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# Phosphorylation-dependent human immunodeficiency virus type 1 infection and nuclear targeting of viral DNA

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**ABSTRACT** **In the replication of human immunodeficiency virus type 1 (HIV-1), gag MA (matrix), a major structural protein of the virus, carries out opposing targeting functions. During virus assembly, gag MA is cotranslationally myristoylated, a modification required for membrane targeting of gag polyproteins. During virus infection, however, gag MA, by virtue of a nuclear targeting signal at its N terminus, facilitates the nuclear localization of viral DNA and establishment of the provirus. We now show that phosphorylation of gag MA on tyrosine and serine prior to and during virus infection facilitates its dissociation from the membrane, thus allowing it to translocate to the nucleus. Inhibition of gag MA phosphorylation either on tyrosine or on serine prevents gag MA-mediated nuclear targeting of viral nucleic acids and impairs virus infectivity. The requirement for gag MA phosphorylation in virus infection is underscored by our finding that a serine/threonine kinase is associated with virions of HIV-1. These results reveal a novel level of regulation of primate lentivirus infectivity.**

Following the infection of $CD4^+$ host cells by the lentivirus human immunodeficiency virus type 1 (HIV-1), reverse transcription of viral nucleic acids occurs within the context of a high molecular weight nucleoprotein complex (hereafter referred to as preintegration complex). This complex contains all functions necessary for reverse transcription and nuclear localization of viral DNA and additionally for integration of viral with host cell DNA (1–4). The preintegration complex of HIV-1 is localized to the host cell nucleus by an active transport process which requires ATP but which is independent of host cell division (4). In contrast, nuclear localization of viral preintegration complexes following infection by retroviruses such as murine leukemia virus will occur only after cells have passed through mitosis (5, 6). The active nuclear import of HIV-1 preintegration complexes is facilitated by the nucleophilic structural protein gag MA (matrix) and by the accessory viral protein R (Vpr). Following virus infection, gag MA (7, 8) and Vpr (9) remain associated with viral nucleic acids within the preintegration complex and facilitate nuclear transport of viral nucleic acids in nondividing cells (10, 11).

In addition to the nuclear targeting function of gag MA during virus infection, myristoylation of gag MA is required for efficient membrane localization of gag polyproteins prior to virus assembly (12–14). Thus, the gag MA protein carries out opposing targeting functions in the replication of HIV-1. In this study, we show that gag MA is phosphorylated prior to and during virus entry into the target cell and that this phosphorylation is required for regulation of its membrane targeting and nuclear targeting properties.

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## MATERIALS AND METHODS

**Cells, Antibodies, and Viruses.** HeLa-CD4-LTR/β-gal cells (15) were maintained in RPMI medium supplemented with 20% fetal calf serum. MT4 and 293T cells (16) were maintained in RPMI medium and in DMEM medium, respectively (supplemented with 10% fetal calf serum). All virus experiments utilized the HIV-1 molecular clone, $HIV\text{-}1_{MF}$ (17), a Vpr-negative IIIB-derived clone (18). The HIV-1 gag MA myristoylation mutant (HIV-1 gag MA $myr^-$) contains an $S^6$–A substitution within the myristoylation site at the N terminus of gag MA. $HIV\text{-}1_{MF}$ wild-type and HIV-1 gag MA $myr^-$ virions were obtained following transfection of 293T cells as detailed elsewhere (16). For production of gradient-purified virions, clarified culture supernatants were layered on a 15–60% (wt/vol) continuous sucrose gradient and virions were banded at 200,000 × *g* for 4–6 hr. The amount of virus in each fraction was measured by reverse transcriptase (RT) activity. Virions in peak gradient fractions were harvested (10,000 × *g*, 2 hr) and resuspended in 50 mM Hepes, pH 7.4/10 mM $MgCl_2$ at 0.01× volume.

