JOURNAL OF VIROLOGY, Sept. 1996, p. 6384–6389 Vol. 70, No. 9
0022-538X/96/$04.00+0


# Identification of Domains in the Simian Immunodeficiency Virus Matrix Protein Essential for Assembly and Envelope Glycoprotein Incorporation

SILVIA A. GONZÁLEZ,[1]* ARSÈNE BURNY,[2] AND JOSÉ L. AFFRANCHINO[1]

*Centro de Virología Animal, 1414 Buenos Aires, Argentina,[1] and Department of Molecular Biology, University of Brussels, 1640 Rhode-St.-Genèse, Belgium[2]*

Received 14 February 1996/Accepted 20 May 1996

**The matrix domain (MA) of the simian immunodeficiency virus (SIV) is encoded by the amino-terminal region of the Gag polyprotein precursor and is the component of the viral capsid that lines the inner surface of the virus envelope. To define domains of the SIV MA protein that are involved in virus morphogenesis, deletion and substitution mutations were introduced in this protein in the context of a *gag*-protease construct and expressed in the vaccinia virus vector system. The MA mutants were characterized with respect to synthesis and processing of the Gag precursor, assembly and release of virus-like particles, and incorporation of the envelope (Env) glycoprotein into particles. We have identified two regions of the SIV MA which are critical for particle formation. Both domains are located in a central hydrophobic α-helix of the SIV MA, according to data on the structure of this protein. In addition, we have characterized a domain whose mutation impairs the incorporation of SIV Env glycoproteins with long transmembrane cytoplasmic tails into particles. Interestingly, these mutant particles retained the ability to associate with SIV Env proteins with short cytoplasmic tails.**

The human immunodeficiency viruses (HIV) and the closely related simian immunodeficiency viruses (SIV) assemble their capsids from the viral Gag protein, which is first synthesized as a polyprotein precursor ($Pr55^{Gag}$). Concomitant with or soon after virions bud from the plasma membrane of the infected cells, the virions undergo morphological maturation. The Gag polyprotein is proteolytically processed by the viral protease into the matrix (MA [or p17]), capsid (CA [or p24]), nucleocapsid (or p7), and p6 mature Gag proteins (12, 20).

The HIV and SIV MA proteins form the outer shell of the core of the virion, lining the inner surface of the viral membrane (8). Cotranslational myristylation of the N terminus of the MA protein provides a targeting signal for Gag polyprotein transport to the cell surface membrane (26). For both HIV type 1 (HIV-1) (25, 30, 31) and SIV (11), evidence has been presented indicating that in addition to myristylation, a polybasic region within the N terminus of the MA protein may also be necessary for proper membrane targeting of the Gag precursor. The MA proteins of HIV-1 and SIV have been implicated in a number of key roles during the virus life cycle. The HIV-1 MA is thought to be involved in virus entry (27), transport of the viral preintegration complex to the nucleus (1), envelope (Env) glycoprotein incorporation into virus particles (4, 5, 28), and virus particle assembly (3, 7, 28). In SIV, we have shown that when the MA protein is expressed in the absence of all other viral components by means of a vaccinia virus vector system, it assembles into lentivirus-like particles which are released into the medium of the infected cells (11). This observation, which has been independently confirmed by others (18), indicates that the SIV MA has intrinsic information for both self-assembly and particle release. We have also provided evidence that domains within the SIV MA are involved in interactions with Env glycoproteins, since gp120-gp41 Env complexes were found to be incorporated into the SIV MA-made particles (11).

Because of the important roles described for the HIV-1 and SIV MA proteins, identification of the amino acid regions responsible for these functions seems relevant. To map and characterize domains of the SIV MA that are involved in virus morphogenesis, we have generated a series of mutations in this protein. The mutations were analyzed for their effects on Gag expression and processing as well as on particle assembly and release. In addition, the ability of the MA mutants to incorporate the viral Env glycoproteins into particles was investigated. The results are interpreted in the light of the three-dimensional structure of the SIV MA protein (22).

**Mutagenesis of the SIV MA protein.** We have previously performed mutagenesis studies assessing the role in virus morphogenesis of the SIV MA polybasic region (residues 26 to 32) and the MA conserved cysteine residues (9, 11). To further map domains within the SIV MA that are critical for virus assembly and Env glycoprotein incorporation, a series of mutations was introduced into this protein. The specific regions targeted for mutagenesis are depicted in Fig. 1. Most of the mutations affected two or three residues within domains of high sequence homology between the MA proteins of SIV and HIV-1. The exception was an internal in-frame deletion removing 18 amino acids in the C terminus of the SIV MA. The analysis of this region, where the SIV and HIV-1 MA proteins diverge, was performed to investigate its contribution to viral morphogenesis. Changes in the coding sequence of the $SIV_{smmPBj}$ MA were introduced in a construct (Gag-PR) encompassing the complete *gag* gene in addition to the *pol* region coding for the protease domain (PR) (Fig. 2A). Both substitution and deletion mutations were carried out on a *Sal*I-*Pst*I restriction fragment (nucleotides 828 to 1646) cloned into the pUC19 vector, which corresponds to the first 273 amino acids of the SIV Gag precursor. Substitution mutagenesis was per-

* Corresponding author. Mailing address: Centro de Virología Animal (CEVAN), Serrano 665, 1414 Buenos Aires, Argentina. Phone: (54-1) 857-0012. Fax: (54-1) 856-4495. Electronic mail address: sag@cevan.sld.ar.

