recombinant virus infection of BSC-40 cells. Monolayers of BSC-40 cells were grown to confluency in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum. Cells were infected with v-*env5*, v-*env5* + v-*gag2*, or v-*gag2* recombinants, or with v-NY parental virus at a multiplicity of infection of 10 PFU per cell of each virus. At 12 h postinfection, unless otherwise specified, the cells were radiolabeled for 2 h with [^{35}S]methionine and [^{35}S]cysteine (100 μCi per ml) (Amersham Corp.) in methionine- and cysteine-free media. The media were then collected, and the cells were washed with phosphate-buffered saline (PBS), harvested, and lysed in RIP buffer (1% Nonidet P-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate [SDS] in PBS). The postnuclear cell lysates, in parallel with the culture media, were assayed for HIV proteins by radioimmunoprecipitation (RIP) (16) with human polyclonal anti-HIV-1 sera (Trimar Inc.), followed by fractionation by SDS-polyacrylamide gel electrophoresis (PAGE) (24) in an 11.5% acrylamide matrix. The radioactive bands were visualized by fluorography and autoradiography.

Cell surface iodination. BSC-40 cells were infected with v-*env5* or coinfected with v-*env5* and v-*gag2* recombinants, in parallel with parental v-NY virus, at a multiplicity of infection of 10 PFU per cell of each virus. At 16 h postinfection, the culture media were aspirated and the cells were washed and radioiodinated with 0.5 mCi of ^{125}I by the lactoperoxidase-catalyzed reaction, as described previously (17). Cell lysates were assayed for HIV protein content by RIP and SDS-PAGE in an 8.5% acrylamide matrix.

Isolation and analysis of the HIV-like particles from the cell culture growth media. BSC-40 cells infected with v-*gag2* and v-*env5* as described above were radiolabeled at 5 h postinfection with [^{35}S]methionine and [^{35}S]cysteine (60 μCi per ml) for 10 h. The culture media from each infection condition (14 ml) were collected and clarified of cells by centrifugation at 1,500 rpm (model TJ-6; Beckman Instruments, Inc.) for 10 min. A 2-ml portion of the resulting supernatants was collected as starting, or total secreted (TS), material. The remaining 12 ml from each sample was fractionated by ultracentrifugation in an SW 41Ti rotor at 120,000 × *g* for 3 h into a pellet (P) and supernatant (S). The P was rinsed, suspended in PBS, overlaid onto a 2-ml 15% sucrose cushion, and sedimented again by ultracentrifugation in an SW 55Ti rotor at 120,000 × *g* for 1.5 h. The supernatant was discarded, and the P was suspended in RIP lysis buffer. The P, S (2-ml), and TS (2-ml) fractions were assayed for HIV proteins by RIP and were fractionated by SDS-PAGE in 11.5% acrylamide gels.

Sucrose gradient fractionation. The P from ultracentrifugation of the coinfected cell culture supernatant was suspended in 5% sucrose in PBS, overlaid onto a continuous sucrose gradient (15 to 60%), and sedimented at 120,000 × *g* in an SW 55Ti rotor for 1.5 h. The gradient fractions were collected from the bottom in 200-μl portions. The collected material was assayed for *gag* p24 content by capture enzyme immunoassay (EIA) (20) by using p24-specific monoclonal antibodies (MAbs).

Analysis of assembled particles by EM. The sedimented particulate material (P) from infected culture supernatants was washed several times by gently overlaying the pellet with PBS and then aspirating it off. The pellet was then fixed with 4% paraformaldehyde for 20 min and washed again with PBS. In a similar procedure, monolayers of BSC-40 cells (10^4 to 10^5) were infected with v-*gag2* and v-*env5* recombinant viruses. After 15 h, the growth medium was discarded and the cell monolayers were washed several times with PBS and fixed with 4% paraformaldehyde for 20 min at 22°C.