**Subcellular Fractionation.** Subcellular fractions of radiolabeled HIV-1-infected MT4 cells comprising cytosolic, cell membrane, and nuclear chromatin and matrix extracts were prepared as detailed elsewhere (19). Viral preintegration complexes were isolated from nuclear extracts at 4–6 hr postinfection as detailed previously (7, 9). Nuclei were extracted with a high salt buffer (20 mM Hepes/0.5 mM $MgCl_2$/500 mM potassium acetate/0.5 mM dithiothreitol/0.1 μM phenylmethylsulfonyl fluoride). Nuclear extracts were resolved on a 30–60% (wt/vol) sucrose gradient as detailed elsewhere (7). Gradient fractions (0.5–1 ml) were collected and dialyzed overnight (6000 molecular weight cutoff, Bethesda Research Laboratories microdialysis chamber). $^{32}P$-labeled proteins were visualized following separation on 15% SDS/PAGE gels and autoradiography. Gag MA in cell fractions was determined by measurement of radioactivity in each gradient fraction and in gag MA from gel-fractionated cell extracts.

**Phosphoamino Acid (PAA) and Phosphopeptide Analysis.** $^{32}P$-labeled gag MA immunoprecipitates were resolved on 15% SDS/PAGE gels. The gel fragment containing gag MA was excised and digested with 30 μg of L-1-tosylamido-2-phenylethyl chloromethyl ketone-trypsin (Worthington) in 300 μl of 0.25% ammonium bicarbonate, pH 8.0. Phosphopeptides were resolved in two dimensions on thin-layer cellulose plates

Abbreviations: HIV-1, human immunodeficiency virus type 1; Vpr, viral protein R; MA, matrix; CA, capsid; PAA, phosphoamino acid; RT, reverse transcriptase; LTR, long terminal repeat.

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as detailed elsewhere (20). The samples were separated by electrophoresis at 1000 V for 35 min in formic acid/acetic acid/water (25:78:897), pH 1.9. After electrophoresis each plate was allowed to dry thoroughly; then chromatography was performed at a right angle to the direction of electrophoresis in 1-butanol/pyridine/acetic acid/water (15:10:3:12) as described (20). After chromatography the plates were dried and exposed to x-ray film (XAR-5; Kodak) to localize phosphopeptides.

For PAA analysis (20), dried phosphopeptides were reconstituted in 0.2 ml of 6 M HCl. Partial hydrolysis of the phosphopeptides was performed at 110°C for 1 hr, after which the hydrolysate was diluted 6-fold with water, lyophilized, reconstituted with 100 μl of $H_2O$, and lyophilized a second time. Each sample was spotted on a 20 cm × 20 cm thin-layer cellulose plate in 5 μl of 30% formic acid containing phosphothreonine, phosphoserine, and phosphotyrosine (1 mg/ml each). Samples were electrophoresed at 1300 V for 25–35 min in formic acid/acetic acid/water (25:78:897), pH 3.5. The plates were dried and PAA standards were visualized with ninydrin. Radiolabeled PAAs were identified by autoradiography.

**Recombinant Kinases.** The hematopoietic protein-tyrosine kinases, Hck and Fes, were expressed in the baculovirus/Sf-9 cell system as described elsewhere (21). Hck and Fes were immunoprecipitated from clarified lysates of infected Sf-9 cells and the immune complexes were incubated with permeabilized gradient-purified HIV-1 virions in a 20-μl reaction mixture containing 20 μCi of [γ-$^{32}$P]ATP, 0.5% Nonidet P-40, and 10 mM $MgCl_2$ in 50 mM Hepes, pH 7.4 (1 Ci = 37 GBq). Following incubation at 30°C for 30 min, the reactions were stopped by the addition of SDS sample buffer and heating to 95°C for 5 min. Phosphorylated proteins were separated by SDS/PAGE and visualized by autoradiography.

**Single Cycle Transactivation Assay.** Nonirradiated or γ-irradiated (5000 rads; 1 rad = 0.01 Gy) HeLa-CD4-LTR/β-gal cells were infected with HIV-1 at a multiplicity of infection of 0.1. At 48 hr postinfection, infected cells were scored after visualization of β-galactosidase activity *in situ* by staining cells with 5-bromo-4-chloro-3-indolyl β-galactopyranoside.