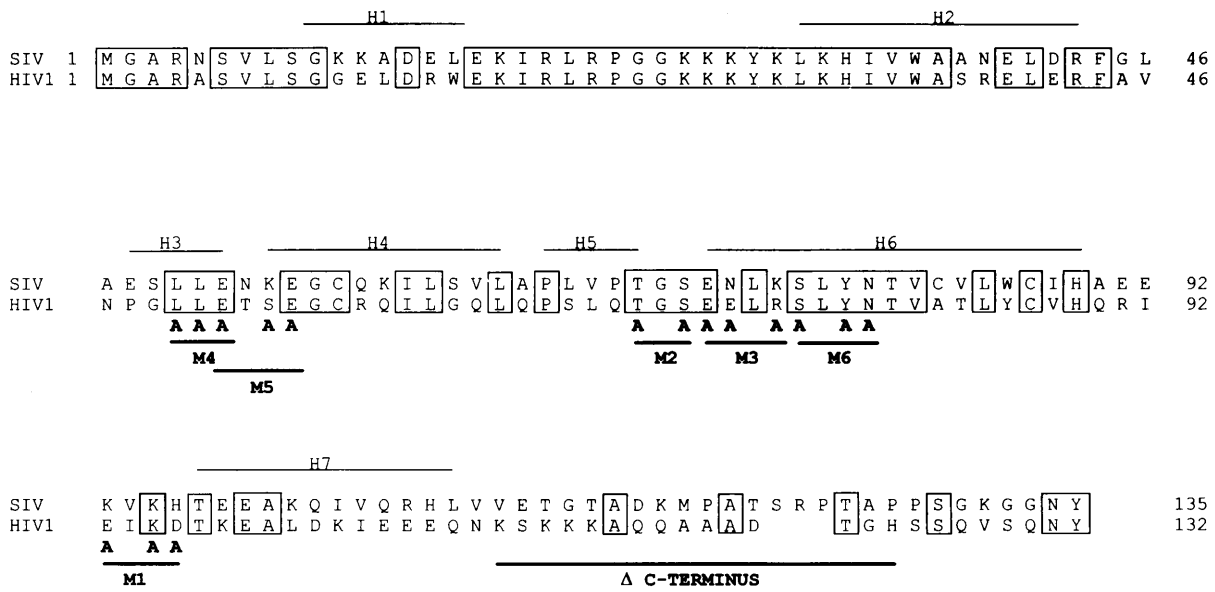


FIG. 1. Alignment of the MA protein sequences of SIV (PBj) and HIV-1 (HXB2) isolates. Identical amino acids are boxed. The helices of the SIV MA structure, H1 to H7 (22), are indicated above the alignment. The alanine substitutions (M1 to M6) and the C-terminus internal deletion (ΔC-terminus) introduced into the SIV MA sequence are shown in boldface below the alignment.

formed by asymmetric PCR-based site-directed mutagenesis as described before (11). The codons targeted for mutagenesis were changed to codons specifying alanine. Alanine was chosen as the replacement residue because it eliminates only the side chain beyond the β-carbon without altering the main-chain conformation. Furthermore, alanine is frequently found in both buried and exposed positions in all kinds of secondary structures (24). To create the C-terminus deletion mutant, the *Sal*I-*Pst*I clone was digested with *Sal*I and *Dra*III restriction enzymes to remove the region coding for the N-terminal 127 amino acids of the SIV MA. The DNA region encoding the first 109 MA residues was PCR amplified using the wild-type (wt) *gag* gene as the template. The PCR sense and antisense primers introduced *Sal*I and *Dra*III restriction sites at the 5′ and 3′ ends of the PCR product, respectively. After digestion with *Sal*I and *Dra*III, the amplified fragment was ligated to the above-described plasmid lacking the first 127 MA codons. The resultant clone thus carries a deletion comprising MA codons 110 to 127. All the mutations introduced into the *Sal*I-*Pst*I clone were confirmed by sequencing. The *Sal*I-*Pst*I fragments carrying the desired mutations were substituted for the wt counterpart in the pMJ601 vaccinia virus transfer vector containing the SIV wt *gag* and PR genes (11). Obtainment, selection, and purification of recombinant vaccinia viruses were performed as described previously (11). Several independent recombinant vaccinia viruses of each MA mutant were initially screened to ensure that their Gag products exhibited the same phenotype.

**Characterization of mutant SIV Gag proteins.** To determine whether the mutations introduced into the SIV MA domain had any effect on the synthesis and processing of Gag polyproteins, CV-1 cells were infected at a high multiplicity (10 PFU per cell) with the recombinant vaccinia viruses and metabolically labeled for 3 to 4 h with [$^{35}$S]methionine (200 μCi/ml; >1,000 Ci/mmol) (Dupont, NEN Research Products) (9, 11). The Gag-specific polypeptides were immunoprecipitated from cell lysates with pooled sera from SIV-infected macaques as previously described (9, 11).