The fixed cells and P fractions were blocked with 0.8% bovine serum albumin–0.1% gelatin–5% normal goat serum in PBS. MAbs, specific for either gp120 (110-4) (27, 36) or gp41 (41-1) (13), were added as ascites fluid (1:2,000 in blocking buffer) to the various samples as indicated. After 3.5 h the samples were washed with PBS and incubated with colloidal gold-conjugated goat anti-mouse immunoglobulin G (in a ratio of 1:5) in blocking buffer for 2.5 h. Samples were then washed well with PBS, fixed in 2% glutaraldehyde, and prepared for thin-section immunoelectron microscopy (EM) analysis as follows. Samples were incubated with 1% osmium tertroxide (OsO_4) in 0.1 M cacodylate buffer for 30 min at 22°C. Samples were then rinsed well with PBS and dehydrated by 3-min sequential incubations in 35, 50, and 75% ethanol prior to staining with 3% uranyl acetate in 70% ethanol for 30 min at 22°C. Samples were further dehydrated with sequential incubations, as described above, in 80, 90, 95, and finally 100% ethanol (3 times), at 22°C. After infiltration with methacrylate resin and polymerization, the samples were mounted, sectioned, and viewed (magnification, ×100,000) by transmission electron microscopy, following standard procedures.

Nucleic acid hybridization. The nucleic acid content of the HIV-like recombinant particles was determined by hybridization with [^{32}P]cytosine-labeled RNA probes reactive with either the *gag* or *env* sequences. The probes were synthesized by in vitro transcription of positive-strand DNA templates, carrying restriction fragments of *gag* or *env* sequences, in the presence of radiolabeled nucleotides by using the Promega Riboprobe in vitro transcription kit according to the directions of the manufacturer. Recombinant particles, equivalent to 300 ng of p24, were solubilized in RNA preparation lysis buffer (2 M guanidine isothiocyanate, 125 mM sodium citrate [pH 7.0], 0.125% sarcocinate, 50% dimethyl sulfoxide) by incubation at 22°C for 1 h. The sample was transferred to a nitrocellulose filter, in parallel with various amounts of similarly solubilized psoralen-inactivated HIV virus (equivalent to 2,600, 260, 26, and 2.6 ng of p24). Separate, identical filters were prepared for reaction with the *gag*-specific or *env*-specific probes. The nitrocellulose filters were incubated for 2 h at 42°C in hybridization buffer (3× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 50% formamide, 5× Denhardt solution, and 150 μg of nonspecific RNA) prior to addition of the probes. The filters were hybridized with the probes overnight at 42°C; they were then washed extensively with 0.1× SSC–0.1% SDS solution, air dried, and analyzed by autoradiography.

RESULTS

Expression and compartmentalization of the HIV-1 *gag* and *env* proteins in BSC-40 cells infected by recombinant vaccinia viruses. Cell lysates and growth media from metabolically radiolabeled BSC-40 cells infected with recombinant vaccinia viruses v-*env5* and v-*gag2* were analyzed for HIV-1 proteins by RIP with human polyclonal anti-HIV-1 sera. The gp160 *env* precursor protein and its proteolytic processing products gp120 and gp41 (1) were detected in the cell lysates of v-*env5*-infected cells (Fig. 1, lane A). The gp120 was