**PCR Analysis of Viral cDNA Synthesis.** Prior to cell infection the virus inoculum was treated with DNase (Worthington) in order to remove carryover proviral DNA in the inoculum (22). PCR conditions for amplification of linear and circular forms of viral DNA were exactly as detailed elsewhere (9). Cellular DNA was isolated at various intervals postinfection from aliquots of 2–3 × $10^6$ cells (Iso-Quick DNA extraction kit). Late reverse transcription products of HIV-1 were amplified by primers to long terminal repeat (LTR) R (5′-G$^{485}$GGAGCTCTCTGGCTAACT) and gag (5′-G$^{912}$GATTAACTGCGAATCGTTC), while 2 LTR circle forms of viral DNA were amplified by primers to LTR R′ (5′-G$^{526}$AGGCTTAAGCAGTGGGTTG) and LTR U5 (5′-G$^{566}$TCTGTTGTGACTCTGGT). Primers are numbered according to the HIV-1 HXB2 sequence (18) and only 3′ LTR coordinates are given. HIV-1 cDNA standards and 2 LTR circle standards were generated by PCR on serial 2-fold dilutions of 8E5 cells [each containing a defective provirus (23)] and HIV-1-infected CD4$^+$ MT4 cells, respectively.

## RESULTS

Since gag MA carries out membrane targeting and nuclear targeting functions in HIV-1 replication, we investigated the mechanism by which these opposing targeting functions of gag MA are regulated. Infection of CD4$^+$ T cells with [$^3$H]leucine-labeled virions indicated that gag MA sequestered in the membrane and nucleus of the target cell (Fig. 1*A*). In contrast, gag CA (capsid), the other major structural virion protein,

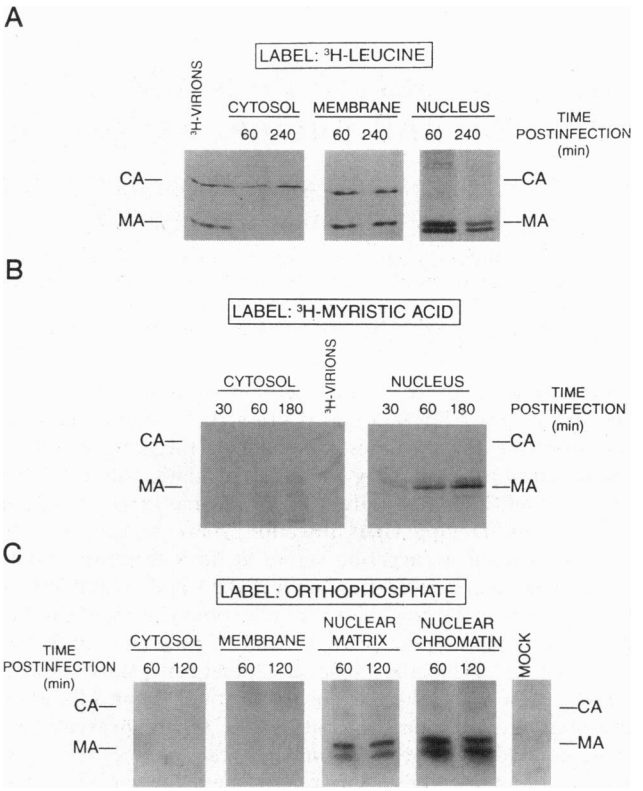


FIG. 1. Subcellular distribution of myristoylated and phosphorylated forms of HIV-1 gag MA following acute infection. (*A*) [$^3$H]Leucine-labeled virions were harvested and concentrated (100×). CD4$^+$ MT4 cells (1–2 × $10^8$) were infected with concentrated radiolabeled virions (1 μg of gag CA) and, at the indicated intervals, 5 × $10^7$–1 × $10^8$ cells were removed for subcellular fractionation after hypotonic cell lysis (19). (*B*) HIV-1 was labeled for 24 hr with [$^3$H]myristic acid (2–5 μCi/ml) as described elsewhere (24). (*C*) HIV-1 gag MA was labeled following infection of CD4$^+$ MT4 cells in the presence of 0.5 mCi of ortho[$^{32}$P]phosphate per ml in phosphate-free medium containing 0.1% calf serum. Labeled viral proteins within cell fractions were visualized by electrophoresis on 15% SDS/PAGE gels and autoradiography. The mock lane depicts nuclear chromatin extract prepared from MT4 cells immediately after addition of $^{32}$P-labeled HIV-1.