The recombinant vaccinia viruses expressing the MA mutants directed the synthesis of essentially wt levels of the $Pr55^{Gag}$ precursor protein as well as of the processed products, CA and MA polypeptides (Fig. 2B). The recombinant MA protein expressed by the mutant ΔC (with a C-terminal internal deletion) migrated with the expected apparent molecular mass, taking into account the deletion introduced into this protein. All these polypeptides are specific to SIV, since none of them were detected in cells infected with a vaccinia virus expressing only β-galactosidase which was used as a negative control (data not shown).

**Effect of the MA mutations on particle production and viral Env incorporation.** To determine the effect of the MA mutations on particle assembly and release, CV-1 cells were infected in parallel with the recombinant vaccinia viruses expressing wt Gag or MA mutants. After metabolic labeling with [$^{35}$S]methionine, the particulate material released into the culture medium of the infected cells was pelleted from the clarified supernatants through a 20% (wt/vol) sucrose cushion as described before (9, 11). The pelleted particles were lysed in TNN lysis buffer (50 mM Tris-HCl [pH 8.0], 150 mM NaCl, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 10 μg of aprotinin per ml) and assayed for SIV proteins by immunoprecipitation (9, 11). Mutants ΔC, M1, and M4 produced particles with efficiencies similar to that of wt Gag (Fig. 3B). This result indicates that the ability to form and release particles was not significantly affected as a consequence of these mutations. A minor effect on particle formation was observed for M2 and M5 mutants. As measured by densitometry, these mutations caused about a 2.5-fold reduction in the levels of particle-associated Gag-specific bands with respect to those of wt Gag. In contrast, mutations M3 and M6 caused a major defect in particle release (15- to 20-fold reduction compared with wt Gag levels) (Fig. 3B). The fact that the steady-state levels of intracellular M3 and M6 Gag products were found to be similar to those of wt Gag (Fig. 3A) suggests that the decrease in particle production exhibited by these mutants is not due to a defect in Gag synthesis or stability.

