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Functional Exchange of an Oncoretrovirus and a Lentivirus Matrix Protein

CAROL A. DEMINIE AND MICHAEL EMERMAN*

Program in Molecular Medicine and Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104

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To map functional domains in the retroviral Gag protein we have constructed chimeric viruses where regions of the murine leukemia virus (MuLV) Gag protein have been replaced with analogous sequences from human immunodeficiency virus type 1 (HIV-1). Here we describe the chimeric virus MuLV(MA_{HIV}) which contains the HIV-1 matrix (MA) protein in place of the MuLV MA. MuLV(MA_{HIV}) is infectious but grows at a reduced rate compared with wild-type MuLV. We found that the partial defect in replication of the chimeric virus is at a late stage in the viral life cycle. The MuLV(MA_{HIV}) Gag proteins are distributed aberrantly within cells and are not associated with cellular membranes. Unlike MuLV, HIV-1 is able to integrate into growth-arrested cells. Incorporation of the HIV-1 MA, which is known to play a role in infection of nondividing cells, does not enable MuLV(MA_{HIV}) to be expressed in growth-arrested cells. While it possesses no amino acid homology, we found that the HIV-1 MA can efficiently replace the MuLV matrix protein in infection.

The retroviral *gag* gene encodes a polyprotein which plays roles in both virion assembly and infection. The Gag protein is made up of matrix (MA), capsid (CA), and nucleocapsid (NC) proteins and, in most viruses, also contains small additional peptides which vary in size and location. Processing of the Gag polyprotein into individual proteins is required in order for them to carry out their roles in infection. As a polyprotein, Gag possesses information directing membrane targeting and protein interactions needed for viral assembly and release (for a review see reference 31). After virus is released from the cell, the polyprotein is cleaved and the Gag proteins can then facilitate virion entry, uncoating, and integration of the reverse-transcribed viral DNA (2, 6, 33).

Domains within the Gag matrix region direct membrane binding of the polyprotein prior to assembly (1, 13, 16, 32, 35). Mutational analyses reveal that in addition to specific primary sequence requirements, proper folding of the Gag polyprotein is necessary for efficient transport and membrane binding (9, 16, 17).

In addition to directing membrane binding, the matrix protein is necessary for viral infectivity at some postassembly step (6, 17, 28, 35). Also, the human immunodeficiency virus type 1 (HIV-1) MA protein is located in preintegration complexes and can mediate viral integration in growth-arrested cells (3). This phenotype, unique to lentiviruses, distinguishes them from the oncoretroviruses.

We have used two well-characterized viruses, murine leukemia virus (MuLV) and HIV-1, to make chimeras which may be used to locate domains in Gag with specific roles in assembly and infection. We previously found that chimeric MuLV virions containing either the HIV-1 MA or CA domain were inefficiently released in the absence of wild-type MuLV (7). Since wild-type MuLV could coassemble with either chimera, both the MA and CA regions are able to mediate Gag protein interactions in assembly. We previously showed that a clonal cell line containing the MuLV(MA_{HIV}) proviral DNA secreted reverse transcriptase (RT) activity which banded at the same density as wild-type MuLV RT in a sucrose gradient (7). We have further characterized MuLV(MA_{HIV}) and now show that it is infectious but assembles inefficiently because of improper intracellular targeting. Incorporation of the HIV-1 MA did not allow MuLV(MA_{HIV}) to replicate in growth-arrested cells. Since MuLV(MA_{HIV}) is infectious, the HIV-1 MA can carry out any roles required of the MuLV MA in the early stages of infection.

MATERIALS AND METHODS

Nomenclature. To distinguish between the matrix (MA) and capsid (CA) proteins of HIV-1 and MuLV Gag, they are also referred to according to their apparent molecular weight. Thus, the HIV-1 MA is p17, the MuLV MA is p15, the HIV-1 CA is p24, and the MuLV CA is p30. The MuLV specific protein pX is referred to as p12.

Cells, infections, and transfections. The NIH3T3tk$^-$ (mouse embryo fibroblast), D17 (dog osteosarcoma), 143Btk$^-$ (human osteosarcoma), and 208F (rat embryo fibroblast) cell lines were maintained in Dulbecco's modified Eagle medium (DMEM) with 10% calf serum (DMEM-10). HeLa CD4-LTR/β-galactosidase cells are grown in DMEM-10 with 0.2 mg of G418 per ml and 0.1 mg of hygromycin B per ml, and their derivation has been described (19).

Construction of the chimeric provirus MuLV(MA_{HIV}) has been described (7). Transfections of 3T3 cells with MuLV (MA_{HIV}) proviral DNA were done by calcium phosphate coprecipitation (5). COS-7 cells, which were maintained in DMEM plus 10% fetal calf serum, were transfected by using DEAE-dextran followed by a dimethyl sulfoxide shock (22).