which is not contained within the HIV-1 preintegration complex (7), was localized in the cytosol and membrane but was excluded from the nucleus (Fig. 1*A*). Infection of cells with virions in which gag MA was labeled with [$^3$H]myristic acid revealed that nuclear forms of gag MA were myristoylated (Fig. 1*B*). Infection of CD4$^+$ T cells with HIV-1 in the presence of ortho[$^{32}$P]phosphate demonstrated that phosphorylated forms of gag MA were present only in the nucleus of infected cells (Fig. 1*C*). The presence of two $^{32}$P-labeled gag MA species (Fig. 1*A*), which both reacted with gag MA antibodies, was most likely due to altered electrophoretic mobility of phosphorylated gag MA in SDS gels as has been described for duck hepatitis B virus capsid protein (25). Although a substantial fraction of gag MA (60–70% of total, based on measurement of $^3$H-labeled gag MA in cell fractions) was present in membrane extracts of infected cells (Fig. 1*A*), phosphorylated gag MA was not detected in that fraction (Fig. 1*C*). In addition, gag MA appeared to be specifically phosphorylated during virus entry since gag CA, which also contains phosphate acceptor residues (26, 27), was not phosphorylated at this time (Fig. 1*C*). Taken together, these results indicate that myristoylation of gag MA does not restrict its nuclear targeting function, and phosphorylated gag MA, within the context of the viral preintegration complex, is selectively localized to the nucleus.

We next determined at which point during virus production and infection gag MA was phosphorylated and whether a serine/threonine or tyrosine kinase was involved. Gag MA from virus particles produced in the presence of ortho[$^{32}$P]phosphate contained predominantly phosphotyrosine and, to a lesser extent, phosphoserine (Fig. 2*A, Left*). In contrast, gag MA obtained from nuclear extracts of CD4$^+$ T cells infected with HIV-1 in the presence of ortho[$^{32}$P]phosphate contained predominantly phosphoserine (Fig. 2*A, Center*). Surprisingly, addition of [$\gamma$-$^{32}$P]ATP to permeabilized, density gradient-purified virus particles resulted in specific phosphorylation of gag MA in gradient fractions with a density of $\approx$1.16 g/ml, which is the expected density for intact virions of HIV-1 (Fig. 2*B*). No other structural proteins of the virus were phosphorylated under these conditions (Fig. 2*B*). Phosphorylation occurred predominantly on serine and, to a lesser extent, on threonine but not on tyrosine (Fig. 2*A, Right*). Tryptic digests of gag MA phosphorylated within permeabilized virions revealed five phosphopeptides (Fig. 2*C*). Since gag MA within virions contained exclusively phosphoserine (Fig. 2*C Inset*), at least five serine phosphate acceptor sites were utilized by the virion-associated kinase. Addition of highly purified recombinant tyrosine kinases including Hck and Fes to permeabilized virions resulted in phosphorylation of gag MA on tyrosine (Fig. 2 *B–D*). Addition of Hck or Fes mutants devoid of tyrosine kinase activity (21) to permeabilized virions resulted in phosphorylation of gag MA (Fig. 2*D*) predominantly on serine and, to a lesser extent, on threonine presumably due to the action of the virion-associated serine/threonine kinase (not shown). Taken together, these results indicate that a cellular serine kinase is selectively incorporated into virus particles and gag MA is a specific substrate for the kinase(s).

Although phosphorylation of gag MA protein has been described (28), the biological significance of gag MA protein phosphorylation or, indeed, of phosphorylation of any structural HIV protein has not been established. Therefore, we determined whether phosphorylation of gag MA was required for its nuclear localization and consequently for HIV-1 infectivity. Initially, we introduced individual amino acid substitutions at all conserved serine residues of gag MA (including S$^{38}$, S$^{67}$, S$^{72}$, S$^{77}$, S$^{111}$). In addition, we mutated the Y$^{132}$ residue of gag MA (Y$^{132}\rightarrow$F), since this residue was shown to be phosphorylated and mutations at this site markedly attenuated HIV-1 infectivity in primary macrophages (29). All single gag MA serine mutants infected CD4$^+$ MT4 cells with kinetics approaching or equal to that of wild-type HIV-1 (not shown). In addition, the MA Y$^{132}\rightarrow$F mutant was as infectious as wild-type HIV-1 in T cells and in primary macrophages (not shown). Gag MA isolated from cells infected with the MA Y$^{132}\rightarrow$F mutant was phosphorylated on tyrosine (Fig. 3*A*), indicating that removal of the Y$^{132}$ phosphate acceptor site led to differential phosphorylation at another tyrosine residue on gag MA.