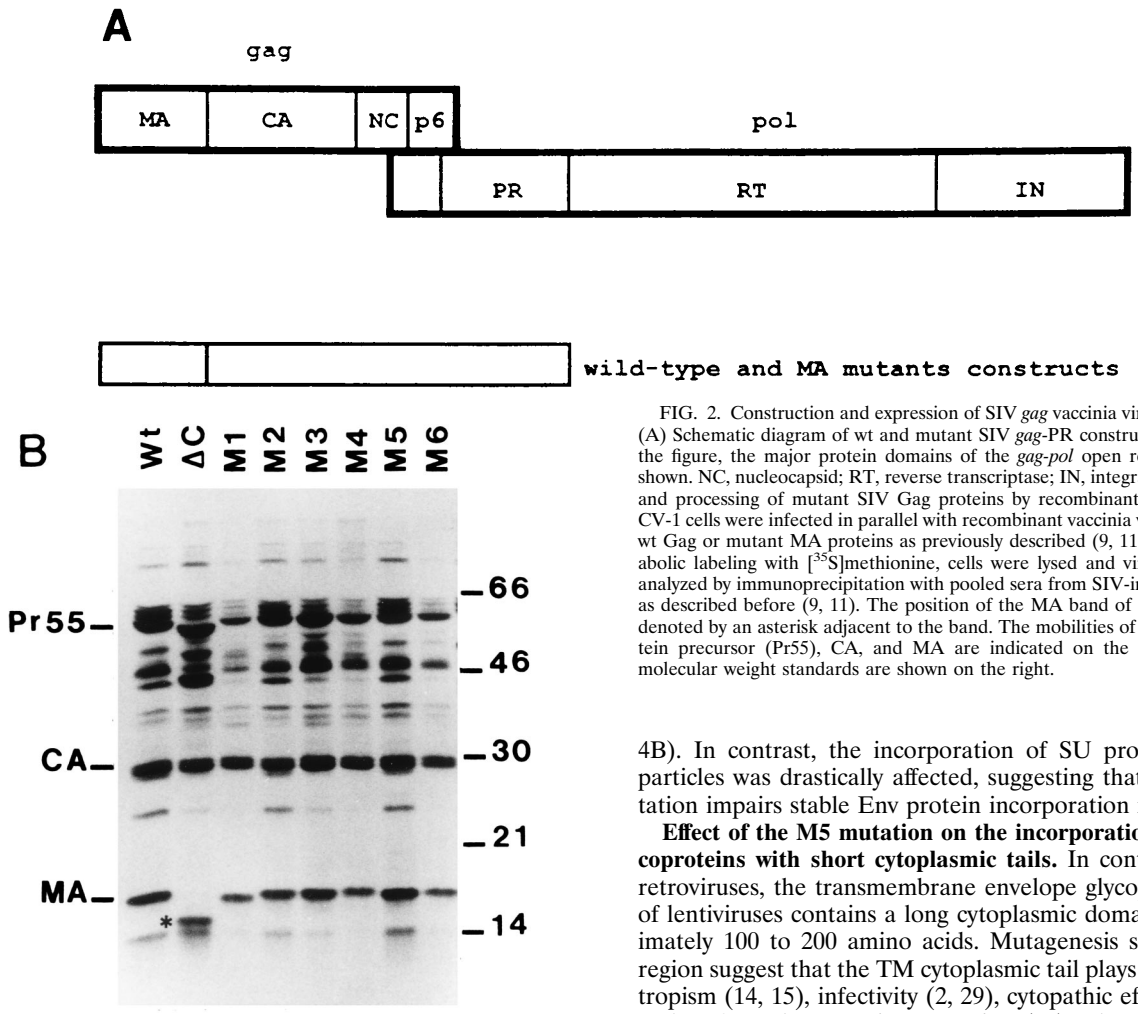


FIG. 2. Construction and expression of SIV *gag* vaccinia virus recombinants. (A) Schematic diagram of wt and mutant SIV *gag*-PR constructs. At the top of the figure, the major protein domains of the *gag-pol* open reading frame are shown. NC, nucleocapsid; RT, reverse transcriptase; IN, integrase. (B) Synthesis and processing of mutant SIV Gag proteins by recombinant vaccinia viruses. CV-1 cells were infected in parallel with recombinant vaccinia viruses expressing wt Gag or mutant MA proteins as previously described (9, 11). Following metabolic labeling with [$^{35}$S]methionine, cells were lysed and viral proteins were analyzed by immunoprecipitation with pooled sera from SIV-infected macaques as described before (9, 11). The position of the MA band of the ΔC mutant is denoted by an asterisk adjacent to the band. The mobilities of the Gag polyprotein precursor (Pr55), CA, and MA are indicated on the left. Positions of molecular weight standards are shown on the right.

The phenotype observed for M3 and M6 mutants could be due to the assembly of Gag polyproteins into intracytoplasmic capsids without subsequent release into the medium. To investigate this possibility, cells infected with these mutants were analyzed by thin-section electron microscopy. No evidence of intracellular assembly was found (data not shown), which suggests that M3 and M6 mutations do not redirect particle assembly to intracellular sites. Consistent with our previous report (11), cells infected with the recombinant vaccinia virus expressing wt Gag showed high levels of typical lentiviral particles budding at the plasma membrane (data not shown).

To examine whether the MA mutants that were competent in particle production retained the ability to interact with the Env glycoproteins, cells were coinfected with each MA mutant and a vaccinia virus recombinant expressing $SIV_{smmPBj}$ Env glycoproteins (10). After metabolic labeling with [$^{35}$S]methionine, both cell lysates and the particulate material released into the culture supernatants were assayed for the presence of the SIV Gag and Env proteins by immunoprecipitation (9, 11). As shown in Fig. 4A, coinfection experiments yielded similar levels of Env glycoproteins and Gag polypeptides in all cell lysates. In the particulate fraction of the culture medium of cells infected with wt Gag, significant amounts of the gp120 surface protein (SU) were detected (Fig. 4B). Similarly, the SU protein was readily detected in all but one mutant particle (Fig. 4B). In contrast, the incorporation of SU protein into M5 particles was drastically affected, suggesting that the M5 mutation impairs stable Env protein incorporation into particles.

**Effect of the M5 mutation on the incorporation of Env glycoproteins with short cytoplasmic tails.** In contrast to other retroviruses, the transmembrane envelope glycoprotein (TM) of lentiviruses contains a long cytoplasmic domain of approximately 100 to 200 amino acids. Mutagenesis studies of this region suggest that the TM cytoplasmic tail plays a role in viral tropism (14, 15), infectivity (2, 29), cytopathic effects (17, 23), and surface glycoprotein expression (16). These lines of evidence indicate that a long cytoplasmic tail has evolved in lentiviruses to fulfill essential functions during the viral life cycle.

It has been shown that mutations in the HIV-1 MA protein that block virion incorporation of the wt HIV-1 Env glycoprotein do not affect the association of particles with Env products bearing short cytoplasmic tails (5, 19). We therefore investigated whether the domain of the SIV MA, identified through the M5 mutation as being critical for SIV Env glycoprotein incorporation, was also necessary for the association of particles with Env proteins bearing short cytoplasmic tails. To this end, CV-1 cells were coinfected with vaccinia viruses expressing M5 Gag products and either $SIV_{smmPBj}$ or $SIV_{macBK28}$ Env glycoproteins. BK28 is a molecular clone of the $SIV_{mac251}$ viral isolate obtained after passage in human cells; the TM glycoprotein of BK28 exhibits a truncated cytoplasmic tail lacking the C-terminal 146 amino acids (13). Virus-like particles were purified from cell culture supernatants by ultracentrifugation through a sucrose cushion, and SIV-specific proteins were detected by immunoprecipitation with pooled sera from SIV-infected macaques (9, 11). For comparison, coinfection experiments with vaccinia viruses directing the synthesis of wt Gag and the different Env glycoproteins were done in parallel. A comparison of the abilities of the Env products with either long or short cytoplasmic tails to associate with wt Gag and MA mutant particles is shown in Fig. 5. Analysis of the cell lysates revealed that PBj and BK28 Env glycoproteins were synthesized and processed with similar efficiencies (Fig. 5A). It