The alkaline phosphatase (AP)-expressing virus was made by infecting a 3T3 clonal cell line stably expressing DOL-AP (10) with wild-type MuLV or MuLV(MA_{HIV}). About 3 weeks postinfection cell-free supernatant was collected and virus was quantitated by RT assay. Virus expressing the HIV-1 *tat* gene was made by infecting 3T3 cells expressing either wild-type MuLV or MuLV(MA_{HIV}) with LtatSN virus (11) made in the PE501 ecotropic packaging cell line and selecting for G418 resistance. The virus produced from the resulting clones was

* Corresponding author. Mailing address: Fred Hutchinson Cancer Research Center, Room C2-023, 1124 Columbia St., Seattle, WA 98104. Phone: (206) 667-5058. Fax: (206) 667-6523.

titered by using the MAGI cell assay (19). Infection of 3T3 cells with MuLV-based viruses was done in the presence of 10 μg of Polybrene per ml for 2 h.

For irradiation experiments, HeLa CD4-LTR/β-galactosidase cells were trypsinized, resuspended in 1 ml of DMEM-10 in a 15-ml tube, and exposed to a ^{137}Cs source (calibrated to 400 rad/min) for 10 min as described previously (20). After irradiation, cells were diluted to 2×10^5/ml and plated. The next day, proliferating and gamma-irradiated cells were infected with dilutions of virus stocks in the presence of 20 μg of DEAE-dextran (HIV-1 infections) per ml or 10 μg of Polybrene (MuLV infections) per ml.

Western blot analysis. Secreted viral proteins were isolated by centrifugation through a 20% sucrose cushion as described previously (7). Thirty milliliters of cell culture medium from 3T3 cells infected with wild-type MuLV or MuLV(MA_{HIV}) was collected for analysis. After centrifugation, the viral proteins were run on a 15% sodium dodecyl sulfate (SDS)-polyacrylamide gel. Following electrophoresis, the proteins were transferred to nitrocellulose by semidry transfer (Hoeffer), and proteins were reacted with sheep anti-p17 serum or goat anti-p15/p12 serum which was detected with a horseradish peroxidase-conjugated anti-goat immunoglobulin G (Cappell). Goat serum reactive with MuLV viral proteins was obtained from Quality Biotech Inc.

RT assays. To quantitate virus stocks and virus released from infected cells, RT activity was measured as previously described (7). The resulting dot blot was analyzed by quantitative PhosphorImaging analysis, and the RT activity was measured in relative imaging units (15).

Radiolabelling and immunoprecipitations. For metabolic radiolabelling of viral proteins, infected 3T3 cells were starved for 20 min in DMEM without methionine, cysteine, or serum. The medium was removed and replaced with medium containing 150 μCi of Trans^{35}S-Label (70% ^{35}S-methionine and 30% ^{35}S-cysteine; ICN Biochemicals, Inc.) per ml and 0.5% dialyzed calf serum. For the isolation of radiolabelled virus, the cells were labelled for 16 h and the virus was concentrated by centrifugation. The pellet was resuspended in NP40-DOC (1× NP40-DOC is 0.5% Nonidet P-40, 1% deoxycholate, 0.1% SDS) and viral proteins were immunoprecipitated with anti-MuLV serum in the presence of protein A-Sepharose beads for 2 h at 4°C as described previously (8). After being washed in high-salt buffers, the proteins were detached from the beads by boiling and resolved by SDS-polyacrylamide gel electrophoresis (PAGE). The gel was fixed in 30% methanol–10% acetic acid, dried, and exposed to film at room temperature.

For the pulse-chase assay, after the 40-min labelling, cells and media were harvested (time 0) or chased with DMEM-10 for 2, 4, 8, 16, or 24 h. The viral proteins were immunoprecipitated with anti-MuLV serum as described above from the cells, after lysis with NP40-DOC, and from the culture supernatant after the addition of 1/10 volume of 10× NP40-DOC.

Subcellular fractionation. Cell fractionations were performed by standard techniques (23). Cells were labelled for 40 min as described above, washed with cold phosphate-buffered saline (PBS) containing proteinase inhibitors (0.2 mM phenylmethylsulfonyl fluoride and 1 μM aprotinin), and scraped into 1 ml of swelling buffer (10 mM KCl, 20 mM Tris-HCl [pH 7.8], 1 mM EDTA, 0.1% β-mercaptoethanol, and 1 μM aprotinin). After a 20-min incubation on ice, the cells were lysed with 50 strokes of a glass Dounce homogenizer. NaCl was added to 0.15 M, and the nuclei were pelleted at 1,000 × *g* for 5 min. The supernatant was spun at 100,000 × *g* for 30 min. The pellet (P) fraction was resuspended in NP40-DOC and 1/10 volume of 10× NP40-DOC was added to the supernatant (S) fraction.

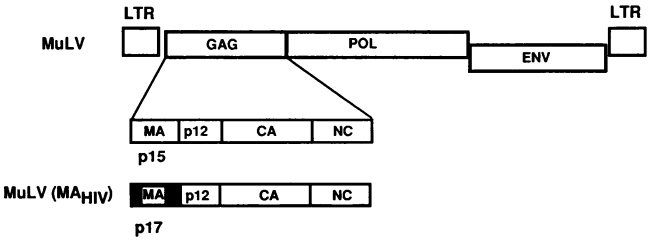

FIG. 1. Schematic representation of MuLV and the chimeric MuLV(MA_{HIV}) Gag protein. The MuLV Gag polyprotein consists of MA (p15), p12, CA (p30), and NC. MuLV (MA_{HIV}) contains the MA of HIV-1 precisely substituted for the MA of MuLV. The remaining proviral sequence of MuLV (MA_{HIV}), including the LTRs, is that of MuLV. To allow complete processing of the chimeric polyprotein, the last 4 amino acids of p17 in MuLV (MA_{HIV}) are MuLV residues. MuLV (MA_{HIV}) was constructed by PCR and was previously described (7).