Given the limitations of the genetic approach, we utilized kinase inhibitors to examine the role of gag MA phosphorylation in HIV-1 replication. To exclude indirect effects of kinase inhibitors on target cells, the inhibitors were applied to HIV-1 producer cells so as to derive HIV-1 virions hypophosphorylated on either serine or tyrosine. Production of virions in the presence of genistein (30) with or without herbimycin (31) prevented gag MA phosphorylation on tyrosine but not on serine (Fig. 3*A*), while the serine/threonine kinase inhibitor H-7 (32) prevented MA phosphorylation on serine but not on tyrosine (Fig. 3*A*). HIV-1 virions were labeled with [$^3$H]myristic acid in the presence of inhibitors and normalized to RT activity prior to infection of CD4$^+$ MT4 cells. For wild-type HIV-1, 12% of $^3$H-labeled gag MA localized in the nucleus following infection while the remainder was distributed in the membrane/cytosol fraction (Fig. 3*B*). For cells infected with HIV-1 hypophosphorylated on serine and tyrosine, 3% and

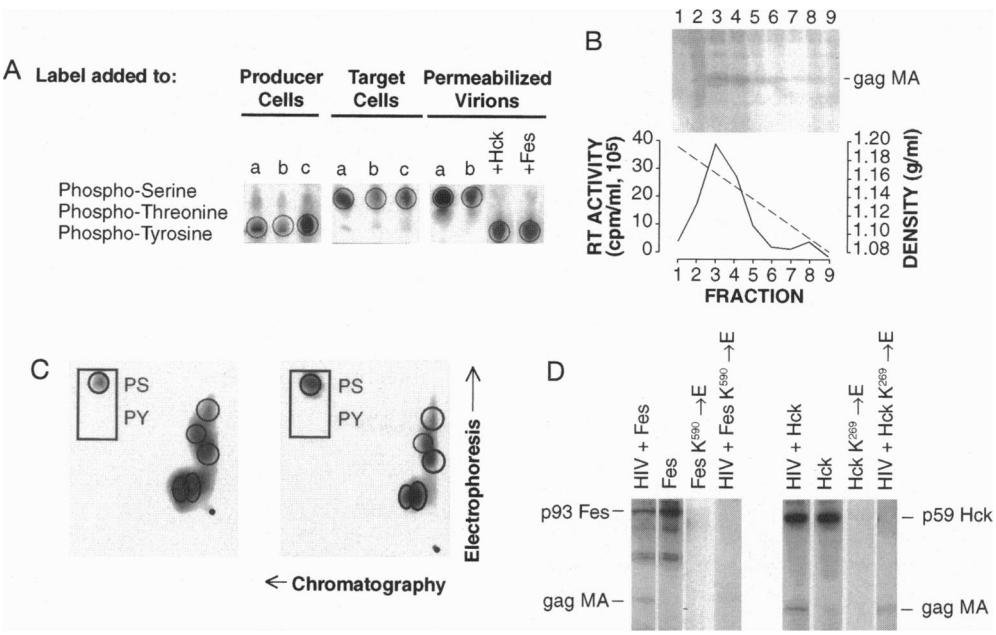


FIG. 2. Differential HIV-1 gag MA phosphorylation prior to and during virus infection. (*A*) PAA analysis of HIV-1 gag MA labeled with ortho[$^{32}$P]phosphate during virus production (*Left*), during virus infection (*Center*), or after addition of [$\gamma$-$^{32}$P]ATP to permeabilized gradient-purified virions (*Right*). Analysis of $^{32}$P-labeled gag MA obtained in independent experiments is denoted a, b, and c. (*B*) Detection of serine/threonine kinase activity in gradient-purified virions of HIV-1. Virions from each gradient fraction were permeabilized by Nonidet P-40 (0.5%) and two freeze/thaw cycles. Phosphorylation of gag MA in permeabilized virions proceeded for 30 min in the presence of 20 $\mu$Ci of [$\gamma$-$^{32}$P]ATP. (*C*) Two-dimensional phosphopeptide analysis of gag MA labeled with [$\gamma$-$^{32}$P]ATP in permeabilized gradient-purified virions. The five phosphopeptides contained phosphoserine since PAA analysis indicated exclusively phosphoserine in gag MA obtained from *in vitro* labeled nascent virions (*Inset*). Two independent virus preparations are shown. (*D*) Phosphorylation of gag MA *in vitro*. Gradient-purified virions were permeabilized as in *B* and incubated with the protein-tyrosine kinases Hck and Fes or with their catalytically inactive (Fes, K$^{590}\rightarrow$E; Hck, K$^{269}\rightarrow$E) mutants. PAA of *in vitro* phosphorylated gag MA is shown in *A*.