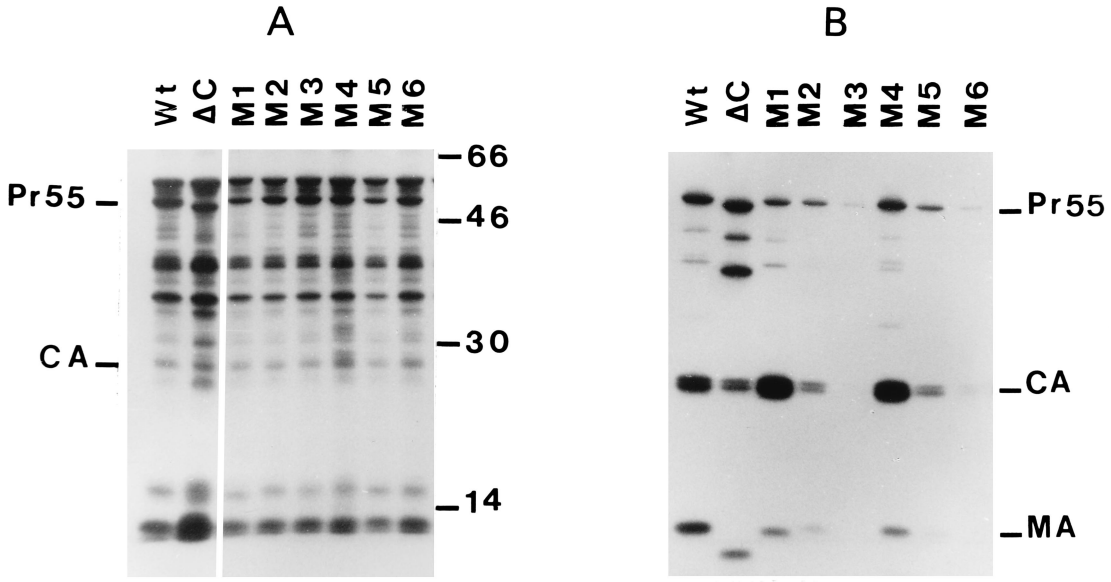


FIG. 3. Effect of MA mutations on particle production. Cells infected with vaccinia viruses expressing wt Gag and the mutant MA proteins were metabolically labeled with [$^{35}$S]methionine for 3 to 4 h (9, 11), and viral proteins were immunoprecipitated from either cell lysates (A) or the particulate fraction of culture supernatants (B). To purify particles, the medium from each infected culture was collected 8 to 9 h postinfection, clarified three times by centrifugation at low speed, and then pelleted through a 20% (wt/vol) sucrose cushion (9, 11). The pellet was resuspended in TNN lysis buffer and assayed for SIV proteins by immunoprecipitation (9, 11). The mobilities of the Gag polyprotein precursor (Pr55), CA, and MA are indicated, as are the positions of molecular weight standards.

should be noted at this point that the gp120 glycoprotein of PBj migrates more slowly than that of BK28. This might be due to an amino acid insertion known to be present within the PBj Env extracellular domain (21) and/or a higher degree of glycosylation of this protein. Fig. 5B shows that both PBj and BK28 Env proteins (with long and short cytoplasmic tails, respectively) were incorporated into wt Gag particles, as determined by the presence of the corresponding SU glycopro-

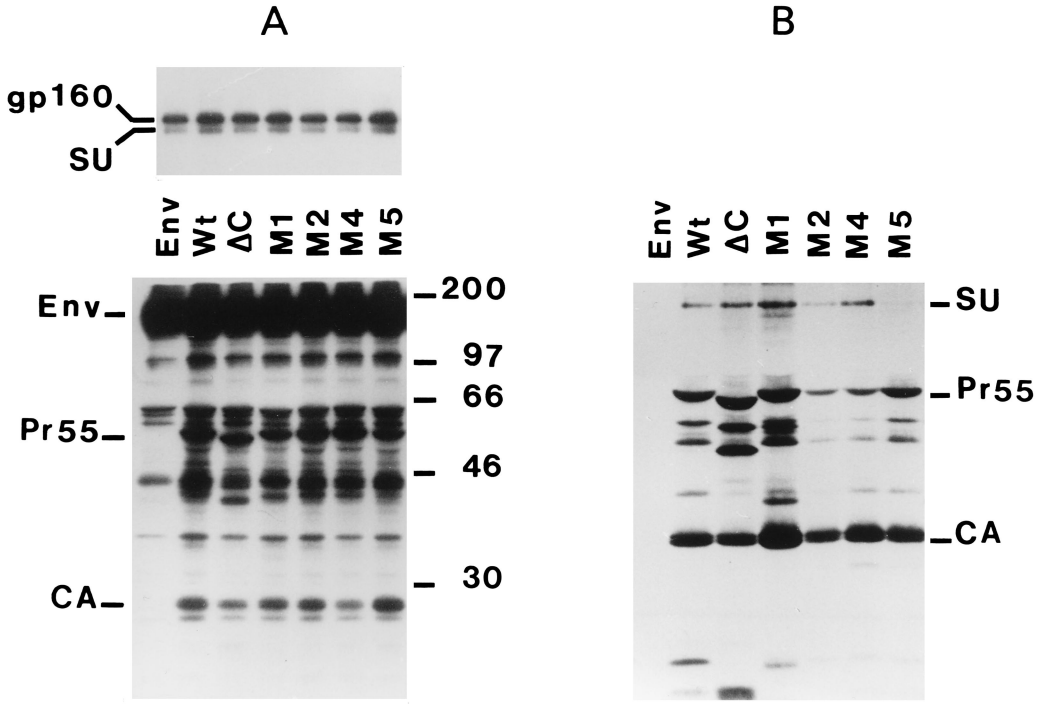


FIG. 4. Effect of MA mutations on incorporation of viral Env glycoproteins into particles. CV-1 cells were either infected with a vaccinia virus expressing $SIV_{smmPBj}$ Env protein (Env) or coinfected with this Env-expressing virus and recombinant vaccinia viruses directing the synthesis of either wt Gag or mutant MA proteins. Infected cells were metabolically labeled, and viral proteins were immunoprecipitated from either cell lysates (A) or the particulate fraction of the culture supernatants (B). In panel A, a shorter autoradiographic exposure showing the positions of the Env proteins is shown at the top. The mobilities of the Gag polyprotein precursor (Pr55), Env, CA, and SU are shown, as are the positions of molecular weight standards.