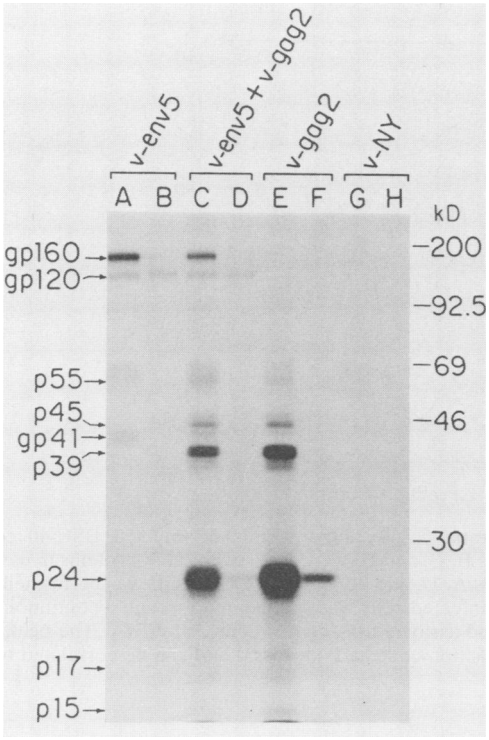

FIG. 1. Expression of HIV-1 proteins by recombinant vaccinia viruses. BSC-40 cells infected with recombinant vaccinia viruses, as indicated, were metabolically radiolabeled with [^{35}S]methionine and [^{35}S]cysteine. The postnuclear cell lysates (lanes A, C, E, and G) in parallel with the culture media (lanes B, D, F, and H) were assayed for HIV proteins by RIP with human polyclonal anti-HIV-1 sera followed by fractionation by SDS-PAGE in an 11.5% acrylamide matrix. The radioactive bands were visualized by fluorography and autoradiography. Molecular size markers are listed at the right (kD).

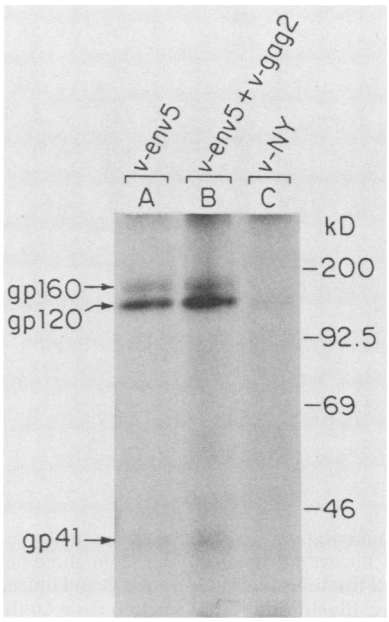

FIG. 2. Cell surface compartmentalization of the HIV envelope proteins. Intact BSC-40 cells were infected with v-*env5* (lane A) or coinfected with v-*env5* and v-*gag2* recombinants (lane B), in parallel with parental v-NY virus (lane C), and were radioiodinated with ^{125}I by the lactoperoxidase-catalyzed reaction. The postnuclear cell lysates were then assayed for HIV proteins by RIP and SDS-PAGE in an 8.5% acrylamide gel matrix. Molecular size markers are listed at the right (kD).

similarly immunoprecipitated from the culture supernatant (lane B) (21). The p55 *gag* polyprotein, synthesized in v-*gag2*-infected cells, was also proteolytically processed, giving rise to mature *gag* proteins p24, p17, and p15, as well as two intermediate precursor species p45 and p39 (lane E) (15). Consistent with previous results (S.-L. Hu et al., in press), the *gag* proteins were also detected in the culture supernatant (lane F). Importantly, cells coinfected with v-*env5* and v-*gag2* yielded processed *env* and *gag* proteins identical to those found in cells infected with the individual recombinant viruses (lane C versus A and E). The mature *env* and *gag* proteins generated from the coinfections were similarly localized to the culture supernatants (lane D versus B and F) and accumulated with similar kinetics (data not shown). Furthermore, iodination of plasma membrane-associated proteins by the lactoperoxidase reaction revealed that cell surface localization of gp160, gp120, and gp41 was the same in doubly infected as in v-*env5*-infected cells (Fig. 2, lane B versus A).

Therefore, taken together, the data suggested that coinfection of cells with the v-*gag2* and v-*env5* recombinant viruses did not alter either the processing or intracellular transport of HIV *env* and *gag* proteins.

HIV-1 *gag* and *env* proteins assemble into particles. Extracellular gp120 and p24 could be derived by release of soluble proteins from cells and/or as constituents of budding particles. To investigate this question, culture supernatants of infected, metabolically radiolabeled BSC-40 cells were separated by ultracentrifugation into particulate (P) and postparticulate (S) fractions. The HIV protein content of each fraction was then determined by RIP in parallel with unfractionated culture supernatants (TS). As seen in Fig. 3 (lanes G through I), the extracellular *gag* proteins p24 and the low levels of p55, p45, p39, and p17, from v-*gag2*-infected cell cultures, separated exclusively with the particulate material (lane H versus I). In contrast, extracellular gp120, derived from v-*env5*-infected cells (lanes A through C), was present primarily in the S fraction (lane C versus B). Upon coinfection with both recombinant viruses, the extracellular gp120 distributed between the P and S fractions (Fig. 3, lanes E and F, respectively). However, the particulate-associated gp120 (lane E) represented a small fraction of the total levels of extracellular gp120 (lane D). Importantly, the RIP analysis of P material from doubly infected cultures revealed the presence of gp41 as well as gp160. Neither of these membrane-associated proteins was detected in the unfractionated material (lane E versus D).