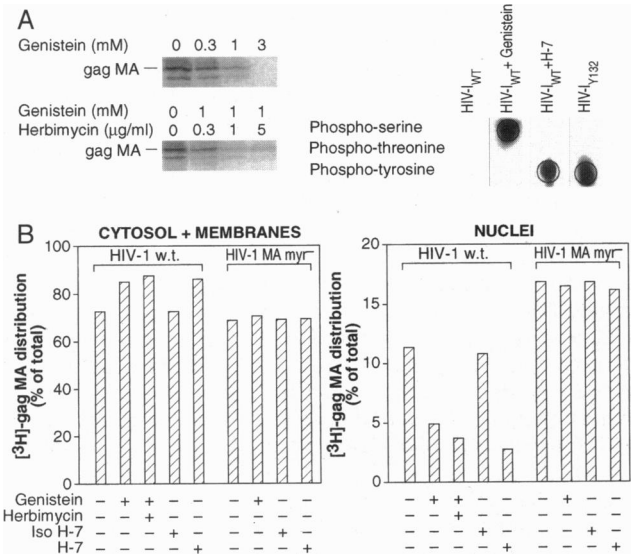


FIG. 3. Protein-tyrosine and serine kinase inhibitors reduce nuclear localization of gag MA. (*A*) Phosphorylation of gag MA in the presence of protein-tyrosine and serine/threonine kinase inhibitors. HIV-1-infected MT4 cells were labeled with ortho[$^{32}$P]phosphate in the presence of protein kinase inhibitors for 6 hr. Gag MA in virions obtained from tyrosine kinase inhibitor-treated producer cells was resolved on 15% SDS/PAGE gels, visualized by autoradiography (*Left*), and confirmed by PAA analysis (*Right*). PAA analysis of gag MA from virions produced in the presence of a serine/threonine kinase inhibitor is shown (*Right*). (*B*) Subcellular distribution of gag MA following infection with protein kinase inhibitor-treated HIV-1. The data shown are representative of four similar experiments. HIV-1-infected MT4 cells were labeled with [$^3$H]myristic acid in the presence of protein kinase inhibitors (genistein, 1 mM; herbimycin, 5 μg/ml; H-7/Iso H-7, 150 μM) for 6 hr. Virions were harvested (10,000 × *g*, 30 min) and normalized to RT activity. Concentrated virus (100×) was added to fresh MT4 cells in the absence of protein kinase inhibitors and, at the indicated times postinfection, distribution of $^3$H-labeled gag MA in subcellular fractions was determined by liquid scintillation spectroscopy as outlined in Fig. 1. The HIV-1 gag MA S$^6$→A mutant does not incorporate [$^3$H]myristic acid, and virions were labeled with [$^3$H]leucine between 42 and 48 hr posttransfection and treated with kinase inhibitors.

4% of gag MA localized in the nucleus, respectively, with concomitantly greater amounts of gag MA in membrane/cytosol fractions (Fig. 3*B*). Iso H-7, an H-7 isomer which does not affect serine/threonine kinase activity (33), had no effect on the subcellular distribution of gag MA within acutely infected cells (Fig. 3*B*). Neither serine nor tyrosine kinase inhibitors influenced subcellular distribution of a nonmyristoylated (gag MA S$^6$→A) gag mutant of HIV-1 (Fig. 3*B*). Taken together, these results indicate that gag MA phosphorylation facilitates its nuclear localization. Inhibition of gag MA phosphorylation reduces dissociation of gag MA from the membrane but only in the context of gag MA, which contains a membrane anchoring signal.