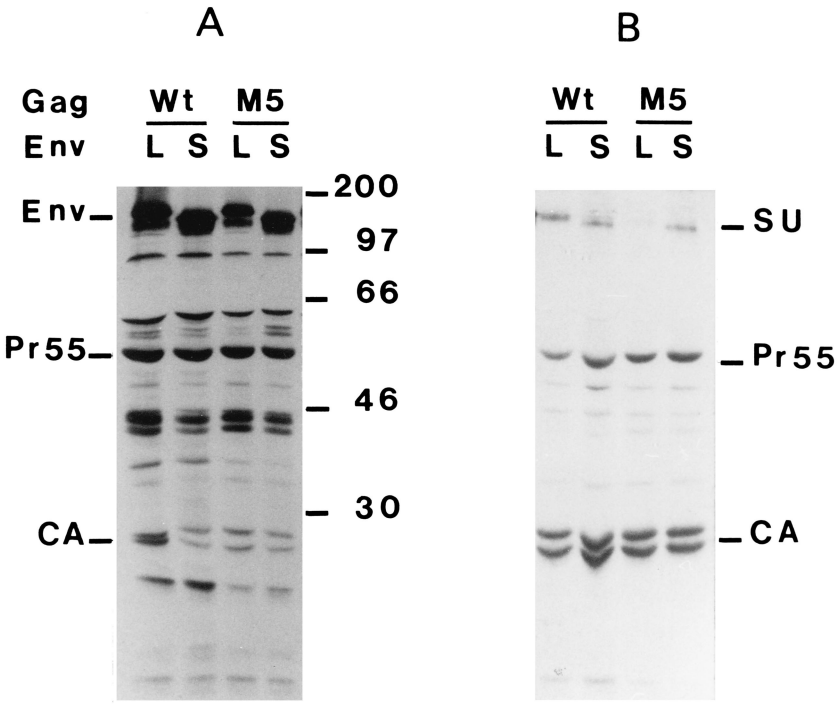


FIG. 5. Analysis of the incorporation of Env glycoproteins with long and short TM cytoplasmic tails into M5 mutant particles. CV-1 cells were coinfected with recombinant vaccinia viruses expressing M5 Gag products and either PBj (L, long cytoplasmic tail) or BK28 (S, short cytoplasmic tail) Env glycoproteins. Coinfection experiments using the vaccinia virus expressing wt Gag were included for comparison. Cells were metabolically labeled, and cell-associated (A) and particle-associated (B) viral proteins were analyzed by immunoprecipitation. The mobilities of the Gag polyprotein precursor (Pr55), Env, CA, and SU are shown, as are the positions of the molecular weight standards.

teins in the particulate fraction. As expected, M5 Gag particles failed to associate with the PBj Env protein. However, the SU protein of BK28 could be readily detected in the particulate material produced by the M5 construct. Thus, the M5 mutation does not affect the ability of particles to incorporate Env glycoproteins with short cytoplasmic tails.

**Conclusions.** The MA mutants generated in this study were used to identify domains of the protein that are important for particle assembly and Env glycoprotein incorporation. The M3 and M6 mutations, which substituted conserved residues in α-helix H6 of the SIV MA (22), impaired the production of virus-like particles. It is likely that the defect in particle formation caused by these mutations is at the level of assembly rather than release, since no evidence of intracellular particles was obtained. H6 spans residues Glu-73 through His-89 and provides a hydrophobic core around which all the other helices are packed. Therefore, mutations in this region might disrupt the overall structure of the SIV MA, affecting the presentation of the interacting domains that participate in protein multimerization. The recently reported crystal structure of the SIV MA reveals that the MA molecules assemble as a trimer (22). In order to form trimers, monomers interact through hydrogen bonding between the carbonyl oxygen of Gly-45 and the backbone nitrogen of Ser-72 and between the backbone nitrogen of Ala-47 and the carbonyl oxygen of Thr-70 (22). Interestingly, the residues altered by the M3 mutation lie in close proximity to amino acids 70 and 72. Thus, this mutation may alter the positions of residues 70 and 72, thereby affecting the ability of these amino acids to establish intermolecular contacts. It has been described that the contacts between the SIV MA monomers in the trimer involve the main-chain groups of these interacting residues (22). It has been speculated that amino acid changes at these positions would have no effect on assembly (22). In keeping with this prediction, mutation M2, in which Thr-70 and Ser-72 were replaced by Ala, had a minor effect on particle production.