Viral proteins were immunoprecipitated as above by using anti-MuLV serum, and the proteins were resolved by SDS-PAGE (10%).

Indirect immunofluorescence assay. Forty-eight hours after transfection, COS-7 cells were fixed for 20 min with 4% paraformaldehyde as described previously (26). After permeabilization for 5 min with 0.2% Triton X-100, the cells were incubated with anti-MuLV serum diluted 1:750 in PBS–1% calf serum. After 1 h at 37°C, the cells were washed in PBS–1% calf serum, and the primary antibody was reacted with fluorescein isothiocyanate-conjugated anti-sheep immunoglobulin G diluted 1:400.

AP and β-galactosidase staining. For detection of AP activity or β-galactosidase expression, cells were fixed and stained as described previously (10, 19, 24).

RESULTS

MuLV(MA_{HIV}) is infectious. We have previously described the construction and initial characterization of several MuLV/HIV-1 chimeric viruses (7). To create the chimera MuLV (MA_{HIV}), shown in Fig. 1, we precisely replaced the MuLV matrix protein with the HIV-1 matrix protein (7). To determine if MuLV(MA_{HIV}) is infectious, we transfected 3T3 cells with the proviral DNA and passaged the cells for several weeks. The RT levels were monitored and were observed to steadily increase (data not shown), indicating that the chimeric virus is infectious.

After about 3 weeks, the virus produced by the MuLV (MA_{HIV})-transfected 3T3 cells was examined by Western blot (immunoblot) analysis. Viral particles from MuLV(MA_{HIV})-transfected cells, along with wild-type MuLV and HIV-1, were concentrated by centrifugation through a 20% sucrose cushion and were run on an SDS-polyacrylamide gel. The resulting protein blot was probed first with antiserum which recognizes the MuLV MA (p15) and p12 proteins. In the MuLV lane, large amounts of MA (p15) and p12 can be seen, while only p12 is found in the MuLV(MA_{HIV}) lane (Fig. 2A, lanes 1 and 2). The blot was stripped and reprobed with anti-p17 serum (Fig. 2B). A protein of the correct size, which comigrates with the MA protein from wild-type HIV-1, is found in the MuLV (MA_{HIV}) lane (Fig. 2B, lanes 2 and 3). This shows that the virus released from the MuLV(MA_{HIV})-transfected cells contains the HIV-1 matrix protein and is processed properly. We cannot detect MuLV MA protein in the MuLV(MA_{HIV}) lane; this shows that wild-type MuLV is not present as a contami-

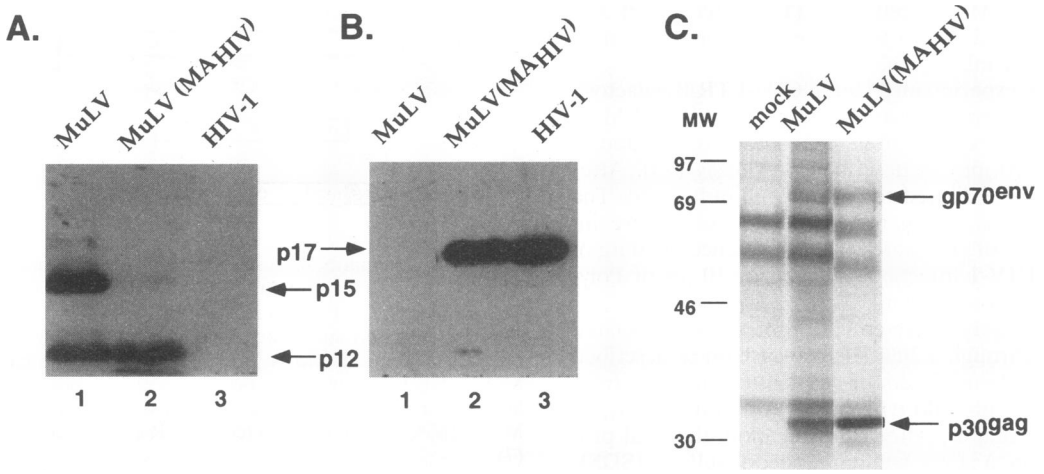

FIG. 2. Analysis of wild-type MuLV and MuLV(MA_{HIV}) secreted viral proteins and Env incorporation. Virus was concentrated by centrifugation from the medium of 3T3 cells infected with wild-type MuLV or transfected with MuLV(MA_{HIV}) proviral DNA. Western blot analysis was used to examine secreted virus proteins. The protein blot was probed with (A) anti-p15/p12 serum or (B) anti-p17 serum. Lanes 1, MuLV; lanes 2, MuLV(MA_{HIV}); lanes 3, HIV-1. The positions of the virus proteins p12, p15, and p17 are marked. (C) 3T3 cells infected with wild-type MuLV or transfected with MuLV(MA_{HIV}) proviral DNA were labelled with ^{35}S-methionine, and virus proteins were isolated by centrifugation. The labelled virus proteins were immunoprecipitated with anti-MuLV serum and were resolved by SDS-PAGE (10%). Lanes 1, uninfected 3T3 cells; lanes 2, wild-type MuLV; lanes 3, MuLV(MA_{HIV}). The p30 and gp70^{env} are noted and also the positions of the molecular mass markers (in kilodaltons).