We next examined the infectivity of hypophosphorylated HIV-1 virions in an integration-dependent transactivation assay (34), which provides an indication of the ability of HIV-1 to translocate to and integrate within the host cell nucleus. Inhibition of gag MA phosphorylation on tyrosine using genistein, or on serine using H-7 but not using Iso H-7, impaired HIV-1 infectivity in dividing and in nondividing ($G_2$ arrest by γ-irradiation) target cells (Fig. 4*A*). In contrast, the infectivity of the HIV-1 gag MA myristoylation minus mutant was unaffected by either tyrosine kinase or serine kinase inhibitors in dividing or in $G_2$-arrested target cells (Fig. 4*A*). Effects of the kinase inhibitors on HIV-1 infectivity were not altered in the presence or absence of a functional vpr gene, which cooperates with gag MA in nuclear import (9).

We next examined the stage at which the kinase inhibitors were attenuating HIV-1 infectivity. PCR and primers to LTR R/gag and LTR R/LTR U5 regions of the viral genome were used to specifically amplify full-length viral cDNA (22) and 2 LTR circle forms (10) of viral DNA, respectively. Since viral DNA circles are formed specifically in the nucleus (9, 35), they represent a marker with which to follow nuclear localization of viral DNA. Kinetics of viral cDNA synthesis following infection of CD4$^+$ MT4 cells with hypophosphorylated (genistein) HIV-1 was equivalent (Fig. 4*B*). Thus, at 48 hr postinfection, viral cDNA synthesis (as quantitated by volume integration of PCR products) in cells infected with hypophosphorylated HIV-1 was 78% of that in cells infected with untreated virus. Since the LTR R/gag primers amplify predominantly full-length and almost full-length viral cDNA, neither the rate nor the extent of viral DNA synthesis was markedly reduced following infection with hypophosphorylated HIV. This indicated that inhibition of gag MA phosphorylation did not significantly influence virus binding, fusion, uncoating, or reverse transcription *per se*. In contrast, nuclear forms of viral