Subfractionation of the P material from coinfected cell culture supernatants by sedimentation through a continuous sucrose density gradient (15 to 60%) revealed that the p24 antigen, as determined by EIA, separated as a single prominent peak within the 36 to 40% sucrose fractions (Fig. 3, illustration). The fractions constituting the top of the gradient did not contain any detectable p24, as confirmed by Western blot (immunoblot) analysis (data not shown). These results suggested that the *gag* proteins were released as constituents of particles. Furthermore, these data (Fig. 3, autoradiogram) also suggested that particles released from coinfected cells appear to have incorporated the gp120-gp41 complexes.

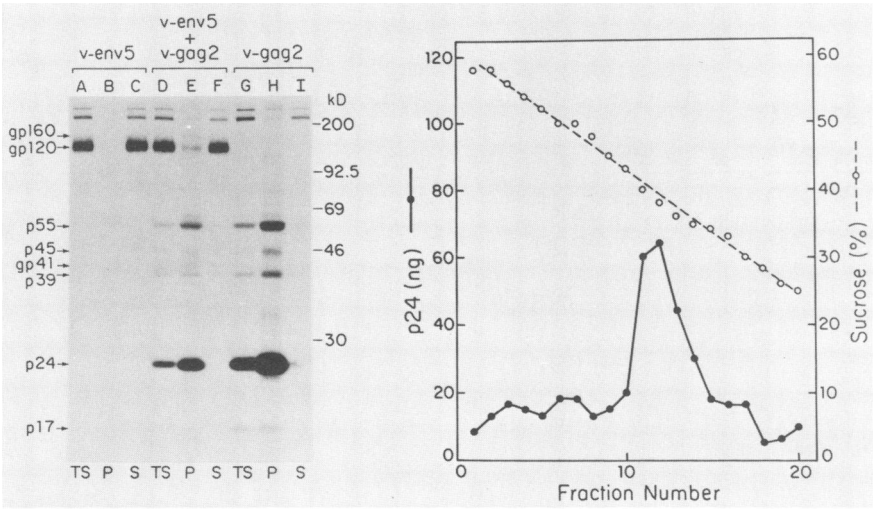

FIG. 3. Sedimentation of particles from culture supernatants. Autoradiogram: culture supernatants of infected BSC-40 cells were fractionated by ultracentrifugation. The sedimented particulate fractions (P) (lanes B, E, and H) were assayed for HIV proteins in parallel with unsedimented S fractions (lanes C, F, and I) and unfractionated TS material (lanes A, D, and G) by RIP. SDS-PAGE was performed in 11.5% acrylamide gels. Illustration: the P fraction from culture supernatants of doubly infected cells was sedimented through a continuous sucrose gradient (15 to 60%). The gradient fractions were collected in 200-μl portions and assayed for *gag* p24 content by EIA. The peak fractions of the EIA are presented (nanograms of p24, closed circles, left ordinate; per fraction collected, abscissa) and can be correlated to sucrose concentration (open circles, right ordinate).

Gross morphology of the particles is identical to that of mature virions. To analyze the morphology of the assembled particles directly, the P material isolated from culture supernatants of doubly infected cells, as described above, was reacted first with MAb 110-4 or 41-1 (as indicated in Fig. 4) and then reacted with secondary antibody conjugated with colloidal gold. Components of the particulate fractions were visualized by EM. Particles of 100 to 120 nm in diameter were detected. They contained an electron-dense core that appeared either as a rod (Fig. 4a and c) or as a sphere (Fig. 4b). These images were similar to those of mature particles isolated from HIV-1-infected cells (9, 10). Furthermore, the recombinant-made particles were labeled with antibody-colloidal gold conjugates, suggesting that they displayed both gp120 (Fig. 4a and b) and gp41 (Fig. 4c) on their surfaces.