nant. Additionally, to ensure that the released MuLV(MA_{HIV}) did not sustain any mutations after transfection, Hirt DNA was isolated and amplified by PCR by using primers that flanked the MA coding sequence. A single PCR product was detected. This product corresponded to the MA of HIV-1, confirming that no wild-type MuLV was present in the transfected cell culture. Four different subclones were sequenced and no mutations were found. Therefore, the chimeric virus is infectious in the absence of additional mutations in the HIV-1 MA.

Within the viral particle the matrix protein is closely associated with the envelope glycoprotein and may play a role in envelope incorporation (12, 34). Therefore we asked if MuLV (MA_{HIV}) efficiently incorporated MuLV envelope glycoproteins. 3T3 cells producing wild-type MuLV or MuLV(MA_{HIV}) were labelled with ^{35}S-methionine for 16 h. Labelled virus in the media was concentrated by centrifugation, and the pelleted viral proteins were immunoprecipitated with anti-MuLV serum. Both wild-type MuLV and MuLV(MA_{HIV}) contained gp70^{env} (Fig. 2C). The ratios of gp70^{env} to CA (p30) were determined by quantitative PhosphorImage analysis and revealed a difference of less than twofold in envelope incorporation between wild-type MuLV and MuLV(MA_{HIV}).

MuLV(MA_{HIV}) shows slower growth kinetics than does wild-type MuLV. To compare the growth kinetics of MuLV (MA_{HIV}) with wild-type MuLV, cell-free media containing equivalent amounts of RT activity, corresponding to 0.25 infectious U of wild-type MuLV per cell, were used to infect several different cell types. At 3-day intervals, cell medium was collected and the cells were split at a 1:10 ratio. The spread of infectious virus was monitored by RT assay.

The wild-type virus grew very quickly in the 3T3 cells, giving about 10^4 U of RT activity at day 3 postinfection and remaining at that high level for the next 2 weeks (Fig. 3). The medium from the MuLV(MA_{HIV})-infected 3T3 cells showed lower amounts of RT activity early after infection. By 12 days after infection, MuLV(MA_{HIV}) production peaked, giving about 2 $\times$ 10^3 U of RT activity in the cell medium. After infection of a dog cell line, D17, we observed a similar difference in virus release early after infection (Fig. 3). The D17 cells infected with wild-type MuLV secreted greater than 10-fold more virus at day 3 postinfection than did the chimera. As in the 3T3 cells, MuLV(MA_{HIV}) grew more slowly and released less virus than did wild-type MuLV. Similar results were obtained with two other cell types, a rat cell line, 208F, and human 143B cells (data not shown).

3T3 cells infected with wild-type MuLV or MuLV(MA_{HIV}) were also examined by electron microscopy. While the chimeric virus showed fewer budding virions, it was similar in morphology to wild-type MuLV (data not shown). Also, the MuLV(MA_{HIV}) virus particles observed had mature condensed cores indistinguishable from wild-type virions.

MuLV(MA_{HIV}) is as infectious as wild-type MuLV. The slow growth kinetics of MuLV(MA_{HIV}) could result from defects either in the early stages of infection, at entry, reverse transcription, or integration or at a late stage in replication, at particle assembly, or at release. To assess the efficiency of MuLV(MA_{HIV}) in the early steps of infection, we introduced the transmissible retroviral vector DOL-AP (10) into wild-type MuLV and MuLV(MA_{HIV}) (Fig. 4) for use in a one-step growth assay. Infection of cells with virus containing the DOL-AP vector is detected by staining fixed cells for expression of the AP gene.

The MuLV and MuLV(MA_{HIV}) viruses containing the DOL-AP vector were used to infect 3T3, D17, and 208F cells at several dilutions. Forty-eight hours after infection, the cells were stained for expression of the AP protein, and the number of infected (purple) cells was counted. The two viruses infected about the same number of cells (Table 1), indicating that MuLV(MA_{HIV}) can carry out the early steps of infection about as efficiently as wild-type MuLV. In addition, this result shows that MuLV(MA_{HIV}) can incorporate the retroviral vector DOL-AP. Therefore, the slow growth of MuLV(MA_{HIV}) is probably not the result of a defect in an early step after infection.