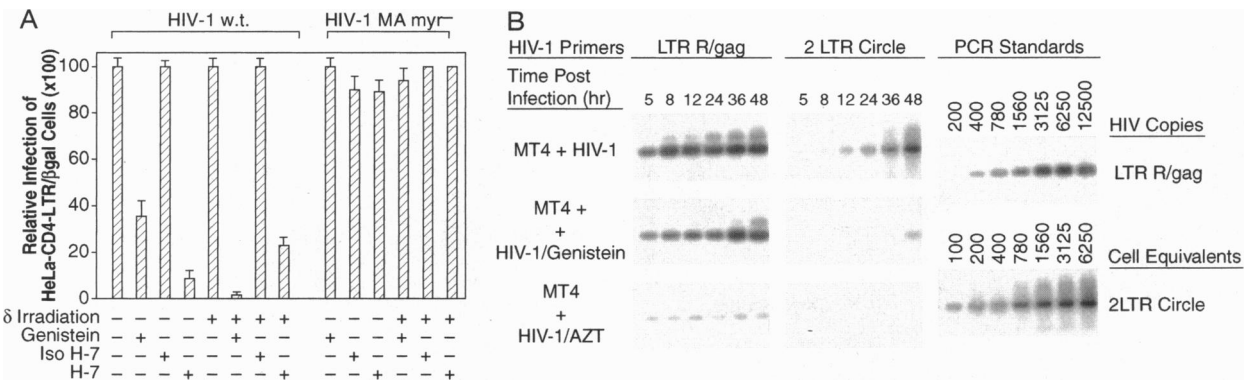


FIG. 4. Gag MA phosphorylation is required for HIV-1 infectivity. (*A*) HIV-1 was prepared in the presence of the indicated kinase inhibitors as outlined in Fig. 3. HeLa-CD4-LTR/β-gal target cells (15) were infected with equal amounts of HIV-1 (based on RT activity) produced in the presence of the respective protein kinase inhibitors. In this assay, upon HIV-1 infection and integration, tat production leads to transactivation of a β-galactosidase gene under control of the HIV-1 LTR. Infected cells are scored after visualization of β-galactosidase activity *in situ* by staining cells with 5-bromo-4-chloro-3-indolyl β-galactopyranoside (34). Relative cell infection is the number of cells infected by hypophosphorylated virions divided by the number of cells infected by wild-type HIV-1 (mean ± SD, $n = 6$). (*B*) Phosphorylation of gag MA is required for nuclear localization of viral DNA. MT4 cells were infected with hypophosphorylated and wild-type HIV-1 as outlined in Fig. 3. At the indicated times postinfection, cell aliquots were withdrawn for isolation of total cellular DNA and analysis of full-length and nucleus-specific 2 LTR circle forms of viral DNA by PCR. Infections carried out in the presence of 3′-azido-3′-deoxythymidine (AZT; μM) were used to verify *de novo* synthesis of viral cDNA.

DNA, as evidenced by the abundance of 2 LTR circle forms, were markedly reduced in cells infected with hypophosphorylated HIV (Fig. 4*B*). By volume integration (9), the level of 2 LTR circle products in cells at 48 hr following infection with hypophosphorylated HIV-1 was 6% of that in cells infected with untreated virus. Taken together, these results suggest that inhibition of gag MA phosphorylation prevented nuclear localization of viral cDNA following virus infection.

## DISCUSSION

Our studies reveal a previously unsuspected level of regulation of primate lentivirus infectivity. Phosphorylation of gag MA appears predominantly to regulate the membrane targeting of gag MA by allowing dissociation of gag MA from the target cell membrane. An analogous mechanism of phosphorylation-dependent membrane dissociation has been implicated in translocation of the myristoylated alanine-rich protein kinase C substrate (MARCKS) between the membrane and the cytoplasm (36). Phosphorylation of MARCKS protein increases the negative charge on the molecule, which causes its dissociation from the negatively charged membrane (37). In our study, single amino acid substitutions within highly conserved serine residues did not markedly influence HIV-1 infectivity either in dividing or in nondividing cell systems (not shown). Since multiple residues within gag MA were phosphorylated, it is likely that the additive effect of these multiple phosphate acceptor sites imparts a strong negative charge on MA, thus promoting its dissociation from the negatively charged membrane of the target cell. Recently, Gallay *et al.* (29) demonstrated that a single C-terminal tyrosine ($Y^{132}$) of gag MA was critical for phosphorylation-dependent translocation of gag MA from the membrane to the nucleus and for HIV-1 infectivity of nondividing cells. In our hands, a gag MA $Y^{132}$ mutant, either with or without a functional Vpr, was fully infectious in dividing (MT4) and nondividing (macrophages) cell systems and was differentially phosphorylated on tyrosine. Thus, our studies suggest a greater complexity in the phosphate acceptor sites that regulate gag MA localization in the target cell and that influence HIV infectivity.

Our results provide evidence for the association of a serine/threonine kinase with virions of HIV-1. Although association of protein kinases with virions has been described for several enveloped and nonenveloped viruses, to our knowledge, the presence of a serine/threonine kinase in virions has not been described for other retroviruses. While gag MA was phosphorylated predominantly on tyrosine prior to and during virus assembly, gag MA in native virions and in preintegration complexes isolated from target cells was phosphorylated exclusively on serine. It is possible that tyrosine phosphorylation and serine phosphorylation of gag MA operate independently in virus infectivity. Phosphorylation of gag MA on tyrosine during and immediately following virus production may facilitate dissociation of gag MA from the membrane phospholipids of the nascent virus particle. This would allow MA to enter a compartment of the virion (for example, the virus core) where it can subsequently be incorporated into viral preintegration complexes. Serine phosphorylation of gag MA within nascent virions and within viral preintegration complexes would facilitate dissociation of viral preintegration complexes from the cell membrane at the site of virus entry and, additionally, prevent reassociation of viral preintegration complexes with cytosolic membranes as the complex localizes to the host cell nucleus. Studies aimed at the identification of kinases involved in phosphorylation of gag MA should aid our understanding of the regulation of HIV-1 entry and may point to novel targets for inhibition of HIV-1 infection.

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