Similar analysis of coinfected intact cells revealed numerous 100- to 120-nm particles budding from the cell surface that were positive for gp120 (Fig. 4d and e). The cell-associated, 110-4 MAb-labeled particles assumed primarily two distinct morphologies. The first represented a diffuse vesicle form (Fig. 4d, star), while the second was distinguished by an eccentrically localized, thickened double-membrane region (Fig. 4d, closed arrow). Presumably, these structures represented various forms of immature particles. Occasionally, a particle containing a fully formed rod-shaped capsid could be discerned proximal to the cell surface (Fig. 4e, open arrow). Vaccinia virus particles budding from the cell surface did not incorporate gp120 (Fig. 4d, double arrow). Parallel experiments in which cells infected with the v-NY parental virus were used indicated that formation of the MAb-colloidal gold conjugate complexes detected by EM was specific for the presence of the HIV *env* proteins (data not shown).

Nucleic acid content of the HIV-like particles. The morphology of the HIV-like particles together with their protein content suggested that their assembly occurred by a process similar to that of the HIV virion. Therefore, we examined the ability of these particles to also package nucleic acid molecules. Recombinant particles sedimented from the culture supernatants of v-*gag2* and v-*env5* doubly infected cells were solubilized in RNA denaturation buffer and transferred to a nitrocellulose filter in parallel with known amounts of psoralen-inactivated HIV-1 virus. Two identical filters were then analyzed for HIV nucleic acids by hybridization with [^{32}P]cytosine-labeled RNA probes reactive with either the *gag* or *env* sequence. Only the *gag*-specific probe reacted with nucleic acids in the recombinant-made particle sample (Fig. 5A versus B, lanes 1). In contrast, both *gag*- and *env*-specific probes reacted with the inactivated HIV samples (panels A and B, lanes 1 through 4). The data suggested that the HIV-like particles packaged the *gag* mRNA but not the *env* mRNA molecules. However, comparison of samples containing equivalent amounts of p24 protein showed that the incorporation of HIV nucleic acid molecules into the recombinant-made particles (panel A, lane 1) was less efficient than into the HIV-1 virions (panel A, lane 2).

DISCUSSION

We have shown in this study that coexpression of the HIV-1 core and envelope proteins in mammalian cells infected with recombinant vaccinia viruses resulted in the assembly and release of HIV-like particles. Biochemical analysis showed that the budding particles incorporated both *env* gp120-gp41 complexes and the *gag* proteins p24, p17, p55, p45, and p39. Moreover, Western blot analysis with *env*-specific MAbs as well as polyclonal anti-HIV sera suggested that the core and *env* proteins in the recombinant-made particles were present in ratios similar to those in HIV-1 virions (data not shown). RIP analysis of isolated particles further revealed the presence of a 160-kDa *env* protein at low levels. It was previously shown that in HIV-1 virions, the transmembrane protein gp41 was arranged into multimeric 120- and 160-kDa structures that represented trimers and tetramers, respectively (31). However, the 160-