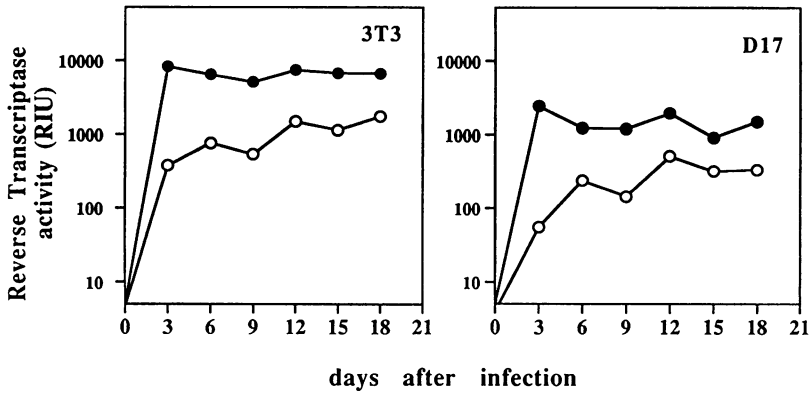

FIG. 3. Growth of MuLV(MA_{HIV}) in 3T3 and D17 cells. The replication kinetics of wild-type MuLV and MuLV(MA_{HIV}) were compared by using 3T3 and D17 cells. Cells were infected with equal amounts of wild-type MuLV (closed circles) or MuLV(MA_{HIV}) (open circles) on the basis of quantitation of RT activity. The cells were passaged every 3 days, and the culture medium was tested for RT activity. The levels of RT activity in the medium were determined by quantitative PhosphorImaging.

MuLV(MA_{HIV}) Gag precursor has a long half-life in infected cells. To assess the efficiency of MuLV(MA_{HIV}) in the late stages of virus production, the rates of release of MuLV and MuLV(MA_{HIV}) Gag proteins from infected cells were examined by a pulse-chase assay. Chronically infected 3T3 cells were pulse-labelled with ^{35}S-methionine and then chased for 2, 4, 8, 16, and 24 h. Cells and medium were harvested immediately after the labelling (time 0) and at each time point. Viral proteins were immunoprecipitated with anti-MuLV serum and were resolved by SDS-PAGE.

The resulting autoradiograph showed that similar levels of wild-type and chimeric $pr65^{gag}$ proteins were labelled within the cells during the short pulse (Fig. 5A and B, lane 0).

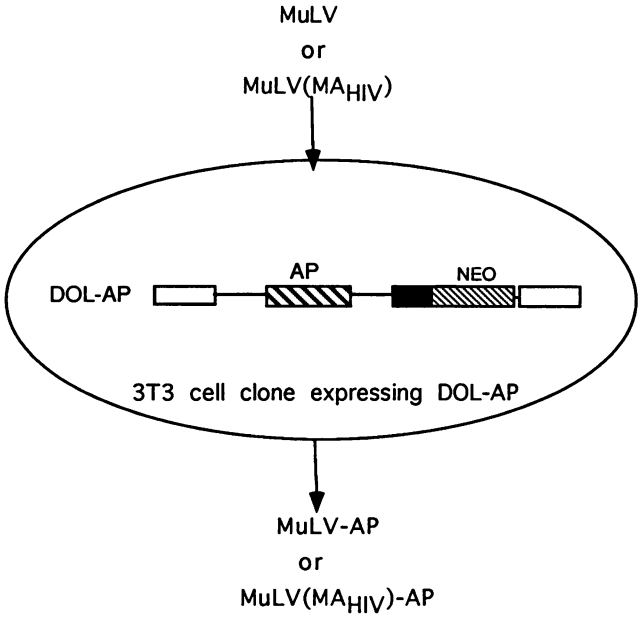

FIG. 4. Wild-type MuLV and MuLV(MA_{HIV}) were used to infect a 3T3 cell clone which stably expresses the retroviral vector DOL-AP. Virus produced will have packaged the DOL-AP RNA. Expression of the human AP gene is driven by the MuLV LTR, and the *neo* gene is expressed from the SV40 early promoter.

Therefore, both types of Gag proteins are being synthesized at equivalent rates. With the wild-type virus, CA (p30) is detected in the cell medium after the 2-h chase, and $pr65^{gag}$ is dramatically decreased in the cell fraction (Fig. 5A). Therefore, most of the labelled wild-type virus is assembled and processed within a 2-h period. In contrast, with MuLV(MA_{HIV}), CA (p30) is readily detected in the cell medium only after the 16-h chase (Fig. 5B). The $pr65^{gag}$ band is only slightly decreased in the cell fraction after the 24-h chase (Fig. 5B). This shows that the chimeric Gag protein is very stable in infected cells and is assembled and released very slowly. Inefficient release of the chimeric virus would account for its slow spread in infected cells. In agreement with the Western blots, the absence of precursor Gag protein in the cell medium (Fig. 5B) shows that MuLV(MA_{HIV}), once committed to assembly and release, is fully processed.