In this report, we identified a domain of the SIV MA that is critical for the incorporation of the SIV Env glycoproteins into particles. This result is in agreement with our previous observation that virus-like particles produced upon expression of recombinant SIV MA had the ability to associate with the Env glycoproteins (11). Taken together, these studies indicate that the SIV MA domain mediates the incorporation of Env proteins into viral particles. Mutation M5, in which Glu-52, Lys-54, and Glu-55 were replaced by Ala, impaired the stable association of Env glycoproteins with particles. This mutation altered residues located at the end of helix H3 and the beginning of helix H4 in the SIV MA, as determined on the basis of the three-dimensional structure of this protein (22). The location of this region at an exposed side of the SIV MA trimer may allow its interaction with the TM cytoplasmic domain, and these intermolecular contacts may lead to the incorporation of Env proteins into Gag particles. In HIV-1, it has been reported that mutations at MA residue 13, 31, or 35 blocked Env glycoprotein incorporation into particles (5, 6). It can be speculated that the SIV MA region defined by M5 mutation acts in concert with other MA domains, including residues located at the N terminus, to mediate Env glycoprotein association with particles. Further mutagenesis studies of the SIV MA will help elucidate this point.

In this study, we also showed that the defect in Env glycoprotein incorporation caused by the M5 mutation could be reversed by coexpression of M5 Gag with the $SIV_{BK28}$ Env bearing a short TM cytoplasmic tail. This result, which is con-

sistent with recent reports on HIV-1 (5, 19), suggests the existence of a specific interaction between the SIV MA and the C-terminus of TM gp41 which mediates the incorporation of Env glycoproteins into virus particles. Two possibilities may account for the observation that a mutation in the SIV MA impairs the incorporation of Env proteins with long but not short TM cytoplasmic tails. The altered MA domain may still be able to interact with the truncated gp41, or alternatively, the incorporation of this type of Env protein may be MA independent. Further studies will be necessary to clarify this issue.

We are indebted to D. Hockley (NIBSC, South Mimms, United Kingdom) for electron microscopy studies and for helpful discussions on retrovirus assembly. We thank P. N. Fultz for the anti-SIV sera, B. Moss for the pMJ vectors, and J. I. Mullins for the SIV PBj1.9 and BK28 proviral clones.

S.A.G. and J.L.A. are Career Investigators of the National Research Council of Argentina (CONICET).