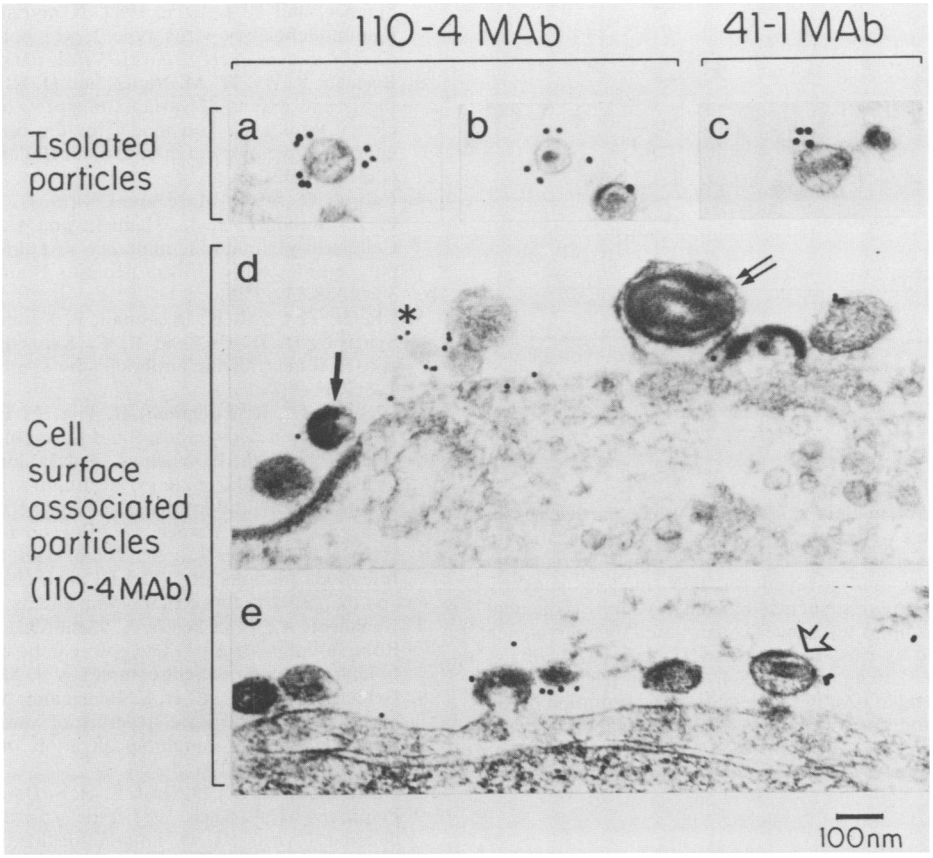

FIG. 4. Analysis of assembled particles by EM. Intact cells coinfected with v-*gag2* and v-*env5* as described above (d and e), in parallel with P fractions sedimented from the culture supernatants (a, b, and c), were reacted with MAb 110-4 (a, b, d, and e) or 41-1 (c) followed by incubation with gold-conjugated goat anti-mouse immunoglobulin G. The samples were prepared for EM analysis. Bar scale, 100 nm.

kDa protein contained in the particles reacted with MAb specific for gp120 (data not shown), suggesting that it was, at least in part, composed of unprocessed precursor protein.

Thin-section electron microscopy revealed that the recombinant-made particles were similar to HIV-1 virions in that they were 100 to 120 nm in diameter, contained a cylindrical core structure, and budded from the plasma membrane of infected cells (10). EM analysis of the immunolabeled particles suggested that they displayed the envelope glycoprotein complexes on their surfaces. This complex is known to mediate the binding (28) and entry (23) of virions into $CD4^+$ target cells. However, the HIV-like particles failed to generate a productive infection and did not mediate syncytium formation in CEM lymphoblastoid culture (data not shown). The apparent inability of particles to replicate in cells was predicted, since in addition to absence of a reverse transcriptase, the packaged *gag* mRNA lacked the necessary HIV promoter and regulatory sequences.

The apparent preferential incorporation of the *gag* mRNA could not be accounted for by random packaging, since analysis of RNA species in lysates of v-*env5* and v-*gag2* doubly infected cells revealed the presence of equivalent amounts of *env* and *gag* RNA. Moreover, this packaging correlated with the presence of the HIV viral RNA packaging signal (coordinates 300 to 319; Fig. 5C, arrow) (26) in the HIV sequences (coordinates 258 to 3317, Fig. 5C) used to generate the v-*gag2* vaccinia recombinant. However, it is conceivable that small amounts of *gag* mRNA as well as *env* mRNA not detectable by our assay may be randomly packaged into the assembled particles. The inefficiency in packaging of nucleic acid molecules into the recombinant-made particles relative to virus may be due to impairment in the formation of the ribonucleoprotein complex. This may result from a number of factors, such as an altered secondary structure of a truncated transcript (*gag* mRNA) relative to the viral genomic RNA as well as absence of a complete reverse transcriptase protein. Importantly, the low levels of HIV nucleic acids detected suggested that a significant proportion of the recombinant-made particles assembled either empty core structures or nonspecifically encapsidated cellular tRNA molecules. Both phenomena have been previously described for other retrovirus systems (6).