Intracellular localization of wild-type MuLV and MuLV (MA_{HIV}) Gag proteins. Since aberrant intracellular targeting may result in slow particle assembly, we asked how our chimeric Gag polyprotein was distributed within infected cells. We used an indirect immunofluorescence assay to examine in situ the intracellular localization of the MuLV(MA_{HIV}) Gag proteins. If intracellular targeting or transport of the chimeric Gag proteins is defective, we would expect to see a staining pattern different from that obtained with wild-type MuLV Gag. For the indirect immunofluorescence assay staining, COS-7 cells were transfected with either MuLV or MuLV (MA_{HIV}) proviral DNAs. The cells were fixed 48 h after

TABLE 1. Infectivity of MuLV(MA_{HIV})

Virus	Cell line	No. of AP^+ cells per ml of virus[a]
MuLV	3T3	6.7×10^3
MuLV(MA_{HIV})	3T3	2.0×10^4
MuLV	D17	7.6×10^3
MuLV(MA_{HIV})	D17	1.1×10^4
MuLV	208F	5.6×10^3
MuLV(MA_{HIV})	208F	9.8×10^3

[a] Cells, infected with 10-fold dilutions of MuLV-AP and MuLV(MA_{HIV})-AP, were stained for AP expression 48 h after infection. The amount of virus in the two stocks was quantitated by RT assay, and MuLV(MA_{HIV})-AP gave 1.5-fold more activity than did MuLV-AP.

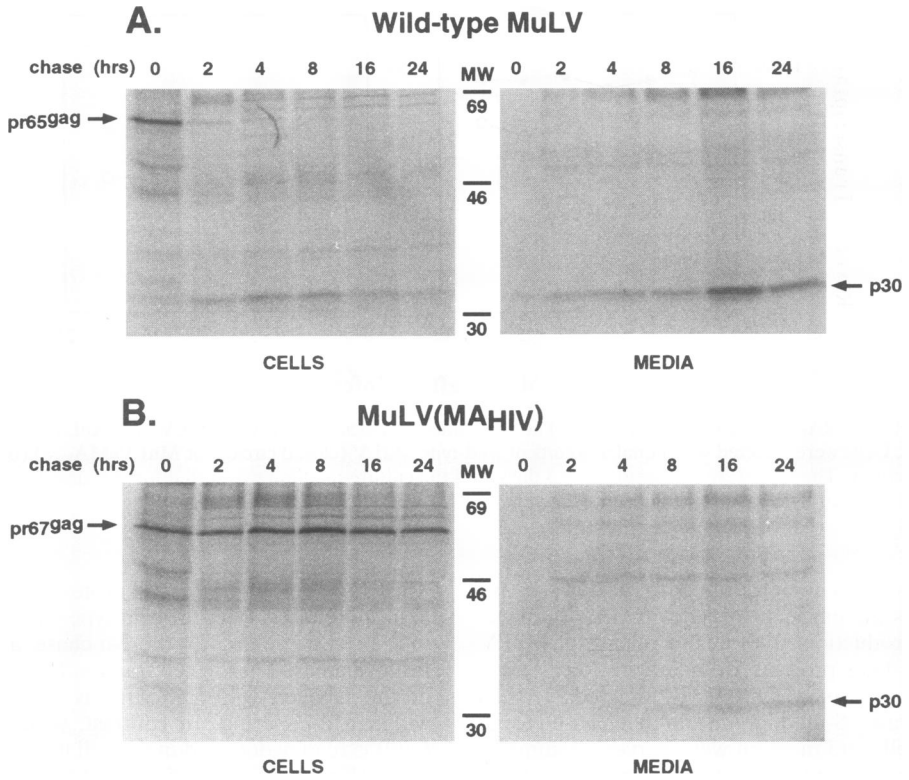

FIG. 5. Pulse-chase analysis of wild-type MuLV and MuLV(MA_{HIV}) polyproteins. 3T3 cells chronically infected with wild-type MuLV or MuLV(MA_{HIV}) were labelled with ^{35}S-methionine (lane 0) and were then chased for 2, 4, 6, 8, 16, and 24 h. Radiolabelled proteins were isolated from the cells and medium by immunoprecipitation with anti-MuLV serum, separated by SDS-PAGE (10%), and visualized after being exposed to film for 5 days. (A) Wild-type MuLV; (B) MuLV(MA_{HIV}). The positions of $pr65^{gag}$ and p30 are noted and also the positions of the molecular mass markers (in kilodaltons).

transfection and were reacted with anti-MuLV serum followed by a fluorescein isothiocyanate-conjugated antibody. Cells transfected with the wild-type MuLV vector showed the expected dense patches of staining and, in particular, intense perinuclear cytoplasmic staining (Fig. 6A). This pattern likely represents the accumulation and oligomerization of Gag proteins. In contrast to this, the MuLV(MA_{HIV})-transfected cells showed only diffuse, cytoplasmic staining (Fig. 6B). No fluo-