## REFERENCES

1. **Bukrinsky, M. I., S. W. Haggerty, M. P. Demsey, N. Sharova, A. Adzhuvei, L. Spitz, P. Lewis, D. Goldfarb, M. Emerman, and M. Stevenson.** 1993. A nuclear localization signal within the HIV-1 matrix protein that governs infection of nondividing cells. Nature (London) **365:**666–669.
2. **Chakrabarti, L., M. Emerman, P. Tiollais, and P. Sonigo.** 1989. The cytoplasmic domain of simian immunodeficiency virus transmembrane protein modulates infectivity. J. Virol. **63:**4395–4403.
3. **Chazal, N., B. Gay, C. Carrière, J. Tournier, and P. Boulanger.** 1995. Human immunodeficiency virus type 1 MA deletion mutants expressed in baculovirus-infected cells: *cis* and *trans* effects on the Gag precursor assembly pathway. J. Virol. **69:**365–375.
4. **Dorfman, T., F. Mammano, W. A. Haseltine, and H. G. Göttlinger.** 1994. Role of the matrix protein in the virion association of the human immunodeficiency virus type 1 envelope glycoprotein. J. Virol. **68:**1689–1696.
5. **Freed, E. O., and M. A. Martin.** 1995. Virion incorporation of envelope glycoproteins with long but not short cytoplasmic tails is blocked by specific, single amino acid substitutions in the human immunodeficiency virus type 1 matrix. J. Virol. **69:**1984–1989.
6. **Freed, E. O., and M. A. Martin.** 1996. Domains of the human immunodeficiency virus type 1 matrix and gp41 cytoplasmic tail required for envelope incorporation into virions. J. Virol. **70:**341–351.
7. **Freed, E. O., J. M. Orenstein, A. J. Buckler-White, and M. A. Martin.** 1994. Single amino acid changes in the human immunodeficiency virus type 1 matrix protein block virus particle production. J. Virol. **68:**5311–5320.
8. **Gelderblom, H. R., E. H. S. Hausmann, M. Ozel, G. Pauli, and M. A. Koch.** 1987. Fine structure of human immunodeficiency virus (HIV) and immunolocalization of structural proteins. Virology **156:**171–176.
9. **González, S. A., and J. L. Affranchino.** 1995. Mutational analysis of the conserved cysteine residues in the simian immunodeficiency virus matrix protein. Virology **210:**501–507.
10. **González, S. A., J. L. Affranchino, and A. Burny.** 1994. Expression of biologically active envelope glycoprotein from the acutely pathogenic simian immunodeficiency virus $SIV_{smmPBj}$. Virus Genes **8:**75–78.
11. **González, S. A., J. L. Affranchino, H. R. Gelderblom, and A. Burny.** 1993. Assembly of the matrix protein of simian immunodeficiency virus into virus-like particles. Virology **194:**548–556.
12. **Henderson, L. E., R. E. Benveniste, R. Sowder, T. D. Copeland, A. M. Schultz, and S. Oroszlan.** 1988. Molecular characterization of *gag* proteins from simian immunodeficiency virus ($SIV_{Mne}$). J. Virol. **62:**2587–2595.
13. **Hirsch, V. M., P. Edmondson, M. Murphy-Corb, B. Arbeille, P. R. Johnson, and J. I. Mullins.** 1989. SIV adaptation to human cells. Nature (London) **341:**573–574.
14. **Johnston, P. B., J. W. Dubay, and E. Hunter.** 1993. Truncations of the simian immunodeficiency virus transmembrane protein confer expanded virus host range by removing a block to virus entry into cells. J. Virol. **67:**3077–3086.
15. **Kodama, T., D. P. Wooley, Y. M. Naidu, H. W. Kestler III, M. D. Daniel, Y. Li, and R. C. Desrosiers.** 1989. Significance of premature stop codons in *env* of simian immunodeficiency virus. J. Virol. **63:**4709–4714.
16. **LaBranche, C. C., M. M. Sauter, B. S. Haggarty, P. J. Vance, J. Romano, T. K. Hart, P. J. Bugelski, M. Marsh, and J. A. Hoxie.** 1995. A single amino acid change in the cytoplasmic domain of the simian immunodeficiency virus transmembrane molecule increases envelope glycoprotein expression on infected cells. J. Virol. **69:**5217–5227.
17. **Lee, S.-J., W. Hu, A. G. Fisher, D. J. Looney, V. F. Kao, H. Mitsuya, L. Ratner, and F. Wong-Staal.** 1989. Role of the carboxy-terminal portion of the HIV-1 transmembrane protein in viral transmission and cytopathogenicity. AIDS Res. Hum. Retroviruses **5:**441–449.
18. **Liska, V., D. Spehner, M. Mehtali, D. Schmitt, A. Kirn, and A. M. Aubertin.** 1994. Localization of viral protein X in simian immunodeficiency virus macaque strain and analysis of its packaging requirements. J. Gen. Virol. **75:** 2955–2962.
19. **Mammano, F., E. Kondo, J. Sodroski, A. Bukovsky, and H. G. Göttlinger.** 1995. Rescue of human immunodeficiency virus type 1 matrix protein mutants by envelope glycoproteins with short cytoplasmic domains. J. Virol. **69:**3824–3830.
20. **Mervis, R. J., N. Ahmad, E. P. Lillehoj, M. G. Raum, R. F. H. Salazar, H. W. Chan, and S. Venkatesan.** 1988. The *gag* gene products of human immunodeficiency virus type 1: alignment within the *gag* open reading frame, identification of posttranslational modifications, and evidence for alternative *gag* precursors. J. Virol. **62:**3993–4002.
21. **Myers, G., S. Wain-Hobson, B. Korber, R. F. Smith, and G. N. Pavlakis (ed.).** 1993. Human retroviruses and AIDS. A compilation and analysis of nucleic acid and amino acid sequences. Los Alamos National Laboratory, Los Alamos, N.Mex.
22. **Rao, Z., A. S. Belyaev, E. Fry, P. Roy, I. M. Jones, and D. I. Stuart.** 1995. Crystal structure of SIV matrix antigen and implications for virus assembly. Nature (London) **378:**743–747.
23. **Ritter, G. D., Jr., M. J. Mulligan, S. L. Lydy, and R. W. Compans.** 1993. Cell fusion activity of the simian immunodeficiency virus envelope protein is modulated by the intracytoplasmic domain. Virology **197:**225–264.
24. **Rose, G. D., A. R. Geselowitz, G. J. Lesser, R. H. Lee, and M. H. Zehfus.** 1985. Hydrophobicity of amino acid residues in globular proteins. Science **229:**834–838.
25. **Spearman, P., J.-J. Wang, N. Vander Heyden, and L. Ratner.** 1994. Identification of human immunodeficiency virus type 1 Gag protein domains essential to membrane binding and particle assembly. J. Virol. **68:**3232–3242.
26. **Veronese, F. M., T. D. Copeland, S. Oroszlan, R. C. Gallo, and M. G. Sarngadharan.** 1988. Biochemical and immunological analysis of human immunodeficiency virus *gag* gene products p17 and p24. J. Virol. **62:**795–801.
27. **Yu, X., Q.-C. Yu, T.-H. Lee, and M. Essex.** 1992. The C terminus of human immunodeficiency virus type 1 matrix protein is involved in early steps of the virus life cycle. J. Virol. **66:**5667–5670.
28. **Yu, X., X. Yuan, Z. Matzuda, T.-H. Lee, and M. Essex.** 1992. The matrix protein of human immunodeficiency virus type 1 is required for incorporation of viral envelope protein into mature virions. J. Virol. **66:**4966–4971.
29. **Yu, X., X. Yuan, M. F. McLane, T.-H. Lee, and M. Essex.** 1993. Mutations in the cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein impair the incorporation of Env proteins into mature virions. J. Virol. **67:**213–221.
30. **Yuan, X., X. Yu, T.-H. Lee, and M. Essex.** 1993. Mutations in the N-terminal region of human immunodeficiency virus type 1 matrix protein block intracellular transport of the Gag precursor. J. Virol. **67:**6387–6394.
31. **Zhou, W., L. J. Parent, J. W. Wills, and M. D. Resh.** 1994. Identification of a membrane-binding domain within the amino-terminal region of human immunodeficiency virus type 1 Gag protein which interacts with acidic phospholipids. J. Virol. **68:**2556–2569.