It was previously reported that HIV-1 *gag* proteins spontaneously assembled into budding particles when expressed in insect cells (12), COS 7 cells (14), or CMT3 cells (35a). Myristoylation but not proteolytic processing was necessary for the assembly to occur. Consistent with these results, we have previously shown that processed as well as unprocessed *gag* proteins expressed by recombinant vaccinia viruses in BSC-40 cells also assembled into budding particles (S.-L. Hu et al., in press). However, the novelty of the HIV-like particles reported on in this study is the incorporation, in addition to the *gag* proteins, of processed gp120-gp41 *env* complexes. Similar to virions, the *env* complexes were also present on the surface of particles, suggesting that the assembly of the HIV-like particles mimics the process of

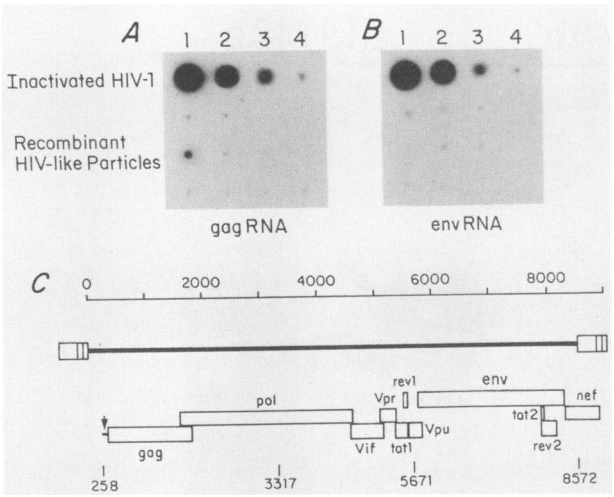

FIG. 5. Packaging of nucleic acids by HIV-like particles. Isolated HIV-like particles were prepared for dot blot hybridization in parallel with psoralen-inactivated HIV. The samples were transferred to nitrocellulose and assayed for HIV nucleic acids by hybridization with either *gag* sequence-specific (A) or *env* sequence-specific (B) probes. Samples tested were quantitated by equivalence of p24 *gag* determined by EIA. Inactivated HIV: lanes 1, 2,600 ng; lanes 2, 260 ng; lanes 3, 26 ng; and lanes 4, 2.6 ng of p24. HIV-like particles: lanes 1, 300 ng of p24. (C) Schematic representation of the HIV-1 genome showing the coordinates for the fragments used to generate v-*gag2* (258 to 3317) and v-*env5* (5671 to 8572) recombinant viruses. The arrow indicates the position of the viral RNA packaging sequence.

HIV-1 virus maturation. Therefore, the recombinant system we have described presents a unique approach for studying the process of virus encapsidation and maturation. Furthermore, the versatility that it provides for the use of various mutagenized forms of the structural proteins will allow the direct analysis of *gag* and *env* protein interaction.

Recent findings indicate that both the envelope glycoproteins gp120 and gp41 (5, 18, 35, 38; D. Tyler, S. Zolla-Panzer, M. Gorny, D. Stanley, B. Bolognesi, and K. Weinhold, Abstr. 5th Int. Conf. AIDS, 1989, T.C.O. 33, p. 521) as well as core structural proteins (29) of HIV-1 are targets of humoral (5, 18, 35) and cell-mediated (29, 38; Tyler et al., Abstr. 5th Int. Conf. AIDS) immune responses. Moreover, viral antigens presented as components of membrane structures were previously shown to be more immunogenic than their soluble counterparts (4, 19). Since the HIV-like particles incorporated both of the major structural antigens of HIV and since they did not contain a complete viral genome and were noninfectious, they represent a novel and attractive vaccine candidate.

ACKNOWLEDGMENTS

The authors thank J. Lewis, P. Linsley, E. Thomas, A. Senear, and P. Sridahr for their suggestions and critical evaluation of the manuscript and S. Roberts for technical assistance.

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