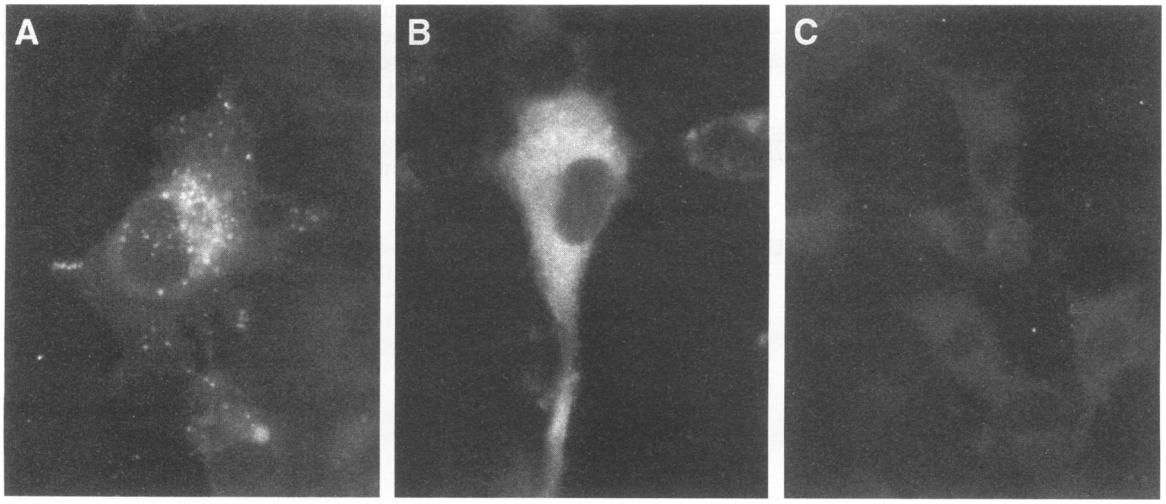

FIG. 6. Immunofluorescent localization of wild-type MuLV and MuLV(MA_{HIV}) proteins in transfected cells. Forty-eight hours after transfection of COS-7 cells with wild-type MuLV (A), MuLV(MA_{HIV}) (B), or mock (C) DNA, the cells were fixed and reacted with anti-MuLV serum which was detected with a fluorescein isothiocyanate-conjugated antibody. The cells were viewed with a Bio-Rad MRC-600 scanning laser microscope, and images were collected at a magnification of ×750.

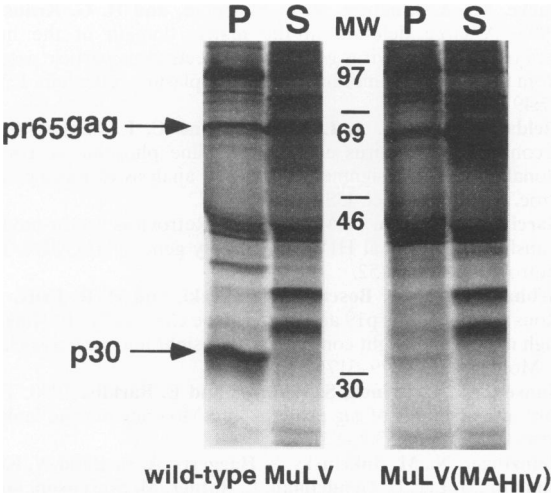

FIG. 7. Subcellular localization of wild-type MuLV and MuLV (MA_{HIV}) Gag proteins. Chronically infected 3T3 cells were labelled with ^{35}S-methionine, and the proteins were separated into cytosolic (S) and membrane (P) fractions under low ionic strength conditions (0.15 M NaCl) by a 30-min high-speed (100,000 × *g*) spin. Virus proteins were immunoprecipitated with anti-MuLV serum, resolved by SDS-PAGE (10%), and visualized after being exposed to film for 5 days.

rescent staining was seen in mock-transfected COS-7 cells (Fig. 6C). This result clearly indicates that, unlike the wild-type Gag, MuLV(MA_{HIV}) Gag proteins are not properly targeted within cells and are unable to accumulate to high densities at discrete sites to initiate assembly.

The intracellular distribution of the chimeric Gag protein was also examined by biochemical fractionation. 3T3 cells, chronically infected with wild-type MuLV or MuLV(MA_{HIV}), were labelled with ^{35}S-methionine, and the cell proteins were separated into soluble (S) and membrane-bound (P) fractions. Viral proteins were immunoprecipitated with anti-MuLV serum and were resolved by SDS-PAGE. The resulting autoradiograph shows a dramatically different distribution of wild-type and chimeric Gag polyproteins (Fig. 7). The wild-type $pr65^{gag}$ is found primarily in the membrane (P) fraction. In contrast, the majority of the MuLV(MA_{HIV}) Gag protein is seen in the soluble (S) fraction (Fig. 7). This shows that the chimeric protein, unlike the wild-type $pr65^{gag}$, is not tightly membrane bound. Association with cell membranes is clearly required for efficient particle assembly.

Incorporation of HIV-1 MA into MuLV virions does not allow growth in nondividing cells. Unlike oncoretroviruses such as MuLV, HIV-1 is able to productively infect nonproliferating cells (20, 21). A region in the matrix protein of HIV-1, which has a nuclear localizing function, is important for the ability of HIV-1 to integrate into nondividing cells (4). Since MuLV(MA_{HIV}) contains the matrix protein of HIV-1, we asked if this would allow the virus to be expressed in growth-arrested cells.

MAGI cells, which contain an integrated copy of the β-galactosidase gene driven by the HIV-LTR (19), can be arrested in the G_2 stage of the cell cycle by irradiation (20). In order to use these cells in a one-step growth assay, Tat, the LTR transactivator, was introduced into MuLV and MuLV (MA_{HIV}) and was used to infect irradiated and nonirradiated MAGI cells. Forty-eight hours after infection, fixed cells were treated with X-Gal, and infected cells were identified by the presence of blue nuclei.

TABLE 2. Infection of proliferating and growth-arrested MAGI cells[a]

Expt.	Virus	No. of blue cells per ml of virus		
		Untreated	Irradiated	Relative infection of arrested cells[b]
1	HIV-1	9.1×10^3	3.5×10^3	38
2	HIV-1	6.0×10^3	1.3×10^3	22
1	MuLV-tat	1.7×10^4	80	0.5
2	MuLV-tat	1.2×10^5	1.1×10^3	0.9
1	MuLV(MA_{HIV})-tat	2.8×10^3	30	1.1
2	MuLV(MA_{HIV})-tat	2.2×10^3	40	1.8

[a] Results are those of infecting untreated and irradiated MAGI cells in two separate experiments.
[b] Infected irradiated cells divided by infected untreated cells times 100.

As previously shown (20), we find that HIV-1 is expressed in a large number of irradiated cells, about 30% of the number of infected untreated cells (Table 2). Neither MuLV nor MuLV (MA_{HIV}) was able to infect a significant number of irradiated MAGI cells. Therefore, incorporation of the HIV-1 matrix protein into MuLV is not sufficient to confer the ability to integrate into nondividing cells.

DISCUSSION

This report describes the characterization of a chimeric virus, MuLV(MA_{HIV}), in which the matrix region of MuLV was replaced with the MA from HIV-1. MuLV(MA_{HIV}) is able to infect several different cell types as efficiently as does wild-type MuLV (Table 1). Since the HIV-1 MA can substitute for the MuLV MA, they must possess domains that have equivalent roles early in infection, for example at entry and uncoating. Also, in contrast to findings with bovine leukemia virus (18), the MuLV MA must not be necessary for specific packaging of viral RNA.

We observed delayed growth kinetics of MuLV(MA_{HIV}), compared with wild-type MuLV (Fig. 3) and determined that this was due to a slow rate of release of virus from infected cells (Fig. 5). Biochemical fractionation showed that the MuLV (MA_{HIV}) Gag polyprotein localizes almost exclusively to the soluble (S) cell fraction (Fig. 7), while generally only 15% of wild-type MuLV Gag is non-membrane bound (23). The need for membrane binding in retroviral assembly has been shown by the rescue of assembly-defective Rous sarcoma virus Gag proteins with the membrane-binding domain of $p60^{src}$ (32) or the amino terminus of HIV-1 Gag (1). Clearly, myristylation-defective (Myr^-) MuLV and spleen necrosis virus are not infectious, because they are unable to assemble because of a lack of membrane binding (23, 27, 30). Since MuLV(MA_{HIV}) does produce infectious particles, it is unlikely that the Gag polyprotein is not myristylated.

By indirect immunofluorescence assay we observed diffuse cytoplasmic staining of MuLV(MA_{HIV}) Gag proteins, while wild-type Gag proteins gave darkly staining perinuclear patches (Fig. 6A and B). Myr^- Gag proteins also give a diffuse staining pattern and have been shown biochemically to exist as monomers within cells (13, 30). The punctate staining of wild-type Gag proteins is believed to represent oligomeric forms of the polyprotein in the process of assembly. The MuLV(MA_{HIV}) Gag proteins may be primarily monomeric within infected cells, because they are not targeted effectively to cellular membranes, where oligomerization and assembly occur. Several insertion and deletion mutations in MuLV and HIV-1 MA disrupt intracellular targeting and assembly, sug-

gesting that proper folding of the Gag protein is important (9, 13, 29). Since MuLV(MA_{HIV}) contains the putative HIV-1 membrane-binding sequences, the chimeric Gag may have lost the ability to be efficiently targeted to membranes because of an altered secondary structure.

Once we had determined that MuLV(MA_{HIV}) was infectious, we asked if it possessed the ability to integrate into nondividing cells, a phenotype specific to lentiviruses. A sequence in the HIV-1 MA is known to play a role in nuclear entry of preintegration complexes into growth-arrested cells (3), along with the HIV-1 Vpr protein (14). Oncoretroviruses such as MuLV are limited to growth in proliferating cells, as integration occurs only after the cells have passed through mitosis (21, 25). Using a one-step assay, we found that MuLV(MA_{HIV}) was not expressed in cells arrested at the G_2 stage of the cell cycle (Table 2). This result demonstrates that while the MA of HIV-1 is necessary for HIV infection of nondividing cells, this signal is not sufficient for that phenotype. Possibly, since the MuLV preintegration complexes contain the Gag CA protein, the HIV-1 MA protein was excluded and therefore unable to mediate nuclear entry.

Analyses of chimeric Gag proteins have revealed that the MA of HIV-1 can functionally replace the MuLV MA in infection. The high level of infectivity of MuLV(MA_{HIV}) shows that the MA of MuLV is not needed for viral RNA packaging. Although the MA of HIV-1 did not allow MuLV to grow in nondividing cells, it will be interesting to see if other HIV-1-specific characteristics are present in MuLV(MA_{HIV}).

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